Enzymatic treatment of wastewater sludge in presence of a cation binding agent

- improved solubilisation and increased methane production

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Master of Science Thesis

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Ronja Beijer
Master of Science Thesis performed at Stockholm Water AB
2008-03-19

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Preface

This Master of Science Thesis is performed at Henriksdal wastewater treatement plant belonging to Stockholm Water AB and is part of my Master of Science in Engineering Biology at Linköping University.

I would like to thank my supervisor at Stockholm Water Doctor Daniel Hellström for the opportunity to perform this thesis work at Henriksdal wastewater treatment plant (WWTP) and for commenting on my written thesis. Also, thank you Lena Johnsson for reading and commenting my thesis.

Further, I will specially thank Doctor Joanna Wawrzyńczyk, Kemira Helsingborg for all her guidance and for being so friendly. All your help has been invaluable to me. Also thank you to Armina Mustafic and Doctor Olof Norrlöw, Kemira Helsingborg for letting me visit and learn more about enzymatic treatment of wastewater sludge and Mikael Hansson, JTI for performing the batch laboratory digestion tests.

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THANK YOU!

Abstract

Stockholm Water is a water and sewage company with Henriksdal as one of two wastewater treatment plants (WWTPs). At Henriksdal wastewater sludge generated in the wastewater treatment process is digested which generate biogas; a mixture of mainly methane and carbon dioxide. If purified to methane content of 96 - 98 % this gas is called biomethane.

Biogasmax is a project aiming to reduce the use of fossile fuels in Europe by providing that biogas is a good technical, economical and environmental alternative as a vehicle fuel. The specific aim for Stockholm Water is to increase the biogas production at the existing plant in Henriksdal. Enzymatic treatment of wastewater sludge is an innovative technique earlier proofed to increase the biogas production from wastewater sludge with up to 60 %. The enzyme activity is in turn proven to significantly increase in the presence of a cation binding agent.

One aim with this thesis was to investigate if the sludge from Henriksdal wastewater treatment process at all is affected of enzymatic treatment in presence of the cation binding agent sodium citrate since this has shown to have some significance. The chemical oxygen demand (COD) was measured in the liquid phase of sludge after treatment and used as a measurement of treatment effect. Another aim of this thesis was to look into the possibility to increase the methane production from sludge at Henriksdal WWTP through enzymatic treatement in presence of sodium citrate. This was investigated through batch laboratory digestion tests.

The sludge from Henriksdal WWTP was shown to be a good substrate for the enzymes added. COD in the liquid phase was increased with 17 – 32 % depending on the dose of enzymes and sodium citrate added. Digestion of sludge with a total addition of 18.6 mg enzymes per 1 g total solids (TS) and a concentration of 5 mM sodium citrate increased the methane production with almost 18 % compared to untreated sludge. This equals an increase of 18.3 % when converted to represent a totally blended and continuous digestion chamber at Henriksdal WWTP. The increased methane production also results in a sludge reduction out from the digestion chambers. The increased methane production and sludge reduction though does not fulfil the increased costs for the enzymes and sodium citrate applied. These doses must be decreased and the costs for both enzymes and sodium citrate must be reduced for this technique to be economically feasible in a full scale operation.

Keywords: Anaerobic digestion, biogas, methane, hydrolytic enzymes, cation binding agents

Sammanfattning

Stockholm Vatten är ett vatten- och avloppsföretag och Henriksdal är ett av två avloppsreningsverk som tillhör Stockholm Vatten. Vid Henriksdal bryts slam från reningsprocessen av avloppsvatten ned till biogas i en anaerob process. Biogas består huvudsakligen av metan och koldioxid och när denna gasblandning renas till en metanhalt på 96 – 98 % kallas den biometan.

Biogasmax är ett projekt vars mål är att reducera användandet av fossila bränslen i Europa genom att visa att biogas är ett bra tekniskt, ekonomiskt och miljömässigt alternativ som fordonsbränsle. Stockholm Vattens specifika mål inom detta projekt är att öka biogasproduktionen vid den befintliga anläggningen i Henriksdal. Enzymatisk behandling av avloppsslam är en innovativ teknik som tidigare visats öka biogasproduktionen från avloppsslam med upp till 60 %. Tidigare studier har också visat att den enzymatiska aktiviteten höjs i närvaro av en katjonbindare.

Ett av målen med detta projekt var att undersöka om slammet från Henriksdal överhuvudtaget påverkas av enzymatisk behandling i närvaro av katjonbindaren natriumcitrat. COD (chemical oxygen demand) mättes i slammets vätskefas efter behandling och användes sedan som ett mått på hur slammet påverkades av behandlingen. Ett annat mål var att se på möjligheterna att öka metangasproduktionen från Henriksdalsslam vid tillsats av hydrolytiska enzymer och natriumcitrat. Detta undersöktes genom satsvis utrötning.

Slam från Henriksdals avloppsreningsverk visade sig vara ett bra substrat för enzymerna. COD i vätskefasen kunde ökas med 17 – 32 % beroende på vilken dos av enzymer och natriumcitrat som användes. Utrötningsförsöket av slam med totalt 18.6 mg enzymer per 1 g torrsubstans vid en 5 mM koncentration av natriumcitrat gav nästan 18 % ökning av metangasproduktionen jämfört med obehandlat slam. Detta motsvarar 18.3 % ökning omräknat till att gälla en totalomblandad och kontinuerlig rötkammare vid Henriksdal. Denna ökade metangasproduktion resulterade också i en minskad rötslamsmängd ut från rötkamrarna. Det är dock så att den ökade metangasproduktionen och reducerade rötslamsmängden inte täcker upp kostnaderna för de tillsatta enzymerna och natriumcitratet. Tillsatserna av enzymer och natriumcitrat måste minskas och kostnaderna för dessa måste reduceras för att denna teknik ska vara ekonomiskt lönsam i fullskala.

Nyckelord: Anaerob nedbrytning, biogas, metan, hydrolytiska enzymer, katjonbindare

Definitions and abbreviations

The abbreviations used within this thesis are listed below together with some short definitions.

AD	**Anaerobic Digestion** *Degradation of organic matter in an oxygen free environment*
COD	**Chemical Oxygen Demand** *Oxygen required oxidising a specific amount of organic matter*
EAS	**Excess Activated Sludge** *Sludge generated in the biological treatment of wastewater*
EOM	**External Organic Material** *External incoming organic material to the anaerobic digestion process not generated in the wastewater treatment process; for example fat from provision productions and restaurants*
FAE	**Fatty alcohol ethoxylate** *Surface active substance which lowers the interfacial tension in a mixture with hydrolytic enzymes*
HRT	**Hydraulic Retention Time** *Average time an aqueos system is present in a digestion chamber*
OL	**Organic Load** *Amount of degradable substrates pumped in to a digestion chamber*
PPG	**Polypropylene glycol** *Improves the stability of hydrolytic enzymes during storage*
SGP	**Specific Gas Production** *Methane production per amount of organic substance expressed in terms of standard temperature and pressure (STP)*
SRT	**Solids Retention Time** *Average time solid matter is present in a digestion chamber*
TS	**Total Solids** *The remaining solids in the sludge after removal of water*
VFA	**Volatile Fatty Acid**
VS	**Volatile Solids** *The organic part of TS*
WWTP	**Wastewater Treatment Plant**
	Methane potential *Methane produced when time goes to infinite in an anaerobic digestion process.*

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1 Introduction

This section introduces the reader to the subject of interest within this thesis. The problems ending up in the specific aim of the thesis is further presented together with the methods used to find answers to the problems. These sections are followed by the delimitations in the thesis work and finally a disposition of the thesis project.

1.1 Background

Henriksdal wastewater treatment plant (WWTP) is one of two WWTPs belonging to Stockholm Water AB. Sludge from the wastewater treatment process in Henriksdal is digested in an anaerobic process which generates biogas. The biogas contains mainly methane and carbon dioxide and if purified from carbon dioxide this gas is called biomethane and can be used as a vehicle fuel. The energy content in 1 Nm^3 biomethane is about the same as in 1 liter petrol. The biogas can also be used as a source of heating of the own plant or for production of electricity and heat by gas engines. (Stockholm Water 1, 2008)

The growing environmental consciousness and the followed interest in using biomethane as a vehicle fuel has put a pressure on WWTPs to produce more biogas. Henriksdal WWTP is in a period of transition to using district heating instead of heating with the own produced gas. The produced gas is more valuable as vehicle fuel then as a source of heating of the own plant. (Hellström, personal communication)

Stockholm Water is taking part of a project called Biogasmax run by seven city regions in Europe; Lille, Stockholm, Torun, Gothenburg, Zielona Góra, Berne and Rome. The aim with this project is to reduce the use of fossil fuels in Europe by increasing the use of biogas as an alternative fuel. 1 liter petrol generates 2.5 kg fossil carbon dioxide while biogas contributes to no net increase in discharge of carbon dioxide. The discharge of carbon dioxide during biogas combustion is the same amount as the carbon dioxide bound in the plants months before. The project Biogasmax proceeds from 2006 to 2009 and should in the end show that biogas is a good technical, economical and environmental alternative as a vehicle fuel. Stockholm Water has a task together with Swedish Biogas to apply tools for increased biogas production at the plant in Henriksdal. (Vallin et al., 2008; Stockholm Water 1, 2008) The biogas production has been proofed to enhance when using different disintegration methods. These methods can be mechanical, chemical or biological. (Wawrzyńczyk, 2007)

1.2 Definition of the problem

Previous studies performed by Borggren (2008) have shown that the sludge used in the biogas process at Henriksdal WWTP have a bigger methane potential than the methane being utilized today. Davidsson (2007), Davidsson et al. (2007) and Wawrzyńczyk et al. (2003) have shown that biological treatment; treatment of sludge with hydrolytic enzymes could increase the methane potential up to 60 %. The enzymes are shown to improve the hydrolysis of organic matter in sludge which is the rate limiting step in the anaerobic digestion (AD) process. This brings up questions of the possibility to implement this

technique on the sludge at Henriksdal WWTP and utilize some of the remaining methane potential. The efficiency of the enzymatic treatment depends among other factors on the composition of the sludge. The organic matter in sludge is hardbound in flocs maintained by cations which complicate the action of the enzymes. Wawrzyńczyk (2007), Wawrzyńczyk et al. (2003) and Davidsson et al. (2007) have shown that a part of the enzymes became entrapped in the sludge matrix and therefore became inactivated. Addition of a cation binding agent prior to enzymatic treatment of sludge was shown to improve the enzyme action in the studies performed by Wawrzyńczyk et al. (2007a and 2007b) The cation binding agents made it easier for the enzymes to reach the organic matter through binding to the cations maintaining the floc structure of organic matter. The treatment process of the wastewater in Henriksdal involves iron and the use of a cation binding agent in the enzymatic treatment of sludge from Henriksdal WWTP is therefore probably important.

One question is if the sludge at Henriksdal WWTP is at all affected of the enzymatic treatment in presence of a cation binding agent? A resulting question is if an increasing dose of enzymes and cation binding agents improves the possible effect? Another question is if the utilization of methane gas from the sludge at Henriksdal WWTP increases with the addition of hydrolytic enzymes and cation binding agents? If so, how big is this increase and is it big enough to fulfill the increasing costs corresponding to the enzymes and cation binding agents if the technique is implemented in a full scale operation at Henriksdal WWTP?

1.3 Aim of the thesis

The specific aim of this thesis was to look into the possibility to increase the methane production from the sludge in Henriksdal WWTP through the addition of hydrolytic enzymes and cation binding agents. If the methane gas was shown to increase with this treatment a lower dose of enzymes and a lower concentration of cation binding agents should be tried out to reduce the costs. Further an estimation of the profits corresponding to the possible increase in methane production should be done and an approximate calculation of the costs for the enzymes and sodium citrate applied.

1.4 Methods

To provide answers to the questions that ended up in the aim of this thesis a lot of literature has been collected and read and many consultations with competent persons has been held. A visit in Lund at the disputation of the thesis *Enzymatic treatment of wastewater sludge; sludge solubilisation, improvement of anaerobic digestion and extraction of extracellular polymeric substances* performed by Joanna Wawrzyńczyk was made at the beginning of this project. Further a visit at Kemira in Helsingborg was made to get further knowledge of the performance of the enzymatic treatment process.

Batch laboratory digesion tests were performed to evaluate the possibility to increase the methane production in sludge from Henriksdal WWTP treated with hydrolytic enzymes and a cation binding agent. Because of the long time required to determine the methane potential with batch laboratory digestion the selection of the lower enzyme dose and cation binding agent concentration should be done with a faster measurement of the biodegradability of

the sludge. The increase in solubilisation of organic matter as a result of enzymatic treatment in presence of cation binding is such a measurement. Solubilisation of the sludge showed if the sludge was a good substrate for the enzymes and cation binding agent applied. The technique and equipment used in the solubilisation experiments was tried out during a few weeks before the real trial could begin.

1.5 Delimitations

At the beginning of this project the purpose was to perform the batch laboratory digestion tests at Henriksdal WWTP with newly bought equipment using continuous measurements of the produced methane. However, problems with delivery and function of equipment made it practically impossible to perform these test within the time frame of this project. The batch laboratory digestion tests were therefore performed at Swedish Institute of Agricultural and Environmental Engineering (JTI). Because of the time limits only one cation binding agent was tried out and this was the one shown to be most effective in previous studies performed by Wawrzyńczyk et al. (2007a). The enzyme mixtures used were also a result of previous studies. The time limits also lead to that the effect of the enzymes and cation binding agents themselves on the solubilisation of organic matter in sludge was not investigated. Wawrzyńczyk et al. (2007a) has shown that the combination of enzymes and cation binding agents has the greatest impact on the solubilisation of organic matter and therefore this selection was made.

1.6 Outline of the thesis

This thesis work is divided in several chapters which in turn are divided in many subchapters. *Theoretical background* is the first chapter and the main theme in this chapter is AD. The microbiology, investigation methods of AD and previous studies are discussed in this chapter. The following chapter, *Stockholm Water*, is a presentation of the WWTP where this project was carried out, Henriksdal WWTP. This chapter is an overview of the wastewater treatement process and anaerobic digestion process at Henriksdal WWTP. Further in the *Experimental* part there is a presentation of the chosen methods within this thesis and the used substrates and reagents. The *Results* chapter presents the obtained results in figures and tables, the *Discussion* chapter discuss the most interesting results with the improvement of AD as a result of enzymatic treatment of sludge in presence of a selected cation binding agent as the centre of gravity and the *Conclusions* chapter summarizes the most important conclusions from this master thesis work.

2 Theoretical background

The main theme in this chapter is AD. First this chapter provides with an introduction to AD and the microbiology meaning the involved microorganisms in AD and the conversion of organic matter in wastewater sludge to biogas. Further the environmental factors and process parameters in AD are described and a presentation of the methods used in the investigation of AD is made. At last the reader is introduced to the techniques of using hydrolytic enzymes and/or cation binding agents in the treatment of wastewater sludge and the advantages and difficulties of such techniques.

2.1 Anaerobic digestion

The need for adequate treatment and disposal of sludge from WWTPs is an increasing problem (Davidsson, 2007). The problem involves large sludge volumes because of water binding to organic matter in the sludge. AD is a technology for treatment and handling of waste and is carried out in digestion chambers. The AD is used to stabilize solids in the sludge, meaning degrade the organic matter and reduce the sludge volume. The organic matter is degraded through the action of microorganisms that occur naturally in the sludge. This takes place in the absence of oxygen through parallel metabolic pathways. The main products are carbon dioxide and methane. (Gurgo e Cirne, 2006)

2.1.1 Microorganisms involved in anaerobic digestion

Organic matter in wastewater sludge consists of lipid, carbohydrate and protein molecules, often very complex. Complex, big molecules can not penetrate the cell membrane and are therefore not directly available as substrates for the microorganisms to digest. Microorganisms in the sludge produce enzymes to degrade these substrates to smaller molecules which then enter the cells and are digested. The microorganisms which produce these enzymes are obligate or facultative anaerobes. (Gurgo e Cirne, 2006; Davidsson, 2007)

Two types of enzymes are involved in the substrate degradation; exoenzymes and endoenzymes. Exoenzymes are produced inside the microorganism cells but released to solubilise particulate insoluble substrates attached to the cell walls. Once solubilised, these substrates enter the microorganism cells where the degradation takes place. Endoenzymes, also produced inside the microorganism cells, are the ones responsible for degradation of these and other soluble substrates within the cell. Endoenzymes are produced by all microorganisms but exoenzymes are not. Each endo- and exoenzyme does only degrade a specific substrate or group of substrates and no microorganism produce all the enzymes needed to degrade the large variety of substrates in sludge. Therefore a large variety of microorganisms is needed to ensure an adequate degradation of wastewater sludge. (Gerardi, 2003)

The metabolism of the solubilised organic matter into methane is performed by several groups of microorganisms in the sludge. In anaerobic digestion chambers there are three important groups of microorganisms named after the substrates being utilized. These groups are the acetate forming bacteria, the sulphate reducing bacteria and the methanogens. As the name reveal, the acetate forming bacteria are a producer of acetate. It also grows in a symbiotic relationship with the methanogens. Methanogens consume the hydrogen

produced when ethanol for example is converted to acetate by the acetate forming bacteria. Acetate forming bacteria can only survive at very low concentrations and pressure of hydrogen and the generation time for these organisms is usually greater than 3 days. Therefore this symbiotic relationship is very important. (Davidsson, 2007; Gerardi 2003)

Methanogens are the microorganisms producing methane and they are grouped in the domain archae microorganisms. Archae means ancient and these microorganisms are some of the oldest. Methanogens are oxygen sensitive and the only organism producing methane. They have a long generation time which requires a high retention time in an anaerobic digestion chamber to ensure a high population of methanogens for the digestion of the organic compounds. The methanogens can be divided in two groups with respect to the utilized substrates. These groups are the hydrogenotrophic methanogens and the acetotrophic methanogens. (Davidsson, 2007; Gerardi 2003)

Sulphate reducing bacteria are found in the presence of sulphate and they reproduce using hydrogen and acetate. Hydrogen is also consumed when sulphate is used in the degradation of an organic compound. This causes a competition between sulphate reducing bacteria and methanogens for the hydrogen and acetate present. The sulphate reducing bacteria obtain hydrogen and acetate more easily so with a high sulphate concentration the sulphate reducing bacteria win the competition of hydrogen and acetate. On the other hand if the concentration is low, the methanogen are favoured. (Gerardi, 2003)

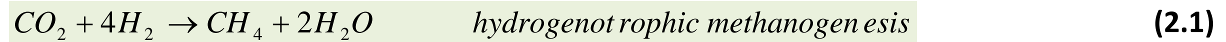
2.1.2 Microbiology in anaerobic digestion

AD is often divided in three stages. These stages are *hydrolysis*, *acid forming* and *methanogenesis*. An overview of these stages can be seen in Figure 1. An efficient AD is when the degradation rates of all reactions are equal. (Gerardi, 2003)

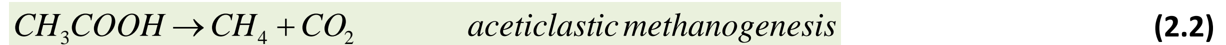
As described in section 2.1.1 the need for enzymes to degrade the complex organic molecules into smaller soluble molecules are important. Hydrolytic enzymes produced by the facultative and anaerobic microorganisms degrade the complex protein, carbohydrate and lipid molecules to amino acids, sugars and fatty acids in the first step of AD, the hydrolysis. (Davidsson, 2007) These smaller molecules are soluble and quickly go into solution. Hydrolysis is known to be the rate limiting step in the AD especially when sludge contains a lot of complex substrates. (Gurgo e Cirne, 2006)

The acid forming stage is degradation of the compounds produced in the hydrolysis, by facultative anaerobes and anaerobes. This stage can be further divided in acidogenesis and acetogenesis. Acidogenesis is often the fastest step in the AD and the products are acetate, hydrogen, carbon dioxide, alcohols and volatile fatty acids (VFA). Example of VFAs is acetic, propionic, butyric and valeric acid. In the acetogenesis acetate, hydrogen and carbon dioxide is formed from long chain fatty acids and the VFA produced in the acidogenesis. (Davidsson, 2007; Wawrzyńczyk, 2007) The main substrates in the methanogenesis are acetate, carbon dioxide and hydrogen. About 2/3 of the produced methane in an anaerobic digestion chamber originates from a conversion of acetate in the methanogenesis. Two different methanogens are responsible for the methane production in the methanogenesis; hydrogen utilizing methanogens and aceticlastic methanogens. Hydrogen utilizing methanogens form methane from carbon dioxide and hydrogen in the hydrogenotrophic methanogenesis

(equation 2.1 below) while aceticlastic methanogens cleave acetate to form methane and carbon dioxide in the aceticlastic methanogenesis (equation 2.2 below). The general composition in biogas produced from wastewater sludge is 50 - 60 % methane and 40 - 50 % carbon dioxide. (Davidsson, 2007; Wawrzyńczyk, 2007)



$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \textit{hydrogenotrophic methanogenesis} \qquad \textbf{(2.1)}$$



$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \textit{aceticlastic methanogenesis} \qquad \textbf{(2.2)}$$

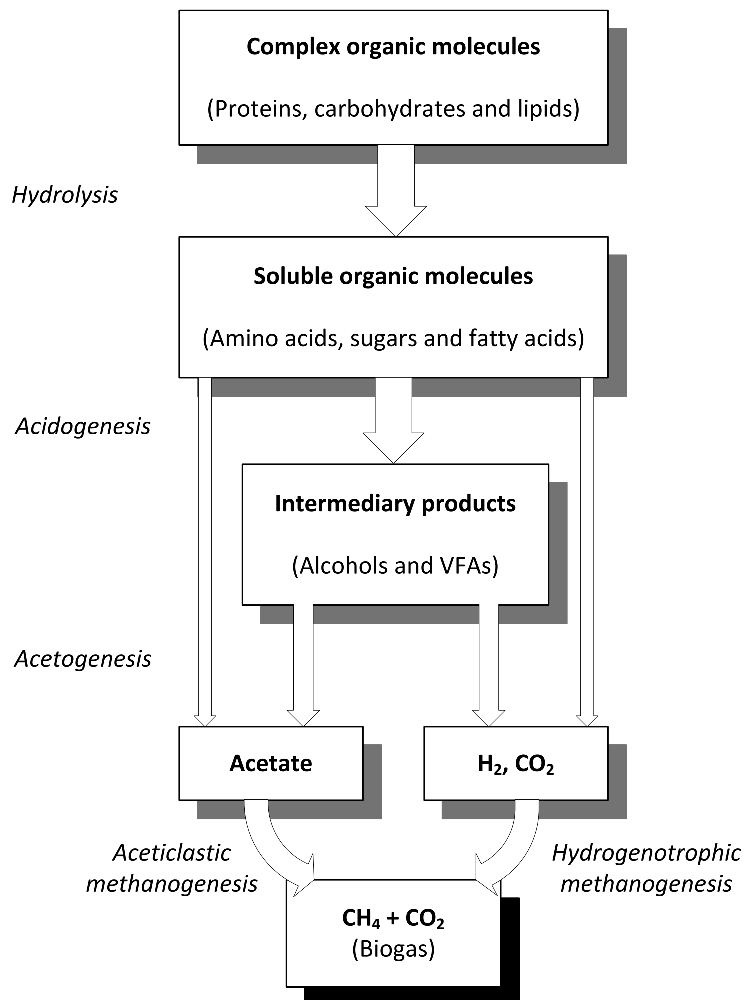


Figure 1: A schematic view of the degradation steps of carbon in the AD process. The figure is modified from Davidsson (2007).

2.1.3 Environmental factors in anaerobic digestion

The methanogens in wastewater sludge are very sensitive and it is therefore important to provide for good environmental factors in the AD chambers. This is not an easy task because one condition may affect another and the microorganisms in the sludge have different optimums. The AD process is influenced by conditions like temperature, pH, toxicants and nutrients available in the sludge. The text in this section is composed from Davidsson (2007).

Temperature

AD can take place at different temperatures. Regardless of temperature it is important to keep the temperature constant and uniform throughout the whole sludge volume. This is for example accomplished through thorough mixing. Variations in temperature can lead to undesired activity and/or inhibition of bacteria.

The two most common temperature intervals used in large scale applications are the mesophilic and thermophilic intervals (see Table 1). Mesophilic digestion is performed at temperatures between 15°C and 45°C with an optimum of 35°C and thermophilic digestion is performed at temperatures between 45°C and 75°C with an optimum of 55°C. Most of the methanogens are active in these intervals principally in the mesophilic interval.

The rate of AD and the methane production is proportional to the temperature in the digestion chambers. A higher temperature results in a higher destruction rate of volatile solids (VS) meaning a higher methane production. The greater destruction of pathogens in thermophilic conditions also benefits the reuse of wastewater treatment sludge. One disadvantage of thermophilic digestion because of the high reaction rates are the accumulation of acids produced in the acidogenesis. If the production rate of these acids is greater than the rate of which methanogens can convert them, there is a risk of imbalance in the reactor. Another disadvantage of thermophilic digestion when compared to mesophilic digestion is that thermophilic digestion is more sensitive to ammonia produced when the sludge treated has high nitrogen content.

Table 1: Temperature range and temperature optimum; the two most common divisions of AD. The table is modified from Davidsson (2007).

	Temperature range [°C]	Temperature optimum [°C]
Mesophilic digestion	15 – 45	35
Thermophilic digestion	45 – 75	55

Nutrients

The microorganisms in the sludge responsible for the conversion of organic matter in sludge to methane require a number of substances to maintain an adequate AD process. Carbon, nitrogen and phosphor are the most important substances needed in the growth of these microorganisms. The nitrogen quota is important to balance with the quota of carbon.

Production of the enzymes needed to utilize the carbon is hindered when there is too little nitrogen available for the microorganisms. On the other hand a too large amount of nitrogen can inhibit the growth of the microorganisms. The optimum C: N ratio for AD is often suggested to be in the range 20:1 to 30:1.

Toxicants

A part from the importance to provide the microorganisms with the nutrients needed in the growth it is also important to prevent inhibition of the methanogens by toxic substances such as for example volatile fatty acids. This substance can either originate from the feed of the sludge to the digestion chambers or be produced during the AD process.

pH

pH in the sludge is also an important factor for an adequate AD process. The microorganisms in the sludge have growth optima at different pH values. The pH optima for the acidogens are at 6 and for the methanogens and acetogens around 7. A pH between 6 and 7 is therefore desirable for AD.

2.1.4 Process parameters

The process parameters of AD differ among processes and are important to control (Vallin et al., 2008). Some of the parameters; retention time, total solids (TS), volatile solids (VS), chemical oxygen demand (COD) and organic load (OL) are described in this section. If not stated in the text this section is reviewed from Vallin et al. (2008).

Retention time

The retention time can be measured either as the hydraulic retention time (HRT) or as the solids retention time (SRT). The HRT is the average time an aqueous system is present in a digestion chamber and the SRT is the average time solid matter is present in a digestion chamber. The SRT is of big importance in the microorganism growth. Today in Sweden most of the digestion chambers are continuously and fully blended and the aqueous phase is not separated from the solid phase meaning the HRT is equal to the SRT. If the solid matter is instead separated from the aqueous phase and recirculated to the digestion chambers the HRT and SRT can be controlled independently. The adequate microorganism growth is then maintained even if the HRT is kept low to reduce the volume of the digestion chambers.

Total solids, volatile solids and chemical oxygen demand

The content of all wastewater sludge is extremely complex and differs among treatment plants. All wastewater sludge though contains proteins, lipids, carbohydrates and nondigestible substances. (Davidsson, 2007)

The sludge is referred to as a substrate when used in the AD and can be divided in two parts, water and TS. TS are consequently the remaining solids in the sludge after removal of water. In the TS determination sludge is heated to 105 °C for at least 12 hours. TS are determined;

$$TS\ [\%] = \frac{Weight\ after\ heating\ to\ 105°C}{Weight\ before\ heating} \cdot 100 \qquad (2.3)$$

TS can be further divided into VS and fixed solids (FS). The VS is the organic part of TS whereas FS is the inert part. In the VS determination sludge already heated to 105 °C is further heated to 550 °C for 2 hours. The VS in the sludge is determined;

$$VS\ [\%] = \frac{Weight\ after\ heating\ to\ 105°C - Weight\ after\ heating\ to\ 550°C}{Weight\ before\ heating} \cdot 100 \quad \textbf{(2.4)}$$

COD is the amount of oxygen consumed in the oxidation of organic matter in wastewater sludge and is measured as mg O_2/l. COD can be measured in the total sludge or in the liquid or solid phase of separated sludge. (Borggren, 2008) In this thesis the COD in the liquid phase is the only one measured and is referred to as soluble COD (COD_{sol}).

<u>Organic load</u>

The OL is the amount of degradable substrates pumped in to a digestion chamber every day and is determined;

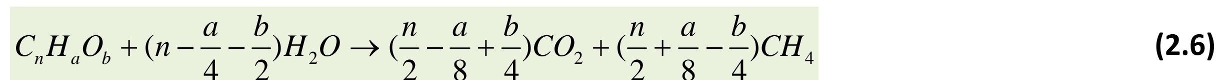
$$OL\ [kg\ VS/m^3 \cdot day] = \frac{kg\ VS\ pumped\ in\ to\ a\ digestion\ chamber\ every\ day}{Volume\ of\ digestion\ chamber} \quad \textbf{(2.5)}$$

2.2 Investigation of anaerobic digestion

Sludge from WWTPs is referred to as a substrate when utilized in AD and has an inherent methane potential. The methane potential is the methane produced when time goes to infinite in an anaerobic digestion process. In this section suggested calculation models of the theoretical methane potential in the substrate is presented, a method to determine the real methane potential are described and two equations for the determination of the degree of degradation of the substrate is shown. Also a short trial method which gives an indication of the forthcoming real methane potential of the sludge is described.

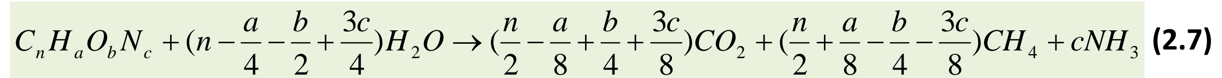
2.2.1 Theoretical methane potential

The text in following section is composed from Davidsson (2007). The theoretical methane potential can be determined when the elemental composition of a substrate is known. The equation used is the Buswell formula (2.6) when expressing the organic compound as $C_nH_aO_b$.



$$C_nH_aO_b + (n - \frac{a}{4} - \frac{b}{2})H_2O \rightarrow (\frac{n}{2} - \frac{a}{8} + \frac{b}{4})CO_2 + (\frac{n}{2} + \frac{a}{8} - \frac{b}{4})CH_4 \quad \textbf{(2.6)}$$

Equation 2.7 below is an extended version of the Buswell formula including nitrogen. The organic compound is now expressed $C_nH_aO_bN_c$.



$$C_nH_aO_bN_c + (n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4})H_2O \rightarrow (\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8})CO_2 + (\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8})CH_4 + cNH_3 \quad \textbf{(2.7)}$$

The component composition can also be used to determine the theoretical methane potential with the Buswell formula. This is implemented by using the average chemical formulas for the components in the substrate. Protein, carbohydrate and fat are useful components when calculating the theoretical methane potential from wastewater sludge. Table 2 shows the average chemical formulas for the corresponding components.

Table 2: Average chemical formulas for protein, fat and carbohydrates. The table is modified from Davidsson (2007).

Component	Chemical formula
Fat	$C_{57}H_{104}O_6$
Protein	$C_5H_7NO_2$
Carbohydrate	$(C_6H_{10}O_5)_n$

Davidsson (2007) suggest that even the COD content can be used to calculate the theoretical methane potential from a substrate when expressing the organic compound as $C_nH_aO_bN_c$. The methane produced from such a compound is given above (equation 2.7):

$(\frac{n}{2} + \frac{a}{8} + \frac{b}{4} - \frac{3c}{8})$ mole CH_4 per mole organic substance and the oxygen demand required to oxidise the organic substance is according to Davidsson (2007) given by:

$(n + \frac{a}{4} - \frac{b}{2} - \frac{3c}{4})$ mole O_2 per mole organic substance.

The result from these calculations is a methane potential of 350 Nm^3CH_4 per tonne COD in the sludge. This value is given at 0°C and 1 atm pressure.

2.2.2 Real methane potential

The real methane potential within a substrate can be determined through digestion tests. These tests can be in small scale (a few ml) up to full scale (thousands of m^3). The AD is a rather slow and sensitive process and therefore a long time is needed for start up. Before a new technique is to be implemented in an existing full scale AD process (change in temperature, introduction of a new incoming substrate or implementation of a sludge treatment method such as for example enzymatic treatment, described below in section 2.4.2) this technique should first be tried out in a small scale operation. The batch laboratory digestion is a small scale method easy to implement and below is a short introduction to this method. (Davidsson, 2007)

There are several batch methods used to determine the methane potential of waste. Common for these methods is incubation of a small amount of waste together with an inoculum under anaerobic conditions and measurement of the produced gas volume and its composition. The inoculum originates from an existing digestion chamber and is used in digestion tests to obtain an adequate environment of microorganisms to degrade and metabolise the waste into methane. The differences between batch methods are the technical approaches meaning the properties of the inoculum, the incubation, the gas measurement technique and the pretreatment of the sludge. The technical approaches are determined out of the purpose of measuring the methane potential and the properties of the waste used. (Hansen et al., 2003)

The methane potential of waste is often expressed in terms of standard temperature and pressure (STP) ml CH_4 per 1 g organic substance in the waste and the organic substance is often VS. This is referred to as the specific gas production (SGP) and is express below (equation 2.8). The use of SGP renders the possibility to compare the performance of different AD processes. (Hansen et al., 2003; Davidsson et al., 2007)

$$SGP = \frac{V_{CH_4}}{m_{org.subs.}} \tag{2.8}$$

where

SGP is the specific gas production [Nm^3/g VS]

V_{CH_4} is the produced volume of methane gas [Nm^3]

$m_{org.subs.}$ is the mass of organic substance [g VS]

2.2.3 *Degree of degradation in anaerobic digestion*

The degree of degradation (DD) is a measure of the effectiveness in the AD process and in which extend the substrate is degraded. This can be determined through comparison between the incoming and outgoing organic substances in the digestion chamber. The text in this section is composed from Vallin et al. (2008).

$$DD = \frac{organic\,substance_{in} - organic\,substance_{out}}{organic\,substance_{in}} \cdot 100 \quad \textbf{(2.9)}$$

The measurement of an organic substance can be for example VS or COD. In these calculations the organic substances lost during the AD process is assumed to be degraded and transformed to gas. Equation 2.9 is therefore equal with;

$$DD = \frac{Real\ methane\ production}{Theoretical\ methane\ potential} \cdot 100 \quad \textbf{(2.10)}$$

Equation 2.10 is used within this thesis when determining the DD of a substrate.

2.2.4 *Solubilisation of sludge*

Because of the long time required to determine the biodegradability of a substrate using laboratory digestion (described in section 2.2.2) there is a strong need for faster but yet reliable methods for the estimation of the biodegradability of a substrate. The determination of soluble COD in sludge when used as a substrate is such a method. When comparing different treatment methods of the sludge the release of soluble COD as a result of the sludge treatment is an indication of the forthcoming methane production. The highest release of soluble COD from the same sludge treated differently probably gives the highest methane potential. This is because the sludge solubilisation is improved which enables for the microorganisms to easier degrade the solubilised organic matter. (Wawrzyńczyk, 2007)

2.3 Extra cellular polymeric substances

Wastewater sludge is generated during the treatment process of wastewater. Different sludge are generated at different steps in the treatment process. One step involves treatment using microorganisms. The generated sludge in this step is referred to as active sludge and consists of activated sludge flocs. The wastewater treatment process in Henriksdal is described more detailed in section 3.1.1. Activated sludge flocs compose of different living microorganisms, dead cells, an inorganic fraction and large organic fragments not digested because of entrapment in the flocs. If not stated the text in this section is reviewed from Wawrzyńczyk (2007).

Extra cellular polymeric substances (EPS) are major components in the activated sludge flocs and compose of a matrix of carbohydrates, proteins (including enzymes) and humic substances mainly but also lipids, uronic and deoxyribonucleic acids. Interactions between EPS, multivalent cations, hydrophobic interactions and hydrogen bonds enable the formation of the network of polymeric substances in activated sludge.

EPS originate from active secretions of bacteria and from the organic and inorganic debris present in the activated sludge. The formation of EPS depends on a variety of functions and the composition and quantity of the EPS therefore vary markedly between sludges. Some of the factors affecting the composition and quantity of the EPS are the type and age of the sludge, the types of microorganisms present in the flocs and the cations available.

The name reveals that EPS are located at or outside the cell surface and the two separate forms of EPS are therefore bound or soluble. EPS form the space between the microbial cells in the activated sludge flocs (Chrysie et al., 2002).

The exact function of the EPS matrix is still uncertain because of its extremely heterogeneous nature. Some of the confirmed functions are; adhesion to surfaces, aggregation of bacterial cells in flocs and formation of a protective barrier that provides resistance to harmful affects such as biocides. One idea of the EPS function is that the EPS matrix allows microorganisms to live continuously at high-cell densities in stable mixed population communities. (Chrysie et al., 2002) An excess of EPS may on the other hand hinder the bioflocculation and dewatering of sludge because of the ability of EPS to bind a large volume of water.

2.4 Hydrolytic enzymes

An enzyme is a molecule which catalyzes several biological reactions. The catalysis takes place at a particular site on the enzyme called the active site. Nearly all known enzymes are proteins. (Berg et al., 2002)

There are six basic classes of enzymes; oxidoreductases, transferases, hydrolysases, lyases, isomerases and ligases. Hydrolases or *hydrolytic enzymes* used within this thesis are the second largest group. These enzymes require water to break down a chemical compound. (Wawrzyńczyk, 2007)

2.4.1 The effect of hydrolytic enzymes on the solubilisation of sludge

Hydrolytic enzymes are released from the microorganisms present in the sludge and enable the solubilisation of EPS through the act of hydrolysis, described previous. This reaction can be improved with an external treatment. Microorganisms producing hydrolytic enzymes can be added to the sludge to increase this limiting hydrolysis reaction but external added hydrolytic enzymes offer several advantages over the use of microorganisms. They are cell free, small and soluble and are therefore able to reach the substrate easier. The enzymes can also function in the presence of microorganism predators and inhibitors of microbial metabolism and they function under a wide range of environmental conditions such as temperature and pH. The enzymes also reduce the volume of the waste while

microorganisms added contribute to a large amount of biomass which increases the sludge volume. (Wawrzyńczyk et al., 2007)

The increase in soluble COD is a direct measurement of the degradation of suspended matter in the sludge. Wawrzyńczyk et al. (2003) have shown that enzymatic treatment of sludge from Källby WWTP in Lund with four glycosidic enzymes, one lipase and one protease increase the release of soluble COD with increasing enzyme dose. The duration of a typical experiment was four hours and the temperature was kept at 45 °C with a pH adjustment to 7. TS in the sludge and the enzyme concentration varied but the ratio between these was kept constant. It was shown that increasing TS content in the sludge released more COD but the relative release was rather constant.

The temperature and duration dependence on the release of soluble COD was also investigated by Wawrzyńczyk et al. (2003). A treatment in 45 °C improved the solubilisation of sludge significantly compared to treatment in room temperature and a longer treatment time lead to increased solubilisation. However the most of the release takes place within the first hours.

2.4.2 Improvement of anaerobic digestion

Below is a presentation of previous studies of the improvement of AD with the use of hydrolytic enzymes. The studies are performed in laboratory scale, pilot scale or in a full scale operation.

<u>Laboratory and pilot scale operation</u>
Wawrzyńczyk et al. (2003) have also shown that a total four hours pretreatment of sludge with four glycosidic enzymes, one lipase and one protease lead to improved biogas production in both liquid and solid phase of sludge in laboratory digestion tests at 35 °C. The largest improvement is achieved in the liquid phase and the improvement in total sludge was 60 % compared to untreated sludge when 60 mg of each enzyme was added per 1 g TS in the sludge. This makes it possible to separate the liquid and solid phase and utilize the liquid phase in a high rate digestion process.

Davidsson et al. (2007) showed that the increase of methane production from enzyme treated sludge in general was higher in pilot scale continuous digestion than in batch laboratory digestion. This was suggested to depend on the increasing amount of sludge available for the enzymes due to the increasing stirring rate in the continuous tests or the fact that fresh enzymes were added every day in the continuous test compared to the batch tests where all enzymes were added at the beginning of the process. In the continuous experiments the variations in the raw sludge are also included.

Another advantage in the continuous tests shown by Davidsson et al. (2007) was that a higher enzyme dose resulted in a significant higher methane potential while in the batch tests about the same methane potential was reached regardless of enzyme dose. Davidsson et al. (2007) suggested that this was because in the batch laboratory digestion tests the lower dose is an optimal dose and with a higher dose the enzymes are active but simply there is no substrate available for them. They further explained that in a continuous digestion test fresh sludge is transferred to the digestion chamber every day so this problem does not occur.

Full scale operation

A full scale operation with the use of two glycosidic enzymes of technical grade in an AD process was performed by Recktenwald et al. (2007). The operation was continuous with a sludge feed of primary sludge mixed with biological sludge and the performance was carried out during a six months period. The dosage of enzymes was 2.5 kg of each enzyme solution per tonne feed TS to the digestion chamber. Both the enzyme treated and the reference digestion chamber was fed via a pump and valve system, the feed sludge load was approximately 45 m^3/day and the retention time was 24 days. The two digestion chambers were fed with the same amount and quality of sludge mixture from the buffer tank. The dosage point of enzymes was at a heat exchanger system which was run every fourth hour for 30 to 40 minutes. The digestion was carried out at 35 °C but the heat exchange loop heated the sludge to 55 °C. The enzymes applied had a temperature optimum between 45 - 60°C so this gave the enzymes an extra time of activation and mixing. A schematic view of the full scale operation can be seen in Figure 2 below.

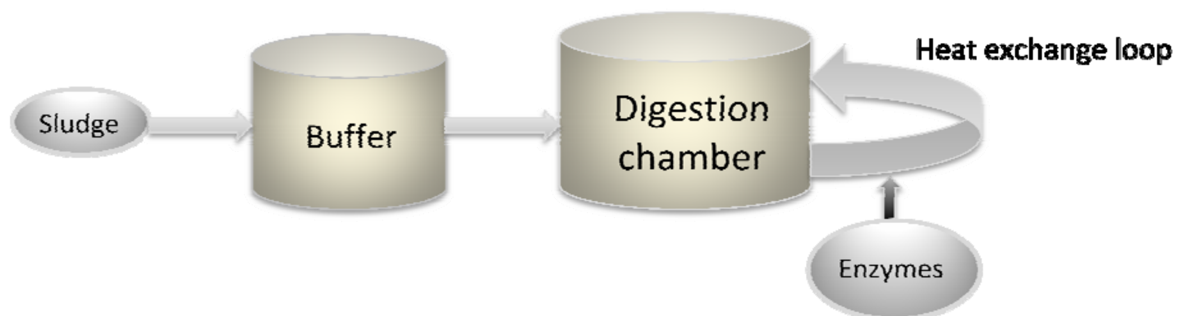


Figure 2: A schematic view over a full scale operation of AD with added hydrolytic enzymes. The operation is performed by Recktenwald et al. (2007).

The results from the full scale operation was improved gas production in the enzyme treated digestion chamber by 10 – 20 % compared to the reference digestion chamber. No increase in VFAs was shown which was a positive result. The VFAs decrease the pH in the digestion chamber which inhibits the methanogens and as this did not occur there is a possibility of practical application. Analysis of the reject water back to the plant showed no difference for the enzyme treated digestion chamber compared to the reference. This is also a positive result.

2.5 Cation binding agents

A chemical substance capable to form a complex compound with another substance in a solution is called a complexing agent. Because these agents have negatively charged ligands, they attract positively charged metal ions and forms stable compounds. Such a cation binding agent disturb the structure of the sludge flocs by removing cations such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} maintaining the floc structure. (Wawrzyńczyk, 2007)

2.5.1 The effect of cation binding agents on the solubilisation of sludge

When enzymes are added to the sludge they often adsorb to the sludge matrix and is distributed uneven in the sludge. The enzymes binding to the sludge can also lead to inactivation. When treating the sludge with a cation binding agent prior to enzymatic treatment the organic matter in the sludge are released which potentially could lead to improved AD and improved dewatering abilities. The organic matter in the sludge is now suggested to be a better substrate for the enzymes meaning components previously protected by the EPS structure are more available to be degraded. (Wawrzyńczyk, 2007)

Wawrzyńczyk et al. (2003) have shown that the addition of cation binding agents to biosludge lead to a marked release of organic matter in the sludge. A positive relationship between the released organic matter and the defined concentration of cation binding agents was also found.

2.5.1.1 The effect on solubilisation of sludge with cation binding agents and enzymes combined

Wawrzyńczyk et al. (2007b) showed that enzymatic treatment of wastewater sludge was significantly improved in the presence of cation binding agents. The adsorption of the enzymes to the sludge matrix was reduced. A low dose of each enzyme (12 mg/g TS) was shown to be more effective in the presence of cation binding agents than a high dose of each enzyme (60 mg/g TS) alone.

The most effective cation binding agent was proven to be citric acid for the tested substrates and there is also a potential for a practical application of this cation binding agent since citric acid is fully biodegradable.

2.5.2 Anaerobic digestion of sludge with cation binding agents and enzymes combined

AD of sludge treated with enzymes and the cation binding agent citric acid was improved in a laboratory digestion test performed by Wawrzyńczyk (2007) but the data are not presented.

3 Stockholm Water

Stockholm Water is a water and sewage company owned by the municipality. The main task is to deliver drinking water to Stockholm, Huddinge and nine adjacent municipalities. Stockholm Water owns two WWTPs; Henriksdal and Bromma. Together these two plants pure about 135 million m^3 *wastewater from about 1 000 000 persons every year. This chapter is a presentation of Henriksdal WWTP where this project was carried out. The wastewater treatment process and the AD process are described because of their importance in this thesis work. (Vallin et al., 2008)*

3.1 Henriksdal wastewater treatment plant

The WWTP in Henriksdal is completely suited inside the rock. The WWTP was inaugurated 1941 and the capacity was later doubled in 1953 through an expansion. Henriksdal is now the biggest WWTP in Stockholm town and one of the biggest in Sweden. Wastewater from almost 700 000 persons is purified here which correspond to about 250 000 m^3 wastewater every 24-hour. (Stockholm Water 1, 2008)

During the treatment process of wastewater suspended matter and water is separated, which generate sludge. This sludge is together with an external sludge digested in digestion chambers at Henriksdal WWTP which generates digested sludge and biogas. The biogas can for example be used as a vehicle fuel and the digested sludge as a deposit or fertilizer. (Vallin et al., 2008)

A schematic view of the wastewater treatment process in Henriksdal, the incoming sludge to the digestion chambers and the outgoing products is presented in Figure 3.

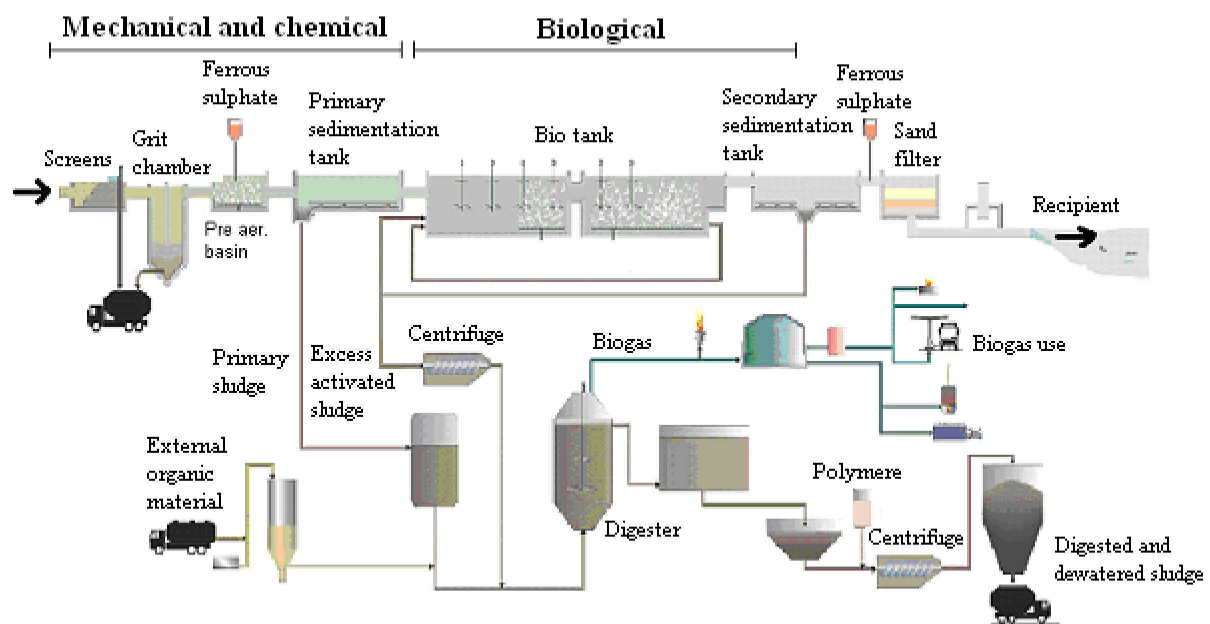


Figure 3: A schematic view of the wastewater treatment process in Henriksdal. The figure is modified from Vallin et al. (2008).

3.1.1 The wastewater treatment process in Henriksdal

The wastewater treatment process is carried out through different procedures; mechanical, chemical and biological treatment and a last step of filtration (Stockholm Water 2, 2008; Vallin et al., 2008). These four steps are described below and showed in Figure 3 above.

Mechanical treatment

The first procedure in the cleaning process is the mechanical treatment. The main task is to remove solid impurities in the received water. This is accomplished by use of two different treatment methods, one cleaning the water from impurities bigger than 3-10 mm (screen) and another cleaning the water from impurities bigger than 0.15 mm (grit removal). The water is now cleaned from sand, seed and coffee ground but not from sludge and smaller particles. This cleaning procedure, removing large particles such as sand, is very important for the coming cleaning procedures. Without the mechanical treatment large particles would accumulate at the bottom of the reactors and the equipment would be exposed to wear.

Chemical treatment

Chemical treatment is a precipitation procedure. A chemical precipitation carried out before a biological treatment is called a pre-precipitation. As precipitation substance different metal salts can be used. Ferrous sulphate is the precipitation substance used at Henriksdal WWTP. Ferrous sulphate added to the wastewater precipitate dissolved phosphor as a ferrous phosphate. Ferrous hydroxide is at the same time precipitated and form flocs. These flocs bind the ferrous phosphate and other suspended substances in the water. The chemical precipitation therefore does not only reduce dissolved phosphor but also organic bound phosphor and suspended substances. In Henriksdal WWTP there are primary settlings tanks where sludge from this chemical cleaning sediment at the bottom together with the large remaining sludge from the mechanical treatment. The generated sludge is called primary sludge.

Biological treatment

Cleaning of the wastewater from organic matter and nitrogen is carried out through biological treatment in a reactor containing microorganisms. The microorganisms are mainly bacteria which arrive to the reactors with the incoming wastewater. The bacteria generate an active sludge in the reactors and some of the sludge is recirculated to the reactors. The recirculated sludge is mixed with incoming water to maintain an adequate sludge and microorganism content in the reactors. The sludge being removed is called excess activated sludge (EAS).

Filtration

The last step in the cleaning process of wastewater is a filtration step. The filter consists of small crushed balls of ceramic material and sand which keeps the remaining small particles away before the water is let out in the recipient.

3.1.2 Anaerobic digestion at Henriksdal WWTP

The AD process in Henriksdal WWTP is carried out in seven digestion chambers. All of the seven chambers are located inside the rock which constitutes the walls of the chambers. The sludge is pumped in at the bottom of the chambers and flows out over a weir at the top. Below is a presentation of the utilized substrates and produced products together with a presentation of the process parameters in the existing AD process in Henriksdal. The text is reviewed from Stockholm Water2 (2008) and Vallin et al. (2008).

Substrate in the anaerobic digestion at Henriksdal WWTP

Henriksdal WWTP generates two different kinds of sludge during the treatment process, primary sludge and EAS. The primary sludge is generated in the presedimentation step and EAS is generated in the biological treatment step as described above (section 3.1.1). The sludge generated at Henriksdal WWTP is used as a substrate in the AD process.

Around 1400 m^3 primary sludge is produced at Henriksdal WWTP every day. The sludge is pumped out from the reactors and collected in a sludge silo. TS in the primary sludge at Henriksdal WWTP are in means 3.6 % and VS are in means 73 % of TS.

At Henriksdal WWTP the EAS has a TS content of about 0.5 percent and is relatively homogen when pumped from the reactors. The centrifuges have a capacity to thicken this sludge up to a TS content between 6 and 8 %**,** but year 2006 TS in the EAS were around 4.0 %. This was mainly because too high TS content complicated the pumping of the sludge to the AD chambers. The production of EAS is about 470 m^3 every day and the VS content is almost 63 % of TS.

Henriksdal WWTP receives about 80 m^3 external organic material (EOM) every day via a separate reception. The EOM is also used in the production of biogas and is therefore pumped to the digestion chambers. 96 % of the EOM consist of fat from provision productions and restaurants. Because of the high fat content the sludge is pumped together with primary sludge and EAS to the digestion chambers to prevent clogging of the pipes. The organic matter in the received EOM varies a lot and the TS lie between 0.3 and 38.6 % with a mean value of 10 %. The VS is in average about 95 % of TS.

The anaerobic digestion process at Henriksdal WWTP

The seven digestion chambers at Henriksdal WWTP have a total volume of 39 000 m^3. They are mainly blended with a stirrer consisting of three blades; one bigger blade in the bottom of the chambers and two smaller in the middle and the top respectively. These three blades are located on a common long stirring axis. Some stirring also occur when the sludge is recirculated over the external placed heat exchangers. This sludge is transferred out at the top of the bottom cone of the digestion chambers and brought back at the bottom together with the incoming substrate. The AD process is performed at mesophilic conditions with an average retention time of 20 days.

Products in the anaerobic digestion at Henriksdal WWTP

The biogas produced in the AD at Henriksdal WWTP is used for heating of the own plant and electricity production. Since year 2004 the gas is also purified from carbon dioxide through a recirculating water scrub. When the gas is purified to a methane content of 96-98 % it is called biomethane and can be used as a vehicle fuel. In year 2009 almost all of the produced

gas will be purified and sold as a vehicle fuel. 1 125 N m^3 biogas is produced every hour which corresponds to 749 N m^3 CH_4 when the methane content is 66.5 % as it was year 2005.

The sludge generated in the digestion chambers is called digested sludge. Henriksdal WWTP generates about 700 000 tonne digested sludge every year and this sludge is dewatered to 50 000 tonne, collected in dry sludge silos and mainly used to establish vegetation on mine deposits. The TS content of the dewatered digested sludge is about 30 %.

4 Experimental part

This chapter will report of the experimental setups and used substrates within this thesis. The possibility that enzymatic treatment of sludge from Henriksdal WWTP in the presence of a cation binding agent could increase the solubilisation of organic matter was investigated and implemented in a factorial design. Two batch laboratory digestion tests were also performed with selected enzyme doses and concentrations of the cation binding agent. The same sludge and reagents was used in both the sludge solubilisation experiment and the batch laboratory digestion tests but the handling of the sludge and the treatment pattern differed.

4.1 Sludge and reagents

EAS and primary sludge from Henriksdal WWTP were used as substrates. TS and VS in the sludge were determined with the method described previously in section 2.1.4. TS varied between 3.11 - 4.65 % in the primary sludge and between 5.54 - 6.12 % in the EAS. VS in the primary sludge varied between 2.45 - 2.83 % (between 72.70 – 77.11 % of TS) and in the EAS between 3.92 - 4.03 % (between 65.11 – 65.85 of TS).

The enzymes were divided into two mixtures, mixture A and mixture B and both mixtures were used in all experiments. The choice of enzymes in the both mixtures was made after consultation with Wawrzyńczyk and Norrlöw.

Mixture A consisted of one lipase (Lipolase) and three glycosidic enzymes; endo-xylanase (Pulpzyme HC), cellulase (Celluclast 1.5L) and α-amylase (Termamyl 300L). The commercial names of the enzymes are shown in brackets. Lipase removes or emulsifies fatty matter in the sludge which enhances the access to the sludge for the three glycosidic enzymes (Dey et al., 2006). These four enzymes were mixed with the polypropylene glycol (PPG) and fatty alcohol ethoxylate (FAE). FAE is a surface active substance which lowers the interfacial tension in the mixture and improves the activity of the lipase as lipase acts on interface between water-lipid (Wawrzyńczyk, personal communication). PPG was added to the mixture to improve the stability of the enzymes during the storage time and did not affect the enzymatic activity or the hydrolysis process. The relationship between the enzymes, PPG and FAE in mixture A was also determined after consultation with Wawrzyńczyk. The composition of enzyme mixture A can be viewed in Table 3.

Table 3: The composition of enzyme mixture A used within this thesis. The commercial names of the enzymes are shown in brackets.

Component	Share [% in the solution]
Lipase (Lipolase)	24.3
Cellulase (Celluclast 1.5L)	24.3
α-amylase (Termamyl 300L)	24.3
Endo-xylanase (Pulpzyme HC)	24.3
Polypropylene glycol (PPG)	2.4
Fatty alcohol ethoxylate (FAE)	0.24

Mixture B contained one protease (Alcalase 2.4L). This was added to the sludge for hydrolysis of proteins. Mixture B was separated from enzymes in mixture A to prevent hydrolysis of these enzymes during storage (Wawrzyńczyk, 2007).

All used enzymes were of technical grade and the reagents FAE and PPG were of analytical purity. Both enzymes and the reagents FAE and PPG were supplied by Kemira, Helsingborg. The used costs of enzymes in the calculations are 14 USD/kg for the protease, 11 USD/kg for the glycosidic enzymes and 30 USD/kg for the lipase. This was approximate costs obtained from Andersson. The exact cost of enzymes is not possible to obtain. If this technique is to be implemented in a full scale this must be an agreement between the supplier and Stockholm Water. The cost for the FAE and PPG is neglected because they are assumed to be very low.

Tri-sodium citrate (referred to as just sodium citrate in the continuation) was the selected cation binding agent. Sodium citrate is the salt from citric acis which was the cation binding agent showing to be most effective in previous studies performed by Wawrzyńczyk et al. (2007c) with different cation binding agents on solubilisation of sludge. Sodium citrate was of analytical purity and added to the sludge as a powder. The used cost of sodium citrate is 10 SEK/kg. This was an approximate cost obtained from Unnefors.

Table 4 shows the specificity of the five enzymes used concerning enzyme commission (EC) number and cleavage specificity (Dey et al., 2006).

Table 4: Specificity of the hydrolytic enzymes used within this thesis for the release of soluble COD and increase in methane production. The enzyme commission (EC) numbers were assigned by the International Union of Pure and Applied Chemistry. The commercial names of the enzymes are in brackets. The table is modified from Dey et al., 2006.

Enzyme Name	EC Number	Preferential Cleavage Specificity
Protease (Alcalase 2.4L)	3.4.4.16	Peptide bond
Lipase (Lipolase)	3.1.1.3	Ester linkages
Cellulase (Celluclast 1.5L)	3.2.1.4	1, 4-(1,3;1,4)-β-D-Glucan-4-glucanohydrolase
Xylanase (Pulpzyme HC)	3.2.1.8	Endo-1,4-βD-xylalosidic linkage
Amylase (Termamyl 300L)	3.2.1.1	1, 4-α-D-glucosidic linkage

4.2 Solubilisation of sludge with enzymes in the presence of sodium citrate

In this thesis soluble COD was used as a measurement of sludge solubilisation when looking into the possibility to solubilise the sludge at Henriksdal WWTP with enzymatic treatment in the presence of sodium citrate.

4.2.1 Sludge handling

EAS and primary sludge was collected from Henriksdal WWTP between October and December 2007. A fresh batch of sludge was collected for each new trial and stored in 4°C.

The primary sludge and EAS were mixed out of TS content with a relationship resembling the one in the existing digestion chambers at Henriksdal WWTP and then diluted to a TS content of 2 %. 73 % of TS originated from the primary sludge and 27 % of TS from the EAS in agreement with Vallin et al. (2008) for the sludge composition in the digestion chambers year 2005. The mixture was then stored in 4°C before use. The maximum storage time of the sludge was 6 days.

4.2.2 Treatment pattern

Treatments with three different concentrations of sodium citrate and five different enzyme doses were combined in a factorial design. This design was implemented in two trials. The combination of the concentrations of sodium citrate and enzyme doses was chosen after consultation with Wawrzyńczyk and the combinations can be viewed in Figure 4. Enzyme dose 11 corresponds to 11 mg of each enzyme final concentration in mixture A per 1 g of the sludge mixture TS. The addition of the enzyme in mixture B is always 70.8 % of the dose of each enzyme in mixture A per 1 g TS. The relationship between the enzymes in mixture A and mixture B agree with the one in Wawrzyńczyk et al. (2007b).

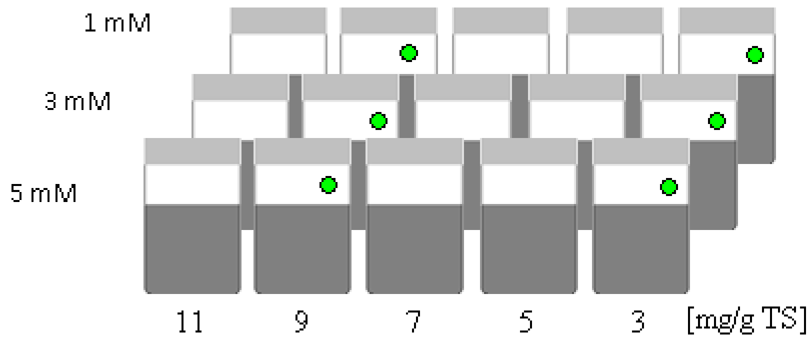


Figure 4: A factorial design of five enzyme doses (horizontal) and three concentrations of sodium citrate (vertical). Enzyme dose 11 corresponds to a final concentration of 11 mg of each enzyme in mixture A per 1 g TS in the sludge mixture. The addition of each enzyme in mixture B is always 70.8 % of the each enzyme in mixture A per 1 g TS. The marked dose combinations are used in an additional short trial.

One of the implementations of the factorial design was to determine the total soluble COD ($COD_{sol,\ tot}$) in the sludge after four hours treatment in 37 °C during stirring (treatment pattern 1) and the other was to determine the direct contribution to soluble COD ($COD_{sol,\ contr}$) from the enzymes and sodium citrate added (treatment pattern 2). The treatment pattern for the two implementations is described later in this section. The final aim was to determine the effect on the release of soluble COD from the treatment with enzymes and sodium citrate ($COD_{sol,\ treat\text{-}effect}$). The calculation is presented later in section 4.2.4.

Because of the limitations in laboratory equipment several experimental setups were done at different occasions and therefore different sludge batches were used. The treatments were performed in duplicate or triplicate and the order in which they were carried out was randomized to minimize the effect of variations in the sludge batches.

Because of the large variations in starting soluble COD between the cold sludge batches ($COD_{sol,\ cold}$) a short trial was made using only one sludge batch. In this trial treatment pattern 1 was used. The treatments were performed just once because of lack of enzymes. The enzyme doses and sodium citrate concentrations used in this shorter trial is marked in Figure 4 and the relationship between mixture A and mixture B was the same as before.

<u>Treatment pattern 1</u>
For each combination of enzyme dose and sodium citrate concentration a 1 litre glass bottle was filled with 800 g of the sludge mixture and placed in water baths with a temperature of 37°C. Sodium citrate was added to the sludge and stirring was started. Stirring of the sludge was achieved with magnetic stirrers. Three different magnetic stirrers were used. Two of them were constructed to be placed in the water bath (Variomag, Munich & Stem, VWR) and one had to be placed under the bath (FALC instruments, Italy)

To obtain comparable setups and the best environment for the enzymes, the enzymatic treatment was not started until the sludge had reached 37°C. The enzymatic treatment procedure was divided into two steps. The first two hours the sludge was hydrolyzed by enzyme mixture A and then further hydrolyzed for two hours by mixture B. The enzymes were added directly to the sludge treated with sodium citrate. For each setup one bottle with untreated cold sludge was heated and stirred exactly as the others. The soluble COD (COD_{sol}) in the sludge after heating is referred to as $COD_{sol,\ heat}$. The heating of the sludge is also a source to release of COD_{sol} so this sludge was used to determine $COD_{sol,\ heat\text{-}effect}$ for each sludge batch used. (See section 4.2.4) The treatments were performed in duplicate or triplicate.

Treatment pattern 2

In this treatment 800 g of the sludge mixture was used for each dose combination as in treatment pattern 1. These treatments were performed in duplicate and the enzymes and sodium citrate was added at the same time to the cold sludge. No noticeable time was passed between the addition of enzymes and sodium citrate and the COD_{sol} determination so the enzymes and sodium citrate were not allowed to react with and release COD_{sol} from the sludge. COD_{sol} in sludge was determined both before treatment ($COD_{sol,\ cold}$) and after ($COD_{sol,\ cold,\ tot}$) and used to calculate the $COD_{sol,\ contr}$ from the enzymes and sodium citrate added (see section 4.2.4).

4.2.3 Determination of soluble COD

All sludge samples were first past through folded filters (595 ½, Whatman) to retention particles between 4 and 7 µm. This facilitates for the further filtration through a membrane filter (Membrane Filter, ME25, pore size 0, 45 µm, Whatman). This was easiest achieved through vacuum filtration. COD was determined in the received liquid phase using test kits from Dr. Lange (COD LCK 514 and COD LCK 014). The obtained COD determination is referred to as soluble COD (COD_{sol}). COD_{sol} in the cold untreated sludge for every new sludge batch was also determined and this is reffered to as the starting COD_{sol} of the sludge batches ($COD_{sol,\ cold}$).

4.2.4 Calculations of the effects on the release of COD_{sol}

Below are the equations determining the $COD_{sol,\ contr}$, $COD_{sol,\ heat\text{-}effect}$, $COD_{sol,\ treat\&heat\text{-}effect}$ and $COD_{sol,\ treat\text{-}effect}$ together with a discussion of the belonging standard deviations (stdv). The stdv is always determined

$$stdv = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \bar{y})^2}{n-1}} \qquad \textbf{(4.1)}$$

A short description of the parameters used in the calculations is given below;

$COD_{sol, contr}$	is the contribution to COD_{sol} from the addition of enzymes and sodium citrate added
$COD_{sol, cold, tot}$	is the total COD_{sol} in the cold sludge after addition of enzymes and sodium citrate
$COD_{sol, cold}$	is the COD_{sol} in the cold sludge before addition
$COD_{sol, heat}$	is the COD_{sol} in the sludge after heating to 37 °C for four hours
$COD_{sol, tot}$	is the COD_{sol} in the sludge after heating to 37 °C for four hours in the presence of enzymes and sodium citrate

$\underline{COD_{sol, contr}}$

The contribution to COD_{sol} from the addition of enzymes and sodium citrate added ($COD_{sol, contr}$) was important to for the forthcoming calculations of the effects of the treatment with enzymes and sodium citrate. $COD_{sol, cold, tot}$ and $COD_{sol, cold}$ is determined in triplicates and the same sludge batch is used for each determination of $COD_{sol, contr}$. The stdv for the $COD_{sol, contr}$ is a summary of the corresponding $COD_{sol, cold, tot}$ and $COD_{sol, cold}$ stdvs. The $COD_{sol, contr}$ is in turn determined twice for each dose combination using different sludge batches. Between these two samples another stdv is set and is referred to as the sample stdv. $COD_{sol, contr}$ is determined;

$$COD_{sol,contr} = COD_{sol,cold,tot} - COD_{sol,cold} \quad \textbf{(4.2)}$$

$\underline{COD_{sol, heat\text{-}effect}}$

$COD_{sol, heat}$ and $COD_{sol, cold}$ is both set in triplicates on the same sludge batch. The $COD_{sol, heat\text{-}effect}$ is determined for each sludge batch. The stdv in the determination of $COD_{sol, heat\text{-}effect}$ is a summary of the two stdvs originating from the determination of $COD_{sol, heat}$ and $COD_{sol, cold}$ for the corresponding sludge batch. The heating effect on the release of COD_{sol} is determined;

$$COD_{sol,heat-effect} = COD_{sol,heat} - COD_{sol,cold} \quad \textbf{(4.3)}$$

$\underline{COD_{sol,\ treat\&heat\text{-}effect}\ \text{and}\ COD_{sol,\ treat\text{-}effect}}$

$COD_{sol,\ tot}$, $COD_{sol,\ cold}$ and $COD_{sol,\ heat}$ was set in triplicate on the same sludge batch for each determination of $COD_{sol,\ treat\&heat\text{-}effect}$ and $COD_{sol,\ treat\text{-}effect}$. $COD_{sol,\ contr}$ is a mean value of two parallels of the corresponding dose combination of enzymes and sodium citrate. The stdv for each $COD_{sol,\ treat\&heat\text{-}effect}$ (or $COD_{sol,\ treat\text{-}effect}$) is a summary of the three stdv originating from the determination of $COD_{sol,\ tot}$, $COD_{sol,\ cold}$ (or $COD_{sol,\ heat}$) and the stdv between the two parallels determining the $COD_{sol,\ contr}$ for the corresponding dose combination. The stdv in the $COD_{sol,\ contr}$ determination for each parallel is neglected to obtain a calculation easy to implement. This stdv was later shown to be very small which justify the neglection. The $COD_{sol,\ treat\&heat\text{-}effect}$ and $COD_{sol,\ treat\text{-}effect}$ is in turn determined twice or three times using different sludge batches and the sample stdv is set between these.

The combined effect on the release of COD_{sol} from the heating and treatment with sodium citrate and enzymes ($COD_{sol,\ treat\&heat\text{-}effect}$) is determined;

$$COD_{sol,\,treat\&heat-effect} = (COD_{sol,\,tot} - COD_{sol,\,cold}) - COD_{sol,\,contr} \qquad \textbf{(4.4)}$$

The effect on the release of COD_{sol} as a result of the enzymatic treatment in presence of sodium citrate ($COD_{sol,\ treat\text{-}effect}$) is determined;

$$COD_{sol,\,treat-effect} = (COD_{sol,\,tot} - COD_{sol,\,heat}) - COD_{sol,\,contr} \qquad \textbf{(4.5)}$$

4.3 Batch laboratory digestion tests

In this project two batch laboratory digestion tests were performed at Swedish Institute of Agricultural and Environmental Engineering (JTI), Ultuna. The methane production in the sludge mixture with and without addition of enzymes and sodium citrate was monitored.

The handling of the sludge and the treatment pattern used were the same between the two tests but the enzyme dose and sodium citrate added differed.

4.3.1 Sludge handling

EAS and primary sludge was collected from Henriksdal WWTP in November 2007 and January 2008. To get representative batches primary sludge and EAS were collected two times at one day for each of the two trials. Sludge from one digestion chamber at Henriksdal WWTP was also collected and stored together with the primary sludge and EAS in 4°C. The sludge from a digestion chamber is called inoculum and is used in digestion tests to obtain an adequate environment in the samples.

The primary sludge and EAS were mixed out of VS content with a relationship resembling the one in the existing digestion chambers at Henriksdal WWTP. VS were determined in the sludges with the procedure described previously in section 2.1.4. 77 % of VS in the digestion

chambers originate from primary sludge and 23 % of VS from EAS according to Vallin et al. (2008) for the composition in the digestion chambers in Henriksdal year 2005.

The sludge mixture was together with the inoculum transported to JTI and used as a substrate in the laboratory digestion tests. Fresh values of TS and VS were determined in the sludge mixture and the inoculum at JTI before startup. TS and VS in the sludge used in the first digestion test were 3.7 % and 2.7 % respectively and 3.4 % and 2.4 % respectively for the second digestion test. In the first digestion test 72.97 % of TS was VS and in the second digestion test this number was 70.59 %. The maximum storage time of the sludge was 6 days in 4°C.

4.3.2 Sample treatments

The first digestion test was performed to see how the combination of enzymes and sodium citrate affect the methane production. Since the sodium citrate is a carbon source and therefore also functions as a substrate for methane production one sample was made with addition of sodium citrate alone. This renders the possibility to see the enzyme effect. 2.7 mg of each enzyme in mixture A and 7.8 mg of the enzyme in mixture B per 1 g TS in the sludge mixture (a total amount of 18.6 mg enzyme per 1 g TS) was added and the concentration of sodium citrate was 5 mM (1.46 g/l) which corresponds to an addition of 0.88 g. The thought was to have the same relationship between the enzymes in mixture A and B as in the solubilisation experiment but it did not turn out this way because of a misunderstanding. This dose was therefore not possible to entirely compare with the solubilisation experiment. In total the enzymes represent 0.026 g VS per g VS in the sludge mixture and the sodium citrate represent 0.303 g VS per g VS in the sludge mixture.

In the second digestion test a smaller amount of total enzymes and a lower concentration of sodium citrate were used. A lower dose of enzymes was used to reduce the costs and the same thought was for the decrease in sodium citrate concentration. The use of smaller doses was also strengthened in the solubilisation experiment. Even in this digestion test 2.7 mg of each enzyme in mixture A was added per 1 g TS in the sludge mixture but the addition of the enzyme in mixture B was only 1.9 mg per 1 g TS in the sludge mixture (a total amount of 12.7 mg enzyme per 1 g TS). This relationship is in agreement with the solubilisation experiment. The concentration of sodium citrate in digestion test two was lowered to 1 mM (0.29 g/l) which corresponds to an addition of 0.18 g. Now, in total the enzymes represent 0.017 and g VS per g VS in the sludge mixture and the sodium citrate represents 0.060 g VS per g VS in the sludge mixture. The affect of enzyme treatment alone was also examined here.

The added VS originating from the sludge substrate, the different treatments, concentrations of sodium citrate and enzymes, in the two digestion test can be viewed in Table 5 below.

Table 5: The concentration of sodium citrate and addition of enzymes in the treated sludge in the two batch laboratory digestion tests. The sludge treatments in the first digestion test is SC (Addition of Sodium citrate alone), and E + SC (Enzymes together with sodium citrate). The same treatments are found in digestion test two with an additional treatment E (Treatment with enzymes alone). The VS content in the samples originating from the sludge substrate is shown as an average of the three parallels.

Sludge treatment	Digestion test [No.]	VS substrate [g]	Mix A enzyme [mg/g TS]	Mix B enzyme [mg/g TS]	Sodium citrate [mM]
SC	1	3.0	-	-	5
	2	3.0	-	-	1
E +SC	1	2.8	2.7	7.8	5
	2	3.0	2.7	1.9	1
E	2	3.0	2.7	1.9	-

4.3.3 The procedure

The digestion tests were performed in 1 litre glass bottles with a rubber septum and a cap to prevent gas leakage. The substrate represented approximately 1/3 of the total VS in the sample. For digestion test one this corresponded to an average amount of 107 ml substrate (2.9 g VS) and in test two 120 ml substrate (3.0 g VS). The remaining approximately 2/3 originated from the inoculum. Water was added to get a working volume of 600 ml. The enzyme mixtures and the sodium citrate were added directly to the sludge at the beginning of the procedure. One sample contained only inoculum and water. Measurement of the produced methane in this sample was important for calculation of the methane production originating from the substrate. All samples were run in triplicate and contained the same amount of inoculum to obtain samples easy to compare. The sample bottles were flushed with nitrogen gas at the startup to obtain an anaerobic environment. The bottles were then placed on a vibrating table and incubated in a room with constant temperature 37°C.

The gas pressure in the bottles was determined with a digital meter (GMH 3110), equipped with a pressure sensor (GMSD 2BR; -1000 to 2000 mbar), connected to a cannula which was allowed to penetrate the rubber septum. A small amount of gas (2 ml) was then taken out from the bottles using a cannula and the methane content was monitored by gas chromatography (PerkinElmer ARNEL , Clarus 500 ,column : 7’ HayeSep N 60/80, 1/8” SF ;

FID detector 250 °C; carrier gas: Helium , flow 31 mL/min ; temp injector: 60 °C, Headspace, sampler Turbo Matrix 110). The pressure in the bottles was equalized and the procedure was repeated at several occasions. The bottles were kept in the temperate room until the methane production ceased.

The measured gas pressure in the bottles together with the determined methane content of the gas was used to calculate the methane production from the samples. The calculation model can be viewed in Appendix F.

4.4 Degree of degradation determination

There are two main factors influencing the degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP ($DD_{Henriksdal}$). One is the fact that all of the organic substances added to the digestion chamber are not degraded. The share of the substrate being degraded in the batch laboratory digestion tests was assumed to be the maximal methane potential in a substrate. The degree of degradation of the substrate in the digestion test is therefore referred to as DD_{max}. DD_{max} was calculated using equation 2.10, section 2.2.3 for each of the different treatments in the first digestion test.

The retention time of the substrate is another factor influencing the $DD_{Henriksdal}$. The longer the retention time the more substrate is available to be digested. This was taking in account through calculations of the share of DD_{max} being fulfilled at every moment ($DD_{share\ of\ max}$) in the batch laboratory digestion tests. $DD_{share\ of\ max}$ (t) is the share of the maximal degree of degradation reached after t days in the batch laboratory digestion tests. A detailed principle for the calculation of the $DD_{share\ of\ max}$ can be viewed in Appendix I. The $DD_{share\ of\ max}$ at each moment was further used to calculate the total share of the DD_{max} when times goes to infinity in the digestion chamber ($DD_{share\ of\ max,\ total}$). The equation used was;

$$DD_{share\ of\ \max, total} = \int S(t) \cdot DD_{share\ of\ \max}(t)\ dt \qquad \textbf{(4.6)}$$

where

S(t) is the substrate amount at a given time

t is the time

The $DD_{Henriksdal}$ is given when the two factors are multiplied;

$$DD_{Henriksdal} = DD_{share\ of\ \max,\ total} \cdot DD_{\max} \qquad \textbf{(4.7)}$$

One mean value for the three parallels of each sample in the first digestion test was calculated.

5 Results

In this chapter there is a presentation of the solubilisation of sludge from Henriksdal WWTP as a result of enzymatic treatment in presence of sodium citrate. The results from the two batch laboratory digestion tests within this thesis with applied enzymes and sodium citrate concerning accumulated methane production and the real methane potential and degree of degradation for the first digestion test is also presented.

5.1 Solubilisation of sludge with hydrolytic enzymes and sodium citrate

Enzymatic treatment of sludge from Henriksdal WWTP in presence of sodium citrate and heating of the sludge resulted in a release of soluble COD (COD_{sol}) after four hours treatment in 37 °C. The addition of enzymes and sodium citrate also contributed direct to COD_{sol}.

In the following section are the results given from the determination of the total soluble COD in sludge after enzymatic treatment in presence of sodium citrate and heating to 37 °C for four hours ($COD_{sol,\ total}$). The effect of enzymatic treatment in presence of sodium citrate on the release of COD_{sol} and the heating effect on the seven sludge batches used are also presented ($COD_{sol,\ treat\text{-}effect}$ and $COD_{sol,\ heat\text{-}effect}$). The combined effect of the heating and treatment is also shown ($COD_{treat\&heat\text{-}effect}$). Five additional sludge batches were used in the determination of the contribution to COD_{sol} from the sodium citrate and enzymes added ($COD_{sol,\ contr}$). These results are also presented in this section.

The equations used in the determinations presented above can be found in section 4.2.4.

All treatments were carried out on a mixture of primary sludge and EAS with a TS content of 2 %. All COD_{sol} determinations were set in triplicates for each sample using test kits from Dr Lange (COD LCK 514 and COD LCK 014).

5.1.1 Contribution to soluble COD from enzymes and sodium citrate

The sodium citrate and enzymes added to increase the solubilisation of organic matter in the sludge are carbon sources and therefore directly contribute to COD_{sol}. In section 4.2.2 a method for determination of the contribution to COD_{sol} from these additions is described and equation 4.2 used in the calculations is presented in section 4.2.4. The value of $COD_{sol,\ contr}$ was important in the determination of the *effect* corresponding to the addition of enzymes and sodium citrate. This effect is presented later in this chapter. Figure 5 shows an increasing $COD_{sol,\ contr}$ with increasing enzyme dose and sodium citrate concentration. The treatments were performed in duplicates on different sludge batches and Figure 5 therefore shows the mean value. The stdv in each $COD_{sol,\ contr}$ determination varied between 6 – 33 mg/l (1 – 5 %) and the sample stdv varied between 1 – 62 mg/l (0 – 10 %). Appendix B presents the $COD_{sol,\ cold,\ tot}$ used in the calculation of $COD_{sol,\ contr}$ and the number of the sludge batch used in the corresponding treatment. The $COD_{sol,\ cold}$ for each sludge batch also used in the calculation of $COD_{sol,\ contr}$ can be viewed in appendix A.

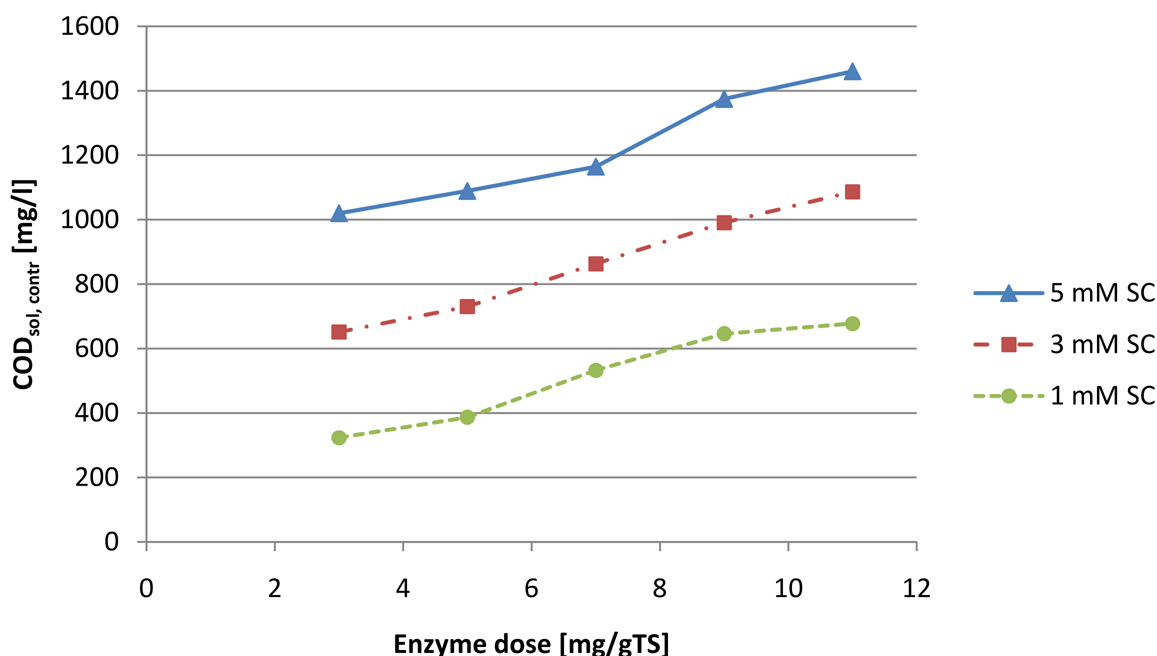


Figure 5: Direct contribution to COD_{sol} from enzymes and sodium citrate added ($COD_{sol, contr}$) to the sludge. Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per 1 g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A. Three different concentrations of sodium citrate (SC) are combined with five enzyme doses and the data represent the $COD_{sol, contr}$ for each combination. The addition of each dose combination was performed in duplicates and the figure therefore shows the mean value of these parallels. The $COD_{sol, contr}$ was determined in triplicates in the liquid phase for each sample. The stdv in the $COD_{sol, contr}$ determinations varied between 6 - 33 mg/l (1 – 5 %) and the sample stdv varied between 1 – 62 mg/l (0 – 10 %).

5.1.2 Heating effect on the release of soluble COD

The release of COD_{sol} from the different sludge batches used as a result of heating to 37 °C for four hours ($COD_{sol, heat-effect}$) was calculated. Equation 4.3 used in the calculation of the $COD_{sol, heat-effect}$ is presented in section 4.2.4 and the data used in the calculations can be viewed in Appendix A. No relationship between $COD_{sol, heat-effect}$ and $COD_{sol, cold}$ was detected.

The stdv in the $COD_{sol, heat-effect}$ determination for the different sludge batches varied between 7 – 81 mg/l (1 – 9 %) and between 2 – 7 mg/l (0 – 1 %) in the $COD_{sol, cold}$ determination. $COD_{sol, heat-effect}$ was together with the $COD_{sol, contr}$ determination in 5.1.1 used to determine the *effect* on the release of COD_{sol} from enzymatic treatment in presence of sodium citrate presented later in this chapter.

5.1.3 Soluble COD in sludge after enzymatic treatment in presence of sodium citrate

The total COD_{sol} in the sludge after enzymatic treatment, addition of sodium citrate and heating of the sludge ($COD_{sol, tot}$) is shown in Figure 6. This includes the $COD_{sol, cold}$, the $COD_{sol, heat-effect}$, the $COD_{sol, treat-effect}$ and the $COD_{sol, contr}$. This was the initial value used in the

calculation of the $COD_{sol,\ treat\text{-}effect}$ and $COD_{sol,\ heat\&treat\text{-}effect}$. The treatments were performed in duplicates or triplicates on different sludge batches and Figure 6 therefore shows the mean of the parallels. The order of the treatments was randomized to minimize the effect from the differences in the sludge batches.

The stdv in each $COD_{sol,\ tot}$ determination varied between 6 - 63 mg/l (0 – 2 %) and the sample stdv varied between 13 – 425 mg/l (1 – 12 %). Section 4.2.2 presents a method for determination of this total $COD_{sol,\ tot}$ and the corresponding stdv was calculated using equation 4.1 in section 4.2.4. The data presented in Figure 6 can also be viewed in appendix C together with the number of sludge batch used in each sample.

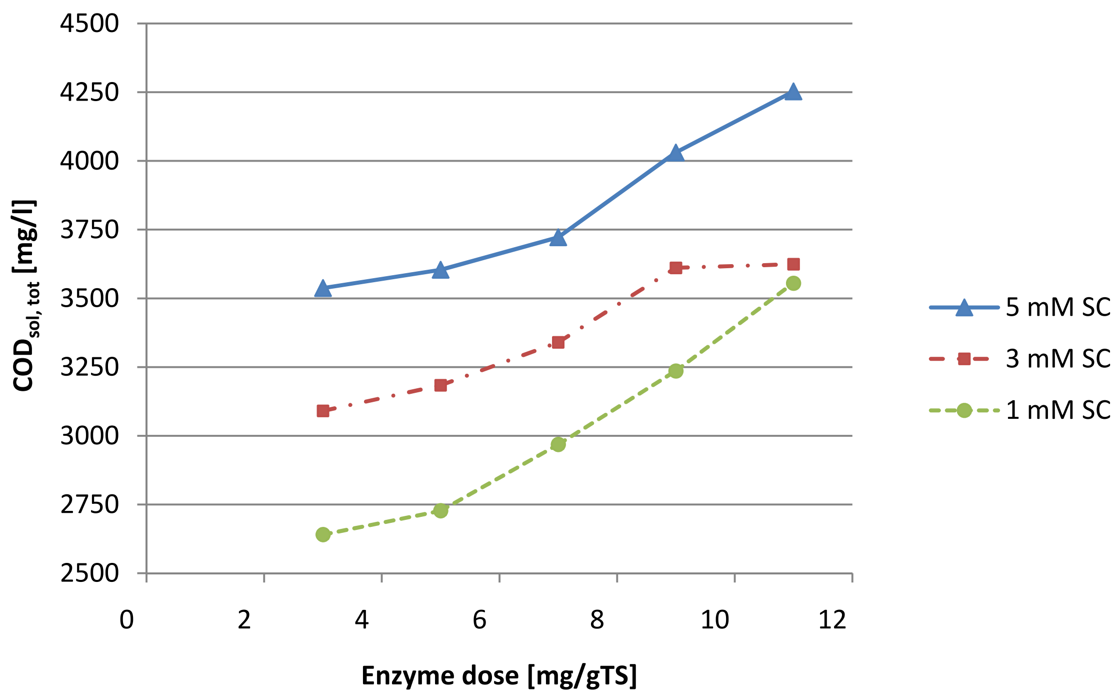


Figure 6: $COD_{sol,\ tot}$ in the sludge for the different dose combinations. Different sludge batches with a mixture of primary sludge and EAS (TS 2 %) was used. Each treatment was performed in duplicates or triplicates and the figure therefore shows the mean value of these parallels. The sample stdv varied between 13 – 425 mg/l (1 – 12 %) $COD_{sol,\ tot}$ in each parallel was determined in triplicates with a variation in stdv between 6 – 63 mg/l (0 – 2 %). Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A.

Figure 7 shows the $COD_{sol, tot}$ in the sludge presented in Figure 6 after the $COD_{sol, contr}$ presented in Figure 5 are subtracted for the corresponding samples. The figure therefore includes the $COD_{sol, cold}$, $COD_{sol, heat-effect}$ and the $COD_{sol, treat-effect}$. Data used in the $COD_{sol, contr}$ determinations can be viewed in appendix A and B. $COD_{sol, tot}$ can be viewed in appendix C for the corresponding dose combinations. The stdv in the soluble COD determination varied between 15 – 102 mg/l (1 – 4 %) and between 13 – 425 mg/l (1 – 17 %) in the sample stdvs.

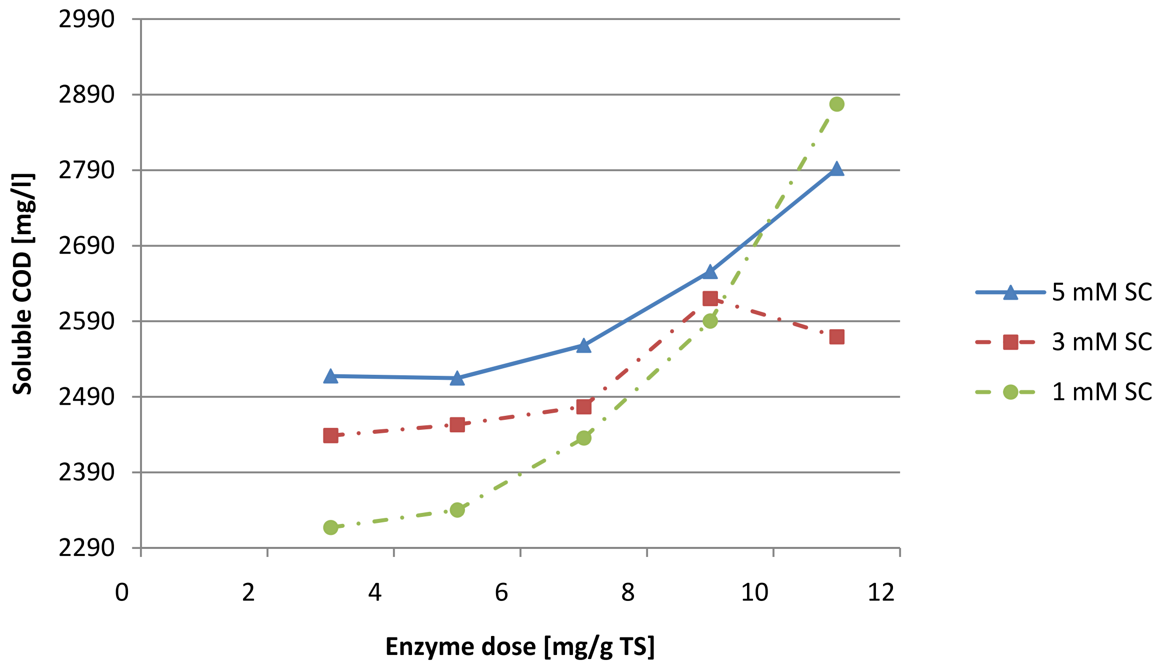


Figure 7: A subtraction of $COD_{sol, contr}$ from the corresponding values of the $COD_{sol, tot}$ presented in Figure 6. Different sludge batches with a mixture of primary sludge and EAS (TS 2 %) were used. Each treatment was performed in duplicates or triplicates and the figure therefore shows the mean value of these parallels. COD_{sol} from each treatment was determined in the liquid phase in triplicates. Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A. The stdv in the soluble COD determination varied between 15 – 102 mg/l (1 – 4 %) and between 13 – 425 mg/l (1 – 17 %) in the sample stdvs.

5.1.3.1 *Effect of heating and addition of enzymes and sodium citrate on the release of soluble COD*

Figure 8 shows the *effect* of heating to 37 °C for four hours combined with the effect of enzymatic treatment in presence of sodium citrate on the release of COD_{sol} ($COD_{sol,\ treat\&heat\text{-}effect}$). $COD_{sol,\ treat\&heat\text{-}effect}$ is a subtraction of the direct contribution to COD_{sol} from the enzymes and sodium citrate added ($COD_{sol,\ contr}$) and the starting COD_{sol} in the cold sludge ($COD_{sol,\ cold}$) from the total COD_{sol} in the sludge after heating and treatment, meaning the effect on the release of COD_{sol} as an effect of both heating and enzyme and sodium citrate treatment is shown in Figure 8. The equation used in the determination of $COD_{sol,\ treat\&heat\text{-}effect}$ (equation 4.4) and the determination of the belonging stdv is presented in section 4.2.4 and the data used in the calculation can be viewed in appendix A, B and C. The treatments were performed in duplicates or triplicates on different sludge batches and Figure 8 therefore shows means of these parallels. The stdv in each $COD_{sol,\ treat\&heat\text{-}effect}$ determination varied between 19 – 107 mg/l (1 – 8 %) and between 10 – 337 mg/l (1 – 24 %) in the sample stdv. Figure 8 shows no clear difference between the sodium citrate concentrations, especially not between the dose 1 and 3 mM. A difference between all three sodium citrate concentrations is though confirmed in Figure 10, later in this chapter.

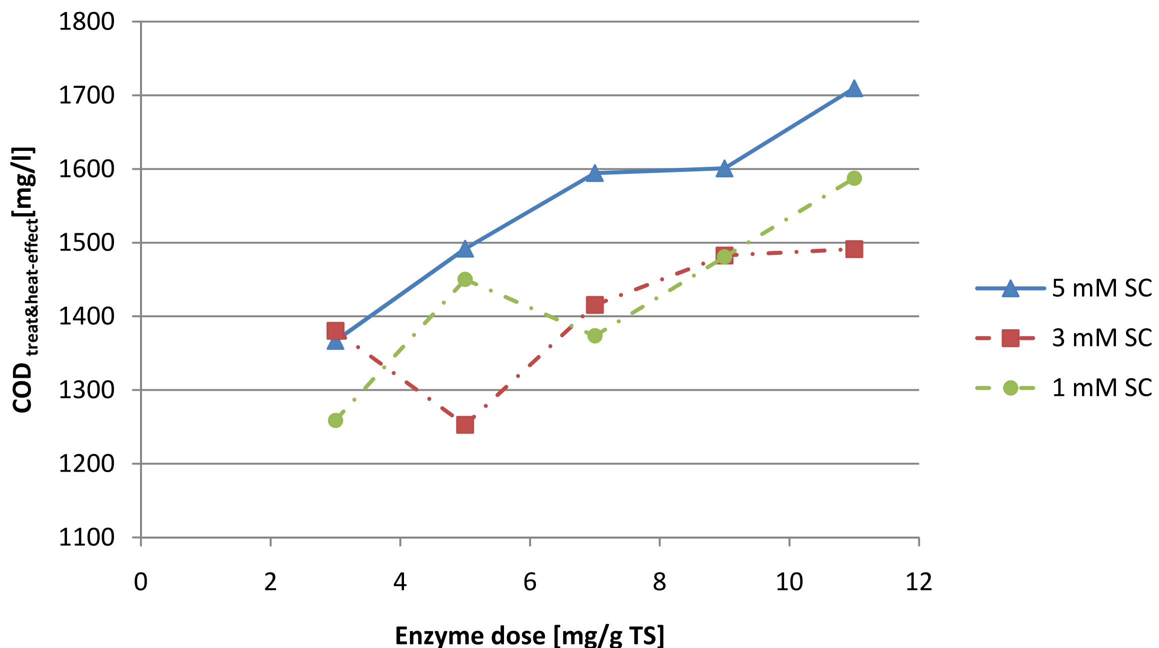


Figure 8: $COD_{sol,\ treat\&heat\text{-}effect}$ for the different dose combinations. Different sludge batches with a mixture of primary sludge and EAS (TS 2 %) were used. Each treatment was performed in duplicates or triplicates and the figure therefore shows the means of the parallels. All COD_{sol} determinations were set in triplicates. Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A. The stdv in the $COD_{sol,\ treat\&heat\text{-}effect}$ determination varies between 19 – 107 mg/l (1 – 8 %) and between 10 – 337 mg/l (1 – 24 %) in the sample stdv.

5.1.4 *Effect of enzymes in presence of sodium citrate on the release of soluble COD*

Figure 9 shows the *effect* of enzymatic treatment in presence of sodium citrate on the release of COD_{sol} from the sludge ($COD_{sol,\ treat\text{-}effect}$). The equation used in this determination (equation 4.5) together with the belonging stdv is shown in section 4.2.4. The data used in the calculations can be viewed in appendix A, B and C.

The treatments were performed in duplicates or triplicates on different sludge batches and Figure 9 therefore is means of the parallels. The stdv in each $COD_{sol,\ treat\text{-}effect}$ determination varied between 17 – 165 mg/l (3 – 32 %) and the sample stdv varied between 9 – 205 mg/l (1 – 37 %).

The increase in release of soluble COD as a result of enzymatic treatment corresponded to between 17 – 32 %. Figure 9 shows just as Figure 8, no clear difference between the sodium citrate concentrations. As told before, these differences are confirmed in figure 10, later in this section.

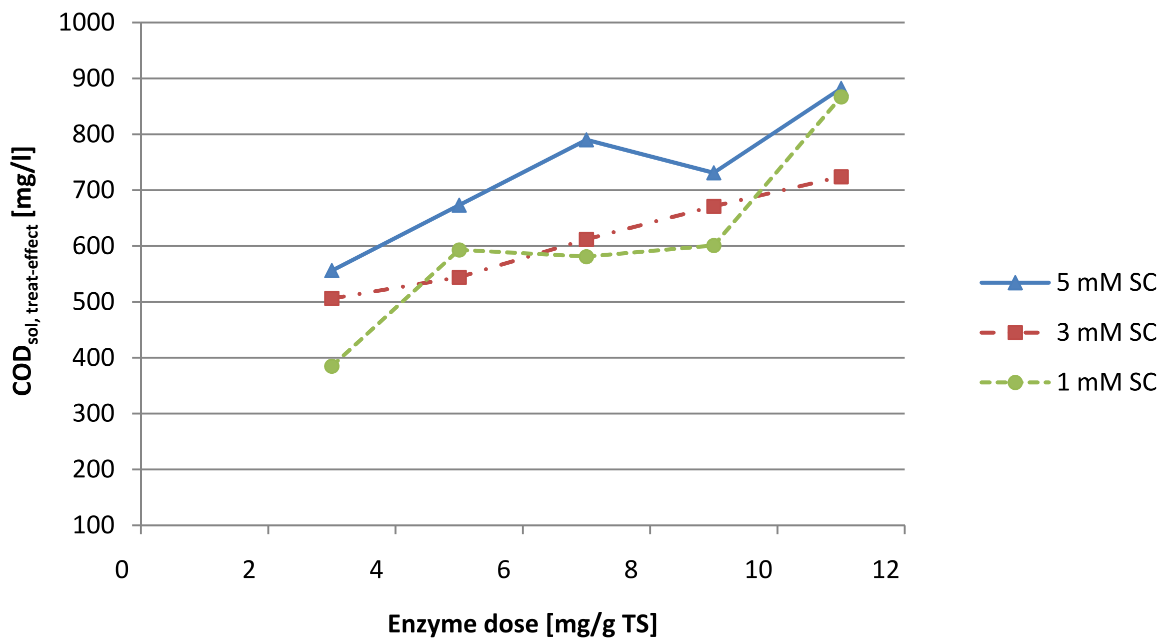


Figure 9: $COD_{sol,\ treat\text{-}effect}$ for each dose combination presented as the effect on enzyme dose. Different sludge batches with a mixture of primary sludge and EAS (TS 2 %) were used. Each treatment was performed in duplicates or triplicates and the figure therefore shows the means of the parallels. All COD_{sol} determinations were set in triplicate. Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per 1 g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A. The stdv in the $COD_{sol,\ treat\text{-}effect}$ determination varied between 17 -165 mg/l (3 – 32 %) and between 9 – 205 mg/l (1 – 37 %) in the sample stdv determination.

5.1.5 Effect of sodium citrate together with enzymes on the release of soluble COD

The difference in effect between the three sodium citrate concentrations used within this thesis is presented in Figure 10. The data presented in Figure 10 is the $COD_{sol,\ treat\text{-}effect}$ determined in the short trial described in section 4.2.2 but the effect between the three different sodium citrate concentrations is shown instead of the difference between the enzyme doses as in Figure 9. Two different concentrations of enzymes were utilized in this trial. The treatments were performed once and the sludge batches 6 and 7 were used. This is the same sludge batch but the treatments were performed at two different occasions and the batches are therefore referred to as two different batches. The use of this sludge batch should eliminate almost all the effects from the difference between batches.

Equation 4.5 in section 4.2.4 was used in the determination of $COD_{sol,\ treat\text{-}effect}$. The $COD_{sol,\ tot}$ used in the calculation can be found in appendix C, the $COD_{sol,\ contr}$ can be calculated from the data presented in appendix B as before and $COD_{sol,\ cold}$ and $COD_{sol,\ heat}$ can be viewed in appendix A. The stdv in each $COD_{sol,\ treat\text{-}effect}$ determination varied between 43 – 125 mg/l (7 – 25 %).

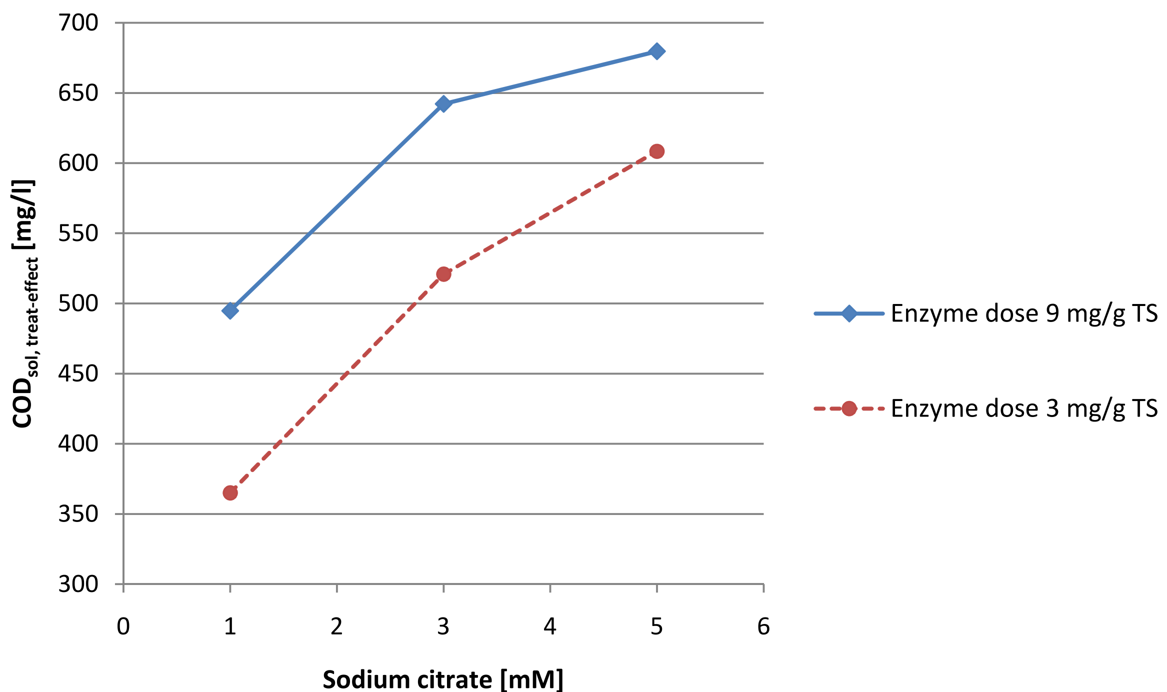


Figure 10: $COD_{sol,\ treat\text{-}effect}$ for each dose combination presented as the effect of sodium citrate concentration. A mixture of primary sludge and EAS (TS 2 %) was used as a substrate. Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per 1 g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A. Each treatment was performed once and all COD_{sol} determinations were set in triplicates. The stdv in the determination of $COD_{sol,\ treat\text{-}effect}$ varied between 43 – 125 mg/l (7 – 25 %).

5.2 Batch laboratory digestion

In this section the results concerning methane production from the two batch laboratory digestion tests are presented. A mixture of primary sludge and EAS was used in both digestion tests but the treatment of the samples differed. The tests were carried out at 37 °C on a vibrating table to ensure a good mixing of enzymes and substrate.

The first digestion test was run with three samples. In two of the samples sodium citrate was added to a concentration of 5 mM. One of these samples was also treated with 2.7 mg of each enzyme in mixture A and 7.8 mg of the enzyme in mixture B per 1 g TS in the sludge (totally 18.6 mg enzyme per 1 g TS). In the third sample there was no addition at all. The second digestion test was run with four samples. As before two of the samples contained sodium citrate but this time with a concentration of 1 mM. One of these plus an additional sample without sodium citrate was treated with 2.7 mg of each enzyme in mixture A and 1.9 mg of the enzyme in mixture B per 1 g TS in the sludge (totally 12.7 mg enzyme per 1 g TS). In the fourth sample there was no addition at all.

For both digestion tests the accumulated methane production from day one until the production ceased are shown in figures. For the first digestion test the theoretical and real methane potentials are presented in tables and the degree of degradation are calculated and presented together with the total degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP. In the second digestion test no increase in methane potential was observed and the margin of error was large so the theoretical methane potential and the degree of degradation were not interesting to determine.

5.2.1 Methane potential

The accumulated methane production for the different samples in the two digestion tests can be viewed in Figure 11 and Figure 12. The real methane potential in the different samples is defined as the methane production when the methane production in the two figures has ceased. The methane potential are presented as the produced amount of methane gas per amount organic material originating from the substrate (Nml/g VS) meaning the methane production from the inoculum is subtracted. The samples were run in triplicates so the values are mean values of the three parallels.

For the first digestion test (Figure 11) the real methane potential was reached after 20 days in the sludge treated with sodium citrate (SC) and after 33 days in the enzyme and sodium citrate treated sludge (SC + E) and untreated sludge. The methane production in the SC treated sludge ceased already after 20 days probably because of a different setup. The stdv in the real methane potential determination varied between 0.5 - 8.8 N ml CH_4/g VS_{sludge} (1 – 5 %).

91 % of the methane potential in the sludge treated with both enzymes and sodium citrate in the first digestion test was reached already after 14 days. This is less than half the time for 100 % to be reached. Less than 94 % of the methane potential was reached after 19 days. For the untreated sludge 91 % of the methane potential was reached after nearly 17 days. The treatment with enzymes and sodium citrate in test one decrease the retention time needed to reach 91 % of the methane potential with almost three days.

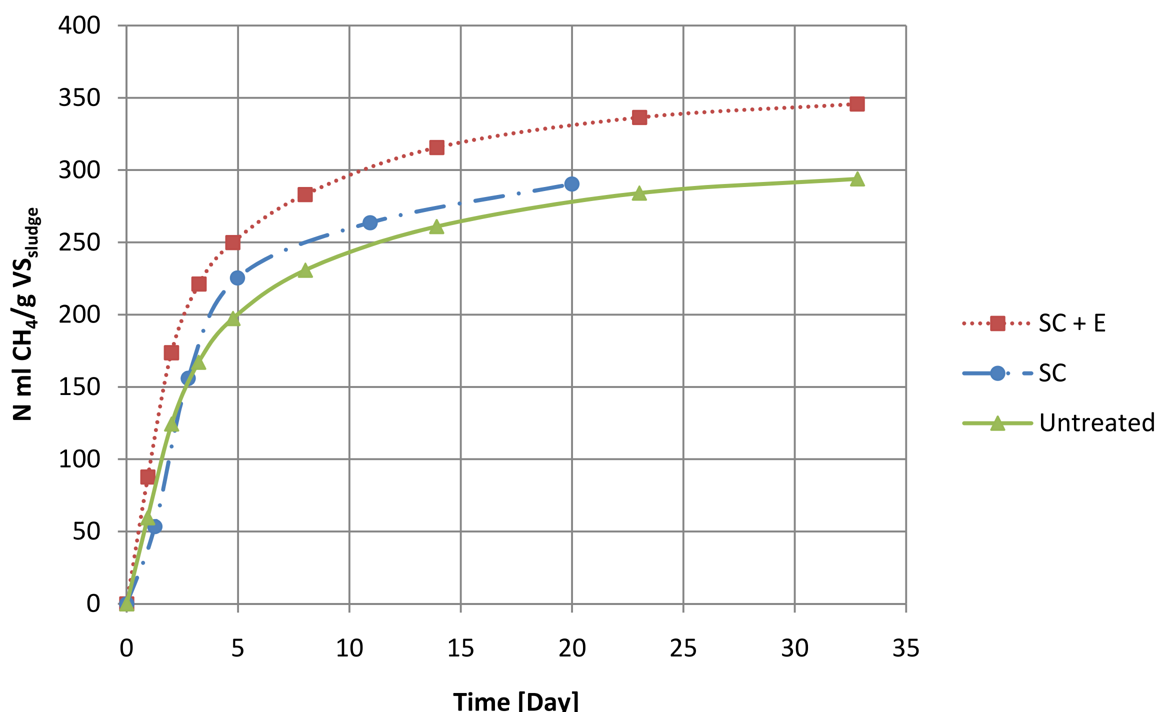


Figure 11: Accumulated methane production in the first batch laboratory digestion test. A mixture of primary sludge and EAS was used as a substrate. The addition of enzymes was a total amount of 18.6 mg per 1 g TS and the sodium citrate concentration was 5 mM. One sample was treated with enzymes and sodium citrate (SC + E), one sample with sodium citrate alone (SC) and one sample remained untreated. The methane production in the SC treated sludge ceased already after 20 days, probably because of a different setup.

The exact values of the real methane potentials reached in Figure 11 for the different samples in the first digestion test are presented in Table 6 together with the theoretical methane potential of the volatile solids in the sludge substrate. The calculations of these theoretical methane potentials are described in section 2.2.1 and the data used can be found in Appendix E. The theoretical methane potentials of the enzymes and sodium citrate applied is also shown in Table 6. These methane potentials were calculated using Buswell formula (equation 2.4 section 2.2.1), the assumption that all enzymes are proteins and the average composition for proteins shown in Table 2 section 2.2.1.

Over all the reached real methane potentials in this first digestion test are lower than previous studies of the methane potential in sludge from Henriksdal WWTP performed by Borggren (2008). After 35 days in the studies performed by Borggren (2008) an untreated sludge mixture of primary sludge and EAS similar to the one in digestion test one had reached a real methane potential of 310 – 320 Nml CH_4/g VS_{sludge} compared to 294 Nml CH_4 as in digestion test one.

Table 6: The methane potential in the different samples in the first batch laboratory digestion test performed within this thesis. The first digestion test contained one untreated sample, one sample treated with sodium citrate (SC) alone and one treated with both sodium citrate and enzymes (E + SC). The concentrations of sodium citrate and enzymes can be viewed in Table 5, section 4.3.2. The treatments were run in triplicate and the values are therefore means of the three parallels. The stdv in the real methane potential determination varied between 0.5 - 8.8 Nml CH4/g VS_{sludge} (1 – 5 %).

Treatment	Test	Real methane potential	Theoretical methane potential		
	[No.]	[Nml CH_4/g VS_{sludge}]	[Nml CH_4/g VS_{sludge}]	[Nml CH_4/g VS_E]	[Nml CH_4/g VS_{SC}]
Untreated	1	294	1521.3	-	-
SC	1	290	1628.0	-	23.1
E + SC	1	346	1519.4	5.2	23.1

Table 6 shows an increase of more than 19 % for the sludge treated with both enzymes and sodium citrate compared to the treatment with sodium citrate alone. When comparing with the untreated sludge the increase was almost 18 % for the enzyme and sodium citrate treated sludge. As can be seen in Figure 11 the methane production ceased already after 20 days for the sludge treated with sodium citrate alone so probably this result is not reliable.

For the second digestion test the stdv in the real methane potential determination varied between 0 - 36 Nml CH_4/g VS_{sludge} (0 – 15 %). These stdv were bigger than for the first digestion test and arised because of some diverging values. These diverging values originated from bottle 7 (enzyme treated sludge) and bottle 12 (enzyme and sodium citrate treated sludge) and these bottles were therefore excluded and new methane potentials were determined. For the samples in the second digestion test the real methane potentials was reached after 45 days for all the four samples (see Figure 12).The new stdv now varied between 0 – 16 Nml CH_4/g VS_{sludge} (0 – 4 %).

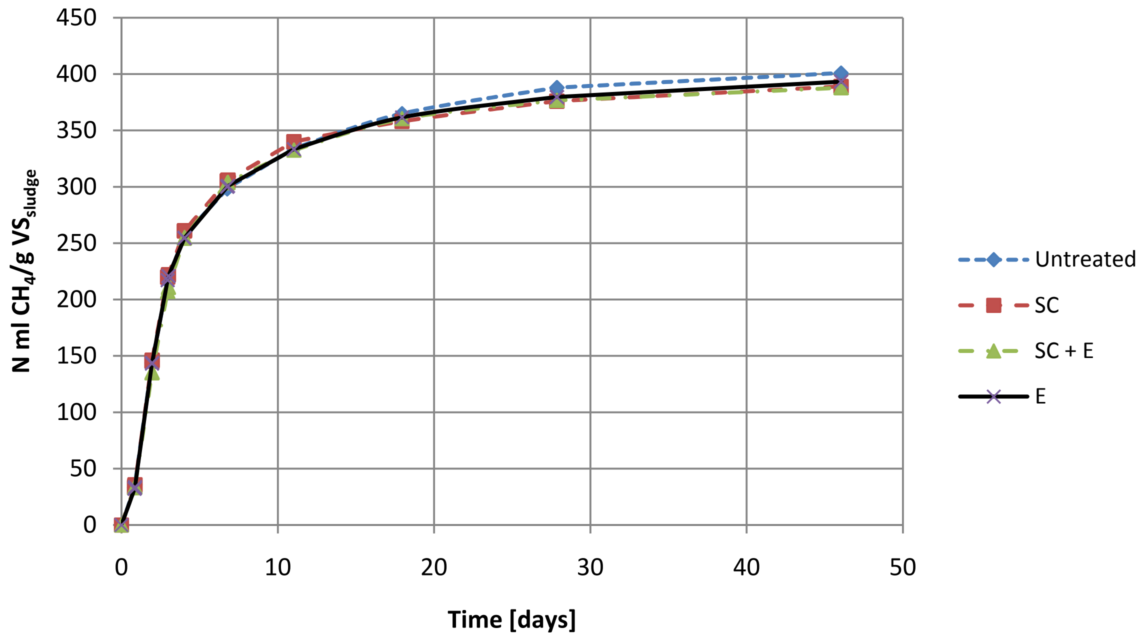


Figure 12: Accumulated methane production in the second batch laboratory digestion test. A mixture of primary sludge and EAS was used as a substrate. The enzymes added was a total amount of 12.7 mg per 1 g TS and the sodium citrate concentration was 1 mM. One sample was treated with sodium citrate and enzymes (SC + E), one sample with enzymes or sodium citrate alone (E or SC) and one sample remained untreated. The values are means of three parallells except for enzyme treated sludge (E) and enzyme and sodium citrate treated sludge (SC + E) were one bottle for each treatment was excluded in the calculations because of a diverging value.

As can be seen in Figure 12 the untreated sludge reached a higher methane potential than sludge treated with enzymes and sodium citrate which not is possible in the reality. This also means that no increase of the real methane potential could be set for the treated sludge compared to the untreated in the second digestion test. Maybe this is not a correct image of the reality because of the large stdvs. If the first digestion test was a correct image of the increase in real methane potential from a sludge mixture of primary sludge and EAS treated with enzymes in presence of sodium citrate and the increase in the release of soluble COD is directly related to the increase of the methane potential, the predicted increase of the real methane potential in the second digestion test can be determined. This was done for the increase of real methane potential for sodium citrate and enzyme treated sludge compared to the untreated sludge. The determination was accomplished through calculations of the decrease in the release of soluble COD between the two doses used in the two digestion tests. This decrease was directly translated as the decrease in real methane potential increase and the increase in real methane potential for sludge treated with enzymes and sodium citrate in the second digestion test was thereafter determined with the use of the increase in real methane potential for the sludge treated with enzymes and sodium citrate in the first digestion test. These calculations can be viewed in Appendix H. The predicted increase of real methane potential for the sodium citrate and enzyme treated sludge in the second digestion test was 7.4 %.

The accumulated methane potential in the second digestion test for the untreated sludge is larger than the methane potential reached in previous studies performed by Borggren (2008). As the untreated sample in the first digestion test reached a lower methane potential than previous studies performed by Borggren (2008) the results from the second digestion test were used to predict the real methane potential reached in the different samples in the first digestion test if the substrate was better utilized as it probably was in the second digestion test. Through extrapolation of Figure 11 with the use of Figure 12 the accumulated methane potential for the untreated sludge would be 301 Nml CH_4/g VS_{sludge} and the apperence of the accumulation of methane can be viewed in Figure 13. The increase in reached real methane potential for the sodium citrate and enzyme treated sludge (SC + E) compared to the untreated sludge is still almost 18 % but now the sodium citrate treated sludge reaches a higher methane potential than the untreated sludge which should agree more with the reality. This increase would be almost 5 %.

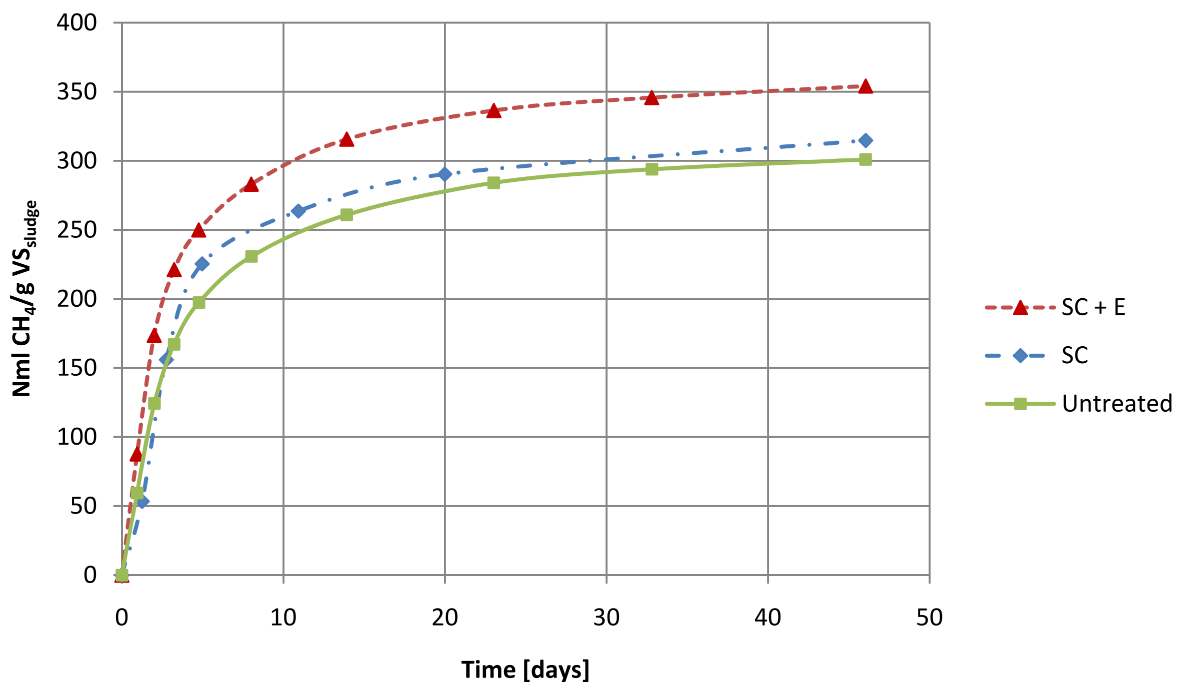


Figure 13: Accumulated methane production in the first batch laboratory digestion test. A mixture of primary sludge and EAS was used as a substrate. The addition of enzymes was a total amount of 18.6 mg per 1 g TS and the sodium citrate concentration was 5 mM. One sample was treated with enzymes and sodium citrate (SC + E), one sample with sodium citrate alone (SC) and one sample remained untreated. Some values are obtained through extrapolation using Figure 12.

5.2.2 Degree of degradation

As no increase in real methane potential could be set for the treated samples in the second digestion test no further comparision between the different samples were done.

The methane produced in the batch laboratory digestion tests was assumed to be the maximal methane potential of the substrate. The degree of degradation in the samples within the first batch laboratory digestion test is therefore referred to as DD_{max} in

Table 7.

Table 7 also shows the calculated degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP ($DD_{Henriksdal}$) for each sample in the first digestion test. The values are means of the three parallels of each sample. The degree of degradation was improved with 18.3 % for sludge treated with both enzymes and sodium citrate compared to untreated sludge. A detailed principle for the calculation of the degree of degradations is presented in Appendix I. Values of the used parameters can be viewed in Appendix E and G together with a more detailed result summary in Appendix J.

Table 7: Degree of degradation in the laboratory digestion chambers (DD_{max}) in the first digestion test and the theoretical degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP $DD_{Henriksdal}$. The untreated sample and the samples treated with sodium citrate (SC) or sodium citrate and enzymes (SC + E) are shown.

Treatment	Digestion test [No.]	DD_{max} [%]	$DD_{Henriksdal}$ [%]
Untreated	**1**	54.1	43.1
SC	**1**	53.5	44.2
SC + E	**1**	62.0	51.0

As a result of the improved degree of degradation in the digestion chambers at Henriksdal WWTP with enzymatic treatment in presence of sodium citrate the outgoing organic substances (originating from the primary sludge and EAS) from the digestion chambers could be reduced meaning there can be a smaller amount of digested sludge to be taken care of every year. The reduction in dewatered digested sludge (TS 30 %) would be about 4 700 tonne every year for the sludge treated with sodium citrate and enzymes compared to the untreated sludge. The calculation of this reduction can be viewed in Appendix J.

6 Discussion

This chapter provides the reader with a discussion of the most interesting results obtained within this thesis. The centre of gravity in the discussion is the benefits from the improved AD of sludge as a result of the addition of sodium citrate prior to enzymatic treatment. The chapter ends with a discussion of the approximate costs and revenues arising with a full scale application of the enzyme technique.

6.1 Solubilisation of sludge

In Figure 9 there was a clear increase in COD_{sol} with increasing enzyme dose. The same trend could be seen for all of the three concentrations of sodium citrate with some values diverging from the increasing trend. The treatments were performed at different times and on different sludge batches which can explain the diverging values. The effect corresponding to the treatment of sludge with enzymes and sodium citrate (Figure 9) improved the solubilisation of organic matter in the sludge with 17 - 32 % depending on the enzyme dose and sodium citrate concentration. The increase in COD_{sol} with increasing enzyme dose indicates that the sludge from Henriksdal WWTP is a good substrate for the enzymes added. None of the dose combinations of enzymes and sodium citrate was too low to affect the solubilisation of sludge so the lower doses of enzymes and sodium citrate used in the solubilisation experiments is justified to use in the batch laboratory digestion for a possible increase in methane production.

Figure 9 indicates that there is a difference in effect on the release of COD_{sol} between the three concentrations of sodium citrate as well. The clearest difference was seen between the two concentrations 5 and 3 mM. The short trial presented in Figure 10 strengthened this theory. This trial was performed on the same sludge meaning the effects from the differences in the sludge batches suggested in Figure 9 were almost eliminated. The sample stdv in this determination was relatively large though and this was because of the stdv originating from the determination of $COD_{sol,\ contr}$ used in the calculation of $COD_{treat\text{-}effect}$. Figure 10 showed a clear difference between all three of the sodium citrate concentrations for both the enzyme doses used. The increase in COD_{sol} with increasing enzyme dose was also clear in this figure. More reliable results similar to the once in Figure 9 could probably be obtained with the use of the same sludge batch. To obtain results corresponding to the ones in this thesis this would have required more laboratory equipment.

The increase in COD_{sol} from the heating of the sludge was in the same range as the increase from the treatment effect. (See appendix A, appendix B and appendix C) The heating effect was even greater than the treatment effect for low doses of enzymes and sodium citrate. Maybe it is possible to release more COD_{sol} from the sludge with a higher temperature instead of enzymatic treatment. Another possibility is that the enzymes and sodium citrate added release another fraction of organic matter in the sludge than the heating of the sludge does.

6.2 Benefits from sludge treatment with enzymes and sodium citrate

Below is a discussion of the benefits from the sludge treatment with enzymes and sodium citrate concerning increase in real methane potential and a resulting increased degree of degradation. The increase in real methane potential strengthens the theory stated above that sludge from Henriksdal WWTP is a good substrate for the enzymes applied.

6.2.1 Increase in methane potential

The first digestion test showed that the methane potential in the sludge increased with enzymatic treatment in presence of sodium citrate in batch laboratory digestion. When discussing the increase in methane potential originating from the effect of the enzymes in presence of sodium citrate, sludge treated with sodium citrate alone must be referred to as a reference to the sludge treated with both enzymes and sodium citrate. This is because sodium citrate itself is a source to methane production. The increase in methane potential corresponding to the effect of the enzymatic treatment in presence of sodium citrate was 19 % in the first batch laboratory digestion test when the sludge was treated with a total amount of 18.6 mg enzymes per 1 g TS in the sludge. The concentration of sodium citrate was 5 mM. The enzymes are also carbon sources but the theoretical methane potential of the enzymes were maximum 5.2 Nm^3 CH_4/tonne VS_{sludge} which if the enzymes were totally utilized to methane still should give an increase of the methane potential in the the first digestion test with more than 17 %.

As can be seen in Figure 11 the methane production in the sludge treated with sodium citrate alone ceaseed already after 20 days compared to the other two samples whose methane production continued for another 13 days. The untreated sludge also reached higher methane potential than the sludge treated with sodium citrate alone which should not be possible. This was obtained in all of the three parallels (data not shown) which arise questions of the possibility that the treatment with sodium citrate alone inhibited the methane production. This is though not probable because the sample with both enzymes and sodium citrate were not inhibited. The samples with sodium citrate alone were started the day after the untreated samples and the samples with sodium citrate together with enzymes but the same substrate and inoculum were used. Another possible explanation is therefore that the activity of the microorganisms within the inoculums was reduced. Because of the strange behavior of the sodium citrate treated samples these results are not reliable. All of the samples in the first digestion test reached lower real methane potentials than untreated sludge in previous studies performed by Borggren (2008). The methane potential also ceased earlier than in previous studies. The results from the second digestion test where the reached real methane potentials instead were higher than previous studies performed by Borggren (2007) were used to extrapolate Figure 11.

If the increase in methane potential is going to be used in a calculation of the costs and revenues the increase in methane potential from the sludge treated with enzymes and sodium citrate must be compared to the untreated sludge. This increase was about 18 % in the first batch laboratory digestion test for both the extrapolated (Figure 13) and not extrapolated (Figure 11) figures. This was a lower increase than compared to the sludge treated with sodium citrate alone for the not extrapolated figure which not is possible in the

reality as discussed above. The increase compared to untreated sludge is though more reliable.

In the second digestion test a total amount of 12.7 mg enzymes per 1 g TS in the sludge and a concentration of 1 mM sodium citrate was used. The solubilisation experiment justifies the lowered doses as described previous. No increase in the methane potential in this digestion test could be set. A possible increase would have been diminshed by the stdvs which made the reached real methane potential overlap for the different samples. Determination of methane potential in laboratory scale is probably too insecure to discover such a small possible increase. It is impossible to secure that the sludge is completely homogen and in a small scale such as batch laboratory digestion this factor is of greater importance than in a larger scale. If a smaller amount of enzymes and sodium citrate is to be tried out for the sludge at Henriksdal WWTP this is probably necessary to implement in a pilot or full scale operation with larger sludge volumes to obtain more reliable results. The increase in real methane potential for the enzyme and sodium citrate treated sludge in the first digestion test together with the relationship between the release of COD_{sol} and increasing enzyme dose and sodium citrate concentration was used to predict a possible increase in real methane potential for the enzyme and sodium citrate treated sludge in the second digestion test. This predicted increase was 7.4 % in the second digestion test compared to 17.6 % as in the first digestion test. This assumes that the first digestion test was reliable. This prediction also indicates that the stdvs diminshed the possible increase and that lower doses of enzymes and sodium citrate is justified.

6.2.2 Enhanced degree of degradation in the digestion chambers at Henriksdal WWTP

The calculated degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP was increased with 18.3 % for the sludge treated with both enzymes and sodium citrate compared to the untreated sludge when the results from the first batch laboratory digestion test was used. This theoretical increase was slightly greater than the increase determined in the digestion chamber because the first 20 days were the once being repeated in the calculation. This increase mostly corresponds to the effect of the enzymes in presence of sodium citrate on the solubilisation of organic matter in the sludge. Sodium citrate probably released the organic matter in the flocs which enables for the enzymes to degrade the complex organic molecules within the flocs to smaller molecules. These molecules were probably further converted to methane gas through the action of microorganisms in the sludge during AD.

The increased degree of degradation was also seen in Figure 11, section 5.2.1. The time needed to reach 91 % of the methane potential was decreased with almost three days in the sludge treated with enzymes and sodium citrate compared to the untreated sludge. This creates an opportunity to receive more wastewater sludge or EOM to the WWTP in Henriksdal every day. An enhanced degree of degradation also leads to a reduction of the digested sludge volume out from the digestion chambers as presented in section 5.2.2.

If the increase in degree of degradation is assumed to be equal with the increase of methane production the methane production at Henriksdal WWTP is increased with 18.3 % when sludge is treated with both enzymes and sodium citrate with a total amount of 18.6 mg enzyme per 1 g TS in the sludge and a concentration of 5 mM sodium citrate.

6.3 Enzyme and sodium citrate costs vs. revenues

This section presents the approximate costs and revenues arising if the technique with addition of sodium citrate prior to enzymatic treatment of wastewater sludge is to be implemented in a full scale operation at Henriksdal WWTP. The doses of enzymes and sodium citrate concentrations used in the first batch laboratory digestion test are used in the calculations. The section ends up with an economical conclusion from the calculations of the costs and revenues.

6.3.1 Costs

With the load of primary sludge and EAS every day presented in section 3.1.2 to the digestion chambers at Henriksdal WWTP the load of TS originating from these sludges are 67 800 kg every day. If the combined treatment of the sludge with enzymes and sodium citrate in batch laboratory digestion test one was to be implemented in a full scale operation this would correspond to an addition of 183 kg of each enzyme in mixture A and 529 kg of the enzyme in mixture B every day. With the approximate costs for the enzymes presented in section 4.1 this corresponds to approximately 19 000 USD every day which in turn corresponds to approximately 120 000 SEK every day at the moment of writing (Yahoo, 2008).

The total amount of sludge loaded to the digestion chambers in Henriksdal (primary sludge, EAS and EOM) is 1950 m^3 every day. If the same concentration of sodium citrate is to be used as in digestion test one (5 mM) this corresponds to approximately a total amount of 2 900 kg sodium citrate every day. With the approximate cost of sodium citrate presented in section 4.1 (10 SEK/kg) the daily cost of sodium citrate is 29 000 SEK.

The total costs every day would be approximately 150 000 SEK.

6.3.2 Revenues

The increased methane potential in the digestion chambers at Henriksdal WWTP (18.3 %) discussed in section 6.2.2 for sludge treated with a total amount of 18.6 mg enzyme per 1 g TS and with a concentration of 5 mM sodium citrate corresponds to an increase in methane production with 137 Nm^3 CH_4 every hour when the average methane production every hour at the existing plant in Henriksdal is 749 Nm^3. With a methane content of nearly 100 % in the purchased biogas and an assumption that the credit value (revenue minus margin cost) of this biogas is 5 SEK/Nm^3 this corresponds to an increasing income of 685 SEK every hour or approximately 16 440 SEK every day.

The reduction in digested sludge out from the digestion chambers calculated in section 5.2.2 also leads to a reduction in costs. With the average disposal cost of digested sludge presented in section 3.1.2 (250 SEK/tonne dewatered (TS 30 %) digested sludge) and the reduction in digested sludge calculated in section 5.2.2 (4 700 tonne dewatered digested sludge/year) this gives a cost reduction of approximately 1 175 000 SEK every year or 3 220 SEK every day.

The total revenues every day would be approximately 20 000 SEK.

6.3.3 Economical conclusions

As can be seen from the discussion above the increasing revenues from the improvement in AD and belonging increase in biogas production and reduce in digested sludge does not cover the costs for the enzymes and sodium citrate applied. The costs of the enzymes are though just a guideline in the calculation. If this technique is to be implemented in a full scale operation at Henriksdal WWTP the costs for the enzymes must be an agreement between Stockholm Water and the supplier. This would probably decrease the costs remarkably. Another important aspect is the existing demand of the enzymes. Enzymes to be applied in full scale operations are not at the moment a usual product. Before this technique is to be used in full scale operations more research has to be done to find lower doses of enzymes still affecting the methane production positively. If WWTPs in the future implement the enzyme technique this would lead to an upscale of the production of technical enzymes which in turn leads to decrease of the enzyme costs.

7 Concluding Remarks

The most important concluding remarks are listed below in this chapter.

- Sludge from Henriksdal WWTP is a good substrate for the hydrolytic enzymes used within this thesis when the cation binding agent tri-sodium citrate is presence.

- A mixture of primary sludge and EAS (73:27 TS) released 17 – 32 % more COD to the liquid phase when treated with enzymes and sodium citrate. An increased release of COD was observed for an increasing enzyme dose and sodium citrate concentration.

- A total amount of 18.6 mg enzymes per 1 g TS applied to a mixture of primary sludge and EAS (77:23 VS) from the existing WWTP in Henriksdal with a sodium citrate concentration of 5 mM resulted in an almost 18 % increase of the methane potential in a batch laboratory digestion test when compared to untreated sludge. If the sludge is to be treated with this dose of enzymes and sodium citrate concentration the retention time can be decrease with more than three days compared to the untreated sludge loaded in the digestion chambers today.

- The theoretical degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP based on the results from the digestion test presented above is increased with 18.3 % for sludge treated with the same enzyme dose and sodium citrate concentration as above.

- The need for a reduction in the enzyme costs is necessary for a profitable full scale operation using the enzyme technique.

8 Future research

The absence of improvement in AD in the second digestion test indicates that the concentration of sodium citrate used in the first digestion test (5 mM) is the lowest concentration enhancing the activity of the enzymes when the sludge from Henriksdal WWTP is used as a substrate. It is possible that a concentration of 5 mM sodium citrate affect the AD process positively even when the lower concentration of enzymes (12.7 mg/g TS) is used. The prediction of increase in real methane potential in the enzyme and sodium citrate treated sludge compared to untreated sludge in the second digestion test though show that the dose and concentration used in the second digestion test should give a 7.4 % increase of the methane potential. Batch laboratory digestion test is an insecure method due to the large margin of error in the methane potential determination if small increases are to be detected. The increase in methane potential must exceed the margin of error to obtain a significant result. Therefore small increases in methane production are difficult to detect with laboratory digestion tests.

Since the sludge from Henriksdal WWTP is shown to be a good substrate for the enzymes this technique should be applied in a larger scale e.g. pilot scale. In a pilot scale application even small increases in the methane production can be detected because larger sludge volumes are used.

With respect to the cost one possibility is to reduce the amount of lipase in the sludge or even eliminate the addition of lipase. Lipase is the most expensive enzyme and this could be worth a try. The affect of the elimination of lipase could be investigated using the solubilisation experiment described in section 4.2.2 but to obtain more reliable results a shorter trial should be done if possible with the use of the same sludge batch. This eliminates the fact that variations in the sludge batches are a big factor affecting the outcome of the investigation.

9 References

Below is the used reference within this thesis listed as literature, digital sources and personal communication.

9.1 Literature

Berg, J.M., Tymoczko, J.L., Stryer, L. (2002) *Biochemistry fifth edition*, W.H. Freeman and Company, (8), 189-213.

Borggren, C. (2008). *Mätning av metanpotentialen hos slam på Henriksdal och Bromma, metodutveckling och utvärdering av labutrustning ”BCS - CH_4 Biogas”, Master`s thesis,* Royal Institute of Technology.

Chrysi, S., Laspidou, Bruce, E. & Rittmann (2002). *A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass.* Water Research, 36, 2711-2720.

Davidsson, Å. (2007). *Increase of biogas production at wastewater treatment plants; addition of urban organic waste and pre-treatment of sludge.* Water and Environmental Engineering, department of chemical engineering, Diss. Lund University ISBN: 978-91-7422-143-5.

Davidsson, Å., Wawrzyńczyk, J., Norrlöw, O. & La Cour Jansen, J. (2007). *Strategies for enzyme dosing to enhance anaerobic digestion of sewage sludge.* Journal of Residuals Science & Technology, 4 (1).

Dey Szwajcer, E., Szewczyk, E., Wawrzyńczyk, J. & Norrlöw, O. (2006). *A novel approach for characterization of exopolymeric material in sewage sludge.* Journal of residuals science & technology, 3 (2), 97 – 103.

Gerardi, M.H. (2003). *The microbiology of anaerobic digesters,* Wiley Interscience ISBN: 0-471-20693-8.

Gurgo e Cirne, D. (2006). *Evaluation of biological strategies to enhance hydrolysis during anaerobic digestion of complex waste,* Diss. Lund University ISBN: 91-89627-41-5.

Hansen, T.L., Schmidt, J.E., Angelidaki, Il, Marca, E., Jansen, J.C, Mosbaek, H. & Christensen, T.H. (2004). *Method for determination of methane potentials of solid organic waste.* Waste Management, 24(4), 393-400.

Recktenwald, M., Wawrzynzczyk, J., Dey Szwajcer, E. & Norrlöw, O. (2007). *Improved industrial scale anaerobic digestion by the addition of glycosidic enzymes.* Water Research, submitted.

Vallin, L., Hellström, D., **Jonsson, L., Hagland, E., Medoc, M. & Petterson, F.,** (2008). *Real life case in Henriksdal wastewater treatment plant in Stockholm, Sweden.*

Wawrzyńczyk, J. (2007). *Enzymatic treatment of wastewater sludge; sludge solubilisation, improvement of anaerobic digestion and extraction of extracellular plymeric substances,* Divison of pure and applied biochemistry, Diss. Lund University ISBN: 978-91-628-7246-5.

Wawrzyńczyk, J. Recktenwald, M., Norrlöw, O. & Dey Szwajcer, E. (2007a) *Solubilisation of sludge by combined chemical and enzymatic treatment.* African Journal of Biotechnology, 4 (17).

Wawrzyńczyk, J., Reckentwald, M., Norrlöw, O. & Dey Szwajcer, E. (2008). *The function of cation-binding agents in enzymatic treatment of municipal sludge.* Water Research, 42(6-7), 1555-1562.

Wawrzyńczyk, J, Dey Szwajcer, E., Norrlöw, O. & Jansen, J.C. (2003). *Alternative method for sludge reduction using commercial enzymes.* Published in the proceedings of the 8th European Biosolids and Organic Residuals Conference, Wakefield, West Yorkshire, UK, 1 – 5.

Wawrzyńczyk, J., Szewczyk, E., Norrlöw, O. & Dey Szwajcer, E. (2007c) *Application of enzymes, sodium tripolyphosphate and cation exchange resin for the release of extracellular polymeric substances from sewage sludge. Characterization of the extracted polysaccharides/ glycoconjugates by a panel of lection.* Journal of Biotechnology 130 (2007), 274 – 281.

9.2 Digital sources

Stockholm Water 1:

http://www.stockholmvatten.se/stockholmvatten/, 2008-03-03

Stockholm Water 2:

http://www.stockholmvatten.se/Stockholmvatten/commondata/269/PDF_fordjupning_avlopp.pdf, 2008-03-03

Yahoo:

http://finance.yahoo.com/currency, 2008-02-29 (1 USD = 6,1745 SEK)

9.3 Personal communication

Unnefors, B. Brenntag Nordic AB.

Hellström, D. Head of development and investment, Wastewater treatment, Stockholm Water Co., Sweden.

Wawrzyńczyk, J. Kemira Kemi AB, Helsingborg.

Norrlöw, O. Head of recycling, competence centre, Kemira Kemi AB, Helsingborg.

Appendix A – The sludge mixtures

Below are the TS in the primary sludge and EAS used in the different sludge batches listed together with the exact composition of each sludge batch concering primary sludge, EAS and water per one litre sludge batch.

Primary sludge No.	TS [%]	EAS No.	TS [%]
1	3.92	**1**	5.54
2	3.20	**2**	5.73
3	3.11	**3**	5.86
4	3.26	**4**	5.95
5	3.31	**5**	5.89
6	3.64	**6**	5.97
7	4.50	**7**	5.90
8	4.54		
9	4.65		

Batch	Primary sludge		EAS		Water	Total
No.	**No.**	**Addition [g/l batch]**	**No.**	**Addition [g/l batch]**	**Addition [g/l batch]**	**g/l batch**
1	1	373	1	97	530	1000
2	2	456	2	94	450	1000
3	3	469	2	94	437	1000
4	4	448	3	92	460	1000
5	5	441	3	92	467	1000
6	6	401	4	91	508	1000
7	6	401	4	91	508	1000
8	7	324	5	92	584	1000
9	8	322	6	90	588	1000
10	8	322	6	90	588	1000
11	9	314	7	92	594	1000
12	6	401	4	91	508	1000

Appendix B – $COD_{sol, cold}$ and $COD_{sol, heat}$ in the sludge batches

The COD_{sol} in all of the cold sludge batches used within this thesis ($COD_{sol, cold}$) are presented in the table below. For seven of the twelve sludge batches COD_{sol} was determined after four hours in 37 °C ($COD_{sol, heat}$). The five remaining sludge batches were only used in the determination of the contribution to COD_{sol} from the enzymes and sodium citrate added to the sludge ($COD_{sol, contr}$) and was therefore never heated. All COD_{sol} determinations were accomplished using test kits from Dr. Lange (COD LCK 514 and COD LCK 014) and set in triplicates. The values below are therefore means of three measurements.

Batch No.	$COD_{sol, cold}$ [mg/l]	$COD_{sol, heat}$ [mg/l]
1	1012	1923
2	1290	2011
3	1111	1808
4	834	1699
5	946	1796
6	1207	2056
7	1329	2176
8	715	-
9	739	-
10	787	-
11	1304	-
12	1207	-

Appendix C – $COD_{sol, cold, tot}$ for the dose combinations

Following table shows the total COD_{sol} in the sludge after addition of enzymes and sodium citrate ($COD_{sol, cold, tot}$). The sludge batch used in the individual treatment is also presented. Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per 1 g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A. All $COD_{sol, cold, tot}$ determinations were performed in the cold sludge directly after the addition of enzymes and sodium citrate using test kits from Dr. Lange (COD LCK 514 and COD LCK 014).

Sodium citrate [mM]	Enzyme dose [mg/g TS]	Batch No.	$COD_{sol, cold, tot}$ [mg/l]
5	11	8	2201
		9	2173
	9	11	2664
		12	2596
	7	10	1940
		10	1963
	5	8	1804
		9	1828
	3	8	1747
		11	2312
3	11	10	1832
		11	2433
	9	8	1672
		11	2329
	7	8	1551
		10	1678
	5	9	1476
		10	1511
	3	8	1386
		11	1936
1	11	9	1418
		9	1414
	9	11	1906
		12	1897
	7	8	1223
		11	1860
	5	9	1115
		11	1701
	3	8	1017
		11	1649

Appendix D – $COD_{sol, tot}$ for the dose combinations

Following table shows the total COD_{sol} in the sludge after enzymatic treatment in the presence of sodium citrate ($COD_{sol, tot}$). The sludge batch used in the individual treatment is also presented in the table. Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per 1 g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A. All $COD_{sol, tot}$ determinations were performed in the sludge after four hours treatment in 37 °C and during stirring, using test kits from Dr. Lange (COD LCKA 514 and COD LCK 014).

Sodium citrate [mM]	Enzyme dose [mg/g TS]	Batch No.	$COD_{sol, tot}$ [mg/l]
5	11	1	4200
		2	4427
		5	4132
	9	1	4097
		5	3885
		6	4110
	7	3	3705
		4	3671
		5	3791
	5	1	3674
		3	3574
		5	3564
	3	2	3760
		4	3048
		7	3805
3	11	3	3603
		4	3525
		2	3746
	9	5	3413
		7	3809
	7	1	3529
		3	3151
	5	2	3359
		3	3009
	3	1	3082
		4	2840
		7	3349

Sodium citrate [mM]	Enzyme dose [mg/g TS]	Batch No.	$COD_{sol, tot}$ [mg/l]
1	11	2	3565
		2	3545
	9	1	3276
		6	3196
	7	2	3189
		4	2748
	5	4	2718
		5	2736
	3	1	2664
		4	2392
		7	2865

Appendix E – Substrate content in the digestion tests

Both digestion test one and two was performed with a mixture of primary sludge and EAS treated with sodium citrate alone (SC), sodium citrate together with enzymes (SC + E) and one untreated sludge sample. Digestion test two was performed with an additional sample with enzymatic treatment alone. The VS originating from the primary sludge were 77 % and VS from the EAS were 23 %. The enzyme doses and sodium citrate concentrations used can be found in section 4.3.2.

VS and COD content

The COD content in the sludge was not determined. Therefore theoretical values of the COD content was determined and used in the calculation of the degree of degradation (below). The VS in each sample were though determined and then used in the calculation of the theoretical COD content. The primary sludge consist of 1.56 g COD/g VS in the sludge and the EAS consist of 1.52 g COD/g VS according to Vallin et al. (2008). With the relationship between the primary sludge and EAS presented above in the sludge mixture the evaluation factor for this mixture is 1.55 g COD/g VS. The VS content in the different samples together with the estimated content of COD is shown in Table 8 below.

Table 8: VS and COD added in the different samples used in the two batch laboratory digestion tests performed within this thesis. VS were measured and COD was calculated. Both values are round offs. According to Vallin et al., (2008) 1 g VS corresponds to 1.55 g COD. The substrate used was a mixture of primary sludge and EAS. The treatments in the samples were sodium citrate alone (SC), sodium citrate together with enzymes (SC + E) or enzymes alone (E). One sample remained untreated in both the digestion tests.

Treatment	Digestion test [No.]	VS [g]	COD [g]	VS_{mean} [g]
Untreated	1	2.8	4.3	2.8
		2.8	4.3	
		2.9	4.5	
	2	3.0	4.7	3.0
		3.0	4.7	
		3.0	4.7	
SC + E	1	2.8	4.3	2.8
		2.9	4.5	
		2.8	4.3	
	2	3.0	4.7	3.0
		3.0	4.7	
		3.0	4.7	

Treatment	Digestion test [No.]	VS [g]	COD [g]	VS_{mean} [g]
SC	1	2.8	4.3	3.0
		3.0	4.7	
		3.1	4.8	
	2	3.0	4.7	3.0
		3.0	4.7	
		3.0	4.7	
E	2	3.0	4.7	3.0
		3.0	4.7	
		3.0	4.7	

Appendix F – Calculation principle of methane production

To be able to calculate the produced methane gas in the different samples the volume of produced biogas is first determined. The content of methane set with gaschromatography (PerkinElmer ARNEL, Clarus 500, column: 7’ HayeSep N 60/80, 1/8” SF; FID detector 250 °C; carrier gas: Helium, flow 31 mL/min; temp injector: 60 °C. Headspace, sampler Turbo Matrix 110) is then used to determine the volume of methane produced.

The measured values of total biogas pressure at every measurement together with the knowledge of the gas volume and temperature in the bottles are used to determine the amount of produced biogas with the ideal gas law, equation 1. The space in the bottles not consisting of sludge is referred to as headspace and this is where the gas is produced. With a working volume of 600 ml sludge for every sample the headspace is equal between the bottles.

$$pV = nRT \tag{1}$$

where

p	is the pressure
V	is the volume
N	is the amount of gas (mole)
R	is the gas constant (8.314 $Jmol^{-1}K^{-1}$)
T	is the temperature in Kelvin

The amount of biogas in the bottles at every measurement occasion is determined;

$$n_1 = \frac{p_1 \cdot V_1}{R \cdot T_1} \tag{2}$$

where

n_1	is the amount of biogas at each measurement
p_1	is the total pressure at each measurement (measured + atmospheric pressure)
V_1	is the headspace in the bottles
T_1	is the temperature at each measurement

The amount of biogas in the bottles after each measurement is determined;

$$n_2 = \frac{p_2 \cdot V_2}{R \cdot T_2} \qquad (3)$$

where

n_2	is the amount of biogas in the bottles after equalization of pressure
p_2	is the pressure in the bottles at incubation and after each equalization (assumed to be atmospheric)
V_2	is the headspace in the bottles
T_2	is the temperature in Kelvin at incubation and after each measurement

The amount of biogas produced at each measurement occasion is determined using equation;

$$n_1 - n_2 = n_{biogas} \qquad (4)$$

The produced volume of biogas corresponding to n_{biogas} is determined with the ideal gas law, equation 1. The volume is determined at STP (1 atm, 0°C). With the known content of methane in the biogas, calculated using gas chromatography, the volume of methane gas produced is set.

Appendix G–Accumulated methane production

Below is the accumulated methane production in the two batch laboratory digestion tests. Both the methane production originating from the inoculum and the methane production originating from the different sample substrate is shown. The methane production from the substrates is the accumulated methane production when the methane production from the inoculum is subtracted.

The first digestion test

The first digestion test was carried out with three different samples run in triplicates. The methane production from the inoculum was also measured in triplicates and this data is shown below.

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Inoculum 1**	**Inoculum 2**	**Inoculum 3**	**Means**	**Stdv [%]**
0	0	0	0	0	0
0.95	0.94	0.75	0.67	0.78	17.41
2.00	2.26	2.14	2.09	2.17	4.08
3.26	4.24	3.97	3.75	3.99	6.19
4.75	6.54	6.05	5.99	6.19	4.91
7.97	11.35	10.46	10.40	10.74	4.98
13.91	22.09	19.84	19.80	20.58	6.37
22.98	37.17	35.82	36.22	36.40	1.90
32.77	48.82	46.59	47.90	47.77	2.34

The first sample (bottles 1 - 3) was untreated sludge (data shown below).

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Bottle 1**	**Bottle 2**	**Bottle 3**	**Means**	**Stdv [%]**
0	0	0	0	0	0
0.94	59.68	59.93	58.95	59.52	0.85
2.01	123.78	122.77	126.56	124.37	1.58
3.23	167.52	165.96	167.89	167.12	0.61
4.77	196.25	196.57	199.26	197.36	0.84
8.02	228.62	230.88	232.84	230.78	0.91
13.92	259.36	261.55	261.93	260.95	0.53
23.02	282.17	284.93	285.21	284.10	0.59
32.81	292.05	296.31	293.32	293.89	0.74

The second sample (bottles 4 – 6) was sludge treated with a 5 mM concentration of sodium citrate and a total amount of 18.6 mg enzyme per 1 g TS in the sludge (data shown below).

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Bottle 4**	**Bottle 5**	**Bottle 6**	**Means**	**Stdv [%]**
0	0	0	0	0	0
0.95	83.93	87.40	91.92	87.75	4.57
2.01	170.77	175.96	174.27	173.67	1.52
3.24	216.99	224.41	222.18	221.20	1.72
4.76	245.00	254.44	250.24	249.89	1.89
8.01	278.05	288.04	283.07	283.05	1.76
13.92	313.11	322.48	311.27	315.62	1.91
23.03	331.37	343.01	334.87	336.42	1.78
32.80	339.54	353.39	344.20	345.71	2.04

The third sample (bottles 7 – 9) was treated with a total amount of 18.6 mg enzymer per 1 g TS in the sludge (data shown below).

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Bottle 7**	**Bottle 8**	**Bottle 9**	**Means**	**Stdv [%]**
0	0	0	0	0	0
1.26	55.39	53.95	51.04	53.46	4.15
2.76	161.39	156.37	150.17	155.98	3.60
4.97	232.95	225.01	218.23	225.40	3.27
10.93	272.55	262.64	255.67	263.62	3.22
19.99	297.96	291.86	281.22	290.35	2.92
29.78	289.18	284.78	272.23	282.06	3.12

The second digestion test

The second digestion test was carried out with four different samples run in triplicates. The methane production from the inoculum was also measured in triplicates and this data is shown below.

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Inoculum 1**	**Inoculum 2**	**Inoculum 3**	**Means**	**Stdv [%]**
0	0	0	0	0	0
0.87	2.60	2.44	2.25	2.43	7.16
1.94	4.78	4.50	4.21	4.50	6.27
2.94	8.10	7.83	7.32	7.75	5.10
4.02	12.00	11.52	10.45	11.32	7.00
6.82	20.49	20.61	19.93	20.34	1.79
11.03	33.22	33.60	32.00	32.94	2.55
17.94	50.23	51.79	49.58	50.53	2.25
27.87	62.24	64.22	60.69	62.38	2.84
46.06	80.46	82.62	80.02	81.03	1.72
58.03	91.50	93.47	90.47	91.81	1.66

The first sample (bottles 1 – 3) was untreated sludge (data shown below).

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Bottle 1**	**Bottle 2**	**Bottle 3**	**Means**	**Stdv [%]**
0	0	0	0	0	0
0.83	33.34	33.82	33.14	33.43	1.04
1.96	144.89	144.89	144.89	144.89	0.00
2.95	216.60	215.85	214.26	215.57	0.56
4.02	257.56	256.46	255.59	256.54	0.38
6.82	299.07	295.53	303.69	299.43	1.37
11.03	337.41	326.99	337.93	334.11	1.85
17.95	370.17	355.05	368.46	364.56	2.27
27.85	393.29	375.81	394.18	387.76	2.67
46.04	406.47	388.04	409.17	401.23	2.87

The second sample (bottles 4 – 6) was sludge treated with a 1 mM concentration of sodium citrate (data shown below).

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Bottle 4**	**Bottle 5**	**Bottle 6**	**Means**	**Stdv [%]**
0	0	0	0	0	0
0.85	34.47	33.52	33.59	33.86	1.57
1.96	145.83	143.88	149.06	146.26	1.79
2.95	220.78	215.86	223.27	219.97	1.71
4.03	263.86	256.82	262.12	260.93	1.41
6.81	306.57	307.41	305.45	306.48	0.32
11.03	341.39	343.89	334.01	339.76	1.51
17.95	360.20	362.51	351.84	358.18	1.57
27.86	378.06	379.95	369.56	375.86	1.47
46.05	390.68	392.85	382.71	388.75	1.37

The third sample (bottles 7 – 9) was sludge treated with a total amount of 12.7 mg enzymes per 1 g TS in the sludge (data shown below).

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Bottle 7**	**Bottle 8**	**Bottle 9**	**Means**	**Stdv [%]**
0	0	0	0	0	0
0.85	27.78	32.40	33.29	31.15	9.48
1.95	108.23	142.90	143.23	131.45	15.30
3.00	182.36	221.97	219.51	207.95	10.67
4.03	214.63	258.03	251.51	241.39	9.70
6.81	263.60	302.94	298.63	288.39	7.48
11.03	298.4	335.91	331.00	321.78	6.34
17.95	325.54	364.32	359.04	349.63	6.01
27.86	345.00	381.59	377.88	368.16	5.47
46.05	358.3	396.23	390.00	381.67	5.35

The forth sample (bottle 10 – 12) was sludge treated with a total amount of 12.7 mg enzymes per 1 g TS in the sludge and a concentration of 1 mM sodium citrate (data shown below).

	Acc. Methane [Nml CH4/g VS]				
Time [days]	**Bottle 10**	**Bottle 11**	**Bottle 12**	**Means**	**Stdv [%]**
0	0	0	0	0	0
0.86	30.88	37.53	33.54	33.98	9.85
1.95	139.96	129.93	114.07	127.99	10.20
3.01	218.23	204.35	173.16	198.58	11.62
4.04	263.61	246.56	213.84	241.34	10.48
6.76	312.00	295.10	255.01	287.37	10.19
11.04	345.01	320.21	276.77	314.00	11.00
17.95	372.11	348.74	303.28	341.38	10.25
27.86	388.34	365.08	318.29	357.23	9.99
46.05	398.97	376.98	330.68	368.87	9.45

Appendix H – Predicted real methane potential in the second digestion test

No increase in real methane potential was set for sludge treated with enzymes and sodium citrate in the second digestion test compared to untreated sludge. The stdvs could have diminshed a possible increase.

If the increase in real methane potential for the sludge treated with enzymes and sodium citrate in the first digestion test was a correct image of the reality and the increase in the release of soluble COD is directly related to the increase of the real methane potential, the predicted increase in real methane potential for the enzyme and sodium citrate treated sludge in the second digestion test compared to untreated sludge can be determined.

First the decrease in release of COD_{sol} between the different enzyme doses and sodium citrate concentrations must be determined. Figure 14 shows the relationship between enzyme dose and sodium citrate concentration for the two sodium citrate concentration 5 and 3 mM and five different enzyme doses. Figure 14 is a subtraction of $COD_{sol,\ heat}$ from $COD_{sol,\ tot}$.

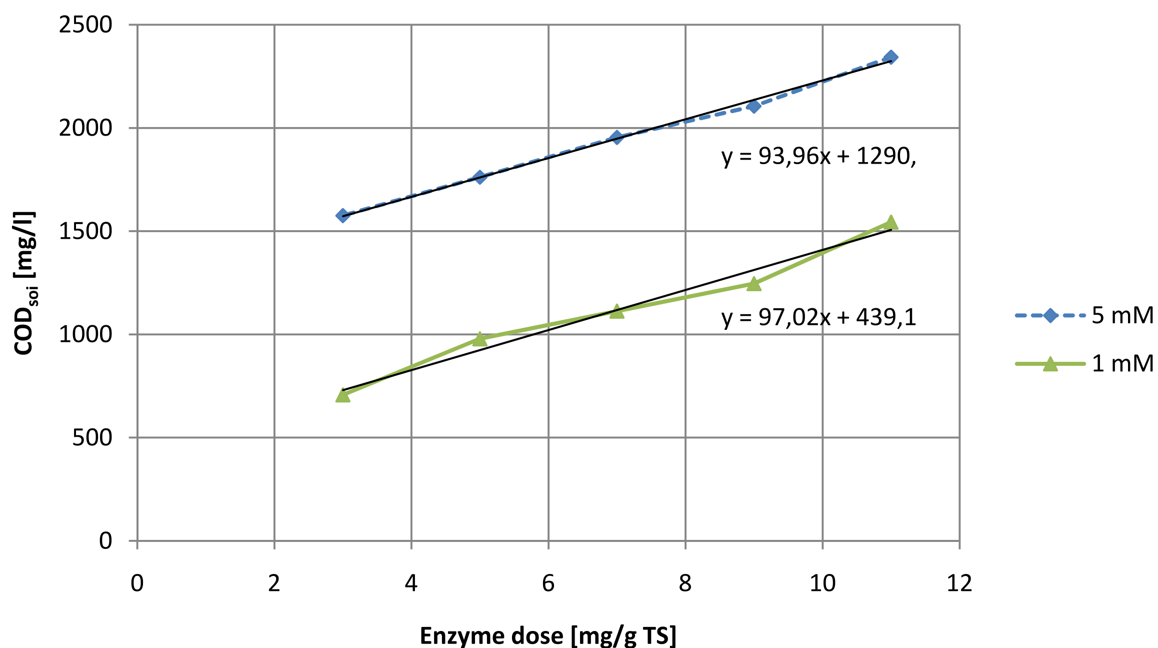


Figure 14: COD_{sol} in sludge after subtraction of $COD_{sol,\ heat}$ from $COD_{sol,\ tot}$. Different sludge batches with a mixture of primary sludge and EAS (TS 2 %) were used. Each treatment was performed in duplicates or triplicates and the figure therefore shows the mean value of these parallels. COD_{sol} from each treatment was determined in the liquid phase in triplicates. Enzyme dose 11 corresponded to 11 mg of each enzyme in mixture A per g TS in the sludge. The addition of the enzyme in mixture B was always 70.8 % of the dose of each enzyme in mixture A.

With the use of equations shown in Figure 14 for the increase in release of COD_{sol} with increasing enzyme dose for the two sodium citrate concentrations, the release of COD_{sol} for the corresponding enzyme doses and sodium citrate concentrations used in the two digestion tests can be determined. As the relationship between the enzymes in mixture A and mixture B in the first digestion test did not agree with the relationship in the solubilisation experiment the addition of enzymes in the first digestion test had to be translated to agree with the solubilisation experiment to be able to compare these results. Approximately 3.95 mg enzyme of each enzyme in mixture A per 1 g TS in the sludge was added to the sludge in the first digestion test together with a concentration of 5 mM sodium citrate. This corresponds to a release of 1661 mg COD per 1 liter using the equation for the concentration of 5 mM sodium citrate in Figure 14. In the second digestion test 2.7 mg of each enzyme in mixture A per 1 g TS in the sludge was added to the sludge togheter with a concentration of 1 mM sodium citrate. This corresponds to a release of 701 mg COD per 1 liter using equation for the concentration of 1 mM sodium citrate in Figure 14. The decrease in release of COD_{sol} was 57.8 % between the doses used in the first and second digesion test. As the increase in real methane potential for the first digestion test was 17.6 % the increase in real methane potential in the second digestion test should be 7.4 % if the decrease in release of COD_{sol} could be directly translated to the decrease in real methane potential increase.

Appendix I – Principle for calculation of the degree of degradation in a fully blended and continuous digestion chamber

Given below is a principle for an estimation of the degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP ($DD_{Henriksdal}$). The fact that the digestion chamber is fully blended is an assumption making it possible to implement this principle.

The results from the batch laboratory digestion tests were used in the calculation. The batch laboratory digestion tests were run in triplicate with a mixture of EAS and primary sludge from Henriksdal WWTP. The methane production was determined in the digestion tests until the methane production ceased.

The degree of degradation of the added substrate in the batch laboratory digestion test (DD_{lab}) was calculated and the part of the incoming amount of substrate being degraded in a digestion chamber at Henriksdal WWTP was determined. Together these two values give an estimation of the $DD_{Henriksdal}$.

Degree of degradation in batch laboratory digestion, DD_{lab}

DD_{lab} is determined using formula below. The methane production after t days is determined in the digestion test and the theoretical methane production is the theoretical amount of methane a given substrate can produce. According to Davidsson 2007, 350 Nm^3 CH_4 is produced per tonne COD in the added substrate.

$$DD_{lab}(t) = \frac{(Methane\ production\ after\ t\ days)}{(Theoretical\ methane\ production\ after\ t\ days)} \cdot 100$$

Degradation of incoming substrate in a fully blended and continuous digestion chamber

To be able to calculate the share of the incoming substrate being degraded in a fully blended continuous digestion chamber there must be assumptions. One assumption is that the *maximal methane production* a substrate can produce is reached in batch laboratory digestion tests when the methane production ceases. The degree of degradation in a batch laboratory digestion test is therefore in the continuation referred to as the maximal degree of degradation, DD_{max}. This is an assumption which probably gives an underestimation of the maximal methane production. This is though of less importance when the calculated values are relatively compared.

The share of the maximal degree of degradation reached after t days in the batch laboratory digestion test, $DD_{share\ of\ max}$ (t), is calculated using formula below;

$$DD_{share\ of\ \max}(t) = \frac{DD_{lab}(t)}{DD_{\max}} = \frac{Methane\ production\ after\ t\ days}{Maximal\ methane\ production} \cdot 100$$

A given amount substrate added to the fully blended digestion chamber at t = 0 is diluted according to the following equation;

$$C(t) = C_0 * e^{-t/\theta}$$

Where

C(t)	*is the current concentration of substrate*
C_0	*is the starting concentration of substrate*
θ	*is the mean retention time for sludge in the digestion chamber*
	= Volume/mean flow=V/Q
t	*is the current time*

The total amount of substrate that has passed the digestion chamber after time t is defined;

$$\int A(t)\,dt = \int Q(t) \cdot C(t)\,dt = \int \frac{V}{\theta} \cdot C(t)\,dt$$

Where

A(t)	*is the amount of substrate passed at a given time*
Q(t)	*is the mean flow of substrate at a given time*
V	*is the volume in the digestion chamber*

The assumption that $\rho_{substrate} = \rho_{water}$ gives that the amount of substrate in the digestion chamber at a given time per unit of volume is;

$$S(t) = \int \frac{1}{\theta} \cdot C(t)\, dt$$

Where

S(t) is the amount of substrate at a given time per unit of volume.

The share of the total incoming substrate available to be degraded in a fully blended and continuous digestion chamber is determined;

$$DD_{share\ of\ \max, total} = \int S(t) \cdot DD_{share\ of\ \max}(t)\, dt$$

Degree of degradation in a fully blended and continuous digestion chamber

There are two main factors influencing the degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP ($DD_{Henriksdal}$). Both are described and calculated above. One is the fact that all of the organic substances added to the digestion chamber are not degraded. The share of the substrate being degraded was determined with batch laboratory digestion tests and is referred to as DD_{max}. The dilution of the substrate is another factor. This is determined through calculations of the share of DD_{max} being fulfilled at every moment ($DD_{share\ of\ max}$). A sum of the share at each moment gives the total share ($DD_{share\ of\ max,\ total}$). The $DD_{Henriksdal}$ is given when the two factors are multiplied;

$$DD_{Henriksdal} = DD_{share\ of\ \max, total} \cdot DD_{\max}$$

Appendix J – Result summary of digestion test one

Given below is a summary of the results concerning degree of degradation, methane potential and theoretical reduction in digested sludge for the first batch laboratory digestion test.

Degree of degradation and methane potentials

The degree of degradation was determined for the substrate added in the different samples in the first batch laboratory digestion test. As described in Appendix I this is assumed to be the maximal methane production and is therefore referred to as DD_{max} in Table 9 below.

DD_{max} is determined as described in Appendix I from the real methane production reached in the batch laboratory digestion test and the theoretical methane production calculated from the COD content in the sludge substrate as described in section 2.2.1 using Buswells formula. The calculated values of the theoretical methane production are shown in Table 9 together with the total methane production in the different samples in the batch laboratory digestion test referred to as real methane. All values in Table 9 are means of three parallels.

Table 9: Real methane potential, theoretical methane potential and DD_{max} for the samples in the first digestion test. The real methane potential is determined through batch laboratory digestion tests with the use of a mixture of primary sludge and EAS as a substrate. The sludge was treated with sodium citrate alone (SC) or sodium citrate together with enzymes (SC + E). One sample remained untreated. The theoretical methane production is determined from the COD content in the different samples. Together these two values determine the degree of degradation of the substrate (DD_{max}). All values are means of three parallels of each sample treatment.

Treatment	Digestion test [No.]	Real methane [Nml CH_4]	Theoretical methane [Nml CH_4]	DD_{max} [%]
Untreated	1	823	1521.3	54.1
SC	1	871	1628.0	53.5
SC + E	1	942	1519.4	62.0

The share of the substrate applied in the first batch laboratory digestion test available to be degraded in a fully blended and continuous digestion chamber at Henriksdal WWTP was also determined using the principle described in Appendix I. This degree of degradation is referred to as $DD_{share\ of\ max,\ total}$ in Table 10 below. In this table the calculated degree of degradation in one of the fully blended continuous digestion chamber at Henriksdal WWTP ($DD_{Henriksdal}$) from the calculated values of DD_{max} and $DD_{share\ of\ max,\ total}$ is also shown. This calculation is also presented in Appendix I.

Table 10: $DD_{share\ of\ max,\ total}$ and $DD_{Henriksdal}$ for the samples in the first digestion test. The part of the substrate applied in the batch laboratory digestion test available to be degraded in a fully blended and continuous digestion chamber at Henriksdal WWTP ($DD_{share\ of\ max,\ total}$) is presented together with the total degree of degradation of a substrate in the same digestion chamber ($DD_{Henriksdal}$). The treatments in the samples were sodium citrate alone (SC) or sodium citrate together with enzymes (SC + E). One sample remained untreated and the substrate used within each sample was a mixture of primary sludge and EAS.

Treatment	Digestion test [No.]	$DD_{share\ of\ max,\ total}$ [%]	$DD_{Henriksdal}$ [%]
Untreated	1	79.7	43.1
SC	1	82.7	44.2
SC + E	1	82.3	51.0

Digested sludge reduction

Incoming substrate

The amount of incoming primary sludge and EAS to the seven digestion chambers at Henriksdal WWTP is also presented previous in section 3.1.2.

Primary sludge

1400 m^3/day

TS = 3.6 % => **50 400 kg TS/day**

VS = 73 % of TS => **36 792 kg VS/day**

EAS

470 m^3/day

TS = 4 % => **18 800 kg TS/day**

VS = 63 % of TS => **11 844 kg VS/day**

TOTAL

- ⇨ **69 200 kg TS/day = 25 258 000 kg TS /year**
- ⇨ **48 636 kg VS/day = 17 752 140 kg VS/year**

Digested sludge

When calculating the theoretical amount of digested sludge out from the digestion chambers at Henriksdal WWTP (originating from primary sludge and EAS) the VS in the sludge is assumed to be the organic substance being reduced.

Untreated sludge

As presented in Table 10 above, in the first digestion test the calculated degree of degradation in a fully blended and continuous digestion chamber at Henriksdal WWTP ($DD_{Henriksdal}$) for the untreated sludge was 43.1 %.

If the total VS from the incoming primary sludge and EAS is reduced with 43.1 % this gives a reduction of 7 651 172 kg VS/year. As described in section 2.1.4 TS is composed of VS and inert material. This reduction in VS gives a reduction in TS to **17 606 828 kg TS digested sludge/year.**

Sludge treated with sodium citrate and enzymes

Presented in Table 10 above is also the calculated $DD_{Henriksdal}$ for the sludge treated with sodium citrate and enzymes in the first digestion test. The $DD_{Henriksdal}$ is 51.0 %. This gives a reduction in VS of 9 053 591 kg VS/year and consequently a reduction in TS to **16 204 409 kg TS digested sludge/year.**

Reduction in digested sludge

The reduction in digested sludge is (17 606 828 – 16 204 409) = 1 402 419 kg TS digested sludge/year **= 1 402 tonne TS digested sludge/year.** With a TS content of 30 % in the dewatered digested sludge this corresponds to a reduction of **4 675 tonne dewatered digested sludge/year.**