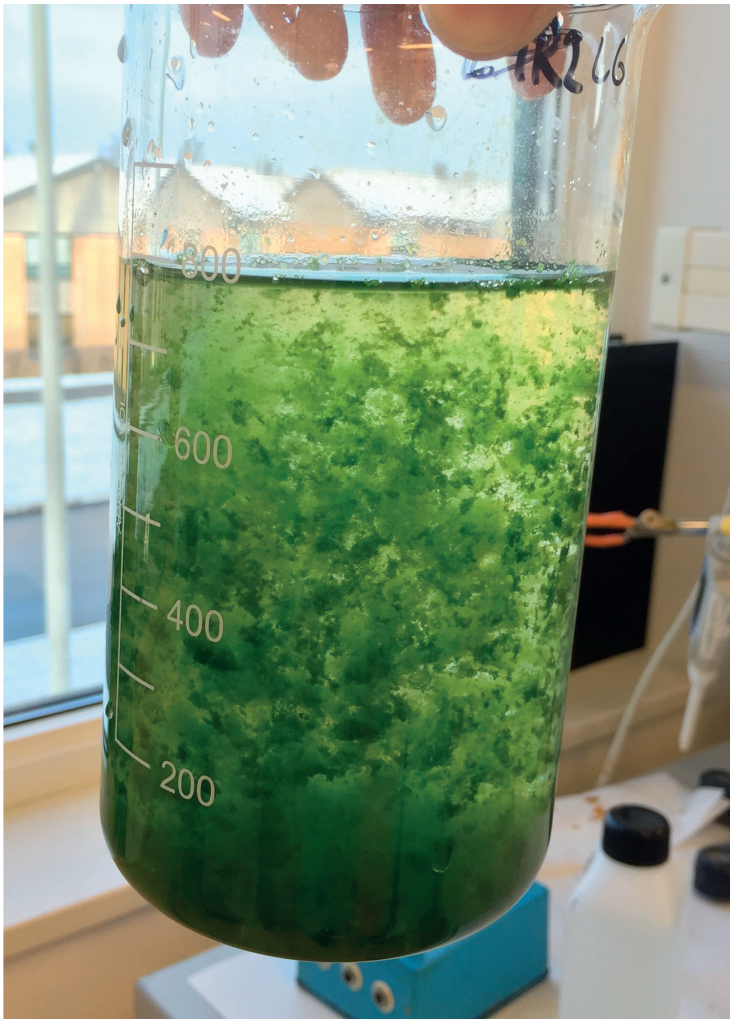
Renewable energy from wastewater grown microalgae

A concept for nutrient recycling and sustainable energy recovery



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by

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Picture on front page: Algae during flocculation experiments. Photo by Martina Uldal.

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Preface

This master thesis has been performed at AnoxKaldnes in Lund in cooperation with Water and Environmental Engineering, Department of Chemical Engineering at Lund University.

First and foremost I would like to express my thankfulness to my supervisors, Åsa Davidsson and Martina Uldal for giving me the opportunity to perform this interesting master thesis and for your continuous support and help during this project.

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Stina Lidén

Abstract

Wastewater is an excellent source of nutrients and energy. By treating the wastewater in a clever way, it is possible to both recycle nutrients and recover energy, and thereby create opportunities for a sustainable system. In conventional wastewater treatment where the activated sludge process is used, aeration is needed and a lot of biomass is produced. In regions with warmer climate, or if low-valuable heat is available for heating this technique could be replaced with an anaerobic one. With anaerobic techniques, less sludge is produced, the need for aeration is decreased and potentially more energy can be recovered.

An increased interest in renewable energy sources has put demand on finding suitable substrates for production of biobased fuels. For this purpose, algal biomass presents interesting characteristics. Algae use sunlight, carbon dioxide and nutrients for growth. One of the hurdles with production of algal biomass is nutrient supply but this can be solved by growing algae in wastewater. In this way two problems are solved: the algae are supplied with nutrients at the same time as nutrient reduction in the wastewater is accomplished.

In this study, the feasibility of integrating an algal step in a wastewater treatment system was evaluated based on a series of laboratory experiments. Further, a concept for wastewater treatment including an Anaerobic Moving Bed Biofilm Reactor (AnMBBR) and algal cultivation was compared to an existing wastewater treatment plant (WWTP).

Nutrient reduction over the algal cultivation showed more than 97% reduction of phosphate and more than 84% reduction of ammonium. Algal harvesting experiments showed that it was possible to efficiently separate algal biomass and treated water by sedimentation for 30 minutes after flocculation by addition of ferric chloride and cationic polymer. These experiments also showed that it was possible to meet the discharge limits for P-tot (0.3 mg/L), N-tot (10 mg/L) and COD (70 mg/L). Harvesting efficiency of up to 96% was achieved.

Methane potential from primary sludge was found to be 295 NmL/gCOD and for untreated microalgae, dominated by *Scenedesmus* sp., 95-108 NmL/gCOD. In batch tests, no synergistic effects could be seen for co-digestion of algae and primary sludge. The methane yield for algal biomass was increased by 46% when pretreated at 120°C for 30 minutes and by 74% when pretreated at 170°C for 30 minutes.

Evaluation of the proposed concept showed that the ratio between primary sludge and algae would be 32:68 on volatile suspended solids basis, if algae are grown 12 months per year. Compared to a conventional WWTP which uses the activated sludge process, the yield of methane was 35% higher without pretreatment, and up to 75% higher if pretreatment is applied. Finally, it was found that microalgae have a great potential for biogas production compared to some energy crops. The energy potential of algae was found to be 60-160 MWh/(ha·year) depending on pretreatment and cultivation period (8-12 months/year).

Keywords: AnMBBR, biogas, microalgae, nutrient recycling, nutrient reduction, renewable energy, sustainable wastewater treatment

Sammanfattning

Avloppsvatten utgör en utmärkt källa för näring och energi. Genom att behandla avloppsvattnet på ett smart sätt, är det möjligt att både återvinna näringsämnen och utvinna energi och därigenom skapa möjligheter för ett hållbart system. Vid konventionell avloppsvattenrening där aktivslamprocessen används, krävs luftning och det bildas mycket biomassa. I regioner med varmare klimat, eller om spillvärme finns tillgänglig för uppvärmning, skulle denna teknik kunna ersättas med anaerob teknik. Med anaeroba tekniker produceras mindre slam, behovet av luftning minskas och mer energi kan potentiellt utvinnas.

Ett ökat intresse för förnybara energikällor har ställt krav på att hitta lämpliga substrat för produktion av biobaserade bränslen. För detta ändamål har det visat sig att alger har många intressanta egenskaper. Alger använder solljus, koldioxid och näringsämnen för tillväxt. Ett problem med produktion av algbiomassa är näringstillförsel, men detta kan lösas genom att odla alger i avloppsvatten. På detta sätt kan två problem lösas: algerna förses med näringsämnen på samma gång som näringsreduktion i avloppsvattnet uppnås.

I denna studie genomfördes en rad labförsök för att undersöka möjligheten att integrera ett algsteg i ett system för rening av avloppsvattnen. Dessutom jämfördes ett koncept för rening av avloppsvatten, med hjälp av en ”Anaerobic Moving Bed Biofilm Reactor” (AnMBBR) och algodling med ett befintligt avloppsreningsverk (ARV).

Näringsreduktionen över algodlingen visade på mer än 97 % reduktion av fosfat och mer än 84 % reduktion av ammonium. Experiment för att skörda alger visade att det var möjligt att effektivt separera algbiomassan och det behandlade vattnet genom sedimentering i 30 minuter efter flockning med tillsats av järnklorid och katjonisk polymer. Dessa experiment visade också att det var möjligt att uppfylla utsläppskraven för P-tot (0,3 mg/L), N-tot (10 mg/L) och COD (70 mg/L). Skördningsutbyte på upp till 96 % uppnåddes.

Metanpotentialen för primärslam visade sig vara 295 NmL/gCOD och för obehandlade mikroalger, som domineras av *Scenedesmus* sp., 95-108 NmL/gCOD. I satsvisa utrötningsförsök upptäcktes ingen synergieffekt för samrötning av alger och primärslam. Metanutbytet för alger ökade med 46 % när de förbehandlats vid 120°C under 30 minuter och med 74 % när de förbehandlats vid 170°C under 30 minuter.

Utvärdering av det föreslagna konceptet visade att förhållandet mellan primärslam och alger skulle vara 32:68 baserat på vikt (organiskt material), om alger odlas 12 månader per år. Jämfört med ett konventionellt ARV som använder aktivslamprocessen, blev utbytet av metan 35 % högre utan förbehandling av algerna, och upp till 75 % högre om förbehandling appliceras. Slutligen konstaterades det att mikroalger har en stor potential för biogasproduktion jämfört med vissa energigrödor. Energipotentialen för alger bestämdes till 60-160 MWh/(ha·år) beroende på förbehandling och odlingsperiod (8-12 månader/år).

Nyckelord: AnMBBR, biogas, förnybar energi, hållbar avloppsvattenrening, mikroalger, näringsreduktion, återvinning av näringsämnen

List of abbreviations

Anammox	Anaerobic ammonium oxidation
AnMBBR	Anaerobic moving bed biofilm reactor
BMP	Biomethane potential
CA (in material balances)	Concentrated algae
COD	Chemical oxygen demand
D (in material balances)	Digestate
HPTH	High pressure thermal hydrolysis
HRT	Hydraulic retention time
LCFA	Long chain fatty acids
OLR	Organic loading rate
PS (in material balances)	Primary sludge
R (in material balances)	Reject
SCOD	Soluble chemical oxygen demand
SRB	Sulfate reducing bacteria
SRT	Solids retention time
SS	Suspended solids
STP	Standard temperature and pressure
TCOD	Total chemical oxygen demand
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

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1 Introduction

Wastewater, resulting from human activities, is an excellent source of nutrients and energy. If wastewater is released without treatment it can affect the receiving water bodies negatively. By treating the wastewater in a clever way, it is possible to recycle nutrients and recover energy at the same time as negative effects are avoided. Production of mineral fertilizers requires energy and by recycling nutrients from wastewater, energy consumption can be decreased (McCarty *et al.*, 2011). Another important aspect is that the phosphorus reserves are declining, thus phosphorus recycle is crucial (Naturvårdsverket, 2010). Conventional wastewater treatment often includes an aerated step which is very energy consuming. Also, during aerobic treatment a lot of biomass is produced. One way to treat this biomass is to digest it anaerobically which leads to biogas production. However, using this treatment procedure, only a portion of the energy stored in the wastewater is recovered since a lot is lost in the aerobic step. By applying anaerobic treatment directly to the wastewater, potentially more of the energy can be recovered and since the need for aeration is decreased, less energy input is needed. Another benefit of anaerobic treatment is that the production of biomass is low, thus the need for sludge handling is reduced (McCarty *et al.*, 2011).

Further, an increased interest in renewable energy sources has put demand on finding suitable substrates for production of biobased fuels (Ras *et al.*, 2011). Biofuels produced from edible feedstocks (first generation biofuels) are not recommended since it could lead to increase in food prices. Instead, second generation biofuels have been investigated. These include biofuels from lignocellulosic feedstocks and waste but have showed great resistance to degradation. Following the first and second generation biofuels are the third generation which are produced from algae (Montingelli *et al.*, 2015). Algae use sunlight, carbon dioxide (CO_2) and nutrients for growth and because of the fast growth rate of algae, this biomass presents an interesting feedstock for biofuel production. To make the production of algae for biofuel production economically feasible, one way is to use nutritious wastewater as growth medium. In this way, two problems are solved: the algae are supplied with nutrients and the wastewater is purified which allows for safe release into the surrounding environment (Udom *et al.*, 2013). One way of producing biofuels is through anaerobic digestion. Anaerobic digestion of algae was studied as early as in the 1950's by Golueke *et al.* (1957) and in 1960, a system for sewage treatment by algae and further biogas production from the produced algal biomass was proposed (Oswald & Golueke, 1960). Algae remove nutrients in wastewater mainly by assimilation (Xin *et al.*, 2010). This means that if algae are used for nutrient reduction followed by anaerobic digestion, a lot of the nutrients in the wastewater will end up in the digestate. By using the digestate as fertilizer, nutrient recycle is achieved. Carbon dioxide emissions from biogas upgrading or power plants can potentially be decreased by supplying it to algal cultivations (Montingelli *et al.*, 2015). However, before effective energy generation from algal biomass is possible, some concerns must be solved such as high protein content, harvesting difficulties and resistance to degradation (Ward *et al.*, 2014).

1.1 The warm and clean city

The warm and clean city ("den varma och rena staden") is a project funded by Vinnova and aims at investigating new, innovative systems for wastewater treatment. Some of the goals with the project are to create opportunities for energy recovery and nutrient recycle. Alfa Laval, AnoxKaldnes, Aquaporin, BioMil, EkoBalans, ESS, Heliospectra, Hydrotech, Kraftringen, Lund University, Norups gård, Purac, Swedish University of Agricultural

Sciences and Trelleborgs municipality are involved in the project which is coordinated by Lunds municipality.

Currently, two different concepts are evaluated in pilot scale; one compact line, with mechanical and chemical treatment and one energy positive line with mechanical and biological treatment. In the energy positive line, the biological treatment will be focused on anaerobic treatment for organic carbon reduction and for nutrient reduction both algal cultivation and anaerobic ammonium oxidation, anammox, will be evaluated. By utilizing sludge and algae as substrate in anaerobic digestion, valuable biogas will be produced and the digestate might be used as fertilizer. The system is suitable for regions with warmer climate, or if low-valuable heat is available for heating.

1.1.1 The energy positive concept

The goal of this concept is to treat wastewater in a way that the amount of energy recovered is higher than the input energy. The outline of the concept is shown in Figure 1.1. Water is first treated mechanically by screens and a grit chamber where large particles are removed. The treatment continues with a presedimentation basin where a lot of the suspended solids are removed. This is followed by an optional hydrolysis step to decrease the concentration of dissolved oxygen in the water. After this, biological treatment is achieved by an anaerobic moving bed biofilm reactor (AnMBBR) where organic carbon is converted to biogas. In the MBBR technology microorganisms grow as a biofilm on carriers that are retained in the reactor by a screen. This technology leads to higher treatment capacity since the biomass concentration can be higher (Qiqi *et al.*, 2012). Finally, nutrient reduction is achieved by algal cultivation in the summer and an anammox reactor in the winter. By excluding extensive aeration during biological steps, the energy demand is decreased. From the presedimentation basin sludge is separated and further digested, in order to produce biogas. The algae are separated after the cultivation and can be digested for production of biogas. The digestate from the digester is dewatered and might be used as fertilizer since it contains a lot of nutrients. The reject water can be returned to the algal cultivation or anammox reactor. Since the water from the biological step will be high in nutrients, carbon dioxide might become limiting in the algal step. This can be solved by using the carbon dioxide found in the produced biogas. The biological treatment and anammox will be tested in pilot scale at Källby wastewater treatment plant (WWTP) in Lund whereas the algal cultivation and anaerobic digestion will be evaluated in laboratory scale. Some of the questions to be answered are how much biogas that can be produced, how the algal separation will be achieved and what quality the treated water will have.

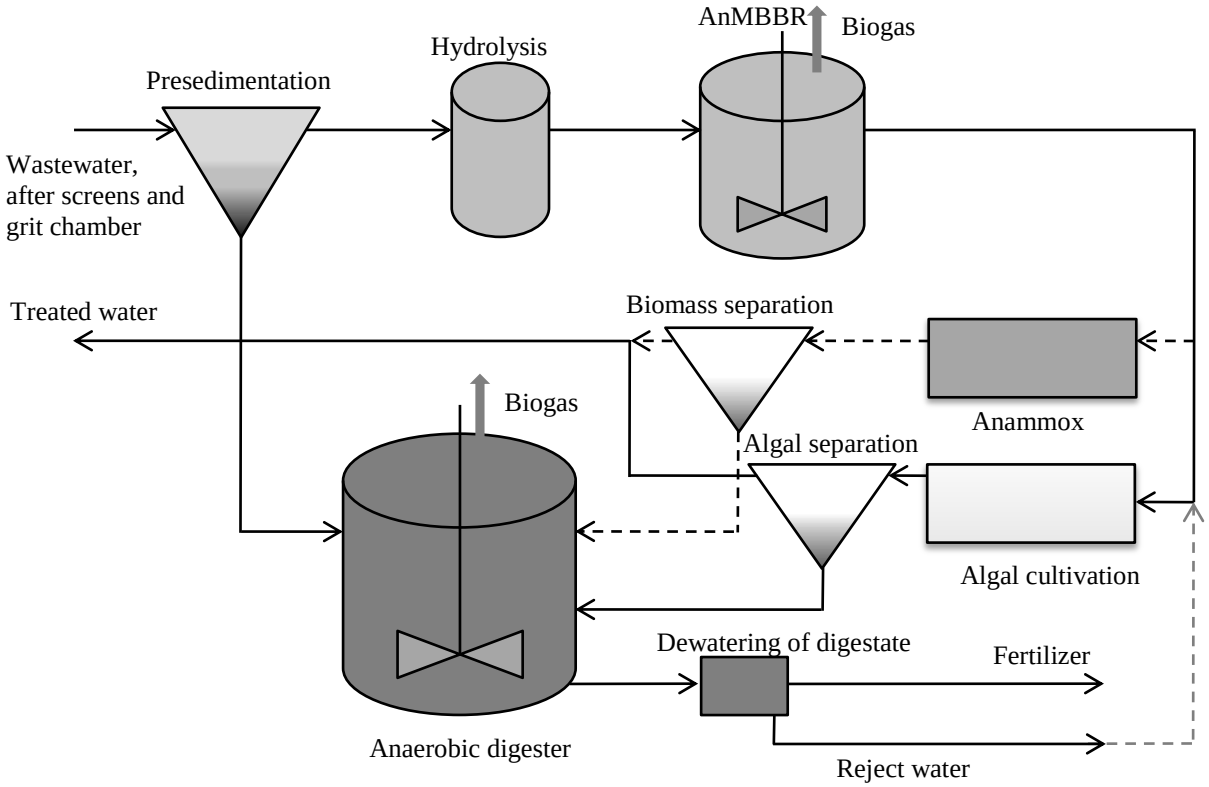

Figure 1.1. Overview of the energy positive concept. Black dotted lines represent anammox treatment during winter and gray dotted line show possible recirculation of reject water.

1.2 Aim

In this study, the feasibility of integrating an algal step in a wastewater treatment system was evaluated. This included nutrient reduction potential of algae, biogas potential of algae and primary sludge, and effects of co-digestion of algae and primary sludge. Further, algal harvesting and possible pretreatment methods to increase the biogas yield were evaluated. These evaluations finally resulted in a possible treatment concept which was evaluated based on energy recovery and treatment efficiency. The main goals of this study were to conclude:

- The nutrient reduction over the algal cultivation.
- If it is possible to effectively separate algae and treated water.
- If it is possible to meet the discharge limits using the proposed concept.
- The methane potential of algae and if it is possible to increase the yield by applying a pretreatment method.
- How much methane that can be recovered using the proposed concept.
- How the contribution to the total methane production is divided between primary sludge, algae and AnMBBR.

1.3 Delimitations

As far as possible, the experimental planning focused on methods that would be possible for full scale implementation. Because of time limitation, continuous digestion experiments were excluded. The amount of available substrate limited which evaluations that were possible to perform. Further, no evaluations of the anammox treatment or different cultivation techniques for algae were explored. Because of limitation in time and amount of available substrate it was not possible to perform a statistical evaluation.

2 Theory

2.1 Wastewater treatment

Wastewater treatment aims at reducing the amount of suspended solids, organic material and nutrients such as nitrogen and phosphorus in the wastewater. This is to avoid negative effects on the surrounding environment when the water is released (Gillberg *et al.*, 2003).

2.1.1 Primary treatment

Water that is entering the treatment plant is first treated mechanically to remove large particles and suspended solids. This is accomplished by passing the water through screens (3-20 mm width) to remove larger contaminants. After this, heavier contaminants and grease are removed in a grit chamber and finally, a lot of the remaining suspended solids can be removed by sedimentation in a sedimentation basin (Gillberg *et al.*, 2003).

2.1.2 Secondary treatment

This step is often biological, which can be aerobic, anoxic, anaerobic or a combination of them and aims at reducing the organic material. During this step only a part of the nutrients are taken up by the microorganisms. In general, aerobic systems, such as the activated sludge process, are faster than anaerobic systems (Gillberg *et al.*, 2003). A drawback of aerobic systems is the need for air supply which can account for a major part of the energy consumption of the whole treatment plant (McCarty *et al.*, 2011).

In aerobic degradation of organic matter, around 40% of the energy in the substrate is converted to heat, compared to 5% in anaerobic degradation. When treating the wastewater anaerobically, up to approximately 90% of the energy can be stored in methane (CH_4) (Jonstrup *et al.*, 2011). However, anaerobic systems might need heating to increase the degradation rate (Gillberg *et al.*, 2003). Since a lot of the energy in anaerobic systems is stored in methane less biomass is produced compared to aerobic systems where a lot more of the energy in the substrate can be used for growth (Jonstrup *et al.*, 2011).

2.1.3 Nutrient reduction

The nutrient reduction can be either biological or chemical and aims at removing enough nutrients for safe release of the water into the environment. Chemical processes are often more expensive than biological and could lead to secondary pollution (Abdel-Raouf *et al.*, 2012). The nutrient reduction step is in many cases combined with the secondary treatment (Metcalf & Eddy, 1991).

Biological nitrogen and phosphorus removal

Nitrogen can be removed biologically by nitrification and denitrification which means that ammonium is converted by microorganisms to nitrogen gas by oxidation of ammonium to nitrate in nitrification and further reduced to nitrogen gas in denitrification. Nitrification is performed under aerobic conditions whereas denitrification is performed in anoxic conditions (Jonstrup *et al.*, 2011). Another process for biological nitrogen removal is anammox where ammonium is oxidized to nitrogen gas with nitrite as electron acceptor (Ahn, 2006). Phosphorus can be removed by enhanced biological phosphorus removal where bacteria capable of storing high amounts of phosphorus are favored by the conditions in the reactor. The phosphorus is removed from the system through removal of excess bio-sludge (Jonstrup *et al.*, 2011).

Flotation and flocculation
Chemical removal of phosphorus can be achieved by precipitation with for example ferric- or aluminum salts, which also can remove suspended particles in the water. This works since the positively charged flocculants neutralizes the repelling force between negatively charged particles or phosphate. When chemical treatment is used, the flocculant is added to the water in a mixing stage which is followed by a step where flocs are formed. These flocs are then removed from the water by for example sedimentation of flotation (Gillberg *et al.*, 2003). Flotation is a unit operation where fine gas bubbles are introduced to the water and the particles attach to the bubbles. In this way, particles start to float on the surface of the water and can be skimmed off. Flotation can effectively remove even small or light particles that settle very slowly in contrast to sedimentation which would require longer separation time. When flotation and flocculation are used together the effectiveness can be enhanced since the flocculants binds to the particles and a structure that can entrap gas bubbles more efficiently is created (Metcalf & Eddy, 1991). Further, by addition of polymers during the floc-forming phase stronger flocs are formed by cross-linking with the polymer (Gillberg *et al.*, 2003). Both flotation and flocculation can be used for other purposes than phosphorus removal, for example for removal of suspended solids (Metcalf & Eddy, 1991).

2.2 Sludge

During the treatment of wastewater, sludge is produced in the different steps. At the bottom of the sedimentation basin, sludge is collected and this sludge is referred to as “primary sludge”. During biological treatment the sludge produced is referred to as “secondary sludge” or “biological sludge” (Gillberg *et al.*, 2003). When the activated sludge process is used the sludge is referred to as “waste activated sludge” (WAS) (Wang *et al.*, 2013). The quantity of biological sludge produced depends on which kind of biological treatment that is applied. If chemicals are used in the treatment process “tertiary sludge” or “chemical sludge” is produced (Gillberg *et al.*, 2003).

Sludge typically contains a lot of water and to reduce the cost for further treatment sludge is thickened by for example sedimentation or flotation (Gillberg *et al.*, 2003). Sludge contains biodegradable material which means that it continues to be biologically active and might cause problems with for example offensive odors and putrefaction, if not further treated. Treatment involves stabilization of the sludge and can be accomplished by for example aerobic or anaerobic digestion, lime stabilization, heat treatment and composting (Metcalf & Eddy, 1991). Anaerobic digestion is a widespread stabilization method which not only leads to stabilization of the sludge but also, energy is recovered in the form of biogas (Caporgno *et al.*, 2015). During this process, the amount of inorganic material remains constant but 40-60% of the organics are broken down which leads to volume reduction of the sludge. After anaerobic digestion, water is removed from the sludge in a step called dewatering. This is done to further reduce the volume of the remaining sludge (Gillberg *et al.*, 2003).

The origin of the sludge will affect its characteristics. Primary sludge, which to a great extent consists of suspended solids, is for example much easier to dewater compared to secondary sludge, which mainly consists of biomass (Turovskiy & Mathai, 2006). Also, the biodegradability of the sludge will vary. According to Parkin and Owen (1986) the degradability of for example WAS is lower than for primary sludge. They state that it is possible to achieve a volatile solids reduction of 40-70% when primary sludge is anaerobically digested whereas when WAS is anaerobically digested a volatile solids reduction of 20-50% can be achieved (Parkin & Owen, 1986).

2.3 Anaerobic digestion

Anaerobic digestion is a process where several different groups of microorganisms are involved in the degradation of organic matter into primarily carbon dioxide and methane. Anaerobic means that neither oxygen nor nitrate is present as electron acceptor, instead carbon dioxide or sulfate is used. The microorganisms responsible for the degradation live in a syntrophic relationship which means that they cooperate to degrade substrates neither of them could degrade by them self (Jonstrup *et al.*, 2011). The process is very complex, but can be divided into four major steps, as can be seen in Figure 2.1.

Hydrolysis, step 1

The substrates used for anaerobic digestion consist of, among others, large molecules such as proteins, fats and sugars which are too large to be taken up by the microorganisms. To enable the uptake, the first step in anaerobic digestion is the hydrolysis of large molecules into smaller units. This is done by extracellular enzymes which are produced by hydrolytic fermentative bacteria (De Lemos Chernicharo, 2007). The enzymes involved are mainly amylases for carbohydrate degradation into sugars, lipases for the degradation of fats into fatty acids and proteases for the degradation of proteins into amino acids (Jonstrup *et al.*, 2011).

Acidogenesis, step 2

In acidogenesis, the products from hydrolysis are further metabolized by fermentative bacteria or anaerobic oxidizers. The main products in a stable process are acetate, carbon dioxide and hydrogen which can be used directly in the last step, methanogenesis. A small part of the products from hydrolysis is however converted into intermediary products such as volatile fatty acids (VFAs) (other than acetate) which cannot be used directly in methanogenesis (Jonstrup *et al.*, 2011). During acidogenesis, hydrogen is produced which in a stable process is consumed in the methanogenesis. If the methanogenesis for some reason is not working properly, there might be an accumulation of hydrogen which could lead to an accumulation of VFAs. Acidogenesis is considered to be the fastest step in anaerobic digestion and the growth rates of the bacteria responsible for this step are between 10 and 20 times higher than for the methanogens (van Lier *et al.*, 2008).

Acetogenesis, step 3

During acetogenesis, VFAs produced during acidogenesis are converted into substrates for methanogens by obligate hydrogen producing acetogenic bacteria (Jonstrup *et al.*, 2011). The acetogens are slow growing and are dependent on a low partial pressure of hydrogen, since hydrogen inhibits their metabolism. A low partial pressure of hydrogen is accomplished by the hydrogen consuming methanogens which usually consumes hydrogen very efficiently. This means that the degradation of VFAs is closely coupled to the activity of the methanogens. If the methanogens are inhibited in some way, the partial pressure of hydrogen will increase and inhibit the acetogens, leading to an accumulation of VFAs (van Lier *et al.*, 2008).

Methanogenesis, step 4

In the final step of anaerobic digestion, methane is formed from either acetate by aceticlastic methanogens or from carbon dioxide and hydrogen by hydrogenotrophic methanogens. The aceticlastic methanogens are very slow growing, with a doubling time of several days whereas the hydrogenotrophic methanogens have doubling times of a few hours. It has been found that approximately 70% of the methane is produced by the aceticlastic methanogens. When the substrate used is easy to hydrolyze, the rate-limiting step in anaerobic digestion is usually the aceticlastic methanogenesis (van Lier *et al.*, 2008; Jonstrup *et al.*, 2011).

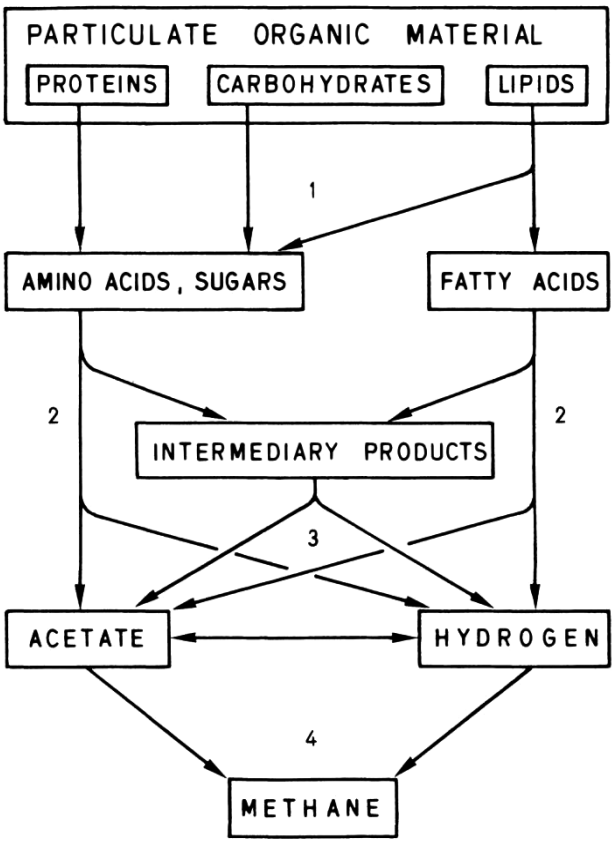

Figure 2.1. Overview of the anaerobic digestion process (Gujer & Zehnder, 1983). Step 1 is hydrolysis, step 2 acidogenesis, step 3 acetogenesis and step 4 methanogenesis. Reproduced with kind permission from the copyright holders, IWA publishing.

2.3.1 Important process parameters

In anaerobic digestion, it is of great importance to keep the balance between the different microorganisms to avoid process instability and to achieve a high biogas production (Jarvis & Schnürer, 2009). The organisms are affected by many different parameters, some of them described below.

Temperature

Anaerobic digestion is mostly performed in the mesophilic (25-40°C) or the thermophilic (>45°C) range, but is also possible in the psychrophilic (<20°C) range. The microflora will be affected by the temperature range, since the optimal temperature differs between microorganisms (Jonstrup *et al.*, 2011). Generally, the digestion process is faster in the

thermophilic range since the reaction rate increase with temperature. However, anaerobic digestion at thermophilic temperature has sometimes been found to be more sensitive to disturbances. Further, the microflora has shown to be more diverse in mesophilic reactors compared to thermophilic which could affect how well the process can cope with changes and disturbances (Jarvis & Schnürer, 2009). In the psychrophilic range, methanogenesis is possible but at lower rates and the hydrolysis step is slow (Jonstrup *et al.*, 2011). The temperature affects the growth rate of microorganisms, the solubility of methane in water (Bandara *et al.*, 2011), the enzymatic activity and the diffusivity of compounds (De Lemos Chernicharo, 2007). The temperature also affects the dissociation of some compounds, such as ammonia. When the temperature is increased, at a specific pH, the equilibrium between ammonium and ammonia is pushed towards ammonia, which is inhibitory to methanogens. This means that mesophilic processes might be more tolerant to higher ammonia concentrations than processes in the termophilic range (Jarvis & Schnürer, 2009).

When the desired temperature is chosen, it is important to keep the digester at this temperature since anaerobic processes are very sensitive to temperature changes. Preferably, the temperature should not change more than 0.5°C (Jarvis & Schnürer, 2009).

pH and alkalinity

As for temperature, the microorganisms have different optimal pH regions. The optimal pH for methanogens and acetogens is approximately 7, and if the pH drops below 6.6 the growth of methanogens is significantly inhibited. The acidogens prefer a pH around 6, but functions at neutral pH as well. To ensure growth of all these organisms, the pH should be kept at neutral. An important factor when dealing with pH is the alkalinity, or buffering capacity of the medium. This refers to the system's ability to avoid pH changes and depends on the carbonate system and the composition of the substrate. If accumulation of VFAs occurs, and the alkalinity is low, it could lead to a drop in pH and further process instability (Jonstrup *et al.*, 2011). One way to improve the alkalinity of a substrate with poor alkalinity is to mix it with a substrate with higher alkalinity (Ward *et al.*, 2014).

Solids retention time, hydraulic retention time and organic loading rate

The solids retention time (SRT) and the hydraulic retention time (HRT) are the mean retention time for the sludge and the liquid in the system, respectively. If the digester is totally mixed, the SRT and the HRT are equal but if sludge is recirculated or retained in the system in some way the SRT is longer than the HRT (Jonstrup *et al.*, 2011). To avoid wash out of methanogens the SRT needs to be longer than the doubling time for the organisms (Gerardi, 2003). The retention time in the reactor will affect how much of the organic material that is broken down, longer retention time means a more complete degradation. The time needed in the digester is determined by the composition of the substrate, an easy degradable substrate requires a shorter retention time than a substrate that is difficult to degrade (Jarvis & Schnürer, 2009).

Organic loading rate (OLR) is a measurement of how much organic material that is introduced to the process per unit time. During start up of an anaerobic reactor, the OLR needs to be quite low, but as the microorganisms increase in number, the loading can be increased. If too much substrate is added compared to the amount of microorganisms, instability of the process can occur due to accumulation of undigested material (Jarvis & Schnürer, 2009).

Mixing
Mixing during anaerobic digestion is important for several reasons; it creates better contact between the substrates and the bacteria and bacterial enzymes, decreases the risk of foaming, dilutes toxic compounds and creates a uniform environment throughout the reactor (Parkin & Owen, 1986; Abbasi *et al.*, 2012).

Substrate
To achieve a successful process it is important that the substrate is suitable for anaerobic digestion. The substrate can be characterized by means of total solids (TS), volatile solids (VS), chemical oxygen demand (COD) and the fraction of carbohydrates, proteins and fats. The composition will affect the amount of biogas that is produced, the composition of the gas, the rate of decomposition and the quality of the digestate (Carlsson & Uldal, 2009; Jarvis & Schnürer, 2009). Further, suspended solids (SS) and volatile suspended solids (VSS) could be analyzed. SS is the part of TS that is retained by a glass-fiber filter during filtration and VSS the part of SS that is lost when the sample is ignited. VS and VSS give an estimation of the amount of organic content that is present in the sample (Metcalf & Eddy, 1991).

During the degradation of proteins, ammonium is released and as will be discussed later, high protein content can cause instability in the digestion process through ammonia inhibition. Carbohydrates include simple sugars which are degraded fast and more complex polymers such as cellulose and hemicellulose which are more difficult to degrade. If the substrate contains high amounts of easily degradable carbohydrates the methanogenesis might become rate-limiting, instead of the hydrolysis and there is a risk of VFA accumulation. On the other hand, if the substrate contains a lot of cellulose it might be required to apply some form of pretreatment in order to increase the rate of hydrolysis. Substrates rich in fats will yield high amounts of methane but during degradation problems can arise due to formation of long chain fatty acids (LCFAs). Investigations have also shown that foaming may occur due to high amount of fats (Jarvis & Schnürer, 2009).

Nutrients
To enable degradation of organic matter, nutrients are needed. In addition to nitrogen and phosphorus, which are most important, the microorganisms need iron, nickel, cobalt, sulfur, calcium and trace elements but in lower concentrations (Parkin & Owen, 1986). It is important that all nutrients are present in sufficient amounts to achieve a successful process (Carlsson & Uldal, 2009).

C/N ratio
The ratio between carbon and nitrogen in the substrate is another parameter to consider. If the ratio is too high, meaning a lot of carbon compared to nitrogen, nitrogen might become limiting and hence reduce the carbon reduction. On the other hand, a low ratio can lead to ammonia inhibition as nitrogen is released from the substrate in the degradation process (Abbasi *et al.*, 2012). According to Montingelly *et al.* (2015) the optimal ratio for anaerobic digestion is between 20/1 and 30/1. The ratio may give an indication on how well suited the substrate is for anaerobic digestion but it should be kept in mind whether the carbon and nitrogen are available for the microorganisms or not. Carbon in the form of lignin is not available at all whereas carbon in the form of sugars is very easily available. Further, the optimal ratio may depend on the substrate and process conditions (Jarvis & Schnürer, 2009).

Inhibitory substances
Several substances are known to cause instability in the process and some of them are ammonia, sulfate, LCFAs, VFAs and oxygen (Gerardi, 2003).

Oxygen form free radicals which can cause oxidation of cell components if the organism lacks defense mechanisms against this. Among the organisms that are present in the anaerobic digestion process some can tolerate oxygen, some grow better in the presence of oxygen whereas for example the methanogens are inhibited by oxygen. Small, temporary amounts of oxygen could be tolerated since some of the microorganisms are able to rapidly consume the oxygen and in this way spare the methanogens (Jarvis & Schnürer, 2009).

In the digestion process, a small amount of sulfate reducing bacteria (SRB) are also present among the other organisms. The SRB use sulfate as electron acceptor to produce hydrogen sulfide. More energy is released when sulfate is used as electron acceptor compared to carbon dioxide which means that the SRB could out-compete the methanogens if sulfate is present in sufficient amounts (Jarvis & Schnürer, 2009). Also, the SRB use hydrogen and acetate as substrate just as the methanogens which causes competition between these organisms. Hydrogen sulfide can cause severe problems in piping and storage tanks as well as inhibit the microorganisms in the process (Ward *et al.*, 2014). One way of addressing the problem with sulfide is by addition of iron to the digester, which leads to precipitation of iron sulfide (Gerardi, 2003).

When nitrogen compounds are degraded, ammonia and ammonium are released. Ammonia is toxic to several organisms and especially the methanogens. The equilibrium between ammonia and ammonium is dependent on the temperature and pH and as the pH and temperature rises, the equilibrium is pushed towards ammonia. Since it is the unionized form that is most toxic, ammonia inhibition is often more pronounced in processes in the thermophilic range (Jarvis & Schnürer, 2009).

Accumulation of VFAs can occur when easily degradable material is used as substrate. With these types of material, the hydrolysis and acidogenesis proceeds faster than the methanogenesis, leading to an accumulation of VFAs and consequently, alkalinity is consumed. This causes the pH to drop with possible inhibition of methanogens as a result (Geradi, 2003; Jarvis & Schnürer, 2009).

During degradation of materials that are high in fats, LCFAs such as stearic and oleic acid, are released (Jarvis & Schnürer, 2009). LCFAs are able to dissolve in the cell wall of the methanogens and in this way inhibit their activity (Gerardi, 2003). In the same way as for VFAs, degradation of LCFAs requires the simultaneously activity of hydrogen consuming organisms and if disturbances in the process occur, there is a risk for LCFA accumulation (Jarvis & Schnürer, 2009).

2.3.2 Biogas
Biogas is commonly referred to as the mixture of methane and carbon dioxide (and small amounts of other gases) that is produced when organic matter is degraded in an anaerobic environment. The volumetric amount of methane in the produced gas varies between 40 and 70% given that the process functions optimally (Abbasi *et al.*, 2012). One problem with production of methane is that some of the gas will be dissolved in the effluent from the treatment step. In the subsequent steps, the gas could be stripped and if not collected, released into the atmosphere (Noyola *et al.*, 2006). Methane is a greenhouse gas and it is therefore

important to limit its release into the environment. In addition, if the dissolved gas is not collected in some way, some of the energy that could have been gained is lost. The solubility of methane increases as the temperature decreases which means that the lower the treatment temperature, the higher amount of methane is lost in the effluent (Bandara *et al.*, 2011).

Applications of biogas

There are several applications of biogas, such as generation of electricity, heating and as vehicle fuel. Both the economic and environmental benefits are often optimized by using the biogas as vehicle fuel and thereby replacing fossil fuels. However, to be able to use the biogas for this purpose, upgrading is needed meaning that carbon dioxide and trace gases are removed and that the remaining gas is pressurized. The upgrading process is expensive which means that it may be difficult to achieve economical profitability in this area (Jonstrup *et al.*, 2011). In 2013, 53 plants for biogas upgrading were in use in Sweden. The methods used in these plants were pressure swing adsorption, water scrubber or chemical absorption. In pressure swing adsorption, the carbon dioxide is removed from the biogas by adsorption to active carbon. In a water scrubber, pressurized biogas gets in contact with water and due to that carbon dioxide has a higher solubility than methane in water, the carbon dioxide is removed. Chemical absorption is similar to the water scrubber technique, but instead of water, chemicals are used for the removal of carbon dioxide (Statens energimyndighet, 2014).

Estimation of methane yield

The amount of biogas that is produced and the composition of the gas depend on the composition of the substrate (Jarvis & Schnürer, 2009). Production of methane is often related to standard temperature and pressure (STP) which corresponds to 0°C and 1 atm. Further, the produced amount is related to the amount of added substrate expressed as for example COD or VS. The theoretical methane yield can be estimated in different ways, for example based on the COD of methane, giving a theoretical yield of 0.35 Nm^3/kgCOD, where "N" stands for normalized to STP. Another way is to predict the methane yield from the composition of lipids, carbohydrates and proteins in the substrate (Angelidaki & Sanders, 2004). The respective yield and methane content is presented in Table 2.1.

Table 2.1. Teoretical methane yield and methane content for degradation of lipids, carbohydrates and proteins (Angelidaki & Sanders, 2004).

Substrate	**Methane yield (Nm^3/kgVS)**	**Methane content (%)**
Lipids	1.014	70
Carbohydrates	0.415	50
Proteins	0.496	50

2.3.3 Digestate

The remaining material after anaerobic digestion is called digestate. This can be used as fertilizer in agriculture since it is high in nutrients. Especially from an environmental point of view, recycling of nutrients is important, since the use of mineral fertilizers can be reduced (Jonstrup *et al.*, 2011). The remaining digestate contains almost all the nutrients that enter the digester, but in addition other less favorable substances will also remain. It is important to analyze both the nutrient levels and the amount of unwanted substances to ensure that the digestate is suitable for use on for example farmland (Carlsson & Uldal, 2009).

At the moment, there are no regulations that demands hygienization of sludge before use in agriculture. However, the Swedish Environmental Protection Agency has proposed that all sludge should be treated before distribution, to decrease the amount of released pathogens. Different methods of hygienization have been proposed, such as pasteurization at 70°C for at least one hour or digestion under termophilic conditions (Naturvårdsverket, 2010). In a study by Kjerstadius *et al*. (2012) pasturization prior to anaerobic digestion, mesophilic digestion and thermophilic digestion at both 55°C and 60°C was evaluated for reduction of pathogenic bacteria. In this study, they found that energetically, pasteurization was the most suitable method for hygienization provided that the pretreatment leads to at least 20% increase in methane yield.

2.4 Microalgae

Microalgae are microscopic organisms which uses inorganic nutrients and carbon for growth. Through photosynthesis, microalgae are capable of assimilating carbon dioxide by using solar energy and in the process, oxygen gas is released as a by-product. Essential for algal growth are sufficient amounts of nutrients, available carbon source and light (Larsdotter, 2006). The productivity of microalgae is high, and large amounts of biomass can be produced in a short time. When cultivating algae the need for high amounts of nutrients is a challenge (Ward *et al.*, 2014). This opens up for the use of algae for nutrient reduction in wastewater treatment. Algae reduce nutrients in water mainly by assimilation during growth but some algal species are able to take up phosphorus and nitrogen in excess which later can be used for growth during nutrient limited conditions (Larsdotter, 2006). In wastewater, lack of available carbon and/or light are the most likely reasons for growth limitations since the amount of nutrients often are more than enough for algal growth. Light limitation might occur as a consequence of internal shading in high dense cultures or due to presence of particulate matter. One way of preventing light limitation is to use shallow cultivation vessels. Further, good mixing helps by allowing at least short periods of light exposure for each algal cell. The risk of carbon limitation can be avoided by supply of carbon dioxide to the water. In addition, some algal species such as *Chlorella* and *Scenedesmus* have shown to be able to shift their carbon metabolism from inorganic carbon to organic carbon when different carbon sources are available (Larsdotter, 2006).

2.4.1 Cultivation

There are several available techniques for cultivation of algae, for example in raceway ponds, wastewater treatment ponds and photobioreactors. Wastewater treatment ponds are used for reduction of biological oxygen demand aerobically. By including algae, nutrient reduction can be accomplished simultaneously and since algae produce oxygen, the need for expensive aeration of the ponds is eliminated. Photobioreactors are closed systems such as for example tubing or bags which allows maximum exposure to sunlight. The productivity is high in these systems compared to open pond systems, but with higher capital and operational costs. Raceway ponds (shown in Figure 2.2) are built individually from each other or in series and are operated in a continuous mode. A paddlewheel is used for mixing, and drives the water around in a closed loop recirculation channel. Raceways are quite inexpensive, easy to operate compared to photobioreactors and have higher productivity than wastewater treatment ponds. In open pond system, contamination of other organisms can occur. Also, for water high in nutrients, *Chlorella* sp. and *Scenedesmus* sp. are likely to dominate which means that species selection and control can be difficult (Wiley *et al.*, 2011).

Especially *Scenedesmus* and *Chlorella* are two algal genera that have been found to grow in wastewater (Golueke & Oswald, 1959; Rusten & Sahu, 2011; Yuan *et al.*, 2012; Wang *et al.*, 2013; Hidaka *et al.*, 2014; Olsson *et al.*, 2014). Further, using microalgae for reduction of nutrients in wastewater have been investigated by several researchers with promising results (Rusten & Sahu, 2011; Yuan *et al.*, 2012; Sahu *et al.* 2013; Udom *et al.*, 2013; Ficara *et al.*, 2014). The nutrient reduction depends on several factors, such as light, available carbon source, retention time and algal species. Ficara *et al.* (2014) achieved total nitrogen reduction of between 77 and 82% and Udom *et al.* (2013) achieved a 95% reduction in ammonium and a total phosphorus reduction of 72%, when treating wastewater using algae. Growth limitation can occur due to low light transmittance of the water. For example, reject water can have a very low light transmittance, and without pretreatment light might become limiting to algal growth (Rusten & Sahu, 2011). The light dependency can also limit the possibility of using algae in wastewater treatment at higher latitudes due to light limitations in the winter (Larsdotter, 2006).

Figure 2.2. Pilot scale raceway pond for treatment of wastewater using algae, in Spain (picture taken by Lars-Erik Olsson, AnoxKaldnes).

During algal growth, dissolved carbon dioxide is used and the depletion leads to an increased pH in the water. By supplying carbon dioxide to the algal cultivations the productivity can be increased (Park & Craggs, 2010). During upgrading of biogas to vehicle fuel, carbon dioxide is removed. This could be supplied to the algal cultures to increase the productivity (Wiley *et al.*, 2011). If instead, the produced biogas is burned for generation of heat and electricity, the released carbon dioxide can be supplied to the cultivations (Caporgno *et al.*, 2015).

2.4.2 Biofuel potential

There is an interest in replacing fossil fuels by more sustainable alternatives such as renewable energy from biomass sources. One way is to use agricultural derived biomass for production of biofuels; however, there are some concerns with this, especially competition with the production of food crops. Due to this, microalgal production has gained a lot of interest since this biomass can be produced in areas unsuited for food production and the biomass yield is high. The fact that algae can fixate carbon dioxide means that there is potential of algal biomass as a source for a carbon neutral biofuel (Ward *et al.*, 2014). There

have been investigations aiming at production of biodiesel, bioethanol, biohydrogen and biogas from algae (Passos *et al.*, 2014). A lot of the recent research is based on the production of biodiesel from algae, however, biodiesel is produced by extracting the lipids from algae which is a very costly operation. Sialve *et al.* (2009) found that production of biodiesel from algae was not energetically beneficial if the lipid content of algae was below 40%. It is possible to induce lipid accumulation in algae by creating an environmental pressure, for example nutrient limitation. This will lead to decrease in growth rate and lipid accumulation (Xin *et al.*, 2010). As mentioned before, the amount of nutrients in wastewater are high, and nutrient limitation will probably not occur (Larsdotter, 2006). Biogas production from algae can proceed regardless of the lipid content in the biomass (Wiley *et al.*, 2011), thus, biogas production from wastewater derived algae might be more beneficial. Another advantage of biogas production is that the anaerobic digestion process can handle higher water content of the substrate than the biodiesel process can. This means that less effort is needed in the concentration step if algae are used for biogas production instead of biodiesel production (Ras *et al.*, 2011; Wiley *et al.*, 2011; Passos *et al.*, 2014).

The composition of microalgae has shown to be dependent on both environmental conditions and to be species dependent (Sialve *et al.*, 2009). Consequently, since the biogas yield is dependent on substrate composition, the biogas yield from algae varies with algal species and culture conditions. Further, digestion conditions such as temperature, digestion time and inoculum will affect the yield. As mentioned, *Scenedesmus* and *Chlorella* are two algal genera frequently found to grow in wastewater. Degradability of these genera have been investigated by Mussgnug *et al.* (2010) who found that degradability was low, and intact cells of *Scenedesmus* were found even after six months in the fermenter. The biogas yield was reported to be 178 NmL/gVS for *Scenedesmus* and 218 NmL/gVS for *Chlorella* in batch experiments after 32 days (Mussgnug *et al.*, 2010). In batch experiments performed by González-Fernández *et al.* (2012) the methane yield from *Scenedesmus* was found to be 76 NmL/gCOD after 33 days whereas Olsson *et al.* (2014) achieved a yield of 367 NmL/gVS when a mix of *Scenedesmus* and *Chlorella* was digested in batch experiments.

2.4.3 Challenges with anaerobic digestion of algae

Some of the obstacles with anaerobic digestion of algal biomass are ammonia inhibition, rigid cell wall, and problems with harvesting.

Ammonia inhibition

Microalgae can contain large amounts of proteins and nitrogen resulting in a low C/N ratio. As algae are degraded in anaerobic digestion, release of nitrogen in the form of ammonium may cause ammonia inhibition depending on the pH and temperature in the digester. To overcome this problem, mixing the algal biomass with a carbon rich substrate has been proposed (Ward *et al.*, 2014; Montingelli *et al.*, 2015).

Harvesting

Although anaerobic digestion of microalgae does not require the same high concentration as biodiesel production does, some form of concentration step is still needed. Even when algae are grown in systems which generate high cell-densities, the moisture content is more than 99% (Wiley *et al.*, 2011). Microalgae are small in size, have negative surface charges and low specific gravity and this creates problems in the separation of algae from the growth medium (Wiley *et al.*, 2011; Vandamme *et al.*, 2013). Also, the growth phase of the algae and the pH of the solution will affect the separation (Udom *et al.*, 2013; Vandamme *et al.*, 2013). Separation techniques for algal biomass can be divided into primary and secondary harvesting

methods. Primary methods are able to achieve TS content of between 0.5 and 6% and include sedimentation and flotation techniques. The algal slurry obtained by primary methods can be further concentrated to between 10 and 20% by secondary harvesting methods, such as for example centrifugation (Wiley *et al.*, 2011). The harvesting step can be very expensive and energy demanding which should be kept in mind when designing the process. For example, centrifugation might not be suitable in full scale due to the high energy requirement (Ras *et al.*, 2011; Ward *et al.*, 2014).

Different methods have been proposed for harvesting of algae, such as gravity sedimentation, centrifugation, filtration, flotation and screening (Rusten & Sahu, 2011). Also, by addition of flocculants, surface charges can be neutralized and larger aggregates can be created. In this way, improvement of sedimentation can be achieved (Wiley *et al.*, 2011). In addition, autoflocculation has been described which is a spontaneous type of flocculation that can occur in algal cultures at high pH. This requires high concentrations of phosphate and addition of phosphate is not desirable because of the declining reserves. If however, high amounts of phosphate is present in the wastewater this could be an option (Vandamme *et al.*, 2013).

Several researchers have tried to harvest different species of *Chlorella* with varying result. Ras *et al.* (2011) successfully harvested 60% of *Chlorella vulgaris* in one hour, by natural sedimentation in a settling tank whereas Park and Craggs (2010) were able to recover 70% of algal biomass in 6 hours in gravity algal settling cones. Wang *et al.* (2013) used centrifugation to recover *Chlorella* sp. whereas when Rusten and Sahu (2011) tried both sedimentation and centrifugation to recover *Chlorella* sp. neither method was successful without any addition of flocculants. Olsson *et al.* (2014) successfully harvested a mix of *Scenedesmus* and *Chlorella vulgaris* by centrifugation and sedimentation. Further, Udom *et al.* (2013) were able to recover 93% of algae by addition of ferric chloride and 98% by using cationic polymer. These different results, even when working with the same genus, is in agreement with Rusten and Sahu (2011) who implies that the most suitable harvesting method depend on cell density, algal species and the culture conditions.

Cell wall resistance

In the study by Mussgnug *et al.* (2010) it was found that the amount of biogas produced in the degradation of different species of algae was dependent on the composition of the cell wall. They found that species lacking cell wall, or had a cell wall which consisted of only proteins were the most easily degradable species whereas for species with a cell wall high in hemicellulose no, or very little, cell degradation could be seen. They also found that the amount of biogas produced was lower for the species with hemicellulose-containing cell wall but the biogas production was not directly linked to the extent of cell wall degradation. One possible explanation could be that the methanogenesis was inhibited by compounds released in the degradation (Mussgnug *et al.*, 2010). As mentioned before it is difficult to control the algal culture in open pond systems. Hence, it may not be possible to choose algal species well suited for anaerobic digestion. Instead, some form of pretreatment method could be used to facilitate the degradation if the mixture of algae obtained by natural selection in the nutrient reduction step is found to be resistant to degradation.

2.4.4 Pretreatment

Several methods, such as thermal, chemical, biological and mechanical, have been tested for pretreatment of algae to increase the biogas production during anaerobic digestion. The feasibility of a method will depend on the increase in biogas yield in relation to the energy

required for the pretreatment method. The method cannot be more energy consuming than the gain in methane yield can account for.

Passos *et al.* (2014) reviewed several methods for pretreatment of algae but emphasizes the difficulties of comparing different methods because of variations in conditions and algal species. Their evaluation was based on comparing the increase in methane yield and the input energy for pretreatment. The amount of water in the algal biomass will affect the energy needed for pretreatment and to account for this, Passos *et al.* (2014) evaluated the energy requirement based on VS content. The results showed that thermal methods were the most promising (Passos *et al.*, 2014). It should be noted that only the energy required for temperature increase was accounted for which means that the energy requirement will be the same regardless of the time of treatment in their evaluation. Thermal pretreatment methods can be divided into thermal (temperatures below 100°C, at atmospheric pressure), hydrothermal (temperature over 100°C and gradual pressure release) and hydrothermal with steam explosion (temperature over 100°C and sudden pressure drop) (Passos *et al.*, 2014).

Cambi™ and Exelys™ are two commercially available techniques for biomass treatment, prior to anaerobic digestion. Cambi™ is operated in batch mode including a flash step whereas Exelys™ on the other hand is operated in continuous mode, without a flash step. Both are operated at approximately 165°C and 8-9 bars for 30 minutes. These methods have shown to increase both the digestibility and the dewaterability of sludge. In both methods, steam is used for heating but steam recycling is only applied in Cambi™ (Abu-Orf & Goss, 2012). It is unclear if algal biomass have been treated using these techniques. Nevertheless, high pressure thermal hydrolysis (HPTH) have been applied to algal biomass with increased biogas yield as a consequence (Keymer *et al.*, 2013).

It should be kept in mind that if a pretreatment method is used, the need for heating of the anaerobic digester decreases and there is a possibility that hygienization is achieved during pretreatment. Also, energy can be recovered when treated biomass is cooled to digestion temperature. Finally, the higher the water content of the biomass that is treated, the more energy is needed per kg of VS (Passos *et al.*, 2014). Thus, the effectiveness of the algal thickening will ultimately affect the energy balance of the system.

2.5 Co-digestion

Co-digestion in general means that two, or more, different substrates are digested together. In this way, many problems with mono-digestion can be avoided and the biogas production can be improved. Some of the positive aspects with co-digestion are optimized C/N-ratio and improved availability of nutrients and trace substances. Further, by mixing one easily degradable substrate with a more difficult substrate, the process can be stabilized by slowing down the initial degradation steps and the risk for instability is decreased. Synergistic effects have been found in co-digestion, which means that more biogas is produced during co-digestion than when the substrates are digested separately (Mata-Alvarez *et al.*, 2014). To circumvent problems associated with anaerobic digestion of algae, co-digestion has been proposed. Several different substrates have been tested; such as pig or cow manure, corn stalks, lipids, soybean oil, glycerol and waste paper (Ward *et al.*, 2014). In addition, research have been made where different species of microalgae have been co-digested together with waste activated sludge (Yuan *et al.*, 2012; Wang *et al.*, 2013), mixed primary and secondary sludge (Rusten & Sahu, 2011; Olsson *et al.*, 2014; Caporgno *et al.*, 2015) or primary domestic sewage sludge (Samson & LeDuy, 1983) with varying results.

To evaluate if synergistic effects are present, equation 2.1 can be used as an indication, adapted from Wang *et al.* (2013). The calculated methane yield is compared with the yield achieved experimentally and if the experimental value is higher than the calculated, a synergistic effect might be present. In equation 2.1, Y_s and Y_a are the methane yield from sludge and algae, respectively and C_s and C_a are the respective added fractions on VS basis for sludge and algae.

$$\text{Calculated methane yield} = Y_s C_s + Y_a C_a \quad \text{(Eq. 2.1)}$$

In Table 2.2 the results from some co-digestion experiments are presented. The "improved methane yield" is calculated by dividing the experimental value presented with the yield calculated according to equation 2.1.

Table 2.2. Summary of co-digestion experiments with algae and different types of sludge. The "improved methane yield" is calculated dividing the experimental value presented with the yield calculated according to equation 2.1.

Algal species (% of VS)	**Co-substrate (% of VS)**	**Temperature (°C)**	**Mode of operation**	**Improved methane yield (%)**	**Reference**
Selenastrum capricornutum (50%)	Primary and secondary sludge (50%)	33	Batch	8.6	Caporgno *et al.* (2015)
Scenedesmus sp. and *Chlorella vulgaris* (37%)	Primary and secondary sludge (63%)	37	Batch	18	Olsson *et al.* (2014)
Chlorella sp. (41%)	WAS (59%)	37	Batch	19	Wang *et al.* (2013)
Spirulina maxima (50.6%)	Primary domestic sewage sludge (49.4%)	35	Continuous	12.5	Samson and LeDuy (1983)

In addition to increased biogas production, research have showed that co-digestion of algae and sludge resulted in greater VS reduction compared to sludge alone (Samson & LeDuy, 1983; Yuan *et al.*, 2012; Wang *et al.*, 2013) and improved dewaterability of the digestate (Yuan *et al.*, 2012; Wang *et al.*, 2013) when sludge was mixed with algal biomass.

2.6 Biomethane potential test

Biomethane potential (BMP) test is a batch method for determining the maximum methane potential and degradability of a substrate. The goal is to determine the maximum possible yield by creating optimal conditions for methane production (Carlsson & Schnürer, 2011). The procedure consists of incubation of the substrate of interest with a methanogenic inoculum, in closed bottles in anaerobic environment and measure the produced methane over time (Angelidaki & Sanders, 2004). Even though the test is designed to give the maximal possible potential, several factors have been found to influence the outcome such as choice of inoculum, loading and equipment (Carlsson & Schnürer, 2011).

The inoculum used should consist of a variety of microorganisms and be adapted to the intended temperature range. To avoid overload (and acidification) it is important that sufficient amount of inoculum is added, however, if too much is added the methane production from the inoculum could be too high compared to the substrate resulting in

uncertain results. To decrease the methane contribution from the inoculum, it should be incubated at the intended experimental temperature for a few days to ensure that easily degradable matter is degraded (Carlsson & Schnürer, 2011). During the test, it is recommended that the quality of the inoculum is tested using a control substance. Further, test bottles with only inoculum are needed to determine the methane production from the inoculum alone. Finally, the methane production from the substrate can be determined by subtracting the methane produced from the inoculum at each sampling point. From one sampling point to another, the yield of methane should be the same or higher, but in some cases the value is lower than the previous measuring point. This can be due to uncertainty in the measuring method or a consequence of that the methane production from the inoculum in the blanks differs from the production from the inoculum in the other bottles. The produced methane is related to the added amount of substrate and the BMP is determined when the methane production has leveled out (Carlsson & Schnürer, 2011).

From BMP tests, it can be hard to conclude effects of co-digestion and inhibition by different substances. Substances that in a continuous experiment would be inhibitory might be diluted to a concentration which is not inhibitory since the substrate is mixed with a lot of inoculum. Also, the inoculum contributes with nutrients which mean that nutrient limitations in the substrate tested can be hard to detect. Significant nutrient limitation, inhibition or synergy effects could be detected, but continuous experiments are more suited for detection of less pronounced effects (Carlsson & Schnürer, 2011).

3 Materials and methods

3.1 Collection of materials

As a part of the warm and clean city project, a pilot plant for the energy positive line was installed at Källby WWTP, Lund, in November 2014. Figure 3.1 shows a simplified version of the pilot plant (presedimentation and AnMBBR) and algal cultivation. At the time, the algal cultivation was performed at the Swedish University of Agricultural Sciences (SLU) in Alnarp and not at the pilot plant. The incoming water to the presedimentation has passed screens and a grit chamber, located at Källby WWTP. Primary sludge was collected from the presedimentation basin at sample point 1 by placing a container at the outlet. Water for algal cultivation was collected from the outflow of the AnMBBR and delivered to SLU, Alnarp where a filtration of the water was performed before algal cultivation. Samples for analysis of the outflow of the AnMBBR were taken at sample point 2. One time, sample was also taken after the filtration, sample point 3. Algal suspension was collected at sample point 4, and delivered to AnoxKaldnes Laboratory in Lund. In Table 3.1 a summary of the terms used are described.

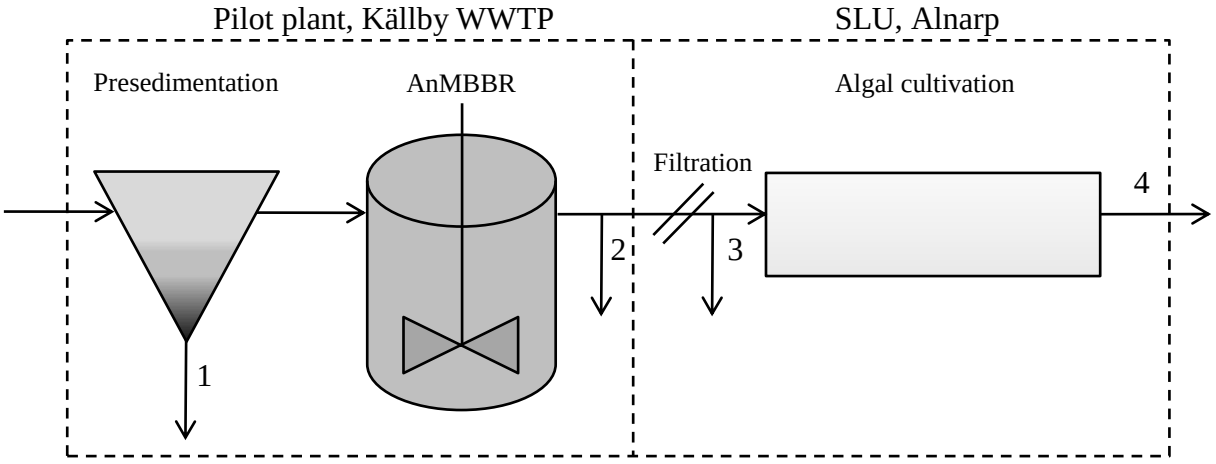

Figure 3.1. Simplified version of the pilot plant and algal cultivation showing where samples were collected.

Table 3.1. Description of terms used and summary of where samples were collected from.

Term	Description
Primary sludge	Sludge collected from the presedimentation (sample point 1) at the pilot plant.
Outflow AnMBBR	Water exiting the AnMBBR (measurements done at sample point 2).
Growth medium	Water used for cultivation of algae. Obtained after filtration (25 µm) of the outflow from the AnMBBR (measurement done at sample point 3).
Algal suspension	The homogenous suspension of algae and water that was obtained after cultivation. In a full scale plant, this would be the outflow from the nutrient reduction step before separation of algae from the treated water (sample point 4).
Concentrated algae	Algae obtained after the harvesting step.
Digestate	The remaining material after the anaerobic digester.

3.2 Analytical methods

Total solids (TS) was determined as the residue after drying samples at 105°C and volatile solids (VS) was determined as the loss of ignition at 550°C, according to Swedish standards SS 028113. Suspended solids (SS) and volatile suspended solids (VSS) were measured by filtering a defined volume of the sample through a glass microfiber filter (GF/A) and determine the residue after drying at 105°C and the loss of ignition at 550°C, respectively, according to Swedish standards SS-EN 872:2005 (SS) and SS 028112 (VSS). According to the method used for TS and VS analysis, the resulting weight of the sample after drying needs to be higher than 20 mg for the method to be valid.

For measurement of total COD (TCOD), soluble COD (SCOD), total organic carbon (TOC), ammonium nitrogen (NH_4^+-N), total nitrogen (N-tot), phosphate phosphorus (PO_4^{3-}-P) and total phosphorus (P-tot) HACH LANGE cuvettes were used, see Appendix I for measuring range and methods used. Prior to analysis of SCOD, NH_4^+-N and PO_4^{3-}-P samples were filtered through MUNKTELL glass microfiber filter MGA (1.6 µm). Primary sludge was homogenized for 3 minutes using X10/25 homogenizer from Ystral®**,** prior to analysis of TCOD and TOC because of the heterogeneity of the substrate. For the TOC analysis, inorganic carbon was removed using HACH LANGE TOC-X5 and all COD, N-tot, P-tot and TOC samples were digested using HACH LANGE LT200. All HACH LANGE cuvettes were measured spectrophotometrically using HACH LANGE DR2800.

Algal suspension and primary sludge were analyzed for total lipids (SS EN 1483), total Kjeldahl nitrogen (TKN) (SS-EN 25663), P-tot (SS-EN ISO 15681-1:2005) and the following metals; Lead, Cadmium, Copper, Chromium, Zinc, Nickel (ICP-MS/AES) and Mercury (SS EN 1483) at external laboratories.

3.3 Algal cultivation

Water was collected from the outflow of the AnMBBR pilot plant at Källby WWTP (Figure 3.1) at several occasions and used as growth medium for algae. The water was analyzed for TCOD, SCOD, NH_4^+-N, PO_4^{3-}-P, N-tot, SS and VSS and handed to SLU in Alnarp. The water was filtered through a filter cloth (pore size 25 µm) prior to the cultivation, in order to remove coarse particles since these could affect the light transmittance of the water. At one occasion, samples were taken before and after filtration to evaluate how much that is removed by filtration.

The water was inoculated with 10% (v/v) of a five day old microalgal culture which had grown under the same conditions as described below. The inoculum originated from a wastewater treatment plant where microalgae are used for treatment of wastewater. The cultivations were performed in batch mode in open plastic containers (Figure 3.2), with a working volume of 6 L, in a greenhouse. The plastic containers had a depth of 14 cm and a surface area of 11 dm^2. To avoid evaporation, the cultivations were covered with a plastic lid but this was assumed to not affect the amount of added light. The temperature in the greenhouse was kept at 25°C and the cultures were exposed to an added light intensity of 100 µmol/(m^2s), 16 hours/day. The cultivations were performed between 12th of January and 2nd of March and the natural light exposure was low. In order to avoid settlement of algae the cultivations were continuously aerated at a rate of 0.3 vvm (gas volume flow/unit of liquid volume/minute) and continuously stirred at a speed of 100 rpm using magnetic stirrers and magnetic stirbars. Algal cultivations were kept for five days except for one occasion when the

cultivation was ended after three days because of high pH and fast growth of the algae. The microalgal population was dominated by *Scenedesmus* sp. (Figure 3.3). The algal suspension was analyzed for TCOD, SCOD, NH_4^+-N, PO_4^{3-}-P, N-tot, SS and VSS.

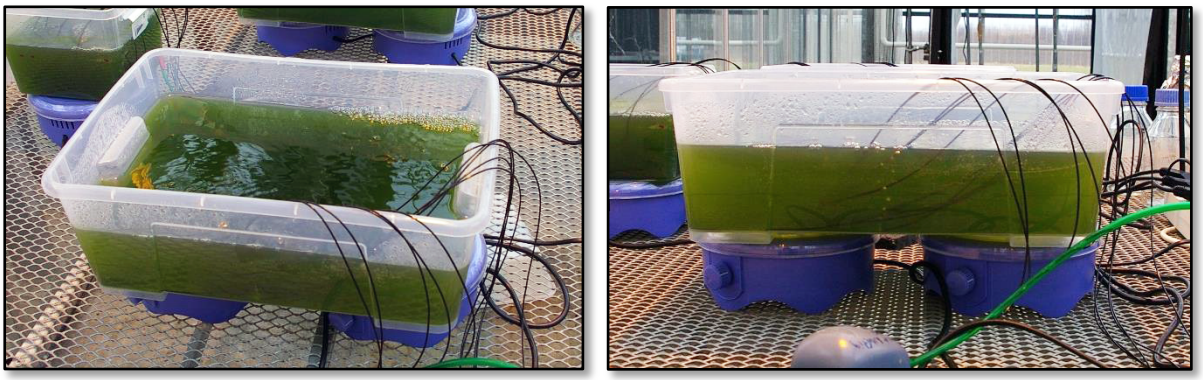

Figure 3.2. Set up of algal cultivations performed at SLU, Alnarp (plastic lid removed).

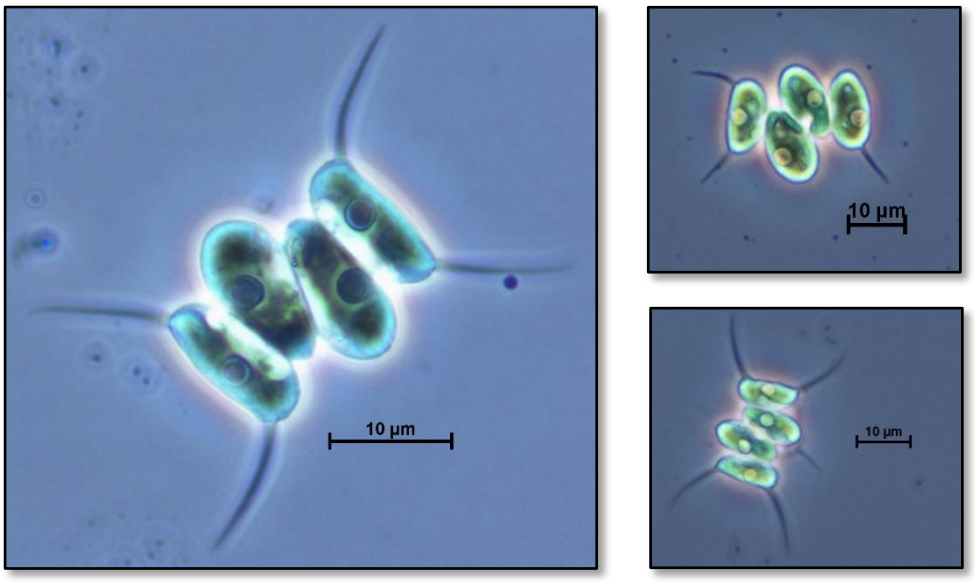

Figure 3.3. Pictures taken during microscopy of samples taken from the last cultivation showing the dominating species which was Scenedesmus.

3.4 Algal harvesting

Two harvesting methods, gravitational sedimentation and flocculation were evaluated. Even though centrifugation has shown promising results in previous studies, this was not evaluated simply because it would be very expensive in full scale.

3.4.1 Chemicals used

As mentioned in chapter 2.3.1, there are benefits of adding iron to the digester and for this reason PIX 111, provided by Kemira was chosen as one of the flocculants. PIX 111 is ferric chloride ($FeCl_3$) in sulphuric acid with an iron content of 13.8%. The solution has a density of 1.42 g/cm^3 and a pH below 1 (Kemira, 2014). Zetag® Cationic Polymer - Solid grade flocculant (nr 8190), provided by BASF, was also used (further referred to as polymer). Polymer was prepared by mixing dry powder with distilled water to obtain a 0.2% w/w solution. Prepared polymer was kept in room temperature for a maximum of three weeks, after that new polymer was prepared. In addition to these, one aluminum salt (poly aluminum chloride, PlusPAC 1800 provided by BASF) was also tested. For pH adjustments hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used.

3.4.2 Initial trials

Flocculation was evaluated by addition of only PIX 111, only polymer and a combination of them under rapid stirring. After a short while, the stirring was decreased to induce floc formation and after a while, the stirring was ended and the flocs were allowed to settle. At first, both PIX 111 and polymer were added in large amounts to evaluate whether or not flocculation was possible. Thereafter, the dosage was gradually lowered and it was decided to use 0.05 mL of PIX 111 and 1 mL of polymer for each liter of algal suspension, when harvesting algae for the BMP tests. During the initial trials, the sedimentation time was not monitored, instead, the clear phase was discarded when most of the flocs had settled. Also, further concentration was done by allowing the concentrated algae to sediment overnight in a cold room. In the morning, the clear phase was discarded and the concentrated phase was kept in a cold room until use.

During the initial trials, a few attempts of flotation were also conducted by adding very small air bubbles to the bottom of the beaker after flocculation was performed. The equipment for lab-scale flotation was a bit hard to handle and separation of the two phases was found to be tricky since no method for skimming off the algae were available. Therefore, no further evaluation using lab-scale flotation was performed.

3.4.3 Experimental set up – optimization

In order to determine the optimal dosage of chemicals, the experiments stated in Table 3.2 were performed. At room temperature (22°C), 180 mL of algal suspension was poured into a beaker and stirred with the help of a magnetic stirrer and a stir bar (Figure 3.4, left). The pH was monitored using a pH-meter (HI 991001, HANNA® instruments) and was adjusted to neutral before each experiment. PIX 111 or PlusPAC 1800 was added according to Table 3.2 at high stirring speed, pH was adjusted to neutral again, and polymer was added, still at high stirring speed. After 15 seconds the stirring speed was lowered to induce floc formation. After 5 minutes of flocculation stirring was ended and the suspension was gently poured into a 175 mL Falcon tube (Figure 3.4, right) and the flocs were allowed to settle for 30 minutes. In addition, 180 mL of algal suspension, without addition of chemicals, was poured into the same kind of tube and algae were allowed to settle for 15 hours in darkness in a cold room (experiment 24).

Table 3.2. Added amounts of PIX 111, PlusPAC 1800 and polymer in each of the experiments in the optimization.

Experiment	PIX 111 (µL/L)	PlusPAC 1800* (µL/L)	Polymer (mL/L)
1	0	0	0.50
2	0	0	1.0
3	0	0	1.5
4	0	0	2.0
5	0	0	2.5
6	0	0	3.0
7	0	0	3.5
8	0	0	4.0
9	50.0	0	0.50
10	25.0	0	0.50
11	12.5	0	0.50
12	50.0	0	1.0
13	25.0	0	1.0
14	12.5	0	1.0
15	50.0	0	1.5
16	25.0	0	1.5
17	12.5	0	1.5
18	50.0	0	2.0
19	25.0	0	2.0
20	12.5	0	2.0
21	0	78.0	1.0
22	0	39.0	1.0
23	0	19.0	1.0
24	0	0	0

**Volumes are based on adding the same amount of Al^{3+} ions as the amount of Fe^{3+} ions that were added for PIX 111.*

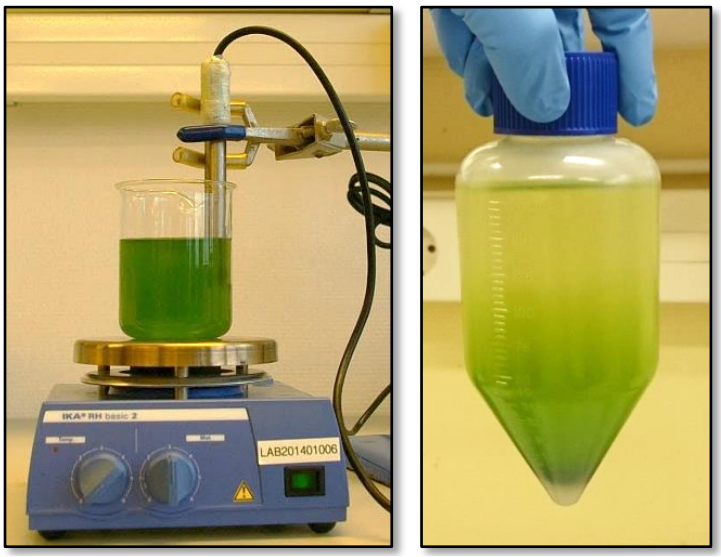

Figure 3.4. Experimental set up used in the flocculation experiments (left) and the Falcon tube used for sedimentation (right).

3.4.4 Evaluation of harvesting efficiency

After 30 minutes of sedimentation 15 mL was withdrawn carefully, from the bottom of the tube, using a pipette. The rest (clear phase) was analyzed for TCOD, P-tot and N-tot. The volume of the clear phase was not enough to perform SS analysis, instead a correlation between TCOD and SS was derived and was used to assess the amount of SS in the clear phase in each experiment. Harvesting efficiency was calculated according to equation 3.1 where $SS_{initial}$ is the measured concentration of SS at the beginning and SS_{end} is the concentration of SS in the clear phase after sedimentation (calculated using the correlation between TCOD and SS).

$$\text{Harvesting efficiency } (\%) = \frac{SS_{initial} - SS_{end}}{SS_{initial}} \cdot 100 \qquad \text{(Eq. 3.1)}$$

3.4.5 Economical evaluation

For the economical evaluation the prices for PIX 111 and Zetag® Cationic Polymer - Solid grade flocculant was needed. The price depends on which quantity that is bought, and since this depends on the size of the treatment plant, only approximate prices could be found. In the evaluation a price of 1440 SEK/ton of PIX 111 and 35 SEK/kg of Zetag® Cationic Polymer - Solid grade flocculant was assumed (Olsson, 2015). This corresponds to 0.002 SEK/mL PIX 111 and 0.00007 SEK/mL 0.2% Zetag® Cationic Polymer - Solid grade flocculant. The cost for harvesting was assessed using equation 3.2, where PIX111 and polymer is the respective volume of added substance (in mL/m^3 algal suspension).

$$\text{Cost } (\text{SEK/m}^3) = \text{PIX111} \cdot 0.002 + \text{Polymer} \cdot 0.00007 \qquad \text{(Eq. 3.2)}$$

3.5 Pretreatment of algal biomass

In order to try to increase the biogas yield three pretreatment methods of algae were evaluated. The first one aimed at mimicking hygienization by pasteurization which can be regarded as a quite sensitive method in this context. In contrast to pasteurization, microwave treatment at 120°C and 170°C for 30 minutes was also evaluated in order to see how much the methane yield can be increased if a more aggressive treatment is used. Before and after pretreatment TCOD, SCOD and NH_4^+-N were measured. Increase in SCOD was calculated according to equation 3.3.

$$\text{Increase}_{\text{SCOD}}(\%) = \frac{(SCOD/TCOD)_{after}}{(SCOD/TCOD)_{before}} \cdot 100 \qquad \text{(Eq. 3.3)}$$

3.5.1 Pasteurization

One hygienization method is to heat the substrate at 70°C for one hour, called pasteurization. To accomplish this, a shaking water bath was used. Concentrated algae were divided into two glass bottles, 87.5 mL each. To minimize evaporation of water but avoid pressure build up, the bottles were closed with an air-tight rubber septum with a needle through the cork (Figure 3.5, left). When the water bath had reached 70°C, the bottles were put in the water bath (Figure 3.5, right), and were continuously shaken. To monitor the temperature, one bottle was filled with 87.5 mL of tap water and the temperature was measured in this bottle. The assumption was made that the concentrated algae followed the same heating pattern as the water. The desired temperature was reached after 11 minutes and the temperature was kept at

72 ± 1°C for one hour. After this, the bottles were put in cold water to cool the suspension. The pretreated algae were kept in a cold room until use.

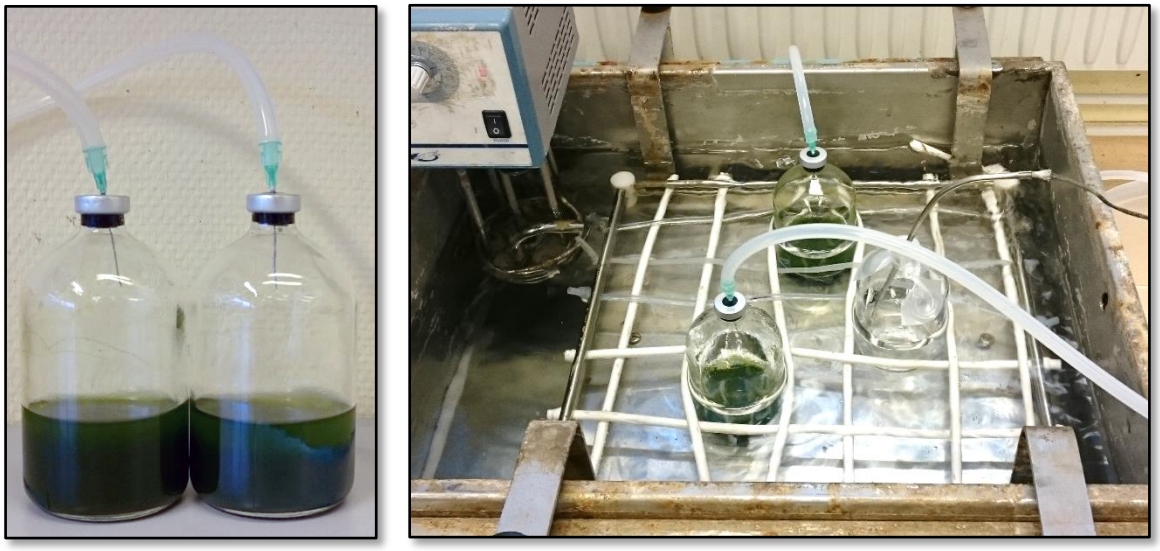

Figure 3.5. The bottles (left) and water bath (right) used in pasteurization.

3.5.2 Microwave treatment

In order to achieve higher temperatures EthosPlus microwave labstation was used. Concentrated algae were poured into the digestion vessel (Figure 3.6, upper left) and the lid was closed. In the lid, a thermocouple was placed. The vessel was put into the holder and secured with the help of three screws. In order to tighten the screws a torque wrench was used, which was set to 22 Nm, and the screws were tightened until a "click" sound was achieved (Figure 3.6, upper right). The holder, with the digestion vessel, was put into the microwave and the temperature transmitter was connected to a port inside the microwave oven (Figure 3.6, lower left) and the door was closed. With the help of a controller, connected to the microwave and equipped with MLS-easyWAVE (version 3.3.0.0) software, a digestion program could be designed. Sample volume, temperature, pressure and digestion time for each experiment are shown in Table 3.3, the time for heating to the desired temperature was set to 2 minutes for both experiments. After heating to the desired temperature, the temperature of the sample was kept at the set-temperature ± 1°C. When the program was finished, the holder with the digestion vessel, was put into a water bath to cool the suspension (Figure 3.6, lower right), and when the suspension had cooled, the digestion vessel was opened by releasing the screws and removing the lid. The pretreated algae were kept in a cold room until use.

Table 3.3. Sample volume, treatment temperature, treatment pressure and digestion time for each microwave treatment experiment.

	Experiment 1	**Experiment 2**
Sample volume (mL)	195	190
Temperature (°C)	170	120
Digestion time (min)	30	30
Pressure* (bar)	9.6	3.4

**Calculated, see Appendix II for calculations.*

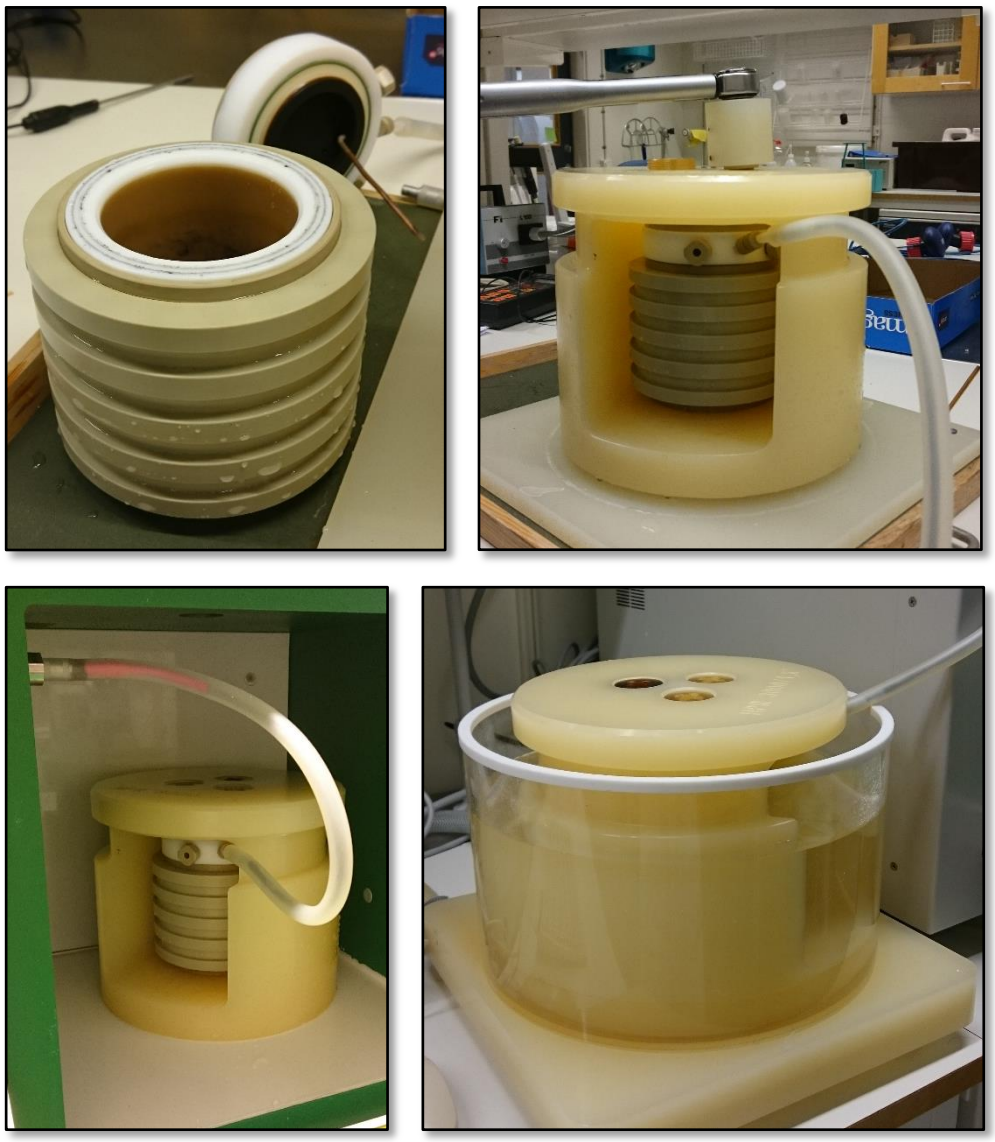

Figure 3.6. Equipment used in microwave treatment. Digestion vessel (upper left), securing the digestion vessel into the holder (upper right), placement of holder and temperature transmitter in the microwave (lower left) and water bath for cooling after treatment (lower right).

3.5.3 Energy requirement

The energy (E) required, in kJ/day, for pretreatment was approximated using equation 3.4, modified from Passos *et al.* (2014). This estimation accounts for the energy needed for raising the temperature of the algal suspension from initial temperature (T_i) to pretreatment temperature (T_p) whereas the energy needed for keeping the suspension at this temperature during the treatment time is neglected. Further, it is assumed that energy is recovered using a heat exchanger (with efficiency, η=85%) when the algal suspension is cooled to digestion temperature (T_d) before entering the digester. It is assumed that density (ρ) and specific heat capacity (c_p) of the suspension is equal to water and average values are used for the

temperature interval of interest. The flow (Q) in m^3/day that is treated will depend on the SS content in the concentrated algae. This means that the more concentrated the algae is, the less energy is needed for pretreatment.

$$\mathrm{E\,(kJ/day)} = \rho Q c_p (T_p - T_i) - \rho Q c_p (T_p - T_d)\eta \qquad \text{(Eq. 3.4)}$$

3.6 Biomethane potential test

The experimental procedure of the BMP tests was based on the method described by Hansen *et al.* (2004) with some adjustments that was necessary because of the limitation in available substrate and the nature of the algae. The reactor volume was decreased and the loading was based on COD (for substrate). Two sets of BMP tests were conducted and both lasted for 49 days. The first one aimed at investigating the BMP of primary sludge, algae and co-digestion of these substrates in different ratios. The second aimed at evaluating if, and how much the pretreatment methods (described in 3.5) could increase the biogas yield from algae.

3.6.1 Estimation of theoretical BMP

Theoretical BMP was calculated based on the composition of substrate used in the first BMP test. The composition was determined as described by Carlsson and Uldal (2009) where protein content is determined as the organic nitrogen content of the sample multiplied with a conversion factor (6.25, used for protein structures), the lipid content is directly measured and the carbohydrate content is determined as the part of VS that is not proteins or lipids. From the composition, the theoretical BMP and the methane content were determined using the values in Table 2.1.

3.6.2 Substrate

BMP test 1

Primary sludge was collected from the presedimentation at the pilot plant at Källby WWTP two days before the start up and kept in cold room. Algae were delivered two days before the start up, was concentrated the day before the start up and kept in a cold room.

Based on a material balance over a theoretical treatment plant (see Appendix III), the ratio between algae and primary sludge was set to 70% algae and 30% primary sludge on COD basis. In addition, two more mixtures were chosen, with lower amount of algae (40 and 15%, respectively). These are further referred to as mixture 1 (70% algae), mixture 2 (40% algae) and mixture 3 (15% algae).

BMP test 2

Algae were delivered at two occasions, nine and five days before the start up. The two batches were mixed and concentrated four and five days before the start up. A portion of the concentrated algae was pretreated using pasteurization (described in 3.5.1) two days before the start up and two portions of algae were pretreated using microwave treatment (described in 3.5.2) one day before. Algae were kept in a cold room until start up.

3.6.3 Inoculum

Inoculum for each BMP test was collected from an anaerobic digester at Källby WWTP which treats mixed primary and secondary sludge and is operated in the mesophilic range. Sample was taken for TS and VS analysis and the rest of the inoculum was put in an incubator at 37 ± 1°C for four days, to allow remaining easily degradable matter to be degraded, and

thereby minimize the contribution of biogas formation from the inoculum during the BMP test.

3.6.4 Experimental procedure

For the experiments, 245 mL glass bottles were used with a working volume of 100 mL and thus a head space of 145 mL (shown in Figure 3.7). In order to avoid a too high pressure in the bottles the experiment was designed for production of 75 NmL (BMP test 1) or 80 NmL (BMP test 2) methane per bottle. By assuming the theoretical yield of 350 NmL CH_4/gCOD the amount of COD needed in each bottle could be calculated. Based on the TCOD concentration in the substrates, the VS concentration in the inoculum and the decision of having the ratio between substrate and inoculum of 0.5 gCOD/$gVS_{inoculum}$, the amount of each component could be calculated (see Appendix IV for calculations). In addition to the bottles with the investigated substrates, blanks with only inoculum and controls with inoculum and sodium acetate (NaAc) were prepared.

For substrates, mixtures, blank and control triplicate bottles were prepared. This was done by preparing 400 mL by weighing inoculum, substrate and water according to the calculations. After mixing, 100 mL was distributed into each bottle. To create anaerobic environment in the bottles, nitrogen gas was flushed through the liquid phase for at least 2 minutes and in the head space for at least 1 minute before the bottles were sealed with an air-tight rubber septum and aluminum crimps caps. All bottles were put in an incubator at 37 ± 1°C.

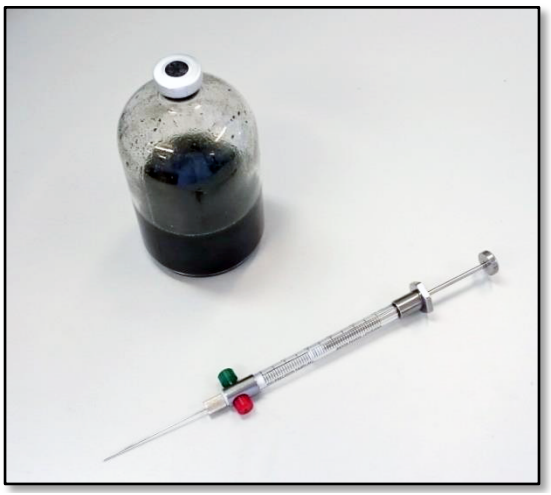

Figure 3.7. Bottle used in BMP tests and the glass syringe used for collection of gas samples.

Methane measurements

Every weekday, the bottles were shaken and production of methane was measured between one and three times per week using gas chromatography. The gas chromatograph (GC) used was Clarus® 480 from PerkinElmer® equipped with thermal conductivity detector and a 2 m · ⅛ inch · 2.1 mm column. For calibration and collection of gas samples a 0.5 mL glass syringe with pressure lock (PRESSURE-LOK® Gas syringe from VICI Precision Sampling) was used (shown in Figure 3.7). Calibration of the GC was performed by injection of 0.2 mL of standard gas containing 75 vol% methane, 15 vol% carbon dioxide and 10 vol% nitrogen. Measurement of produced methane in each bottle was performed by inserting the syringe through the rubber septum and collecting a 0.2 mL sample from the head space of the bottle

and injecting it to the GC. Data was processed using the program TotalChrom Navigatior software from PerkinElmer® in which the peak areas for carbon dioxide and methane in the sample were related to the standard gas, and further normalized to STP. It was assumed that the headspace (145 mL) was completely occupied by nitrogen gas at the beginning, and that the volume of nitrogen was constant during the test. Further, some of the produced gas will dissolve in the liquid. To account for this the solubility of methane and carbon dioxide in water at 35°C was used, and is 25.5 mL/L and 592 mL/L, respectively (Dean, 1999). Methane production in the blanks was subtracted from the production in the other bottles to give the methane potential from the actual substrate.

4 Results and discussion

4.1 Algal cultivation

Water used for algal cultivation was filtered to remove particles that could affect the light transmittance of the water. Analysis of the water, before and after filtration were performed once, and showed an 8% loss in total nitrogen and a 33% loss in TCOD for water collected on the 23rd of February (Table 4.1). For implementation in full scale this must be considered since filtration of the water coming in to the nutrient reduction step will add cost to the plant, and it might not be very convenient. Another method for removal of particles after the AnMBBR would be desirable. One possibility could be sedimentation where the particles are removed and transferred to the anaerobic digester. It would however be interesting to see if the particles actually affect the light transmittance in an extent that inhibits algal growth. Otherwise no pretreatment of the water would be needed, and the cost for this separation step is avoided.

Table 4.1. Comparison of TCOD and N-tot before and after filtration of the water used for the 4th batch of algae, water was collected on the 23rd of February.

	Before filtration	**After filtration (25 µm)**	**Loss (%)**
TCOD (mg/L)	159	106	33%
N-tot (mg/L)	47.4	43.6	8%

Four batches of algal suspension were received from SLU. Table 4.2 shows a summary of when water from the outflow of the AnMBBR was collected, when the algal suspension was collected and the cultivation time for each of the batches. It should be noted that on the 2nd of February, a large amount of water was collected. One part was used for the 2nd batch and the other stored (in a cold room) until the 2nd batch was done and then used for the 3rd batch. Analysis of the water was done directly after collection, and no measurements were done after storage of the water used for the 3rd batch. The 1st batch was used for BMP test 1, analysis of heavy metal content and composition analysis. The 2nd and 3rd batch were used for BMP test 2 and the 4th batch was used for optimization of harvesting.

Table 4.2. Summary of collection dates for the outflow of the AnMBBR and the algal suspension and the cultivation time for each batch.

Algal batch	**Collection date**		**Cultivation time (days)**
	Outflow AnMBBR	**Algal suspension**	
1	26 January	2 February	5
2	2 February	9 February	5
3	2 February	12 February	3
4	23 February	2 March	5

Nutrient reduction

Results from measurement of NH_4^+-N, N-tot and PO_4^{3-}-P before and after algal cultivation are shown in Table 4.3. In none of the batches, PO_4^{3-}-P could be detected after cultivation which corresponds to a reduction of more than 97%. Precipitation of phosphate can occur at high pH which, besides phosphorus assimilation, could have contributed to the reduction. The

ammonium concentration was low in all batches after cultivation. However, since the pH was 10 or higher in all algal suspensions there is a great possibility that nitrogen, in the form of ammonia have been lost to the atmosphere since the equilibrium between ammonia and ammonium is pushed towards ammonia at high pH. This theory is in accordance with the measurement of total nitrogen, which should be the same before and after cultivation, but as can be seen in Table 4.3, total nitrogen concentration has dropped in all batches during cultivation. The most likely reason for the high pH is depletion of CO_2 and as have been investigated by Park and Craggs (2010), it is possible to regulate the pH in algal cultivations by addition of CO_2. It is also crucial to regulate the pH in algal cultivations to avoid inhibition by free ammonia (Azov & Goldman, 1982).

Table 4.3. Results from measurement of NH_4^+-N, N-tot and PO_4^{3-}-P before and after each algal cultivation. The pH in each batch and the reduction of NH_4^+-N, N-tot and PO_4^{3-}-P is also shown.

Algal batch		pH	NH_4^+-N (mg/L)	N-tot (mg/L)	PO_4^{3-}-P (mg/L)
1	Growth medium	7.5	27.7	35.1*	2.62
	Algal suspension	10.0	1.52	22.6	<0.05
	Reduction (%)		**94.5**	**35.7**	**>98**
2	Growth medium	7.4	24.0	27.2*	1.70
	Algal suspension	10.9	0.02	18.7	<0.05
	Reduction (%)		**99.9**	**31.2**	**>97**
3	Growth medium	7.4	24.0	27.2*	1.70
	Algal suspension	11.1	3.67	19.3	<0.05
	Reduction (%)		**84.7**	**29.0**	**>97**
4	Growth medium	7.4	39.6	43.6**	3.31
	Algal suspension	10.5	2.98	29.2	<0.05
	Reduction (%)		**92.5**	**33.0**	**>98**

**Calculated, assuming that 8% is lost in the filtration before cultivation.*
***After filtration (25 µm).*

Since both nitrogen and phosphorus are needed for growth, lack of one of them could lead to growth limitations. Assuming that only ammonium and phosphate were available for algal growth, the ratio between nitrogen and phosphorus in the growth medium was between 10/1 and 14/1. According to Larsdotter (2006) P-limitation occurs at a N/P-ratio of above 30/1 whereas N-limitation occurs at a ratio of below 5/1. This indicates that neither N- nor P-limitation should occur with this water. Limitation in carbon could occur due to that the carbonate equilibrium is pushed towards carbonate at high pH, and microalgae are incapable of assimilating carbonate (Larsdotter, 2006).

Further investigations with pH regulation, particularly using CO_2 addition, are needed to investigate the possibilities with using microalgae for this treatment step. By regulating the pH it might be possible to decrease the loss of nitrogen to the atmosphere and avoid possible precipitation of phosphate.

Algal biomass production
The concentrations of TCOD and SCOD in the algal suspension and the growth medium are shown in Table 4.4. Increase in TCOD is highest in the last batch, which also had the highest amount of incoming ammonium and phosphate (Table 4.3). The increase in TCOD is lowest in the 3rd batch which probably can be explained by the short cultivation time. The amount of

SCOD is approximately the same before and after algal cultivation except for the 3rd batch. One possible explanation of the increase in SCOD in the 3rd batch is that during storage of the water, particles have been hydrolyzed and in this way increased the portion of SCOD. Since no measurements were done after storage, it is not possible to conclude this but as water will not be stored in a full scale plant, this will not be a problem. As these experiments both vary in incoming concentration of nutrients, COD and cultivation time more controlled experiments are needed to conclude optimal conditions and maximum COD increase. It is also possible that the microalgal culture used as inoculum have been adapting during the course of this project and thereby giving varying results.

Table 4.4. Concentrations of TCOD and SCOD in the growth medium and the algal suspension as well as the increase in TCOD over the algal cultivation for all batches.

Algal batch	Growth medium		Algal suspension		Increase
	TCOD (mg/L)	SCOD (mg/L)	TCOD (mg/L)	SCOD (mg/L)	TCOD (mg/L)
1	157*	29.2	365	31.0	208
2	77*	30.4	382	36.8	305
3	77*	30.4	272	60.2	195
4	106**	48.2	433	43.4	327

**Calculated, assuming that 33% is lost in the filtration before cultivation.*
***After filtration (25 µm).*

The concentration of SS in each of the batches is presented in Table 4.5 as well as the productivity. The concentration of biomass achieved in batch 1, 2 and 4 is close to 0.3 g/L that Larsdotter (2006) reported as possible in open pond systems. The highest productivity was achieved in the 3rd batch which however had the lowest concentration. This indicates that the production rate of biomass is higher in the beginning, but there is still potential for production of more biomass by prolonging the cultivation time. According to Majid *et al.* (2014) the productivity of algal biomass in open pond systems depends on pond configuration and algal species. For the system used in this project, the productivity is low compared to the range of 0.06 to 0.42 g/(L · day) that was reported by Majid *et al.* (2014) as average for open pond systems. However, there are opportunities for improvement to increase the biomass productivity. If algal growth has been inhibited by presence of free ammonia, the productivity might be increased by regulating the pH. Also, if pH regulation is performed by addition of CO_2 additional carbon is provided to the algae which hopefully would increase the productivity. Continuous cultivation could also lead to further adaptation of the algal culture which could increase the nutrient reduction potential and biomass productivity.

Table 4.5. Concentration of SS after algal cultivation and productivity for each batch.

Algal batch	SS (g/L)	Productivity (gSS/(L · day))
1	0.275	0.0551
2	0.279	0.0559
3	0.180	0.0601
4	0.284	0.0568
Average		0.0570

Comparison of TS, SS, VS and VSS
In some cases it was desired to know the TS and VS content of the algal suspension and the concentrated algae. Because of the low biomass concentration in the algal suspension and the shortage of concentrated algae from the 1st batch it was only possible to measure SS and VSS.

Further on, when the 2nd and 3rd batch had been concentrated, enough material was obtained to perform analysis of TS, VS, SS and VSS to investigate the correlation between them. The results are shown in Table 4.6, and as can be seen, there is only a small difference between TS and SS and between VS and VSS. Since the difference is small, TS and SS as well as VS and VSS for algae are assumed to be equal through the rest of this report, both for algal suspension and for concentrated algae.

Table 4.6. Comparison of TS, SS, VS and VSS of concentrated algae, mix of 2nd and 3rd batch. Results are shown as average of triplicates with standard deviation.

	Concentrated algae (mix of 2nd and 3rd batch)
TS (g/L)	5.61 ± 0.02
SS (g/L)	5.35 ± 0.03
VS (g/L)	3.77 ± 0.01
VSS (g/L)	3.88 ± 0.07

4.2 Algal harvesting

After the nutrient reduction step, the algae are completely mixed with the water, which means that before releasing the water into the surrounding environment, harvesting of the algae is necessary. It is also important to reduce the water content of the algal slurry that is entering the anaerobic digester to decrease the needed size of the digester, and also to decrease the needed energy input if a pretreatment step is used.

4.2.1 Initial trials

During the initial trials it was concluded that addition of only PIX 111 was not enough for successful flocculation of the algae. Addition of only polymer showed more promising results, but to be sure that enough algae could be harvested for the BMP tests a combination of polymer and PIX 111 was used. The initial trials also showed that it would probably be possible to lower the concentrations of both PIX 111 and polymer and still be able to achieve sufficient separation.

Trials using the lab-scale flotation system showed very promising results. As can be seen in Figure 4.1 (left), almost all of the algal flocs are found in the concentrated phase on the top and the clear phase is almost completely clear. In Figure 4.1 (right) corresponding experiment using sedimentation is shown. In both these experiments flocculation was achieved by addition of 0.05 mL PIX 111 and 1 mL polymer for each liter of algal suspension. What should be noted are the difference between the clear phases and the size of the concentrated phases. In both experiments, algal suspension from the same batch was used, thus the amount of algae was the same. As can be seen from the figures, the algae are more concentrated in the experiment where flotation was used.

In some cases, the algal flocs started to float by themselves without any addition of air bubbles in the bottom of the beaker. The results obtained during the initial trials strongly suggest that flotation would be preferable compared to sedimentation. As can be seen in

Figure 4.1 (left) the lab-scale flotation system caused dilution of the sample, thus representative sampling was considered difficult. Also, the practical difficulties mentioned before (chapter 3.4.2) excluded further evaluation of flotation.

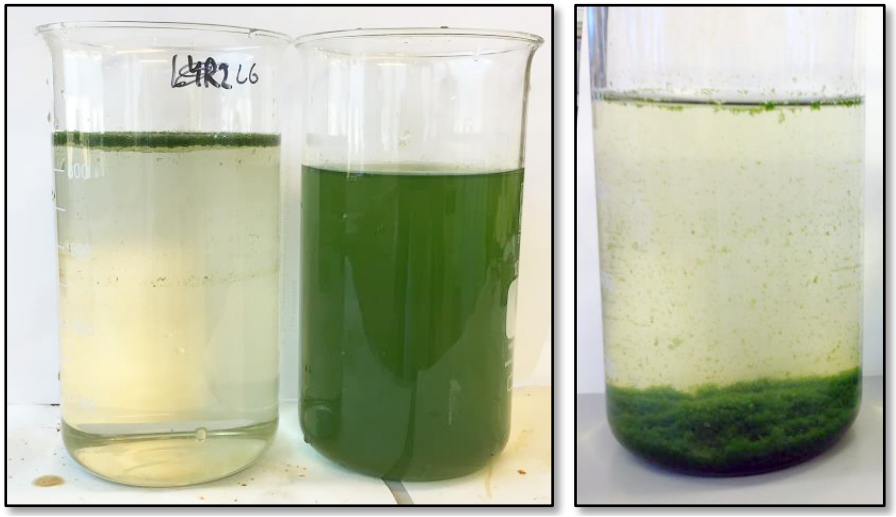

Figure 4.1. Results from flotation (left) and sedimentation (right) experiments. Flocculation was achieved by addition of 0.05 mL PIX 111 and 1 mL polymer for each liter of algal suspension. In the middle, the algal suspension before addition of chemicals is shown for comparison.

4.2.2 Optimization

It is crucial to optimize the amount of chemicals that are to be added in the harvesting step since this will not only affect the economics of the treatment but also the quality of the released water and the digestate. If too much chemicals are used the price will be high, there is a risk of residual chemicals in the water and the amount of chemicals in the digestate might compromise its further use. On the other hand, if the amounts are too low, the amount of biomass in the water will be too high. Consequently, the water will be too high in nitrogen, phosphorus, COD and SS to be released into the environment. Also, if a lot of biomass is lost in the effluent, less biogas can be produced.

Measurement of NH_4^+-N and PO_4^{3-}-P in the algal suspension were performed prior to the optimization experiments. Ammonium concentration was assumed to be unaffected by flocculation, thus measurement before experiment is enough. The concentration of NH_4^+-N was 2.98 mg/L in the algal suspension before flocculation. The phosphorus concentration can be decreased during flocculation. The concentration of PO_4^{3-}-P in algal suspension used (batch 4) was undetectable, thus it was unnecessary to measure it after flocculation experiments.

The results from measurement of P-tot, N-tot and TCOD in the clear phase after the flocculation experiments are shown in Figures 4.2-4.4, experiments 21-23 (addition of PlusPAC 1800) and experiment 24 (no chemicals added) are excluded. Results from all experiments are shown in Appendix V. For Källby WWTP the limit for P-tot in the discharged water is 0.3 mg/L and for N-tot and TCOD the recommended highest

concentrations are 10 mg/L and 70 mg/L, respectively (VA SYD, 2014), represented as a black line in each figure. For NH_4^+-N the recommended limit is 3 mg/L (VA SYD, 2014) which is met in all experiments assuming that the concentration is the same before and after flocculation (in this batch 2.98 mg/L).

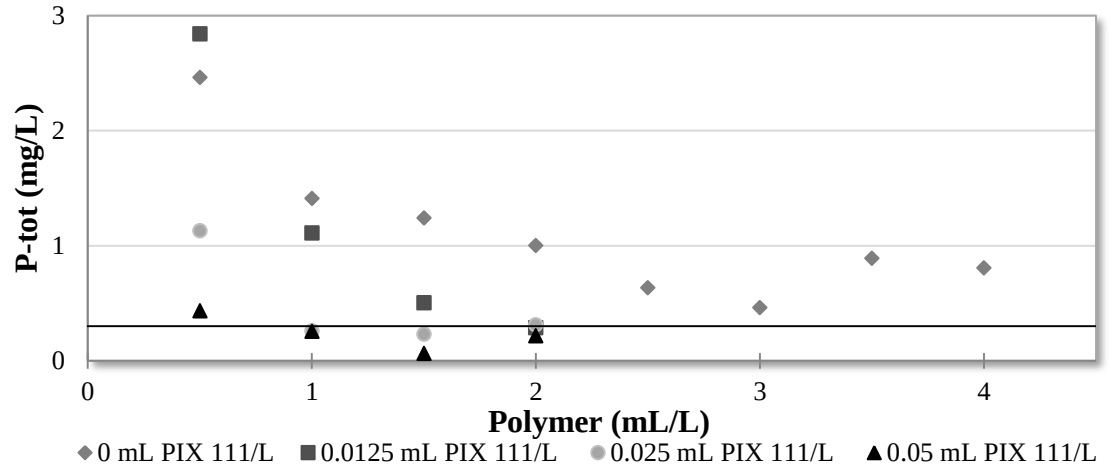

Figure 4.2. Results from measurement of P-tot in the clear phase in flocculation experiments 1-20. Solid black line shows the limit for P-tot concentration in discharged water at Källby WWTP.

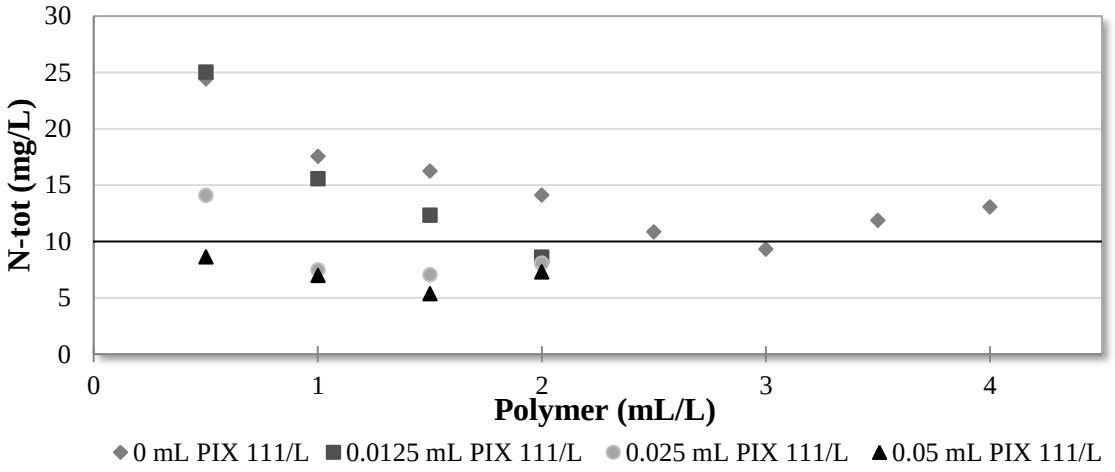

Figure 4.3. Results from measurement of N-tot in the clear phase in flocculation experiments 1-20. Solid black line shows the recommended lowest concentration of N-tot in discharged water at Källby WWTP.

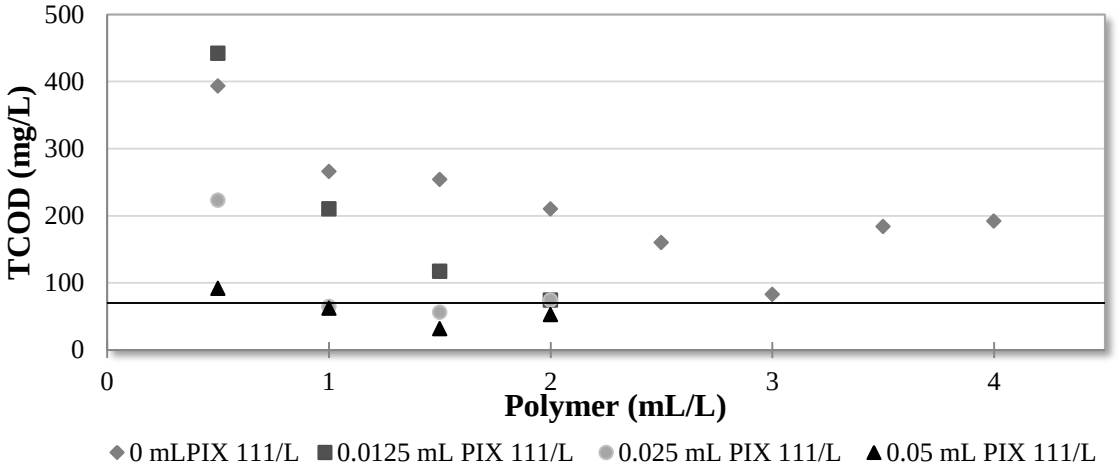

Figure 4.4. Results from measurement of TCOD in the clear phase in flocculation experiments 1-20. Solid black line shows the recommended lowest concentration of TCOD in discharged water at Källby WWTP.

From the experiments it was found that it is possible to meet the discharge limits by flocculation followed by sedimentation for some combinations of PIX 111 and polymer. For the highest addition of PIX 111 (0.05 mL/L) it was possible to meet both the limit for P-tot and the recommended limits for N-tot and TCOD except for the lowest polymer addition (0.5 mL/L). For the experiments with PlusPAC 1800 it was possible to meet the limits only when the highest amount of PlusPAC 1800 was added (see Appendix V). For the lowest addition of PIX 111 (0.0125 mL/L) it was not possible to meet the limits regardless of the polymer additions tested.

In neither of the experiments with only polymer the limit for P-tot or recommended limit for TCOD was achieved. For N-tot, only addition of 3 mL/L gave water which met the recommended limit. As can be seen, the concentration of P-tot, N-tot and TCOD decreases for increasing polymer addition up to 3 mL/L, after this, the concentrations are higher. At higher polymer addition (<3 mL/L) the created flocs were very sticky and got stuck on everything, which probably affected the analysis. Based on this observation, the polymer addition should be kept lower to avoid sticky flocs. Also, it was concluded that addition of only polymer was not sufficient.

Cost evaluation

Other than meeting the discharge limits, the cost is essential. Summarized in Table 4.7 are the results from those experiments where the clear phase met both the limit for P-tot and the recommended limits for N-tot and TCOD. The cost per m^3 of algal suspension is presented as well as the harvesting efficiency, based on SS. Experiment 13 (which corresponds to addition of 0.025 mL PIX 111 and 1 mL polymer per liter of algal suspension) is the one that would be cheapest and still meet the discharge limits. The cost per year, for a daily flow of 25 000 m^3, would be 1.1 MSEK. The harvesting efficiency is lowest for this case, 87%, but as can be seen it is possible to increase the harvesting efficiency up to 96% and achieve better quality of the water regarding P-tot, N-tot and TCOD, but at a higher cost.

Table 4.7. Summary of those experiments where the clear phase met both the limit for P-tot and the recommended limits for N-tot and TCOD. The cost for flocculation chemicals needed per m^3 of algal suspension and the corresponding harvesting efficiency are also shown. Experiment number corresponds to the added amounts of chemicals listed in Table 3.2.

Experiment	Cost (SEK/m^3)	N-tot (mg/L)	P-tot (mg/L)	TCOD (mg/L)	SS* (mg/L)	Harvesting efficiency (%)
12	0.17	7.0	0.26	62	34	88
13	0.12	7.5	0.26	65	36	87
15	0.21	5.4	0.063	32	13	96
16	0.16	7.0	0.23	56	30	89
18	0.24	7.1	0.22	52	27	90
21	0.23**	6.1	0.11	41	19	93

**Calculated using the correlation between TCOD and SS, see Appendix V.*
***Calculated assuming the same price for PlusPAC 1800 as for PIX 111.*

Udon *et al.* (2013) were able to recover 93% of microalgae by addition of only $FeCl_3$ and 98% by addition of only a cationic polymer and found a linear relationship between optimal flocculant dose and algal concentration. Comparing the added dose of $FeCl_3$, considering the starting algal concentration the amount used is 50% lower in experiment 13 than was stated as the optimal dose by Udon *et al.* (2013). In experiment 13, polymer was used in combination with $FeCl_3$ but since Udon *et al.* (2013) did not evaluate the combination of $FeCl_3$ and polymer it is difficult to compare the results. Also, Udon *et al.* (2013) based the optimal addition of flocculant on when the lowest turbidity and SS concentration was achieved and did not consider the concentrations of N-tot and P-tot.

Based on experiments in the present study, using both polymer and a metal salt in combination seems to be very promising for the harvesting of microalgae, especially when it is crucial that the separated water meets specific discharge limits.

Additional remarks
By using flotation, the separation can probably be improved and it might be possible to reduce the amount of added flocculants. Also, the harvesting efficiency could be improved, yielding more substrate for biogas production. Additional experiments using flotation will unravel how much the separation can be improved. An economical evaluation should be conducted to compare the cost of chemicals against the cost for flotation since this can be a quite energy consuming operation (Wiley *et al.*, 2011).

During the optimization experiments it was observed that flocs adhered to the walls of the tube and were thus included in the analysis of the clear phase. In full scale, the contribution from this adhering would be lower since the ratio between the wall area of the reactor and the total volume would be much lower. Hence, more of the algae would be harvested, and the quality of the water would be improved. Further, a lot of small flocs that did not settle were observed in the clear phase. If flotation had been used during the optimization, more of these small flocs would probably have been removed, improving both the harvesting efficiency and the quality of the water. Thus, in full scale using flotation, an increased harvesting efficiency can be expected.

As discussed in chapter 4.1 the nutrient reduction step needs to be investigated further. Even though it was possible to achieve good quality of the water using flocculation it must be taken

into consideration the quality of the water exiting the nutrient reduction step. Since ammonium will not be affected by addition of for example PIX 111 and/or cationic polymer it is crucial that the concentration of ammonium is low enough before entering the harvesting step. Phosphate can be precipitated using PIX 111 but the amount of added PIX 111 might need to be adjusted. What can be said is that, as long as the nutrient reduction step is working, it will be possible to achieve water which meets the discharge limits and recommendations.

4.2.3 Only sedimentation

It would be desirable to harvest the algae without any addition of chemicals. As can be seen in Table 4.8 (experiment 24), neither of the limits were met and the harvesting efficiency was low although a very long sedimentation time was applied. Park and Craggs (2010) were able to achieve 70.6 and 68% recovery of algal biomass (as SS) using gravitational sedimentation in gravity algal settling cones with a retention time of six and three hours, respectively. Rusten and Sahu (2011) reported that depending on which algal species that is present, different harvesting methods must be applied. In the study by Park and Craggs (2010), the algal culture was a mixture of *Scenedesmus*, *Microactinium*, *Pediastrum* and *Ankistrodesmus* which could explain the discrepancy between their results and the results found in this project, where the algal culture was dominated by *Scenedesmus*. In conclusion, sedimentation without addition of chemicals showed very poor results, and would not be feasible in large scale with the microalgal culture used in this project.

Table 4.8. Results from experiment 24 where only sedimentation was applied, without addition of chemicals.

Experiment	Sedimentation time (h)	N-tot (mg/L)	P-tot (mg/L)	TCOD (mg/L)	SS* (mg/L)	Harvesting efficiency (%)
24	15	19	1.8	263	179	37

**Calculated using the correlation between TCOD and SS, see Appendix V.*

4.3 Pretreatment of algal biomass

The results from measurement of TCOD, SCOD and NH_4^+-N before and after pretreatment are shown in Table 4.9. To get an estimation of the solubilization of COD, the soluble fraction was related to the total amount of COD in each case. As can be seen, the soluble fraction of COD increased with treatment temperature, which was also the case for ammonium release.

Table 4.9. Results from measurement of TCOD, SCOD and NH_4^+-N before and after pretreatment. Increase in SCOD is calculated according to equation 3.3.

	Untreated	Pasteurized	T=120°C, 30 min	T=170°C, 30 min
TCOD (mg/L)	6 112 ± 62	6 789 ± 23	6 307 ± 130	6 432 ± 80
SCOD (mg/L)	40.8	388	446	902
SCOD/TCOD (%)	0.67	5.71	7.07	14.0
Increase in SCOD (%)	-	856	1 060	2 100
NH_4^+-N (mg/L)	0.026	3.2	3.5	12.8

The increase in SCOD compared to untreated algae was quite high but the concentration of SCOD is still low compared to what was found by Cho *et al.* (2013) and Keymer *et al.* (2013). Cho *et al.* (2013) were able to increase the SCOD to 33% of TCOD when treating a mix of *Chlorella* and *Scenedesmus* at 120°C for 30 minutes. Their result indicates that *Chlorella* biomass could be more susceptible to thermal treatment than *Scenedesmus* biomass. Keymer *et al.* (2013) were able to increase the SCOD to 55% of TCOD when treating *Scenedesmus* enriched biomass using HPTH at 170°C and 8 bars for 30 minutes. It should be noted that the starting concentration of SCOD in both these studies was much higher than it was in the present study, which probably affected the outcome. Further, for pasteurized algae the SCOD concentration (as % of TCOD) after pretreatment is comparable to results found by González-Fernández *et al.* (2012) who achieved a final SCOD concentration of 5.4% of TCOD when treating algal biomass (dominated by *Scenedesmus* sp.) at 70°C for 60 minutes. Nevertheless, increasing the treatment temperature seems to have a positive effect on solubilization of both COD and NH_4^+-N.

Although all the pretreatment methods led to increase in SCOD this cannot be used as certain indication of increased biogas production. In the work by González-Fernández *et al.* (2012), it was found that increase in SCOD did not directly imply increased biogas production. In fact, they found that the amount of ammonium and COD that was released at 90°C was only slightly higher than at 70°C whereas there was a much higher increase in biogas yield at 90°C then at 70°C when compared to untreated algae. The reason for the higher biogas yield was attributed to disintegration of the cell wall to a greater extent at the higher temperature (González-Fernández *et al.*, 2012). Thus, digestion experiments are needed to conclude if the pretreatment methods were successful.

During the pretreatments, TCOD concentration increased (see Table 4.9) but no reason for this could be found. One reason could have been evaporation of water during pretreatment but the volume difference before and after pretreatment was only a few mL. Another reason could have been the uncertainty in the HACH method for determination of COD, this is however unlikely since all samples were measured in triplicates and the variation within the triplicates was smaller than between samples.

Energy requirement

The energy requirement for each pretreatment method was calculated using equation 3.4 (T_d=37°C and T_i=25°C), and the results are presented in Table 4.10. Since the energy requirement per day is dependent on the flow, the results are presented as MJ/m^3. As will be discussed later, the higher the concentration of algae the less energy is required per kg of dry mass of algae.

Table 4.10. Energy requirement for each pretreatment method, calculated according to equation 3.4 assuming T_i=25°C and T_d=37°C.

Method	**Energy requirement (MJ/m^3)**
Pasteurization	70.5
120°C, 30 min	101
170°C, 30 min	132

4.4 Biomethane potential

4.4.1 Theoretical biogas potential

Results from the composition analysis and theoretical methane potential calculation based on the composition are shown in Table 4.11. Both the theoretical methane yield and the methane content is almost the same for both algae and primary sludge. Since the analysis was only made once for each substrate, the results are quite uncertain. The composition of algae varies a lot with cultivation conditions and because of the heterogeneity of primary sludge, more samples should be taken in order to give reliable results. Microalgae are known to be able to accumulate high amounts of lipids, but this occurs when algae are grown during nutrient limitation, especially nitrogen limitation (Wiley *et al.*, 2011). Since one of the aims of using algae in this project is to reduce the nutrients in wastewater, lipid accumulation will probably not occur, at least not as a consequence of nitrogen limitation. The high protein content in the algal suspension (53% of SS) is in accordance with what is reported by Olsson *et al.* (2014) who claim that *Scenedesmus* often contains 50-60% protein.

Table 4.11. Composition of algal suspension (1st batch) and primary sludge (from 2nd of February) and the corresponding theoretical methane yield and methane content.

Algal suspension		**Primary sludge**	
SS (% of total)	0.028	TS (% of total)	0.90
VSS (% of SS)	79	VS (% of TS)	74
Lipids (% of VSS)	5	Lipids (% of VS)	8
Carbohydrates (% of VSS)	28	Carbohydrates (% of VS)	50
Proteins (% of VSS)	67	Proteins (% of VS)	42
Nm^3 methane/kgVSS	0.50	**Nm^3 methane/kgVS**	0.50
Methane content (%)	51	**Methane content (%)**	52

4.4.2 BMP test 1

Figure 4.5 shows the accumulated methane production from the different substrates and mixtures in BMP test 1. Results are presented as average of triplicate bottles with standard deviation. Methane production from the blank is subtracted. The inoculum used was assessed to be of good quality since the methane production from the control bottles reached the theoretical value of 350 NmL/gCOD within five days.

From Figure 4.5 it can be seen that methane production from algae did not increase very much after 15 days and was much lower than the methane yield from primary sludge. The methane yield increased with increasing portion of primary sludge, compared to pure algae.

After day 29, there was an unexpected increase in methane yield from all bottles containing primary sludge. This behavior could be described by the fact that the primary sludge contained a lot of particles which took some time to hydrolyze. It was noted that the standard deviation between triplicates were highest for bottles containing primary sludge which can be explained by the heterogeneity of this substrate. In order to achieve smaller variations between bottles, it would be desirable to use larger sample volumes. In this case it was not possible to increase the amount of substrate used since there was limitation in the amount of available algae. Another option was to homogenize the primary sludge before the BMP test but this would probably affect the outcome since homogenization is one treatment method that can be used for increasing the biogas yield. What also can be seen is that the standard

deviation is higher towards the end of the experimental period which can be explained by the presence of particles. If the distribution of particles between the bottles is a bit uneven this will be more pronounced when the hydrolysis of these particles has taken place. The highest standard deviation between triplicates was 6.6% (mixture 3, day 40) which is in the acceptable region according to Carlsson and Schnürer (2011).

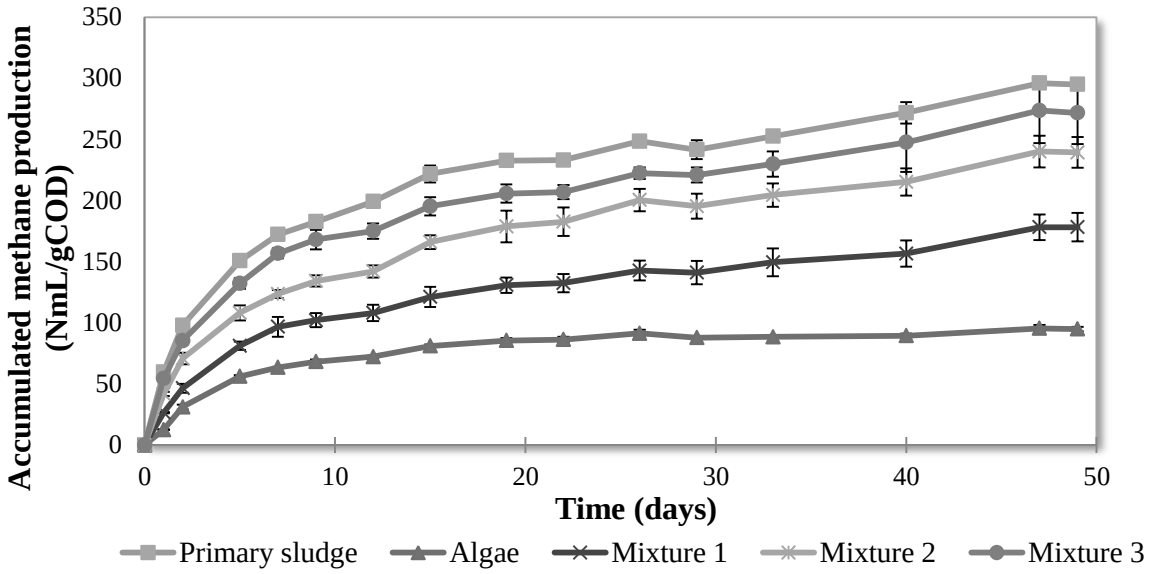

Figure 4.5. Accumulated methane production from the different substrates and mixtures in BMP test 1. Results are presented as average of triplicate bottles with standard deviation. Methane production from the blank is subtracted.

In Table 4.12, the final methane yield and content in the gas is shown (day 49). Also, the yield is shown on VS basis using the ratio of 1.77 gTCOD/gVS for primary sludge (measured for primary sludge from 2nd of February) and 1.62 gTCOD/gVSS for algae (measured for concentrated algae, 1st batch). The yield of methane from algae was quite low, but still comparable to both González-Fernández *et al.* (2012) (76 NmL/gCOD) and Mussgnug *et al.* (2010) (178 NmL/gVS). Even though the BMP test should give the maximum theoretical yield different experimental conditions can affect the results. Also, conditions during algal growth will affect the composition, hence also the methane yield. The methane yield from primary sludge is higher than reported for mixed primary and secondary sludge (Olsson *et al.*, 2014; Carpongo *et al.*, 2015) but since primary sludge is considered to be more easily degradable than secondary sludge (Parkin & Owen, 1986) the result is reasonable. As for algae, the composition of the sludge will affect the yield and the composition will ultimately depend on its origin (Metcalf & Eddy, 1991).

Compared to the theoretical value of 350 NmL CH_4/gCOD, 84% was achieved for primary sludge whereas only 27% was achieved for algae. This strongly suggests that primary sludge is more easily degradable than algae. The theoretical methane yield based on composition was 500 NmL/gVSS for algae and 500 NmL/gVS for primary sludge (see Table 4.11). Compared to this, a slightly higher yield (105%) was achieved for primary sludge, and the methane content in the gas was higher than predicted. This could be explained by that the method used for characterization only gives a rough estimation of the methane yield. For algae, only 31% of the theoretical yield was achieved but the methane content in the gas was higher than

predicted. Compared to primary sludge, the microalgae used in this experiment was substantially more difficult to degrade.

Table 4.12. Final methane yield and content in the gas (day 49) on both COD basis and VS basis. For calculation of yield on VS basis the ratio of 1.77 gTCOD/gVS for primary sludge and 1.62 gTCOD/gVSS for algae were used.

	Methane yield (NmL/gCOD)	**Methane yield (NmL/gVS*)**	**Methane content (%)**
Primary sludge	295	523	65
Algae	94.8	154	68
Mixture 1	178	297	66
Mixture 2	239	410	66
Mixture 3	272	476	66

**VSS was used for algae.*

During BMP tests, it is desirable to reduce the methane production from the inoculum, especially when production from the substrate is low (Carlsson & Schnürer, 2011). For pure algae, the contribution from the inoculum was quite high (58% of the total methane production). It would thus be desirable to incubate the inoculum for a few more days to reduce this contribution. However, since the variation between bottles was low the result is regarded as reliable.

C/N-ratio

The C/N-ratio was determined as the amount of organic carbon divided by the amount of organic nitrogen in the substrates. Organic carbon was determined as TOC and organic nitrogen as TKN. In the TKN analysis, also ammonium present in the sample from the beginning is measured and by subtracting the amount of ammonium in the sample, the organic nitrogen content was determined. As shown in Table 4.13 the ratio is higher for primary sludge than for algae. The C/N-ratio for algae is a bit lower than in the work by Yen and Brune (2007) who used a mix of *Chlorella* and *Scenedesmus* which had a C/N-ratio of 5.3. The C/N-ratio for primary sludge is in the reported region of 6-16 (Yen & Brune, 2007). By mixing the available substrates it will not be possible to increase the C/N-ratio to the optimal between 20 and 30 (Montingelly *et al.*, 2015). Although both substrates had a low C/N-ratio, no signs of ammonia inhibition could be observed. However, this must be evaluated in continuous mode since it can be difficult to discover inhibition problems in BMP tests.

Table 4.13. Measured values for TKN, NH_4^+-N and TOC for the 1st batch of algal suspension and primary sludge (from 2nd of February) and the calculated C/N-ratio for both.

	Algal suspension	**Primary sludge**
TKN (mg/L)	25	480
NH_4^+-N (mg/L)	1.52	30.2
N-org (mg/L)	23.5	450
C-org as TOC (mg/L)	108.5 ± 8.6*	4 555 ± 205**
C/N-ratio	4.6	10

**Based on triplicates.*
***Based on duplicates.*

Co-digestion

To detect if pronounced synergistic effects were present for the mixtures in BMP test 1, equation 2.1 was used. The results, together with the experimental values are shown in Figure 4.6. If a synergistic effect was present, the experimental values (darker lines) should be higher than the calculated ones (brighter lines). For the first 25 days, the values correspond very well with each other; hence no effect can be seen. After 25 days there is a slight difference between the experimental values and the calculated ones. However, due to the variations within triplicates no synergistic effect could be concluded. Synergistic effects are difficult to evaluate just by performing BMP tests. Thus it would be desirable to evaluate possible effects in continuous mode especially since synergistic effects have been found by others when primary sludge and algae were co-digested in continuous mode (Samson & LeDuy, 1983).

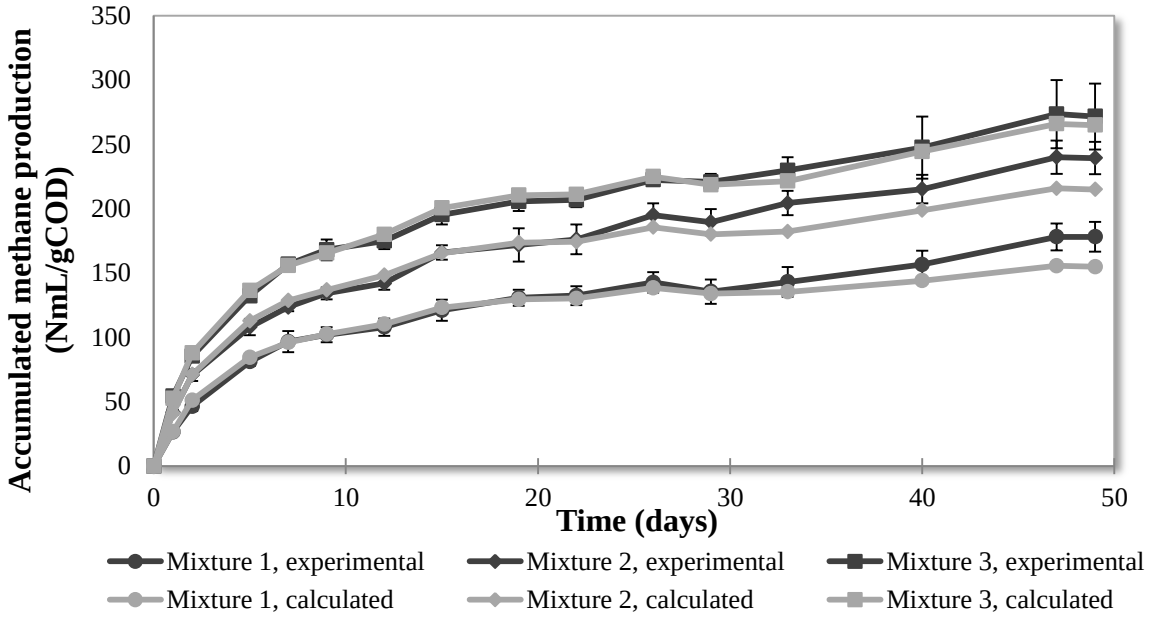

Figure 4.6. Evaluation of synergistic effects in BMP test 1. Brighter lines represent calculated yield according to equation 2.1 and darker lines represents experimental values.

Heavy metal content

The digestate resulting after anaerobic digestion might be used as fertilizer. According to the Swedish regulation 1998:944, it is not allowed to use it in agriculture if the concentration of some specified metals exceeds a certain level (Svensk författningssamling, 1998). To evaluate the suitability of using digestate resulting from digestion of algae and sludge the 1st batch of algal suspension and primary sludge collected on the 2nd of February was analyzed for concentration of the specified metals. These are: lead, cadmium, copper, chromium, zinc, nickel and mercury. The regulation defines the limits as "mg/kg dry solids" and the result from the analysis was given as "mg/L" (see Appendix VI). Using the result from SS analysis of algal suspension and TS analysis of primary sludge the concentrations were re-calculated. Further, it must be taken into consideration that during the digestion process, the concentration of metals will increase due to a decrease in VS content. Assuming a starting VS content of 70%, a VS reduction of 50% and that all of the metals remain in the sludge during concentration, the resulting concentration of metals can be determined. The results, together with the limits are shown in Table 4.14.

Table 4.14. Calculated concentrations of heavy metals in the 1st batch of algal suspension (based on SS) and primary sludge, collected on the 2nd of February before and after an anaerobic digester with a VS reduction of 50% and a starting VS content of 70%. The regulated limit of each metal is also shown (Svensk författningssamling, 1998).

Heavy metal (mg/kg TS)	**Algae***		**Primary sludge**		**Limits**
	Before	After	Before	After	
Lead	<1.8	2.9	12	19	100
Cadmium	<0.36	0.59	0.47	0.74	2
Copper	34	56	170	260	600
Chromium	<3.6	5.9	11	18	100
Zinc	93	150	350	560	800
Nickel	6.8	11	6.9	11	50
Mercury	0.36	0.59	0.23	0.37	2.5

**Based on SS instead of TS.*

None of the metals investigated are over the regulated limits which indicates that the metal content will not prevent the use of the digestate in agriculture, regardless of the ratio between algae and primary sludge. However, as only one measurement was done, variations cannot be discovered and regular measurements should be conducted if the digestate is going to be used.

4.4.3 BMP test 2

Figure 4.7 shows the accumulated methane production in BMP test 2. Results are presented as average of triplicate bottles with standard deviation. Methane production from the blank is subtracted. The inoculum used was assessed to be of good quality since the methane production from the control bottles reached 99% of the theoretical value of 350 NmL/gCOD within five days.

The degradation rate can be evaluated by comparing when for example 80% of the final yield is achieved. For untreated algae and algae treated at 120°C, 80% of the final yield was achieved after 12 days whereas for algae treated at 170°C this was achieved after nine days. Hence, the degradation was somewhat faster for algae treated at 170°C but it is not possible to determine the needed retention time in a continuous process just based on these results.

Notable is the shape of the curve for algae pretreated at 120°C. In the beginning the yield was lower than for untreated algae but after 12 days, the yield got higher. This could be explained by possible presence of inhibitory substances released during pretreatment, leading to slower methane production.

The most unexpected result was that the methane yield from pasteurized algae was approximately half of the yield from untreated algae. González-Fernández *et al.* (2012) used the same pretreatment method and found that the methane yield, compared to untreated algae was 12% higher. Based on their findings, it was not expected that the yield would increase substantially by pasteurization but at least the same yield as untreated algae should have been achieved. Attempts were made trying to sort out what could have been the reason for this unexpected result, but nothing could be found. Before concluding that pasteurization is unsuitable, it is recommended that the experiment is performed again.

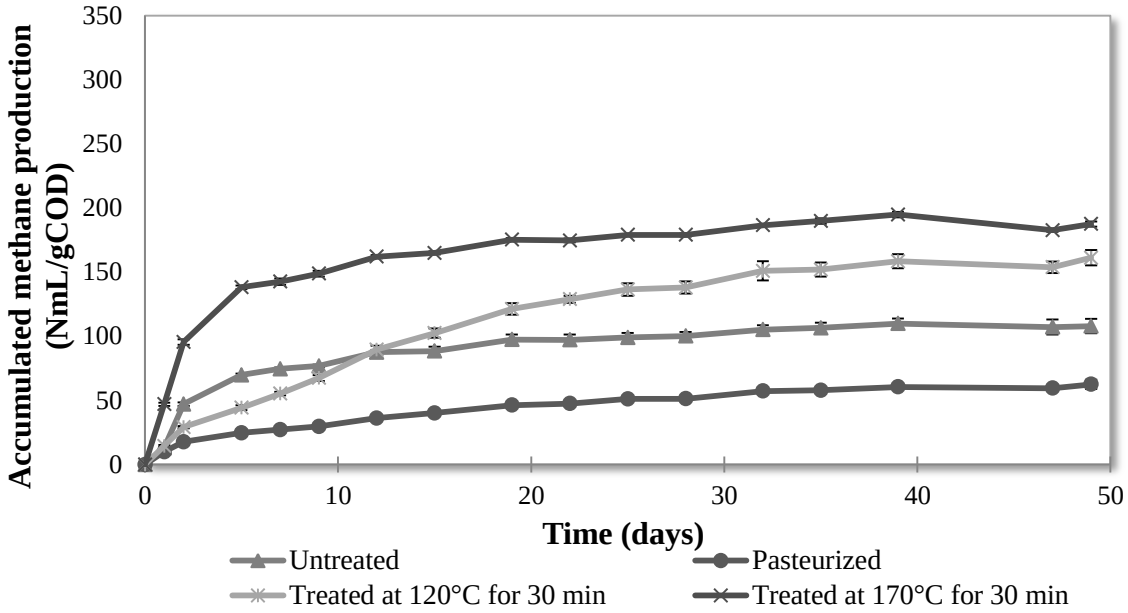

Figure 4.7. Accumulated methane production in BMP test 2. Results are presented as average of triplicate bottles with standard deviation. Methane production from the blank is subtracted.

In Table 4.15, the final methane content in the gas is shown together with the yield on COD basis and on VSS basis using the ratio of 1.57 gTCOD/gVSS for algae (measured for concentrated algae, mix of 2nd and 3rd batch). The yield is presented as average of the last three measurements. Further, the increase in methane yield for pretreated algae compared to untreated algae is shown.

The final yield for untreated algae is slightly higher than in BMP test 1. Since the algae used are from different batches there might be a difference in composition. Also, since the difference in yield is small it could be explained by the uncertainty in the BMP test itself.

Table 4.15. Final methane yield and content in the gas on both COD basis and VSS basis. The yield is presented as average of the last three measurements. For calculation of yield on VSS basis the ratio of 1.57 gTCOD/gVSS for algae was used. The increase in methane yield for pretreated algae compared to untreated algae is also shown.

	Methane yield (NmL/gCOD)	**Methane yield (NmL/gVSS)**	**Increase (%)**	**Methane content (%)**
Untreated algae	108	170	-	69
Pasteurization	60.8	95.7	- 44	70
120°C, 30 min	158	248	46	70
170°C, 30 min	188	296	74	70

Due to time limitation it was not possible to measure TCOD in the pretreated algae before the 2nd BMP test was started. Instead, it was assumed that the TCOD content did not change during the pretreatment and the same amount of algae (based on mass) was added in each bottle. When the experiment had been started, the pretreated algae were analyzed to confirm the assumption. As discussed in chapter 4.3, the TCOD had increased during pretreatment,

which means that the loading ($gCOD/gVS_{inoculum}$) in the bottles with pretreated algae were a bit higher than in the others. The difference was however assumed to not affect the experiment significantly. When relating the produced methane to amount of added substrate the difference is accounted for.

Evaluation of pretreatment

During BMP test 2, the methane content in the gas did not seem to be affected by the pretreatment (Table 4.15). Pretreatment at 120°C caused an increase in methane yield of 46% compared to untreated algae. Cho *et al.* (2013) found a 20% increase in methane yield for treatment at 120°C. The difference could be explained by that their yield for untreated algae was much higher; hence there was little room for improvement. Also, they used algal biomass which consisted of mostly *Chlorella* and less *Scenedesmus* which could have affected the outcome. Pretreatment at 170°C caused an increase in methane yield of 74% which is comparable to what was achieved by Keymer *et al.* (2013) who used HPTH at 170°C and 8 bars. Their treatment increased the methane yield with 81% compared to untreated algae. The increase in yield is a bit higher but this could be explained by more extensive cell disruption due to the flash step included in HPTH. Using a microwave in full scale would probably not be feasible, however, commercial methods for thermal pretreatment of sludge is available. Further investigation of high temperature treatments of algae using these methods would be interesting both from an economical and an energy perspective.

González-Fernández *et al.* (2012) suggest that there is a threshold temperature where cell breakage occurs. This is dependent on how strong the bonds in the cell structure are, hence the threshold temperature would depend on algal species. Based on this, it might be possible to increase the methane yield substantially by treating algae at a temperature just above its threshold. In this way, it could be possible to increase the methane yield without unnecessary energy input.

In order to evaluate if the increase in methane yield would cover the energy input for pretreatment it is necessary to do this with regard to the concentration of the algal suspension that is pretreated. To do this, the gain in energy released as methane for biomass pretreated at 120°C and 170°C was calculated assuming 9.97 kWh/Nm^3 CH_4 (Statens energimyndighet, 2014), and are shown as the dotted lines in Figure 4.8. It is assumed that the energy released is independent of SS concentration. Further, the energy needed for pretreatment (per kg VSS) was calculated using equation 3.4 (T_d=37°C and T_i=25°C) and the assumption that VSS is 73% of SS. As can be seen in Figure 4.8, the energy needed for pretreatment decreases as the SS concentration increases. The crosses in Figure 4.8 represents where the energy input needed for pretreatment is covered by the extra energy released as methane, as a result of pretreatment. It can be concluded that at the higher pretreatment temperature, 170°C, the SS concentration does not need to be as high as for the lower temperature, in order for the gain in methane yield to cover the energy required for pretreatment. It should be noted that this is only a rough estimation since a lot of other aspects will affect the energy balance, such as for example the method used for heating and how the energy in the produced gas is recovered.

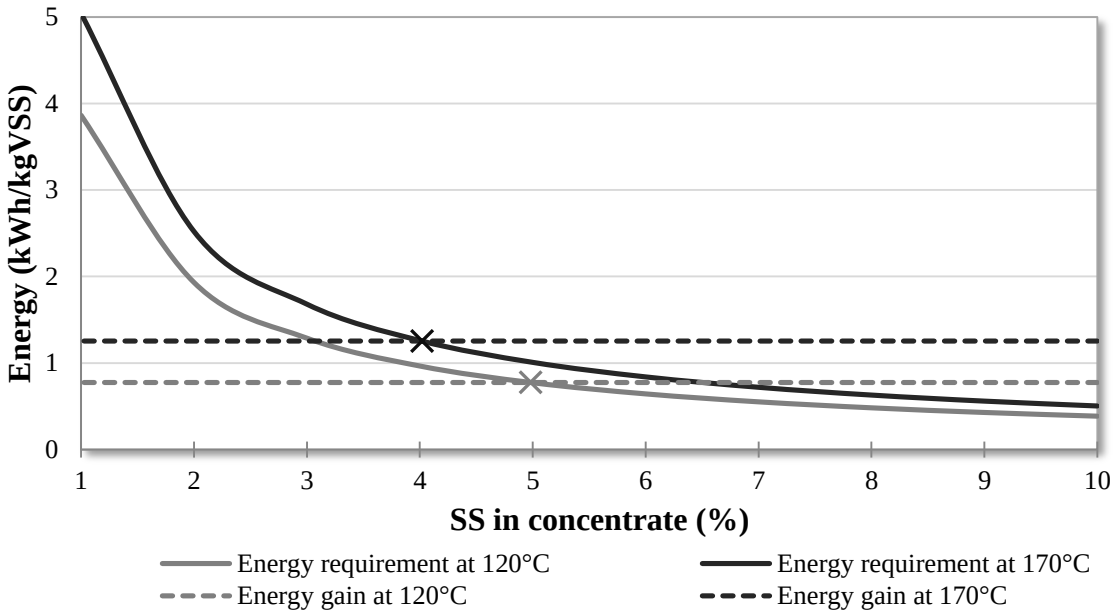

Figure 4.8. The energy needed for pretreatment of algal biomass as a function of SS concentration. Dotted lines represent the increased methane yield achieved owing to the pretreatment, assuming that 100% of BMP can be achieved. Crosses represents at which SS concentration the energy requirement is balanced with the increase in methane yield.

In this project, anaerobic digestion in the thermophilic range was not investigated. Thermophilic processes are considered to be faster which means that a shorter retention time is needed and thus a smaller reactor. However, digestion at higher temperatures would require higher heating demand which only can be justified if the methane yield is increased sufficiently. Thermophilic digestion of algae have shown lower methane yield than mesophilic digestion (Olsson *et al.*, 2014; Caporgno *et al.*, 2015). This could be due to the high protein content of algae and that thermophilic processes are more sensitive to ammonium. Consequently, digestion of algae at thermophilic temperatures does not seem feasible.

4.5 Full scale

To get an idea about how the energy recovery from a treatment plant with AnMBBR and algal step would look like, a material balance was made. In Figure 4.9, the proposed plant is shown, notations are used as subscripts in tables and stands for concentrated algae (CA), primary sludge (PS), digestate (D) and reject (R).

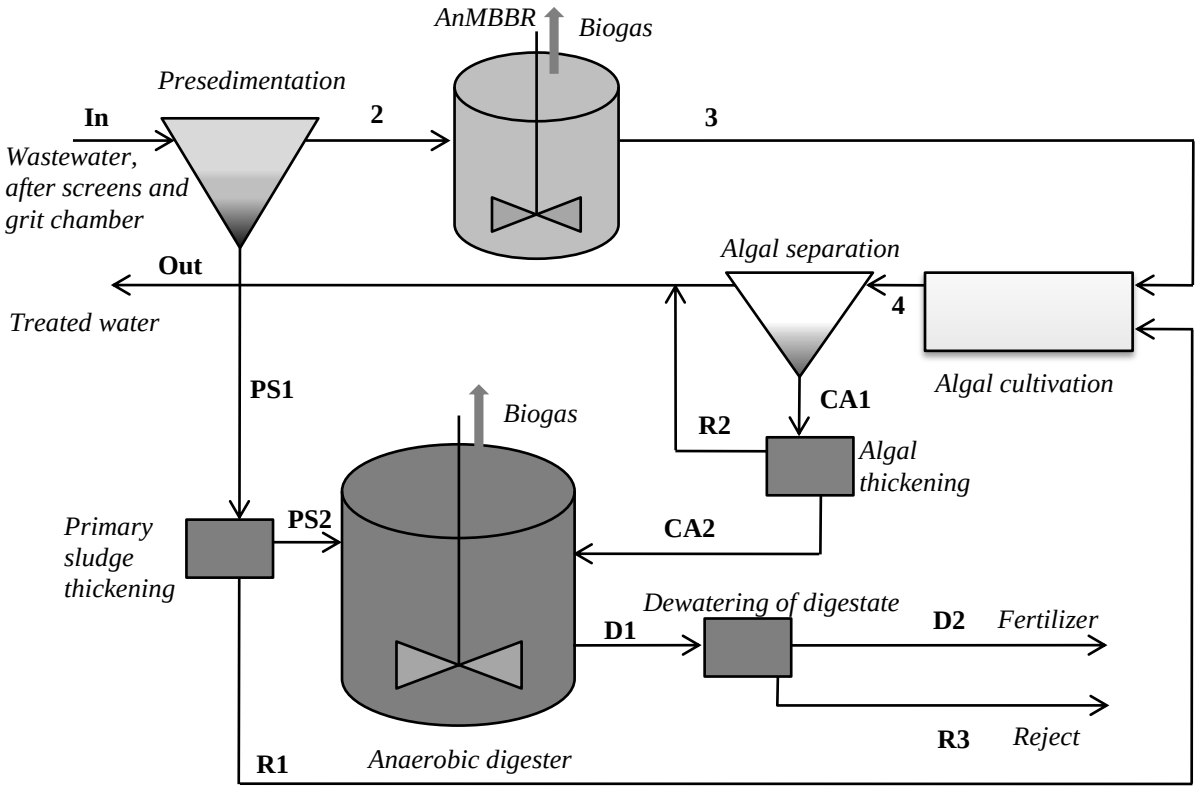

Figure 4.9. Outline of proposed treatment plant, notations are used as subscripts in tables and stands for concentrated algae (CA), primary sludge (PS), digestate (D) and reject (R).

For comparison Källby WWTP is used, since the water used in the pilot plant in this project is taken from the grit chamber at Källby WWTP. During 2013 Källby WWTP had an average flow of 27 500 m^3/day. At this plant, the water is first treated mechanically (screens, grit chamber and presedimentation), after this the activated sludge process is used together with predenitrification and finally chemical precipitation is used for phosphorus removal. Sludge is anaerobically digested for production of biogas. During 2013 the total production of biogas was 777 162 Nm^3 (VA SYD, 2014). If a methane content of 64% is assumed, the corresponding yearly production of methane was 497 384 Nm^3.

The material balance is based on a combination of some measuring values, average values from Källby WWTP, results from experiments done in laboratory scale and some assumptions. Presented in Table 4.16 are the values that the material balance is based on together with where the values are derived from.

Table 4.16. Values that the material balance is based on together with where the values are derived from. Q_i is volume flow, SS_i is concentration of suspended solids, $TCOD_i$ is concentration of total COD, $N\text{-}tot_i$ is concentration of total nitrogen and $P\text{-}tot_i$ is concentration of total phosphorus, subscripts correspond to notations in Figure 4.9.

Notation	Value	Source
$\mathbf{Q_{in}}$	27 500 m^3/day	Average for Källby WWTP 2013
$\mathbf{SS_{in}}$	186 g/m^3	Average from Källby WWTP (2013-07-30 to 2014-07-22)
$\mathbf{SS_{PS1}}$	9 000 g/m^3	Measurement from 2 Feb*
$\mathbf{SS_3}$	49.2 g/m^3	Average from four measurements**
$\mathbf{SS_4}$	280 g/m^3	Average from batch 1, 2 and 4
$\mathbf{SS_{out}}$	36 g/m^3	Result from flocculation experiment 13
$\mathbf{SS_{PS2}}$	70 000 g/m^3	Assumed
$\mathbf{SS_{CA2}}$	40 000 g/m^3	Assumed
$\mathbf{TCOD_{in}}$	403 g/m^3	Average from Källby WWTP (2013-07-30 to 2014-07-22)
$\mathbf{TCOD_{PS}}$	11 800 g/m^3	Measurement from 2 Feb
$\mathbf{TCOD_{out}}$	65 g/m^3	Result from flocculation experiment 13
$\mathbf{N\text{-}tot_{in}}$	44 g/m^3	Average for Källby WWTP 2013
$\mathbf{N\text{-}tot_3}$	38 g/m^3	Average from four measurements**
$\mathbf{N\text{-}tot_{out}}$	7.5 g/m^3	Result from flocculation experiment 13
$\mathbf{P\text{-}tot_{in}}$	6.1 g/m^3	Average for Källby WWTP 2013
$\mathbf{P\text{-}tot_{out}}$	0.26 g/m^3	Result from flocculation experiment 13

**Measurement was done for TS, it is assumed that SS=TS in this case.*

*** Measurements performed 2015-01-26, 2015-02-02, 2015-02-09 and 2015-02-23.*

It is assumed that the profit for biogas is 6 SEK/Nm^3 CH_4, the cost for PIX 111 is 1440 SEK/ton and the price for Zetag® Cationic Polymer - Solid grade flocculant is 35 SEK/kg (Olsson, 2015).

The methane production from the AnMBBR is 100 NmL CH_4/g$SCOD_2$ and 45% of TCOD in the inflow to the AnMBBR is assumed to be SCOD. Of the produced methane, between 70-80% is found in the water (at 25°C) which means that it is necessary to include a step for methane removal (Uldal, 2015). The methane production in the anaerobic digester is based on the experimental BMP but it is assumed that it is not possible to achieve 100% of BMP, instead 85% is used. For primary sludge the yield after 30 days is used instead of after 49 days based on the assumption that the gain in yield during the last days of BMP test 1 will not have time to happen because of the shorter HRT in the continuous digester. In Table 4.17, the values are summarized.

Table 4.17. Summary of values used for methane yield and ratio between SCOD and TCOD and between VSS and SS.

	Methane yield (NmL/gSCOD)	**SCOD/TCOD (%)**
AnMBBR	100	45
	Methane yield (NmL/gVSS)	**VSS/SS (%)**
Primary sludge	369	74*
Untreated algae	140	73**
Algae treated at 120°C	211	73**
Algae treated at 170°C	251	73**

**Assuming that VSS/SS is equal to VS/TS.*
***Average from three measurements (concentrated batch 1, 2 and 3).*

Using material balances over each step (described in Appendix VII), all flows and some concentrations were calculated. Further, energy recovery and energy demand for pretreatment was also derived. The results that are of interest for further discussion is presented in the following sections and all results from the material balance are presented in Appendix VII. If not stated, values for algae correspond to untreated algae, produced during the whole year (12 months).

AnMBBR

In Table 4.18, the results needed for calculation of energy recovery from the AnMBBR are shown. The methane produced corresponds to 23% of the total methane production at Källby WWTP. The activated sludge process requires aeration which can be very expensive, also a lot of biomass is produced. At Källby WWTP, the WAS is anaerobically digested to produce biogas. If an anaerobic step would replace the activated sludge process no energy for aeration will be needed. However, since less of the energy in the wastewater is converted to heat in anaerobic systems, heating of the reactor might be needed. This could probably be solved by using low-valuable heat from surrounding industries. Further, by replacing the aerobic step, less sludge will be produced and thus loss in energy recovery from the sludge treatment. On the other hand, by treating the water anaerobically, potentially more of the stored energy can be converted to methane since very little of the energy is converted to heat (5% in anaerobic conversion compared to 40% in aerobic conversion).

Table 4.18. Values describing the flow into the AnMBBR, yearly production of methane and corresponding energy content in the produced gas.

Q_2 (m^3/day)	**$TCOD_2$ (g/m^3)**	**$SCOD_2$ (g/m^3)**	**Methane (Nm^3/year)**	**Energy (GWh/year)**
27 159	260	117	116 000	1.16

Anaerobic digester

In Table 4.19 the results needed for calculation of energy recovery from the anaerobic digester are shown. The distribution between primary sludge and algae is 32:68 on VSS basis. Although the quantity of algae is a lot bigger than primary sludge, the yearly production of methane from algae is lower. In total, the yearly production of methane from the anaerobic digester is 11% higher than the methane production at Källby WWTP 2013.

Table 4.19. Values describing the flow of primary sludge and concentrated algae into the anaerobic digester, yearly production of methane and corresponding energy content in the produced gas.

	Q (m^3/day)	**SS (g/m^3)**	**VSS (g/m^3)**	**VSS (%)**	**Methane (Nm^3/year)**	**Energy (GWh/year)**
PS2	43.8	70 000	51 800	32	306 000	3.05
CA2	167	40 000	29 200	68	248 000	2.48

For the whole treatment line, including AnMBBR and anaerobic digester (both primary sludge and algae), 670 000 Nm^3 of methane is recovered according to the calculations. This is 35% higher then what was produced at Källby WWTP in 2013.

Algal separation

In Table 4.20 the flow into the separation step and the yearly cost for addition of flocculation chemicals is shown (cost for pH adjustment is not included). To justify the use of algae, sufficient nutrient reduction is essential. Further, the separation of algae from the wastewater cannot be too costly. For comparison, the chemical phosphorus removal at Källby WWTP will be used. In 2013, 712 ton of "Plusjärn S314" from Feralco (which is comparable to PIX 111 in density and iron content (Feralco, 2010)) was added for phosphorus removal (VA SYD, 2014). This corresponds to approximately 14 mgFe^{3+}/L of wastewater. In the separation of produced algae, only 5 mgFe^{3+}/L of algal suspension would be needed, but in combination with polymer. Further, consideration regarding nitrogen reduction must be included since both phosphorus and nitrogen will be removed simultaneously if algae are used. Also, if the pH is regulated, limited amounts of nitrogen will be lost and a more complete recycle of nutrients can be achieved. Consequently, even though the cost for flocculants is notable, it should be compared with the cost for equivalent nitrogen- and phosphorus removal as well as the increase in nutrient recovery.

Another aspect is to evaluate if the produced methane from algae alone can cover the cost of flocculants. The profit from selling the biogas produced from algae alone would be approximately 1.49 MSEK/year. Thus, cost for flocculation chemicals (1.21 MSEK/year) could be covered.

Table 4.20. Flow into the separation step, addition of flocculation chemicals and the yearly cost for chemicals (cost for pH adjustment is not included).

Q_4 (m^3/day)	**PIX 111 (L/m^3)**	**Polymer, 0.2% (L/m^3)**	**Cost (MSEK/year)**
27 456	0.025	1.0	1.21

Flotation

If flotation would be used as separation step, it might be possible to achieve sufficient solids concentration in Q_{CA1} without a second concentration step. This would mean that Q_{R2}=0 and SS_{CA1}=SS_{CA2} and the algal thickening step can be removed. If flotation is used it is also probably possible to recover more algal biomass. By assuming that it is possible to achieve SS_{CA1}=SS_{CA2}=40 000 g/m^3, and 95% recovery of algae using flotation the results in Table 4.21 are obtained. Using this approach, the total methane yield would be 39% higher than at Källby WWTP.

Table 4.21. Values describing the flow of concentrated algae into the anaerobic digester, yearly production of methane and corresponding energy content in the produced gas assuming that 95% of the algae can be recovered using flotation.

Q_{CA2} (m^3/day)	$TCOD_{CA2}$ (g/m^3)	$SCOD_{CA2}$ (g/m^3)	Methane (Nm^3/year)	Energy (GWh/year)
182	40 000	29 200	271 000	2.70

Several aspects must be taken into consideration when deciding the suitable separation method. Wiley *et al.* (2011) reports that it is possible to achieve solids content between 3-5% by using dissolved air flotation (DAF) which is a quite energy consuming process. There are other flotation systems reported, such as suspended air flotation (SAF) which requires less energy and is able to achieve the same solids content. However, in SAF, additional chemicals are needed which will affect the cost of the whole plant. Even though the energy recovery from algae only is increased with 9% it should be noted that if the harvesting efficiency increases (as was assumed) the quality of the outgoing water will be improved. It would also be possible to remove the thickening step for algae if sufficient solids content can be achieved. As discussed in chapter 4.2.2, it might also be possible to reduce the amount of added chemicals, especially if flotation is used. In conclusion, a more thorough energy and cost analysis is needed to conclude the best option for separation.

Pretreatment

Pretreatment of algal biomass would be applied right before the anaerobic digester, to CA2 in Figure 4.9. In Table 4.22, the yearly methane yield from untreated and pretreated algae is shown, together with the corresponding energy and the energy increase caused by pretreatment. In this evaluation, the recovery of algae was set to 87% as in the case without flotation. Compared to Källby WWTP the total yearly methane yield would thus be 60% higher at 120°C or 75% higher at 170°C.

Table 4.22. The yearly methane yield from untreated and pretreated algae is shown, together with the corresponding energy and the energy increase caused by pretreatment. Recovery in algal separation is set to 87%.

	Methane (Nm^3/year)	Energy (GWh/year)	Energy (kWh/kgVSS)	Energy increase (kWh/kgVSS)
Untreated	248 000	2.48	1.39	-
120°C, 30 min	375 000	3.74	2.10	0.710
170°C, 30 min	446 000	4.45	2.50	1.11

As discussed in chapter 4.4.3, the energy needed for pretreatment depends on the solids concentration. Taken into consideration that it is not possible to achieve 100% of BMP in a continuous reactor the needed solids concentration in the algal slurry that is going to be pretreated needs to be higher, compared to what was found in chapter 4.4.3, to account for the energy input for pretreatment. From Figure 4.10 it can be seen that the solids concentration needs to be higher than 5% if pretreatment temperature is set to 120°C. According to Wiley *et al.* (2011) it is only possible to achieve up to 5% solids concentration using DAF, thus an additional concentration step would be needed if treatment at 120°C is applied, even if flotation is used. Treatment at 170°C can be energetically beneficial if the solids concentration is above 4.6%. Even though this reasoning indicates that it would be possible to

increase the net energy recovery by applying pretreatment it is important to evaluate how much energy that actually is needed for pretreatment and the cost for investment and maintenance of pretreatment apparatus. Regard should also be taken for heating of the digester. In the energy calculations recovery of energy during cooling of biomass to digestion temperature is accounted for but if no pretreatment method is used additional energy is needed for heating of the digester. Further, pretreatment could increase the amount of ammonium and phosphate available in the digestate which would increase its value. Additionally, it is crucial to evaluate if the pretreatment leads to instability in the digestion process due to increased concentration of ammonium.

If the regulations regarding sludge distribution is changed and hygienization becomes a requirement, the sludge could also be pretreated. If one of the proposed methods is used, the demand for hygienization would be met and the yield of methane from sludge could also be increased.

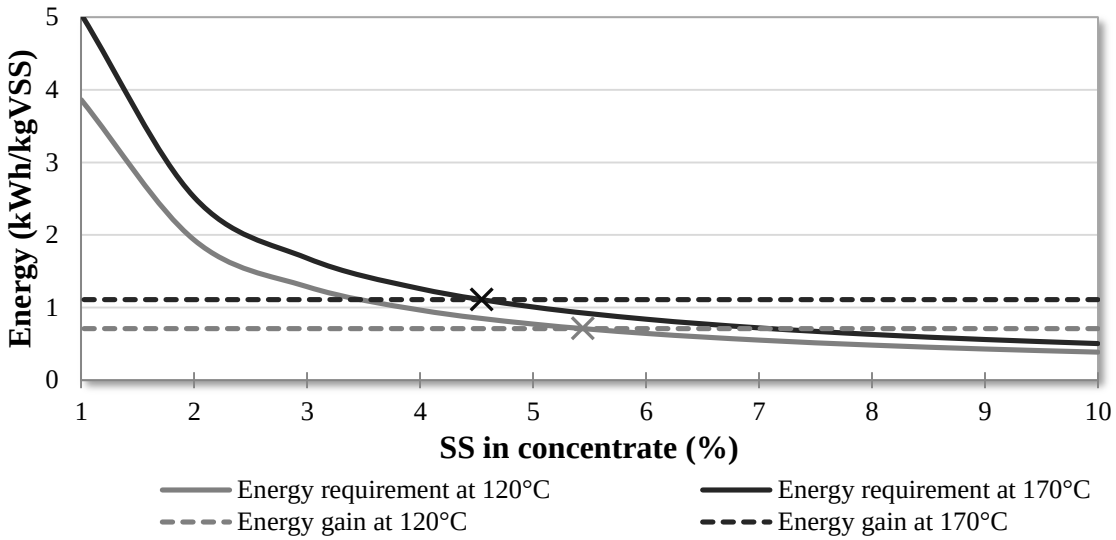

Figure 4.10. The energy needed for pretreatment of algal biomass as a function of SS concentration. Dotted lines represent the increased methane yield achieved owing to the pretreatment, assuming that 85% of BMP can be achieved. Crosses represents at which SS concentration the energy requirement is balanced with the increase in methane yield.

Nutrients

Algae reduce nutrients in water mainly by assimilation during growth (Larsdotter, 2006). Thus the amount of nutrients recovered will depend on the effectiveness of the algal cultivation and the algal separation. Since a lot of nitrogen was lost during algal cultivation it is not possible to evaluate where the nitrogen would end up in reality. However, if the algal cultivation is working properly and the loss of nitrogen is limited it can be assumed that almost all nitrogen will end up in the digestate. Compared to conventional nitrogen removal, nitrogen will not be lost in the form of nitrogen gas, but it is crucial to limit the loss of ammonia in order to recover the nitrogen. Since iron is used in the harvesting of algae, hopefully all phosphorus will be recovered even if the reduction in the algal step is not complete. If a pretreatment method is applied and the amount of ammonium and phosphate released increases it would result in higher concentrations in the reject water from dewatering of digestate.

Annual variation

In the previous discussions, it is assumed that production of algae is the same throughout the year. This could be possible in areas where the climate allows it (enough temperature and sufficient sunlight). In areas, such as Sweden, where the climate changes during the year, the production would vary. Assuming that algal cultivation can be performed from March to October (8 months per year) with the productivity stated above and that 87% of algae can be recovered in the separation, the methane yield and energy presented in Table 4.23 are obtained. Even if algae only are grown 8 months per year and no pretreatment is applied, the yearly methane production from the whole plant would exceed the production obtained at Källby WWTP in 2013 with 18%.

Table 4.23. Yearly methane yield and energy if algae cultivation is performed 8 months per year.

	Methane (Nm^3/year)	**Energy (GWh/year)**
Untreated algae	165 000	1.65
Pretreated algae (120°C, 30 min)	250 000	2.49
Pretreated algae (170°C, 30 min)	297 000	2.96
Anaerobic digester (Untreated algae)	471 000	4.70
Whole treatment line (Untreated algae)	587 000	5.86

If algae cannot be used the whole year, the nutrient reduction must be achieved in another way. As proposed in the "energy positive concept" anammox treatment can be used during winter and could also be used for treatment of reject water from dewatering of digestate. An alternative phosphate reduction method would also be needed. During the months where algae cannot be grown, the flocculants could be used for precipitation of phosphate instead.

Disregarding the methane yield from algal biomass, the yearly methane yield from the proposed plant would be 85% of what was reported for Källby WWTP in 2013. Theoretically, the yield from the proposed plant (without algae) should still be higher, since less energy is lost in anaerobic treatment compared to aerobic. This discrepancy could be due to difference in COD reduction during the anaerobic treatment compared to aerobic or that the methane yield from primary sludge is underestimated. If the methane yield from primary sludge is underestimated, this would mean that even more methane could be recovered from the proposed concept. Further evaluation is needed to conclude the actual yield. Nevertheless, taken the aeration need in aerobic treatment into consideration, replacing the aerobic treatment with an anaerobic one seems promising.

Land requirement

Assuming that raceway ponds are going to be used for algal cultivation the area needed can be calculated. Assuming a HRT of three days and a depth of 0.3 m the total area for the algal step would be 27.5 hectares (0.275 km^2). Because of the large surface area evaporation/rainfall as well as heat losses will probably be significant. Using algae for nutrient reduction as proposed here would probably not be suited for WWTPs of this size because of the land requirements. The use of algae seems more appropriate for smaller WWTP or if another cultivation technique is used.

If a closed system is used, the evaporation can be minimized and productivity is increased. It also allows for more species control (Wiley *et al.*, 2011) making it possible to select an algal

species more suitable for anaerobic digestion. One of the main drawbacks of closed systems is the cost of installation and maintenance which must be taken into consideration. An option for WWTPs along coast lines is the NASA OMEGA system. OMEGA stands for "offshore membrane enclosure for growing algae" and uses semi-permeable bags for cultivation of algae. The idea is to create algal blooms inside the bags instead of in the open water, as would happen if nutritious wastewater is released. Mixing is provided by the natural waves in the water, temperature is controlled by the surrounding water and CO_2 is supplied by using for example gas filled bladders with gas-permeable membranes. Filters which retain algae and nutrients allow treated water to diffuse out of the bags (Trent, 2009).

Another option would be to make use of the mixotrophic metabolism observed in some microalgal species (Larsdotter, 2006). This would allow for deeper ponds to be used but would probably require addition of some form of carbon source, which consequently would add cost to the plant.

Regardless of which mode of operation that is used the need for light leads to the conclusion that algal treatment seems more suitable in areas with more sunlight hours or if treatment is not applied all year around.

From another point of view, the production of biogas from algae using wastewater as growth medium can be compared with biogas production from other feed stocks. Summarized in Table 4.24 is the energy generated as biogas per hectare and year for some energy crops. As can be seen, even for untreated algae, the energy generation is double compared to beets. Taken into consideration that it would not be possible to grow algae all year around in Sweden due to lack of natural sunlight, the energy generation per year would decrease. Assuming that it is possible to grow algae from March to October (8 months/year) the energy generation for untreated algae would be 60 MWh/(ha·year) which still is higher than for beets. Not only would the energy recovered from algae exceed the energy recovered from energy crops (per hectares and year) but nutrient reduction in wastewater would also be achieved. Another benefit of algal biomass production is that raceway ponds could be installed in areas unsuitable for cultivation of crops.

Table 4.24. Energy generation from some feed stocks used for biogas production (Björnsson, 2013). Presented is also the corresponding energy yield from microalgae found in this project.

Substrate	**Hemp**	**Beet**	**Corn**	**Rye wheat**	**Microalgae***
Energy (MWh/(ha·year))	21	45	29	25	90-160

**Results from this project. Lower limit is for untreated algae and upper limit is for pretreated algae at 170°C. Cultivation 12 months per year and 87% recovery is used for both cases.*

5 Conclusion

In this study, several aspects of integration of an algal step in a wastewater treatment system were evaluated. The proposed concept for wastewater treatment including AnMBBR and algal cultivation was compared to Källby WWTP. The lab work and analysis finally resulted in the following conclusions:

- Over the algal cultivation the reduction of phosphate was found to be over 97% and reduction of ammonium was over 84%.
- It was possible to efficiently separate algae and treated water by flocculation followed by sedimentation. Flocculation using ferric chloride and cationic polymer resulted in up to 96% recovery of algae.
- Lab-scale experiments showed that flotation after flocculation is promising as a harvesting method. Experiments also showed that if flotation is used instead of sedimentation, potentially more of the algae could be recovered.
- It was possible to meet the discharge limit of P-tot (0.3 mg/L) and the recommended limits for N-tot (10 mg/L) and COD (70 mg/L) using flocculation followed by 30 minutes of sedimentation. The lowest cost for achieving this was 0.12 SEK/m^3 of algal suspension. This corresponds to addition of 0.025 L of PIX 111 and 1 L of polymer per cubic meter of algal suspension and resulted in 87% recovery of algal biomass.
- BMP tests showed no pronounced synergistic effects for co-digestion of algae and primary sludge. No signs of ammonia inhibition could be seen in BMP tests although the protein content of algae was high (67% of VSS).
- BMP of primary sludge was 295 NmL/gCOD.
- BMP of untreated algal biomass, dominated by *Scenedesmus* sp. was found to be low (95-108 NmL/gCOD) but was increased by applying a pretreatment method. Pretreatment at 120°C for 30 minutes increased the BMP with 46% and pretreatment at 170°C for 30 minutes increased the BMP with 74%.
- A concept for wastewater treatment using AnMBBR and algal cultivation (12 months per year, no pretreatment of algal biomass) showed a yearly methane recovery of 670 000 Nm^3. Compared to Källby WWTP this corresponds to 35% higher methane recovery. In this concept 46% of the methane resulted from digestion of primary sludge, 37% from digestion of algae and 17% from organic reduction in the AnMBBR. By applying pretreatment, the yearly methane production compared to Källby WWTP was up to 75% higher.
- The ratio between primary sludge and algae was determined to be 32:68 on VSS basis, provided that algae are produced during the whole year.
- The energy recovery as methane from wastewater grown microalgae in raceway ponds (0.3 m depth) was found to be between 90 and 160 MWh/(ha·year) for full year production or between 60 and 107 MWh/(ha·year) for production 8 months per year, depending on pretreatment. Thus, great potential for biogas production can be found at WWTPs by using algae for nutrient reduction followed by anaerobic digestion.

6 Suggestions for future work

Although promising results were found in the present study, further evaluations would contribute to the understanding of this kind of system. Also, to be able to determine if the goals of the energy positive concept can be achieved, further evaluations are needed. Below are suggestions for future work:

Nutrient reduction
Further evaluation of using microalgae for nutrient reduction is needed. To conclude the actual nutrient reduction capacity of algae, pH regulation is essential.

Improvement of algal cultivation
The effect of CO_2 addition to algal cultivation should be investigated both for pH regulation and supply of carbon. In this way, positive aspects could be achieved such as higher productivity and reduced loss of nitrogen. Further the needed HRT should be evaluated since this affects the needed size of the raceway. Other options than raceway ponds should be considered as well as how the light limitations in northern countries will affect the algal cultures during the winter. Depending on if a closed system is used for algal cultivation, it could be possible to choose another algal species, more suited for anaerobic digestion since *Scenedesmus* is one of the species shown to be very resistant to degradation. Finally it would be interesting to investigate if the mixotrophic metabolism that has been found for some microalgal species can be utilized to decrease the light requirement.

Improvement of harvesting
Flotation showed very promising results in this study, but additional harvesting experiments, including energy evaluation should be performed to unravel the best option for harvesting, the maximum possible harvesting efficiency and needed amounts of flocculants.

Methane production
Continuous digestion experiments are needed to evaluate the actual yield from algae and primary sludge, possible synergistic effects from co-digestion and possible ammonia inhibition. Pasteurization of algae should be performed again, and other pretreatment methods could also be evaluated.

Full scale
To get a more reliable picture of a full-scale plant, a lot more measuring points are needed. Also, to get an idea about if it is possible to achieve energy positive wastewater treatment, a thorough energy evaluation must be performed.

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Appendix I

HACH LANGE

Table A.1. List of HACH LANGE methods used and the measuring range of each method. Samples were diluted with distilled water in order to fall into the measuring range.

Analysis	HACH LANGE method	Measuring range
COD	LCK 414	5-60 mg/L
	LCK 114	150-1000 mg/L
	LCK 014	1000-10000 mg/L
	LCK 914	5-60 g/L
NH_4^+-N	LCK 304	0.015-2.0 mg/L
	LCK 303	2.0-47.0 mg/L
N-tot	LCK 138	1-16 mg/L
	LCK 238	5-40 mg/L
PO_4^{3-}-P	LCK 349	0.05-1.5 mg/L
	LCK 350	2.0-20.0 mg/L
P-tot	LCK 349	0.05-1.5 mg/L
	LCK 350	2.0-20.0 mg/L
TOC	LCK 385	3-30 mg/L

Appendix II

Calculation of pressure during microwave treatment

Table A.2.Values used in calculation of the pressure during microwave pretreatment. Partial pressures and densities are collected from Mörtstedt & Hellsten (2010).

	120°C		170°C	
	Start (20°C)	End	Start (20°C)	End
Temperature (K)	293.15	393.15	293.15	443.15
Partial pressure water (bar)	0.023368	1.9854	0.023368	7.9202
Density water (g/L)	998.2	943.5	998.2	897.3*
Sample volume (L)	0.190	-	0.195	-

**Interpolated between 160°C and 180°C.*

Total volume of the digestion vessel is 0.385 L and is assumed to be constant. In the following equations, subscript "1" stands for starting temperature, 20°C, and subscript "2" stands for treatment temperature, 120°C and 170°C, respectively.

$$p^*_{air,1} = P_{tot,1} - p^*_{water,1}$$

$$V_{air,1} = V_{vessel} - V_{sample,1}$$

$$m_{sample,1} = \frac{V_{sample,1}}{\rho_{water,1}}$$

The mass of the sample is constant, $m_{sample,1}=m_{sample,2}$, and therefore the volume of the sample at the new temperature can be calculated.

$$V_{sample,2} = m_{sample,1} \cdot \rho_{water,2}$$

Further, the volume of the gas phase at the new temperature can be calculated

$$V_{air,2} = V_{vessel} - V_{sample,2}$$

The partial pressure of air can be calculated using the ideal gas law

$$p^*_{air,2} = \frac{T_2}{T_1} \cdot \frac{V_{air,1}}{V_{air,2}} \cdot p^*_{air,1}$$

The total pressure inside the digestion vessel can be calculated

$$P_{tot} = p^*_{air,2} + p^*_{water,2}$$

Table A.3. Results from calculation of pressure during microwave pretreatment.

	120°C		170°C	
	Start (20°C)	End	Start (20°C)	End
Partial pressure air (bar)	0.990	1.41	0.990	1.69
Sample volume (L)	0.190	0.201015	0.195	0.217
Sample mass (g)	190	190	195	195
Air volume (L)	0.195	0.184	0.190	0.168
Total pressure (bar)	1.01325	**3.39**	1.01325	**9.61**

Appendix III

Material balance for BMP test 1

Values and assumptions
Material balance used for determination of expected ratio between primary sludge and algae. The notations in Figure A.1 are used as subscripts in tables and calculations. Used values are shown in Table A.4. Further, Q_i is volume flow in m^3/day, SS_i is concentration of suspended solids in g/m^3 and COD_i is concentration of total COD in g/m^3.

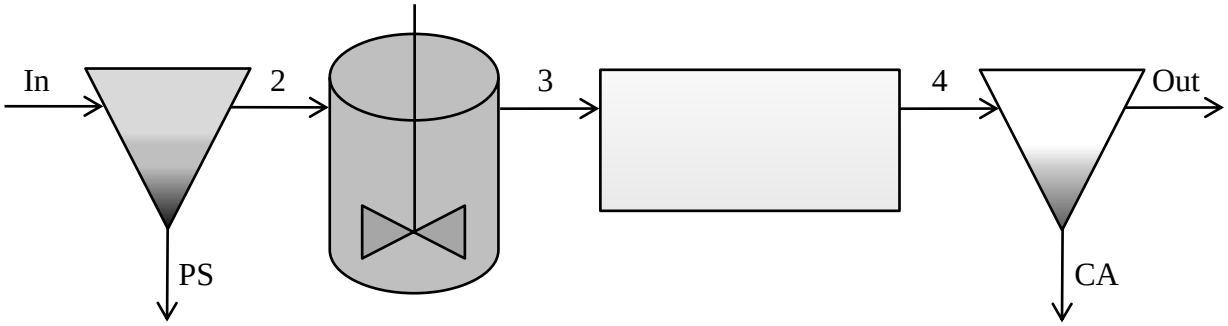

Figure A.1. Overview of the plant used for the first material balance. Notations are used as subscripts in tables and calculations.

Table A.4. Values that the material balance is based on together with where the values are derived from. Q_i is volume flow, SS_i is concentration of suspended solids, COD_i is concentration of total COD, subscripts correspond to notations in Figure A.1.

Notation	Value	Source
Q_{in}	25 000 m^3/day	Assumed
SS_{in}	186 g/m^3	Average from Källby WWTP (2013-07-30 to 2014-07-22)
SS_{PS}	9 000 g/m^3	Measurement from 2 Feb*
SS_3	45 g/m^3	Measurement from 2 Feb
SS_4	275 g/m^3	Measurement of 1st batch
SS_{CA}	6 460 g/m^3	Measurement of concentrated algae, 1st batch
COD_{in}	403 g/m^3	Average from Källby WWTP (2013-07-30 to 2014-07-22)
COD_{PS}	11 800 g/m^3	Measurement from 2 Feb
COD_3	115 g/m^3	Measurement from 2 Feb
COD_4	369 g/m^3	Measurement of 1st batch
COD_{CA}	7 700 g/m^3	Measurement of concentrated algae, 1st batch

**Measurement was done for TS, it is assumed that SS=TS in this case.*

Since this material balance was done only to get an idea of the ratio between primary sludge and algae, some assumptions were made.

- 60% of SS is removed in the presedimentation (Gillberg *et al.*, 2003).
- 95% of SS is removed in the separation step after the algal cultivation. This is based on that the recovery in the first harvesting was close to 100%, but since the

sedimentation time was longer than what it would be in reality, the removal efficiency will be lower.

- No account has been taken regarding evaporation and/or rainfall in the algal cultivations meaning that Q_3 is equal to Q_4.

Results

Table A.5. Results from the material balance calculations.

Notation	Calculated value
Q_2	24600 m^3/day
Q_{PS}	310 m^3/day
Q_3	24700 m^3/day
Q_4	24700 m^3/day
Q_{out}	23700 m^3/day
Q_{CA}	998 m^3/day
SS_2	75.3 g/m^3
SS_{out}	14.3 g/m^3
COD_2	260 g/m^3
COD_{out}	60.0 g/m^3

Based on SS, the ratio between primary sludge and algae would be:

$$R_{CA} = \frac{Q_{CA}SS_{CA}}{Q_{CA}SS_{CA} + Q_{PS}SS_{PS}} = \frac{998 \cdot 6460}{998 \cdot 6460 + 310 \cdot 9000} = 0.7$$

$$R_{PS} = \frac{Q_{PS}SS_{PS}}{Q_{CA}SS_{CA} + Q_{PS}SS_{PS}} = \frac{310 \cdot 9000}{998 \cdot 6460 + 310 \cdot 9000} = 0.3$$

Based on TCOD the ratio between primary sludge and algae would be:

$$R_{CA} = \frac{Q_{CA}COD_{CA}}{Q_{CA}COD_{CA} + Q_{PS}COD_{PS}} = \frac{998 \cdot 7700}{998 \cdot 7700 + 310 \cdot 11800} = 0.7$$

$$R_{PS} = \frac{Q_{PS}COD_{PS}}{Q_{CA}COD_{CA} + Q_{PS}COD_{PS}} = \frac{310 \cdot 11800}{998 \cdot 7700 + 310 \cdot 11800} = 0.3$$

Appendix IV

Experimental setup for BMP test 1 and 2

Based on the desired amount of maximal methane production in each bottle (75 NmL in BMP test 1 and 80 NmL in BMP test 2) and the theoretical yield of 350 NmL/gCOD the needed amount of COD in each bottle was calculated:

$$m_{COD,bottle}(gCOD/bottle) = \frac{V_{CH_4,bottle}}{350 NmL/gCOD}$$

The working volume of each bottle is set to 100 mL and by assuming a density of 1 kg/L for all solutions, the needed mass (m) of substrate and inoculum for preparation of 400 mL could be calculated based on the concentration (C) of COD in the substrate and VS in the inoculum. The results from measurement of TS and VS in the inoculum used in the BMP tests and measurement of TCOD in substrates used are shown in Table A.6 and A.7, respectively. Further, experimental set up for BMP test 1 and 2 are shown in Table A.8 and A.9, respectively.

Amount of substrate in 400 mL:

$$m_{substrate}(g) = 4 \cdot \frac{m_{COD,bottle}}{C_{COD,substrate}}$$

Amount of inoculum in 400 mL:

$$m_{inoculum}(g) = 4 \cdot \frac{2 \cdot m_{COD,bottle}}{C_{VS,inoculum}}$$

Table A.6. Results from analysis of TS and VS in the inoculum used in each BMP test.

BMP	Collection date	TS (%)	VS (%)	VS (g/L)
1	2015-01-30	5.30	3.32	33.2
2	2015-02-13	5.29	3.31	33.1

Table A.7. Measurement of TCOD in substrates used for BMP test 1 and 2.

BMP test	Substrate	TCOD (g/L)
1	Primary sludge	11.8
1	Concentrated algae (1st batch)	7.70
2	Concentrated algae (mix of 2nd and 3rd batch)	6.11

Table A.8. Experimental set up for BMP test 1 showing amount of inoculum, control substrate ($NaAc \cdot 3H_2O$), substrate and water that were used when preparing 400 mL of solution.

	Proportion of algae (%)	Inoculum (g)	$NaAc \cdot 3H_2O$ (g)	Primary sludge (g)	Algae (g)	Water (g)
Blank	-	51.7	-	-	-	348.3
Control	-	51.7	1.83	-	-	346.5
Primary sludge	0	51.7	-	72.6	-	275.7
Algae	100	51.7	-	-	111.3	237.0
Mixture 1	70	51.7	-	21.8	77.9	248.6
Mixture 2	40	51.7	-	43.6	44.5	260.2
Mixture 3	15	51.7	-	61.7	16.7	269.9

Table A.9. Experimental set up for BMP test 2 showing amount of inoculum, control substrate ($NaAc \cdot 3H_2O$), substrate and water that were used when preparing 400 mL of solution.

	Inoculum (g)	$NaAc \cdot 3H_2O$ (g)	Algae (g)	Water (g)
Blank	55.2	-	-	344.8
Control	55.2	1.95	-	342.9
Algae	55.2	-	149.6	195.2
Pasteurization	55.2	-	149.6	195.2
170°C for 30 min	55.2	-	149.6	195.2
120°C for 30 min	55.2	-	149.6	195.2

Appendix V

Results from flocculation experiments

Table A.10. Results from all flocculation experiments during optimization.

Experiment	PIX 111 (µL/L)	Polymer (mL/L)	N-tot (mg/L)	P-tot (mg/L)	TCOD (mg/L)	SS* (mg/L)
1	0	0.5	24.4	2.46	393	273
2	0	1	17.5	1.41	266	181
3	0	1.5	16.2	1.24	254	173
4	0	2	14.1	1.00	210	141
5	0	2.5	10.8	0.634	160	105
6	0	3	9.30	0.462	82.8	49.3
7	0	3.5	11.9	0.890	184	122
8	0	4	13.1	0.805	192	128
9	50.0	0.5	8.62	0.433	91.6	55.6
10	25.0	0.5	14.1	1.13	223	150
11	12.5	0.5	25.0	2.84	442	308
12	50.0	1	6.96	0.256	62.2	34.5
13	25.0	1	7.46	0.259	64.6	36.2
14	12.5	1	15.6	1.11	210	141
15	50.0	1.5	5.36	0.0630	31.8	12.6
16	25.0	1.5	7.04	0.231	56.0	30.0
17	12.5	1.5	12.3	0.502	117	73.9
18	50.0	2	7.28	0.217	52.4	27.4
19	25.0	2	8.06	0.310	70.4	40.4
20	12.5	2	8.58	0.285	74.0	43.0
	PlusPAC 1800 (µL/L)					
21	78.0	1	6.28	0.113	40.8	19.1
22	39.0	1	8.40	0.309	78.0	45.9
23	19.0	1	8.24	0.269	81.2	48.2
24	0	0	18.9	1.79	263	179

**Calculated using the correlation between TCOD and SS, Figure A.2.*

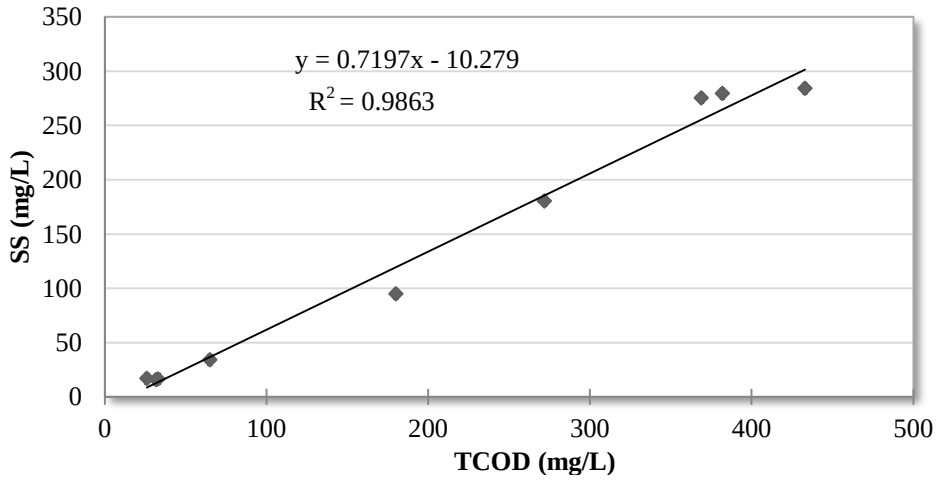

Figure A.2. Correlation between TCOD and SS including equation from linear regression.

Appendix VI

Heavy metal analysis

Table A.11. Result from analysis of heavy metals in primary sludge and algal suspension.

	Algae	**Primary sludge**
Lead (mg/L)	<0.00050	0.11
Cadmium (mg/L)	<0.00010	0.0042
Copper (mg/L)	0.0096	1.5
Chromium (mg/L)	<0.0010	0.10
Zinc (mg/L)	0.026	3.2
Nickel (mg/L)	0.0019	0.062
Mercury (mg/L)	<0.00010	0.0021

Appendix VII

Full scale

Below is a description of how the material balance was created. Since thickening of primary sludge and algae was not investigated it was assumed that the SS concentration in Q_{R1} and Q_{R2} is zero. This is not likely, however to simplify the calculations a bit this assumption was made. Further, the whole balance is based on SS. Thus SS is assumed to be the same as TS. Also, rainfall and/or evaporation have been neglected. Q_i is volume flow, SS_i is concentration of suspended solids, $TCOD_i$ is concentration of total COD, $SCOD_i$ is concentration of soluble COD, N-tot_i is concentration of total nitrogen and P-tot_i is concentration of total phosphorus, subscripts correspond to notations in Figure 4.9.

Presedimentation
To calculate Q_2 and Q_{PS1} it is assumed that 60% of SS is removed in the presedimentation (Gillberg *et al.*, 2003). Also, it is assumed that the TS concentration in the primary sludge is equal to the SS concentration. From this, $TCOD_2$ can be derived knowing $TCOD_{PS1}$.

Primary sludge concentration
In this step it is assumed that all suspended solids end up in the concentrate and that SS_{PS2} is 7%. From this, Q_{R1} and Q_{PS2} are calculated.

AnMBBR
Nothing accumulates in the AnMBBR, thus $Q_3=Q_2$. For calculation of methane production, it is assumed that 45% of TCOD is SCOD in Q_2. Also, the hydrolysis step is not considered; instead it is merged with the AnMBBR.

Algal step
To decrease the loading on the algal step, the reject water from dewatering of digestate is not recirculated. Q_{R1} is introduced into the algal step which leads to that $Q_4=Q_3+Q_{R1}$. The concentration of N-tot and P-tot in Q_{R1} is assumed to not affect the concentration in the algal step. The SS concentration in Q_4 is based on measurement in algal batch 1, 2 and 4.

Algal separation
Based on flocculation experiment 13 the concentration of SS in the outflow and the recovery based on SS are known. From experiment 13 it was possible to calculate SS_{CA1} and from this and the recovery, Q_{out} and Q_{CA1} were determined.

Algal concentration
As for primary sludge concentration it was assumed that all SS end up in the concentrate and that SS_{CA2} is 4%. From this, Q_{R2} and Q_{CA2} are calculated. Since it is assumed that all SS ends up in the concentrated algae, Q_{R2} is almost completely clean, thus is can be discharged.

Anaerobic digester
The volume reduction in the digester is neglected, thus $Q_{D1}=Q_{CA2}+Q_{PS2}$. SS_{D1} is calculated assuming a SS reduction of 37% in the digester, corresponding to a VSS reduction of 50%.

Dewatering
It is assumed that 3.5% of the SS ends up in Q_{R3} and that the concentration in the dewatered digestate is 25%.

Nutrients
N-tot is assumed to be unchanged before and after AnMBBR, thus $N\text{-}tot_2 = N\text{-}tot_3$.

Since no experiments were conducted where the pH was regulated in the algal step, the balance is made based on cultivation without pH regulation. Thus, the loss in N-tot in the algal step is set to 33%, which is the average N-tot loss in batch 1, 2 and 4.

In Table A.11, flows and concentrations are shown. Values with grey background are calculated whereas white background means starting values.

Table A.11. All values, both starting values and calculated values, used in the material balance. Notations refer to Figure 4.9.

	Q (g/m^3)	**SS (g/m^3)**	**TCOD (g/m^3)**	**N-tot (g/m^3)**	**P-tot (g/m^3)**
In	27 500	186	403	44	6.1
2	27 159	75.3	260	38.3	
3	27 159	49.2		38.3	
4	27 456	280		25.7	
Out	27 289	36.0	65.0	7.5	0.26
PS1	341	9 000	11 800		
PS2	43.8	70 000			
CA1	2 257	2 959			
CA2	167	40 000			
R1	297	0			
R2	2 090	0			
R3	175	1 860			
D1	210	44 100			
D2	35.8	250 000			

Energy calculations
Energy for pretreatment is calculated as described in chapter 3.5.3. Densities and heat capacities for water are used. These values are collected from Mörtstedt and Hellsten (2010). Since both density and heat capacity are temperature dependent, an average between the relevant temperatures is used. T_d is set to 37°C and T_i is set to 25°C. Energy content in methane was set to 9.97 kWh/Nm^3 CH_4 (Statens energimyndighet, 2014).

Appendix VIII

Populärvetenskaplig sammanfattning

Mikroalger för biogasproduktion och avloppsvattenrening

I dagens samhälle används stora mängder vatten som måste renas innan det släpps tillbaka ut i naturen. Rening av vattnet sker i avloppsreningsverk som ska minska mängden organiskt material och mängden näringsämnen i vattnet innan det släpps ut. Om vattnet inte renas finns det en risk för negativ påverkan på sjöar och vattendrag, t.ex. övergödning.

Fosfor är ett av de näringsämnen som finns i avloppsvatten och som ofta behöver tillsättas som gödsel i jordbruk. Genom att återvinna fosforn som finns i avloppsvatten minskar belastningen på jordklotets ändliga fosforreserver. En annan vinst med att återvinna näringsämnen från avloppsvatten är att energiförbrukningen kan minska. Vanligtvis fixeras kväve från luften, i en energikrävande process, för att användas som gödningsmedel. Om kvävet i avloppsvattnet istället används minskar energiförbrukningen.

Eftersom efterfrågan på förnybara energikällor ökar, krävs det nya system för att möta efterfrågan. Genom att betrakta avloppsvatten som en resurs finns det positiva vinster att göra. Det organiska materialet i avloppsvattnet kan omvandlas till biogas genom att det behandlas i syrefri miljö. Biogasen kan användas för att generera el och värme eller som fordonsbränsle. I konventionell avloppsvattenrening används ofta ett luftat steg där mycket av energin går förlorad. Genom att byta ut detta steg mot ett syrefritt kan mer av energin tas tillvara. Detta är möjligt i lite varmare klimat, eller om spillvärme finns tillgänglig för uppvärmning.

Vidare har det även föreslagits att biobaserade bränslen, t.ex. biogas kan produceras från mikroalger. Mikroalger är mikroskopiskt små organismer som på samma sätt som växter utnyttjar fotosyntesen. Det betyder att de med hjälp av solljus kan omvandla koldioxid och vatten till kolhydrater. Förutom koldioxid och solljus behöver algerna näringsämnen såsom kväve och fosfor. Ett problem har varit att på ett hållbart sätt förse algerna med tillräckliga mängder näringsämnen. Därför har det föreslagits att alger kan odlas i avloppsvatten. På detta sätt uppnås två mål samtidigt; vattnet renas och algerna förses med den näring de behöver. När algerna tagit upp näringsämnena från avloppsvattnet, separeras algbiomassan och används för biogasproduktion. Vattnet har blivit renat och kan släppas ut utan att påverka den omgivande naturen negativt.

Biogasprocessen kallas även rötning. Det som blir kvar efter att det organiska materialet omvandlats till biogas kallas rötrest. Rötresten innehåller de näringsämnen som fanns i materialet från början. Detta betyder att det går att skapa ett kretslopp om alger används för näringsreduktion i avloppsvattenrening. Algerna tar upp näringen från vattnet när de växer. När algerna sedan rötas omvandlas den organiska delen av algerna till biogas och näringsämnena stannar kvar i rötresten. Rötresten kan användas som gödningsmedel och biogasen kan användas till el- och värmeproduktion eller som fordonsbränsle. När biogasen förbränns avges koldioxid men eftersom algerna tar upp koldioxid när de växer, fås även ett kretslopp för kol. För att öka tillväxten hos algerna kan extra koldioxid tillsättas i odlingen.

Syftet med detta examensarbete var att undersöka om det går att rena avloppsvatten med hjälp av mikroalger och sedan använda mikroalgerna för produktion av biogas. Den experimentella

delen av arbetet fokuserade på hur algerna kan separeras från vattnet, hur mycket biogas som kan produceras och hur värmebehandling av algerna påverkar hur mycket biogas som bildas. Resultaten ledde fram till en jämförelse mellan ett befintligt avloppsreningsverk och en möjlig ny design. Resultaten visade att tillräckligt hög reningsgrad gick att uppnå med hjälp av alger och att den totala mängden biogas som kunde utvinnas var högre än vid det befintliga verket som användes vid jämförelsen. Det visade sig också att det gick att utvinna mer biogas genom att värmebehandla algerna. Även om vidare utredningar behövs så verkar avloppsvattenrening med hjälp av mikroalger och biogasproduktion från algerna vara ett mycket lovande koncept.