The feasibility of using algae as a co-substrate for biogas production

Laboratory experiments of the co-digestion of algae and biosludge

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Möjligheten av att använda alger som samsubstrat for biogasproduktion
Laboratoriska experiment av samrötning mellan alger och bioslam

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Prologue

This master thesis represents the last and final part of my education at Karlstad's University, within Industrial Management and Energy- and Environmental Engineering. The thesis coves 30 university points and has been conducted during spring of 2015 at Karlstad's University.

I would like to thank my supervisors Andreas Nilsson and Karin Granström, for their mentorship, support and endless patience. Without their help and expertise this work had been much harder.

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Abstract

Today 88 % of the world energy comes from fossil fuels. Greenhouse gas emissions are increasing and the fossil fuels energy sources will decrease at some point. Other alternatives must be found, to substitute and lower the usage of fossil fuels. Biogas is one of these other options. It is a versatile fossil free fuel that can be used for heat, power and fuel for vehicles. Many different substrates have been used for biogas production over the years, and now algae are examined as a substrate. Algae have advantages over the former substrates used for biogas production. Laboratory experiments were conducted to examine the co-digestion potential of algae and biosludge, which is a rest product from a wastewater treatment plant at a pulp and paper mill. The profitability aspect of using algae and biosludge for biogas production has been examined as well.

The result shows that unmixed algae were the highest methane producing substrate, which produced a maximum of 203,5 Nml/g VS. An interesting result was that both algae and biosludge separately produced more methane gas than the mixtures. The profitability aspect of the thesis showed that it is not profitable to use algae primarily for biogas production, based on the conditions of today.

Sammanfattning

Idag kommer 88 % av världens energi från fossila bränslen. Utsläppen av växthusgaser ökar och de fossila bränslenas energikällor kommer att minska inom en viss tid. Andra alternativ måste hittas, för att ersätta och minska användningen av fossila bränslen. Biogas är ett av dessa alternativ, det är ett mångsidigt fossilfritt bränsle som kan användas för värme, el och bränsle för fordon. Många olika substrat har använts för biogasproduktion under årens lopp, och nu har alger undersöktes som ett nytt substrat. Alger har fördelar jämfört med de tidigare substrat som används för biogasproduktion. Laboratorieförsök genomfördes för att undersöka samrötning av alger och bioslam, som är en restprodukt från en avloppsanläggning vid ett massa- och pappersbruk. Lönsamhetsaspekten av att använda alger och bioslam för biogasproduktion har också undersökts .

Resultatet visar att alger utan inblanding av bioslam gav mest metan och producerade maximalt 203,5 Nml/g VS. Ett intressant resultat var att både alger och bioslam rötade separat producerade mer metangas än blandningarna. Lönsamhetsaspekten av rapporten visade att det inte är lönsamt att använda alger för endast biogasproduktion, baserat på villkoren i dag.

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Nomenclature and list of words

AMPTS[2]	Autometic Methane Potential Test System	
Anaerobic digestion	A process where microorganisms break down organic material in the absence of oxygen	
Anaerobic fermentation	A process where microorganisms break down organic material in the absence of oxygen	
Bioenergy	Renewable energy that derives from biological sources	
Biogas	A gas fuel which is produced during anaerobic digestion	
C/N-ratio	The relation between carbon and nitrogen	
FAN	Free Ammomium Nitrogen (NH_3)	(mg/l)
H_2S	Hydrogen Sulfide	(mg/l)
Lignocellulosic material	Biomass from trees, bushes and grass.	
NH_4^+	Ammonium Nitrogen Ion	(mg/l)
TAN	Total Ammonium Nitrogen (NH_3-N)	(mg/l)
TS	Total Solids	(%), (g VS/g sub), (g)
VFA	Volatile Fatty Acids	(mg/l)
VS	Volatile Solids	(%), (g VS/g sub), (g)

1. Introduction

This chapter presents the background of the research area together with problem discussion, generating the purpose of this thesis.

1.1. Background

Today 88% of the worlds energy demand comes from fossil fuels. The energy demand is increasing rapidly and with that the greenhouse gas emissions increase in the atmosphere, where CO_2 is the important contributor (Weiland 2010). Another effect of the high energy demand is the fact that fossil fuels reserves are decreasing rapidly and this leads to higher energy prices (Mussgnug et al. 2010). To be able to decrease global warming and climate changes, the greenhouse gas emissions needs to be cut in half, based on the emissions in 1990 (Weiland 2010). Many countries try because of this to find a stabile energy supply for future generations. Several different ideas and techniques have been developed, wind energy, geothermal energy, energy from the water and solar irradiation (Mussgnug et al. 2010).

In Sweden 30 % of the energy demand comes from fossil fuels (Naturvårdsverket & Björsell 2015). The transport sector is the biggest user of fossil fuels in Sweden, and is struggling to find alternative fuels. In 2030 Sweden has set a goal that the transport sector should be free from fossil fuels and by the time 2050 climate neutral (Sköldberg et al. 2010).

An alternative to fossil fuels is biogas, which is a versatile energy source. Biogas can be used for power and heat production and also fuel for vehicles (Weiland 2010). Biogas is considered one of the most energy efficient and environmentally beneficial technologies for production of bioenergy. It reduces greenhouse gases rapidly compared to fossil fuels and make use of local resources (Weiland 2010). In 2007 the energy production from biogas in Europe reached 6 million tons oil equivalents. The agriculture in Europe can produce 1.5 billion tons of biomass for biogas production, half of this amount is energy crops (Weiland 2010).

In 2013 Sweden produced 1,7 TWh from 264 biogas plants. The biogas was used as gas for vehicles, heat production and electricity (Biogasportalen

2014a). In figure 1 is the usage of these 1,7 TWh is shown and the distribution between the different applications.

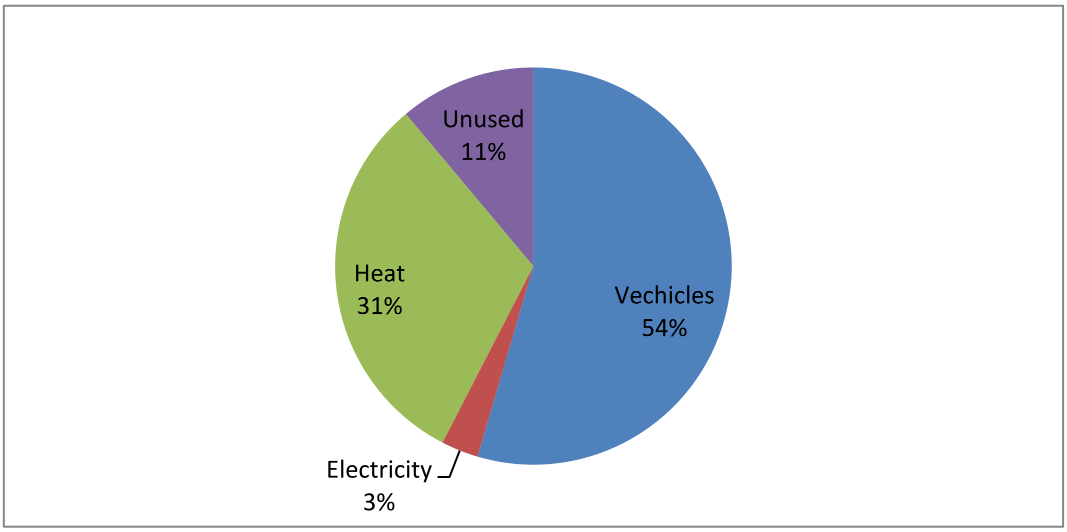

Figure 1 Biogas usage in Sweden 2013 (Biogasportalen 2014a)

The use of local resources when producing biogas reduces the greenhouse gases compared to the use of fossil fuels (Montingelli et al. 2015). Methane production will release a lower amount of atmospheric pollutants and release less carbon dioxide per produced unit. Methane has a higher heating value than biodiesel, bioethanol and biomethanol (Montingelli et al. 2015). Biogas from waste, residues and energy crops will be of importance in the near future. Biogas can be used for heat and power production and fuel for vehicles. Biogas can also be as a substitute for nature gas in production of chemicals and materials (Weiland 2010). Anaerobic fermentation has been used for about 50 years. The peak of the process came in the 1970-1980 when the first oil crisis hit (Mussgnug et al. 2010). Producing biogas through anaerobic digestion is one of the most energy-efficient and environmentally beneficial techniques for bioenergy production (Montingelli et al. 2015).

There are three different generations of feedstock for biofuels, the first was made of corn, soybean, sugarcane and rapeseed. Using these resources for energy made the price for food increase, since they competed for agricultural land. The second generation came from waste and lignocellulosic materials, the benefits of using these are less needed land and higher yields (Montingelli et al. 2015). The lignocellulosic materials are hard to break down into biofuel and not economic in the long run (Montingelli et al. 2015). Micro- and macro-algae are the third generations of biofuel feedstock. Algae have advantages over the two previous generations of feedstock, as no agricultural land is needed and they can produce 2-20 times more biomass than the ordinary energy crops (Montingelli et al. 2015). Algae may also double their biomass in 24 hours. The low amount of lignin makes them easier to break down compared to lignocellulosic materials (Montingelli et al. 2015). The biomass itself can be transformed into many different types of products: bioethanol, biodiesel or biogas (Mussgnug et al. 2010). Microalgae has been taken into consideration when it comes to bioenergy, microalgae can produce about 20 times higher oil yield than other energy crops like soybean, oil palm or sunflower. Due to high costs for lipid extraction and high concentrations of unsaturated fatty acids in biofuels from algal biomass, large scale productions are inhibited (Wang et al. 2013). A more suitable method for energy extraction of algal biomass is anaerobic digestion. This is a good method if the lipid content of the algal biomass is less than 40% (Wang et al. 2013).

1.2. Problem discussion

There are some difficulties of using algae for biogas production. Algae have high water content, other factors are variations in nutrient content because of season variations, low C/N- ratio (Montingelli et al. 2015) and the algae's cell walls can be hard to break down (Ramos-Suárez & Carreras 2014). Some of these difficulties can be handled by using a wet anaerobic digestion or pretreatments.

Introducing this carbon rich material can help to decrease the chance of ammonia toxicity. Carbon rich materials might be; primary or secondary sludge (biosludge), oil-grease, waste paper or food waste (Wang et al. 2013). Previous studies have shown that co-digesting algae with for example waste paper helps to higher the C/N-ratio and also the production of methane (Yen & Brune 2007). For this master thesis, biosludge will be used as a co-substrate to algae. Biosludge has a high C/N-ratio, but the use of biosludge for anaerobic digestion is rare (Hagelqvist 2013; Meyer & Edwards 2014). The benefits of using sludge from a pulp and paper mill is the high content of organic substance, large quantities produced every day, and no pretreatment is needed (Hagelqvist 2013). Future research should use biosludge in the anaerobic digestion process (Meyer & Edwards 2014). The use of algal biomass for bioenergy production is widely studied, but it is rare to find studies of their economic feasibility for bioenergy production (Montingelli et al. 2015).

1.3. Purpose

This master thesis will examine the biogas production by anaerobic co-digestion of algae and biosludge from a pulp and paper mill, aiming at finding a mixture for maximum methane production. An additional question is what can be done with the digestate, after the anaerobic digestion. Research about the use of algae and biosludge together for biogas production is rare, so the economic feasibility of using these substrates for biogas production will also be analyzed.

1.3.1. Research questions:

Under what conditions is co-digestion of algae and biosludge feasible for biogas production?

- What proportion of algae / biosludge for co-digestion, will be the best for producing the highest amount of biogas?
- Is it profitable to use algae as a substrate for biogas production?

1.4. Delimitations

A master thesis is conducted under a limited amount of time, so to find the best mixture of algae and biosludge for biogas production, more laboratory experiment must be conducted. Also other laboratory equipments could be used to validate the results.

Since the algae used for this thesis is cultivated at a test-facility for a pilot-project called "*Algae culturing at pulp- and paper industries for sustainable production of biofuel*" not much economic information has been provided. The economic calculations in this thesis are based on a pre-study conducted by the pilot-project, however not much economic information about the cultivation of algae has been brought forward, which is a limitation.

2. Theoretical framework

In this chapter the theoretical framework explained. The chapter starts with explaining what biogas is and how it is produced. The substrates used in this thesis are also described here, as well as the digestate.

2.1. Biogas

Biogas is a mixture of methane and carbon dioxide which is produced by anaerobic digestion (Mata-Alvarez et al. 2014). Biogas consists mostly of methane (40-75%) and carbon dioxide (15-60%). To meet the standards for usage of the biogas as vehicle fuel or use in the natural gas grid the methane gas needs to be separated from the carbon dioxide. The upgraded biogas is often called bio methane and contains then 97-99% methane and 1-3% carbon dioxide (Ryckebosch et al. 2011). The relative amount of methane determines the quality of the biogas (Mussgnug et al. 2010). If the distribution or the production of the biogas leaks, the methane gas will have 34 times greater environmental impact than carbon dioxide (Naturvårdsverket & Björsell 2015), small leakages can erase the advantages the biogas has against other fuels. Biogas is rising in popularity and infrastructure is now developing in Sweden (Hagelqvist 2013).

2.2. Anaerobic Digestion

Anaerobic digestion is a biological process that occurs in an oxygen free environment (Mata-Alvarez et al. 2014). An anaerobic process uses a cultivation of different bacteria, these bacteria's works as a metabolic unit that will produce methane 60% and carbon dioxide 30% (Montingelli et al. 2015). Single substrate anaerobic digestion experience some difficulties, based mostly on the properties of the substrate. Some unfortunate properties are: low organic loads, high/ low nitrogen concentrations, high concentrations of heavy metals and long chain fatty acids (Mata-Alvarez et al. 2014). These problems can be solved by adding another substrate to the anaerobic digestion process, so called co-digestion. Co-digestion often solves these problems and it also increase the methane production and because of that it becomes more economically viable (Mata-Alvarez et al. 2014). Co-digestion will produce more methane than digesting the substrates individually. Many factors should be considered when co-digesting transport costs, organic loading rate (OLR), blend ratio, best co-substrate etc. The most frequent substrates are animal manures and sewage sludge, while the most frequent co-substrates are industrial waste and agricultural waste (Mata-Alvarez et al. 2014). A full-scale biogas plant is continuously fed with subsrate, the substrate is in the chamber between 15-30 days before it is emptied and fed again (Biogasportalen 2014b).

The anaerobic process is divided into four different biological processes; hydrolysis, acidogenesis, acetogenesis/dehydrogenation and methanogenesis (Weiland 2010; Montingelli et al. 2015), as shown in figure 1. The different degradation steps use different types of microorganisms. These different microorganisms have different requirements on the environment. For the microorganisms to survive and grow some nutrients are important, these are; carbon, phosphor and sulfur (Weiland 2010).

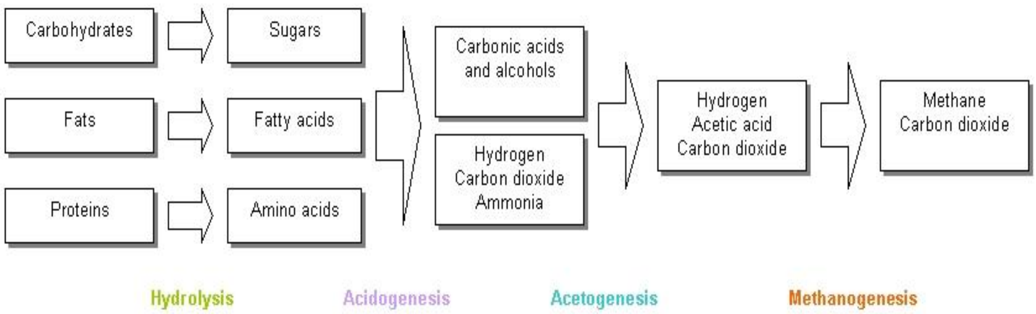

Figure 2 The different stages during anaerobic digestion (Wikimedia Commons 2015)

The hydrolyzing and fermenting microorganisms start the process by using the polymers and monomers to produce acetate and hydrogen. Amount of volatile fatty acids (VFA) can be produced and in that case in the form of propionate and butyrate (Weiland 2010). The VFA will be turned into acetate and hydrogen by hydrogen-producing acetogenic bacteria. Hydrogen may be a substrate that limits the methanogens. In the final step of the degradation methanogenic bacteria produces methane from acetate or hydrogen and carbon dioxide (Weiland 2010). The methanogenic bacteria are anaerobic and only a few species are able to convert acetate into methane and carbon dioxide, but all methanogenic bacteria can turn hydrogen into methane. The four different microbes are linked together, because of that the process can be performed in two stages (Weiland 2010).

For the anaerobic digestion to be stabile the two stages need to be equal, the rate of degradation needs to be the same in the two stages (Weiland 2010). If the degradation in the first step runs to fast the acid concentration raises, because of that the pH level decreases to below 7 and this inhibits the methanogenic bacteria. If it is vice versa and the second degradation runs to fast the production of methane is limited by the hydrolytic stage. These rates are determined by the substrates and its compounds (Weiland 2010).

The anaerobic digestion can be mesophilic with a temperature at 35-42 °C or thermophilic with a temperature at 45-60°C (Weiland 2010). The temperature during the process must be kept constant, otherwise it will affect the biogas production negatively. Thermophilic processes are more sensitive to changes in the temperature, it takes longer time for the process to adapt if the temperature changes. Mesophilic bacteria tolerate changes in the temperature better without reducing the biogas yield (Weiland 2010). The methanogenic bacteria grow faster in thermophilic conditions, which make the process faster and efficient. Due to this the thermophilic digester can be loaded more or have a lower hydraulic retention time (HRT) than a mesophilic process, but the temperature of thermophilic conditions can result in imbalance and ammonia inhibition. The ammonia toxicity will increase when the temperature raises, and this can lead to that the population of microorganisms dies (Weiland 2010).

2.2.1. Inhibition of anaerobic digestion

Anaerobic digestion isn't just beneficial, anaerobic digestion can be inhibited causing lower biogas yields. The inhibition can be caused by: ammonia, pH, volatile fatty acids or hydrogen sulfide. These inhibition factors are often depending on each other (Kayhanian 1999).

Ammonia

Ammonia inhibition can be caused of many things, ammonium concentration, pH, temperature, other present ions and acclimation. Ammonia can show itself in two different inorganic formations, ammonium nitrogen ion (NH_4^+) and free ammonia nitrogen (NH_3). When it comes to ammonium nitrogen ion levels above 1500 mg/l will lead to toxic conditions (Montingelli et al. 2015). Meanwhile levels above 80 mg/l free ammonium nitrogen will be inhibitory (Montingelli et al. 2015; Weiland 2010). Previous studies have shown that it is free ammonia nitrogen concentration that inhibits the methanogenesis stage and not the total ammonia nitrogen (Kayhanian 1999). NH_3 will increase with temperature and pH. Free ammonia nitrogen depends on pH (Kayhanian 1999). If the ammonia increases because of an increase in pH, this will cause process instability and VFA accumulation. This will lead to a decrease in pH and a decreasing concentration of NH_3 (Montingelli et al. 2015). An inhibited steady state can occur and this will produce a smaller methane yield under a stabile process, this state is determined by the interactions between NH_3, pH and VFA (Montingelli et al. 2015).

pH

The methane is produced within a pH interval of 6.5-8.5 with an optimum level at 7.0-8.0. The process will be inhibited if the pH is below 6 or higher than 8.5. The pH value will increase with the accumulation of ammonia, but the pH value will decrease with the accumulation of VFA (Weiland 2010).

Volatile fatty Acids (VFA)

The inhibition levels for VFA lies about 6000 mg/l. The OLR is critical factor when it comes to VFA accumulation. VFAs will increase with the increasing loading rate (Montingelli et al. 2015). If ammonia inhibits the process the concentration of VFA increases and therefore the pH will decrease (Weiland 2010).

Hydrogen sulfide (H_2S)

The production of hydrogen sulfide may reduce the methane yield by the competition between methanogens and sulfate-reducing bacteria. The inhibitory level lies between 100-800 mg/l for dissolved sulfide or 50-400 mg/l for disintegrated hydrogen sulfide. Microalgae don't contain of sulphureted amino acids and because of this they produce a lower amount of hydrogen sulfide than other organic materials (Montingelli et al. 2015).

2.3. Substrate

Different types of substrates can be used for anaerobic digestion: food waste, sludge from municipal waste water treatment, manure, agricultural crops etc (Oilgae 2015a). In this master thesis, algae and biosludge will be the used substrates. Algae used in this master thesis are cultivated in a test-facility outside Kristinehamn, a town in central Sweden. The projects purpose is to investigate different ways to extract the energy of the algae. The biosludge is a rest product that is incinerated for energy production.

2.3.1. Algae

Algae have great potential for biogas production: they don't compete with the food market and they have a low content of lignin. They are fast growing and can be used for nutrient removal in waste water treatment (Mata-Alvarez et al. 2014). The biomass of algae is full of nutrients like carbon, nitrogen and phosphorus. These nutrients are very important for anaerobic microorganisms (Montingelli et al. 2015). Half of the algal biomass contains of carbon dioxide, by producing 100 tonne algae, 183 tonne carbon dioxide has been fixed from the atmosphere. Microalgae can be used to fixate carbon dioxide released from power plants. The best way of producing biofuels from macroalgae is by anaerobic digestion (Montingelli et al. 2015). Microalgae is most of the time used as a substrate for biodiesel, the interest of microalgae for biogas has increased. Some claim that the methane potential in microalgae is about 70 % (Montingelli et al. 2015). Many different attempts using microalgae as substrate for anaerobic digestion has been done, the documented results show a methane production range between 70-600 Nml/g VS (Ward et al. 2014). Different spices of algae, cultivation strategies, pretreatments might explain the wide range in methane production (Ward et al. 2014).

For production of microalgae two different techniques are used: raceways or photobioreactors. A raceway or raceway pond is an open pond in which the algae are cultivated. The raceway is a shallow so the algae always are exposed to the sunlight. The algae in the raceway pond are circulated by paddlewheels, so the nutrients are evenly distributed (Oilgae 2015a). Photobioreactors is a close system, which provides a controlled environment. The closed system provides a controlled cultivation where the requirements for algae can be fulfilled (Oilgae 2015b). The raceways are cheaper to produce but the photobioreactor produces more algal biomass (Montingelli et al. 2015). A raceway can produce about 10-25 g/m^2, d while the photobioreactor can produce 25-50g/m^2, d (Montingelli et al. 2015). A system with photobioreactors has not yet been produced to be cost-effective. By incorporating microalgal production with wastewater treatment it might become feasible. Raceways have been used to treat municipal, agricultural and industrial waste waters (Montingelli et al. 2015). Using algae to remove nutrient from wastewaters is another good way to make use of the algae, they need N, P and C which is present in wastewaters (Montingelli et al. 2015). Microalgae can be cultivated in the final steps of wastewater treatments, where nutrient recovery, water treatment and biomass production can be done simultaneously (Olsson et al. 2014). Microalgae remove nitrogen and phosphorous from the waste water (Olsson et al. 2014). Microalgae use these nutrients in the photosynthesis and produces dissolved oxygen which oxidizes the water (Montingelli et al. 2015).

Algal biomass contains different components that will affect the biogas production; moisture content, lipids, carbohydrates, proteins, ash content and lignin fraction (Montingelli et al. 2015). To solve the moisture issue, a drying step has been evaluated but this will affect the process costs. Lipids have the highest methane value compared to carbohydrates and proteins (Montingelli et al. 2015; Ward et al. 2014). In microalgae the lipid content is approximately 3-20 % of the dry weight. Which make the microalgae suitable for anaerobic digestion, but the lipids fermentation will slow down the hydrolysis. This is why the microalgae are often used for production of oils (Montingelli et al. 2015). Microalgae can also be high in carbohydrates, as starch, sugar, cellulose and polysaccharides, and this makes them suitable for anaerobic fermentation (Ward et al. 2014). The ash content will vary with the season, higher ash content will be found during the winter. Lignin is almost not present in microalgae, 2 % of its dry weight is lignin. A low lignin content provides high hydrolysis rates in both ethanol and biogas production (Montingelli et al. 2015).

The C/N ratio during anaerobic digestion should be about 20-30 according to (Yen & Brune 2007) however, according to (Montingelli et al. 2015) the C/N ratio should be about 20-25. If the ratio is too low, nitrogen will be released and it will turn into ammonium ion (Montingelli et al. 2015). High ammonium ion levels will affect the pH, the pH level increases in the digester. This will lead to a toxic affect on the methanogens population. The C/N ratio of an algal biomass is about 10, which is lower than the desired ratio (Montingelli et al. 2015). Such low C/N ratio during anaerobic digestion could lead to inhibitory high concentration of TAN and VFA (Yen & Brune 2007). By adding carbon-rich materials the inhibiton by ammonium ion might be avoided. (Montingelli et al. 2015) showed that by adding 50 %VS of waste paper, the methane production increased about 100 %. Co-digesting substrates can increase the methane yield, compared to single digestion of the substrates. Co-digestion has some benefits; lower the concentration of the toxic ammonia, increased loading rate and a higher biogas yield. The biomass of the algae represents a big source of fuel and recovered nutrients as nitrogen and phosphor. Algae use the photosynthesis to store solar energy, through anaerobic digestion this energy will be released as methane (Yen & Brune 2007).

Another inhibitor to a high biogas yield may also be the cell walls, if they are thick and strong. Pretreatments can help to increase the digestibility or the algae. Physical-chemical treatments can be; mechanical shearing, sonication, thermal process, alkaline treatment or combination of treatments. Pretreatments can help the digestion of the algae but it is not sure if the increased biogas yield will make up for the pretreatment costs (Wang et al. 2013).

2.3.2. Biosludge

A pulp and paper mill produces many types of wastewater. The wastewaters have different characteristics because of the differences in the pulp and paper process and its raw materials (Bayr & Rintala 2012). In pulp and paper mills wastewater treatment activated sludge plants is often used and before the activated sludge plant comes sedimentation or flotation. It is in the sedimentation or flotation the primary sludge is produced. Primary sludge contains of wood fibers (Cellulose, hemicelluloses and lignin), papermaking fillers, ash etc. While secondary sludge (biosludge) consists of microbial biomass, cell-decay products and non-biodegradable lignin precipitates (Bayr & Rintala 2012). Today the two sludge's mixes and thickened before incineration, which is not energy saving. The sludge can also been used as land fillers (Karlsson et al. 2011), but this has become forbidden due to emissions and it is a waste of resources (Bayr & Rintala 2012).

Anaerobic digestion is commonly used in municipal wastewaters, the usage of wastewater from a pulp and paper mill is low (Bayr & Rintala 2012). Experiments have shown however, that sludge can produce about 100-200 Nml/ g VS (Karlsson et al. 2011). The anaerobic digestion of sludge from a pulp and paper mill can be inhibited by nutrient deficiency and the content of lignin and sulphur (Hagelqvist 2013). Different methods for pretreatment of the biosludge have been developed. The methods is divided into thermal, chemical, biological and physical treatments, these treatment will enhance the anaerobic digestibility of the biosludge (Meyer & Edwards 2014). Ultrasound techniques have been proved to be the best for biosludge but hydrolytic enzyme has also given good results (Karlsson et al. 2011). However pretreatments aren't cheap, according to (Meyer & Edwards 2014) the more improvement in the anaerobic digestion after the pretreatment the more the pretreatments costs.

The pulp and paper mill used for this master thesis produces chemothermomechanical pulp, Kraft pulp and food packaging board. The mill produces sludge annually in their wastewater treatment at 18,000 tones of TS (Hagelqvist 2013). The sludge might contain traces of: sulphite, sulphate, lignin, resin acids, fatty acids, DTPA and terpens, all these components may inhibit the anaerobic digestion process. The mill starts its waste water treatment with a primary clarifier, after that comes an aerated lagoon with SRT at 21 days and the bio sludge is constant removed, the final step is a chemical flocculation (Hagelqvist 2013).

An anaerobic digestion plant on the same site as a pulp and paper mill would bring economic advantages. Lower transport costs for the sludge, maintenance and operations, high supply of low excess- heat and revenues from the production of biogas (Hagelqvist 2013). Also re-circulating the reject water from the anaerobic digestion plant to the activated sludge process could cut costs (Karlsson et al. 2011). The reject water is full of nutrients like nitrogen and phosphorus, which are needed in the activated sludge process (Karlsson et al. 2011). The digestate could be used for soil fertilizer and sold for that purpose. Other of sources of organic waste could be treated at the plant against a fee (Hagelqvist 2013).

2.4. Digestate

The digestate from anaerobic treatment is becoming more important as because of its richness in nutrients as nitrogen, phosphorus and organics (Sapp et al. 2015; Zeshan & Visvanathan 2014). The digestate can be used as fertilizers on arable land or in forests, it can be a substitute for mineral fertilizers (Weiland 2010). Digestate has also proven to be a good soil fertilizer since it contains phosphorus, phosphorus is a finite resource and its production has a high environmental impact based on transports and raw materials (Sapp et al. 2015).

Table 1 Restrictions of heavy metals for digestate usage (mg/kg TS) (SP 2015; Avfall Sverige 2012)

	Lead, Pb	Cadmium, Cd	Copper, Cu	Chrome, Cr	Mercury, Hg	Nickel, Ni	Zinc, Zn
Biofertilizer[1]	100	1	600	100	1	50	800
Fertilizer[2]	100	2	600	100	2,5	50	800

The digestate can also contain heavy metals, pathogens and organic pollutants. Restrictions and standards to keep these hazards to a minimum have been implemented in countries worldwide (Sapp et al. 2015). These restrictions will determine what can be done with the digestate based on trace metal content (Karlsson et al. 2011; Montingelli et al. 2015). Table 1 shows the restrictions of heavy metal content in the digestate as biofertilizer and fertilizer. In Sweden a certification system has been developed to evaluate if digestate can be qualified as usable biofertilizer. SP Technical Research Institute of Sweden and other interests have implemented this optional certification system (Biogasportalen 2014c). Table 2 shows the restrictions of nutrients in the digestate. If the digestate doesn't qualify as biofertilizer it still can be used on arable land, but not under the name of biofertilizer.

Table 2 Restrictions of nutrients in digestate (mg/kg TS) (Avfall Sverige 2012)

NH4-N	Tot-P
150000	22000

The digestate might contain methane formation potential, which can contribute to the climate change (Zeshan & Visvanathan 2014). Emissions like ammonia, carbon dioxide and nitrous oxide could occur. Because of these emissions different ways of managing the digestate has been developed (Zeshan & Visvanathan 2014).

The digestate could either be directly used on agricultural land or be subjected to an aerobic process before land use. Large amounts of digestate are produced every day, due to this all the digestate can't be applied on the land at the same time (Zeshan & Visvanathan 2014). This is because of crop growth, type of soil etc. The digestate is because of this stored for a few months, it can also be dewatered for easier transportation. The liquid can be used as fertilizer for agricultural land, re-cycled back to the anaerobic digestion process or treated in a waste water treatment plant. The dewatered digestate can be composted or cured, and can also be used as land fillers (Zeshan & Visvanathan 2014).

3. Research methodology

To answer the first research question for this thesis laboratory experiments has been used. This chapter aims at explaining why and how the laboratory experiments have been conducted. Based on the results from the laboratory experiments research question two can be calculated and answered.

3.1. Research strategy

This thesis investigated the consequences of co-digesting algae with biosludge för biogas production, which are rare. This goes hand in hand with what Saunders et al. (2009) claims is exploratory research. Exploratory research, described by the authors is to discover new information, what happens in different situations, and to seek understanding.

Laboratory experiments were conducted to find the best proportion of algae and biosludge for anaerobic digestion, and to produce the highest amount of biogas. The economic feasibility was examined and calculated using the results of the laboratory experiments.

This type of research could to some extent be done by modeling and calculations, but to get more realistic results laboratory experiments was chosen as a research design. The experiments had to be done in laboratory scale, because full-scale experiments could not have been conducted. This because it is impossible to build a full-scale biogas plant and using an existing plant is not an option. Both these options are not feasible concerning finances and time. In laboratory scale experiments the conditions can be monitored and controlled (Bryman 2011). The research questions will be answered based on the results from the experiments. The main focus of this thesis is to answer the research questions and provide the field with new knowledge.

3.2. Literature study

This master thesis is conducted within the field of biogas production, and in particular the biogas production by co-digestion algae and bio sludge. The literature study made has focused on research materials and research articles within biogas production, utilization of algae and bio sludge. The body of the literature study lies in the current research of this field.

The literature study provides a constant flow of information on the chosen subject and research field. A well planned and carefully performed literature study can provide you will valuable information about what is already known in the area of interest, concepts and theories, controversies and also unanswered research questions (Bryman & Bell 2011). It is these unanswered research questions or gaps that will bring meaning to the study. A disadvantage of doing a literature study is the limited amount of time to conduct it and the unlimited amount of literature (Saunders et al. 2000). To be able to conduct the literature research, important key words and research parameters must be identified. A good discussion with your supervisor can also be useful during the literature study (Bryman & Bell 2011).

Defined search parameters:
Language: English and Swedish
Subject area: Biogas, algae, bio sludge, co-digestion
Business area: Renewable energy

Literature type: Journals and books
Databases: Business source premium, Scopus and Google scholar, Library database
Keywords: Biogas, biogas production, algae, bio sludge, anaerobic digestion, co-digestion

3.3. Experimental setup

The laboratory experiments were done in three rounds, all rounds contained the same mixtures as shown below:

- Bottle 1-3: only inoculum
- Bottle 4-6: biosludge + inoculum
- Bottle 7-9: 1/3 algae + 2/3 biosludge + inoculum
- Bottle 10-12: 2/3 algae + 1/3 biosludge + inoculum
- Bottle 13-15: algae + inoculum

3.3.1. Substrates and inoculum

To conduct these experiments two different substrates was used. Inoculums come from Sjöstad wastewater treatment plant in Karlstad, the inoculums are the anaerobic microorganisms that will be used to break down the organic materials. The inoculums are stored in 55° C for a few days before the experiments started so the methane can be released. For each round of experiment fresh inoculums must be used, because if the inoculums are not used they will die within a couple of days. Table 3 shows the TS (%) and VS (%) values for the inoculum.

Table 3 TS and VS values for the inoculums

	Round 1	Round 2	Round 3
TS (%)	0,32	2,65	2,22
VS (%)	0,20	1,68	1,41

The algae were collected from a pilot-/testfacility at Bäckhammars bruk outside Kristinehamn. It was stored in a refrigerator after being collected. The algae used for these experiments are of two different mixtures. The first algae (AT3) that was tested in round 1 and 2 contained three different species: S. quadricauda, Monoraphidium and Chlorella. The second mixture (AT5) that was tested in round 3 contained Monoraphidium, Granström[1]. The C/N ratios of the mixtures was 3,9 and 1,5 respectively, Granström[1]. The TS (%) and VS (%) value of the algae is shown in table 4. In previous studies AT3 produced the least amount of biogas and AT5 produced the most biogas.

Table 4 TS and VS values for the algae

	Round 1	Round 2	Round 3
TS (%)	0,76	0,76	1,04
VS (%)	0,64	0,64	0,88

The biosludge that comes from Skoghall pulp and paper mill were stored in a refrigerator. Biosludge were be used in all three rounds, however in round three a new batch of biosludge were used. Table 5 is showing the TS (%) and VS (%) values of the biosludge in each round.

[1] Karin Granström Docent Karlstad University, meeting 20 March 2015.

Table 5 TS and VS values for the biosludge

	Round 1	Round 2	Round 3
TS (%)	2,48	2,48	2,59
VS (%)	2,08	2,08	2,22

3.3.2.AMPTS2

The equipment used to execute the experiments was a AMPTS2 (Automatic Methane Potential Test System), from Bioprocess Control. The bottles were filled with substrates and will act as reaction-chambers during the anaerobic digestion. The system was hooked up with the use of a detailed user manual. In the reaction-chamber the substrates were intermittently stirred, the stirring was active 60 seconds at a time and still for the same amount of time. The gas produced in the reaction-chamber was transported through a gas-pipe to bottles containing a mixture of NaOH, which absorbed the carbon dioxide. After the absorption of carbon dioxide, the methane gas continued through the gas-pipe, where the amount of methane gas was measured and registered. The AMPTS2 was connected to a computer where the accumulated amount of methane gas was showed.

Figure 3 The equipment AMPTS2. The digestion chamber is to the left, in the middle is the CO2-fixation unit and to the right is where the produced gas is measured.

The experiments were divided into three rounds. The equipment included 15 bottles that contain the substrates. Three bottles in each round must contain the inoculum, because later on when analyzing the results the inoculums methane contribution must be withdrawn. In each round four different mixtures of substrates can be tested in the bottles, and each mix will be done in triplicate. Each round will take 20 day to conduct before the results can be analyzed. The bottles were placed in a water bath, using distilled water and at a constant temperature of 55°C. The anaerobic digestion will occur under thermophilic conditions (Weiland 2010).

Before the experiments could start volatile solids (VS %) and total solids (TS %) of each substrate had to be calculated. The calculated VS-value was used when the experiments starts, the value of VS (%) for each substrate shall be logged in the software program Bioprocess control on the computer. The program then calculates how much VS (g) of each substrate that is required in the bottles. Each bottle contains 400 g of substrates. Based on the amount of VS (g) and the VS (g VS/g substrate) in each substrate, the required amount of each substrate could be calculated.

3.3.3. VS and TS

Some preparations before calculating the values of VS and TS has to be done, first an aluminum form has to be weight. The form is then filled with 20 ml of the substrate. The aluminum form is placed in an oven for 24h at 103°C. After 24h the form were weight again, the weighing shall be done directly after taking the form out of the oven, otherwise it soaks up the moisture in the surrounding atmosphere. After weighing the aluminum form were placed in the oven for 20 minutes at 550°C, and then weight again as quickly as possible. This procedure shall to be done for each substrate used in the experiments. After all steps has been done TS (%) and VS (%) can be calculated as shown in equation (1) and (2). To get VS (g VS/g substrate) you don't multiply by 100.

$$TS(\%) = \frac{V_{103} - V_{empty}}{V_S - V_{empty}} \cdot 100 \qquad (1)$$

$$VS(\%) = \frac{V_{103} - V_{550}}{V_S - V_{empty}} \cdot 100 \qquad (2)$$

Where:

V_{103}= The weight of the aluminum form after drying in 103°C during 24h

V_{empty}= The weight of the aluminum form empty

V_S = The weight of the aluminum form with algae/ sludge

V_{550}= The weight of the aluminum form after incineration at 550°C

3.3.4. Round 1

In round one the ratio of substrate and inoculum is 1:1. The software program indicated how much VS (g) of each substrate that were needed. In each bottle with substrate the amount of VS (g) algae/biosludge must be the same as VS (g) inoculums. The calculations for bottles 4-6 and 13-15 are shown in equations (3) and (4).

$$g_{Algae} = \frac{VS(g)}{VS(g_{VS} / g_{Algae})} \quad (3)$$

$$g_{Biosludge} = \frac{VS(g)}{VS(g_{VS} / g_{Biosludge})} \quad (4)$$

The amount of substrate in the bottle should be 400g, so the amount of inoculums is shown in equation (5);

$$g_{Inoculums} = 400 - g_{Algae} - g_{Biosludge} \quad (5)$$

The software program don't take into consideration that the substrates can be mixed, because of that an average concentration must be calculated first. This average concentration of VS (%) is then logged in the software, and the software provides the amount of VS (g) needed for inoculums and substrates. The average concentration of bottles 7-9 is shown in equation (6) and bottles 10-12 in equation (7)

$$VS(\%)_{Average} = \frac{VS(\%)_{Algae} + (2 \cdot VS(\%)_{Biosludge})}{3} \quad (6)$$

$$VS(\%)_{Average} = \frac{(2 \cdot VS(\%)_{Algae} + VS(\%)_{Biosludge})}{3} \quad (7)$$

The amount of algae and biosludge can be calculated based on the amount of VS (g) provided by the program. In bottle 7-9 one third of the substrate were algae, the amount of algae were calculated with equation (8) to (9).

$$VS(g)_{a\lg ae} = \frac{VS(g)}{3} \quad (8)$$

$$g_{\mathrm{Alg}ae} = \frac{VS(g)_{\mathrm{Alg}ae}}{VS(g_{VS} / g_{\mathrm{Alg}ae})} \quad (9)$$

Meanwhile the amount of biosludge was calculated using equation (10) to (11). The amount of inoculums was calculated by equation (5).

$$VS(g)_{Biosludge} = \frac{VS(g) \cdot 2}{3} \quad (10)$$

$$g_{Biosludge} = \frac{VS(g)_{Biosludge}}{VS(g_{VS} / g_{Biosludge})} \quad (11)$$

For the bottles 10-12 the average concentration has been calculated in equation (7). The amount of algae and biosludge can be calculated, it is in a similar way as with bottles 7-9, but the ratio is different. To calculate the amount of algae for bottles 10-12 are calculated using equation (12) and equation (9). The amount of bio sludge used in bottles 10-12 is calculated by equation 13) and equation (11). The inoculums used in these bottles are determined by equation (5). Table 6 is showing how much if each substrate that were used in each bottle.

$$VS(g)_{a\lg ae} = \frac{VS(g) \cdot 2}{3} \quad (12)$$

$$VS(g)_{Biosludge} = \frac{VS(g)}{3} \quad (13)$$

Table 6 The amount of substrate in each of the triplicates (g)

Bottles	Algae	Biosludge	Inoculum
1-3	-	-	400
4-6	-	35	365
7-9	36	22	324
10-12	70	11	319
13-15	94	-	306

When 20 days has gone, the digestate were analyzed before the next round was started. VS and TS were measured for each digestate as well as pH and total ammonium nitrogen (TAN).

3.3.5. Round 2

Based on the result of pH and TAN of the digestate from the first round a decision was made about how to proceed with round two. The pH value of the digestate was a bit high. To increase the alkalinity in the second round sodium carbonate hydrate (Na_2CO_3) was added to the inoculum, 3,29 g Na_2CO_3/l inoculum.

In round two new inoculums were used, the concentration of inoculums for this round was much higher so the ratio between substrate and inoculums were 1:2. In each bottle with substrate the amount of VS (g) inoculums must be twice as much as VS (g) algae/biosludge. The calculations are the same as in round one, determining how much of each substrate should be in each bottle, this is shown in table 7.

Table 7 The amount of substrate in each of the triplicates (g)

Bottles	Algae	Biosludge	Inoculum
1-3	-	-	400
4-6	-	115	285
7-9	115	70	215
10-12	200	31	169
13-15	227	-	173

3.3.6. Round 3

In round three a new mix of algae (AT5) was used. In this round nothing was used to alternate the value of pH, since the use of pretreatment of the inoculums will generate a cost. Calculations for the amount of substrates used in each bottle were the same as in round one and two, as shown in table 8. Also, in this round the ratio between substrate and inoculums was 1:2.

Table 8 The amount of substrate in each of the triplicates (g)

Bottles	Algae	Biosludge	Inoculum
1-3	-	-	400
4-6	-	96	304
7-9	76	60	264
10-12	139	28	233
13-15	177	-	223

3.3.7. pH and TAN

The value of pH and TAN was measured for each substrate and each digestate. To measure the TAN the substrate or digestate first needs to be filtered. The liquid is separated from the dry substance by using a filtration device. When the substrate or digestate is filtered, 200ml of the filtrate is filled into a test tube, type Hach Lange LCK 305 which is used for ammonium concentrations between 2-47 mg/l. After 15 minutes the test tube is placed in a photo spectrometer Hach Lange DR 2800 that will measure the ammonium concentration. The pH value is measured using a ph- meter of the type Mettler TOLEDO.

Then TAN and pH was measured, free ammonium nitrogen (FAN) could be calculated as following (14). FAN is depending on TAN, pH and temperature (Kayhanian 1999).

$$NH_3 = \frac{TAN \cdot \frac{K_A}{[H]}}{\frac{K_A}{[H]} + 1} \quad (14)$$

Where:

NH3 = Free ammonium concentration mg/L

TAN = Total ammonium nitrogen concentration mg/L

K_A = Temperature dependent constant = ($3{,}77 \cdot 10^{-9}$ @550 °C)

[H] = Hydrogen ion concentration = 10^{-pH}

3.3.8. Digestate

The digestate from the three different rounds as analyzed by comparing the digestates nutrition and heavy metal content with the allocated values as noted in table 1 and 2. The amount of heavy metals in the biosludge is shown in table 9. The nutrient content of both the algae and biosludge is shown in table 10. The digestate can be certified as biofertilizer or be used as fertilizer, both can be used on arable land.

Table 9 Heavy metal content in biosludge in 2007 (mg/kg TS) Vieweg[2]

Lead, Pb	Cadmium, Cd	Copper, Cu	Chrome, Cr	Mercury, Hg	Nickel, Ni	Zinc, Zn
10	2	40	39	0	19	250

[2] Lennart Vieweg Stora Enso Skoghall, mail conversation 29 April 2015.

Table 10 Nutrient content of algae and biosludge (mg/kg TS)

	NH4-N	Tot-N	Tot-P
Algae (T3)[1]	6000	50000	15000
Algae (T5)[1]	10000	67000	14000
Biosludge[2]	4200	40000	4700

3.4. Economy

Since the cultivation of algae still is in its test-phase, not much information about the costs for cultivating the algae is available. According to Karlsson[3] the test-facility at Bäckhammars Bruk contains of one raceway and eight pools, which is approximately an area of 41 m^2. Theoretically in Sweden about eight kg of algae per m^2 can be produced (Pettersson 2015). Ekendahl[4] explained that in 2014 the harvesting of algae reached a total of 26 kg TS, which is a low amount of algae produced. To Ekendahl[4] knowledge, the investment cost for this test-facility of one raceway and eight pools is 115 000 kr. According to Ekendahl[4] the current production cost for algae is about 100 000kr/ kg, however this number is all costs of the project divided by the algae produced. For this test-project no actual production cost for the algae have been calculated.

For this master thesis, it was assumed that Stora Enso Skoghall will build a similar facility for cultivating algae, and the biogas-plant also is located there. The biosludge used as a co-substrate is a waste product, so there is no production costs for the biosludge. Today Stora Enso Skoghall produces about 18,000 tonne TS biosludge a year, the biosludge is incinerated and produces 64GWh heat. If this scenario is used transportation costs of substrates can be neglected. Distribution of the biogas will however be taken into consideration.

To be able to calculate the costs and revenues, the biogas produced in the experiments must be re-calculated into different units. The amount of biogas that can be produced during a year were calculating using the following

[3] Carl-Anton Karlsson Nordic Paper, mail conversation 19 March 2015.
[4] Susanne Ekendahl SP, mail conversation 18 March 2015.

equation (15). Where Biogas (Nml/g VS) is the produced biogas from the laboratory experiments and VS (g) is the amount VS of the substrates used during a year. To re-calculate the amount of biogas to the unit Nm^3 equation (16) was used.

$$Biogas(Nml) = \frac{Biogas(Nml/gVS)}{VS(g)} \quad (15)$$

$$Biogas(Nm^3) = Biogas(Nml) \cdot 0{,}000001 \quad (16)$$

The biogas, have an average energy value of 8 kWh/Nm^3, which has been used for further calculations.

Costs for production of biogas are mainly the costs of production of algae and operation costs of the biogas-plant. The investment of the biogas-plant will not be taken into consideration, however it will operate at a cost of 0,3 kr/kWh. Costs for upgrading the biogas and distribution will be added at a cost of 0,45 kr/kWh (Ekendahl et al. 2012). Maintaining the operation of the biogas-plant / algae cultivation requires at least one employee with a monthly salary of 24 000, cost for maintenance about 100 000 a year.

The amount biogas that is produced will be sold to the current market price, another revenue might be the digestate. The algae use CO_2 in their cultivation. The price for emission rights of CO_2 in Europe is about 90kr/tonne. So for every tonne CO_2 the algae uses, 90kr can be saved. Skoghalls Bruk had about 49 855 tonne fossil carbon dioxide emission in 2014 (Stora Enso 2015). According to (Truong et al. 2010) the biogas could be sold for 10 kr/ Nm^3. The digestate can be sold for approximately 0, 15 kr/kg if it is usable (Ekendahl et al. 2012).

Three different scenarios were calculated, one with the 26 kg TS algae harvested 2014, the second with the theoretically possible amount of algae that can be harvested, which is 328 kg TS. The third scenario was a bigger facility at 500 m^2, which can produce theoretically 4000 kg TS algae per year. The bigger facility has an investment cost at 1,300 000 kr (Ekendahl et al. 2012).

Based on the result of the experiments another scenario has been developed. This scenario would investigate the biogas production of just biosludge, since the biosludge alone produces a high amount of biogas. The same production-, and distribution-costs will be used as before and the revenues will be the same.

4. Results

This chapter provides the result of the first research question of the thesis: "What proportion of algae/biosludge for co-digestion, will be the best for producing the highest amount of biogas?". Thereafter, the results of the calculations are presented.

4.1. Experimental results

4.1.1. Round 1

The result from the first round is shown in figure 4. The figure 4 shows differences within the triplicates of the four mixtures. In figure 5, the average methane production is shown. Shown in figure 4 and figure 5 are that biosludge and algae separated produces high amounts of methane respectively, and the mixtures with algae and biosludge produces less.

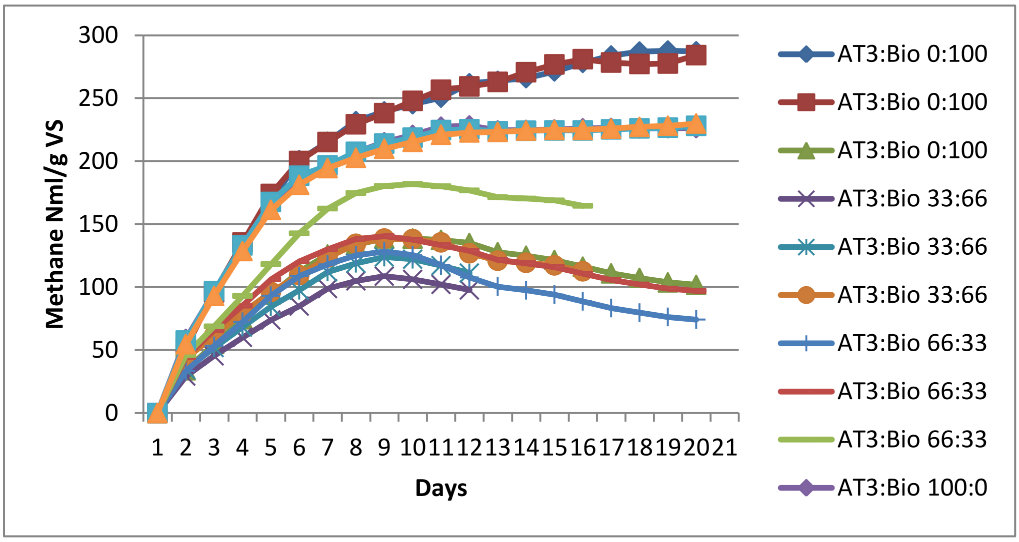

Figure 4 Accumulated methane production without the contribution of inoculums

The algae alone produced a maximum 228 Nml/g VS. The biosludge produces separately a maximum of 225 Nml/g VS, which is really high. Normally biosludge produces between 100-200 Nml/g VS (Karlsson et al. 2011). In figure 5 the methane production reaches a maximum of 150 Nml/g VS, looking at the mixture of 2/3 algae (66:33), meanwhile the ratio with 1/3 algae (33:66) produces a maximum of 124 Nml/g VS.

Some bottles stopped producing methane before the end after 20 days. The anaerobic digestion might have stopped because the substrates had been fully digested or the anaerobic digestion had been inhibited.

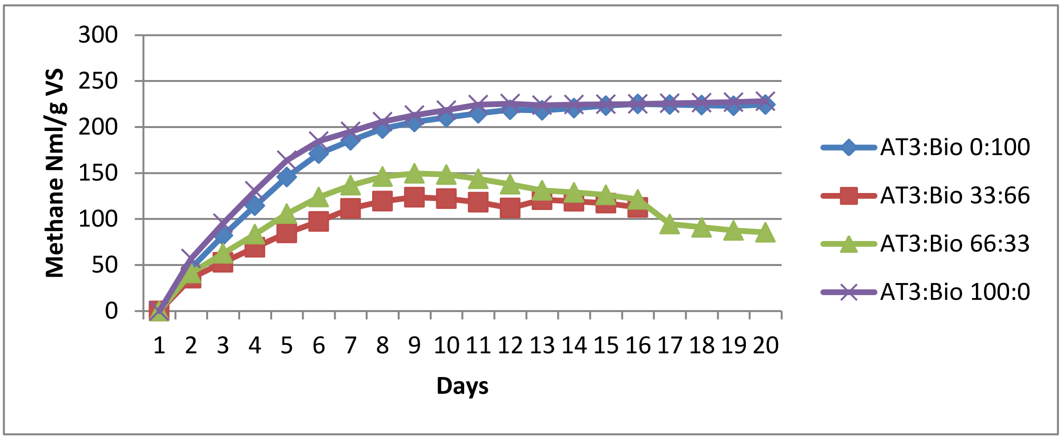

Figure 5 Average methane production of the triplicates without the contribution of inoculums

Results from analyzing the substrates after anaerobic digestion is shown in table 11. The value of pH is closer to 8 than 7. Optimum pH levels during anaerobic digestion should lie between 7- 8 (Weiland 2010). The levels of total ammonium nitrogen (TAN) and free ammonium nitrogen (FAN) are a bit high and over the optimum levels in some bottles, which are 1500 mg/l and 80 mg/l respectively (Montingelli et al. 2015).

Table 11 Analyses of the digestate after anaerobic digestion

Bottles	pH	TAN (mg/l)	FAN (mg/l)
1	7,80	1656	318,2
2	7,90	1216	280,2
3	7,85	1556	327,8
4	7,65	1048	151,0
5	7,72	1172	193,6
6	7,79	1032	194,6
7	7,85	1024	215,7
8	7,84	604	124,9
9	7,81	1592	311,7
10	7,76	564	100,5
11	7,78	1300	240,6
12	7,79	764	144,1
13	7,71	512	83,0
14	7,72	820	135,4
15	7,73	752	126,6

4.1.2. Round 2

In round two the methane produced is shown in figure 6. The algae alone were producing the highest amount of methane in this round. In figure 7 it is easier to see the difference between the substrates, in the figure the average value of the triplicates are shown. The algae produced methane alone at a maximum of 228 Nml/g VS. The biosludge produced a maximum 174 Nml/g VS. The best mixture were the ratio of 2/3 algae (66:33), produced 166 Nml/g VS. The lower ratio with 1/3 algae (33:66) produced 161 Nml/g VS.

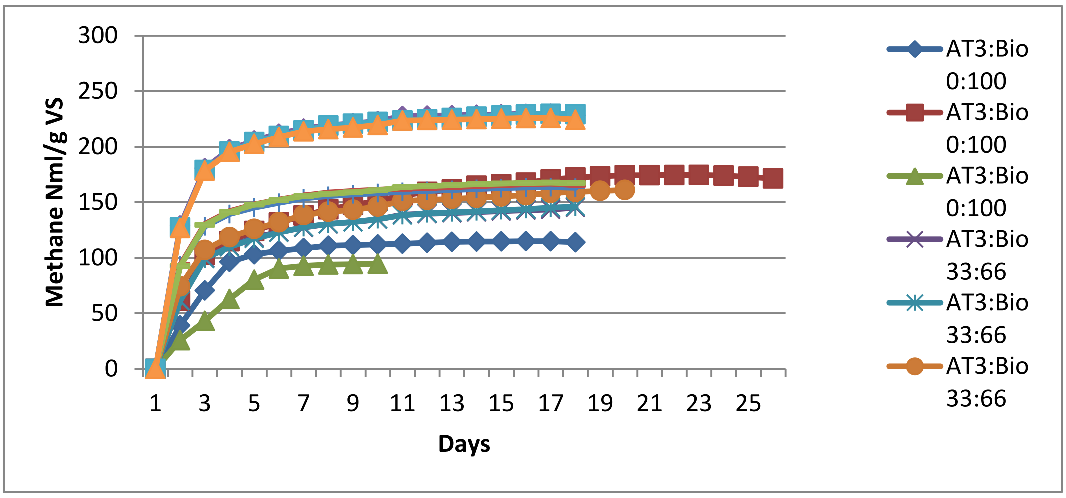

Figure 6 Accumulated methane production without the contribution of inoculums

In figure 7, when focusing on the development until day 10 the algae is the highest methane producing substrate, and the biosludge is at this time the lowest producing substrate. Both mixtures are better than the biosludge, the mixture with 2/3 algae is just slightly better. As in round one some bottles stopped producing biogas within these 20 days, this might be caused by low amount of substrate to digest or inhibition.

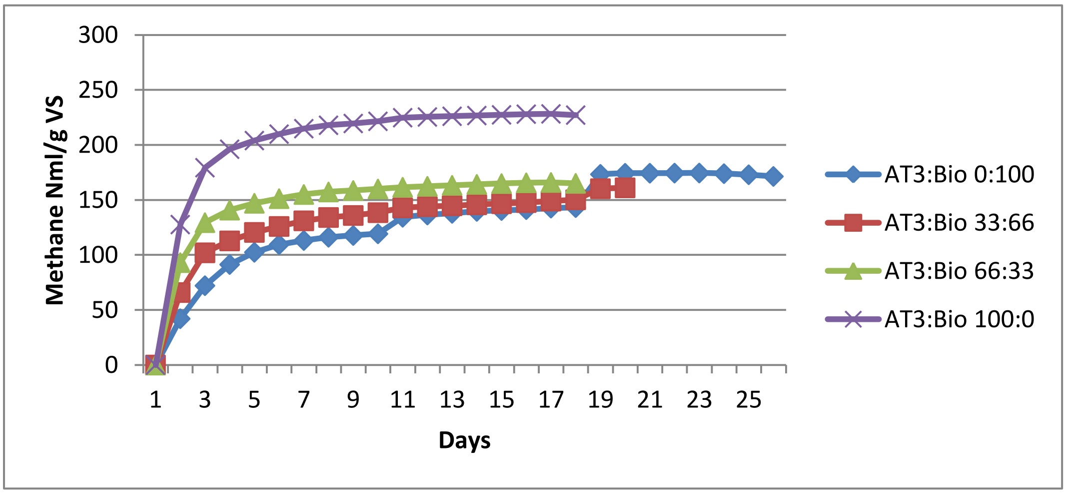

Figure 7 Average methane production of the triplicates without the contribution of inoculums

The result of the digestate analysis is shown in table 12. In this round, round two, the inoculums underwent a pretreatment to lower its pH-value. As a result of that the majority of the total ammonium nitrogen (TAN) and free ammonium nitrogen (FAN) values became lower than in round one. Some of the values are still above the limits for inhibition.

Table 12 Analysis of the digestate after anaerobic digestion

Bottles	pH	TAN (mg/l)	FAN (mg/l)
1	7,95	592	148,89
2	7,99	1040	280,00
3	8,01	768	213,80
4	7,75	1060	185,41
5	7,70	528	83,91
6	7,81	704	137,82
7	7,69	716	111,60
8	7,68	664	101,50
9	7,69	472	73,57
10	7,62	604	82,03
11	7,60	520	67,86
12	7,63	484	67,05
13	7,68	612	93,55
14	7,64	353	49,85
15	7,67	512	76,75

4.1.3. Round 3

In the final and third round another algae mix was used and the result of the anaerobic digestion is shown in figure 8 and 9. The average methane production is lower in this round compared to the first two rounds.

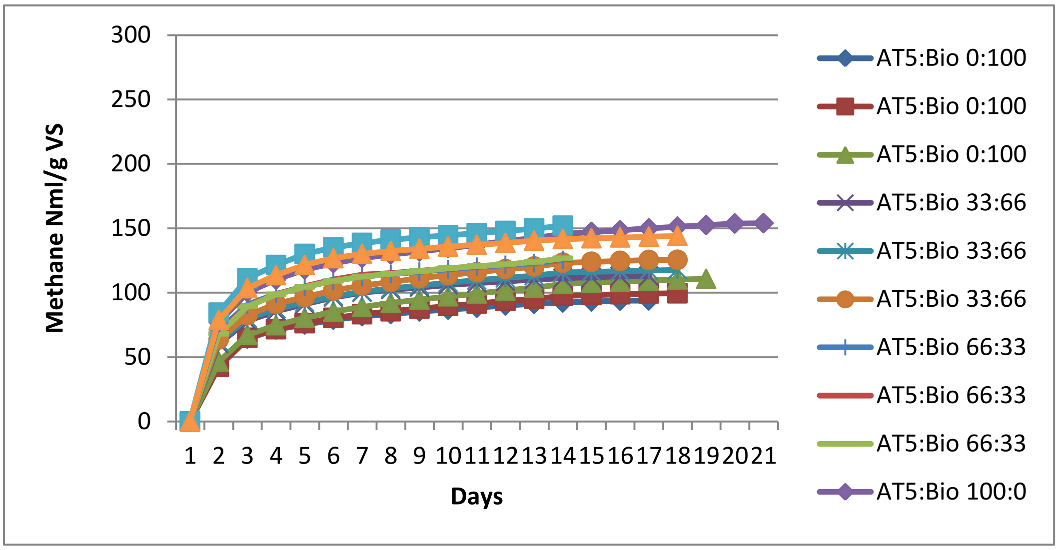

Figure 8 Accumulated methane production without the contribution from the inoculums

In this round the triplicates showed a more similar production if compared to the other two rounds. The algae alone is still the highest methane producing substrate, production is at 154 Nml/g VS. The result of the anaerobic digestion shows that the ratio of 66:33 is slightly better, it produces a maximum of 126 Nml/ g VS methane and the ratio of 33:66 produces a maximum of 121 Nml/g VS methane. The biosludge alone produces methane at a maximum of 111 Nml/g VS.

In this round a similar trend compared to round two can be seen. The algae is the highest producing substrate followed by the mixture with 2/3 algae and 1/3 algae. Biolsudge is the lowest methane producing substrate.

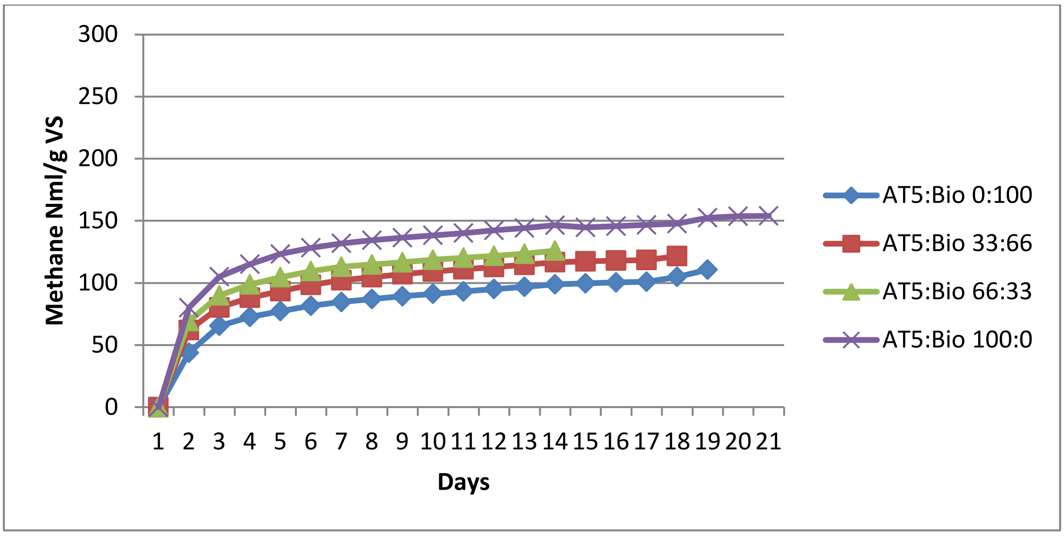

Figure 9 Average methane production of the triplicates without the contribution of inoculums

The results from the analyzis of the substrates after anaerobic digestion is presented in table 7. The values of total ammonium nitrogen (TAN) are laying beneath the inhibition limits of 1500 mg/l however some of the free ammonium nitrogen (FAN) values are over 80 mg/l, and might be inhibitory. It might explain why the methane production stopped for some bottles before 20 days.

Table 13 Analysis of the digestate after anaerobic digestion

Bottles	pH	TAN (mg/l)	FAN (mg/l)
1	7,72	800	132,1
2	7,68	852	130,2
3	7,72	924	152,6
4	7,59	848	108,5
5	7,64	488	69,0
6	7,62	584	79,3
7	7,58	920	115,3
8	7,57	404	49,6
9	7,56	576	69,4
10	7,56	916	110,3
11	7,54	383,2	44,3
12	7,57	604	74,2
13	7,36	644	51,2
14	7,54	328,8	38,0
15	7,54	564	65,2

4.1.4. Summary of the experimental results

Table 14 and 15 below shows a summary of all the result from the experiments in each round. Table 14 shows the average and standard deviation of the methane production in the three rounds. Algae produced the highest amount of methane, 203,5 Nml/g VS, based on all three rounds. Biosludge produced an average of 170 Nml/gVS. The mixtures produced the least amount of methane. The ratio of 1/3 algae and 2/3 algae produced 135,5 Nml/g VS and 147,2 Nml/g VS respectively.

Table 14 Maximum methane production, average production and standard deviation of the mixtures.

	Biosludge	1/3 Algae	2/3 Algae	Algae
Round 1	225,1	123,7	149,6	228,1
Round2	174,1	161,1	166,0	228,3
Round 3	110,7	121,6	126,0	154,0
Average	170,0	135,5	147,2	203,5
Standard deviation	97,0	22,2	20,1	42,8

For each round both average and standard deviation values are calculated. The free ammonium nitrogen (FAN) is in general lower in round three, compared to the other rounds, however round 3 produced less methane shown in table 14. The values of FAN are the highest in round one, followed by round two. Round one and two were the highest producing rounds.

Table 15 Average value and standard deviation of Ph, TAN and FAN

Table 15Average value and standard deviation of pH, TAN and FAN

		Round1			Round 2			Round 3		
Bottles		pH	TAN (mg/l)	FAN (mg/l)	pH	TAN (mg/l)	FAN (mg/l)	pH	TAN (mg/l)	FAN (mg/l)
1-3	Average	7,85	1476	309	7,98	800	214	7,71	859	138
	Stdev	0,05	230,7	25,1	0,03	225,7	65,6	0,02	62,2	12,4
4-6	Average	7,72	1084	180	7,75	764	136	7,62	640	85,6
	Stdev	0,07	76,6	24,9	0,06	271	50,7	0,03	354,6	20,5
7-9	Average	7,83	1073	217	7,69	617	96	7,57	475	78
	Stdev	0,02	495,8	93,4	0,01	128,5	19,7	0,01	262,7	47,8
10-12	Average	7,78	876	162	7,62	536	72	7,56	634	76,3
	Stdev	0,015	380,5	71,7	0,02	61,6	8,4	0,02	267,7	33
13-15	Average	7,72	695	115	7,66	492	73,4	7,48	512	51,5
	Stdev	0,0,1	161,8	28,1	0,02	22	22	0,10	163,8	13,6

4.1.5. Digestate

In table 16 is the result of the comparison of the digestate nutrients and heavy metals content. The result shows that the digestate can be used on arable land but not be certified as biofertilizer, since the cadmium levels are too high in the biosludge. The digestate can then be sold as fertilizer and generate an income.

Table 16 Comparing the substrates against restrictions for usage of digestate

	Restricitions biofertilizer	Restrictions Digestate	Biosludge	Algae (T3)	Algae (T5)
Lead (mg/kg TS)	100	100	10	-	-
Cadmium (mg/kg TS)	1	2	2	-	-
Copper (mg/kg TS)	600	600	40	-	-
Chrome (mg/kg TS)	100	100	39	-	-
Mercury (mg/kg TS)	1	2,5	-	-	-
Nickel (mg/kg TS)	50	50	19	-	-
Zinc (mg/kg TS)	800	800	250	-	-
NH4-N(mg/kg TS)	-	150000	4200	6000	10000
Tot-N (mg/kg TS)	-	-	40000	50000	67000
Tot-P (mg/kg TS)	-	22000	4700	15000	14000

4.2. Economics

Three scenarios have been investigated, looking at the financial aspect of the usage of algae. The scenarios are based on the result from the laboratory experiment and the digestate analysis. The highest biogas production substrate was the algae, the algae produced an average of 203,5 Nml/g VS as shown in table 15.

This value has been used to calculate the economic feasibility. Table 17 is showing the three different scenarios and the difference between them. The result shows that the usage of algae for biogas production might not be feasible. In scenario one the 26 kg TS algae could produce 0,035 MWh of biogas per year, and scenario three could produce theoretically a maximum of 5,5 MWh per year.

Table 17 Revenues and costs from biogas production by algae and biosludge

	Scenario 1	Scenario 2	Scenario 3
Biogas (MWh/år)	0,036	0,5	5,5
Investment (kr)	115 000	115 000	1 300 000
Costs (kr/år)	388 030	388 340	392 130
Revenue (kr/år)	560	7 110	86 750

Based on the laboratory experiments a fourth scenario was created, using only the biosludge. Table 18 shows the result of the calculations. The result in table 18 is based on the average production of biosludge presented in table 15, 170 Nml/g VS. Compared to the mixture of algae and biosludge the biosludge alone would produce significant much more biogas during a year, 21 GWh.

Table 18 Revenues and costs from biogas production by biosludge

	Scenario 4
Biogas (GWh)	21,0
Costs (kr/år)	15 800 000
Revenue (kr/år)	134 500 000

5. Analysis and discussion

This chapter aims to analyze and discuss both the first and second research questions of this thesis and explain this thesis contribution to the research area. This is done by analyzing the results from the laboratory experiments, and the economic calculations.

Shown in the results is that all three rounds produced biogas, round one and two produced the highest amount of biogas. In the two first rounds the biosludge and the algae separated produced the absolute highest amount of biogas, the mixtures did not produce as much. In round three the biogas production was more even than the other two rounds, the triplicates were more equal compared to the two first rounds. The difference in the triplicates in the first two rounds might be explained by different causes, amount of substrate available, inhibition etc. Inhibition strikes when in the total ammonium nitrogen (TAN) and free ammonium nitrogen (FAN) values reaches over 1500mg/l and 80 mg/l respectively (Montingelli et al. 2015).

In round one the mixture that produced the most biogas was mix with 2/3 algae (66:33) at 150 Nml/g VS, as shown in figure 5. Although, the highest methane production came from the algae, at 228 Nml/g VS. In figure 4 the variations in the triplicates of the substrates are shown, some of the triplicates have large variations for example the biosludge (0:100) and 2/3 algae (66:33). These variations might be explained by looking at the table number 11 where the TAN and FAN analyses are displayed. In table 5, large variations in the triplicates are shown. This might be the reason the triplicates act the in the way they do. Another factor might be that the inoculums in this round were much more diluted than the other sound, displayed in table 3.

The second round the inoculums where pretreated by using sodium carbonate hydrate to lower the pH-value. The result of round two showed that the highest biogas yield from the mixtures came from 66:33 at 165 Nml/g VS. As in round one, the algae produces the highest amount of methane, 228 Nml/ g VS. This is not a significant difference compared to the result in round one, a maximum extra of 15 Nml/ g VS was produced. Also in this round the triplicate acted a bit strange and not cohesive. One reason for this might be the difference in TAN and FAN values shown in table 12, another reason might be the fact that it is hard to get exactly the same amount of VS (g) in the triplicates.

In the final and third round the result of the anaerobic digestion was more evenly distributed between the triplicates and the mixtures as shown in figure 8 and 9. In this round the mixture of 2/3 algae (66:33) is the best with a maximum production of 126 Nml/g Vs. This is much lower than the other two previous rounds, almost 40 Nml/g VS compared to round two. In this round the highest biogas producing mixture is the algae alone at 154 Nml/g VS, and the biosludge is the least biogas producing mixture. The values of TAN and FAN in table 13, shows that the TAN values are significantly lower than the inhibiting value at 1500 mg/l. Meanwhile some of the FAN values are still above 80 mg/l.

Algae produced an average of 203,5 Nml/g VS and the biosludge produced an average of 170 Nml/g VS, shown in table 14. These results lie in line with previous studies. Anaerobic digestion of biosludge have resulted in a methane production between 100-200 Nml/g VS (Karlsson et al. 2011). Anaerobic digestion of microalgae only, has in reported studies produced methane gas in a rate of 70-600 Nml/g VS (Ward et al. 2014).

It was unexpected that algae digested separately produced the highest amount of biogas in the three rounds, and the mixtures produces less. In all three rounds algae produces more methane than the biosludge as well, displayed in table 14, comparing the average methane produced. No positive co-digestion effects can be seen.

The explanation cannot lie in the theory based on C/N ratios since algae is compared to a low C/N-ratio which can be an inhibited factor for AD, as the low C/N ratio manifests itself in ammonium ions (Montingelli et al. 2015). By adding a carbon rich material as biosludge the ratio should go up and boost the anaerobic digestion (Yen & Brune 2007), however in these experiments this has not happened.

Looking at the result of the comparison of the digestate and the restrictions of the usage of digestate in table 8, the digestate can be used as fertilizer. The cadmium levels of the biosludge were too high, this is why the digestate can not be certified as biofertilizer. The fertilizer will however generate revenues in the profitability prospect of the thesis (Ekendahl et al. 2012).

Using the mixture of algae and biosludge as co-substrates for biogas production is not effective, since algae produce more methane digested separately. Economically, using algae as a substrate for biogas production is not feasible in these scenarios presented in this thesis. The production costs for producing algae have not been taken into consideration, an additional cost must be added when this information is presented. The revenues presented in table 17 will not be able to pay off the invested money for algae production (Ekendahl et al. 2012).

Another alternative to cultivating algae for biogas production primarily, is to first extract the lipid content of algae and produce oil, as the primary use of algae. The algae biomass that is left might be used as substrate in biogas-production, as a waste product. Combine oil production and biogas production might be a better way to make use of the algae. Other substrates will be needed to be able to produce biogas at a reasonable and profitable level, and making the production feasible. The usage of other substrates will generate revenues as the substrates would be treated against a fee (Hagelqvist 2013).

Biogas production when digesting the biosludge produced about 21 GWh, by using the biosludge for biogas production means that Stora Enso Skoghall will lose 64 GWh heat (Hagelqvist 2013). This fact makes it not feasible to use the biosludge for biogas production instead of the heat production it is used for today.

Algae might have large advantages compared to other substrates: fast growing, large biomasses and they are good nutrient recovery organisms (Mata-Alvarez et al. 2014). They also use carbon dioxide with can help with the global warming (Montingelli et al. 2015). Last year there was a low production of algae, 26 kg TS, when calculating theoretically on the same test-facility the production could be about 328 kg TS per year. To have a feasible biogas production with algae, the cultivation of algae must be efficient and the harvesting may not be this low. The pilot- project at Bäckhammars bruk mission is to determine the best way to extract the energy out of the algae. The pilot–project is focused on producing biodiesel from the cultivated algae, so for this thesis the main focus has been on biogas production. Although, combining the two processes might be a good solution.

This thesis might help them to determine that using the algae primary for biogas production is not the way to go, until the methane production ratio increases or the investment- and production cost decreases. Meanwhile existing substrates should be used for biogas production to help lower the demand of fossil fuels, and lower the increasing carbon dioxide emissions to the atmosphere (Weiland 2010).

6. Conclusions and future research

This master thesis handled the problem of co-digesting of algae and biosludge for production of biogas. The problem had been divided in to two research questions: "*What proportion of algae / biosludge for co-digestion, will be the best for producing the highest amount of biogas?*" and the second "*Is it profitable to use algae as a substrate for biogas production?*"

The first question was answered by the results from the laboratory experiments. The second were answered by economical calculations, the calculations was based on the result from the laboratory experiments.

The answer to research question one is that the mix with 100 % algae was the highest producing proportion. The algae produced the highest methane in all three rounds, in round 2 the highest production reached a maximum of 228,3 Nml/g VS. Both biosludge and algae produced more biogas than the mixtures in all three sounds, which was not the expected result.

To answer research question two the result from research question one was used. The calculations are based on the mixture of 100 % algae, which produced an average of 203,5 Nml/g VS. Based on the result shown in table 17, using algae at present time for biogas production is not feasible.

The conditions change before the biogas production of algae could be feasible. The investment costs and production costs needs to decrease, and the harvesting must be efficient for the biogas production to be feasible. Cultivation of algae for just biogas production is not economically possible or viable. On the other hand cultivation algae has advantages presented in this thesis, however a better way for energy extraction must be determined. Using algae for both oil production and biogas production might be the way to go. First extract the lipid content from the algae for oil production and later use the biomass for biogas production in a biogas plant. Other substrates could be used as well, to make the biogas production more feasible, since the substrates would be treated against a fee. Further research would be to investigate this alternative deeper and see if it might be more economically feasible compared too merely biogas production.

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