Algae Energy

A Perspective on Algae as a Biogas Source in Nordic Climate

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Abstract

Anaerobic digestion of algae has gained high attention recently both as a renewable energy source and for a wastewater treatment. The biodegradability of algae, based on methane yield and biomethane potential (BMP), varies significantly depending upon the species. These properties are key parameters to be considered for economy, design, and operation of a full-scale biogas plant that wants to use algal biomass as substrates.

This Bachelor's thesis is based upon work carried out for Botnia-Atlantica Project TransAlgae with the aim of investigating BMP of different algae species and finding the optimal mixture of algae with municipal organic waste and straw. This work is based, for the theoretical part, on literature research focusing on background information on anaerobic digestion and different perspectives of algae as anaerobic digestion substrate. The practical part consists of laboratory tests conducted with Automatic Methane Potential Test System II (AMPTS II) where different samples of micro-algae consortia and macro-algae species were digested to test their BMP. Furthermore, different species of *Scenedesmus* dominated micro-algae consortia samples and *Laminaria Digitata* species were co-digested with municipal organic waste and straw in different ratios to find the optimal mixture. The results show the BMP of ratio of each substrates with total of average BMP from all substrate.

Language: English **Keywords:** Anaerobic digestion, Algae, Biomethane potential, AMPTS II

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1 Introduction

Energy is essential for the economic development of nations. This has pushed many nations and companies to overuse easily available fossil fuel and drive it to depletion, which has led to environmental degradation and shortage of fossil resources. [1, pp. 1-2]. Among many innovations, biogas production from algae and organic wastes could help to reduce burden on fossil fuel and steer our economy to more sustainable resources. Algae is a third-generation biofuel that can be grown in waste streams and ponds with cost effectiveness and sustainable means. Algae can also be seen as carbon capturer since algae use carbon dioxide (CO_2), light and nutrients to produce biomass, which can be used to produce biogas by anaerobic digestion. Biogas is mostly a mixture of methane (CH_4) and CO_2, which can be used for heating and production of electricity or upgraded into biomethane and used as transport fuel.

These characteristics of algae interest biofuel industry and require more focus and research for the technology to be ready for commercialization. Thus, the focus of this thesis work has been on analyzing algae biomass as an anaerobic digestion substrate, biogas production from different species of algae and their co-digestion. This work has been carried out for the Botnia-Atlantica project TransAlgae in the department of research and development at Novia University of Applied Sciences where the project aims to develop and diffuse the utilization of algae grown in waste streams so it can be transformed into biofuels and valuable products.

1.1 Background

There is a big challenge for industries and municipalities to treat waste streams and reduce carbon dioxide release into the environment. To find a viable way, the Botnia-Atlantica project TransAlgae was initiated to implement different innovative solutions for this problem. Algae absorbs both carbon dioxide and nutrients. Its biomass could also be used to produce energy and other valuable products. Hence, it could presents a realistic solution to overcome this challenge. There are many different species of algae with different properties, which need to be analyzed. A joint effort from Swedish University of Agricultural Sciences, Nattviken Invest AB, Biofuel Region (Bfr) AB, Mid Sweden University, Novia University of Applied Sciences, University of Vaasa, has been started to solve this problem. The focus

of the project is to identify, test and implement innovative solutions related to the cultivation, harvest and anaerobic digestion of algae as well as to develop an efficient way to transform algae biomass to the valuable products.

The department of research and development at Novia University of Applied Sciences is responsible for conducting anaerobic digestion of different species of algae in laboratory using AMPTS II and pilot scale digester. The department is also in charge of providing knowledge on biogas and energy efficiency. Additionally, BMP tests of co-digestion of algae species with regionally available substrates and different pre-treatment methods will be conducted. [2]

The author of this thesis had the opportunity to participate in this project and gave a report on his work. This report has been used as a basis to write this thesis.

1.2 Purpose

The main purpose of this Bachelor's thesis was to provide knowledge in anaerobic digestion, different aspects of algae digestion and to analyze the BMP as well as co-digestion of different algae species with locally available substrates to find the optimal mixture.

In addition, a feasibility study of algae digestion had to be investigated by calculating the preliminary energy balance of anaerobic digestion plant considering realistic scenario. The feasibility study was done primarily to provide knowledge and to identify knowledge gaps in the topic. This study would also serve as a starting point for overall energy balance calculation that will be done later in the project when all necessary data from cultivation, harvest, pre-treatments, anaerobic digestion and transport will be available.

1.3 Limitations

This work presents some limitations. Firstly, the substrates were collected from the partners so it was not possible to control the conditions that the substrates were produced under, which might affect the BMP results. There were also limitations due to time and means constraints. Indeed, the digestion of substrates in reactors approximately takes three weeks plus several days for the preparation and analysis. In addition, only five samples triplicates

could be digested at one batch of experiment. Thus, in the given time and the equipment available three batches of experiments were analyzed. Finally, due to this thesis work being part of a larger project, the exact BMP test results cannot be published at this stage. Hence, it has been decided to present relative values obtained in experiment and compare BMP tests results as a ratio to the average of all of the BMP values.

1.4 Botnia-Atlantica Project TransAlgae

Botnia-Atlantica is a cross-border cooperation programme financing projects between Finland, Sweden and Norway. Their main priorities lie on developing and implementing new innovations, increase cross-border business cooperation, sustainable environment and cultural heritage, and increase different strategies to connect sustainable transport links in east-west direction. The Botnia-Atlantica programme supports projects from the European Regional Development Fund (ERDF). In figure, 1 below the regions covered by Botnia-Atlantica programme can be seen. [3]

Figure 1: Botnia-Atlantica covered regions and municipality [3]

The project goal of TransAlgae is to implement and innovate solutions to develop and diffuse the utilization of micro-algae and macro-algae biomass from waste streams. Which could be

later transformed into biofuels and other valuable products. The project aims to develop a good network of stakeholders within the algae industry in the Botnia-Atlantica region and targets to diffuse these solutions to medium and small size enterprises such as wastewater treatment plants, biogas producers, dairies, power plants and pulp and paper industries. To achieve this goal, the project will also focus on developing a cost and energy efficient method in both the cultivation and harvest of algae, and the products formed from the algae. [4]

1.5 Methods

To achieve above-mentioned goal and to write this thesis work both literature reviews and laboratory experiments were conducted.

A literature review was done to enhance the knowledge and understanding of theoretical background of anaerobic digestion, nature of algae during anaerobic digestion, problems while digesting of algae, pros and cons of different pre-treatment methods, properties and uses of biogas and the digestate produced after digestion as well as feasibility and energy balance of the system.

In laboratory total solid (TS) and volatile solid (VS) of each substrate samples were analyzed before and after digestion. See chapter 2.1.2 for more on TS and VS. Furthermore, the different algae species and mixtures of algae with, straw and municipal organic wastes were digested in AMPTS II. AMPTS II is a system that allows users to conduct anaerobic toxicity assays and determine the true biomethane potential (BMP) and dynamic degradation profile of any biomass substrate [5]. More about AMPTS II can be found in chapter 5.1

2 Theoretical background

Anaerobic digestion is a complex series of microbial processes in which organic matter is decomposed in absence of oxygen (O_2). Mainly bacteria are involved in the process where they convert organic raw materials to biogas and nutrient rich digestate. Anaerobic digestion is completed in four different process steps, which occurs after each other ensuring the stability of the whole digestion process. [6, pp. 104-106]. The figure 2 presents a simplified version of the different phases of anaerobic digestion process.

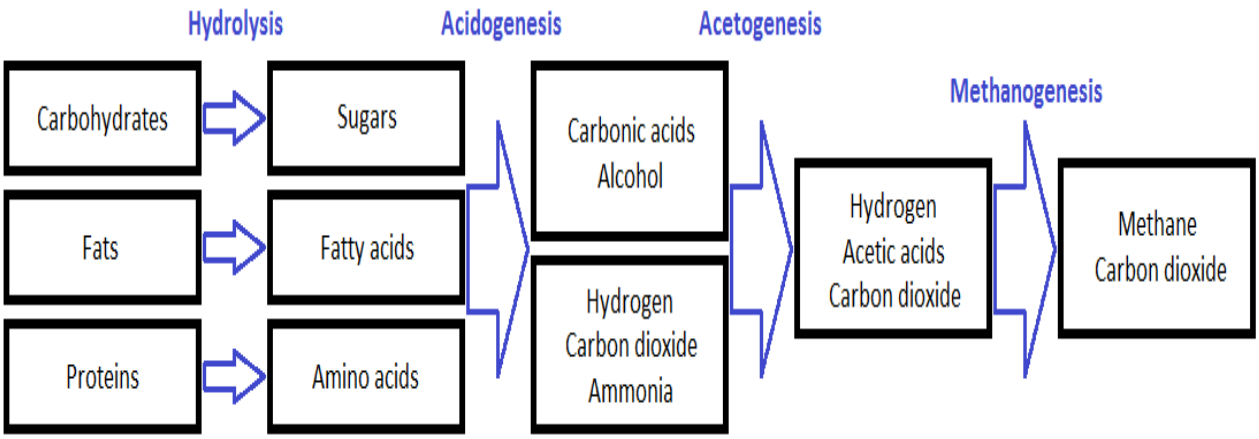

Figure 2: Process steps of anaerobic digestion [6, p. 106]

The first step in anaerobic digestion process is called hydrolysis. During this step, complex organic material such as proteins, carbohydrates, and lipids, are broken down by hydrolytic bacteria into smaller molecules like sugars, amino acids, and fatty acids prior to absorption and further degradation by acidogenic microorganisms. Hydrolysis is one of the most important and rate-limiting step in anaerobic digestion since many organic materials consist of complex structures resulting in a slow degradation and making it less accessible to microorganisms. It is a time consuming step in anaerobic digestion, depending on substrates and processes, thus it may need to be improved. Pre-treating substrates prior to digestion will increase hydrolysis rate (See chapter 2.2 for pre-treatment methods). During the acidogenesis step the sugars, the amino acids, and the fatty acids generated during hydrolysis phase are converted into volatile fatty acids, alcohols, carbon dioxides, and hydrogen. The third step is called the acetogenesis. Here the produced volatile fatty acids are converted into hydrogen (H_2), carbon dioxide (CO_2), acetic acids, and unicarbon compounds such as methanogenic substrates. The final phase of digestion process, the methanogenesis, is also a rate-limiting step that occurs with two bacterial groups, hydrogenotrophic methanogenic bacteria and aceticlastic methanogenic bacteria, where they use substrates to produce methane. It should be noted that the anaerobic digestion process steps are dependent on each other thus disturbances in any phases will affect the other resulting in loss or decrease in methane production. [6, p. 7]

2.1 Conditions and variables influencing anaerobic digestion

As mentioned previously, anaerobic digestion is a complex process and depends on many conditions and variables for proper breakdown of the organic compounds as well as the type of reactor or digester used in the process. The parameters for the operation of digester must be controlled in order to increase microbial activity and thus to increase the efficiency of anaerobic digestion. Some of these parameters are discussed briefly in this section [7, p. 7]. Further details on digester can be found in chapter 2.3

2.1.1 Nutrition composition

Bactria and other microorganisms need different nutrition such as carbon, nitrogen, phosphorous as well as micronutrients and vitamins to survive and grow. Sufficient quantities of these elements in substrate mixture results in high biogas yield. [8]

Carbon (C) and nitrogen (N) ratio or C/N ratio is one of the important parameters for anaerobic digestion. Generally, low C/N ratio leads to increase in production of ammonia, which inhibits the methane production. On the other hand, a very high C/N ratio promotes the growth of methanogens populations that are able to meet their protein requirements and therefore, will no longer react with the remaining carbon content of the substrate, resulting in a lower biogas production. It should be noted that not all the carbon and nitrogen, that is determined or is in the substrate, would be available for digestion. For example, lignin is high in carbon but difficult to digest. Thus, the carbon from lignin is not available for microorganism in anaerobic digestion if it is not pre-treated. For an efficient digestion, available C/N ratio for microorganisms should be in range of 20:1-30:1. The optimum C/N ratio can be achieved by mixing substrates with high and low C/N ratio, for example organic solid waste mixed with manure. [9, pp. 127-128]

2.1.2 Total solid, volatile solids and chemical oxygen demand

Total solid content (TS) or Dry matter (DS) is the amount of solids in substrate, which does not contain any moisture in it. There are three different ranges of solid content in anaerobic digestion system: low solid anaerobic digestion system, where solid contained is less than 10%, medium solid from 15-20%, and high solid system, from 22-40%. The volume of

digester can be reduced by increasing the TS of the substrate due to low water requirement. It should be considered that higher TS could also be a problem as it can affects the mixing of the substrates in bioreactors and the functionality of pumps. However, some material like glycerol present an exception: they have very high TS but can be pumped without any problem. [7, pp. 7-8] The calculation and practical way to determine TS can be found in chapter 5.2.3

Volatile solids (VS) represents the organic matter in the substrate that is biologically available for the bacteria. It is measured by subtracting ash content from TS. It is assumed that everything that departs while burning is volatile, and considered available for biological decomposition. It should be noted that the substances like lignin will go up in smoke but will not be digested. Consequently, it is not an accurate measure to determine biological matter available to bacteria. However, it is the widely used method. Usually higher amount of VS in substrate gives higher yield of biogas. [6, p. 25] More on VS and its calculation and practical way to determine VS can be found in chapter 5.2.3

The chemical oxygen demand (COD) is the amount of oxygen needed to break down specific amount of organic matter. COD is used for calculating the amount of organic matter content in a given substrate. As VS, higher COD concentration gives high yield of biogas. [8, p. 8]

2.1.3 Temperature

Anaerobic digestion can mainly occur by two different bacteria cultures namely mesophilic bacteria and thermophilic bacteria in two different temperature conditions. There are the mesophilic conditions where digestion can take place between 20-40^{0}C, usually 35^{0}C, and the thermophilic condition between 40-60^{0}C, usually 55^{0}C. Since mesophilic or thermophilic bacteria are active in their own temperature range, the process temperature in the reactor should be stable. [10, pp. 31-32] The optimum temperature of digestion can vary depending on substrate composition and type of digester but the temperature should be maintained relatively constant through the digestion process for constant biogas production rate. According to Deublin and Steinhauser (2008), temperature range below or above +/- 2^{0}C can cause up to 30% loss of biogas production. Generally, temperature lower than optimum level increases the hydraulic retention time (HTR) (see chapter 2.1.6). [11, p. 113]

2.1.4 pH

pH plays an important role in digester performance. The bacteria involved in anaerobic digestion process have different optimal pH ranges for well-functioning process. The pH should be maintained at a level where all bacterial groups can flourish. Methanogens are more sensitive to pH than acidogenic bacteria. [10, p. 26] Deublin and Steinhauser (2008) have stated that the optimum pH level for methane forming microorganism is between 6.7-7.5. Thus, it is important to adjust the pH in digester for maximum biogas production. It should also be considered that adding something acidic in the reactor does not always means that the pH is lowered significantly. Indeed, due to buffer capacity of a stable anaerobic digestion process, microorganisms can maintain some changes in pH. There are different ways to control pH in digester if needed. One of them is using ammonia-ammonium buffer system where with falling pH, ammonium ions are formed with release of hydroxyl ions and with rising pH, more free ammonia molecules are formed. Some other ways to solve excessive acidification is to stop supply of substrate so methanogenic bacteria are able to degrade the acid or by adding water to dilute or adding neutralizing substances like sodium carbonate and lime etc. [11, pp. 114-115]

2.1.5 Volatile fatty acids

Volatile fatty acids (VFAs) are compounds having long carbon chains like acetate or lactate [12, p. 26]. These are formed during acetogenic phase of digestion and are the main substrates used by methanogens bacteria to produce methane. An increase in the accumulation of VFAs is an indication that the methanogens are being overloaded, or some other factor is affecting their ability to convert VFAs to methane. An accumulation of VFAs can lead to decreases in pH causing inhibitory environment to methanogens bacteria and a culture crash; therefore, VFAs are an important indicator of process stability. Reducing loading rate can help to avoid this problem. [11, p. 121]

2.1.6 Hydraulic retention time and organic loading rate

Hydraulic retention time (HRT) is an important parameter for dimensioning of biogas reactors. It is the average time interval that the substrate stays inside the digester tank. In

general, longer HRT means larger working volume of reactor and higher investment and operational cost. [12, p. 28]

Organic loading rate (OLR) describes the quantity of substrate to be fed in a digestion process inside reactor at a given time. OLR and HRT is interconnected to each other (OLR= the amount of organic material in daily feed divided by the reactor volume). Usually biogas processes have a threshold limit for OLR, which cannot be increased due to technical limitations like too high TS for the plant, which can result in inefficient mixing and blockage of pumps. Exceeding threshold limit also causes high VS that can causes intermediate inhibition due to microbiological limitation. [13, p. 9]

2.1.7 Agitation

For the stabilization of anaerobic digestion process, a good mixing of reactor content is important. Only small plants can be operated without agitators. The common practice for mixing is mechanical device moving in the bioreactor while pneumatic mixing by bubbling gas from the bottom of the digester is also used. Agitation facilitates heat and mass transfer inside the reactor as well as the release of gas bubbles trapped in the digester slurry. [9, p. 251] According to House (2006 p 52), well stirring of digester slurry can also lead to increase in biogas production and helps in control of foam and maintain constant pH in slurry.

2.2 Pre-treatment

Due to recalcitrant structure of substrates, digestion process in reactor can be difficult and slow. To overcome the problem there are different types to pre-treatment technologies for biogas production has been developed in recent years. Even though pre-treatment of substrate helps in degradation of organic materials faster and facilitate technical functions in digester, they can also be energy intensive. The profitability and effectiveness of the pre-treatment process should be kept in mind while choosing one because not all pre-treatment methods are suitable for every substrate. Some pre-treatment methods are explained briefly below. [6, pp. 89,90]

2.2.1 Mechanical pre-treatment

Mechanical pre-treatment is one of the physical pre-treatment methods, which aims at increasing the specific surface area of biomass to improve biodegradability of lignocellulosic materials. Different kind of mills can be used for chopping or crushing of substrates. Mechanical pre-treatment is expensive process due to the high electrical energy requirements even though it increases and facilitates the biogas production. [6, pp. 90,91]

Ultrasound treatment is also one of the mechanical pre-treatment methods, which can be used to pre-treat sludge or algae biomass for anaerobic digestion. The various frequencies of ultrasound with different power levels can be used for treating organic biomass. Ultrasound frequencies causes cavitation in liquid phase causing disruption on membranes and cell wall of substrate. Thus, the destruction of the cells makes hydrolytic enzymes free and increases the hydrolysis rate of biomass so the digestion process produces more biogas in same HRT. [14] More on ultrasound pre-treatment can be found in chapter 3.4

2.2.2 Thermal pre-treatment

Thermal pre-treatment of the substrate is done by heating the substrate under various pressure and temperatures up to maximum of 220^0C and held for specific time then cooled down and used. Additional water should be mixed for substrates with low water content before thermal treatment. The heat and pressure causes the swelling of biomass by disrupting the hydrogen bonds, which holds together crystalline cellulose and other complex structures. Hemicellulose are also broken down in the process making the substrate easily digestible by bacteria. The other advantage of thermal pre-treatment is that it can be used as a pasteurization for the biomass. [6, p. 92] More on thermal pre-treatment can be found in chapter 3.4

2.2.3 Chemical pre-treatment

Chemical pre-treatment is generally done by adding strong acids, alkalis or oxidant to achieve the destruction of organic compound, and thus, helping them to digest better and faster. The effect of chemical pre-treatment depends on the substrate and the method used to treat. Substrate containing high amount of carbohydrates are not suitable for chemical pre-

treatment due to their accelerated degradation and subsequent accumulation of VFA, which can lead to inhabitation for methanogens bacteria. [15]

Alkali pre-treatment is one of the most common chemical pre-treatment and this measure helps to adjust the pH in anaerobic digestion. During alkali pre-treatment the reactions in substrate induces swelling of solids thus increasing the surface area, which makes it easily accessible to anaerobic microbes. COD is also increased through various reaction during alkali pre-treatment of substrates. [15] Although chemical pre-treatment helps in digestion of substrates, it should be taken into account that the costs of chemicals are generally higher and the need for extra investments in equipment and facilities, which can overshadow the positive effect of this pre-treatment method.

2.2.4 Biological pre-treatment

Biological pre-treatment is done both aerobically and anaerobically in addition enzymes such as lipase can be added for better result. Aerobic pre-treatment such as composting prior to anaerobic digestion can be effective method to increase hydrolysis rate of complex substrates due to the production of hydrolytic enzymes. In general, biological pre-treatment is time consuming and too slow. [15]

2.3 Types of reactors

The main component of a biogas plant is the digester, which is an oxygen proof reactor tank where anaerobic digestion takes place [12, p. 74]. They provide the opportunity to control and monitor conditions throughout the anaerobic digestion process. There are several types and technologies to operate a bioreactor in biogas plant. The proper selection of right type of reactor is very important in process efficiencies, since not all technologies are suitable for every kind of raw materials and one’s purpose. The types and quantity of raw material available should be kept in mind while building a bioreactor.

2.3.1 Continuous digestion

As the name suggests in continuous digestion feedstock is continuously fed into the reactors on daily or hourly basics and same amount of digested material are taken away mechanically or by the pressure of newly feed substrate. In this process, production of biogas is not interrupted due to loading of substrate or unloading of digested. [12, p. 76] These types of reactors are used for commercial scale purpose to handle large volume of raw material without output interruption of biogas production.

2.3.2 Batch wise digestion

In this digestion process, the reactor is filled completely with substrate, which degrades inside reactor without adding or removing anything. The batch feeding process leads to variation in the production and composition of biogas. Typically, in these types of reactors production of biogas increases slowly until it is maximum about half of the residence time and starts decreasing. At the end of process fermenter are removed and new batch is loaded with small amount of inoculum in it, inoculum is a start culture, which is usually taken from the stable running digester. [11, p. 243] Batch wise digestion is commonly used for small-scale biogas production like in agricultural farm and in labs for experimental investigation of BMP and other.

2.3.3 Digestion in several steps

Acid forming bacteria are less affected with low temperature than methane forming bacteria, which gives possibilities to divide the process in several steps and reactor to save large amount of heat energy required. These types of reactor are generally known as hybrid reactors where acid digestion and gas digestion is separated in batch and continuous digestion respectively. Since the hydrolysis takes long time it is better to have in separate reactor so that specific actions can be taken in favor of efficient hydrolysis and increase the overall efficiency of biogas production. Generally, digestion in several steps can be efficiently controlled thus resulting in a very stable process. [10, p. 142]

2.4 Biogas

Biogas produced by anaerobic digestion is a renewable energy source, which can be used to replace fossil fuel in different ways: heating, generating electricity, as vehicle fuel, or injected in natural gas grid after upgrading [12, pp. 10-13]. Biogas mainly contains methane, which is the energy carrier, and carbon dioxide, but also small amount of impurities which can have a negative effect during utilization phase. Methane and carbon dioxide content of biogas produced by anaerobic digestion is about 55-70% and 30-45% respectively [9, p. 86]. The production of other compound or impurities depends on how biogas is produced and the substrate used to produce biogas. For example, ammonia, which has a negative effect in utilization and production phases, is typically generated during hydrolysis of organic materials containing high proteins, such as slaughterhouse wastes. [6, pp. 329-332]

Some common impurities, which are found in biogas, their content, effect, and cause, are listed in Table 1.

Table 1: Common impurities found in biogas and their content, causes and effects. [10, pp. 86-87] [11, p. 52] [6, p. 430]

Compound	Content (Vol %)	Causes	Effects
Carbon dioxide	25 - 50	High C/N, low pH, O_2 contamination.	Reduces heating value, corrosion, damages alkali fuel cells
Hydrogen sulfide	0 - 0.5	Low pH, substrate having protein or sulfate, long digestion time	Emission of SO_2, corrosion, damages catalysts
Ammonia	0 – 0.05	Low C/N ratio, high protein, thermophile temperature	Emission of NO_2 and corrosion
Water vapor	1 – 5	Increases with raise in temperature	Corrosion and condensations
Dust	>5µm	----	Blocks nozzles and fuel cells
Siloxanes	0 – 50 mg/m^3	Siloxanes in substrate	Precipitates during combustion, damage equipment

Biogas can be utilized in different ways as mentioned above. The quality of biogas needs to be maintained according to how and where it is going to be used [6, p. 333]. For example, in Sweden the internationally recommended standard ISO/DIS 15403 is followed. According to standard ISO/DIS 15403, biogas to be used as vehicle fuel needs its methane content to be more than 96% by volume and CO_2 less than 3% by volume. More details can be found in appendix 1 [9, p. 517]. In order to reach the required quality for its purpose, biogas needs to go through cleaning and upgrading processes. The most widely used upgrading systems are scrubbers, filters, membrane separators and chemical additives. In

appendix 1, the regulation in Sweden for biogas as fuel can be seen and these results can only be achieved by upgrading biogas.

2.5 Digestate

Digestate is another product of anaerobic digestion, which is formed along with biogas. It is the digested substrate, which is removed from digester after production of biogas. The main advantage of digestate is that it is rich in nutrients content including nitrogen, phosphorus, potassium and various micronutrients but also organic matter. Digestate contains the same nutrients as that of the substrate but in more concentrated and easily available form to organisms hence making it a good plant fertilizer. To use high quality digestate as plant fertilizer it has to be produce with high quality feedstock. [16]

The substrate may contain different amount of heavy metals like lead and cadmium or persistent organic compounds that are not biodegradable may end up in digestate. For example, animal manure contains heavy metals, which comes from their diet. These elements must be measured carefully and should not be allowed to exceed their legal limits in digestate, when the digestate is recycled to land as bio fertilizer. The digestate can also be used as landfill covers and as raw material for industrial process. [17]

3 Algae and anaerobic digestion

Algae are one of the oldest form of life on earth, they can be autotrophic, heterotrophic or mixotrophic species that can use sunlight or organic carbon. Algae can be found living alone or in colonies. They can be unicellular, multicellular and acellular. These are primarily macro-algae and micro-algae. Macro-algae can grow 0.5 meters per day under favorable conditions, and exist in large quantities in many coastal regions. Micro-algae on the other hand produces large amount of fatty acids and fatty oils, which is interesting from bio-fuel perspective. [18, p. 17] Many different groups have investigated on the anaerobic digestion of algae for various reasons. The oil crisis during 1970s was a reason for the investigation of alternative energy sources, anaerobic digestion of algae was one of them. [19] More recently, environmental degradation and global warming has brought back the interest in anaerobic digestion of different algae species. The food - fuel debate has also changed the

perspective for the use of first generation biofuel and adopt strict measure to limit food source to be used for fuel production. For example in 2014 European Commission agreed to limit the share of biofuels from cereals and other starch rich crops, sugar and oils crops to 7% only. Third generation biofuels do not require agricultural land for production. Generally, third- generation biofuel tends to be produced from algae. These are supposed to have lower area requirement and can be grown in waste streams compared to terrestrial crops like corn, rapeseed, or switch grass. It has also helped to increase the focus on anaerobic digestion of algae. [20]

Even though the idea looks great, biofuel production from micro-algae feedstock has to overcome many challenges before being a mainstream industry capable of producing enough quantity of biofuel required at competitive price. The main challenges faced by the industry are demand for fertilizers due to micro-algae significant utilization of nutrients, high-energy inputs for harvesting and dewatering of biomass and conversion process. Some of the overheads of the production cycle can be eliminated by producing biogas from anaerobic digestion of algae. In addition, the valuable nutrient recovered from the anaerobic digestion of biomass can be essential for sustainability of algae biofuel industries. It is anticipated that cooperation of algal biofuel production, bio-refinery and co-digestion of algae with locally available substrates anaerobically can increase the cost effectiveness of the production phase and can help it to become economically feasible and environmentally sustainable. [21] Figure below shows the conceptual visualization of anaerobic digestion process incorporation into algal biofuels such as biodiesel.

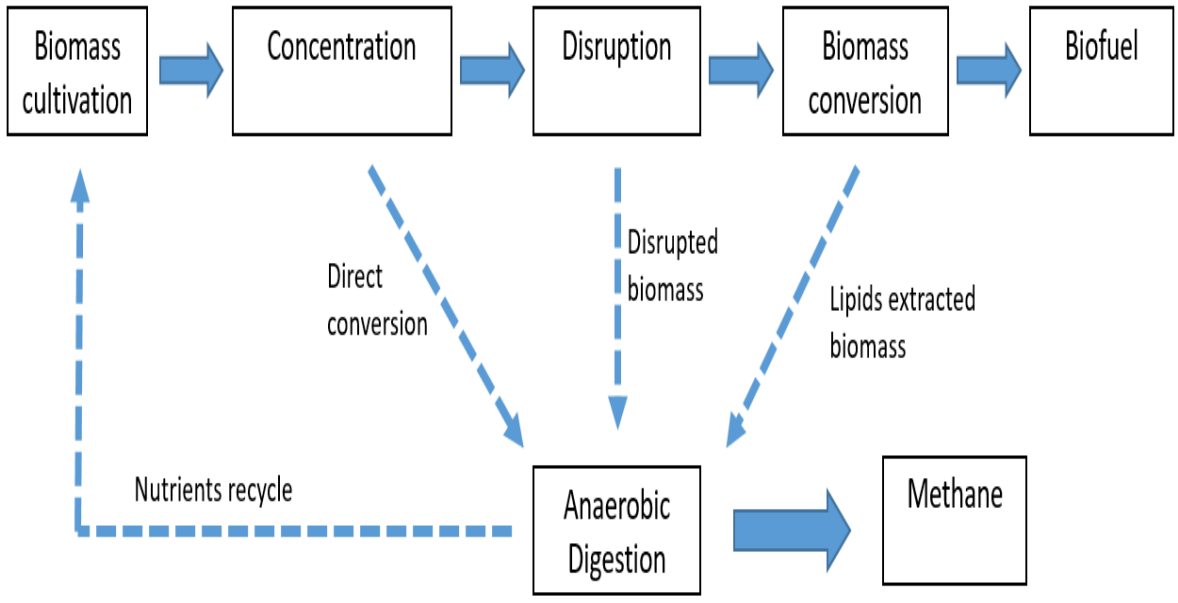

Figure 3: Visualization of anaerobic digestion incorporation into algal biofuel [21]

3.1 Macro-algae or Seaweed

There are many different species of seaweed. They can be generally classified into three broad types: brown seaweeds for example *Saccharina latissima* and *Sargassum muticum*, red seaweeds for example *Palmaria palmate*, and *Asparagopsis armata* and green seaweeds includes *Codium tomentosum* and *Ulva lactuca*. Many seaweeds have a high growth rate allowing it for sustainable harvest. [20] Proliferation of seaweeds, which have caused the eutrophication problems in Baltic Sea can be anaerobically digested and be a viable solution for this problem. Anaerobic digestion of seaweed is not only the solution for the eutrophication problem but it can also be a potential substrate for anaerobic digestion. Commercial seaweed farming has also been assessed in various growth regimes and have been evaluated. [19] Bruhn et al. (2010) cultivated *Ulva lactuca*, which is generally a nuisance species and can be found in abundance. [22] *Ulva* species was initially considered for digestion in TransAlgae project but after knowing the unique chemical properties, which can be more valuable for pharmaceutical, aquaculture and agricultural the plan to digest *Ulva* was replaced by *Laminaria Digitata.*

The composition of seaweeds depends on many factors and varies continuously from place to place, seasons and from species to species. These variations make it complicated while evaluating for substrate as feed sources for anaerobic digestion. They are generally characterized as having no lignin, low cellulose and lipid content. [20]

3.1.1 Protein and carbon to nitrogen ratios

As mentioned in chapter 2.1.2 C: N ratio for anaerobic digestion should be in range between 20:1 to 30:1. Digestion of nitrogenous substrate with C: N ratio less than 15:1 can lead to excess level of ammonia. In seaweed, proteins are the primary sources of nitrogen. Protein concentrations are low in brown seaweed while red and green seaweeds has high concentration. [20] Habig et al. (1984) grew *Ulva* and *Gracilaria* in various nitrogen enrichment and deprivation conditions and anaerobically digested. The highest methane production was by *Gracilaria* with C: N ratio of 8.94:1, in the semi-continuous experiment where methane production ranged from 190-230 Liters per Kilogram (L kg^{-1}) VS. The C: N

ratio for Ulva varied from 8.72 – 30.71 and the methane produced varied from 220 -330 L Kg^{-1} VS. Methane production increased in *Ulva* with increase in C: N ratio. [23].

3.1.2 Harvest of seaweed

Seaweed are mostly collected for human consumption and for hydrocolloid production. Seaweed product for these purpose sustain much higher price for raw material than that for biofuel production. [24] Seaweeds are mainly harvested manually or using special equipment. In addition, traditional hand cutting using sickle and various mechanical harvesting methods are in practice. In case of attached seaweed, it is necessary to cut them, which will raise the energy consumption of harvesting. The free-floating seaweed harvesting is done by raising the installed net in the pond or sea. Species like *Chondrus crispus* and *Mastocarpus stellatus* are difficult to harvest and are usually harvested using raking from boats or with a cutter rake. [25]

Even though seaweeds take longer time to regenerate than micro-algae and require intensive labor for harvesting, they are still more economical to harvest than micro-algae. [25]

3.1.3 Pre-treatments

Various pre-treatment methods like heating, size reduction and/or chemical treatments are commonly used while digesting seaweeds to improve efficiency. Higher temperature in range of 160-180^{0}C and shorter treatment time of around 1-60 minutes can increase methane production whereas increasing pre-treatment temperature to 200^{0}C can reduce the bio convertibility of nitrogenous compounds and carbohydrates with increase in toxicity. [26, pp. 922-924]. Two or more pre-treatment methods can also be used for better results for example ultrasonic plus thermal, thermochemical etc.

3.1.4 Biomethane potential from mono-digestion of *Ulva* lactuca

Studies done by Allen et al. (2013a) where *Ulva lactuca* were collected form West Cork, Ireland showed that the BMP of freshly digested Ulva as 183L/Kg VS. The biodegradability

index was 42% meaning that more than half of substrate was not degraded. The study done in Denmark by Bruhn et al. (2011) also collected similar result as in Ireland, where *Ulva* from Seden beach (Odense Fjord), Denmark were untreated and freshly digested generated 174L/Kg VS. This result suggests that *Ulva* species of Northern Europe will have similar result.

3.2 Micro-algae

In comparison with macro-algae, micro-algae are microscopically small. Many different species of micro-algae can be found. They can exist as solitary cells but formation of colonies of several to many different cells is also common. Micro-algae can be found in highly diverse habitats and grow under strongly varying environmental conditions. [20] Micro-algae are highly productive and produce large amount of biomass more efficiently than terrestrial crops. The photosynthetic efficiency of micro-algae in controlled engineered system can be up to 4-5% of solar energy compared to just 1-2% for terrestrial plants. [21] Micro-algae can be cultivated using wastewater, which reduces both production cost and freshwater requirement. They can also play an important role during wastewater treatment.

3.2.1 Cultivation of microalgae

For sufficient growth of photoautotrophic micro-algae appropriate amount of light, water, carbon and different mineral nutrients are necessary. Elements such as nitrogen, iron, phosphate and silicate are required in large quantity for good growth. Nutrient composition for micro-algae varies according to species. The most efficient way to grow micro-algae biomass economically and environmentally has yet to be defined but there are several approaches for producing micro-algae biomass that differ significantly from each other. [20]

Micro-algae cultivation systems can be divided into indoor and outdoor system. Outdoor system is more economical due to the utilization of sunlight. Other differentiation can be made between open and closed cultivation system. In open cultivations systems algae are grown in open vessels, pond and natural water. These systems have several drawbacks such as insufficient monitoring and control options for parameters like pH, temperature, mixing

and light availability in comparison with closed systems. In table, 2 below advantage and disadvantage of common cultivation are shown.

Table 2: Advantages and disadvantages of open and closed algae cultivation system. [20]

Cultivation system	Advantages	Disadvantages
Open	• Cheap and good gas exchange with the atmosphere • Easy to operate and easy to scale up	• High risk of contamination and high evaporation losses • Large area required and light limitation if thick layers are used
Closed	• Good control of cultivation parameters • Reduced contamination risk • Less CO_2 losses and reproducible cultivation conditions	• Expensive and difficult to scale up

In closed systems, different kinds of photo bioreactors are available for example tubular reactor, laminar reactors, hanging plastic sleeves etc. [20] In figure, 4 below different cultivation systems are shown.

Open cultivation systems for cultivation of microalgae; left: Race way ponds; right: cascade system

Closed cultivation systems for cultivation of microalgae; left: sleeve-bag photobioreactor; right: tubular photobioreactor

Figure 4: Different micro-algae cultivation systems [20]

3.2.2 Harvest of micro-algae

Even though micro-algae can be used for wide range of applications, production of micro-algae is not economically viable yet for biofuel production. One of the reasons for high process cost are the small size of micro-algae and their growth in highly diluted cultures with mass concentration around or less than 1 gram per liters and density close to that of water. In addition, micro-algae surface is negatively charged and their cells contains algogenic organic matter which helps them to keep their dispersed state, thus requiring high energy and sophisticated techniques for separation of the biomass from liquid and raising production cost. [27] According to Christenson and Sims (2011) until now, there is no harvesting method for micro-algae that is both economically viable and efficient [28].

Harvesting of micro-algae currently involves mechanical, chemical, biological and to some extent electrical based methods. In practice, two or more of these methods are combined for better separation rate at lower cost. A good example of this is the combination of flocculation-sedimentation with centrifugation, which reduces the process cost significantly. Mechanical harvesting is the most common and reliable method currently used to harvest

micro-algae. However, these methods are often preceded by a chemical or biological coagulation/flocculation thickening stage to improve efficiency and reduce costs. [27]

3.3 Problems with anaerobic digestion of micro-algae

As anaerobic digestion of micro-algae is still not a mature technology, it faces many different challenges and problems before it can be economically viable. Common problems when using micro-algae biomass as anaerobic digestion substrate are explained below.

3.3.1 Low concentration of digestible substrate

As mentioned in chapter 3.2.2 harvesting micro-algae biomass presents a fundamental challenge to the financial viability of an energy system. Gouleke et al. (1957) has noticed the low volatile solids loading rate, which is associated with micro-algae when used as digestible substrate. The low VS rate is result of low concentration of biomass in large volume of water. [21]

3.3.2 Cell wall degradability

Gouleke et al. (1957) has found that micro-algae are able to pass through an anaerobic digester intact and remained undigested. The author has also noted that the micro-algal cells are known to effectively resist bacterial attacks and were noticed undigested after being in digester for 30-day hydraulic retention time. It has been found that degradation of the cell wall strongly correlates with the amount of biogas production. For example, work done by Mussgnug et al. (2010) has shown that micro-algae species with either no cell walls or cell walls made up of proteins has higher biogas production than those species having rigid cell wall or cell walls made up of carbohydrates. These results suggests the need of pre-treatment method to disrupt the cell wall to increase bacterial hydrolysis prior to anaerobic digestion. [29] More on pre-treatment can be found in chapter 2.2 and chapter 3.4.

3.3.3 Carbon to nitrogen ratio

Low C/N ratio in micro-algae biomass further increases the difficulties in anaerobic digestion. Table 3 below shows the C/N ratio of different micro-algae species, which were investigated for anaerobic digestion, were found to be between 4.16 and 7.82. This low C/N ratio brings imbalance between carbon and nitrogen requirements for anaerobic bacterial groups. When the C/N ratio is below 20 it leads into release of nitrogen in form of ammonia. [21] Ammonia during digestion can be inhibitory to methanogenic bacteria and result in accumulating of VFA in digester, which has negative effect in biogas production as mentioned in chapter 2.1.4 and chapter 3.3.4. To overcome this problem co-digestion of micro-algae with other substrates can be done, more on co-digestion can be found in chapter 3.5.

Table 3: C/N ratio of different micro-algae species [21]

Micro-algae species	**C/N Ratio**
Spirulina maxima	4.16
Chlorella vulgaris	6.00
Arthrospira maxima	4.30-5.33
Scenedesmus Sp. and Chlorella	6.70
Tetraselmis	7.82

3.3.4 Ammonia-Nitrogen toxicity

Ammonia-nitrogen is produced during the breaking down of nitrogenous matter such as proteins and urea. The high level of nitrogen and protein found in micro-algae can lead to significant release of ammonia-nitrogen during digestion. Ammonia toxicity can affect methanogenic bacteria and drop biogas production. The ammonium ions may inhibit the methane-synthesizing enzyme directly and/or the hydrophobic ammonia-nitrogen molecules may diffuse passively in to cells, causing proton imbalance and deficiency of potassium.

[21] According to Parking and Owen (1986), ammonium-nitrogen level above 3000 mg/L has high inhibitive effects and can lead to drop on biogas production [30].

A solution to this problem can be the utilization of two stages anaerobic digestion process where the digestion stages are physically separated with the hydrolytic and acetogenic bacteria in the first stage, and the methanogenic bacteria in the second stage. It has also been found that integration of microbial fuel cells can be beneficial in decreasing ammonia-nitrogen inhibition during anaerobic digestion. Improved performance has been achieved by allowing the ammonium ions to migrate across cation exchange membrane from the anode to the cathode. This use of microbial fuel cell has found to be effective in decreasing the chances of free ammonia-nitrogen inhibition of methanogenic bacteria thus improving the stability of the anaerobic digestion process. [21]

3.3.5 Saline micro-algae and effect of salinity

To avoid the use of agricultural land some of micro-algae are grown in saline environment. The marine species like *Macrocystis pyrifera* and *Tetraselmis sp* have been used as substrate for anaerobic digestion. Low concentrations of alkaline earth metals are required for metabolism of bacteria in anaerobic digestion process but higher concentration can be extremely toxic to methanogenic bacteria. Higher salinity has been seen to be inhibitory because it causes bacterial cell to dehydrate due to increase in osmotic pressure. Salinity can be caused by many different elements like sodium, magnesium and aluminum, which in high levels can be toxic. The sodium ion is the most inhibitory to anaerobic digestion and it makes up a big percentage of the light metal ions found in seawater. However, sodium inhabitation varies depending on many factors such as source of inoculum, element composition of the saline water and substrate used. [21]

3.3.6 Sulfide and its role in anaerobic digestion

Micro-algae biomass from fresh water contains less amount of sulfureted amino acids and their digestion release less amount of hydrogen sulfide. However, micro-algae form saline water and saline substrate can have oxidized sulfur compounds, which can act as electron acceptors for sulfate reducing bacteria that converts organic compounds in an anaerobic

reactors and produce hydrogen sulfide gas. As mentioned in chapter 2.4, hydrogen sulfide, when present in biogas, can cause corrosion and damages machinery such as engines and pipes, which need to be removed if present in high amount leading to higher upgrading costs. Sulfide is needed for cellular metabolism in small concentration by bacteria but high concentration such as 200 mg/L or more can be extremely toxic to methanogenic bacteria. [21]

3.4 Pre-treatment for micro-algae biomass

Several authors such as Mussgnug et al. (2010) and Golueke et al. (1957) have found that degradation of cell walls strongly correlated with the biogas production from anaerobic digestion of micro-algae. Their results indicate the need for pre-treatment steps to disrupt the cell wall of micro-algae biomass before adding to anaerobic digester to increase bacterial hydrolysis.

Gonzalez-Fernandez et al. (2012) investigated the thermal pre-treatment of *Scenedesmus sp*. where at 70^0C for 3 hours resulted in 9% increase in methane production, which increased to 57% when temperature was raised to 90^0C for same duration of time when compared to untreated micro-algae biomass. [31]

Gonzalez-Fernandez et al. (2012) in their further studies investigated ultrasound pre-treatment method for cell disruption of *Scenedesmus* biomass using 20 Hz frequency with varying energy levels for 15 minutes. Due to raise in temperature as high as 85^0C during ultrasound treatment results were also compared with thermal pre-treatment without ultrasound at 80^0C. Result showed that highest methane production was observed with biomass sonicated at 130 MJ/kg, but thermal treatment at 80^0C produced only slightly lower amount. Due to lower energy requirement, thermal pre-treatment methods may be more suitable in this case than sonication. [32]

The various mechanical, thermal, biological and chemical pre-treatment methods can be used to increase biogas production. Several authors have found that the energy consumption for pre-treatment for micro-algae biomass is equal or in some cases higher than the energy produced. Due to these factors careful comparison of expected yield and energy demand before applying it in full-scale digestion plant should be done. [21]

3.5 Co-digestion

Co-digestion is a method to combine two or more substrates and digest them together in digester. The main idea of co-digestion is to mix different substrate and solve issues related to anaerobic digestion of a single substrate. A good example could be mixture of algal biomass and paper. Generally, algae has low C/N ratio, while on the other hand paper has high C/N ratio. If they are mixed and fed into a digester, then parameters will become balanced and thus making the process more stable and efficient. [21]

Yen and Brune (2006) investigated adding paper waste to improve C/N ratio of the micro-algae combination comprised of *Scenedesmus sp.* and *Chlorella sp.* where C/N ratio was increased from 6.7 to 36.4. Their experiment showed that the best co-digestion ratio was 50% paper waste and 50% micro-algae and final C/N ratio of this combination was 18, which has 50% increase in biogas production in compared with digestion of only micro-algae biomass. Yen and Brune (2006) concluded that the best C/N ratio for anaerobic digestion is between 20:1 and 25:1. They also indicated that the increased in C/N ratio decreased the total amount of ammonia-nitrogen as C/N ratio became more favorable for anaerobic digestion thus reducing ammonia-nitrogen inhibition effect. [33]

4 Feasibility of algae digestion

As mentioned in chapter 3 the idea of biofuel from algae looks great but has many challenges to become competitive with fossil fuel. One way to find its profitability is to see energy output from algae species, which is also the focus of the thesis and laboratory work. In this chapter, a general approach to calculate and analyze energy balance in anaerobic digestion biogas plant where algae biomass is used as a substrate can be found. The main goal of this chapter is to investigate the feasibility of algae digestion and identify the possible knowledge gaps in the process. It will serve a starting point for TransAlgae project for the final energy balance of biogas production from algae biomass, which will be conducted later during the project when all the data from cultivation, harvest, pre-treatments, anaerobic digestion and transport are available.

For the determination of energy efficiency of a biogas plant, a calculation of energy balance is necessary. The energy balance summarizes the input and output energy flows of the process. [34] In figure 5 below gives an overview of biogas production process from micro-algae biomass. Energy balance of biogas production can be done by identifying the total energy input for the algae biomass production and anaerobic digestion processes to output energy produced from biogas. Energy input either direct energy or indirect energy depends on many factors such as, species of algae, cultivation techniques, harvest methods and biogas plant technology. [34]

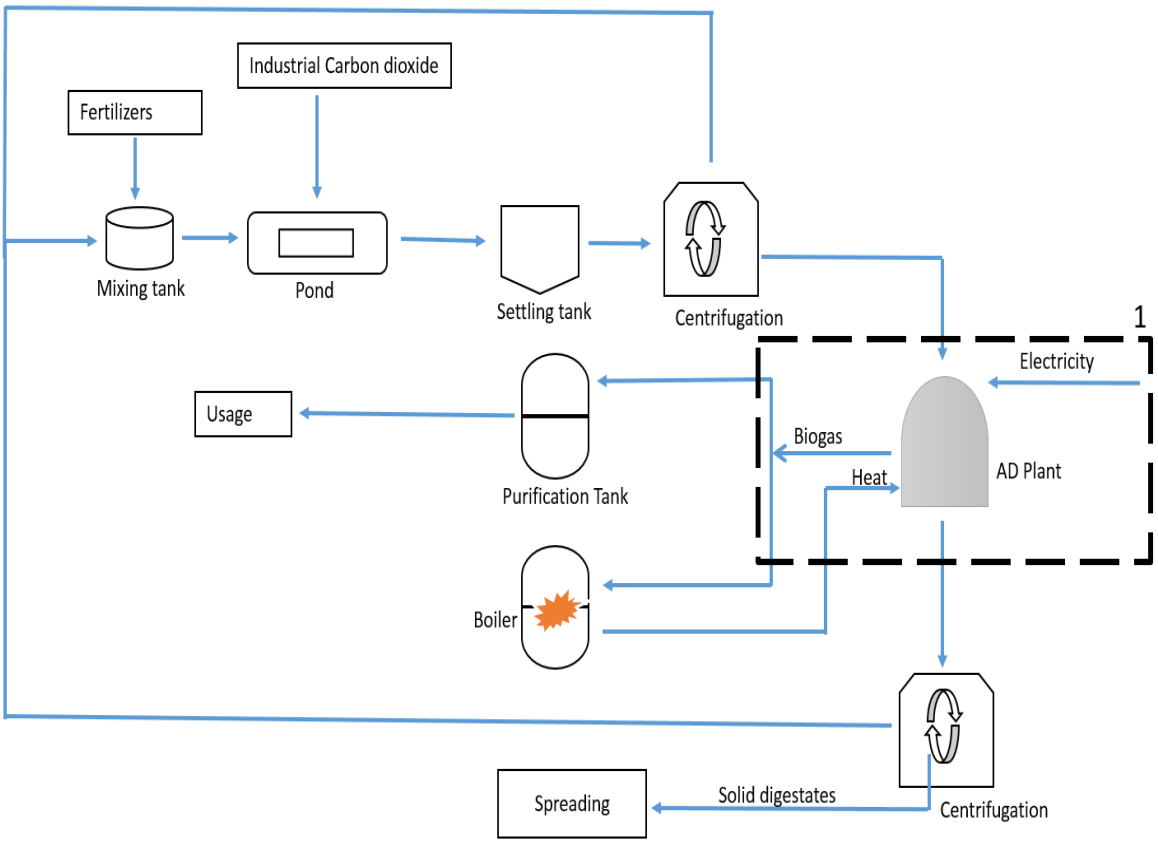

Figure 5: Overview of micro-algae production system with anaerobic digestion

To see the feasibility of biogas plant digesting algae biomass energy performance of biogas production can be analyze, which can be achieved by considering the feedstock coming to the plant as input and the biogas as an output of the plant. The main difficulty lies in finding the energy content of feedstock or algae biomass. It gets more complicated in co-digestion, where the total biogas yield from the feedstock is calculated. [35]

For the preliminary energy balance calculation, data has been assumed from literature that applies to a likely scenario and from the laboratory experiment results. This work includes only direct energy calculation and energy going into anaerobic digestion plant and coming

out of it in form of methane. The system boundary for energy balance is shown with the dotted line represented as 1 in figure 5.

In 2012, at Kymen Bioenergy biogas plant, located in Kouvola (Finland), the energy balance was calculated and gave the following results: to digest 10156 t/a of organic wastes, with average TS% of 27, it required 2600 MWh/a of heat and 450MWh/a of electricity. [35] Assuming the similar energy requirement for algae digestion plant, and comparing biogas production from experiments done in this project, the feasibility of algae digestion plant can be estimated. The highest experimental biogas production among algae species was from *Laminaria Digitata*, which produced approximately 340m^3/t VS. From the experimental result *Laminaria Digitata* has roughly 12% VS so, 10156 ton of wet mass of *Laminaria Digitata* has 1219 ton of VS, giving total production per annum as 414460 m^3. Taking lower heating value of methane as 0,010 MWh/m^3, we get 4145 MWh per annum. Figure 6 shows the energy balance of anaerobic digestion plant digesting *Laminaria Digitata* as substrate. [35]

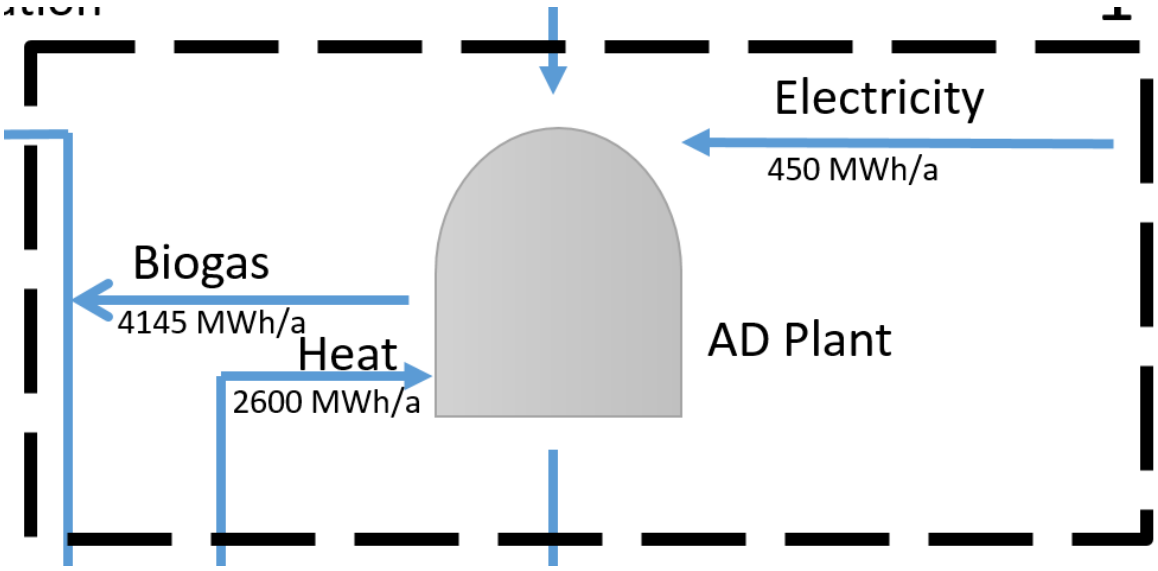

Figure 6: Energy balance of biogas plant digesting Laminaria Digitata as substrate.

From the above scenario, it can be seen that energy balance of *Laminaria Digitata*, which is total output energy subtracted by total input energy, is positive proving that it could be used as substrate for anaerobic digestion. It should be kept in mind that this work does not include cultivation, harvest, transport and pre-treatments methods, which can change the energy balance drastically. Thus producing algae biomass primarily for biogas production is not economically feasible yet due to the high-energy demand and production cost.

The main challenge for calculation of energy balance lies in finding data, since algae species behaves different in different conditions, and, as there are many different methods and technologies for cultivation, harvest and pre-treatment. The creation of a general model for different algae species is thus difficult.

5 Laboratory work

In this chapter, the information on how the practical tests were conducted in laboratory are detailed. The goal of tests was to find out the biogas potential of different algae species and investigate the BMP of algae when mixed with locally available substrates like straw and municipal organic waste. These tests were conducted with AMPTS II from Bioprocess Control, seen in figure 7 below.

Figure 7: AMPTS II from Bioprocess Control [5]

5.1 AMPTS II

AMPTS II is an instrument that allows users to determine the BMP and dynamic degradation profile of any biomass substrate. It consists of three core parts:

1. Sample incubation unit: It contains 15 small reactors where each sample of substrates are mixed with individual agitator during the whole test. Temperature is controlled by a thermostatic water-bath in our case temperature was set to 55^0C.

2. CO_2-fixing unit: The biogas produced in each small reactor passes through individual vials containing 3 M Sodium hydroxide (NaOH) with pH indicator solution Thymolphthalein. CO_2 and Hydrogen sulfide (H_2S) is retained by a chemical interaction with NaOH thus allowing only methane to pass through to flow cell array and data acquisition (DAQ) unit. The color of the solution is blue and when it starts to get colorless this solution need to be changed with the new solution.

3. Gas volume measuring unit: Here methane passed through CO_2-fixing unit is measured using a wet gas flow-measuring device with a multi-flow cell arrangement. This measuring device works according to the principle of liquid displacement and buoyancy and can monitor ultra-low gas flows. An integrated data acquisition system is used to record and analyze the result.

The whole system is then connected to devices, in our case a personal computer using Ethernet. The software that comes with this system is used to monitor and run tests as well as allowing to record, analyze and download reports. [5] More on how to use AMPTS II can be found in user manual, which can be found in bioprocess control website.

5.2 Preparation

To perform laboratory tests for the determination of BMP of the selected substrates following the steps were conducted.

5.2.1 Solutions

The solution used in experiments were prepared with adequate safety and under safe environment. To prepare CO_2- fixing solution first step is to make 3 M NaOH solution, which is prepared by dissolving 240 g of pure NaOH in 2 L of distilled water. Secondly, 0.4% of thymolphthalein pH-indicator solution had to be prepared for that 40 mg of thymolpthalein was dissolved in 9 ml of 99.5% ethanol followed by addition of 1 ml of distilled water. To get final CO_2-fixing solution 10 ml of pH-indicator solution is mixed with 2 L of 3 M NaOH.

5.2.2 Substrates

In this experiment, three different samples of micro-algae consortia, one of them being dominated by different species of *Scenedesmus* and macro-algae species *Laminaria Digitata* were digested to investigate the BMP of these species. For the convenience in this work, the samples of micro algae consortia 1 and 2 are named as algae B1, B2 respectively, and algae consortia dominated by different species of *Scenedesmus* is written simply as *Scenedesmus sp.* Furthermore, *Scenedesmus sp.* and *Laminaria Digitata* macro-algae were co-digested with straw and municipal organic waste. The wheat straw were provided by Yrkesakademin i Österbotten and municipal waste were provided by Stormossen Oy. The municipal waste were already pre-treated and ready to be used. In test 1, the substrates tested were algae B1, B2 and *Scenedesmus sp*. These micro-algae were cultivated in open pond, which were provided by Swedish University of Agricultural Sciences. The substrates were frozen when received and contained large amount of water in it. The TS and VS from these algae sample were too low to get confident result so it was let to sediment and had to be dewatered by pumping thick sedimented layer from the bottom of the container and new TS and VS were done. In test 2, the sample of algae was also provided by Swedish University of Agricultural sciences. It was *Scenedesmus sp.,* which was already dewatered and frozen. In this test, the sample was co-digested with straw and municipal organic waste. For test 3 *Laminaria Digitata* macro-algae sample was provided by Norwegian Institute of Bioeconomy Research, which was washed, ground and frozen. It was also co-digested with municipal organic waste.

5.2.3 Determination of Total solid and Volatile solids

To know the amount of substrates needed in the reactors the TS and VS had to be determined. Every substrate contains some amount of water. VS is usually given as a percentage of TS because if they were measured as a percentage of wet weight, they would vary according to environmental factors such as temperature and humidity. The measurements were done according to European Standard Characterization of sludges- Determination of dry residue and water content EN 12880 and Characterization of sludges- Determination of the loss on ignition of dry mass EN 12879 respectively.

For the determination of dry matter different measurement of substrate were weighed in aluminum tray. They were then dried in an oven at 105^0C for minimum of 18 hours with

40% ventilation. The samples were weighed after they were dried and with the difference in the weight, the TS % was calculated according to equation 1. In figure, 8 below before and after the samples were dried are shown. The TS and VS for each samples were tested before and after digestion.

Equation 1: TS % calculation

$$Ts\% = \frac{(m_c - m_a)}{m_b} \times 100$$

Where,

m_c is the mass of the dish containing the sample's dry matter in grams.

m_a is the mass of the empty dish in grams.

m_b is the mass of the sample in grams.

Figure 8: Samples of algae B1, B2, cellulose, Scenedesmus sp. and Inoculum from left to right respectively; left before drying and right after drying.

The samples from the TS measurement were transferred into ceramic crucible, which was pre-heated for 1 hour and cooled down in desiccator. In this process weight of dry substrate and crucible were also measured. The transferred samples were then burned in 550^0C for two hours then placed into a desiccator to cool down, see figure 9 below.

Figure 9: Samples in desiccator.

After the samples were cooled down, they were weighed again and VS% was calculated according to equation 2 below.

Equation 2: VS % calculation

$$Vs\% = \frac{(m_b - m_c)}{m_b} \times 100$$

Where,

m_c is the mass of the dish containing the sample's dry matter in grams.

m_b is the mass of the sample in grams.

5.2.4 Start up

At first AMPTS II was made ready for the experiment following the user manual. The thermostatic water bath was filled with distilled water and temperature was set to 55^0C. All the necessary connection such as power and motors were connected. Each individual tubing

from reactors to CO_2-fixing unit and from there to flow cell array was done carefully. Flow cell array was also filled with distilled water. Everything was checked before the start of experiment. Flushing of the tubes was conducted by using nitrogen gas to make anaerobic condition. See user manual for more detail on start up for AMPTS II [5]. In figure 10 ready set up for AMPTS II before start up is shown.

Figure 10: Ready set up for AMPTS II before start up.

Three reactors per sample were used for statistical significance so the total of five different substrates could be tested out of these five different substrates one was always a blank or the inoculum. In AMPTS software, each sample was given their unique name and desired values for this experiment were inserted in software, which can be found in table 4, these values remain same for all three batches of experiment. The inoculum to substrate ratio (I/S) was kept at 2/1 for all batch of experiment. The inoculum is the digestate containing already established culture of microorganism to start the process, which was provided by municipal waste treatment company Stormossen Oy.

Table 4: Values inserted in the AMPTS II software

Total sample weight (g)	400
I/S ratio	2:1
Total volume of reactor (ml)	600
Assumed CH_4 content (%)	60
Types of units (VS/COD)	VS
Assumed temperature (^0C)	55

From the filled in values the software calculated the experimental guidelines for amount of substrate and inoculum needed in reactor. Only for co-digested substrate, the amounts were externally calculated. In figure 11 below experiment settings in AMPTS II software program is shown.

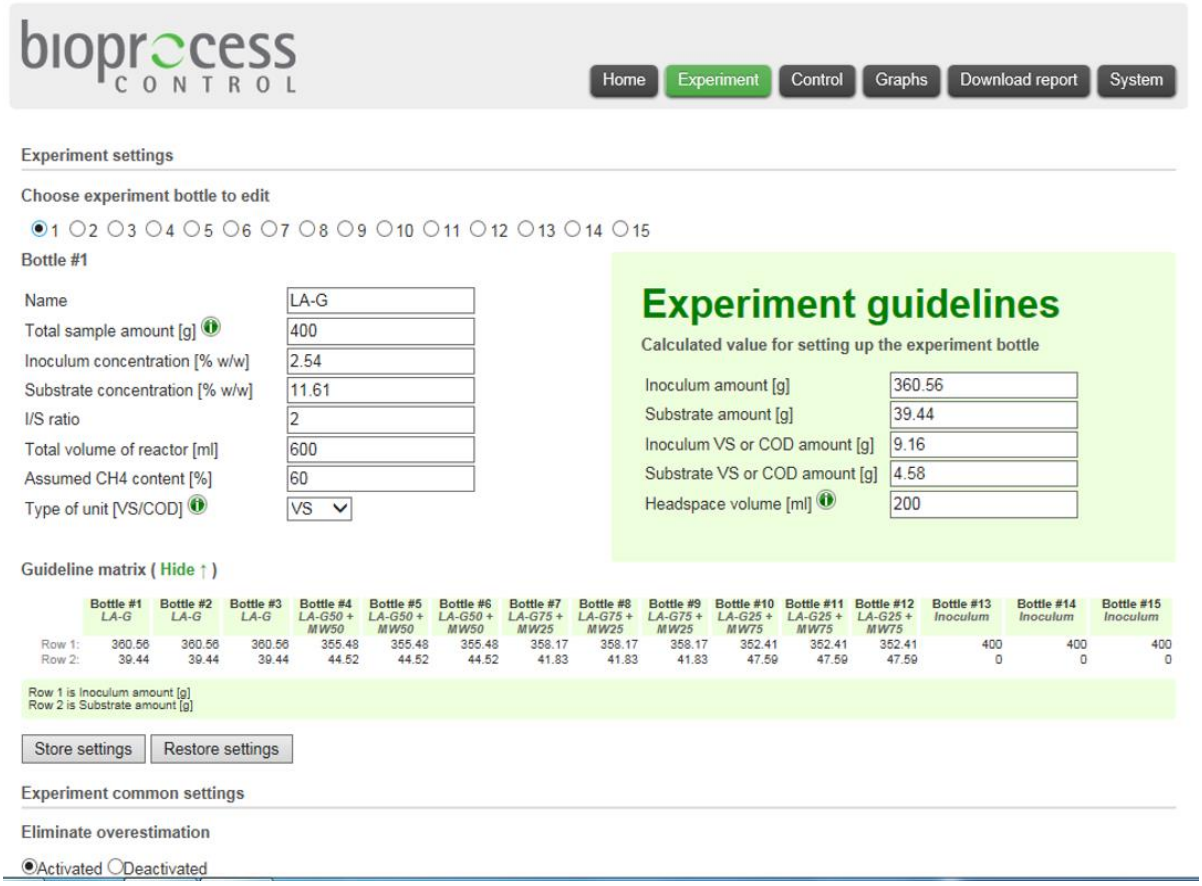

Figure 11: AMPTS II software experiment setting.

5.2.5 Monitoring

Regular monitoring on the experiments were done. The water level in both thermostatic water bath and flow cell array was regularly checked and maintained. NaOH solution in CO_2 fixing unit was replaced with fresh one when the pH indicator turned from blue to colorless. Tubes were also being checked so that they had no bend interrupting the gas flow.

The amount of methane produced as well as the flow rate was constantly monitored and experiments were stopped after the daily flow rate was less than 1% of total accumulated methane production for all samples.

5.2.6 End of operation

Experiments were stopped once all the samples daily production were less than 1% of total methane production. TS and VS of all samples were measured for the determination of degree of degradation. The digestate sample from each reactor were taken and frozen which will be further analyzed for nutrients content in it later in the project.

6 Results and interpretation

Three different batches of tests were conducted. All the experiments ran as expected except *Laminaria Digitata*, it produced more than expected and the digestion was completed faster as well. The biogas produced from each reactor also contains the methane produced by the inoculum, which has to be subtracted from total to get true production form the substrate. Thus, BMP can be expressed as equation 3. In this calculation to find BMP of substrates, the BMP of the blank is subtracted from the BMP of samples on a VS basis.

Equation 3: BMP from substrate

$$BMP = \frac{V_s - V_I}{m_{vs,ss}}$$

In equation 3, V_s is the accumulated volume of biomethane from the reactor containing the sample, which is substrate plus inoculum, V_I is the volume of biomethane coming from the inoculum in the sample bottle and $m_{vs,sS}$ is the amount of VS of the substrate contained in the sample bottle. The blank or only inoculum samples will generate only the amount of biomethane generated from inoculum (V_B) and can further be written as biomethane produced per amount of VS of inoculum, which is $V_B/m_{vs,IB}$ and $m_{vs,IB}$ is the amount of VS of inoculum. The obtained fraction ($V_B/m_{vs,sS}$) has to be multiplied with VS of inoculum in each sample bottle ($m_{vs,IS}$) in order to calculate how much biomethane is coming from the inoculum in the sample reactor, as following. [36]

Equation 4: BMP from substrate per gram VS

$$BMP = \frac{V_s - V_I}{m_{vs,sS}} = \frac{V_s - V_B * \frac{m_{VS,IS}}{m_{VS,IB}}}{m_{vs,sS}} = \frac{V_s - V_B * \frac{m_{IS}}{m_{IB}}}{m_{vs,sS}}$$

Here m_{IS} is the total amount of the inoculum in the sample and m_{IB} is the one in the blank.

In table 5, the BMP as a ratio to the average of all BMP values are shown. The individual biomethane production from substrates are not shown as mentioned in chapter 1.3.

Table 5: Average of total BMP and its ratio with individual production.

Substrate	**BMP NmlCH₄/gVS**	**BMP as a ratio to the average of all BMP values**
Algae B1	*****	0.6
Algae B2	*****	0.6
Scenedesmus sp.	*****	0.4
Cellulose	*****	1.3
Straw	*****	0.7
Municipal organic waste	*****	1.4
Straw 50% + *Scenedesmus sp.* 50%	*****	0.6
Municipal organic waste 50% + *Scenedesmus sp.* 50%	*****	0.9
Laminaria Digitata	*****	1.1
Laminaria Digitata 50% + Municipal organic waste 50%	*****	1.5
Laminaria Digitata 75% + Municipal organic waste 25%	*****	1.3
Laminaria Digitata 25% + Municipal organic waste 75%	*****	1.6
Average of all BMP values	*****	**1**

From the table 5 it can be seen that among three different micro-algae samples, *Scenedesmus sp.* had the lowest BMP which were calculated in normal temperature and pressure, which is 0^0C and 1 atmospheric pressure in milliliter CH_4 per gram VS ($NmlCH_4/gVS$) whereas other two micro-algae consortia samples produced almost the same amount of biomethane. *Scenedesmus sp.,* when mixed with straw and municipal organic waste produced 0.6 and 0.9

times the average of all BMP respectively. The macro-algae species *Laminaria Digitata* produced good results, with production 1.1 times the average of total BMP. *Laminaria Digitata* when mixed with of municipal organic waste 25%and 75% respectively produced the highest BMP in whole test, which suggest that this ratio could be an optimal mixture for co-digestion with municipal organic waste.

BMP test can give the total production of methane but there will always be inner reactions produced by the co-digestion of the different substrates. These reactions are known as synergistic effects. To evaluate the influence of each substrate in the different mixtures and calculate the possible synergistic effects that could be produced in biodegradation process equation 5 below can be used. [37]

Equation 5: Synergistic effects of biodegradation.

$$\alpha = \frac{Experimental\ production}{Theoretical\ production}$$

Here, experimental production is the total biomethane produced in the test by co-digested substrates, and the theoretical production is the sum of percentage of substrate "A" individual production and percentage of substrate "B" individual production. For example, substrate A has 100 unit of biomethane production and B has 40 unit of biomethane production and when they are co-digested with 50% of A and 50% of B then they produce total of 160 unit biomethane. Then, the experimental production is 160 and theoretical production is the sum of 50% of 100 unit and 50% of 40 unit. The result of α indicates that if it is equal to 1 then the substrates work independently from mixture, if it is greater than 1 than the mixture has synergistic effect in final production and if it is less than 1 the mixture has negative effect in the final production. [37] Putting experimental results in equation 5 it has been found that value of α for all three mixture of *Laminaria Digitata* and municipal organic waste was greater than 1 proving that *Laminaria Digitata* has a synergistic effect when mixed with municipal organic waste and mixture ratio 25% *Laminaria Digitata* and 75% municipal organic waste has the highest synergistic effect. It was also found that mixture of straw and *Scenedesmus sp*. has small synergistic effect on digestion. Whereas the co-digestion mixture of *Scenedesmus sp*. and municipal waste has value of α less than 1 suggesting that these mixture had a negative effects on digestion.

7 Conclusion

In this study, different aspects of anaerobic digestion of algae biomass for biomethane production were evaluated. Biomethane production depends on various factors that affect the yields of the biogas from different substrates. Concentration of slurry, temperature, pH, TS, VS, mineral concentration and more importantly, the C/N ratio are the major factors affecting biomethane production. From the results of BMP tests and TS/VS calculation before and after digestion, some of the problems faced by algae biofuel industries are confirmed. Micro-algae species produced less than expected biomethane. Biogas production from these micro-algae species with such a low BMP would be problematic and not feasible for the large-scale biogas production. The main reason for low production can be that they were cultivated in wastewater with high nitrogen and other nutrient which has negative effects on lipids buildup and also that they may be the species with generally low BMP. These micro-algae species samples contained a lot of water and had very low TS and VS in it. VS of all 3 micro-algae was between 1-3% of total wet sample. This shows that dewatering is very essential for better TS and VS % in sample which could be used by microorganisms to produce methane. On the other hand, dewatering is an energy intensive method and raises the costs, which is one of the problem currently faced by algae biofuel industries. From the VS% calculation of digested it was also found that significant percentage VS was still in digestate meaning all the biomass was not fully digested, this shows the need for pre-treatment of biomass or development of new ways and technology that would help in complete digestion of biomass, which increases the biomethane production. From the experiment, it was also found that co-digestion mixture of 25% of *Laminaria Digitata* and 75% of municipal organic waste produced highest biomethane among others making it optimal mixture among rest mixtures and this mixture also had the highest synergistic effect proving that it can be beneficial if mixed with substrates in running municipal waste anaerobic digestion plant.

Based on experimental result and feasibility study at the present time cultivation of algae primarily for biogas production is not economically viable yet. To make it economical, investment and production cost need to decrease. In addition, the cultivation and harvesting methods must be efficient. One way for cost effectiveness could be the cooperation of both industries producing high value products from algae and algae based biofuels with the anaerobic digestion of algae residuals and co-digestion of algae with locally available substrates for maximization of methane-rich biogas. Another way could be to see algae

cultivation as a both carbon capturer from industries and to use nutrients from waste streams for growth of algae, which could otherwise cause eutrophication problem and use that biomass for anaerobic digestion.

8 Limitations and suggestion for further research

Certain limitations were admitted for the results and conclusion presented. First, due to the time and resource constraints the number of samples digested was rather small with four different algae and five different mixtures. It would be interesting to analyze more algae samples but also the co-digestion of samples in other various ratios to test the synergistic effect. The scale of the study has also been limited to AMPTS II. Added value could be brought to the BMP tests results in testing them again on a larger scale digester to confirm and compare the BMP results. Finally, the scope of the study was also limited to anaerobic digestion. Hence, it would be interesting to test the C/N ratio of substrates, pre-treating them by different pre-treatment methods and comparing the biomethane yield.

Further research and experiments could be conducted to fill the above gaps. Moreover, the synergistic effect of algae mixtures has been highlighted by the results presented so it would be interesting to verify these facts with the future studies.

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Appendix 1: Regulation in Sweden for biogas as vehicle fuel.

Parameter	ISO/DIS 15403-2006	Swedish standard SS 15 54 38	
		Normal four-stroke engines	Lean-burn engines
Wobbe index ($kWh\,Nm^{-3}$)	n.s.	12.2–13.1	12.4–12.9
CH_4 content (% by volume)	>96	97 ± 2	97 ± 1
Methane number	n.s.	>130	>130
O_2 content (% by volume)	<0.5	<1	<1
CO_2 content (% by volume)	<3	n.s.	n.s.
$CO_2 + O_2 + N_2$ content (% by volume)	n.s.	<5	<4
Total nitrogen (without N_2) ($mg\,Nm^{-3}$)	<120	<20	<20
Thiols (mercaptans) ($mg\,Nm^{-3}$)	<15		
H_2S content ($mg\,Nm^{-3}$)	<5	<23	<23
H_2O content ($mg\,Nm^{-3}$)	n.s.	<32	<32
Dew point temperature (t_S) for the highest pressure in the gasholder (°C)	At the lowest ambient temperature condensation is not to take place	$t_S - 5$	$t_S - 5$
Dust content	Technically free	n.s.	n.s.
Oil content (ppm)	100–200	n.s.	n.s.