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The Influence of Energy Efficient Cultivation on the Chemical Composition of Algae Biomass for Biodiesel Production

Master's Thesis in Innovative and Sustainable Chemical Engineering

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Division of Life Science
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Department of Chemical and Biological Engineering
Division of Life Science
Chalmers University of Technology
SE-412 96 Gothenburg
Sweden
Telephone: + 46 (0)31-772 1000

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Declaration

The research was conducted at SP Technical Research Institute of Sweden, Borås (SP) under main supervision by the principal investigator Niklas Strömberg and co-supervision by Johan Engelbrektsson. The research is a part of SP's Algae Research Program and the SP Biofuel Competence Platform. All measurements and data processing presented in this report are made under supervision by senior scientists or performed by qualified technical staff at SP. The reactor design and principles of operation has been gradually developed SP scientists since 2011. Algae biomass in this study was cultivated during the period 2011-2013. More information regarding SP's Algae Research Program can be found at www.sp.se.

Abstract

The interest in finding renewable alternative to petroleum-based fuel has increased significantly during the last decades due to global warming and depletion of fossil oil sources. One of the most promising renewable alternatives to petroleum is microalgal biodiesel. Microalgae are advantageous because of significantly higher areal productivity compared to terrestrial plants. However, the main drawback that has hindered the microalgal biodiesel to establish a rigid market position is the energy consumption in the processing steps, which currently is larger than the potential output energy. Life cycle analyses show that the harvesting step may contribute up to 20-30 % of the biomass cost. The overall objective of this research was to investigate efficient ways of harvesting microalgae as well as growing them. The primary aim was to study how the chemical composition of microalgae changes in situation where they are partially degraded and have sedimented to the bottom of the cultivation pond compared with microalgae harvested in the water column. If the sedimented material marginally differs in energy content compared to fresh biomass, there is a great potential in energy savings by harvesting less frequently.

This research project included the determination of the microalgal productivity as well as characterization of two batches of the microalgal strain *Scenedesmus* simris002 that were cultivated in circular, open ponds at *SP Technical Research Institute of Sweden, Borås*. The characteristics that were of main interest was to study how the lipid content as well as the composition of fatty acid methyl esters (FAME) differed between samples harvested at the bottom and in the water column. In addition to these tests, analyses of the elemental composition, concentration of chlorophyll and calorific heating values were also examined. As a complement to the experimental data, theoretical models were constructed to find ways of supplying carbon dioxide, ensure appropriate mixing and harvesting in order to increase the annual productivity at the same time as the energy consumption was minimised.

Without any nutrient and pH control, the biomass productivity in cultures was at least 24 % of the theoretical maximum considering the environmental conditions during the cultivation. The lipid content ranged between 16-32 wt. %. From the characterization of fatty acid methyl ester, it was concluded that palmitic acid methyl esters (C16:0-Me) and oleic acid methyl ester (C18:1-Me) were the most abundant fatty acids in the algae investigated in the first and second batch, respectively.

The calorific heating value was at most 24.5 MJ kg^{-1} for the sedimented algae, i.e. comparable with the energy content of fossil coal (17-27 MJ kg^{-1}). The experimental study also confirmed that chlorophyll is the first component that is degraded, but did not have a significant effect on the energy content of the microalgae.

The theoretical models emphasized the importance of injecting higher concentration of carbon dioxide in order to improve the productivity as well as decreasing the energy consumption. Despite somewhat lower amounts of lipids in the samples harvested at the bottom, it would be advantageous to harvest less frequent by only transferring the sediment to drying ponds.

Key words: *Scenedesmus, FAME, sediment, cultivation, harvesting, energy efficiency*

Acknowledgement

This research would not have been possible without the support of many people. I would like to express my gratitude to my supervisors, Niklas Strömberg and Johan Engelbrektsson, who have enthusiastically guided me through this project and offered invaluable assistance, experience and support. I would also like to convey thanks to the staff of the Chemistry department at *SP Technical Research Institute of Sweden* for all the help during these months.

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1 Introduction

The energy demanding process of deriving biodiesel from microalgae has halted the break-through of an environmental friendly alternative to petroleum-based fuels. Critics claim that until new, less-energy demanding technologies have been developed, microalgal biodiesel will have a hard time establishing a competitive position on the petroleum market. The goal of the research is, therefore, to find and develop a sustainable method of culturing and harvesting microalgae for the production of biodiesel. The research was divided into an experimental study, where the impact of chemical composition on the quality of biodiesel was studied and a theoretical part, where new or modified culturing techniques were evaluated in order to improve energy efficiency.

1.1 Background

Today's limited supply of fossil fuels together with increasing levels of greenhouse gas emissions are two major problems around the globe. It has been projected that the energy demand will increase with 49% by the year 2035, leading to even higher levels of greenhouse gas emissions.[1]

Therefore, there has been a growing interest to find and develop alternatives to petroleum-based fuels. Much of the attention has been directed towards liquid biofuels, especially from terrestrial plants.[1] Currently, there are two generations of bioethanol and one generation of biodiesel on the market. The first generation of bioethanol and biodiesel uses starch and sugars (e.g. from corn and sugar cane) and oil (e.g. from palm tree and oilseed rape), respectively. However, these two fuels have been questioned, mainly due to their competition with arable land for food crops. Regarding the second generation of bio-ethanol in which lignocellulosic material is processed into ethanol, the problem with competing land area is reduced.[2] However, the fermentation of lignocellulosic material requires large amount of enzymes and has been shown to produce undesired byproducts, which interfere with the fermentation and thus, reduces the yield.[3]

These drawbacks for the first two generations of biofuels have led attention to production of biodiesel from algae. An advantage with algae as source of biodiesel is that algae have a high content of lipids from which FAME biodiesel can be produced and together with a rapid biomass production, this gives algal biofuel an interesting potential for the future.[2] Another advantage is that algae have a remarkable ability to fix CO_2. In comparison with terrestrial plants, algal CO_2-fixability are 10-30 times more efficient due to rapid productivity, which makes cultivation of algae nearby large emission sites an application of high potential.[4,5]

The research field of microalgal biodiesel started, however, already in the 1970s as a possible solution to solve the energy crisis by initiative from the administration of American president Jimmy Carter. The Aquatic Species Program (ASP), a sub-group to the National Renewable Energy Laboratory (NREL), investigated the technological and economic potential of deriving biodiesel from microalgae during an 18-year period (1978-1996). Due to diminishing funding from the U.S government, ASP decided to close the program in 1996. Nevertheless, ASP developed a large number of cultivation systems and techniques during this working period leading to an extensive database of information. During the beginning of the 21st century the interest of microalgal biodiesel once again started to bloom due to the desire to find new alternatives within the field of renewable fuels.[6]

Even with the promising potential of microalgae, there are several obstacles that need to be tackled before there will be an algal biodiesel that can contribute to customers' demand on a large scale.[2] One major concern is the energy consumption in the processing steps, which is larger than the energy output. This makes the sustainability concept of this biofuel questionable. Life cycle analyses have shown that the harvesting step may contribute up to 20-30% of the total biomass cost.[7] This is mainly due to removal of large quantities of water, handling of algal biomass and frequent, energy demanding harvesting methods (e.g. filtration and centrifugation).[8]

The overall objective of this research project was to investigate efficient ways of harvesting microalgae as wells as growing them. The primary aim was to investigate how the chemical composition of microalgae changes in a situation where they are allowed to sediment to the bottom of the cultivation pond, subjecting them to partial degradation compared to microalgae harvested in the water column. There is a potential in energy savings by harvesting less frequently.

1.2 Aims

The project aims to find differences in lipid content, fatty acid composition and chlorophyll as well as the chemical composition and calorific heat values between algae harvested at the bottom and at the surface. Extra focus will be set on finding an efficient method for the lipid extraction from algal biomass concerning mass and extraction time.

In addition to the experimental part, this project also aims to find energy effective methods of culturing (e.g. nutrition supply and mixing) as well as harvesting. In combination with the experimental part, the overall aim is to find an economically feasible method of producing biodiesel from microalgae.

1.3 Delimitations

The report concerns evaluation of algae grown in energy efficient batch reactors designed 2011 at SP. For this project, only one species within the algal strain *Scenedesmus* was studied. The research did not focus on finding an optimal algal growth. The cultivation systems were not monitored to obtain information about fluctuations in temperature, light, pH and nutrition. Analyses were performed on two batches with different supply of nutrients. Due to this, comparison between the samples collected from the bottom and from the surface in the ponds was only possible within each batch.

Economic consideration, in its monetary value, will not be taken into account regarding calculations concerning the energy efficiency. Additionally, variables as aeration, supply of CO_2 and harvestings are only considered in the theoretical models calculating the energy efficiency.

2 Theoretical framework

The theoretical framework provided in this report emphasis mainly on algal cultivation and harvesting as well as applications related to the production of algal biodiesel.

2.1 Algae

Algae were previously regarded as any organism that contained chlorophyll *a* and a vegetative body (a thallus) that is not separated into organs. These organisms had also no differentiated roots, stem and leaves. This broad definition includes cyanobacteria as well, although they are defined as a prokaryotic organism nowadays. To be a bit more specific, phycologists choose to refer the term microalgae to the microscopic algae *sensu stricto* and the photosynthetic bacteria.[9]

Microalgae are found all over the world. Most of them are found in marine, brackish and fresh waters, but they are also able to grow on land on the surface of soils.[9] They are characterized by being greatly adapted to live in a broad variety of environmental conditions.[10] The energy process of microalgae is represented by photosynthesis in which inorganic matter and energy in the form of light is converted into organic compounds.[10,11]

The chemical composition of algae (Table 1) varies greatly, not only between different algal groups but also within specific species and depending on environmental conditions (i.e. temperature, nutrition, light, pH etc.).[12]

Table 1. General chemical composition of different algae (% of dry matter).[12]

Alga	Protein	Carbohydrates	Lipids
Chlorella pyrenoidosa	48	26	2
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6
Scenedesmus obliquus	50-56	10-17	12-14
Scenedesmus dimorphus	8-18	21-52	16-40
Spirulina platensis	46-63	8-14	4-9

There is at present some disagreement about the total number of algal species. However, scientists estimate the number of species to approximately 50 000.[10]

For this project, one species within the algal strain *Scenedesmus* was used; simris002. This species was isolated by Simris Alg AB and have close similarities with *Scenedesmus dimorphus*. *Scenedesmus* is a small (ranging from 5-40 μm in length), autotrophic and non-motile strain of microalgae. It can easily be recognized from its oval shape and its tendency to form colonies consisting of approximately 4 to 32 cells. Within the strain of *Scenedesmus*, approximately 200 different species have been identified making it one of the most diverse algal species. They are also known to be cosmopolitan meaning that can be found all over the globe as long as the conditions are acceptable.[13]

Figure 1. Colony formation of a *Scenedesmus*-strain

2.1.1 Physical properties

As described in previous section, microalgal species may include both prokaryotic (Cyanobacteria) and eukaryotic (green algae) microorganisms.[9] However, this report will focus on green algae and thereby the properties of eukaryotic organisms.

The cell organization of microalgae may be different between species. The most common types of organization include unicellular, colonial and filamentous structures in which the unicellular is the most common one when it comes to green algae.[9] The motility of microalgae depends of the presence of flagella. Lacking of flagella, the microalgal cells are non-motile.[9,14]

The eukaryotic microalgae possess a membrane-bound nucleus in which the genome is distributed on chromosomes and nucleolus. For microalgae, the cell contains several membrane-bound organelles (i.e. chloroplasts, endoplasmic reticulum and Golgi body) that are dedicated to execute specific functions.[9,14]

Chloroplast is one of the most important organelle for the survival of an alga. It contains a series of flattened vesicles. These vesicles, also called thylakoids, contain chlorophyll and other pigments. Chloroplasts execute the photosynthesis during daylight. Here, the cells use NADPH and ATP, products of the photosynthesis, to produce organic molecules. These products include sugars that are transported to non-photosynthetic cells of the organisms to meet the metabolic needs.[9,15] This process will be further described in the next section.

The growth of microalgae depends on several chemical and physical factors. Generally, as long as there is a presence of nutrients and no inhibitory influences, the organism remains viable (further described in Section 2.2.). With declining amounts of substrate and energy, or toxic inhibition, the cell declines and finally dies.[9] Sometimes there is also a formation of spores that may survive and thereby giving rise to new individuals. Regarding the reproduction, microalgae are most common reproduced asexually, which involves interphase and mitosis. During the latter, the microalgal cell grows and all cellular components increase in number. This process will ensure that each daughter cell will receive necessary intracellular components and DNA. During the mitosis, the mother cell divides into two daughter cells.[9,16]

2.1.2 Photosynthesis

The photosynthesis, equation (1) is a redox reaction in which terrestrial and aqueous plants convert carbon dioxide and water into carbohydrates and oxygen, supported by light energy. This process not only provides sugars for energy production, but is also the major path in which carbon dioxide returns to the biosphere.[16]

$$6CO_2+6H_2O \xrightarrow{h\nu} C_6H_{12}O_6+6O_2 \quad (1)$$

Light energy, however, cannot directly drive this reaction. The reaction seen in equation (1) can be divided into two sub-processes that occur in all photosynthetic organisms. The first sub-process is called the light reaction, the light energy is used to a photochemical oxidation of the water molecule. In this oxidation, two things are accomplished. Firstly, the oxidizing agent $NADP^+$ is reduced to NADPH and an oxygen molecule is released. Secondly, the light energy is used to phosphorylate ADP to ATP via a proton gradient. In the second sub-process, also called the dark reaction, NADPH and ATP produced in the first sub-process reduces carbon dioxide into carbohydrates.[11,16]

The light reaction has been further studied and reveals that two kinds of photosystem must be involved in the photosynthesis in plants (Figure 2). Both photosystems are localized in the thylakoid membrane. Each photosystem consists of a number of subunits including reaction centres, protein complex and electron transport agents. Chlorophyll and other pigments act like antennas, trapping the energy quanta excited by the absorption of light. Studies of Arnold and Emerson[16] shows that chlorophyll molecules in the cell only absorbs a fraction of the incoming photons. The energy of these are then transferred by resonance to other chlorophyll molecules, which also starts the photochemistry of the light reactions.[16,17]

As the cell density of the culture increases, more photons can be absorbed leading to a strict connection between the presence of light and cell growth. With a dense culture, light cannot penetrate into each individual cell. Thus, the cell growth stagnates.[17]

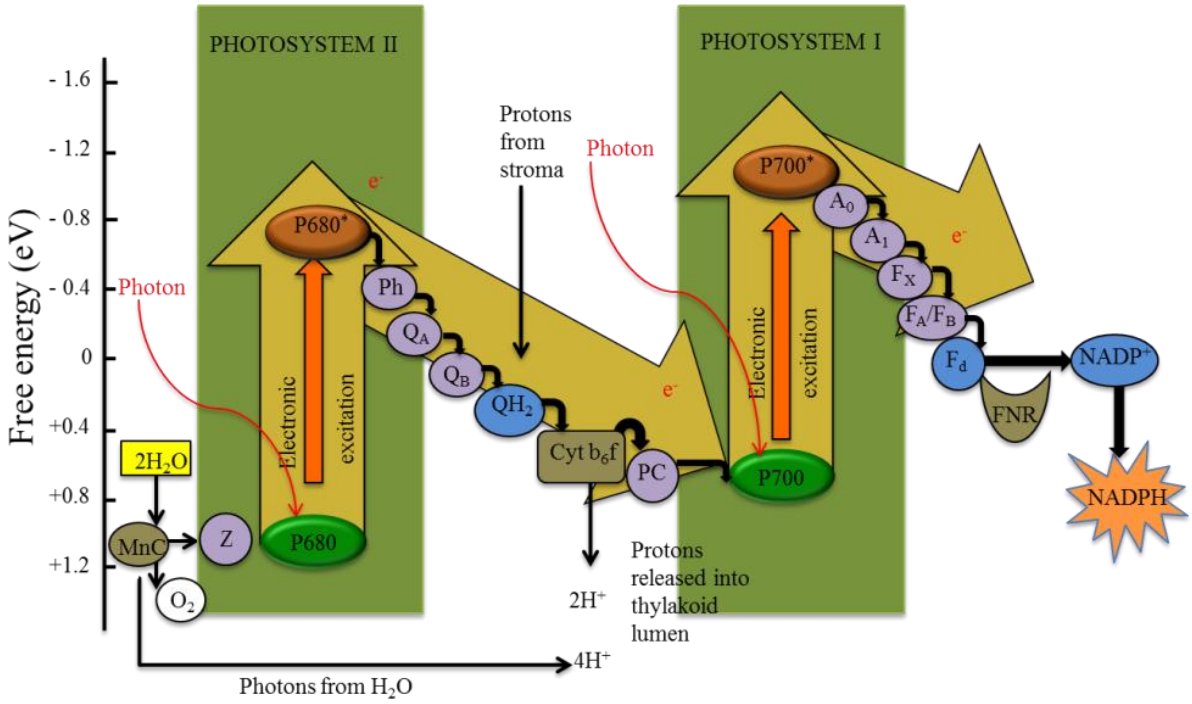

Figure 2. Mechanisms of the two-photosystem light reactions. MnC = manganese center, Z = donor to P680, P680 = photosystem II reaction center, Ph = pheophytin acceptor, Q_A=Q_B = protein-bound plastoquinones, QH_2 = plastquinol in membrane, Cyt b_6f = cytochrome b_6f complex, PC = plastocyanin, P700 = photosystem I reaction center, A_0 = chlorophyll acceptor, A_1 = protein-bound phylloquinone, F_A,F_B,F_x = iron-sulphide clusters, Fd = ferredoxin, FNR = ferredoxin:$NADP^+$ oxidoreductase.

The dark reaction occurs in the stroma of the chloroplasts. The main function of this reaction is to fix available carbon dioxide into carbohydrates. The process starts with adding one carbon dioxide onto an acceptor molecule. The molecule is then passed through a cycle of reactions called the Calvin cycle, which results in formation of hexoses and regeneration of the acceptor molecule.[11,16]

Light is thereby, typically, the main limiting factor for obtaining an effective photosynthesis. For photosynthesis, light in the visible region (400-750nm) is of primarily interest. The small proportion of ultraviolet light that breaks through the ozone layer can only penetrate a short distance into water, making it unavailable for photosynthesis. Photons of a higher wavelength (>750nm) have too low energies to be valuable for photochemical processes.[16]

2.2 Cultivating microalgae

Cultivating microalgae is commonly more expensive than growing terrestrial plants. Except for the presence of light, there are several other important factors influencing the growth of microalgae including supply of nutrients, temperature and mixing.[19,20]

2.2.1 Nutrients

The growth medium is an important aspect in the culturing of microalgae. It needs to include elements as carbon (C), nitrogen (N), phosphorus (P), and sulfur (S).[18] The minimum amount of each element can be estimated by Grobbelaar's[19] approximate molecular formula of microalgal biomass, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$, which describes the elemental ratio in carbon equivalents.

Carbon is by far the most important nutrient in the growth medium.[19] This component is important for obtaining a high autotrophy (most algae belongs to this category) biomass production. On the contrary to terrestrial plants, atmospheric carbon dioxide (CO_2) may not be enough to obtain high biomass productivity. Grobbelaar[19] reports a maximal algae productivity of 10 g m^{-2} day^{-1} if only atmospheric carbon dioxide is provided. This is mainly due to the low diffusion rate of carbon dioxide in water. Thus, it could be necessary to enrich the culture with carbonate (CO_3^{2-}), flue gases with higher concentration of carbon dioxide or improve the mixing in the culture to ensure high productivity.[19,20]

Nitrogen is the second most important element for the biomass production. The content of nitrogen in algal biomass can range between 1-10%. This difference occurs not only between different groups, but also within species. Nitrogen is often present in the growth medium as nitrate (NO_3^-), ammonia (NH_4^+) or urea.[19] It has been shown by Li et. al [21] that nitrogen is connected with the lipid production in algal biomass. By exposing algae for nitrogen starvation, the biomass productivity will decrease, since the protein biosynthesis of new cells is dependent on the availability of nitrogen. In the absence of new cells, algae store lipid reserves instead.[21,22] The increased lipid content is advantageous for biodiesel production, since the lipids are processed into fatty acid methyl ester (Section 2.4.2).[21]

The third major component, phosphorus, is vital for growth and several cellular processes including energy transport, synthesis of nucleic acid, DNA etc. Phosphorus is generally supplied to algae as phosphate (PO_4^{2-}). Even though algal biomass contains less than 1% of phosphorus, it has been considered to one of the most important growth limiting factors. This is mainly due to phosphorus ability to bind to other components, making it unavailable for uptake by algae.[19]

What Grobbelaar does not consider in his molecular formula is sulfur, which may also be an essential element for some microalgal species. Sulfur is found in cysteine and methionine, which both are important amino acids in the protein synthesis. Lacking sulfur does not only interfere with the protein synthesis, but also the repair system in Photosystem II in the photosynthesis.[23] The importance of sulfur in algal biomass has been considered by several researchers, especially for marine algae species.[24]

2.2.2 Temperature

Temperature is one of the most crucial parameters influencing the biochemical composition and growth of microalgae. The temperature range in which algae grows depends greatly on the species. In general, microalgae have a growth optimum in the range between 20-30ºC.[18,25] Goldman and Carpenter[26] show in their study that the temperature dependence can be

described with the Arrhenius-equation in which the doubling rate is doubled for every 10 °C, in the temperature range 5-35 °C.

Studies have also shown that the temperature especially affects the lipid composition in the algal cell. Temperatures below growth optimum stimulate the production of unsaturated lipids. This is probably, according to Hu [25], due to enhance the stability and fluidity of cellular membranes and thereby protecting the photosynthetic machinery from photoinhibition at low temperatures. As the temperature increases, saturated lipids starts to dominate the lipid composition.[25,27]

The growth temperature has also been assumed to affect the cellular carbon and nitrogen ratios together with the volume of the cell, which is characterized by an U-shaped response curve. At an optimal growth temperature, the algal cell has not only the minimum cell size, but also the lowest content of carbon and nitrogen. For temperature above or below the optimal level, the cell increases its volume as well as increasing the cellular content of nitrogen and carbon.[25]

2.2.3 Mixing systems

In order to ensure high productivity, it is of great importance that each microalgal cell has the possibility to consume nutrition and light. As the cell density increases, the microalgal cells prevent each other from the uptake of light energy leading to a decreased photosynthetic efficiency and thereby also stagnation in cell growth. To overcome this problem, mixing systems are installed in the cultivations.[28]

Depending on cultivation system (Section 2.2.4.), different mixing systems can be applied including paddle wheel and airlift systems. A paddle wheel is a simple mechanical device providing a force to cause turbulence and thereby ensure sufficient mixing. Such system also gives a gentle mixing, with low shear forces, which is advantageous for not damaging flocculated algae. However, a paddlewheel system is fairly large compared to other systems making it more suitable for large cultivation systems.[28]

An airlift system is generally much smaller in comparison to paddle wheels. It is advantageous in smaller cultivation systems due to the potential of combining the supply of carbon dioxide and mixing. On the other hand, airlift systems generally produce higher shear forces, which may interfere with autoflocculation.[28]

2.2.4 Cultivation systems

Microalgae are mainly cultivated in two different ways: open (ponds) or closed (photobioreactor, PBR) systems. Both these systems have their advantages and disadvantages. Open pond cultivations are less expensive to operate and maintain in comparison to photo-bioreactors. On the other hand, photobioreactors are easier to control to obtain better cultivation conditions.[8]

Although there are many advantages with PBR listed in Table 2, there is only a small chance that these will have an impact in the nearest future when it comes to algal cultivation. This is mainly due to the difficulty in scaling up PBRs as well as high investment and operating costs.[8]

Table 2. Comparison between open and closed systems.[8]

Culturing aspects	Open systems (pond)	Closed systems (PBR)
Contamination risks	High	Low
Process control	Difficult	Easy
Species control	Difficult	Easy
Mixing	Very poor	Uniform
Population density	Low	High
Investment cost	Low	High
Operating cost	Low	High
Productivity	Low	High
Scale up	Difficult	Difficult

2.2.4.1 Open pond systems

High rate algal ponds (HRAPs) or raceway ponds are the most common large-scale algal production systems. As can be seen in Figure 3, a raceway pond is a closed recirculation system. The depth in such system is typically 0.3 meter and the length of the raceway varies substantially. The recirculation and mixing of algae and nutrition is provided by a paddlewheel. Nutrition is fed in front of the paddlewheel, while harvesting occurs behind it. Theoretically, open systems could have a productivity of 50-60 g $m^{-2}day^{-1}$. However, previous work shows that a productivity of 10-20 g $m^{-2}day^{-1}$ is more common.[8,18,29]

This type of cultivating system has been used since the 1950s and one of the largest facilities today occupies an area of approximately 440,000 m^2 (California, *United States*).[18,29] Although there is a lot of experience in operating open pond systems, there are several obstacles that are hard to handle. A major problem is to control the system. Cooling and heating are mainly achieved with evaporation and sunlight, respectively. This can become a major problem in regions where the temperature changes seasonally. Due to substantial losses to the atmosphere when enriched with carbon dioxide, open systems can fixate significantly less carbon dioxide in comparison to photobioreactors.[18] To prevent these kinds of losses, the ponds are partially covered to maintain high carbon dioxide concentrations.[30]

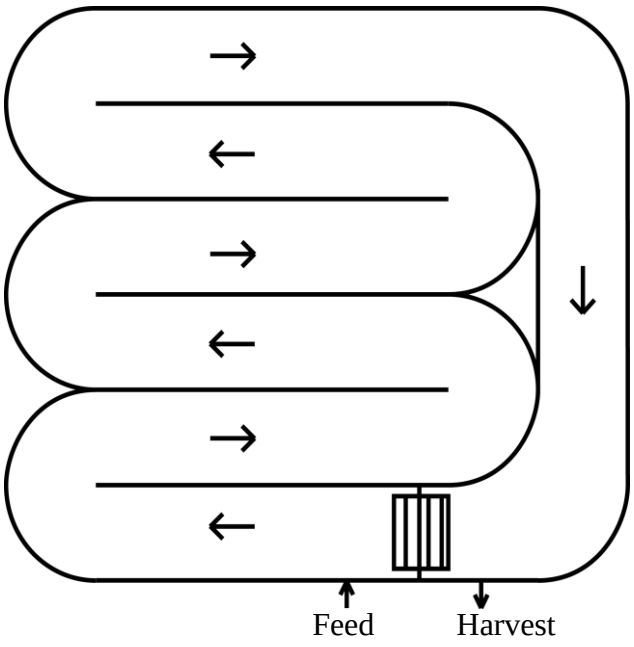

Figure 3. Basic principle of a raceway system.

2.2.4.2 Photobioreactors

Photobioreactors are closed cultivating systems in which solar light is transferred through the transparent reactor's walls to the algal cells. Thus, photobioreactors greatly limit the direct exchange between the environment (gases and contaminants) and the reactor.[31]

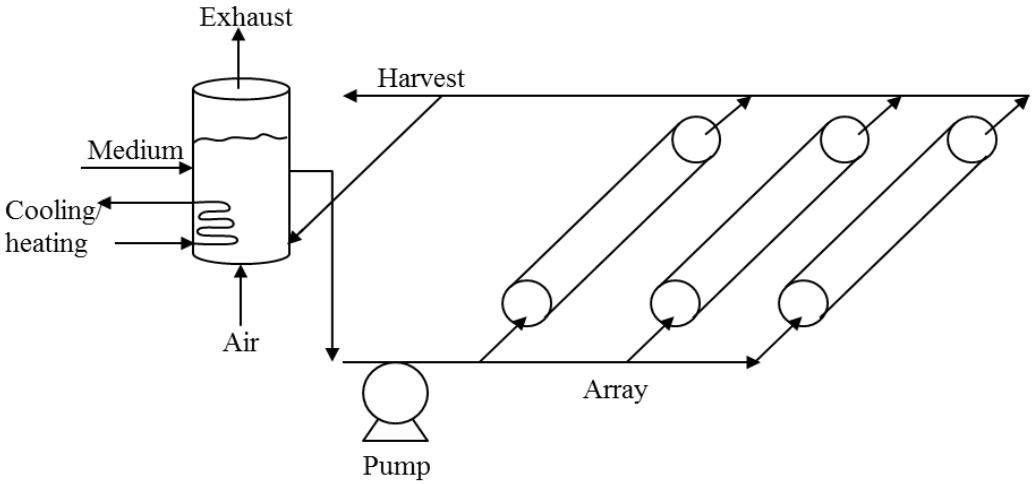

Figure 4. Principle of a photobioreactor construction.

The most common type of photobioreactors is the tubular-shaped reactor. These, typically, consists in a network of tubes (Figure 4) that is directed horizontally or vertically. Recirculation within these systems can either be done mechanically or with an airlift system. The latter allow exchange between the algal culture and needed gaseous nutrients as well as proper mixing, which is essential to obtain high productivity.[32]

Another important aspect of obtaining high productivity is the tube diameter. A consequence of a too wide diameter is that light cannot penetrate through the culture. Thus, the tubes are generally not wider than 0.1m, which on the other hand may cause high temperatures within the tubes.[18] In comparison to open systems, the productivity in a PBR could be more than 13 times greater. Together with the possibility of having a denser algal culture in the system (~ 30 times the concentration obtained in raceways), gives the photobioreactor an interesting position for future applications. However, the production and operating costs are still too large to make this system economically feasible.[18,32]

2.3 Harvesting microalgae

To be able to use the cultivated algae for biodiesel production, the harvesting step is of major importance. It, generally, consists of one or more solid-liquid separations. The number of separation steps depends mainly on the cell density in the cultivation and harvesting technique. Regarding the former, the algal culture is rather diluted (0.5-4 kg m^{-3}) and therefore large volumes need to be processed to obtain a proper amount of algal biomass.[7,32]

2.3.1 Harvesting systems

Currently, there is no universal method for harvesting algal biomass. Depending on the characteristics of microalgae, e.g. species, size, culture density and the purpose of the final products, an appropriate harvesting method needs to be chosen.[7,29,32] In general, harvesting of microalgae can be divided into two steps: bulk harvesting and thickening. The former has the aim of concentrating the broth from the cultivation by a factor of 100-800, resulting in a slurry that contains 2-7% of algal biomass (dry weight). Common technologies for the bulk harvesting involve flocculation, floatation or sedimentation. The second step, thickening, concentrates the slurry and involves technologies such as filtration, centrifugation and

ultrasonic aggregation. Thickening is generally more energy demanding compared to bulk harvesting.[3,32]

2.3.1.1 Flocculation

As mentioned in previous section, flocculation is typically a preparatory step before the thickening procedure. Since the algal culture has a low cell density, it is of economic and technical importance to increase the effective particle size. Thereby, it will be easier to achieve an efficient harvesting.[7,32]

Most microalgae carry a negative charge, which prevents them from autoflocculation. Thus, it is necessary to add ions or polymers with a positive charge to reduce or neutralize the negative surface charge and induce flocculation. These flocculants should be nontoxic, inexpensive and effective in low concentration. It is also of importance to choose a flocculant that does not affect the downstream processing and the final product. Brennan et al.[32] and Molina Grima et al.[7] reports ferric chloride ($FeCl_3$), aluminum sulphate ($Al_2(SO_4)_3$) and ferric sulphate ($Fe_2(SO_4)_3$) as suitable flocculants. In addition to these metal salts, there is also a possibility to use cationic polymers. Positively charged polymers have not only the ability to neutralize the cell surface, but also linking particles together in a process called bridging. The efficiency of polymers depends mainly on the ionic strength, size of particle, polymer and mixing conditions. An influence or a combination of low ionic strength, small particles and low mixing tends to result in an efficient flocculation.[7]

For some algal species (e.g. *Chlorella*), effective flocculation has been achieved by increasing the pH in the culture. This method is advantageous as there is no need of adding other flocculants. However, there is a risk that cells lyse and release their intracellular components for extreme pH values.[7]

2.3.1.2 Filtration

Filtration has been considered to one of the most promising methods when it comes to harvesting algal biomass. It includes methods as microfiltration and ultrafiltration as well as pressure and vacuum filtration.[33] The recovery of biomass, though, depends entirely on the size of the cell. To obtain high filtration efficiency, it is necessary that the cells are relatively large (>70µm). Thus, conventional filtration fails to recover the biomass for algal species of bacterial dimensions (e.g. *Scenedesmus, Dunaliella* and *Chlorella*).[7]

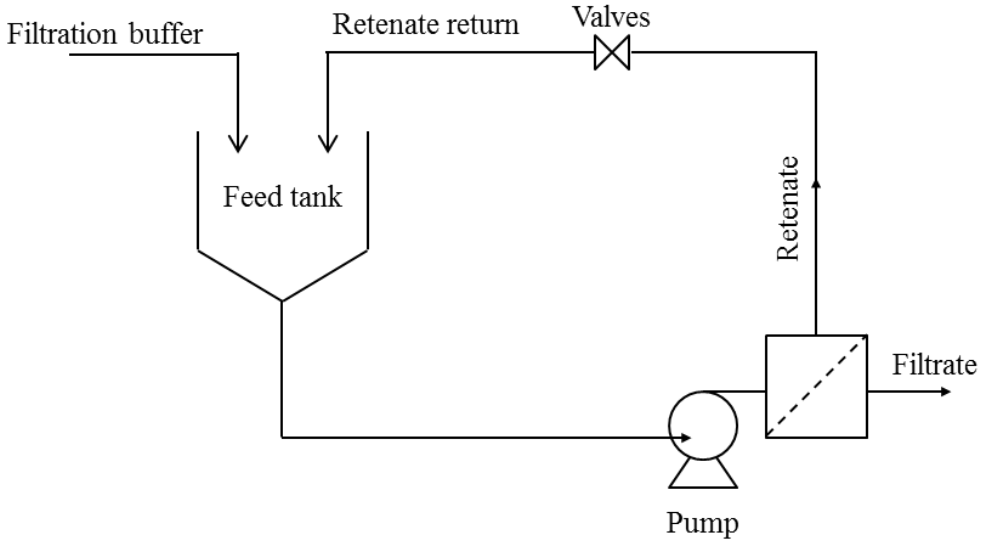

Figure 5. Schematic overview of a tangential flow filtration system.

The general principle of filtration is to allow the culture broth pass through a filter on which the solid matter accumulates while the liquid phase passes through. This process proceeds until the broth is concentrated to an algal paste.[33] The pressure applied across the filter and the pore size are two crucial parameters. The pressure is necessary to maintain a driving force, while the pore size determines separation efficiency. For larger-sized algae, larger openings in the filter can be used and thereby allow higher flow rates and lower costs.[34]

One particular method of filtering that has been shown to be promising, according to Uduman et al.[34], is tangential flow filtration (TFF). Here, the culture broth flows tangentially across the membrane (Figure 5). Unlike conventional filtration, the retentate is recirculated across the membrane. This keeps the algal cells in suspension and minimizes fouling.[34]

2.3.1.3 Centrifugation

Centrifugation is a common and the most preferred method for harvesting algal biomass. It is advantageous since most algal species can be concentrated from the culture using this technique. The separation of solid matter and the liquid phase occurs by applying centrifugal forces. The efficiency of the separation depends on aspects as particle size, density differences of the medium and centrifugal speed. Small particles for instance, which need a high centrifugal speed, have a large impact on separation efficiency.[7,33,34]

Even if centrifugation is an efficient method for algae harvesting, a main drawback is the operating cost due to its high energy demand.[34] Molina Grima et al.[7] investigated different centrifugal speeds and found a rapid decrease of algal recovery with declining speeds. Due to high gravitational and shear forces, there is also a risk that the algal cells can be damaged and thereby releasing their intracellular content.[34]

2.3.2 Dehydration

The final concentration of the slurry after harvesting contains generally 5-15% of dry solids. To be able to process the biomass further, it is necessary to dehydrate the slurry into solid matter. This is of high interest for extracting intracellular components. The type of dehydration or post harvesting process that should be used depends mainly on the final product.[32,34]

For algal biomass in which the oil is the desired end product, there are mainly two available methods: sun drying and lyophilization. The former is definitely the cheapest dehydration method. However, it requires large areas and demands long drying time. The second alternative, lyophilisation, is more expensive but the method is faster and enables a more efficient extraction of intracellular components compared to sun-drying.[32]

2.4 Biodiesel production

Dried or highly concentrated algal biomass is more suitable for processing in to a final product. This is mainly due to algae's otherwise low concentration leading to large amounts of solvents and mechanical work.[35] There are a number of different applications in which algae can be used. Some examples are biofuels, foods and cosmetics.[18] In this report, however, focus is set on methods used for biodiesel production (Figure 6).

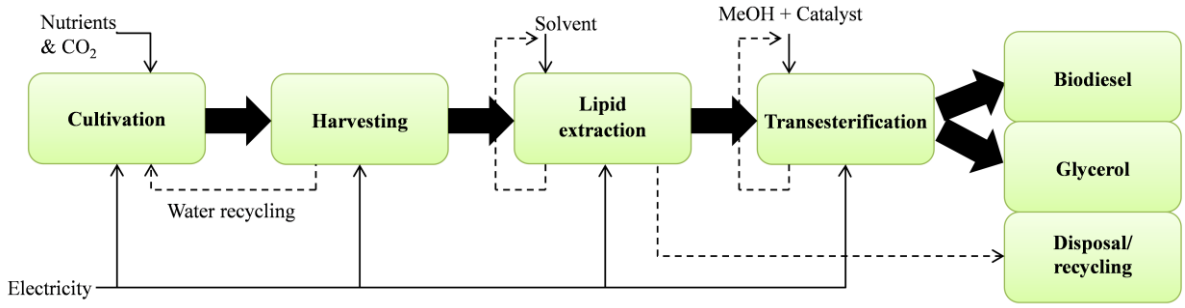

Figure 6. Schematic overview of biodiesel production from microalgae.

2.4.1 Lipid extraction

One major difficulty of processing microalgae in to biodiesel purposes is to efficiently extract the oil from the cell biomass. The lipids or oil are enclosed within the algae's cell wall, which requires cells to burst in order to be able to successfully extract the oil.[35]

Today, there are a rather few methods that are commonly used, including mechanical pressing, milling, solvent extraction, supercritical fluid extraction, enzymatic extraction and osmotic shock. None of these methods is an optimal choice, since they all have their advantages and disadvantages. However, the three former methods are mainly used for lipid extraction, where biodiesel is the desired final product. To make the extraction more efficient, it is common to combine two or several of the methods above.[35]

Mechanical disruption (pressing, milling and homogenization) is an economical method of extracting oil. A mechanical pressure forces the cell walls to break and thereby enables the lipid extraction. This method is rather inefficient on its own. However, it can be used as a pre-treatment step, followed by a solvent extraction.[35]

Extraction methods using organic solvent (i.e. benzene, hexane and chloroform) have shown to be effective.[35] These solvents both degrade the cell wall and extract the oil, mainly due to oil's high solubility in solvents. For large-scale extractions, organic solvents are not to prefer because large quantities of waste solvents are generated leading to increased costs in recycling, as well as raising the safety concerns. Another disadvantage, that might concern the quality of the final product, is the risk of having solvent residues in the final product.[35]

2.4.2 Transesterification

Once the lipids are extracted, they are usually present in the form of triglycerides. To enable these for biodiesel purposes, the triglycerides need to be chemically modified by transesterification. In brief, transesterification is the displacement of alcohol from an ester by another (Eq. 2). The process is similar to a hydrolysis, except alcohol is used instead of water.[36]

$$\underset{\text{Ester}}{RCOOR_1} + \underset{\text{Alcohol}}{R_2OH} \rightleftharpoons \underset{\text{Ester}}{RCOOR_2} + \underset{\text{Alcohol}}{R_1OH} \quad (2)$$

During the transesterification of triglycerides (Figure 7 & 8), the triglycerides are stepwise broken into diglycerides and monoglycerides. The final products after such transesterification are fatty acid alkyl esters and glycerol, where the latter is separated.[36]

As can be seen in Eq. 2, the reaction is reversible. To move the equilibrium towards and accelerate the formation of esters, an excess of alcohol and presence of a catalyst are used. There are several different ways of catalyzing the transesterification including acid, alkaline and enzymatic catalysts, where especially the two former have shown high yields.[36]

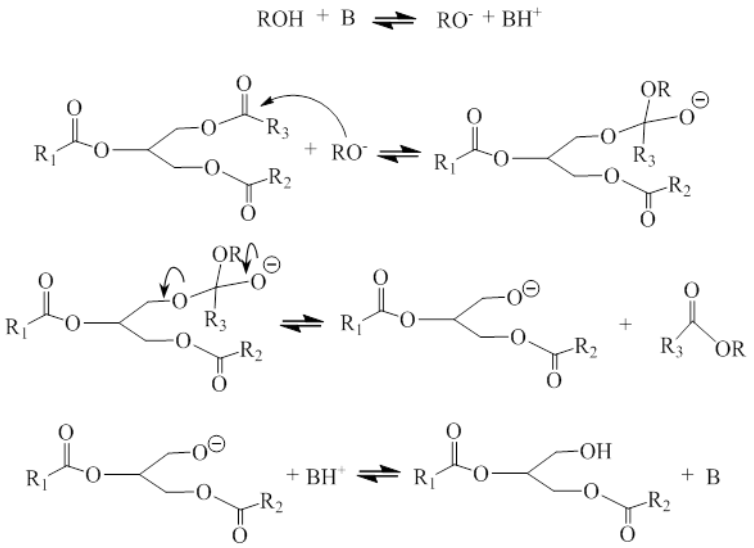

Figure 7. Mechanism for alkaline transesterification. R_1, R_2 and R_3 represent fatty acid carbon chains, while R_4 denotes the alkyl group of the alcohol.

For alkaline transesterification (Figure 7), catalysts such as sodium hydroxide (NaOH), sodium metoxide (NaOMe) and potassium hydroxide (KOH) are typically used.[36] The reaction starts with forming the alkoxide, which in turn makes an attack on the carbonyl carbon of the triglyceride, resulting in a tetrahedral intermediate. The intermediate reacts with an alcohol in which an alkoxide ion is formed. The reaction ends with a rearrangement of the tetrahedral intermediate forming an ester and a diglyceride. Alkaline transesterifications are usually preformed at 60ºC, a catalyst concentration of 1% to the oil content and an alcohol/oil molar ratio of 6:1.[36]

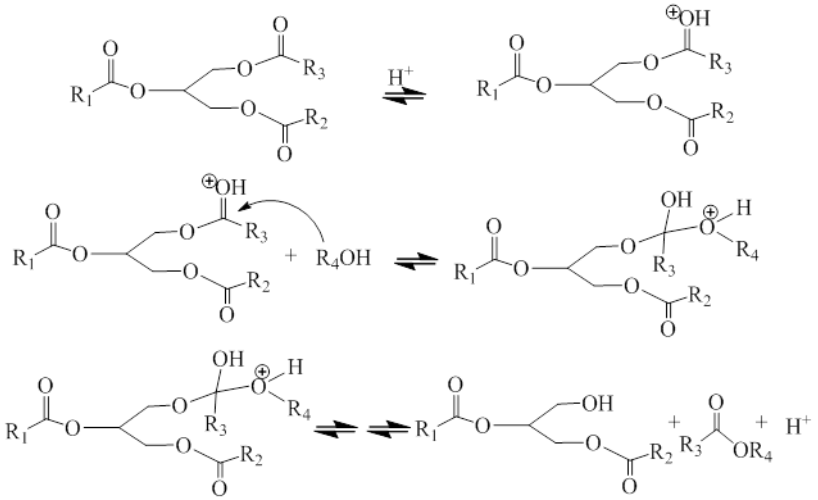

Figure 8. Mechanism for acid transesterification. R_1, R_2 and R_3 represent fatty acid carbon chains, while R_4 denotes the alkyl group of the alcohol

The reaction of acid transesterifications can be catalyzed by Brownsted acids (e.g. hydrochloric and sulfuric acids). The mechanism for the acid transesterification can be seen in Figure 8. In brief, the reaction starts with protonation of the carbonyl group of the ester resulting in a carbocation. A tetrahedral intermediate is formed after a nucleophilic attack of the alcohol. Glycerol is eliminated from the intermediate and a new ester is formed at the same time as the catalyst is regenerated. In this type of transesterification, higher reaction temperature and higher alcohol/oil molar ratio is needed (normally around 80-100ºC and 30:1, respectively).[36]

There are several variables that can affect the efficiency of the transesterification. Two examples are the content of free fatty acid (FFA) and moisture.[37] These factors are particularly crucial for alkaline transesterification. For high content of FFA (>1%), the alkaline catalyst will be used for neutralizing the acids instead of shifting the equilibrium towards formation of fatty acid methyl esters. Presence of water causes soap formation, which also consumes the catalyst. It has also been shown by Ma et al.[37] that a combination of FFA and water has a synergetic negative effect on the reaction.[36-38]

2.4.3 Biodiesel properties

The transesterification reaction, described above, converts triglycerides into fatty acid methyl esters (FAME), which is a core element in biodiesel. The composition of the FAME varies significantly between different algal species.[39] One major concern that affects biodiesel derived from all algal species is that algae are quite rich in polyunsaturated FAME, in contrast to other vegetable oils.[40] The unsaturated state of FAME affects the stability of the fuel. A biodiesel with high amount of polyunsaturated FAME is less resistant to oxidation, leading to complications during storage.[18,39,40]

Viscosity is another crucial parameter for the performance of the microalgal biodiesel. In general, the viscosity increases with the chain length and with increasing degree of saturation.[36] The viscosity affects the injection of the fuel into combustion chamber. With high viscosity there is a great risk of stoppage in the fuel injection system. Thus, it is of great importance to keep the kinematic viscosity in the same range as for conventional diesel fuel (1.6-6.0 mm^2s^{-1}).[36,40] On the other hand, biodiesel from all feedstocks is considered to have excellent lubricity, which can act contrary to the viscosity.[41]

For biodiesel users, the low-temperature performance is of importance. As with conventional diesel, operability in cold climates needs to be considered. At low temperatures, there is a risk of solid formation in the biodiesel causing filter plugging. This leads to engine starvation due to reduced fuel flow. Poor cold flow properties occur in biodiesel mixtures with long, saturated FAME. Generally, FAME longer than C_{12} impairs the cold flow properties.[41]

Due to the various impacts of the FAME composition, it is hard to define an optimum composition. However, of these factors mentioned above, the cold flow properties and the oxidative stability are considered as most important. From this perspective it is possible to say that microalgal biodiesel should contain low proportions of saturated FAME (minimizing cold flow problems), low proportions of polyunsaturated FAME (increasing the oxidative stability) and high proportions of mono-unsaturated FAME.[41] For ensuring the customer of a good quality of biodiesel derived from microalgae, both European and American standards (Table 3) have been constructed.[18]

Table 3. A selection of American and European standards for biodiesel (B100).[18]

Property	U.S (ATSM D6751-08)	Europe (EN 14214)
Water and sediment (vol.%, max)	0.05	0.05
Kinematic viscosity, 40°C ($mm^2 s^{-1}$)	1.9-6.0	3.5-5.0
Polyunsaturated FAME (wt.%, max)	-	1.0
Oxidation stability (hours at 110°C, min)	3.0	6.0
Density ($kg\ m^{-3}$)	-	860-900
Total contamination ($mg\ kg^{-1}$, max)	-	24
Methanol (wt.%, max)	0.20	0.20

2.5 Current situation

Since the first initiative taken by the NREL, *United States* in the 1970s, microalgal biodiesel have started to generate interest around the globe. Singh and Gu reports that, in 2010, there were over 150 companies of ranging size connected to the production of biodiesel from algae. Many of these are located in Europe and the United States (Figure 9) and possess cultivation systems that can produce 25-50 m^3 biodiesel $hectare^{-1}year^{-1}$. This market drive is much due to involvement of large companies, such as ExxonMobil Corp and BP Amoco plc. These companies has contributed with over $600 M to influence the research and production within the field of microalgal biodiesel.[42] These fairly large sums, on the other hand, can be compared with the approximately 50 billion dollars that were spent in 2006 for oil and gas explorations around the globe.[43]

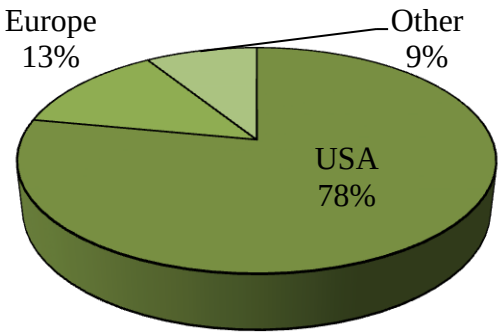

Figure 9. Percentage of region distribution of companies connected to microalgal biodiesel.[42]

Market analysts have outlined a bright future of the usage of microalgae. For the biodiesel production alone, however, current technologies and knowledge are not able to compensate for the high production costs. Therefore, according to Singh and Gu[42], development of high-value co-products, such as pigments and polymers are needed to be extracted and sold to make up for some of the production costs.

3 Materials and methods

The project consisted of the characterization of the microalgal strain *Scenedesmus* simris002, which were harvested both at the surface and at the bottom of the cultivation site. The main focus of the characterization was to determine the amount of lipids and the composition of fatty acid methyl esters within each sample. In addition, elemental analysis, heat value and chlorophyll measurements as well as calculations for obtaining energy efficient systems were examined.

3.1 Cultivation setup

The cultivation site, located at *SP Technical Research Institute of Sweden* in Borås (N 57° 72', E 12° 89'), consisted of two circular, open ponds (Figure 10). The ponds had a constant diameter of 2.1 meter and the water depth was kept at 0.5 meter. Due to the seasonal weather variations, the ponds were kept in a greenhouse in which the temperature was set to maintain at least 20 °C.

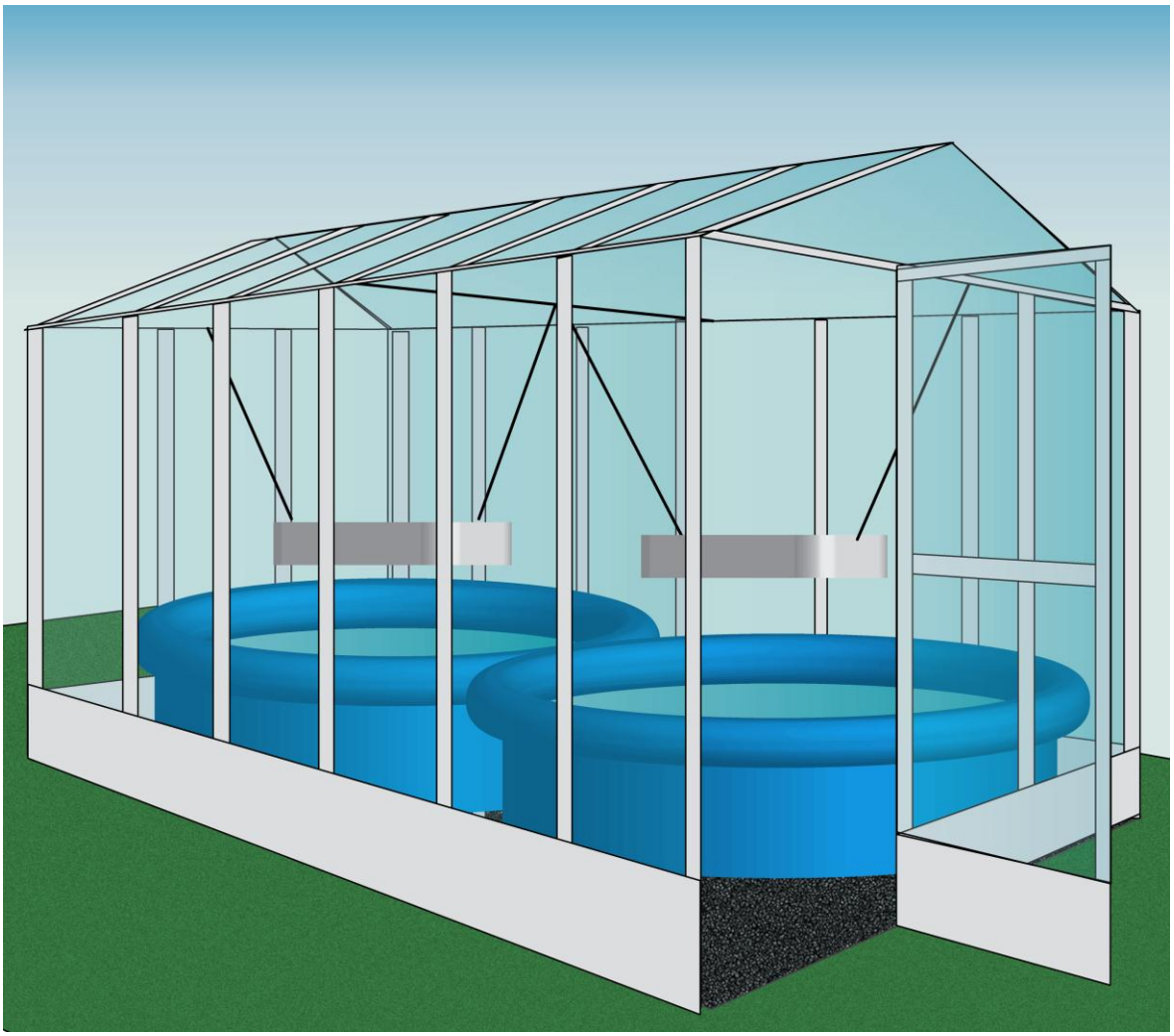

Figure 10. Illustrative sketch representing the cultivation site at SP Technical Research Institute of Sweden.

To prevent contamination, evaporation and temperature losses, the ponds were covered with plastic films. The plastic film also prevented the exchange between air and the water surface meaning that gases were provided by pumping air into the ponds. In order to increase the algal productivity, lamps and airlift systems were installed, which ran for 16 hours per day. During

the remaining eight hours, the algae had sufficient time for photorespiration and auto flocculation, leading to an automatic thinning of culture density at the surface.

For the two batches in which *Scenedesmus* simris002 was cultivated, the water height was kept at 0.5 m. Mixing and the supply of air were provided by a 5 W pump, with a specified flow rate of 2000 $dm^3 h^{-1}$ air, for each pond. The nutrition was provided batch-wise into the ponds. For the first batch, 1.75 kg of chicken manure (undefined composition) was spread into each pond, while in the second batch the same amount of chicken manure was collected in a fine-mesh bag. Leaching then provided the nutrition in the second case.

The method of harvesting also differed between the batches. For the first batch, the pump and growth lamps were turned off on February 7th, 2012. Algae at the surface of the pond were collected first, and algae located at the bottom after the ponds were drained. The slurry from the bottom and the samples from the surface was then allowed to dry using the solar heat within the greenhouse. Once the biomass was dried, it was collected (August 30, 2012) and stored at room temperature. For the second batch, the pumping system and growth lamps were turned off on January 13th, 2013. As for the first batch, algae at the surface were collected. The algae were allowed to settle and the ponds were thereafter drained. Microalgal slurry located at the bottom was collected at February 6, 2013. This time, though, it was not possible, due to time restrictions, to use available heat from the sun. Instead, the microalgae were filtered and then further dried in ovens at 80-100 °C. The harvestings from the batches resulted in a total of four samples of which two of each was collected at the surface and the bottom, respectively.

3.2 Lipid extraction and characterization

The procedure of lipid extraction and its characterization was influenced by the work of Laurens et al.[44]. Dried algal biomass was thoroughly ground using a pestle and mortar to obtain a meal-like powder. To remove residual moisture, samples were placed in an oven at 105 °C for 80 minutes. Once dried, samples of 2 gram per analysis were weighed into a cotton cellulose thimble. The thimbles were covered with glass fibre to ensure that the biomass remained within the thimble.

The thimbles were placed in a regular Soxhlet apparatus in which 210 ml chloroform/methanol (2:1, v/v) solvent was refluxed at a rate of 5-7 times per hour for approximately 72 hours, which ensured complete extraction of the algal biomass. The extract was diluted with chloroform/methanol and transferred to a separation funnel. The extract, at that time, contained lipids, carbohydrates and protein. Thus, a 0.75% NaCl (aq) was added to separate lipids from the other components. The biphasic mixture was let to settle overnight. After settling, the organic phase (the lower phase in the funnel) was emptied into a pre-weighed round bottom flask. The solvent was removed by using a vacuum rotary evaporator in a 35 °C water bath. The flasks were then placed in a 40 °C oven for further drying. The lipid content was determined gravimetrically.

The characterization of the fatty acid composition in the microalgal biomass started with transferring 20 mg, for each sample, of the extracted lipids into vials and 400 µl of HCl/methanol (5%, wt/v) was added to start the transesterification. The transesterification was executed at 80 °C for 80-90 minutes in the presence of 0.1935 g tridecanoic acid (C13:0). Since it is unlikely to find fatty acids with uneven number of carbon atoms, the tridecanoic acid is used as an internal standard to determine the concentration of the other fatty acid methyl esters. To remove unwanted particles and the hydrochloric acid in the sample, n-

heptane was added to each sample to obtain a biphasic mixture. 100 µl of the upper phase (containing the fatty acids) was transferred to a new vial. The characterization then followed *SP Technical Research Institute of Sweden's* protocol for determining fatty acid composition (originally derived from European Standards EN14103:2003 and EN14105:2009). The samples were analysed by gas chromatography-mass spectrometer/flame ionization detector (GC-MS/FID) (Agilent 5975C, VF5HT-column: 30 m x 0.32 mm x 0.10 µm) from which the total concentration of FAME and the concentration for specific FAME could be expressed in mass percentage by the equations below

$$C_{\text{Total FAME}} = \frac{\sum A\text{-}A_{\text{Interal standard}}}{A_{\text{Internal standard}}} \cdot \frac{W_{\text{Internal standard}}}{W} \cdot 100 \quad (2)$$

$$C_{\text{Specfic FAME}} = \frac{A_{\text{Specific FAME}}\text{-}A_{\text{Internal standard}}}{A_{\text{Internal standard}}} \cdot \frac{W_{\text{Internal standard}}}{W} \cdot 100 \quad (3)$$

where A represents the area for the total amount or the specific FAME as well as the area for the internal standards. W and $W_{\text{Internal standard}}$ is the weight (mg sample^{-1}) for the lipids analysed and internal standard, respectively.

Due to poor results in a pre-study using alkaline transesterification, suspicions of the presence of free fatty acids arose. Thus, the samples were also characterized without transesterification. 20 mg of extracted lipids were transferred to vials to which 80 µl of trifluoroacetic acid (TFA) was added. A biphasic mixture was obtained by adding n-heptane to the samples. 100 µl of the upper phase was transferred to a new vial and each sample was then processed in the same way described in the last paragraph.

3.3 CHN-analysis

Dried algal biomass was ground thoroughly and placed in a 105 °C oven to ensure complete removal of moisture. 100-150 mg of algal biomass was weighed into tin foil cups, which were vacuum-packed into a pellet. Without the presence of air, moisture and by using tin cups, there was no development of CO_2, H_2O or NO when the pellet was combusted. The samples were analysed in LECO CHN Elemental Analyzer. From the CHN-analysis it was also possible to estimate the protein content in each sample by multiplying the fraction of nitrogen with a factor of 6.25.[45]

3.4 Chlorophyll

The method for the determination of the chlorophyll content in the algal biomass followed the work of Porra[46]. Ground and dried algal biomass was diluted in 80% aqueous acetone to obtain biomass concentrations ranging from 0.5-0.75 mg ml^{-1}. Samples covered with aluminium foil and were kept in a -20 °C freezer for 70-80 hours before analysis. A UV-VIS absorbance spectrophotometer (Varian Cary 50 Bio) was used for the measurements and concentrations (µg ml^{-1}) of chlorophyll a, b and a+b, was calculated from the following three equations. [46]

$$[\text{Chlorophyll a}] = 12.22\ A^{663.6} - 2.55\ A^{646.6} \quad (4)$$

$$[\text{Chlorophyll b}] = 20.31\ A^{646.6} - 4.91\ A^{663.6} \quad (5)$$

$$[\text{Chlorophyll a+b}] = 17.76\ A^{646.6} + 7.34\ A^{663.6} \quad (6)$$

In equation (4)-(6), A^x represents the absorbance at the x^{th} wavelength. From these three equations it was possible to calculate the weight percentage of chlorophyll a, b and a+b in each sample.

3.5 Heat value and ash content

Dried algal biomass was thoroughly ground using a pestle and a mortar. The samples were placed in a 105 °C to remove residual moisture. Once dried, samples in the range of 0.5-0.6 g were tightly packed into a pellet, which was then placed in a steel container. During combustion, heat is released through the container into a water bath where a calorimeter is located. The analyses were performed in IKA 5003C.

For measuring the amount of ash in the samples, approximately 25 grams of dried algal biomass was ground roughly into 1 mm particles. The ground biomass was transferred into a crucible, weighted and placed in a muffle furnace (Nabertherm N41/H) with following temperature ramp: 20-250 °C (210 min), 250-550 °C (120 min), 550 °C (360 min) and 550-20 °C. The mass percentage of ash was then determined gravimetrically.

3.6 Energy-efficient cultivation systems

In addition to the data collected from the cultivations, calculations were also made with variables affecting the energy consumption at the cultivation site at *SP Technical Research Institute of Sweden, Borås*. The calculations are based on a system of equal measurements compared to the ones described in Section 3.1, with the exception of the water depth, which was set to 0.3 m. Due to malfunction of the pumping system after the second cultivation, new pumps were acquired with a specified flow rate of 720 $dm^3\ h^{-1}$ and an energy consumption of 8 W, which also will constitute the basis for the energy calculations. The plastic films that covered the previous cultivations were swapped to a plastic film dome in order to accumulate more air.

3.6.1 Aeration

To obtain a high productivity, CO_2 has already been declared to be an important factor. The amount of CO_2 needed to obtain certain productivity was estimated from the approximate molecular formula of algae derived by Grobbelaar[19], $C_{100}O_{48}H_{183}N_{11}P$ and from the work of Tredici [47] in which he outlined possible productivity rates around the globe. From Grobbelaar's equation it was possible to determine a ratio between the CO_2 needed and the algal biomass produced equal to 1.88 meaning that approximately 15 kg m^{-2} $year^{-1}$ CO_2 was required to ensure an algal biomass productivity of 8 kg m^{-2} $year^{-1}$, theoretically.

From the statement in the paragraph above, calculations were based on two scenarios: (i) continuous pumping with atmospheric carbon dioxide, (ii) continuous pumping with CO_2 concentration ranging from 0.0396 to 25 vol. %. For these scenarios, the ponds were covered, the pumping system ran for maximum 16 hours per day and ideal mixing was assumed. The volumetric airflow ($dm^3\ h^{-1}$) needed to supply the algal culture with sufficient amount of CO_2 to ensure choosen productivity was calculated with the equation below.

$$\frac{(P_{algae}/M_{algae})\cdot n_{CO_2}\cdot V_{m,CO_2}}{C_{CO_2}\cdot 365\cdot 16}\cdot A_{pond} \quad (7)$$

In equation (7), P_{algae} represents annual productivity (g m^{-2}), M_{algae} the molar mass of microalgae, obtained from Grobbelaar[19] (g mol^{-1}), n_{CO2} the number of C in microalgae, $V_{m,CO2}$ the molar volume of CO_2 (dm^3 mol^{-1}), C_{CO2} the concentration of CO_2 in the supplied air (v/v)

and A_{pond} the surface area of the pond. The annual energy consumption (MJ $year^{-1}$) of the pumping system is estimated with following equation.

$$\frac{(p_{pump}/Q_{pump})\cdot V_{air}\cdot 365\cdot 16\cdot 3.6}{1000} \quad (8)$$

in which p_{pump} represents the energy consumption of the pump (W), Q_{pump} the volumetric airflow (dm^3 h^{-1}) of the pump and V_{air} the volumetric air flow (dm^3 h^{-1}) derived from equation (7).

3.6.2 Mixing

Except from the existing airlift-system (Section 3.6.1.), a paddlewheel system was also examined. To be able to calculate the energy consumption from the paddlewheel, the following equation derived from the work of Green [48] was used

$$9.80\cdot \frac{Q\rho_{H_2O}\left(\frac{v^2n^2L}{R^{\frac{4}{3}}}+\frac{2Kv^2}{2g}\right)}{e} \quad (9)$$

in which Q represents the channel flow (m^3 s^{-1}), ρ_{H_2O} the density of water (kg m^{-3}), v the velocity of water (m s^{-1}), n the Manning's number, L the channel length (m), R the channel hydraulic radius, K the kinetic loss coefficient for 180° bends, e the efficiency of the paddle wheel and g the acceleration of gravity (m s^{-2}). Assumptions were made that Manning's number was equal to 0.008, representing a friction coefficient equal to a plastic film and the kinetic loss coefficient was close the theoretical value of 2.

From equation (9) it was also possible to study how the energy consumption of the paddle wheel changed due to altered length and mixing velocity.

3.6.3 Harvesting

In order to decrease the energy consumption in the harvesting step for future cultivations, calculations were made considering the usage of a pond vacuum cleaner. The vacuum cleaner would be able to pump up the sediment in which most of the algae are located. The algal slurry will be transferred from the cultivation ponds into empty ponds in which they are allowed to dry using solar energy. The annual energy consumption of such harvesting system is described in equation (10)

$$\frac{(V_{water}/Q_{v.c})\cdot P_{v.c}\cdot n_{batches}\cdot 3.6}{1000} \quad (10)$$

in which V_{water} represents the amount of water (containing the algal slurry) that is transferred from the cultivation pond (m^3), $Q_{v.c}$ the pumping capacity of the pond vacuum cleaner (m^3 h^{-1}), $P_{v.c}$ the energy consumption of the vacuum cleaner (W) and $n_{batches}$ is the number of harvestings per year. The result from equation (10) is based on the assumption that further drying, in form of solar energy, is free of charge.

4 Results

In the section below, the results obtained from this project will be presented. In order to prove the differences, if any, between the bottom and the surface samples, independent t-tests were calculated for most of the results using the analytical software tool *Statistical Package for the Social Sciences* (SPSS). In the statistical analysis, unless otherwise noted, the null-hypothesis (H_0) stated $\mu_{Mean,surface} > \mu_{Mean,bottom}$ for all analysed samples at a significance level of p=0.05. The results are presented in their summarized form. For specific results, see proposed Appendices.

4.1 Cultivation

During the research, the two first cultivations (*Scenedesmus* simris002) described in Section 3.1 were harvested. The first batch, cultivated between 2011-11-07 and 2012-02-07, yielded in a total mass of 2400 grams, while the second batch, cultivated during the period 2012-09-26 and 2013-01-13, gave 670 grams of biomass. The harvested biomass for the first and second cultivation represents a productivity of 1308 g m^{-2} $year^{-1}$ and 308 g m^{-2} $year^{-1}$, respectively.

4.2 Lipid extraction

In order to obtain enough lipids for the characterization of fatty acids (see Section 4.3), pre-experiments showed that 2 grams of algal biomass would be needed in the lipid extraction. The pre-experiments indicated a stagnation of lipids extracted after 72 hours.

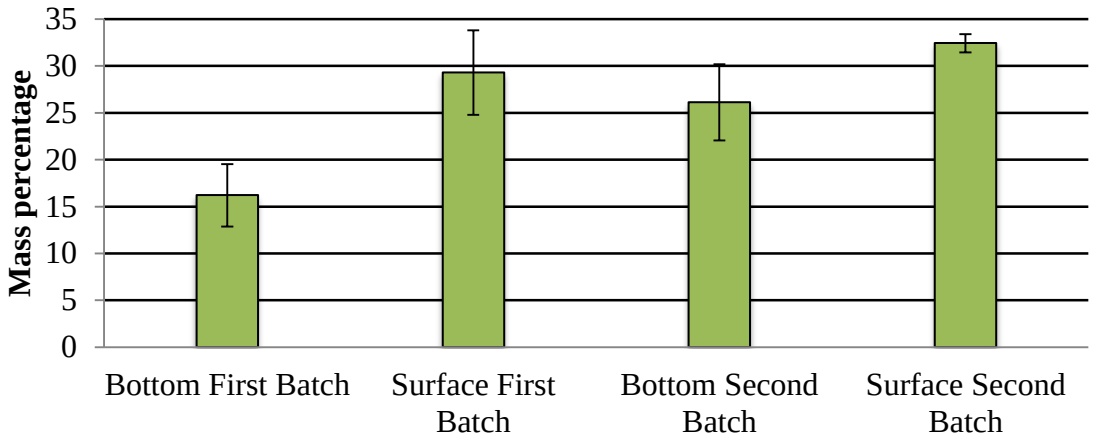

Figure 11. Mean lipid content (wt. %) in each of the four samples including the standard deviation represented by the capped error bars.

The samples were analysed in two to seven replicates due to varying amount of algal biomass. The results for the lipid content (Figure 11 and Table 4) in the four samples indicate higher lipid content in samples harvested at the surface. There is also a larger difference in the lipid content between the bottom and surface samples in the first batch.

Table 4. Summary of analysed samples, showing number of samples, mean values and standard deviation**.**

Lipid content (wt. %)			
Batch	n	Mean	Std. Deviation
Bottom First Batch	4	16.2	3.9
Surface First Batch	2	29.2	3.4
Bottom Second Batch	7	26.1	4.4
Surface Second Batch	2	32.4	0.3

However, due to high values of the standard deviation and few replicates (see Table 4 & 5) in the first batch, it is not possible to state higher lipid content in the samples of the surface, with set confidence level. For the second batch, on the other hand, the statistical analysis reveals that the samples from the surface contain more lipids compared to the bottom samples.

Table 5. Parameters from the statistical analysis using SSPS. Results are based on unequal variances. The significance level is set to p=0.05.

Lipid content					
Batch	t	df	p-value (One-tailed)	Mean diff.	Std. Error diff.
Bottom First Batch vs. Surface First Batch	2.7	1.4	0.1	13.1	4.9
Bottom Second Batch vs. Surface Second Batch	3.8	6.2	0.005	6.3	1.7

4.3 Characterization of fatty acid methyl esters

Due to suspicions of relatively high amounts of free fatty acids in the lipid fraction, the transesterification was performed using an acid catalyst, hydrochloric acid. The characterization of fatty acid methyl esters (Figure 12) revealed that palmitic acid methyl ester (C16:0-Me) dominated for samples in the first batch, while oleic acid methyl esters (C18:1-Me) dominated in the second batch.

From the statistical analysis (Table 6), it can be confirmed that the surface sample in the second batch has a significantly higher amount of oleic acid methyl ester. For the first batch on the other hand, the null hypothesis was modified since the composition in Figure 12 indicates higher content of the palmitic acid methyl ester. Therefore, the null hypothesis for the first batch is set to H_0:$\mu_{Mean,bottom} > \mu_{Mean,surface}$. By testing the null hypothesis, it is concluded that there is a significantly higher amount of C16:0 in the bottom samples.

Table 6. Parameters from the statistical analysis using SSPS. Results are based on unequal variances. The significance level is set to p=0.05.

C16:0-Me					
Batch	t	df	p-value (One-tailed)	Mean diff.	Std. Error diff.
Bottom First Batch vs. Surface First Batch	4.1	4.6	0.006	18.5	4.6
C18:1-Me					
Bottom Second Batch vs. Surface Second Batch	2.7	6.4	0.017	3.0	1.1

During the characterization, two internal standards (tridecanoic acid and heptadecanoic acid methyl ester) were used. By adding a known amount of tridecanoic acid (C13:0) before the transesterification, the fatty acid could be used as an indicator of the recovery was in the separation steps. After the transesterification and gas chromatography analysis, the amount of tridecanoic acid was calculated by using the intensity from the heptadecanoic acid methyl ester (C17:0-Me), which was added after the transesterification. By comparing the theoretical ratio and the measured ratio between the two internal standards, the combined recovery for fatty acids through the several separation steps could be estimated to 90 %.

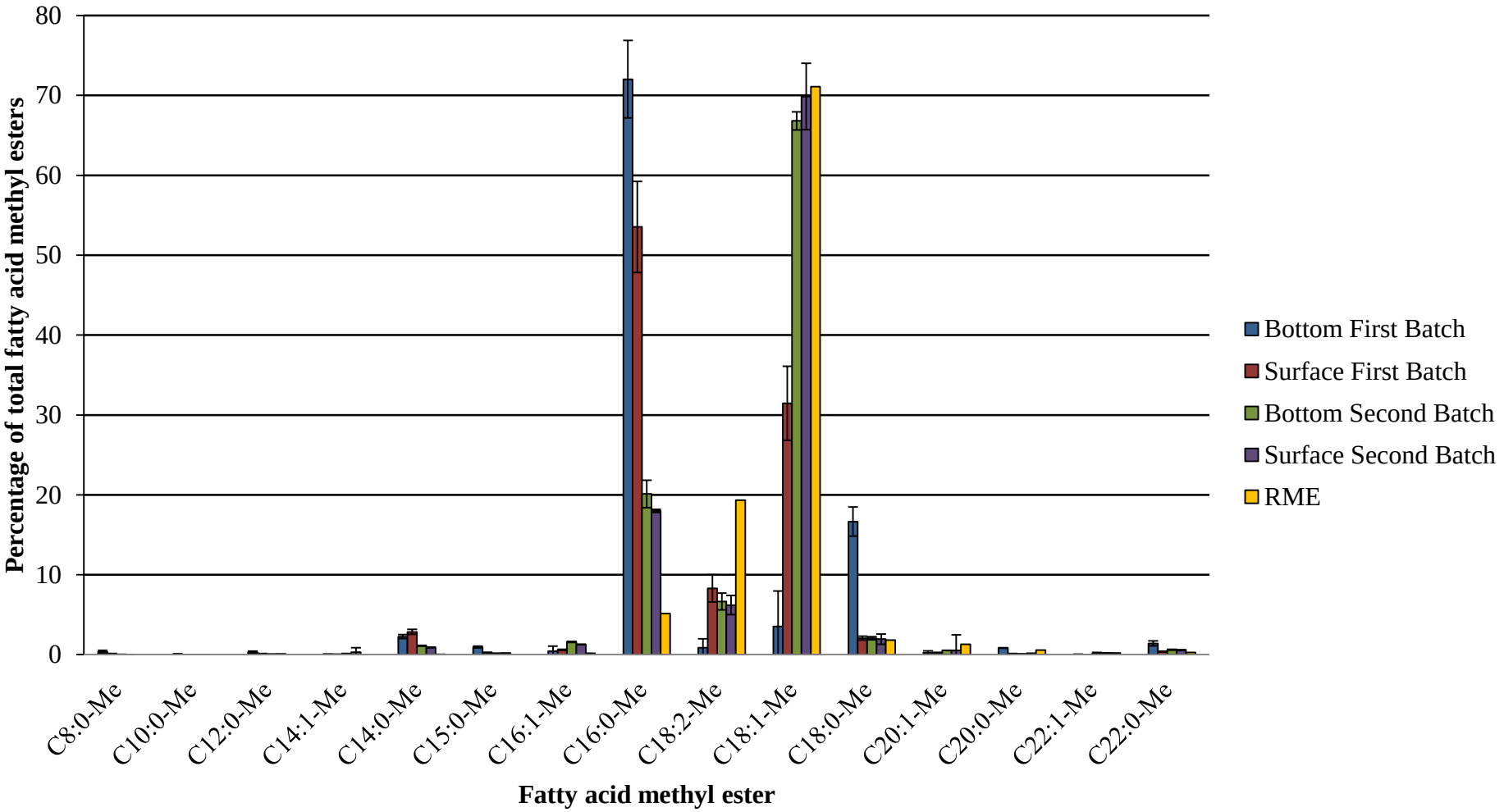

Figure 12. Composition of fatty acid methyl esters presented in percentage of total fatty acid methyl esters including the standard deviation represented by the capped error bars. In addition to the obtained FAME from the microalgae samples, a sample of commercial rapeseed methyl esters (RME) was analysed for comparison.

In order to investigate the amount of free fatty acid present in the algal lipids (Table 7), samples without the transesterification were analysed in duplicates. As for the transesterified samples above, the first and the second batch have high amounts of palmitic acid methyl esters and oleic acid methyl esters, respectively. The higher content of free fatty acids in the bottom samples indicate a degradation of the lipids.

Table 7. Mean amount of free fatty acids present in the algal lipids for each sample.

Sample name	Amount of free fatty acids (wt. %)
Bottom First Batch	11.5
Surface First Batch	4.2
Bottom Second Batch	9.5
Surface Second Batch	3.1

4.4 CHN-analysis

The CHN-analysis (Figure 13) was performed in duplicates for the four samples. The largest percentage difference between the samples is in the amount of nitrogen, which stretches between 4.51 to 8.61 wt. %.

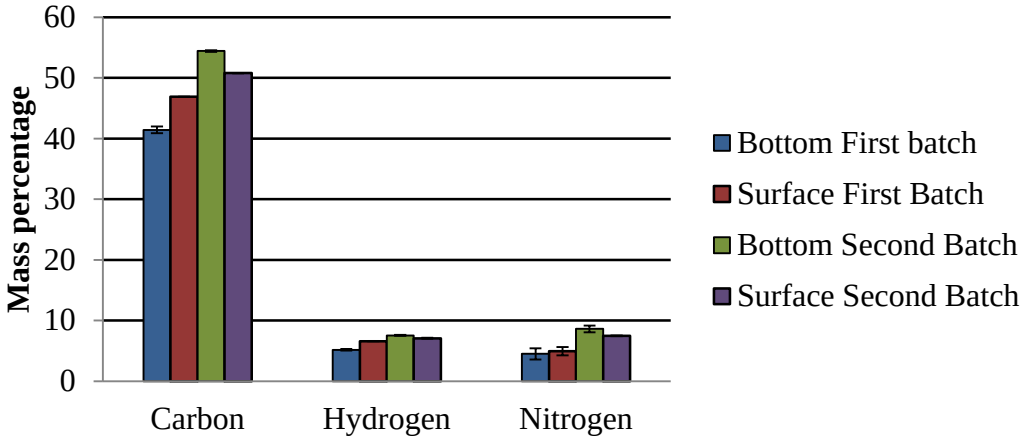

Figure 13. Mean content (wt. %) of carbon, hydrogen and nitrogen for the four samples including the standard deviation represented by the capped error bars.

The result from the analysis indicates a larger amount of carbon, hydrogen and nitrogen in samples from the second batch. There is a difficulty, however, to state general trends between the samples. In the first batch, samples from the surface shows a larger amount of the elements, while the opposite is present for the second batch.

The nitrogen concentration also reveals a relatively high content of protein in the samples. In order to obtain the mass percentage of protein in the sample (Table 8), the nitrogen concentration was multiplied with 6.25 (from Kjeldahl's test).

Table 8. Approximate protein concentration for each sample, derived by multiplying the nitrogen concentration with 6.25.

Sample name	Protein concentration (wt. %)
Bottom First Batch	28
Surface First Batch	30
Bottom Second Batch	53
Surface Second Batch	46

The large percentage differences between the samples concerning the nitrogen concentration make a significant effect on the protein content. For the second batch, the approximate protein content accounts for roughly half of the total mass, which can be compared with earlier reported values in which the protein content ranges between 8-18 wt. % for *Scenedesmus* dimorphus.[12]

4.5 Heat value and ash content

The heat value measurements (Figure 14) varied from 16.9 to 24.5 MJ kg^{-1} and were made as a complement to the lipid analysis in order to prove the advantageous energy that is captured within the cells**.** The analysis shows an overall high value for the heat value, where samples from the second batch contain the highest energy.

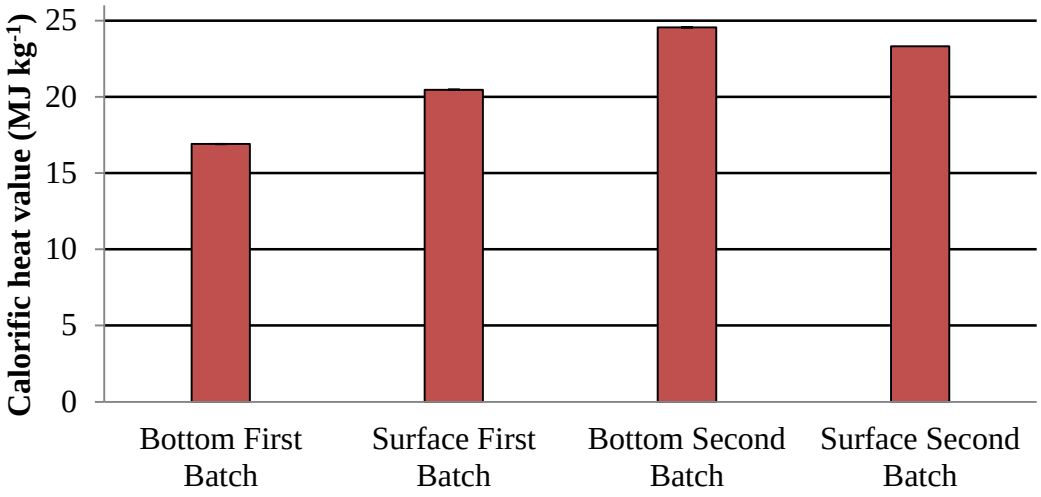

Figure 14. Calorific heating values for the analysed batch including the standard deviation represented by the capped error bars.

Analysis of ash content was only executed for the bottom samples in the two batches. A significant difference could be seen between the batches, with the ash content of 24.46 wt. % and 11.67 wt. % in the first and second batch, respectively.

4.6 Chlorophyll

The chlorophyll measurements (Figure 15 & Table 9) indicates a low concentration of chlorophyll overall, and especially in samples from the first batch in which samples from the bottom only constitutes 0.05 wt. % of chlorophyll a. The low concentration can be compared with earlier reported values stretching between 1-2 wt. % chlorophyll a in algae.[49]

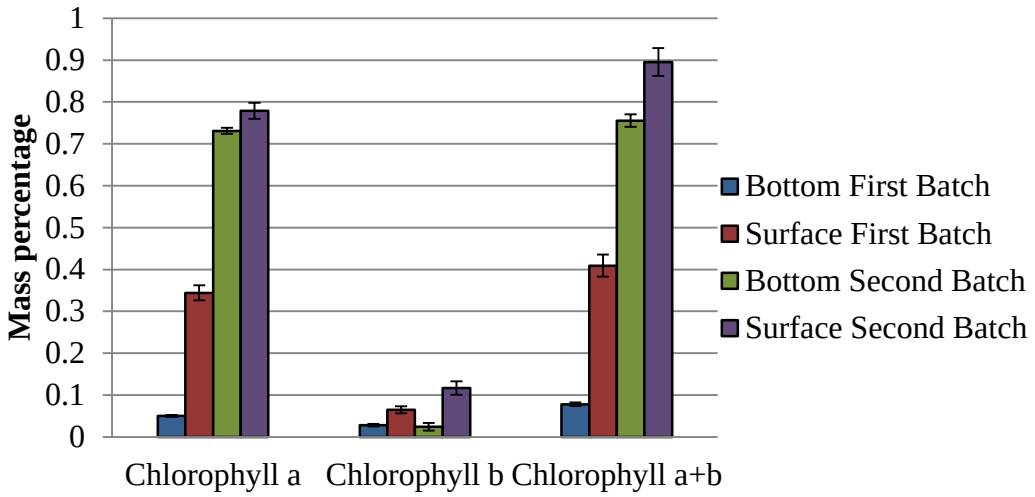

Figure 15. The amount of chlorophyll a, b and a+b expressed in wt. %. The samples were analysed in quadruples.

Due to very low concentrations of chlorophyll b in the samples, statistical analyses were only calculated for chlorophyll a (Table 10).

Table 9. Summary of analysed samples showing number of samples, mean values and standard deviation.

Chlorophyll a (wt. %)			
Batch	N	Mean	Std. Deviation
Bottom First Batch	4	0.05	0.003
Surface First Batch	4	0.7	0.008
Bottom Second Batch	4	0.3	0.02
Surface Second Batch	4	0.8	0.02

High obtained t-values combined with low standard deviations make it possible to state that the surface samples in both batches have significantly more chlorophyll compared to the bottom samples.

Table 10. Parameters from the statistical analysis using SSPS. Results are based on unequal variances. The significance level is set to p=0.05.

Chlorophyll a					
Batch	t	df	p-value (One-tailed)	Mean diff.	Std. Error diff.
Bottom First Batch vs. Surface First Batch	153	3.6	0.000	0.7	0.004
Bottom Second Batch vs. Surface Second Batch	28.8	6	0.000	0.4	0.02

4.7 Energy-efficient cultivation systems

The calculations for the energy efficiency are based on equation (7)-(10) and only concerns applications related to the cultivation of microalgae. Considerations regarding heating, nutrients (e.g. nitrogen and phosphorus) and downstream processing of microalgae into a final product are not taken into account. Unless otherwise noted, the annual productivity is assumed to be 8 kg g m^{-2} where the microalgal biomass has a calorific heating value of 24.5 MJ kg^{-1} and the pond measures an area of 3.64 m^2.

4.7.1 Aeration

The variables concerning the aeration (i.e. energy consumption and volumetric airflow) to the pond are obtained from existing pumping equipment (8 W and 720 dm^3 h^{-1}). Additionally, by introducing the plastic film dome, the mass transport at surface will increase due to the continuous presence of CO_2. As noted in Section 3.6.1, two scenarios were to be examined. In the first scenario (Table 11), the cultivation had continuous pumping with atmospheric carbon dioxide (0.0396 %).

Table 11. The aeration provided by continuous pumping with atmospheric carbon dioxide in order to obtain a productivity of 8 kg g m^{-2} $year^{-1}$.

Variables	
Volumetric airflow (dm^3 h^{-1})	11470
Annual input energy (MJ)	2680
Annual output energy (MJ)	679
Input energy/output energy (%)	394.9

Table 10 states that a continuous pumping system with atmospheric carbon dioxide would not be economical feasible since the annual input energy is approximately four times larger than the output energy. It would not either be possible to operate such scenario, since the volumetric airflow needed, would imply the usage of more than 15 pumps with the described pumping characteristics.

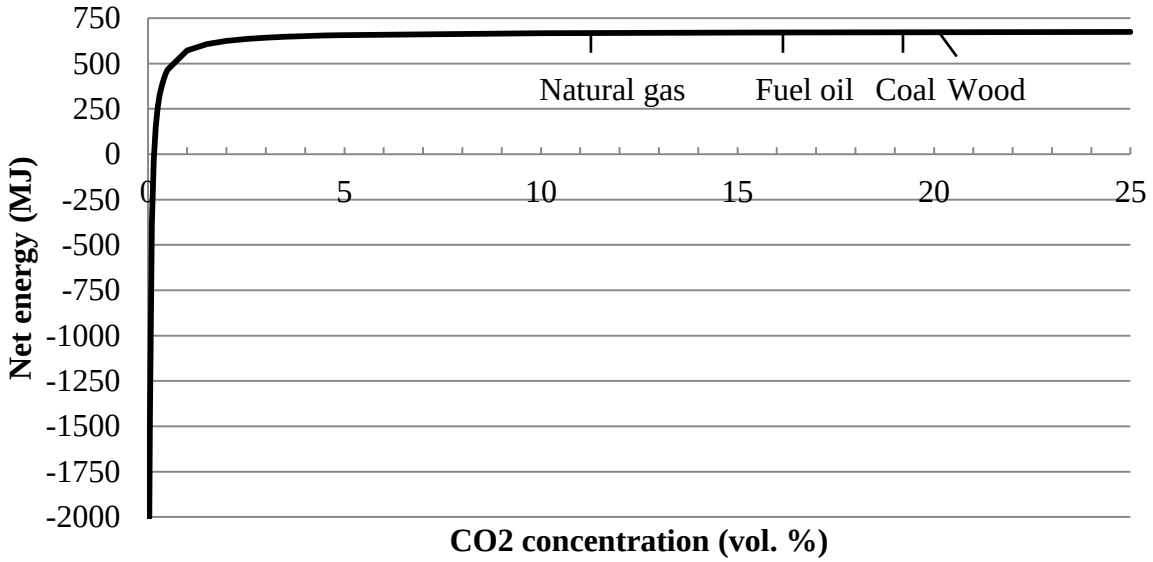

Figure 16. Relationship between altered concentration of CO_2 and the annual net energy. Theoretical values of CO_2 concentration in flue gases from combustion of natural gas, fuel oil, coal and wood are also noted along the curve.

In order to decrease the annual output energy without risking declined productivity, air with higher concentrations of CO_2 could be injected (second scenario). By increasing the concentration of carbon dioxide, less volume of air needs to be pumped into the system and thereby declining the energy consumption. The relation between the concentration of carbon dioxide and annual net energy (Input-Output) is presented in Figure 16.

A break-even in which the annual input energy is equal to the output energy is obtained when a carbon dioxide concentration of approximately 0.2 vol. % is injected into the cultivation. However, to increase the annual net energy higher concentrations of CO_2 are necessary for the described pumping system.

From Figure 16 it is clearly stated that the annual energy consumption, for described aeration system, decreases as the concentration of CO_2 increases. In order to distinguish differences in pump characteristics and energy consumption due to altered concentration, CO_2 concentrations of 1, 5, 10, 15, 20 and 25 vol. % are highlighted in Table 12.

Table 12. Energy consumption for the second scenario using 1, 5, 10, 15, 20 and 25 vol. % CO_2 injected continuously. The annual output energy is based on a productivity of 8 kg m^{-2} $year^{-1}$ and calorific heating value of the biomass equal to 24.5 MJ kg^{-1}

Variables						
CO_2 concentration (vol. %)	1	5	10	15	20	25
Volumetric airflow (dm^3 h^{-1})	454	91	45	30	23	18
Annual input energy (MJ)	106	21	11	7	5	4
Input energy/output energy (%)	15.6	3.1	1.6	1	0.8	0.6

By using higher concentrations of CO_2 (> 5 vol. %), the annual net energy decreases significantly. The data provided in Table 11 also reveals advantages in using flue gases from e.g. fuel oils and wood, as the energy consumption from the aeration system becomes a negligible part of the potential energy consumption. For specific characteristics, see Appendix A

What is worth noticing from Figure 16 and Table 12 is that a higher concentration of CO_2 leading to a lower flow rate, could imply with the mixing and thereby risking a declined productivity.

4.7.2 Paddlewheel

The replacement of the existing airlift system with the paddlewheel system described in Section 3.6.2 could be considered as a potential investment for future cultivations. The model described in equation (9) estimates the energy consumption needed to maintain a given velocity around the track due to variables as friction, viscosity, width and length. For a system of the same measurements as the cultivation ponds in Section 3.1, the energy consumption (Table 13) is large for current cultivation ponds. Green[48] reports a rather low paddlewheel efficiency (e=0.1), but even with an efficiency equal to 0.3, the energy consumption of the paddlewheel alone would account for approximately 45 % of the possible output energy.

Table 13. Mixing characteristics for the energy consumption of the paddlewheel.

Variables	Selected values
Pond area (m^2)	3.46
Water depth (m)	0.3
Mixing velocity (m s^{-1})	0.2
Paddlewheel efficiency	0.1
Annual input energy (MJ)	1085
Annual output energy (MJ)	679
Input energy/output energy (%)	159.8

Even if the paddlewheel increases the mass transport at the surface, it would still be necessary to install an aeration system for the supply of carbon dioxide in order to increase the productivity.

In order to determine a suitable process design in which a paddlewheel can be relevant, the length of the raceway as well as the mixing velocity was altered (Figure 17). Increased lengths of the raceway will give rise to increased cultivation area and thereby also increased annual production of microalgae.

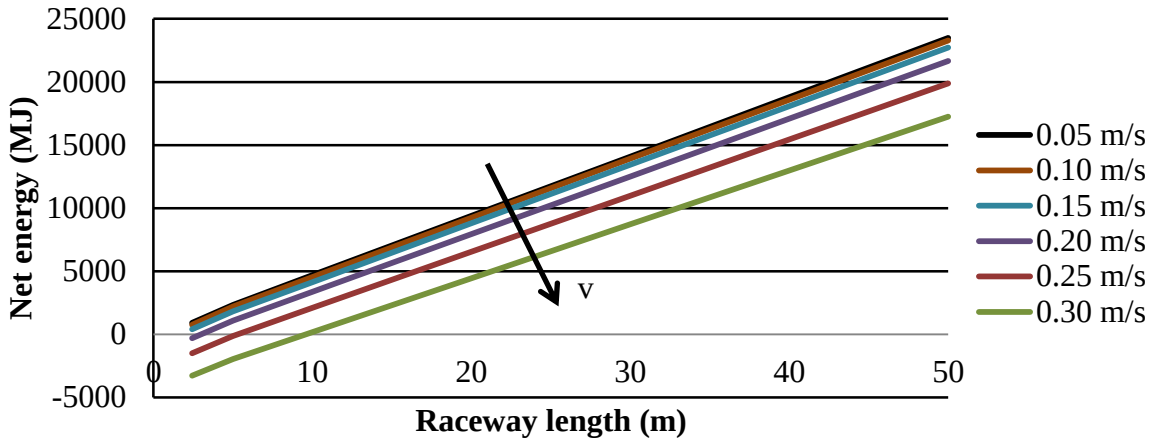

Figure 17. Altered raceway length and mixing velocity in relation to annual net energy. The channel width of the pond is fixed to 1.2 m.

As can be seen in Figure 17, increased length leads to increased annual net energy, while an increasing mixing velocity affects the annual net energy negatively. In a process design with 50-meter raceways in which the mixing (v = 0.20 m s^{-1}) is provided by a paddlewheel, the energy consumption would still account for approximately 8 % of the potential output energy. For specific values, see Appendix B.

4.7.3 Harvesting

The usage of the pond vacuum cleaner in Section 3.6.3 is independent on the concentration of biomass in the cultivation. This is advantageous in comparison to other harvesting methods (e.g. filtration and centrifugation), which are strictly dependent on the amount of biomass harvested as well of the culture volume. Thus, the energy consumption of a pond vacuum cleaner is considered to be constant for every harvest occasion.

However, the energy consumption derived from equation (10) increases linearly with the number of harvestings. In investigated scenario, 200 dm^3 is transferred from the cultivation

pond to a drying pond with a flow rate of 3.5 m^3 h^{-1} by a pond vacuum cleaner (360 W). The energy consumption for such system increases with approximately 0.075 MJ per harvesting

Based on data provided in the paragraph above combined with an annual production of approximately 38 kg algal biomass per pond and five harvestings per year, the energy consumption of the harvesting process would account for approximately 0.01 MJ kg^{-1} DW biomass, which can be compared with 1.4 MJ kg^{-1} and 5.3 MJ kg^{-1} [50,51] for filtration and centrifugation, respectively.

4.7.4 Uncertainty assessment

Since the obtained data from this research show rather varying results, it was of great interest to investigate in which range the theoretical models would be applicable due to altered characteristics.

Based on a continuous aeration system (second scenario in Section 3.6.1) with 10 vol. % CO_2 and harvesting performed with the pond vacuum cleaner (five harvestings per year), the annual net energy from the algae cultivation was examined with altered productivities and obtained heat values from this research (Figure 18).

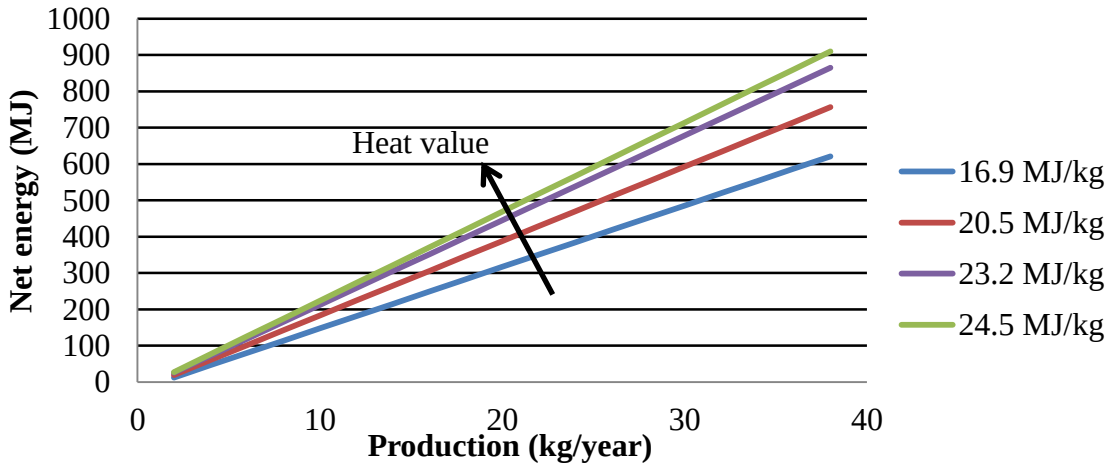

Figure 18. The annual net energy's variation due to altered production. The energy output considers obtained calorific heat value from this research.

The result in Figure 18 indicates an energy efficient cultivation system, covering 3.46 m^2, even at lower production rates for all analysed heat values. The cultivation system could handle production rates low as 0.75 kg per year, with a heat value of 24.5 MJ kg^{-1}, without risking a negative net energy.

If the algal biomass only is used for biodiesel purposes, the system is more sensitive to lower production rates since only the lipid content can be used. With a lipid content ranging between 15-30 wt. % and an assumption of a calorific heating value of 43 MJ kg^{-1}, equal to biodiesel [52], the variation of the annual net energy in relation to altered productivity rates is shown in Figure 19.

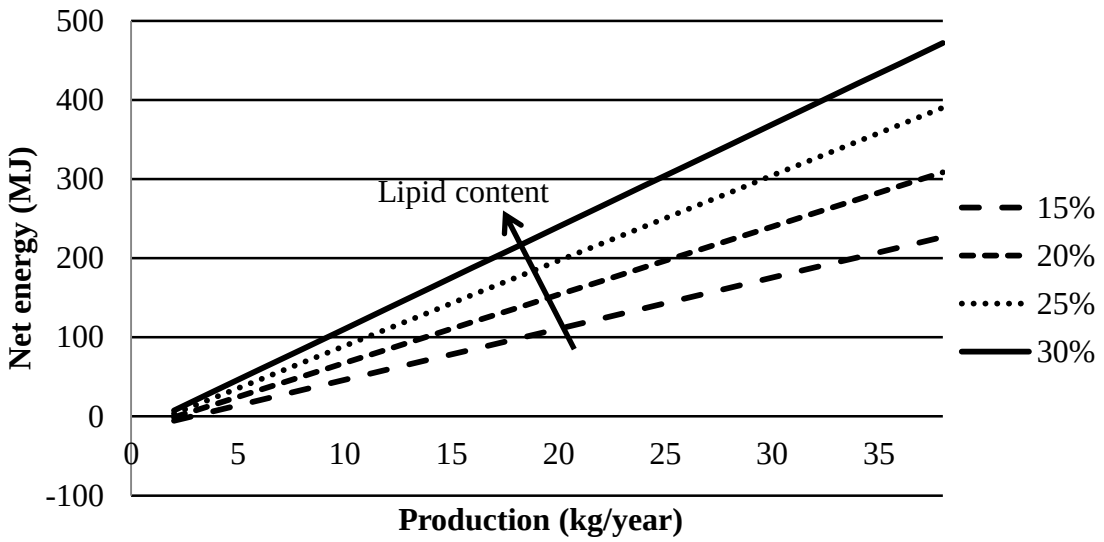

Figure 19. The annual net energy's variation due to altered productivity rates and lipid content. The energy output considers a calorific heating value of 43 MJ kg^{-1} and a lipid content of 15-30 wt. %.

A scenario where only the lipids are used for further process leads to a significantly smaller value of the annual output energy and thus, a lowered annual net energy. The cultivation needs to produce at least 2.8 kg biomass (15 wt. % lipids) to remain efficient from an operational point of view. For specific values, see Appendix C.

5 Discussion

The main purpose of this research was to study the differences, if any, in chemical composition and physical properties between algal samples harvested from the bottom and from the surface in the cultivation ponds. From a cultivation point of view, the analysis could have been further improved by monitoring fluctuations in water temperature, pH and nutrient consumption. On the other hand, this research gives a good indication how to improve current technologies in the field of microalgal biodiesel in order to make it more energy-efficient.

5.1. Cultivation

The two cultivations that were harvested for this project yielded in a significantly lower productivity compared to the 8 kg m^{-2} $year^{-1}$ that Tredici[47] purposed in his work. By observing the cultivation setup, it can be stated that light is definitely not the limiting factor since growth lamps were installed at the cultivation site. The supply of nutrients (nitrogen, potassium, phosphorus and trace elements) was probably not the cause for the low productivity either. The amount of nutrients supplied to each pond was based on the statement from Tredici. It can be questioned, though, how a batch-wise addition of all nutrients affects the algal growth.

Temperature and the supply of CO_2, on the other hand, are probably two of the most crucial factors that have affected the slow growth in the ponds. For the former, temperature was shown by Goldman and Carpenter[26] to be strictly connected with the doubling rate of algae. The doubling rate was halved for every 10 °C decrease in the range between 10-35 °C. The temperature in the greenhouse was set to maintain at least 20 °C. However, during the winter season the heat pump was not able to maintain this temperature. It would be possible to assume that the average temperature in the greenhouse during the cultivations was around 5-10 °C leading to a decrease in growth by a factor of 6. Assuming this, a maximum productivity would then rather be around 1300 g m^{-2} $year^{-1}$.

The pumping system for the analysed batches provided 2000 dm^3 h^{-1} of air 16 hours per day for each pond. Since atmospheric carbon dioxide was used, the maximum annual amount of CO_2 that could be provided for uptake by the microalgae was around 9000 grams leading to a maximal algal productivity of 1328 g m^{-2} $year^{-1}$.

Finally, the maximal productivity is based on a theoretical value meaning that the microalgae would need to consume all of the supplied CO_2 with no losses to the atmosphere. Although ponds were covered, losses to the atmosphere are probably present. Thus, the reported productivities from this research, considering these aspects, are at least 24 % of the theoretical maximum without nutrient control.

Both of the batches were cultivated during the same seasonal time and still differ significantly in the amount of harvested biomass. A reasonable explanation to this difference is the different ways of supplying the nutrients. As described in Section 3.1, chicken manure were spread into the first batch leading to residues at the bottom. Due to slow growth, most of the nutrients were unused and mixed with the sedimented algae. The measured ash content for the two bottom samples can be used as an indication for this impurity. The bottom sample in the first batch had more than twice the amount of ash compared to the second batch. Thus, the purity of the bottom sample in the first batch can be questioned, which will be further discussed in the section below.

5.2 Processing microalgae

Processing microalgae into biodiesel shows promising potential from this research. The lipid extraction yielded in an average stretching from 16-32 wt. % of lipids, which is comparable to previous studies for the *Scenedesmus* dimorphus.[12] The lipid content could probably be even higher if the algae were further stressed with e.g. nitrogen starvation.[21,22]

From Figure 11, it was indicated that the bottom sample for the first batch showed the lowest amount of lipids. Returning to the addition of nutrients; since most of the nutrients in the first batch were unused and mixed with the algae located at the bottom of the ponds, it is reasonable to assume that the chicken manure constitutes a larger part of the extracted biomass and thereby leading to a lower amount of lipids extracted for that sample. In the second batch, the problem with mixing the nutrients with sedimented biomass was solved by collecting the nutrients in a fine-mesh bag. As a result of this, the fraction of lipids for the bottom sample increased significantly, which also indicate a purer biomass.

The characterized FAME (Figure 12) shows the presence of mainly two FAME: C16:0-Me and C18:1-Me. What is interesting from this figure is the fact the former FAME is way more occurring in the first batch, while the latter dominates in the second batch. The addition of nutrients has probably not a direct impact on this difference in this case. What might have happened, however, is an anaerobic degradation of the oleic acid (C18:1) to the palmitic acid (C16:0), which has previously been observed by Lalman and Bagley.[53] Since the algae settle and get packed in the sediment in the ponds, it would be rational to assume anaerobic conditions. On the other hand, the degradation did only occur in the first cultivation indicating that the depth of the sediment may be crucial. The nutrients, in that case, could contribute to increased depth of the sediment at the same time as they bind to available oxygen. For biodiesel applications, this degradation could affect the cold-flow properties negatively. On the contrary, saturated FAME increase the stability for oxidation making it more suitable for storage.[41] In comparison to the commercial RME, analysed in Figure 12 as well, similar relations between the FAME compositions can be observed, especially for the second batch. This can be considered as a positive indication for future usage of microalgal biodiesel.

Table 6 reveals a rather high content (3.1-11.5 wt. %) of free fatty acids (FFA), which also was suspected in the pre-experiments when alkaline transesterification was used. The high content of FFA in algae has been reported in previous studies, which also can lead to problems in the processing steps.[36-38] Therefore, it is important to consider acid catalysts for the transesterification of microalgae leading to smaller risk of soap formation. An extra separation step may be necessary to process the FAME and the FFA individually. However, this will not affect the quality of the biodiesel, since the analysed FFA had similar composition as the analysed FAME. The higher amounts of FFA in the bottom samples indicate a possible degradation of the triglycerides as the cells are packed in the sediment. But if acid catalysts as well as an extra separation step are considered, as mentioned, this would not imply any problems for future processing of microalgae.

As a complement to the rather high lipid content in the samples, the calorific heat value also indicated good features for using algal biomass as a solid biofuel. From Figure 13 and Figure 14, it is possible to see a relation between the content of carbon and the obtained heat values. This relation is reasonable since the carbon, which is present in the lipids majorly, has a significant larger heat value (39-43 MJ kg^{-1}) compared to the heat value of protein (~20 MJ kg^{-1}).[54] Thus, increased content of carbon, and thereby increased amount of lipids, gives a

larger heat value. A maximum measured heat value of 24.5 MJ kg^{-1}, compared to 19 MJ kg^{-1} for wooden material[55], also enables the possibility of using algal biomass for combustion purposes. In such case, the biomass only needs to be dried after cultivation instead of including several processing steps in order to obtain biodiesel.

The CHN-analysis reveals rather high content of carbon, especially in the second batch, which correlates well to the measured heat values (Figure 14). Surprisingly, samples from the first batch show low content of nitrogen. Since it is assumed that the chicken manure constitutes a major part of the dried biomass in the first batch, it would have been more reasonable to observe a rather high content of nitrogen in those samples. A possible explanation of this deviation could be that the nitrogen in the form of nitrate and ammonium has been dissolved in the water. As the ponds were drained, the nitrogen was withdrawn as well and thus, only organic matter from the chicken manure is mixed with the algal biomass.

Since the reproduction of algal cells is strictly dependent on nitrogen for the protein biosynthesis, it is realistic to believe that either too low temperature or the lack of other nutrients (e.g. sulphur) limits the growth.[18,21,23] The approximate protein content (Table 7) derived from the nitrogen concentration is also rather high compared to previous reported values.[12]

Measurements of the chlorophyll content (Figure 15) gives a good indication of the vitality of the algal cell, which shows extensive degradation of the chlorophyll molecules has occurred in the first batch. The large difference between the two batches, except from the supply of nutrients, is the method of dewatering. For the first batch, the biomass was kept outside for over six months leading to a possible degradation, which is also proven by the low concentration of chlorophyll. For the samples of the second batch, the dewatering process was rapid, which seems to retain the chlorophyll molecules within the cell and keeping them from degradation. What is of importance from this analysis is that even if chlorophyll shows tendencies of major degradation, it has not affected the lipid content at large.

5.3 Energy-efficient cultivation systems

In order to obtain productivities in the range nearby values of Tredici[47], CO_2 needs to be added. This was clearly shown in Figure 16, where the concentration of CO_2 was altered in relation to the annual net energy of the cultivation system. For this system in particular, a rapid increase in the annual net energy was obtained for even small changes in the concentration of CO_2. However, to keep increasing the concentration of CO_2 would probably not ensure a more energy-efficient cultivation system. As the supply of CO_2, in this case, is a part of the airflow going into the ponds, an increase of CO_2 would decrease the airflow and thereby risking a proper mixing, which can have just as bad effects as with too low CO_2 concentrations.[28]

There are several variables (e.g. solubility of CO_2, pH changes and mixing properties) that are crucial to consider before choosing one of the aeration system presented in Section 4.7.1. Previous studies, concerning the aeration systems for algae cultivations, state problems of having a sufficient water depth in the cultivation pond in order to ensure that most of CO_2 is dissolved. Stepan et al.[56] claims that at least 1.5 m in water depth is needed to ensure a transfer efficiency of 95 % for 15 vol. % CO_2 injected at a velocity of 0.2 m s^{-1}. A cultivation pond of this depth would both be expensive to build as well as inefficient due to large volumes in which the solar radiation is unable to reach. By having the plastic film dome, described in Section 3.6, there will not be any problems of having shallower ponds. The CO_2

that has not dissolved in the water will accumulate within the dome and will thereby be available for microalgal uptake. Thus, pH changes and mixing properties are considered to be more important for the described cultivation system.

Injecting atmospheric carbon dioxide (first scenario in Section 4.7.1) into the cultivation ponds was clearly unfavourable both from a technical and an economic perspective. The low concentration of CO_2 in atmospheric was discussed in Section 5.1 to be a major problem for ensuring a high algal productivity. Thus, it seems reasonable to believe that higher concentrations of CO_2 need to be supplied for future cultivations.

From Figure 16 and Table 11, representing the second scenario for the modelling of aeration systems, it could be confirmed that the concentration of CO_2 has a substantial impact on the energy consumption of the pumping system. From an energy saving perspective, it would be rational to inject as high concentration of CO_2 as possible, which leads to decreased energy consumption. However, it is important to consider mixing and pH changes. Regarding the former, Weissman[28] emphasises the importance of having rigorous mixing system as the cell density increases and thus, larger airflow from pump is to prefer. On the other hand, due to auto-thinning during night time it is possible to assume relatively low cell densities in the water column making lower airflow a better choice from an energy-saving point of view.

By supplying high concentrations of CO_2, there might be a of risk of acidification in the ponds leading to significant loss in productivity or even cell death.[57] Therefore, it would be of great importance for future cultivations to find a balance between the choice of concentration of CO_2 and mixing.

A further improvement of the aeration system, not presented in this research, would be to combine one stream of air and one stream of pure CO_2 or one of the flue gases presented in Figure 16. In this way, controlling the streams individually can ensure the supply of CO_2 and proper mixing. The potential risk of inhibition due to too high oxygen concentrations, mentioned in 5.1, is avoided by pumping the airstream within the pond. A potential drawback of this idea is the need of having two pumping systems leading to higher investment and operational costs.

An investment of a paddlewheel (Section 4.7.2) to improve the mixing properties in the purposed cultivation system would not be advantageous neither from an energy saving nor an operational point of view. This is mainly due to a low efficiency of the paddlewheel.[48] From equation (10), it was clearly stated that the mixing velocity (Figure 17) also had a large impact on the energy consumption. But a lower mixing velocity in order to save energy is likely to impair the mixing properties in the end of the track. However, for prospective upscaling of pond sizes, the paddle wheel becomes more lucrative. As the pond size increases, kinetic head losses contribute less to the energy consumption compared to the existing system.

The energy consumption for the harvesting step has for a long time been the bottleneck for establishing microalgal biodiesel on the fuel market.[7] But since this research shows promising potential for algae that have been partly degraded, there would not be necessary to have continuous harvesting anymore. Even with a 20 % lower lipid content (Figure 11, second batch) in the bottom samples, it would still be advantageous from an energy perspective to harvest few times per year. This is mainly due to the usage of the pond vacuum cleaner, presented in Section 4.7.3. The vacuum cleaner only withdraws small volumes of water that contains the majority of the algae in the pond and it is not dependent on the harvested mass as

well as it only consumes a fraction of the energy compared to conventional harvesting methods (e.g. filtration and centrifugation).

6 Conclusions

Without any nutrient and pH control the biomass productivity in cultures was at least 24 % of the theoretical maximum considering the environmental conditions during the cultivation.[47]

From this research it can be concluded that there are differences between the biomass harvested at the bottom and in the water column. The differences, however, are varying among the analysed characteristics. For the lipid content, which was of greatest interest in this research, samples collected in the water column showed 1.8 and 1.2 times higher lipid content in the first and second batch, respectively. The significant difference in lipid content for the first batch could be explained large amount of nutrients mixed with the biomass. However, the energy content in the sediment compared to biomass in the water column indicated no or marginal energy losses, thus indicating chemical transformation rather than biomass degradation.

The composition of fatty acid methyl esters shows a possible anaerobic degradation of the oleic acid methyl esters into palmitic acid methyl esters in the first batch, which indicates that the depth of the sediment, supply of nutrients and time of dewatering are critical parameter for the quality of biodiesel. In the second batch there is no clear sign of degradation of the fatty acid methyl esters. The composition of this batch is similar to commercial biodiesel from rapeseeds (RME).

This research also concludes the importance of supplying CO_2 and maintaining optimal cultivation temperature in order to ensure a high productivity. By supplying carbon dioxide into the cultivation ponds higher productivity rates as well as lowered energy consumption could be obtained. From an energy saving perspective, carbon dioxide concentration higher than 10 % should be supplied by a continuous pumping system. Such aeration system would supply sufficient amount of the carbon source to ensure higher productivities and account for less than 2 % of the potential output energy.

Although lower amounts of lipids in the samples harvested from the bottom, a cultivation setup in which microalgae are allowed to settle would still be advantageous from an energy saving perspective. Fewer harvestings per year leads directly to decreased energy consumption and by using a pond vacuum cleaner less water needs to be evaporated as well as this harvesting method is not dependent on the algal productivity. Compared to filtration and centrifugation, the pond vacuum cleaner consumes 140-530 times less energy.

7 Future recommendations

The results from this research shows promising potential for a future use of microalgae as a source to renewable fuels. In order to verify and improve purposed cultivation method, more cultivations and analyses needs to be done. Tests of main interest should focus on supplying the cultivation ponds with carbon dioxide in order to examine the productivity rates. In connection with this, monitoring of nutrient consumption should be further examined to be able to determine an appropriate method of nutrition supply.

Future research should also focus on finding more algal strains with auto flocculation characteristics. Additionally, in order to find smart and energy efficient aeration systems, settling characteristics (i.e. settling time) should be further examined.

From a cultivation setup perspective, upscaling in pond size and possible improvements in the dehydration process by using waste heat is of great interest as well as analysing potential drawbacks in covering the ponds (e.g. oxygen inhibition).

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Appendix A. Aeration

Table 14. Altered concentration of carbon dioxide in relation to annual net energy for a cultivation pond measuring 3.46 m^2.

Sensitivity analysis **Continous pumping. Mixing provided by airlift system** **Altering CO2 concentration. Heat value from algal biomass**						
CO2 conc (v/v)	Production (g m^{-2} $year^{-1}$)	Production (g $year^{-1}$)	Energy Output (MJ)	Energy Input (MJ)	Net energy (MJ)	Input energy/output energy (%)
0.0396	8000	27694.8	678.5226	2679.649209	-2001.126609	394.9240908
0.05	8000	27694.8	678.5226	2122.297577	-1443.774977	312.7821501
0.1	8000	27694.8	678.5226	1061.185817	-382.6632171	156.3965323
0.15	8000	27694.8	678.5226	707.4818971	-28.95929714	104.267993
0.2	8000	27694.8	678.5226	530.6299371	147.8926629	78.20372337
0.25	8000	27694.8	678.5226	424.5187611	254.0038389	62.56516159
0.3	8000	27694.8	678.5226	353.7779771	324.7446229	52.13945374
0.35	8000	27694.8	678.5226	303.2488457	375.2737543	44.69251956
0.4	8000	27694.8	678.5226	265.3519971	413.1706029	39.10731892
0.45	8000	27694.8	678.5226	235.8766705	442.6459295	34.76327398
0.5	8000	27694.8	678.5226	212.2964091	466.2261909	31.28803803
1	8000	27694.8	678.5226	106.1852331	572.3373669	15.64947625
1.5	8000	27694.8	678.5226	70.81484114	607.7077589	10.43662232
2	8000	27694.8	678.5226	53.12964514	625.3929549	7.83019536
2.5	8000	27694.8	678.5226	42.51852754	636.0040725	6.266339182
3	8000	27694.8	678.5226	35.44444914	643.0781509	5.223768397
4	8000	27694.8	678.5226	26.60185114	651.9207489	3.920554915
5	8000	27694.8	678.5226	21.29629234	657.2263077	3.138626826
10	8000	27694.8	678.5226	10.68517474	667.8374253	1.574770648
15	8000	27694.8	678.5226	7.148135543	671.3744645	1.053485255
20	8000	27694.8	678.5226	5.379615943	673.1429841	0.792842559
25	8000	27694.8	678.5226	4.318504183	674.2040958	0.636456941

Appendix B. Paddlewheel

Table 15. Altered raceway length and mixing velocity in relation to annual net energy. The energy output is based on a heat value of the biomass equal to 24.5 MJ kg^{-1}

Width	2.40	2.40	2.40	2.40	2.40	2.40	2.40
Length	2.40	5.00	10.00	20,00	30.00	40.00	50.00
Area (m2)	4.67	12.00	24.00	48,00	72.00	96.00	120.00
Productivity (kg/year)	37.39	96.00	192.00	384,00	576.00	768.00	960.00
Energy output (MJ)	916.02	2352.00	4704.00	9408,00	14112.00	18816.00	23520.00
Energy input (MJ) 0.05 m/s	19.41	19.94	20.95	22,98	25.00	27.04	29.07
Net energy (MJ) 0.05 m/s	896.61	2332.06	4683.05	9385,02	14087.00	18788.96	23490.93
Energy input (MJ) 0.10 m/s	155.27	159.49	167.61	183,84	200.08	216.31	232.55
Net energy (MJ) 0.10 m/s	760.75	2192.51	4536.39	9224,16	13911.92	18599.69	23287.45
Energy input (MJ) 0.15 m/s	524.03	538.28	565.68	620,47	675.26	730.05	784.84
Net energy (MJ) 0.15 m/s	391.99	1813.72	4138.33	8787,53	13436.74	18085.95	22735.16
Energy input (MJ) 0.20 m/s	1242.15	1275.92	1340.86	1470,74	1600.61	1730.49	1860.37
Net energy (MJ) 0.20 m/s	-326.13	1076.08	3363.14	7937,26	12511.39	17085.51	21659.63
Energy input (MJ) 0.25 m/s	2426.08	2492.03	2618.87	2872,53	3126.20	3379.87	3633.54
Net energy (MJ) 0.25 m/s	-1510.06	-140.03	2085.13	6535,47	10985.80	15436.13	19886.47
Energy input (MJ) 0.30 m/s	4192.27	4306.23	4525.40	4963,74	5402.08	5840.41	6278.75
Net energy (MJ) 0.30 m/s	-3276.25	-1954.23	178.60	4444,26	8709.92	12975.59	17241.25

Appendix C. Sensitivity analysis

Table 16. Altered annual production at different concentration of lipids in relation to annual net energy. The energy output is based on a heat value of 43 MJ kg^{-1}, equal to biodiesel.

Production (g/year)	Energy input (MJ)	Energy output (MJ) 15 % lipids	Net energy (MJ) 15 % lipids	Energy output (MJ) 20 % lipids	Net energy (MJ) 20 % lipids	Energy output (MJ) 25 % lipids	Net energy (MJ) 25 % lipids	Energy output (MJ) 30 % lipids	Net energy (MJ) 30 % lipids
2000	29.021	12.9	-16.12	17.20	-11.82	21.50	-7.52	25.80	-3.22
4000	29.021	25.8	-3.22	34.40	5.38	43.00	13.98	51.60	22.58
6000	29.021	38.7	9.68	51.60	22.58	64.50	35.48	77.40	48.38
8000	29.021	51.6	22.58	68.80	39.78	86.00	56.98	103.20	74.18
10000	29.021	64.5	35.48	86.00	56.98	107.50	78.48	129.00	99.98
12000	29.021	77.4	48.38	103.20	74.18	129.00	99.98	154.80	125.78
14000	29.021	90.3	61.28	120.40	91.38	150.50	121.48	180.60	151.58
16000	29.021	103.2	74.18	137.60	108.58	172.00	142.98	206.40	177.38
18000	29.021	116.1	87.08	154.80	125.78	193.50	164.48	232.20	203.18
20000	29.021	129	99.98	172.00	142.98	215.00	185.98	258.00	228.98
22000	29.021	141.9	112.88	189.20	160.18	236.50	207.48	283.80	254.78
24000	29.021	154.8	125.78	206.40	177.38	258.00	228.98	309.60	280.58
26000	29.021	167.7	138.68	223.60	194.58	279.50	250.48	335.40	306.38
28000	29.021	180.6	151.58	240.80	211.78	301.00	271.98	361.20	332.18
30000	29.021	193.5	164.48	258.00	228.98	322.50	293.48	387.00	357.98
32000	29.021	206.4	177.38	275.20	246.18	344.00	314.98	412.80	383.78
34000	29.021	219.3	190.28	292.40	263.38	365.50	336.48	438.60	409.58
36000	29.021	232.2	203.18	309.60	280.58	387.00	357.98	464.40	435.38
38000	29.021	245.1	216.08	326.80	297.78	408.50	379.48	490.20	461.18