



AALBORG UNIVERSITY
STUDENT REPORT

2015

Biodiesel from Microalgae

A Possible Competitor to Petroleum Diesel



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18-12-2015

Title Page

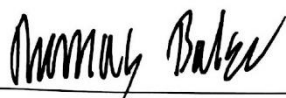
Title: Biodiesel from Microalgae: A Possible Competitor to Petroleum Diesel

Field of study: Chemical Engineering and Biotechnology

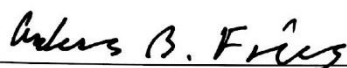
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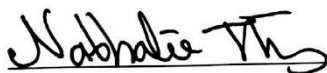
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Abstract

Initially this report explores the production process of biodiesel from microalgae including growth methods, extraction methods and the conversion into methyl esters through transesterification.

The primary focus of this study is on the environmental impact, fuel efficiency and cost of biodiesels from microalgae compared to petroleum diesel.

The biodiesel was made from the microalgae strains *Chlorella vulgaris* and *Dunaliella tertiolecta*, through growth, extraction using hexane, and transesterification using methanol and sodium hydroxide. The product was tested on GC-MS and determined to be biodiesel. The calorific value was found to be comparable to that of petroleum diesel. A discussion of the competitive performance of biodiesel from microalgae is made by combining the results with an investigation of their properties including emissions from the burning of diesels, cetane numbers, gel points and labor costs.

Biodiesel derived from microalgae is found to be superior to petroleum diesel in many of these aspects, however, a major disadvantage is the high cost of biodiesels, making the use of biodiesels as a supplement more realistic.

Preface

This study is made in the period 5/10-2015 to 18/12-2015 by the group CBT15-B311b studying Chemical Engineering and Biotechnology at Aalborg University Esbjerg. The background for the study with the topic of biodiesel is based on a common interest within the group in sustainable energy sources.

A special acknowledgement goes to our supervisors Jens Muff and Torben Rosenørn for clear and useful guidance as well as Dorte Spangsmark and Linda B. Madsen for technical guidance in the laboratory. We would also like to thank Lars M. Husum for providing us with access to algae making our experiments possible.

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1 Introduction

The main topic for this project was “Chemical and Bio industrial Products” and from this we chose the topic of Biodiesel. Biodiesel has been in the public eye for a while because of society’s increasing concern about the environment. Many governments around the world are making an effort to use cleaner energy sources, and biofuels are a good example of this. Using more environmentally friendly fuel sources can make a huge difference since our society is very dependent on fuel for our cars, trains, aircraft, etc. Although there is a great societal awareness of the environmental and health risks associated with fossil fuels, they are still our primary energy source. We want to investigate why a substitute product has not yet emerged as a viable competitor to fossil fuels, and for this purpose we took a particular interest in biodiesel, since it may help reduce the use of petroleum diesel specifically. This could be a good step towards becoming less dependent on fossil fuels.

Biofuel is made from biomass, such as animal fats or plant oils. This of course makes biofuel a very broad topic with many different types to work with, and all of these have different advantages and disadvantages. We shortly investigate the different generations of biofuels in order to choose which type of feedstock we think seems most promising, both in regards to availability, such as its ease of growth, and the environmental impact, such as whether the crops have a negative impact on the surrounding environment. These considerations led to us choosing algae-based biodiesel, since we found it to have the highest potential to compete with petroleum diesel, especially because algae production has no impact on food scarcity.

Our initial research on algae-based biodiesel seems very promising, which has increased our curiosity regarding the subject. The potential for an environmentally friendly and economically sustainable replacement for petrodiesel is very interesting, which is why we have chosen to examine it further in this project.

2 Problem Analysis

We have chosen to focus on the topics we find necessary to be capable of answering the problem statement. To make this more manageable we have made a breakdown chart (see Appendix A) following the process to define our scope of work and gain a better overview of which topics reflect our problem statement, and in the same process eliminating the topics that are not directly identified with it.

2.1 Generations of Biofuel

Through time, different generations of biofuels have been defined to overcome the complications related to previous generations. This, however, does not mean that a newer generation is always better than lower generations in every aspect since factors such as the efficiency of the production process must be taken into account as well [1].

First-generation biofuels refer to biodiesel or bioethanol produced from crops, which are also used as a food source. Examples include wheat and sugar for bioethanol-production and oil seed rape for production of biodiesel. The most notable issue with first-generation biofuels is their competition with food crops for land [2].

Second-generation biofuels are those produced from non-food crops. This solves the initial problem of competition with food crops, but still requires large areas of land to grow the crops needed [2].

Third-generation biofuels include aquatic photoautotrophic¹ organisms such as algae [1]. Their largest benefit is that algae are able to produce more energy per acre than conventional crops and thus help reduce the area needed to cover the same biofuel production [2]. Moreover, biomass for third-generation biofuels can be cultivated anywhere where sufficient amounts of carbon dioxide and water are found [3].

Algae, like corn, soybeans, and other plants store energy in the form of oils, carbohydrates, and proteins. The plant's oil can be converted into biodiesel through different methods. The more efficient a particular plant is at converting solar energy into chemical energy, the better it is from a biodiesel perspective, and algae are among the most photosynthetically² efficient plants on Earth [4].

Fourth-generation biofuels aim to absorb more CO₂ than is emitted back into the atmosphere when burned. This can be accomplished by capturing carbon dioxide throughout the production and geosequestering³ it [2].

¹ Photoautotrophic: An organism, typically a plant, obtaining energy from sunlight as its source of energy to convert inorganic materials into organic materials for use in cellular functions such as biosynthesis and respiration.

http://www.diffen.com/difference/Autotroph_vs_Heterotroph

² Photosynthesis: The synthesis of complex organic material using carbon dioxide, water, inorganic salts, and light energy (from sunlight) captured by light-absorbing pigments, such as chlorophyll and other accessory pigments.

<http://www.biology-online.org/dictionary/Photosynthesis>

³ Geosequestering: The process of capturing carbon dioxide that would otherwise be emitted to the atmosphere, compressing it, transporting it to a suitable site, and injecting it in liquid form into deep geological formations where it is to be trapped for thousands or millions of years. <http://www.landlearnsw.org.au/sustainability/climate-change/research/geosequestration-ccs>



For this project we have chosen to focus on third-generation biofuels, specifically biodiesel produced from algae. The annual productivity and oil content of algae is far greater than seed crops. Soybean can only produce about 450 liters of oil per hectare. Canola can produce 1.200 liters of oil per hectare, and palm trees can produce 6.000 liters of oil per hectare. Algae, on the other hand, can yield 90.000 liters per hectare. Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy [5]. From these values it can be reasonably concluded that algae and microalgae in particular would be the optimal candidate for biofuel. Even though more than 99 % of global biofuel production consists of first- and second-generation biofuels, future technological improvements may help third- and fourth-generation biofuels take a bigger role in the production of biofuels [6] and we think this makes biodiesel from algae an important topic of research.

2.2 Environmental Impact

Fossil fuels have been a part of our everyday lives and our industry for a long time, but they undeniably have significant detrimental effects on the environment, which is becoming something we strive to reduce. In regards to this, the topic of biofuels has gained a lot of interest since they seem to have great potential in reducing this negative environmental impact by acting as a partial or full substitution to fossil fuels. Biofuels tend to be more environmentally friendly than fossil fuels due to a number of factors, such as their renewability and lower pollution, which is why we have chosen to investigate them further.

Biofuel from algae is considered carbon-neutral, which means that the algae consume as much CO₂ when grown as they release when burned. Some companies even claim that the GHG (greenhouse gas) footprint from biodiesel produced by algae is 93 % lower than that of traditional petroleum diesel (petrodiesel) [7]. Earlier sources of biofuel relied on using food crops for oil production, which meant that fuel production would tap into food production, both in regards to the space used for growing crops, and in regards to choosing how to use these crops. This would result in a significant increase in the necessary farmland for crops, although there have been difficulties in reaching an agreement on the amount of required land, since the estimations can be based on different premises and assumptions, leading to many different outcomes. [8] These estimates show that between 0.4 Mha⁴ and 114 Mha⁵ of natural land⁵ could be lost due to biodiesel production. This, of course, depends on which crops are used as well as many other factors [8]. If we instead produce biodiesel from algae, the use of land will be minimized since algae do not share the same soil requirements as land-based crops, and can instead be grown on fields otherwise unsuitable for agriculture, or inside factory facilities [7].

Another problem with the development of biodiesel is that it can have an impact on the natural biodiversity⁶ by ruining habitats in order to make space for agriculture. There are a variety of ap-

⁴ The area of Denmark is approximately 4.3 Mha.

⁵ Land where plants grow freely without human interference

⁶ Biodiversity is a measure of the amount of different species living in an ecosystem



proaches to growing algae, and these do not necessarily require being in fields, or even outside, which means that the production of algae can have a smaller impact on biodiversity than traditional land-based crops [8]. Natural ecosystems are already widely disturbed by the large areas we currently use for agriculture, and the amount of land required for this is expected to increase in the future in accordance with the rising population. If, on top of this, we start growing crops for biodiesel on land currently dedicated to farming for food, then we will make an even greater impact on the natural ecosystems in the different areas.

During early attempts at growing algae for biodiesel, the consumption of water during the growth process was very high. This is a major issue since water is a precious and highly valuable resource, and overuse is already a globally environmental issue, even without the added expenditure from biodiesel production. Attempting to fix the issue of global warming through use of biofuels would make little sense if it resulted in creating a significant shortage of clean water, since this would be a far more urgent issue. The initial methods of growing algae were in shallow ponds, and these ponds required large amounts of water because of the high evaporation rate in the system. But through the continued work and research in regards to growing algae, new methods have been discovered with no (or very low) evaporation issues, where it is even possible to minimize waste of water by recycling up to 85 % of the water [7].

2.2.1 Danish Rules and Regulations on Production, Use and Sale of Biofuels

With the previous chapter's considerations in mind, laws have been made in Denmark that specify the usage of biofuels. When working with the uses of biofuels, it is important to consider the legislation that exists on the topic. Ideally, these laws serve the function of protecting the environment by reducing the emission of greenhouse gasses and other environmental risk factors in order to ensure that the production of biofuels has the lowest possible amount of negative impact on the environment.

The Danish law on biofuels (2009) states that all sales of fuels for land based transport must consist of at least 5,75 % biofuels (measured in energy content) [9].

To count for this requirement the biofuels must meet the following rules for sustainability [10]:

- The savings in greenhouse gas emissions must be at least 35 % (50 % from 2017 and 60 % from 2018 for plants that started operations after 2017).
- The biofuels must not be produced from raw materials from an area with high biodiversity
- The biofuels must not be produced from raw materials from an area with a high carbon storage (defined as areas that were wetlands or dense forests in January 2008, but is not anymore).
- The biofuels must not be produced from raw materials from which were peatlands in January 2008, unless the cultivation will not dewater the soil.
- Raw materials which are produced in Europe for use in biofuels must also follow requirements concerning the protection of [11]:
 - The groundwater from contamination by dangerous chemicals.
 - The environment, especially the soil, in connection with the use of sludge from cleaning facilities.

- Water from contamination caused by nitrates from agriculture.
- Nature as well as wild animals and plants.

Companies must send annual reports to the Danish Energy Agency, explaining how the company has met the requirements above [10].

Companies can apply for funding for projects that promote energy efficiency, for instance by replacing fossil fuels with biofuels [11].

Denmark has no specific laws on the cultivation of algae.

The fact that biofuels must constitute at least 5,75 % of fuels for land based transport reduces competition from fossil fuels against biodiesel. Biodiesel from algae now only has to compete against other biofuels in this limited market share of 5,75 %. Since the production of other biodiesel feedstocks are more restricted by laws than algae production, biodiesel from algae has stronger advantage than it otherwise would have.

2.3 Introduction to Algae Strains and Species

The potential applications of algae have been known for many years, but there have been complications in regards to the development of the technology to use algae on a larger, industrial scale, and it is only in recent years that we have gained enough knowledge about algae to really work with them and utilize them for various purposes, such as the production of biofuels.

In the late 1940s, algae were seriously considered as an alternative food source, particularly the strain *Chlorella*, since it contained a large amount of important nutrients when dried. However, despite seeming very cost-efficient, it turned out that *Chlorella* was difficult to produce reliably with the available technology, since small changes to its environment could cause it to stop growing. The process of harvesting and processing *Chlorella* also turned out to be damaging to the biomass, and as a result, the attempt to turn *Chlorella* into a new food source failed [12]. In 1980, the American Department of Energy created the Aquatic Species Program [13], which attempted to find a way to produce oil from microalgae. In the 16 years the program existed, they tested and analyzed thousands of different species of algae, and helped build a greater understanding of mass-production of algae. The program was shut down in 1996 however, due to financial concerns and the high availability of relatively cheap fossil fuels [14].

Since the days of the Aquatic Species Program there have been a great number of scientific breakthroughs that influence the potential to produce algae to be used for biofuels. Biotechnological advancements allow for genetic engineering of algae species in order to increase efficiency. This includes creating algae with higher concentrations of oil, better conversion of solar energy, more effective or controllable growth systems [15][16]. Algae are appealing as a source of biofuel because they are environmentally friendly, fast-growing, and non-competitive with agriculture [17].

Based on the historical perspective and the general knowledge of production methods, we have chosen to focus on a few select strains of algae that seem to have the best potential for the purposes and parameters that we are considering when the goal is to produce biofuel.



A great benefit to algae is that they can grow anywhere (provided that there is sufficient sunlight and feedstock). Along with some strains of algae's naturally high lipid content of 50 % and upward, these strains can also complete an entire growth cycle every few days. It is the combination of lipid content and growth cycles that forms the basis for evaluating any micro algae used in industrial production. We will therefore primarily focus on lipid content and biomass production in our evaluation of algae strains. Other important considerations that we will only briefly explore are the optimal medium, photosynthetic efficiency and nutritional requirements.

2.3.1 Species of Algae

Marine ecosystems form the largest habitats on Earth, which are primarily dominated by microalgae. Algae exist in numerous forms throughout this ecosystem and have a wide array of properties, such as being capable of photosynthesis. Their ability to produce oil is a topic of interest for chemical engineers, since algae are a renewable energy source as well as CO₂ neutral. Algal organisms can be divided into two distinct groups: macroalgae and microalgae, growing in aquatic environments.

Macroalgae, commonly referred to as "seaweed", are multicellular plants growing in salt or fresh water. Macroalgae can be classified into three groups identified by their pigmentation: brown seaweed (*Phaeophyceae*), red seaweed (*Rhodophyceae*) and green seaweed (*Chlorophyceae*). [18]

Microalgae are unicellular microorganisms that exist in saline or fresh water environments and lives and reproduces by converting sunlight, water and carbon dioxide to algal biomass through aquatic photosynthesis. Aquatic photosynthesis functions by the following chemical equation: $6\text{CO}_2 + 6\text{H}_2\text{O} + \text{light} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6$. Sunlight acts as the initiator of the reaction since it provides the energy necessary for the reaction to occur. The algae absorb light through chlorophyll molecules and conduct photosynthesis. [19]

Microalgae are among the largest producers of biomass. An interesting species is the phytoplankton community, which includes 5000 marine species of microalgae with a broad diversity in cell size, morphology, physiological, and biochemical composition. All phytoplankton are capable of photosynthesis and many have an astounding cell division and growth rate, doubling up to four times per day. Some phytoplankton species also act as bio-indicators reflecting changes in the environment such as pH, temperature, salinity, alkalinity, nutrient composition, solar radiation etc. [18] Exploring new microalgae and synthesizing new algae strains on a larger scale could yield not only advances in algae biodiesel, but also breakthroughs in the medical, cosmetic, and food industry as algae continues to contribute in all these areas.

Only fifteen of the known microalgae species are currently mass cultivated. This is a tiny fraction of the known species of algae and orders of magnitude smaller than the estimated number of unknown species. The classification and exploration of algae remain a relatively unexplored topic in modern science due to ineffective methods of classification. Using primers, which is a strand of nucleic acid sequencing designed to replicate and identify DNA for classification, has proved troublesome due to theoretical construction of strains based on incomplete datasets from cultured organisms [18].

New methods of identification and further studies are warranted, if potentially higher oil yielding algae are to be found.

2.3.1.1 Highest-yielding algae

Microalgae are uniquely rich in lipid content. They consist of proteins, carbohydrates, lipids and nucleic acids in varying amounts. In oil production lipid content is the most important aspect of these characteristics. Among the different micro algae strains we have chosen to focus on the family *Chlorophyceae*. [20]

2.3.1.2 Chlorella

Chlorella is single celled green algae in the class *Chlorophyceae* [21]. *Chlorella* uses photosynthesis to grow. It captures light through special pigments which are called chlorophyll-a and -b. Chlorophyll-a captures the wavelengths associated with photosynthesis, while chlorophyll-b is correlated with the photosynthetic output [22]. *Chlorella* consists of 50 % protein and amino acids, such as tryptophan, 30 % fiber, 10 % mineral salts and 10 % lipids [21]. Various strains of *Chlorella* possess a higher lipid content. *Chlorella vulgaris*, which is an alga that we will be using in our experiments later in this project, consists of 51-58 % proteins and 14-22 % lipids [23]. *Chlorella* grows rapidly in almost any pond or lake worldwide. To grow, it only requires a source of nutrients such as nitrogen, CO₂, sunlight and a stirred medium to ensure that all algae are in a homogenous state [24].

2.3.1.3 Dunaliella

In the family of *Chlorophyceae* another interesting algae exists. *Dunaliella* is also a primary alga with an oval shape and is about 9 to 11 µm long in diameter. The benefit of this alga is that it is simple to cultivate and does not form chains or clump. This strain can have an oil yield of 37 %, while being fast growing and therefore allowing for a high carbon dioxide rate [25].

2.3.1.4 Super Strains

Chlorella and *Dunaliella* are both highly efficient regarding growth-to-lipid ratio, but there are algae that have the ability to outclass them both under the right conditions. One of these strains is the rare algae *Botryococcus braunii*. In 2015 Dr. Jin Qin, from the Flinders University in Australia released an interesting study. In this study, Dr. Qin managed to grow the lipid-rich algae *Botryococcus braunii* in a very short time. *Botryococcus braunii* contains upwards of 75 % lipid content of its dry weight. In the experiment Dr. Qin cycled through a light and dark environment every 12 hours, with a steady temperature of 23 °C in a medium of 8,775 ‰ salinity. Traditionally *Botryococcus braunii* goes through a full growth cycle in 14 days, but in this optimal environment the algae's growth cycle was completed in a mere two days [26].

It would be interesting if we could replicate the remarkable results that Dr. Qin managed to produce with *Botryococcus braunii*. This alga could be a boon in commercial production due to its high lipid content and growth rate. We suspect that optimal growth levels cannot be reached in an open pond system because of the specificity of the requirements for growing the algae. A PBR would be optimal since you can more easily regulate the growth parameters, which we will explore further in section 2.4 regarding growth methods. Due to the temperature requirement of 23 °C it will be extremely difficult to grow this alga outdoor in Denmark with our colder climate. It would instead be

necessary to create an artificial environment, which could make it less attractive from a financial standpoint.

2.3.2 Algae Growth Stages

The growth rate greatly influences the biomass yield of algae. When determining the growth rate of algae it is important to be cognizant of the growth phases they go through. The phases are summarized in the following sections.

2.3.2.1 The Lag Phase

In this phase the sample algae is removed from its medium and placed in a new medium, which may cause the algae to not exhibit any growth for a period. It depends in large measure of the health of the algae sample and the medium in which it is placed. To reduce the lag period, growth conditions from the pre-existing algae and the sample should be equal. [27]

2.3.2.2 Exponential Phase

In this phase the algae increases its biomass over time, which is called “growth rate”. The growth rate determines the ecological success of the species or strain when it comes to adapting to the medium, temperature, salinity, light cycles, and intensity. [27]

2.3.2.3 Decline Phase

During this phase the algae biomass production is declining. This usually happens in cultures when the requirements for production of biomass are limited or something else is limiting cell division. Often biomass will be at a peak in this phase and have exhausted the nutrients in the salt, its CO₂ provision or the culture can be so thick that it restricts incoming light. [27]

2.3.2.4 Stationary Phase

The culture is at a net zero change in growth rate. The culture undergoes extreme changes during this phase. The specifics of the changes depend on the growth limiting factor. If nitrogen is lacking the culture experiences a reduction in protein content and may experience a decrease in lipid content. Light limitation causes pigmentation changes and a change in fatty acid content. Light intensity that was suitable for the first 3 phases may become stressful for the culture and can push it towards the death phase, a condition known as photoinhibition. [27]

2.3.2.5 Death phase

In this phase the vegetative metabolism can no longer be maintained in the culture and it begins to rapidly decay. As the cell membrane integrity of the algae begins to deteriorate, bacteria of various kinds starts to form on the carbon rich algae. The growth rate declines rapidly in this stage until the culture is completely decayed. [27]

In regards to algae production it is critical to identify the exponential growth phase and make certain that the algae do not exit this phase into the stationary phase, or worse yet the death phase. Prolonging the exponential growth phase is usually done by thinning out the culture, so sunlight and nutrients are readily available for the remaining culture. Insight into the growth phases is helpful

when it comes to growing microalgae since they play a part in optimizing the production process. This is one of many factors involved in developing a financially responsible approach to algae production.

2.4 Growth Methods

Several tons of algae are farmed commercially every year. The biomass from these algae is used for human nutritional supplements, specialty animal feeds and pharmaceutical products [28], but in recent years, there has been significant development with new designs of closed circuit systems specifically made for farming microalgae to process into biofuel.

Most microalgae are cultured as photoautotrophic and thereby produce their own food from substances available in their surroundings using photosynthesis. This is one of the primary advantages of farming microalgae as a feedstock for biofuel production. It also creates some limitations to the designs for developing new microalgae production systems able to produce biomass on a commercial level [28]. Commercial plants as of today use one of the following four technologies:

1. *Extensive ponds.*
2. *Circular and raceway ponds.*
3. *Tubular photobioreactors.*
4. *Fermenters (where algae are grown on organic substrates in the dark).*

[28 (p116)]

2.4.1 Open ponds

Today, the most large-scale microalgae biomass production system for commercial purpose is the shallow raceway pond [28]. The reason for this is that they are cheaper to build, clean, and operate than PBR and require a lower energy input [28]. The pond is about 20 to 40 centimeters deep and is designed to copy the conditions of the algae's natural environment (Figure 1). It has a paddlewheel that maintains circulation in the system to mix the algal cells and nutrients, and baffles designed to maintain the flow in the system. These nutrients can be supplied by using wastewater rather than clean water [29]. There are multiple benefits in using this method; it is a low-cost process and a good source of nitrogen and phosphorous, which the algae obtains as nutrients and in the same process cleans the water. The algae are harvested behind the paddlewheel after it has completed a circulation [30]. These open pond systems

are the simplest systems for mass cultivation of microalgae, but they also have great disadvantages. Due to being open-air systems they are exposed to contamination [28] and in contrast to farming, where methods have been developed over hundreds of years to prevent the unwanted impact of external influences, the meth-

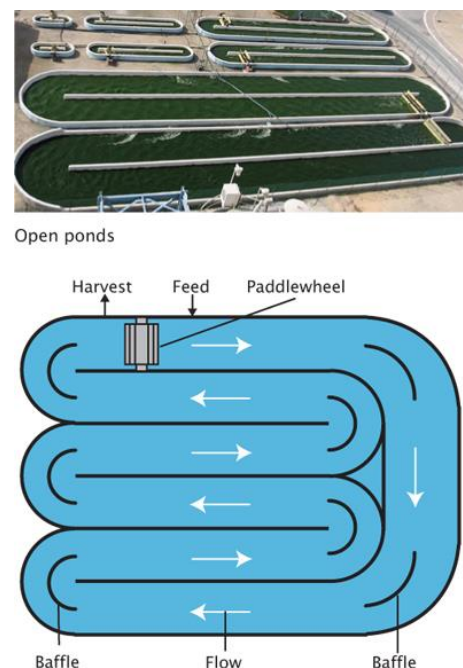


Figure 1 - Open raceway pond [31]

ods within mass algal culture are not even nearly as developed and are still in the very early stages. Another problem is that there is a lot of water loss due to evaporation, which results in the system having an enormous demand for daily water supply in order to compensate for this loss [30]. The climate also presents another major environmental factor for farming microalgae in open ponds, since the strains of algae that are currently used require an optimal temperature of over 20 °C. When the temperature falls below 20 °C, the productivity declines drastically. The optimal climatic conditions for growing algae in open ponds are an annual average temperature above 15 °C [32]. Even in desert regions it can be difficult to maintain a high productivity of algae due to low nighttime temperatures. This impacts the photosynthesis rate because the pond has to warm up again [33]. Different methods have been tried in attempting to circumvent this problem, such as covering the ponds with an isolating top at night or increasing the depth of the pond, but none of these solutions have been sufficiently inexpensive for biofuel production [33].

2.4.2 Photobioreactors

Due to the interest in significantly reducing the risk of contamination and the rate of water evaporation within the open pond systems there has been a lot of research and development going into designing closed circuit systems [28]. These can make it possible to control growth parameters within the system in order to promote biological growth, such as by facilitating better control of CO₂ and water supply, temperature, pH levels, culture density and maximizing the photosynthetic efficiency by creating a sufficient exposure to light [34]. These requirements are collected in the following principles:

1. Adequate mixing to provide a suitable light-dark cycle to the cells and avoid biofouling.
2. High mass transfer capacity to efficiently supply CO₂ and prevent O₂ build-up.
3. High surface-to-volume ratio to increase cell concentration and volumetric productivity.
4. Control of temperature at or near the optimum for the cultivated organism.
5. Accurate control of pH and CO₂ and nutrient concentrations.
6. Adequate harvesting regime to maintain the optimal population density.

[28 (p117)]

Photobioreactors for cultivation of algae have been developed in different designs and variations, but the core process of the system, as seen on figure 2 below, remains the same.

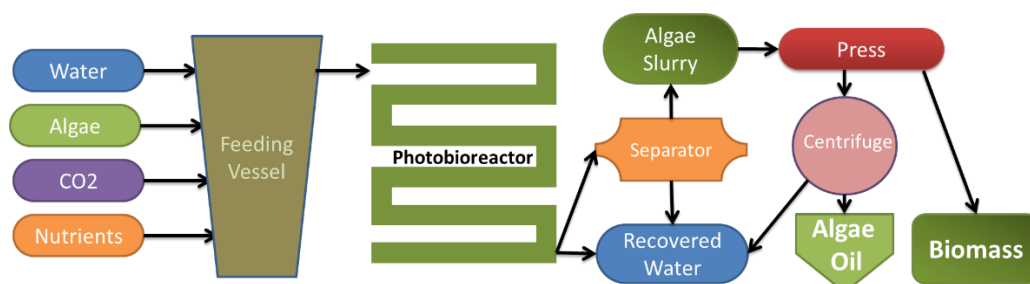


Figure 2 - The process within a photobioreactor. [35]

The most commonly used design for commercial use is the tubular photobioreactors [28], also following the process in figure 2. These are suitable for outdoor bulk cultivation because of their large

surface area for maximizing sunlight capture [36], with tubes made of glass or acrylic, allowing daylight to enter the system for photosynthesis to occur. Nutrients and water are injected to the feeding vessel and are circulated into the system by a pump. When the algae have circulated through the system they flows into the feeding vessel again, monitored by sensors detecting if the algae are ready for harvesting as well as the oxygen level (a product of the photosynthetic process). At this point, the algae are separated and the algae that are ready for harvest will pass through a filter, while the remaining algae flow back into the feeding vessel [34].

Most photobioreactors for algae production built today are small scale and used for research. The reason for this is their high operating cost and reduced scalability [28]. One of the major limitations in larger scale photobioreactors is the build-up of photosynthetically generated oxygen. High levels of dissolved oxygen can be toxic to the algae cultivation and may lead to culture death [37], which poses a major threat when previous studies have showed that very high levels of dissolved oxygen are easily reached in tubular photobioreactors [36]. An example of the drawback of the production cost compared to the yield when



Figure 3 - Tubular photobioreactor. [38]

upsizing photobioreactors is the reactor developed by The Department of Chemical Engineering of the University of Almeria, Spain, with, "... two-layer, 4.000 L horizontal tubular photobioreactor, made of 10 cm diameter Plexiglass® tubes connected by U-joints to form a single 400 m long loop ... used for production of lutein-rich biomass of *Scenedesmus almeriensis*." [28 (p122)]. This system had the potential to yield $16 \text{ t ha}^{-1} \text{ year}^{-1}$ [28] but has since been redesigned to a fully automated system, which "... is now composed of ten $2,8 \text{ m}^3$ vertical serpentine units. Each unit occupies a surface area of about 50 m^2 and consists of 20 m long, 9 cm diameter Plexiglass® tubes running in a fence-like structure ... The culture suspension is circulated by a centrifugal pump ... Adopting a dilution rate of about 35 % a mean volumetric productivity of $0,4 \text{ g L}^{-1} \text{ day}^{-1}$ (corresponding to an area productivity of about $20 \text{ g m}^{-2} \text{ day}^{-1}$) was attained in winter with *Nannochloropsis*." [28 (p122)]. However, the production cost of algae biomass was estimated to be around $\text{€}25 \text{ kg}^{-1}$, much higher than the $\text{€}0,5 \text{ kg}^{-1}$ limit goal. This means that currently the cost of producing algae for the purpose of biofuel is too high, but this is a problem that is being worked on in many ways, which will be explored in the following chapter.

2.4.3 Economical Limitations

One of the main issues in large-scale algae production is the cost involved in cultivation, harvest and extraction. Many start-up companies around the world are working on solution to these issues by experimenting with different approaches that may help find the most cost-effective approach to producing algae.

As discussed earlier, there are various approaches to growing algae, such as open ponds or photobioreactors. There are also options for how to feed the algae, since some depend more on sunlight, while others can be fed sugars instead [39]. There is the photoautotrophic production which is the only method that is technically and economically feasible for large-scale production of algae biomass for non-energy production. The practicability of each system (open ponds and photobioreactors) is influenced by the properties of the selected algae strain, as well as climatic conditions and the costs of land and water. Raceway ponds are the most common systems. Compared to photobioreactors, open pond systems are the cheaper method of large-scale algal biomass production. They also have a lower energy input requirement, and it is easier to perform their maintenance and cleaning routines. Though already in 2008 the cost of producing *Dunaliella salina* in an open pond was too high to justify production for biofuels [40]. Closed photobioreactor systems are designed to overcome some of the major problems with the open pond systems, but come at a much higher cost. Since tubular photobioreactors have a design limit on length of the tubes, they cannot be scaled up indefinitely. Hybrid production systems combine distinct growth stages in photobioreactors and in open ponds, which can reduce the cost of microalgae biomass production. Heterotrophic production has a lower harvesting cost than closed photobioreactor, raceway ponds and hybrid production. The set up cost are also minimal, but the system uses more energy than the production of photosynthetic microalgae. This is because the algae needs a carbon source, which is produced initially by photosynthesis [40].

Since production costs for biofuel from algae are not yet competitive with fossil fuels or with conventional biofuels, efforts are being made to find ways to reduce these costs. According to a 2010 research study by the Lawrence Berkeley National Laboratory, producing fuel from algae grown in ponds at a large scale would cost between \$240 and \$332 per barrel. The current price of a barrel of crude oil is only about \$40⁷, which means that the biofuel simply cannot compete. [39] The researchers at the University of Berkeley think that the biggest potential for reducing the costs is to do some biological improvements, e.g. doubling the algae's productivity through strain selection and genetic modification. There is also room for improvement in regards to cheaper harvesting, reliable cultivation in outdoor ponds, engineering improvements in system components such as in reactor construction, harvesting, dewatering, and oil recovery [41]. To reach those aims the EU has established a "EUROPEAN NETWORK FOR ALGAL-BIOPRODUCTS" named European Cooperation in the field of Scientific and Technical Research or just called short "COST". Their goal is to find an economically feasible model for the commercialization of algae-based bio products. They found out that the production of biofuel requires a coupling between microalgae production and wastes removal in order to be economically and environmentally sustainable. Besides, it demands the de-

⁷ Barrel price: <http://www.oil-price.net/index.php?lang=en>

velopment of a cost-efficient and sustainable microalgae-based bio-economy. This requires though an overcoming of several technological and microbiological limitations plus the complement of several knowledge gaps.

There are some current research niches, where they try to fulfill these requirements:

- Increased microalgae biomass plus high-added value product productivity at decreasing operating cost
 - To make microalgae biodiesel economically viable in low cost open photobioreactors.
 - Development of cost-effective microalgae harvesting
 - Biomass recycling and membrane separation also deserve further investigation.
- [42]

2.5 Extraction Methods

Once a sufficient amount of algae has been produced, the next stage in converting them to biofuel is separating the biomass from the liquid and drying it. This can be executed by centrifuging the algae cultivation to end up with biomass containing minimal amounts of water. When the algae is dry it is ready for extraction of lipids. These lipids mainly consists of mainly triacylglycerols (TAG) [43]. The efficiency of this part of the process plays a large role in the final result, both in regards to cost and the amount of fuel produced by a given amount of biomass. Different extraction methods have different drawbacks and benefits, and these are important when considering which extraction method to use. In our experiment, we chose to use solvent extraction by hexane, which is practical to do in the laboratory without special (and possibly expensive) equipment. However, on a larger scale, different methods may be more beneficial.

2.5.1 Solvent Extraction

A traditional way of extracting lipids from biomass is solvent extraction. The process involves dissolving the TAGs in a solvent. A commonly used solvent for this purpose is hexane, but other molecules have been proposed and tested [44][45]. The extraction can be conducted using a soxhlet extractor as seen in figure 4. In a soxhlet extractor, the sample is placed in the container in the middle of the extractor, while the solvent is in the round-bottomed flask below. When the flask is heated, the solvent evaporates until it hits the condenser at the top. The solvent liquefies and drops into the container with the sample. The lipids are dissolved in the solvent and are filtered through the sides of the container, falling back into the bottom flask. From here, the solvent continues to evaporate while the lipids remain. In time, almost all of the lipids will have been moved into the bottom flask and can then be separated from the solvent through distillation [46].

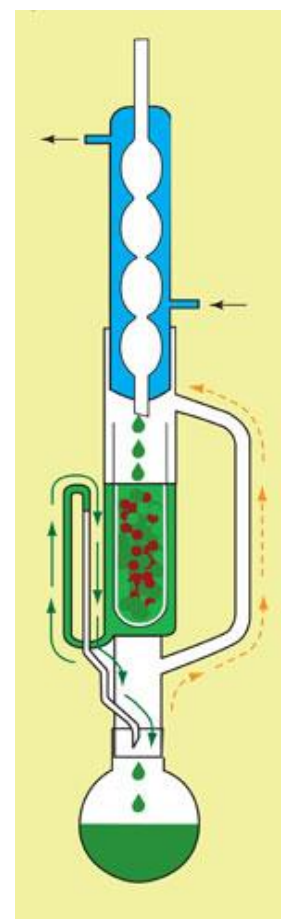


Figure 4 - Overview showing how a soxhlet extractor works. [47]

2.5.2 Supercritical Fluid

An alternative approach to extracting one component from another is by using supercritical fluid (SCF) as the extracting solvent. This is also a form of solvent extraction, and is typically more efficient than for instance solvent extraction by using hexane, but this was unfortunately not a possibility during our current experiment. However, this is still of interest because it could help increase the overall output of biodiesel production from algae, and is also valid for larger-scale industrial production.

When a substance, for instance water or CO₂, is pushed to a certain temperature and pressure, it enters a state where it is no longer possible to distinguish between the liquid and the gas phase because the liquid's density will be equal to the density of the gas. At this point it enters a SCF phase where the substance's density and viscosity levels change when adjusting the temperature or pressure [48]. Some substances even acquire different properties when entering their supercritical phase, which means that it is possible to use a solvent without some of the environmental pollutants like acetone or benzene that are used in chemical processes today for making synthetic materials such as plastic, medicine or fabric [49]. For water, the supercritical phase is induced at the critical temperature of 647 K and the critical pressure of 218 atm⁸ [50] (as seen on figure 5 below). This is due to its high polarity. Water is polar in its liquid state because of its ability to make hydrogen bonds, but when water is supercritical the hydrogen bonds break [49], which means that it becomes non-polar as well as turning acidic [48]. These new properties make it possible to dissolve chemicals or compounds that are insoluble in liquid water. CO₂, however, enters its supercritical state at a much lower critical temperature of 304 K and a critical pressure of 73 atm (as seen on figure 6 below).

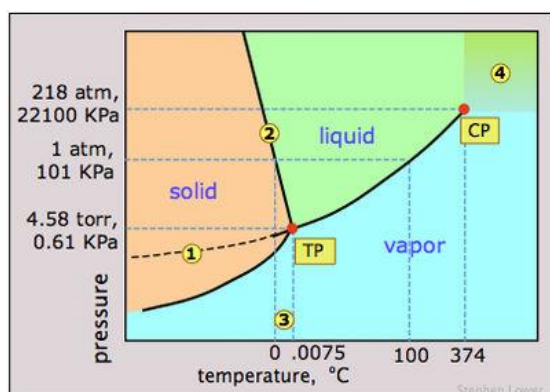


Figure 5 - The phase diagram of supercritical water. [51]

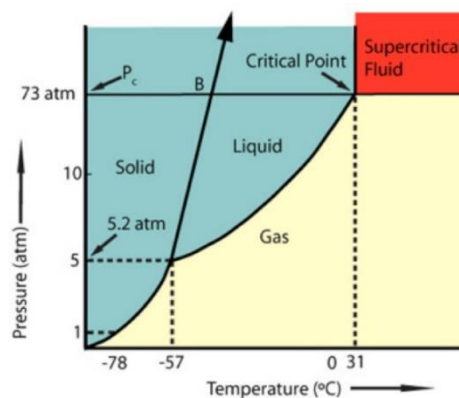


Figure 6 - The phase diagram of supercritical carbon dioxide. [52]

Both supercritical water and CO₂ are considered environmentally safe [53]. Because extraction with supercritical carbon dioxide (SC-CO₂) is efficient at temperatures lower than 323 K it ensures maximum product quality and stability, making it the most commonly used SCF [54][55]. For example, SC-CO₂ is used commercially to decaffeinate green coffee beans by extracting and crystallizing pure caffeine [54] by still maintaining the original quality of the green coffee beans after the process without the use of any toxic substances [55]. Because of the SC-CO₂'s chemical properties to break

⁸ 1 atm = 101,325 kPa



down triglycerides to glycerin and fatty acids by supercritical transesterification, it is a very effective method for extracting the lipids from microalgae [49]. Studies have shown that supercritical fluid extraction is one of the most efficient methods for extracting components from algae [34]. Not only does it yield a high purity and product concentration but it is also extra efficient because the use of supercritical fluid makes it possible to process the wet algae biomass without dewatering the algae first, saving a great amount of energy when cutting out the drying process, compared to traditional solvent separation methods [34].

2.5.2.1 Supercritical Fluid Extraction Process

In the process of supercritical fluid extraction (SFE), it is sometimes necessary to use a co-solvent in order to compensate for traits that the SCF may be lacking. In the case of SC-CO₂ it is common to use ethanol as a co-solvent to compensate for the fact that SC-CO₂ has a low polarity, making it difficult to dissolve polar lipids. There are many possible co-solvents, but ethanol is typically chosen due to being non-toxic, and because it has strong cell wall penetration. Algae tend to have rigid cell walls, which can make it difficult for the SC-CO₂ to reach the lipids, and the ethanol co-solvent helps reduce or eliminate this problem, which can increase the lipid extraction yield by 20-90 % [56][57]. Figure 7 shows the lipid yields from using SFE on various types of microalgae, and in some cases it also displays the impact from using ethanol as a co-solvent.

Summary of published works on supercritical CO₂ extraction of lipids from microalgae.

Species	Solvent	Pressure (MPa)	Temp. (K)	Yield ^a (%)	Extraction yield ^b (%) ^a	References
<i>C. vulgaris</i>	CO ₂	60	333	9	59.2	Safi et al. (2014)
	CO ₂	60	333	10.44	68.7	
	CO ₂ + 5% ethanol	60	333	11.43	75.2	
<i>P. valderianum</i>	CO ₂	35	313	0.85	30.5	Chatterjee and Bhattacharjee (2014)
<i>Scenedesmus</i> sp.	CO ₂	50	333	7.34		Taber et al. (2014)
<i>S. obliquus</i>	CO ₂ + 5% ethanol	30	318	18		Solana et al. (2014)
<i>C. protothecoides</i>	CO ₂ + 5% ethanol	30	318	13		Solana et al. (2014)
<i>N. salina</i>	CO ₂ + 5% ethanol	30	318	30.4		Solana et al. (2014)
<i>H. pluvialis</i>	CO ₂	20	328	7.3		Reyes et al. (2014)
	CO ₂ + 13% ethanol	20	328	28.3		
<i>N. oculata</i>	CO ₂	40	333		100	Crampon et al. (2013)
<i>Nannochloropsis</i> sp.	CO ₂	30	313	33	78	Nobre et al. (2013)
	CO ₂ + 20% ethanol	30	313	45		
<i>N. oculata</i>	CO ₂	40	333	30		Mouahid et al. (2013)
<i>C. closterium</i>	CO ₂	40	333	13.5		
<i>C. vulgaris</i>	CO ₂	40	333	14.5		
<i>S. platensis</i>	CO ₂	40	333	15		
Commercial algae	CO ₂ + ethanol	30	303	66.4		Chen et al. (2013)
	CO ₂ + ethanol	30	303	88.6		
	CO ₂ + ethyl acetate	20	303	63.1		
	CO ₂ + propanol	30	303	91		
<i>Synechococcus</i> sp.	CO ₂	40	333	0.75		Cardoso et al. (2012)
	CO ₂ + 5% ethanol	40	333	3.39		
<i>C. vulgaris</i>	CO ₂	28	313	4.73		Dejoye et al. (2011)
<i>Pavlova</i> sp.	CO ₂	30.6	333	10.4	34	Cheng et al. (2011)
	CO ₂	30.6	333	17.9	98.7	
<i>S. limacinum</i>	CO ₂ + ethanol	35	313	33.9		Tang et al. (2011)
<i>Tetraselmis</i> sp.	CO ₂ + ethanol	35	313	34		Li et al. (2014)
	CO ₂	15	313	10.88		

^a The amount of extracted oil divided by the amount of biomass applied.

^b The amount of extracted oil divided by the amount of oil in the biomass.

Figure 7 – Summary of published works on SC-CO₂ extraction of lipids from microalgae. [58]

The amount of lipids gained from supercritical fluid extraction using SC-CO₂ can vary depending on the level of pressure as well as the temperature. In figures 10 and 11, the yield of the microalgae *Botryococcus braunii* has been observed in regards to these [59].

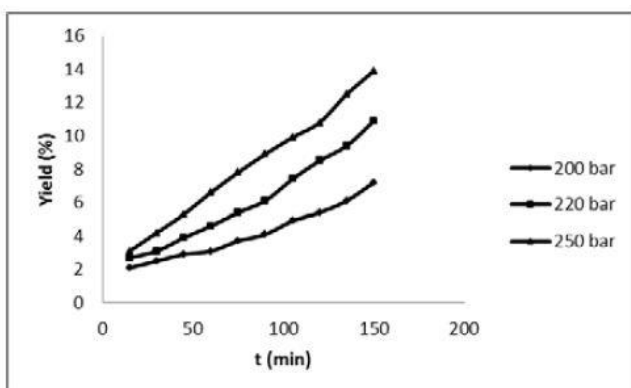


Figure 8 - *Botryococcus braunii* yields at 50°C. [60]

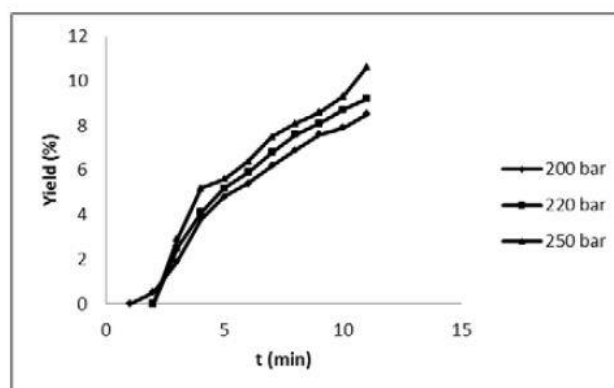


Figure 9 - *Botryococcus braunii* yields at 80°C. [61]

As can be seen on figures 8 and 9, the lipid yield can be significantly increased by applying the right amount of pressure and temperature in regards to the SC-CO₂. Experiments are made to find these values, which can then be applied on a larger scale to increase efficiency in mass production.

Another potential application for SFE in regards to microalgae is the ability to extract materials other than lipids from the same algae, resulting in a higher level of use of the microalgae. These additional materials can be pigments, such as carotenoids, astaxanthin or lutein, which can be used for medical purposes. For instance, astaxanthin reduces the risk of cardiovascular diseases while lutein can prevent age-related macular degeneration. Supercritical fluid extraction of microalgae is thereby not only useful in regards to biofuels, but also for the potential medical applications of the algae [56].

The process of SFE has a certain number of necessary constituents in order to function properly. First of all, creating the supercritical fluid within the system requires a pressure pump and a source of heat in order to reach the supercritical state of the solvent. After this the supercritical solvent is introduced to the initial material, also known as the matrix, in order to extract the desired chemicals, such as the lipids found in microalgae. The final stage involves a separator at a lower pressure, where it is possible to separate the materials from the solvent. In the case of SC-CO₂ it is possible to recycle the CO₂ within the system, which makes the process more efficient and environmentally friendly [62]. However, when the time comes for extraction, for example with lipids, a potential downside to SFE becomes apparent. SFE processes typically operates on batch mode, which means that the material needs to be loaded and unloaded in extractors at atmospheric pressure. This causes increased costs, both in regards to investments, since it requires certain types of opening and closing systems, and in regards to operating expenses, as it requires more manpower and may result in fluid losses and other issues. This can be compensated for by having a series of several extractors, which can then be used in a continuous process so that the SFE system is continuously running, but this is not necessarily a perfect solution. Research is being made to try to find a better solution, but in industrial setups this does work at the current time [57]. Supercritical fluid extraction is a promising approach to extracting the lipids from microalgae for the purposes of creating biofuel. Although there are some obstacles that need to be handled in order to make it sufficiently effective at an industrial scale, it seems like there are options for approaching this and finding a way to utilize the



innate flexibility and efficiency that exists within SFE. This form of extraction also has the added benefit of being able to extract other materials than the lipids, making it appealing for a wider range of potential uses instead of being focused only on lipids. For the purpose of our project, however, lipids are the primary focus and by using the right pressure, temperature and co-solvents, SFE shows a lot of potential for providing the type of efficiency that might be necessary to turn microalgae into a stronger alternative for fossil fuels.

2.5.3 Alternative Extraction Methods

In the search for new extraction methods in order to get as much profit from the algae biofuel production as possible, alternative methods could be of need. Some approaches have already been proven to be more efficient than others, but new methods are still being engineered. Some methods still only exist on a hypothetical level, while others are in the laboratory-testing phase. In order to keep gaining more information on the subject, it is important to consider all of these methods as potentially viable possibilities.

One of the oldest methods of extraction is the Folch Method. This method has laid ground for many of the improved methods. When extracting the oil by the Folch Method you use chloroform-methanol in a 2:1 volume. The cells and the chemical are then thoroughly mixed together. The homogenate⁹ is then filtrated or centrifuged to recover its liquid stage [63]. The liquid is then washed with a 0,9 % sodium chloride solution. The solution is then centrifuged at a low speed in order to separate the two phases, with one phase containing the lipids which can now easily be separated from the rest of the mixture [44]. Many methods have been developed as improvements of the Folch Method that are very sufficient in extracting the oil, but these methods all use chloroform, which is an environmental and health risk in large-scale productions. Reducing the toxicity of the extraction method has unfortunately resulted in newer methods being less effective, but this is still preferable compared to the risks from toxic solvents [44]. Deciding on a less toxic solvent is a challenge, mainly because the effectiveness of the solvent depends on the class of lipids. Many solvents have been tested, such as hexane, ethanol or butanol. A report in 2012 showed that 2-ethoxyethanol was even more superior and less toxic in extraction than other common extraction solvents such as chloroform-methanol. This means that in the future this solvent would be preferable when extracting lipids by the use of chemicals [44]. The solvents made with 2-ethoxyethanol have proved to be efficient at separating both natural lipids, fatty acids, and polar lipids [64].

Mechanical extraction methods are also a possibility as an approach to reducing the use of chemicals. The mechanical methods are less dependent on the type of algae, and less likely to get a contaminated product in the end. The downside of the mechanical extraction methods are their cost for the heat they produce and higher energy consumption. One of the simplest and oldest mechanical extraction methods is Expeller Press. This is a simple yet effective method. Using this method, you apply high mechanical pressure to the dried algal biomass, which then disrupts the cells and squishes the oil out. There are ways to improve this method, such as applying pressure in a particular range, but this technique requires some finesse since too much pressure can actually result in de-

⁹“A tissue that is or has been made homogenous, as by grinding cells into a creamy consistency for laboratory studies. A homogenate usually lacks cell structure.” [8]



creased oil recovery. When using this method, about 70-75 % oil is recovered from the algae, but the structure of the algae do provide an obstacle for the efficacy of it. The algae have a rigid cell wall structure that hinders the oil release, and the presence of pigments, which are pressed out with the oil and must be removed chemically before the oil can be used in biodiesel production [44]. This method is not rendered as highly efficient and has therefore, just like the chemical methods, undergone some development and improvements [44].

Another method called Bead Beating disrupts the cells. Damage to the cells is caused by high speed spinning. All kinds of cells can be disrupted by this method and it could therefore also be used on algae. When using bead mills on algae it is not necessary to provide dry biomass as it was in the Expeller Press method and this makes the bead beating less expensive due to saving energy by leaving out the drying process, but nonetheless it is still an expensive method when considering the energy consumption [44].

One of the newer ideas for extraction is Ultrasonic-assisted Extraction. This method is supposed to have avoided all the larger problems from disruption method (Bead Beating). The process is simple and gives a higher purity of the final product. This method is also more economical and environmentally friendly than many of the other methods that can be used. It is a relatively quick method, has a low energy requirement and can be done at lower temperatures than many other conventional methods. It also does not require any chemicals that would later need to be removed and thereby add to the cost or environmental impact. However, a downside for the method is that too much ultrasonication leads to free radicals and can have a negative effect on the quality of the oil. There are two methods of extracting oil ultrasonically. The first method is by cavitation. Cavitation is made by micro bubbles produced by the appliance of ultrasound, creating a pressure that results in the cells breaking. The other method is acoustic, which utilizes the streaming facilities that mix the algae culture. The ultrasonic waves in these facilities create cavitations due to the compression/decompression cycle that occurs. Unsteady waves can result in transient cavitations and will end with implosion. These implosions create heat shock waves that disrupt the microalgae cells.[44]

Another incredibly useful idea is the use of microwaves. Microwaves are a quick, safe and an economical method of extracting the oil and do not require drying the algal biomass before extraction. Microwaves generate heat and this intercellular heating makes the water evaporate and disrupts the cells from within. Electroporation¹⁰ then opens the cells further and makes room for an efficient extraction. The rapid generation of heat and pressure forces out the content of the cell's matrix. This makes for a very good extraction and better compound recovery. This method has been one of the top picks when extracting oils because it has low cost, very fast recovery (approximately 15-20 minutes) and high efficiency, extracting high quality oil. But on a commercial scale there is some extra maintenance cost, which is a downside to this otherwise promising extraction method. [44]

All of these methods were thought of years ago, some earlier than others, and all of them are still being improved upon. However, a new method has surfaced, although the details surrounding it are

¹⁰ Electroporation: "Electric pulses of intensity in kilovolts per centimeter and of duration in microseconds to milliseconds cause a temporary loss of the semi-permeability of cell membranes, thus leading to ion leakage, escape of metabolites, and increased uptake by cells of drugs, molecular probes, and DNA" [65]



still not shared entirely. The company, OriginOil, has developed a new method that should not require any chemicals or heavy machinery [44]. According to the company they have managed to separate oil, water and biomass by use of gravity alone, while promising a typical efficiency of 94-97 %. Usually recovering the oils from the algae requires more than one step, but OriginOil promises a single step solution. The company has developed a method of feeding the algae with CO₂ while they are growing, which they call Quantum Fracturing. Quantum Fracturing is a fairly simple approach where CO₂ is blown into their algae growth tank creating micro bubbles and letting the algae feed on this CO₂. This technique, combined with electromagnetic pulses and pH modifications, disrupts the cell walls and releases the oil. The oil then rises to the top while the rest of the biomass falls to the bottom [66].

Extraction is a topic that is constantly evolving and new approaches are being developed and tested. By striving to improve the efficiency of extraction methods in regards to cost and yield, the overall cost of biodiesel can be reduced. Extraction methods are an important step in making algae-based biodiesel more competitive with petroleum diesel, though they still need to be combined with cost-effective approaches to algae-growth and conversion of extracted lipids to useable biofuels.

2.6 Transesterification

Once the oil has been extracted from algae, it becomes necessary to look into transesterification, which is a process that can convert the oil into the biodiesel that can now serve a purpose as fuel. This is because it finalizes the process of turning the selected strains of microalgae into biodiesel.

Transesterification is nowadays a well-known process to make biodiesel. Scientists E. Duy and J. Patrick conducted it as early as 1853 [67]. There are plenty of ways to produce biodiesel from transesterification and they all have their benefits. To get high levels of conversion of triglycerides in a short reaction time the alkali-catalysis is used. In order to simplify the purification of fatty acid methyl esters (FAMES) and to recover the glycerol easily, enzymatic transesterification has been used. Then there is the acid-catalyzed transesterification where the reaction is much slower but instead this reaction is more suitable for glycerides that have relatively high free fatty acid contents and more water. Because the alkali-catalysis is the quickest reaction and you get high levels of conversion of triglycerides in a short reaction time it is the most common transesterification method and will be described in further detail in the next section [68].

Transesterification is a nucleophilic substitution reaction that consists of three reversible reactions. First, the triglycerides are converted into diglycerides, which are further converted into monoglycerides. Lastly, the monoglycerides are converted into glycerol. Figure 10 shows the overall reaction and figure 11 shows the three steps involved.

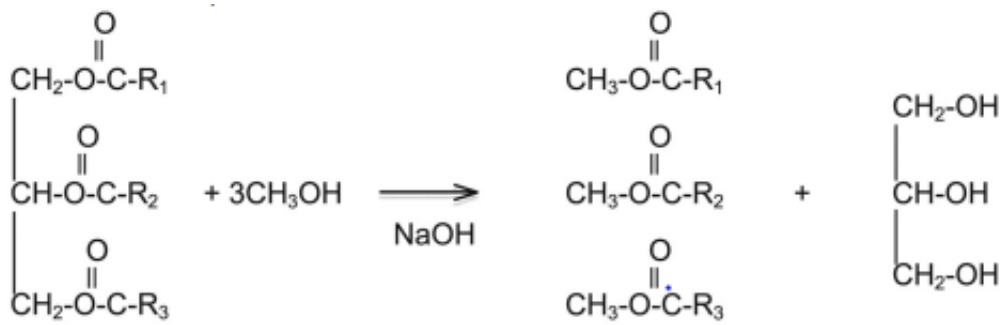


Figure 10 - Overall reaction of transesterification. [69]

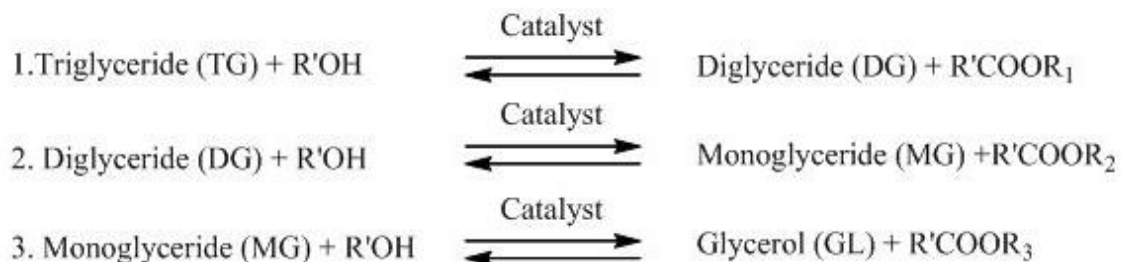


Figure 11 - The separate steps of transesterification reactions. [70]

This is normally done with ethanol and sodium ethanolate. Sodium ethanolate can be produced by reacting ethanol with sodium. It is used as a catalyst. The reaction is between an ester of one alcohol and a second alcohol. The result will be an ester of the second alcohol and an alcohol from the original ester. Chemically, this means taking a triglyceride molecule (or a fatty acid molecule), neutralize the free fatty acids and remove the glycerin to create an alcohol ester. This is done by simply mixing methanol with sodium- or potassium hydroxide to make sodium- or potassium methoxide. This liquid is then mixed with the triglycerides. The mixture settles after some time with the glycerin on the bottom while the methyl esters (or just biodiesel) are left on top. With sodium ethanolate as a catalyst, ethanol reacts with the triglyceride giving the final products of biodiesel, sodium ethanolate, and glycerol. Ether and saltwater are added to the mixture in order to separate it into two phases. After some time the mixture will separate into two layers, the bottom layer containing a mixture of ether and biodiesel. This layer is then separated from the ether via vaporizing under high vacuum [67].

2.7 Fossil Fuel

Fossil fuels are forms of oil, coal and gas resources formed millions of years ago from living organisms like the slow growing micro algae *Botryococcus braunii*, which scientists have linked to be a direct contributor to our natural resources of oil and coal [71]. Fossil fuel is created by fossilized bio-matter in the Earth's crust exposed to pressure and heat over a period of 250-500 million years. Small pockets of gas and unrefined oil are formed through this process, which seeps through the rock and earth to collect in reservoirs. [72]

Oil consists of various hydrocarbons. The groups found in oil are:

- Paraffins- (C_nH_{2n+2}) which could be methane, ethane or propane.
 - Aromatics- ($C_6H_5 - Y$) where Y is a molecule that is connected to one benzene ring.
 - Cycloalkanes- (C_nH_{2n}) where n is a whole number.
- [72]

2.7.1 Petroleum Diesel

Petrodiesel has been utilized since 1897 where Rudolf Diesel invented the first successfully operated diesel engine [73]. Petrodiesel possesses a number of unique benefits compared to gasoline, which is currently the most utilized fuel for engines worldwide. Diesel is less volatile than gasoline and it is more energy dense, providing more energy per unit volume than gasoline [74].

Petrodiesel is produced through distillation of crude oil. Its chemical structure is a combination of hydrocarbons with varying carbon chain lengths and with a boiling point in the range of 163 °C to 357 °C, depending on the structure of the carbons [73]. The biggest determinant for boiling point is the length of the carbon chains. The average chemical structure of petrodiesel is C_{12} , but varies between C_{10} to C_{15} carbons of length [75] while gasoline ranges between C_7 to C_{11} making diesels have higher melting and boiling points along with a thicker consistency [74]. The reason for this variation in boiling point has to do with the properties of various isomers of carbon molecules. A branched isomer of carbons will for instance create a lower boiling point than a straight-line carbon molecule since straight-lined carbons can line up beside each other more easily and therefore have higher intermolecular forces of attraction. The effect of aromatic compounds in the diesel molecule is also an increase in the boiling point, since the molecule will be able to pack tightly into a crystal lattice [76].

Petrodiesel does not have a uniform chemical structure, but does possess similar chemical properties. It varies in purity, molecular structure and carbon chain length. Petrodiesel consists of 75 % saturated hydrocarbons in the form of paraffins and cycloparaffins, and 25 % aromatic compounds such as naphthalenes and alkylbenzenes [75]. The higher the amount of paraffins and paraffin side chains, the lower the ignition temperature has to be in the engine and the higher the cetane number, where the cetane number measures the amount of cetane, a hydrocarbon, in the specific diesel. Cetane number reflects how quickly the diesel ignites after the fuel enters the combustion chamber. As with the octane number for gasoline, the cetane number indicates the performance of the diesel by defining the fuel's ignition quality, cold starting property and the level of idle noise during combustion [77].

2.8 Biodiesel

As mentioned previously, biodiesel is made by transesterification of dry biomass. The product is a FAME as seen on figure 12 below. A mixture of FAMES is commonly referred to as biodiesel. The carboxylic acids are all straight chain molecules with different carbon lengths, most commonly synthesized to range from 16 to 18 carbons [78].

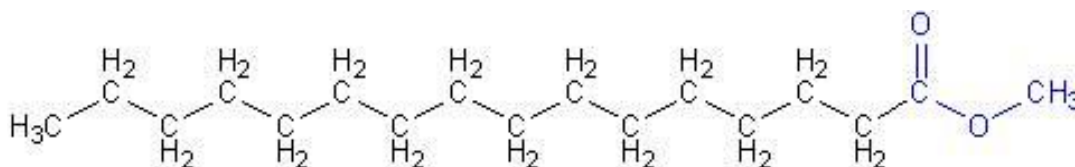


Figure 12 - A C17 FAME molecule. [79]

The physical properties of FAMES are very similar to conventional diesel, but are non-toxic and, as mentioned earlier, biodegradable. It also has higher lubricating properties than petrodiesel, which could extend the lifetime of diesel engines [78]. We will further explore some of the properties of the biodiesel converted from the lipids of the microalgae species *Chlorella vulgaris* and *Dunaliella tertiolecta* in our laboratory experiment.

Biodiesel is meant for use in standard diesel engines without modifications. However, research has shown complications when using B100¹¹ in standard diesel engines, which we will explore in the next section when we compare the physical and chemical properties of biodiesel and petrodiesel as well as looking into limitations and possibilities of various blends.

3 Interim Conclusion

Biodiesel produced from algae has a lot of potential, although our research so far has shown that there are a large number of considerations and obstacles to take into consideration before it can be competitive with petrodiesel. It has become apparent that choosing the right strain of algae can have a large impact on both lipid yield and overall biomass production. The production method also plays a large role in how much algae can be produced and at what cost. Photobioreactors have an advantage in that they create an isolated system, but the high cost prevents them from being a realistic investment choice at this time, as it is not feasible to create them on a large enough scale to be competitive. Instead, it is more viable to pursue open pond systems or hybrids between the two, since these enable a large algae yield while being relatively more affordable. Open pond systems also have other advantages outside of algae production, since they can recycle wastewater as nutrients for the algae, and can reduce CO₂ emitted from nearby power plants. This helps make algae an environmentally friendly approach to fuel production. The laws and regulations made in regards to the production of biofuels are not heavily restrictive, as there is a general consensus that the environmental benefits are worth pursuing. There are requirements for sustainability and quality, however, as these try to of ensuring that the biofuels are actually effective at what they are designed for,

¹¹ B100 consists of 100 % biodiesel.

namely reducing environmental impact. However, the cost still remains too high, compared to the current low cost of fossil fuels.

A way to remedy the high cost of algae production alternative uses for its biomass can be exploited. Algae can be used in a variety of health supplements, medicine and cosmetics. In the algae innovation center in Lolland their primary product is not biodiesel but the previously mentioned products. The supercritical fluid extraction can be useful if pursuing this strategy since it will not damage the algae significantly and allow the further extraction with the same biomass.

All these factors play a part in why algae-based biodiesel is interesting to pursue, even if it may not presently be a strong competitor to petrodiesel. In the following we will be performing a more in-depth comparison of the two, in order to display the inherent strengths and weaknesses of each.

4 Problem Statement

How does the biodiesel made from algae compare to petroleum diesel, in terms of fuel efficiency, cost and environmental impact?

We will answer our problem statement through an analysis of our own unique data gathered through experiments and from the work of other researchers and scholars who we deem to have a proficient understanding of algae and diesel. The goal of this report is to provide the reader with an understanding of what goes into and what comes out of the process of creating biodiesel from algae, as well as a comprehension of the challenges biodiesel faces in competing with other fuels in the areas of fuel efficiency, cost and environmental impact. Through these considerations the reader should gather the necessary information which will allow them to gauge whether biodiesel from algae can play a crucial role in the future energy market.

4.1 Definition of Terms

Fuel efficiency can be defined as a subset of thermal efficiency. More precisely we have defined it as the kinetic energy produced by a fixed amount of chemically stored energy, and the chemically stored energy's readiness to release its energy. To evaluate this property of a fuel we have chosen to test the calorific values of both petrodiesel and biodiesel from algae. The calorific value measures the complete combustion of a fuel and therefore falls within the parameters of our fuel efficiency definition. We have also chosen to evaluate the cetane number, viscosity and gel point as these qualities relate specifically to the readiness or ability of the fuel to release energy.

Environmental impact is a broad term. We have narrowed our scope to an analysis of the emissions produced by the two fuel types as well as their biodegradability. We think emissions are important to mention since future policy restricting or subsidizing in the energy sector will in all likelihood be influenced by a fuel's perceived harm through emissions. We say this because emissions are a primary concern related to the environment for governmental agencies such as the court of justice of the European Union. Biodegradability is included because it is a unique difference between the two

fuel types that helps to clarify the difference when it comes to unintended environmental disasters such as spills.

We have defined cost as purely economic costs. This can include a multitude of factors, however, we will try to identify through our analysis some of the pivotal factors that forms the price of biodiesel and compare it with the price of petrodiesel. This section will only briefly refer to our laboratory data, while heavily relying on studies and market data.

5 Problem Delimitation

Our problem statement branches into three areas of investigation: Environmental, Efficiency and Cost.

In the environmental branch we have chosen not to investigate the production of fossil fuel and its environmental impact. We chose not to do this because the main focus on this report is biodiesel with petroleum diesel as a backdrop only used for comparison.

In our efficiency section we chose not to pontificate on efficiency concerning external factors from biodiesel in the engine. Such areas include mileage and direct fuel injection. Where a higher mileage can result from an efficient fuel but also an efficient engine, which is why we have chosen not to include this efficiency measure in our report. Direct fuel injection is also not exclusive to biodiesel or diesel in general. It is method of increasing the efficiency of the combustion process and thus is not exclusively related to diesel. The measures for efficiency that we have chosen to include all primarily relate to the performance of diesel and not the engine itself.

In our cost section, we have not analyzed all the major components that contribute to the cost like distribution systems, land prices, and economic boom and bust cycles. We did not choose to include these important aspects because it lay outside our jurisdiction as engineers and moves into the venue of other professional occupations such as financial analysts, land inspectors, lawyers and managers. Even though our report has a broad array of topics that we disseminate they all have a link to the considerations that we deem lie closest to the considerations of a chemical engineer.

6 Production of Biodiesel

We chose to grow algae ourselves in order to perform the experiments outlined later in the report. We grew *Chlorella vulgaris*, *Dunaliella tertiolecta* and later *Botryococcus braunii* in simple open pond systems and extracted the lipid with hexane.

We did this because we wanted a deeper understanding of the growth potential of these algae as well as getting a sense of the expected biomass yield after a full growth cycle.

6.1 Methods

We decided on using a simple open pond system that functioned, fundamentally, the same way as described in 3.4.1. For extracting the lipids from the microalgae, the solvent extraction method performed with hexane as the solvent. This is described in detail in 2.5.1. Lastly, the transesterification process was conducted as described in section 2.6 using sodium hydroxide and methanol to yield methyl esters. Guidelines used for extraction and transesterification are found in Appendix B.

6.1.1 Algae Growth

The experiment was developed by the knowledge gained through the problem analysis. Our focus was on producing biodiesel from the microalgae for later tests. The first step was to grow the algae. We started with two types of algae that were not in a state of pure cultivation: *Chlorella vulgaris* and *Dunaliella tertiolecta*. We made a batch (Batch-1) in an open pond system. Batch-1 contained 10 mL fertilizer¹², 20 mL algae and 1 L water in a 2 L conical flask. We installed a light source and a CO₂ source by bubbling atmospheric air into the cultivation, as well as creating a flow with a magnet stirrer. The algae was checked every third day. Figures 13-16 below show the growth progression of the algae for Batch-1 in a period of ten days.

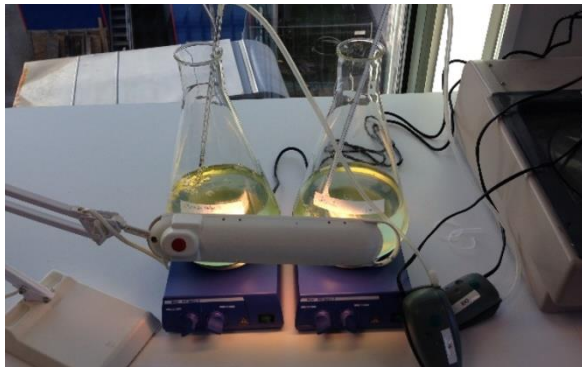


Figure 13 - Batch-1 the first day. *Chlorella vulgaris* on the left and *Dunaliella tertiolecta* on the right.



Figure 14 - Batch-1 on day 3. *Chlorella vulgaris* on the left and *Dunaliella tertiolecta* on the right.

¹² A plant fertilizer named "Substral" was used.

After six days, the algae had turned a dark green, which meant they came close to reaching their stationary stage. We let them grow another four days to make sure they had completed their growth period.

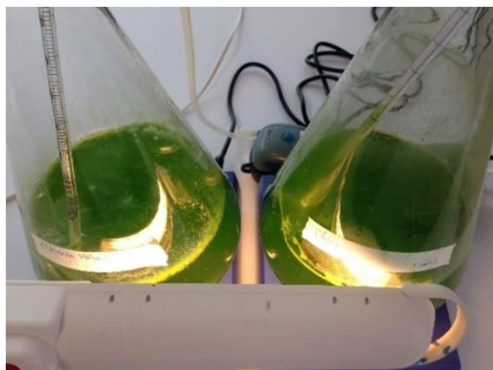


Figure 15 - Batch-1 on day 6. *Chlorella vulgaris* on the left and *Dunaliella tertiolecta* on the right.



Figure 16 - Here you see Batch-1 on day 10. *Chlorella vulgaris* on the left and *Dunaliella tertiolecta* on the right.

After the ten days, a new batch (Batch-2) was made. Because the cultivation seemed very willing to grow, and due to time restrictions, we decided to upscale the batch in order to create a larger batch of algae so that the final yield of biomass would be higher. The volume of water, fertilizer and algae was doubled to 20 mL algae, 2 L water, and 40 mL fertilizer in a 3 L conical flask. Again we checked the algae cultivation every third day. The algae seemed to grow quickly, so on the seventh day, we made three new batches (Batch-3, Batch-4 and Batch-5: shown in the figures below), each the same size as Batch-2.

In these batches, we used two types of algae, *Chlorella vulgaris* and *Botryococcus braunii*, in a state of pure cultivation. We set them to grow in two different media, the normal fertilizer for Batch-3 and Batch-4 (see figure 17 and 18) and a special medium¹³ for Batch-5 (see figure 19).



Figure 17 - Batch-3 the first day. *Chlorella vulgaris* on the right and on the left. (Both from earlier and with the fertilizer "Substral")

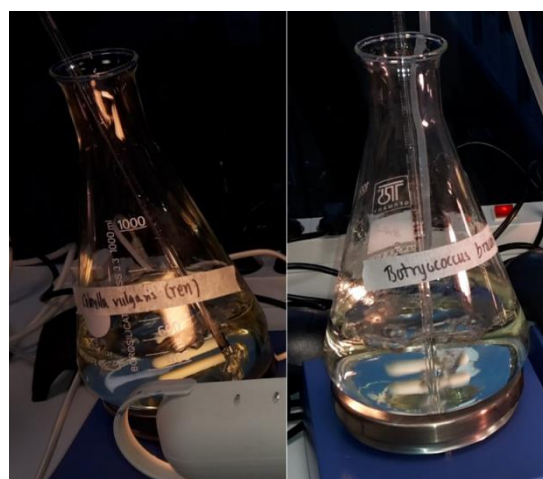


Figure 18 - Batch-4 the first day. *Chlorella vulgaris* on the left and *Botryococcus braunii* on the right. (Both from a state of pure cultivation and with the fertilizer "Substral")

¹³ For *Chlorella vulgaris* MWC+SE and for *Botryococcus braunii* AF-6



Figure 19 - Batch-5 the first day. *Chlorella vulgaris* on the right and *Botryococcus braunii* on the left. (Both from a state of pure cultivation and with a special medium).

Batch 3, 4 and 5 was left for three days. Figure 20 and 21 below shows the different algae.

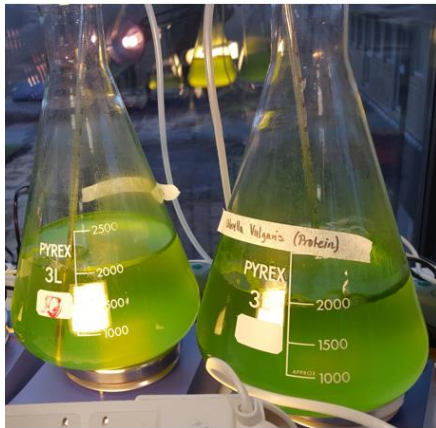


Figure 20 - Batch-3 the third day. *Chlorella vulgaris* on the right and *Dunaliella tertiolecta* on the left. (Both from a state of pure cultivation and with a special medium).



Figure 21 - Batch-4 the first day. *Chlorella vulgaris* on the right and *Botryococcus braunii* on the left. (Both from a state of pure cultivation and with a special medium).

We collected Batch-1 and Batch-2 in order to centrifuge it (as seen on figure 22 and 23 below).



Figure 22 - Batch 1 and batch 2. *Dunaliella tertiolecta* on the left and *Chlorella vulgaris* on the right. Almost three liter.



Figure 23 - Batch-5 the third day. *Chlorella vulgaris* on the right and *Botryococcus braunii* on the left. (Both from a state of pure cultivation and with a special medium).

We did not manage to grow *Botryococcus braunii* (see figure 23) fully in the span of three days because the algae never entered exponential growth phase after the incubation stage because of a temperature shock. We exposed it to 23 °C while it required 15 °C at least in during the incubation stage. Compared to *Chlorella vulgaris* in the same figure it is clear that *Botryococcus braunii* does not have the same ease of growth.

The algae cultivation was centrifuged for 10 minutes, after which most of the water was removed by a pipette so that only the algae sludge was left in the beaker (figure 24 and 25).



Figure 24 - The algae biomass after it was centrifuged.



Figure 25 - The algae was “decentered”.

The algae sludge was dried in an oven at 60 °C for three days. The dry algae biomass was weighed and prepared for extraction of the lipids by grounding it using a pestle and mortar (see figure 26 and 27).



Figure 26 - Dry, ground *Chlorella Vulgaris*.



Figure 27 - Dry, ground *Dunaliella tertiolecta*.

6.2 Extraction

The dried algae mass was smaller than anticipated. We only had 0,69 g dry *Dunaliella tertiolecta* and 0,94 g dry *Chlorella vulgaris*. Each algae type was then transferred to a 100 mL round-bottomed flask. 10 mL hexane was added. The mixture was boiled with refluxing for 30 minutes (see figure 28). After that it was filtrated by suction-filtration and the flask and filter-cake was flushed with 15 mL hexane to be certain that all the oil had been separated from the filter-cake (see figure 29). A new round bottomed flask with a broad bottleneck was weighed, and the mixture was transferred into it. The hexane was removed by a rotary evaporator and the round-bottomed flask was weighed again, giving us the mass of lipids extracted.



Figure 28 - Lipid extraction with hexane using reflux.

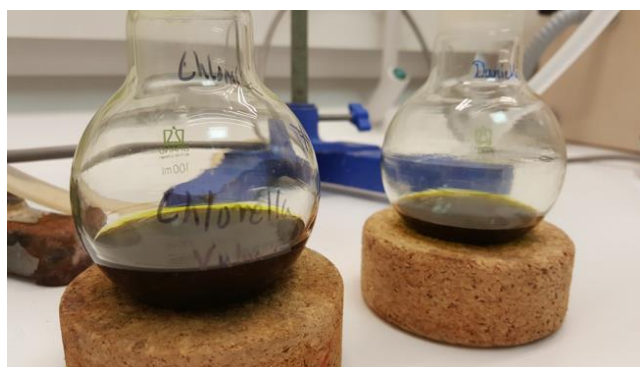


Figure 29 - Lipid compound after filtration.



Figure 30 - Evaporation of hexane using a rotary evaporator.

Now we needed to convert our oil into fatty acid methyl esters. This is done by transesterification, which is explained in the next section.

6.2.1 Biodiesel production

10 mL 0,5 % sodium hydroxide in methanol was added to the oil, which was still in the round-bottomed flask. The mixture was boiled with refluxing for one hour using reflux. The compound after the transesterification is shown in figure 31.



Figure 31 - The compound after transesterification.

This mixture was then transferred to a FAME-glass and left for 15 minutes in order to let the two phases settle. After 20 minutes, only one phase was visible (as seen on figure 32). About 8 mL hexane was added to the FAME-glass and was shaken. This resulted in the compound settling in two phases (figure 33). The upper phase was the biodiesel dissolved in hexane and the lower phase was glycerol and excess methanol. The upper phase was removed with a pipette to be prepared for purifying the biodiesel.

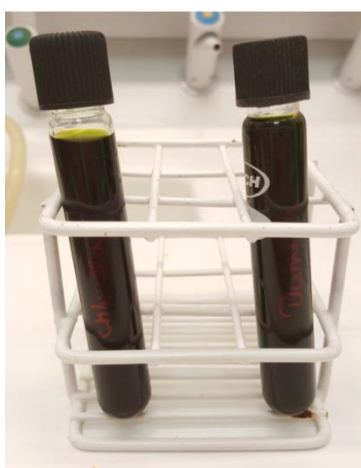


Figure 32 - Biodiesel with glycerol.

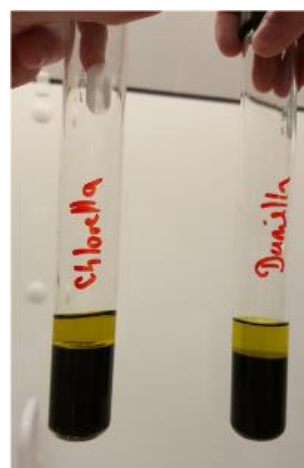


Figure 33 - After adding hexane.

6.2.2 Purification of Biodiesel

The biodiesel was transferred to a 100 mL round-bottomed flask and the oil was evaporated to dryness. Because the biodiesel is soluble in hexane and the impurities are not, a small amount of hexane was added and the mixture filtrated into a new round-bottomed flask. We chose not to filtrate because of the small mass of biodiesel produced, which made us doubt that enough diesel was left after filtration. We chose to transfer our biodiesel to small fame-glass in order to concentrate it. This was done by dissolving it in hexane, transferring it to a fame-glass and evaporating the hexane again, resulting in the mass seen on figure 34 below.



Figure 34 - The final biodiesel (less than 0,03 g).

The biodiesel was now ready to be analyzed on the GC-MS and its calorific value could be measured. Unfortunately, the small amount of algae gave us so little biodiesel that it was hard to work with and resulting in unsatisfying results on GC-MS and did not have enough product to even try to find the calorific value. Because we got so small amounts, we had difficulties weighing it, not resulting in a specific mass.

Luckily we gained access to 10 g of dry *Chlorella vulgaris* algae so that we could do the experiment again with more biomass.

We made a new experiment and followed the same procedure as before. This resulted in a measurable amount of 0,3 g biodiesel (see figure 35 below). However, it was still not enough to measure its calorific value, which requires at least 0,1 g.



Figure 35 - The final biodiesel from the second experiment. (0,03 g).

6.3 Results

In experiment 1 we did not produce enough biodiesel from neither the *Chlorella vulgaris* nor the *Dunaliella tertiolecta*, that we had grown ourselves, to make any measurements.

In experiment 2 we got 0,03 g of biodiesel from 10 g of dry *Chlorella vulgaris*.

6.4 Experiment Discussion

In experiment 1, we had difficulties in gaining the weight of the biodiesel, which we had produced. This is probably because we did not have a large enough amount and the weight was not precise enough.

On the other hand, in experiment 2 our product was recognized as biodiesel. Here we got 0,03 g of biodiesel, which still was not enough to measure the calorific value. Even from the 10 g of algae, which was almost ten times more than in our first experiments, we did not get the expected amount of biodiesel when we take into account that *Chlorella vulgaris* is supposed to contain 14-22 % lipids and that the extraction and transesterification processes both have theoretical yields of 95 %. Having reviewed our process we have not been able to find any sources of error.

7 Biodiesel Compared to Petrodiesel

While biodiesel serves similar purposes and possesses many of the same chemical properties as petrodiesel, there are some differences between the two products. We will explore these differences and discuss the viability of algae-based biodiesel in order to determine whether it can serve as a substitute to conventional diesel.

The content of biodiesel is influenced by the strain of algae used in its production. Differences in cetane number, sulfuric content, aromatics and oxygen can be observed. Because of these differ-

ences it is often difficult to make general statements when comparing biodiesel to petrodiesel unless you hold certain traits constant such as the cetane number.

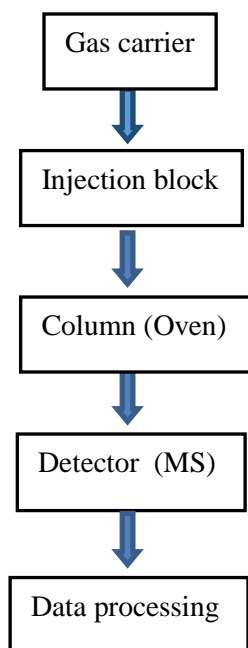
7.1 Environmental Impact

We have chosen to examine the chemical composition of biodiesel and petrodiesel through a GC-MS machine and compare these results with emission and biodegradability theory. These areas are examined to the exclusion of all other environmental concerns because we have evaluated that these areas have significant importance in the eyes of governmental agencies that heavily influence the variability of biodiesel through legal restriction and subsidies.

7.1.1 Methods

Gas chromatography with mass spectrometry is a method where you separate your sample by evaporating it to gas. Using a gas carrier, the sample moves through a column where the different components separate. At last, the sample will reach the mass spectrometer, which operates as a detector and identifies the components of the sample. It is possible to make both quantitative and qualitative measurements. In this paper, we will perform a qualitative measurement. [79]

A gas chromatograph is made of four main parts:



Gas carrier: An inactive gas such as helium or nitrogen is always used. In this project, helium is used.

Injection block: The task of the injection block is to transfer the sample from a liquid state to gas, typically by warming it up to its boiling point. Besides, this is where the gas carrier mixes with the vaporized sample. Depending on the use of a capillary column or a normal column, the split/split-less method can be used. In this paper the split method is used.

Column: The column is placed in an oven. Here the sample separates into its components depending on boiling point and polarity. There are two important expressions: The movable phase (the carrier gas) and the stationary phase (the column material). The separation after boiling point takes place by selection of the temperature in the oven. The stationary phase decides how the sample is separated after polarity. Capillary columns are very long and thin, therefore the split method is often used. [79]

Figure 36 - A sketch from the principle of GC-MS [79].

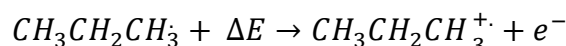
7.1.1.1 Detector (MS)

When you use gas chromatography with mass spectrometry, the mass spectrometer works as a detector. It is a method to determine molecule masses. This takes place by ionizing and mass filtration. Both operate under vacuum. [79]

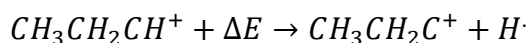
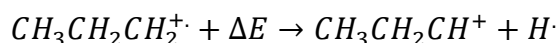
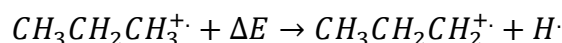
7.1.1.2 Ionization

During ionization, you add energy and the molecules break partly down into fragments. This happens by radiating the molecules, in gaseous form, with an electron-stream. The vacant electrons try, by collision with the molecules, to remove one of the molecule's electrons, which leaves the molecule as a positively loaded ion. [79]

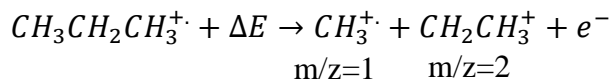
Look at propane as an example, where ΔE is the added energy:



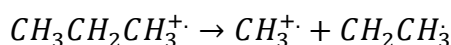
The m/z ¹⁴ for this molecular-ion is 44. The ionized molecule is a radical¹⁵ and some of it will split up like this:



Here the m/z factor drops from 43 to 41. The molecules can also be fragmented in a more fundamental way as shown below:



Radicals are often unstable and will fragment more spontaneously. This will always result in an ion and a neutral fragment.



7.1.1.3 Mass-filtration

The neutral fragment is removed by vacuum and will be lost. When we look at our case, we will now have fragments with m/z 44, 43,42,41,29 and 15. The protons with mass of one and a bigger neutral fragment with mass of 29 are lost. Using this data a spectrum can be drawn. [79]

¹⁴ Mass of the molecule in proton-masses.

¹⁵ M^+ Is a radical as shown by the dot, because there is an unpaired electron left on the molecule.

7.1.1.4 Data Processing

After the ionization, fragmentation and mass filtration the mass of the ionized molecule determines together with the mass of the ionized fragments. From our knowledge from molecule-masses, fragment-masses and their frequency in relation to one another, we can identify the specific molecules. To identify the sample a library¹⁶ is used often. [79]

The whole process of a GC-MS method can be seen in the picture below.

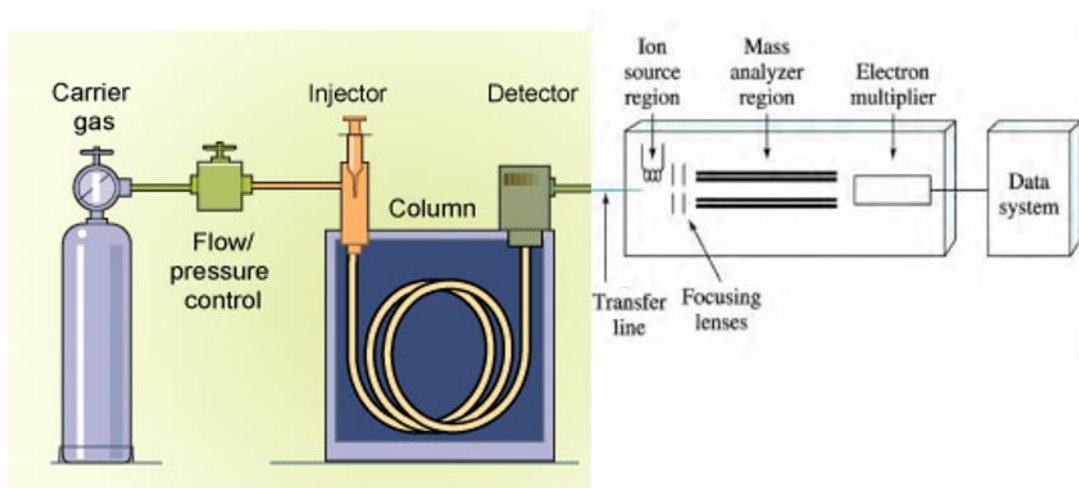


Figure 37 - Sketch of a GC-MS setup. The sample is injected at the injector. With help from the carrier gas, it moves through the column where it separates. The sample is then ionized and fragmented in the mass-spectrometer. Lastly, the computer will detect the data and compare it with a library. [80]

7.1.2 Lab Protocol

A small amount of biodiesel was transferred into a vial with a spatula. The spatula got flushed with isooctane. The vial was filled up to the 1,5 mL mark with isooctane.

In our GC method, we heated the sample up to 240 °C. This was done by raising the temperature in steps. First we started with 150 °C which was hold for a minute then we raised the temperature at 10 °C per minute to 240 °C, which was hold for ten minutes.

Then in the GC-MS we had two methods. Our MS-method had a solvent delay from 0 to 3 minutes. Then the MS began to scan for 20 minutes from mass 75 to 600. In our experiment we used the EI ionization.

¹⁶ Usually "NIST".

7.1.3 Results

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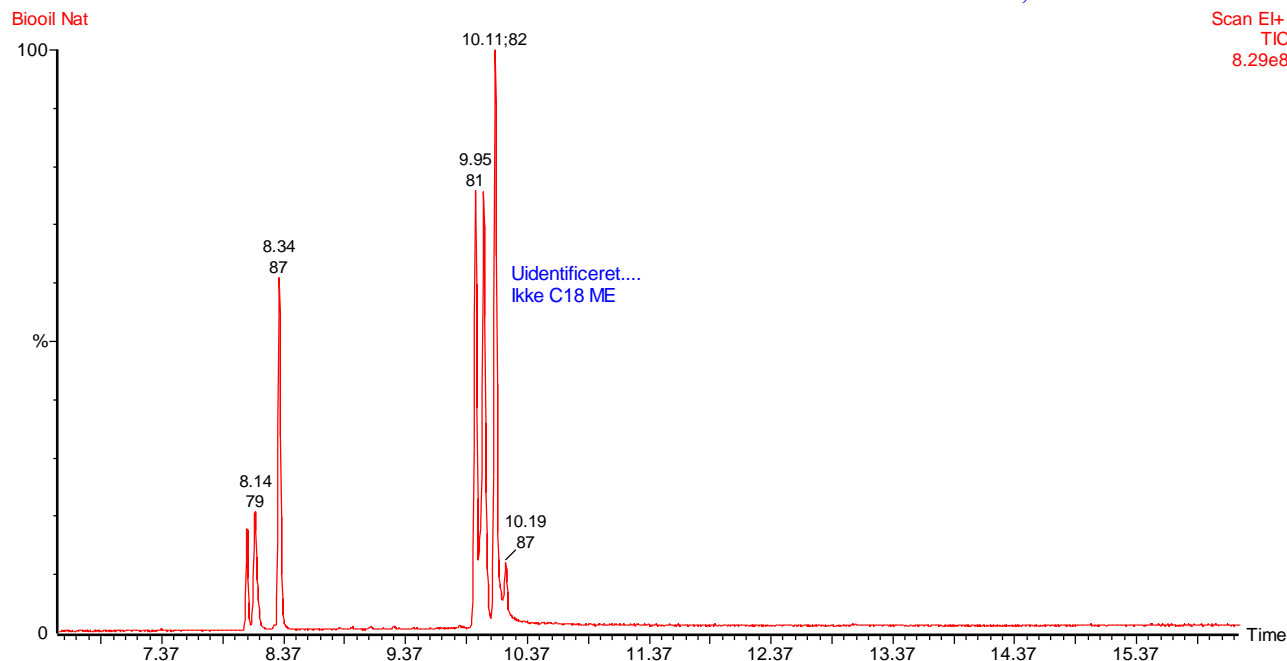


Figure 38 - GC-MS data chart of compound from transesterification of *Chlorella vulgaris*.

As seen on figure 36 above, the product that we produced had all of the characteristic chains for biodiesel, except from the C18:1 ME¹⁷ chain, which signifies that it is indeed biodiesel we produced.

7.1.4 Emissions

An advantage of biodiesel is its emissions compared to fossil fuels. Not only is algae biodiesel CO₂ neutral a much better track record with other emission.

The American environmental protection agency found in their study of biodiesels impact on exhaust emissions that biodiesel runs much cleaner than petroleum diesel. As the figure below shows biodiesel has significantly lower emissions of particulate matter, carbon monoxide and unburned hydrocarbons which makes it an attractive fuel in regards to environmental and legislative aims. [81]

Biodiesel and petrodiesel releases similar amounts of CO₂ when burned, however since biodiesel was captured through photosynthesis in algae that consumes CO₂ it can be said to be CO₂ neutral [82]. This means that they consume as much CO₂ when grown as they release when burned, which results in no net-increase of CO₂ in the atmosphere. Some companies even claim that the GHG (greenhouse gas) footprint from biodiesel produced by algae is 93 % lower than that of traditional diesel [81].

¹⁷ C18:1 ME – A methyl ester where the acid part consists of a chain of 18 carbon atoms with 1 double-bond. Example for a C18:1 ME molecule:





A number of pollutants are regulated by law in Europe and Denmark. These are deemed to have the most adverse health and environmental effects and are restricted by law. The table below has a comparison biodiesel and petrodiesel on the various regulated and non-regulated pollutants.

Average Exhaust Emissions for 100% Biodiesel Compared to Petroleum Diesel Fuel*	
Regulated Exhaust Emissions B100	
Particulate Matter	-47%
Carbon Monoxide	-48%
Total Unburned Hydrocarbons	-67%
Nitrogen Oxides	+/-
Non Regulated Emissions	
Sulfates	-100%
Polycyclic Aromatic Hydrocarbons (PAH)	-80%
Nitrated Polycyclic Aromatic Hydrocarbons (nPAH)	-90%
Speciated Hydrocarbons Ozone Forming Potential	-50%

Figure 39 - A comparison of the emissions between biodiesel and petroleum diesel. [83]

As seen in the table above, biodiesel releases 47 % less particulate matter (PM). PM is a mixture of incredibly small particles and liquids. These particulates can include acids (nitrates and sulfates) [84], but also compounds that we find in the air, such as dust, dirt, soot and smoke. All the components in these are particulates smaller than 2,5 microns [82]. These particulates can be harmful to our health because:

Particulate matter can travel deep into the lungs where it can aggravate asthma, chronic bronchitis, emphysema, and other lung conditions. Our respiratory system filters out larger particles, but smaller particles get trapped in the lungs, while the smallest are so tiny they pass through the lungs into the blood stream. Particles may trigger or cause significant health problems, such as:

- *Coughing and difficult or painful breathing*
- *Aggravated asthma, bronchitis, emphysema*
- *Decreased lung function*
- *Weakening of the heart, heart attacks*
- *Premature death*

[82]

Biodiesel also releases 48 % less carbon monoxide. CO is colorless, odorless, tasteless and toxic gas [85]. CO has gotten much attention in the latest years because of its effect on global warming “Carbon monoxide is only a very weak direct greenhouse gas, but has important indirect effects on global warming. Carbon monoxide reacts with hydroxyl (OH) radicals in the atmosphere, reducing their abundance. As OH radicals help to reduce the lifetimes of strong greenhouse gases, like methane, carbon monoxide indirectly increases the global warming potential of these gases.” [83]. It can cause mild headaches, nausea, fatigue and dizziness in low concentrations and be fatal in high



concentrations. Young infants, fetuses, pregnant women and individuals with cardiovascular diseases are at a higher risk of contracting carbon monoxide poisoning [85]. The main source to this gas today is the emission from vehicles through fuel combustion [82].

The data in the table shows that biodiesel releases 67 % less unburned hydrocarbons. A hydrocarbon is an organic compound made from hydrogen and carbon that can be emitted by an incomplete combustion in the engine. Hydrocarbons react with sunlight and nitrogen oxide turning into photochemical smog. Both the primary and secondary pollutants are highly reactive and have been linked to a variety of negative health outcomes [86]. Furthermore unburned hydrocarbons, such as benzene is a carcinogenic and can disrupt cell growth in humans [87].

NO_x was not measured in the table above, but is a very important pollution. NO_x is a term for compounds with Nitrogen and 1 to 3 oxygen atoms [88]. Some of the compounds in this group is “nitrogen dioxide, nitric acid, nitrous oxide, nitrates, and nitric oxide” [89]. All these gasses are highly reactive and are part of some of our climate changes [82]. Since NO_x is created in high temperatures with available oxygen atoms we would in theory expect biodiesel to lead out more NO_x due to its higher amount of oxygen and more complete combustion [88].

At high temperatures nitrogen and oxygen follows the Zeldovich mechanism: $N_2 + O_2 \rightarrow NO$. The NO yield increases exponentially as the temperature increases. This reaction readily takes place at 1.500 °C, which a combustion engine easily can attain. [90]

Recent studies suggest that biodiesel does indeed have a slightly higher amount of NO_x emissions as seen on the graph below.

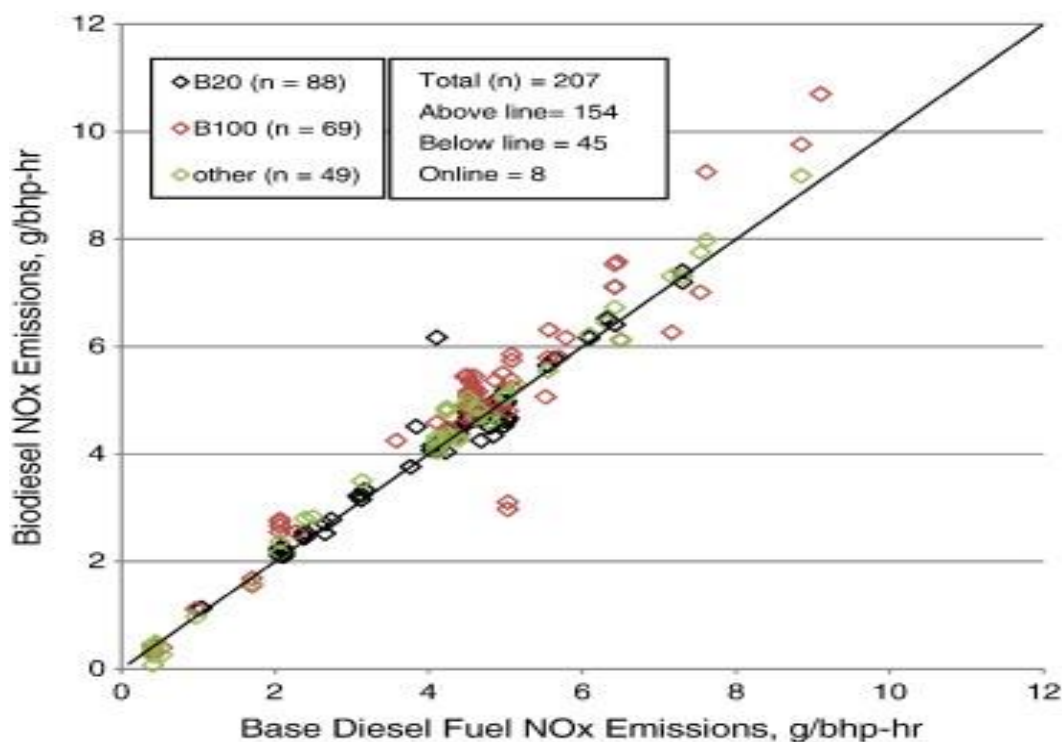


Figure 40 – Graph showing the emissions of NO_x by biodiesel and Petrodiesel in 4 stroke diesel engine. [88]

The graph compares biodiesel with petrodiesel in terms of NO_x emissions. The graph groups its data points above or below a line which separates the biodiesel and petrodiesel NO_x emissions. There is a slight prevalence of data points above the line which indicates biodiesel emits more NO_x.

NO_x gas is seen as a pollutant because it can dissolve in a moist atmosphere and become a component in acid rain, which has deleterious effects on plant life and humans [91]. NO_x is also linked to asthma attacks, other respiratory illnesses and cardiovascular problems [92]. It is estimated by aerosol science professor Ian Colbeck from the University of Essex that NO_x kill 23.500 people per year in the UK [92]. The deaths caused by air pollution in the UK, except for NO_x has recently been estimated to be 30.000 per year [93]. NO_x emission levels may therefore be seen as the most influential pollutant when evaluating the dangers of fuel emissions.

Some debate is still present on the causes of NO_x. Some theory correlates NO_x formation with the iodine number in biodiesel. Since iodine number is an indicator of double bonds. Biodiesel from algae have a high amount of double bonds which would suggest that it would readily produce NO_x once burned. However recent studies from CSU Engines and Energy Conversion Laboratory suggest that biodiesel from algae produce less NO_x than other kinds of biodiesel [94]. A graph with data from the studies can be seen in figure 41 below. It is possible that there are other unknown contributing factors to the emission of NO_x.

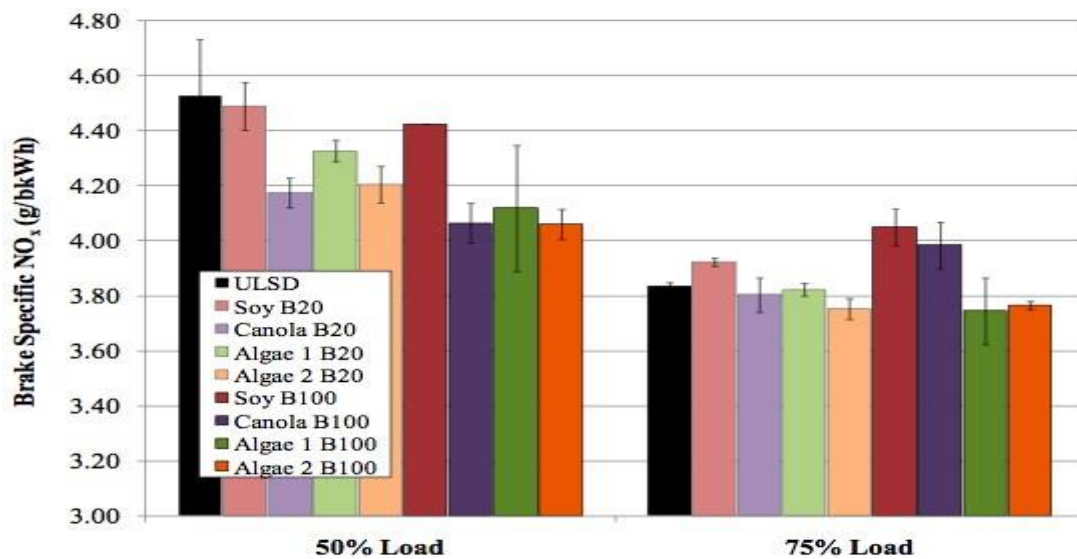


Figure 41 - Graph showing NO_x emission by various biodiesels and blends. [93]

7.1.5 Methods of Limiting Pollution

Measures to combat the emission of undesired pollutants can be taken for both biodiesel and petrodiesel. Two ways of achieving this end is through filters and catalysts.

The most commonly used filter is called a wall flow filter. It works by forcing exhaust gasses into a system of channels made by ceramic material, which is porous to the gasses. The channels are blocked at both ends as the gasses enter the system. The gasses therefor forced to pass through the porous walls, which will block particles from leaving the filter. [95]

Commercially available Wall block filter technology has been successful in blocking upwards of 98% of all particulate emission [96].

As particulate matter builds up inside the filters they become less and less effective. Two categories of cleaning the filters are currently used; active and passive regeneration. In passive regeneration a catalyst inside the filter is continuously being cleansed. Such a catalyst can reduce CO and hydrocarbons by 90 % [95]. Active regeneration takes place periodically. It can be done by raising the temperature of the filter [95].

A Diesel Oxidation catalyst can also be used to reduce emissions. The catalyst is formed like a wall of a beehive coated in precious metals. The hot exhaust gas is funneled through the catalyst. The pollutants react with the metals and are oxidized into harmless gasses. This method is only effective against pollutants that primarily consist of hydrocarbons. It therefore cannot remove black carbon, which is a series of linked pure carbons. Diesel oxidation catalysts are therefor used in conjunction with filters to achieve a more effective reduction of emissions. [95]

This list of pollution limiting methods is not comprehensive, but it does give a peak into the possibilities for limiting the harmful effects of both biodiesel and fossil fuel.

7.1.6 Biodegradability

Biodiesel is biodegradable, which means that it can be broken down into its base compounds by chemical dissolution. A study made in 2008 at *Taylor & Francis Group* by A. Demirbaş compares the biodegradability of biodiesel and petrodiesel fuels [96]. During a period of 30 days, their research showed that biodiesel was degraded by 80,4 % to 91,2 %, depending on the origin of the lipids the biodiesel were made of (see figure 42 below). Their results concluded that biodiesel can be biodegraded in an aquatic environment [96]. It thus possesses no risk to environmental safety since waste products can be readily degraded. They applied the same process to the reference petrodiesel (2-D¹⁸) to test its biodegradability. Their studies showed that after 30 days, petrodiesel had reached only 24,5 % biodegradation (as seen on figure 42 below) [96]. Petrodiesel therefore possesses a much higher risk of creating environmental damage due to complications with waste disposal and potential oil spill. Petrodiesel is not only less biodegradable but also, as mentioned earlier, quite toxic¹⁹.

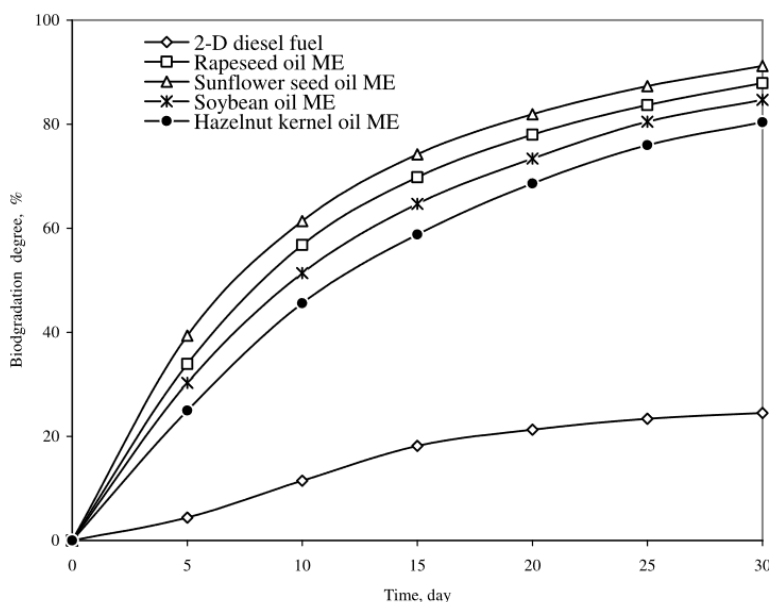


Figure 42 - Plots for biodegradation of biodiesels and 2-D diesel fuel. [96]

To efficiently biodegrade petrodiesel it clearly shows that an aquatic environment is not the best option with its low biodegradation of just 24,5 %. The process cannot break down the complicated petrodiesel compound like the simpler biodiesel molecule. Other studies have showed that to reach satisfactory levels of degradability close to the 90 % of biodiesel, a more complicated process of using microorganisms could be an effective approach. A study made by the Institut français du pétrole [97] shows that using a microflora in an activated sludge²⁰ could degrade petrodiesel up to 60

¹⁸ “Diesel fuels are broken up into 3 different classes: **1-D**, **2-D** and **4-D**. The difference between these classes depends on *viscosity* (the property of a fluid that causes a resistance to the fluid’s flow) and *pour point* (the temperature at which a fluid will flow).” [98]

¹⁹ Petroleum can induce aspiration hazard and cause irritation in lung tissue. [99]

²⁰ “An activated sludge refers to mass microorganisms cultivated in the treatment process to break down organic matter into carbon dioxide, water, and other inorganic compounds.” [100]

% to 73 % of initial diesel oil, depending on the activated sludge used. All the lighter molecules were degraded but the heavier branched hydrocarbons were resilient to the biodegradation process [97].

7.2 Fuel Efficiency

We have chosen to examine the cetane numbers, calorific values and viscosity of both biodiesel and petrodiesel to evaluate the efficiencies of both fuels. These measurements are independent of engine performance and therefore give information only related to the fuel itself which we have chosen to be the basis of our research.

7.2.1 Methods

The calorific value can be measured on a calorimeter. Any calorimeter builds on the principle that the change of energy when matter is heated can be calculated as:

$$Q = m \cdot c \cdot \Delta T$$

where m is the mass of the heated matter, c is its specific heat capacity and ΔT is the change in temperature. [101]

Different types of calorimeters exist; the one we use is a bomb calorimeter. In a bomb calorimeter the sample is placed in a reaction chamber called surrounded by water, with the calorimeter having a constant volume. Using an electrical circuit through the reaction chamber, the sample is ignited. The energy released by the sample during combustion heats the surrounding water, and the change in temperature can be measured with a thermometer. Since we know the mass of water and we know the specific heat capacity of water, we can calculate the energy released by the combustion [101]. Using Hess' law²¹ and the fact that the change of internal energy is 0, the calorific value of the sample can be calculated by:

$$\Delta H_c = -C_v \Delta T + \Delta n RT,$$

where ΔH_c is the calorific value, C_v is the constant heat capacity of the calorimeter and Δn is the change in moles of gas during the reaction. [102]

Since all of these values are either constants or can be measured, we can determine the calorific value.

In our experiment we will compare the calorific values of biodiesel and petroleum diesel. We do this to have a quantitative measurement of the differences between the two, which can help us compare and contrast the relative strengths and weaknesses of both forms of diesel.

²¹ **Hess's Law Definition:** Law which states that the energy change in an overall chemical reaction is equal to the sum of the energy changes in the individual reactions comprising it.

<http://chemistry.about.com/od/dictionariesglossaries/g/bldefhess.htm>

7.2.2 Lab Protocol

Because of the low yield of biodiesel from our algae we were unable to test the calorific value on the calorimeter. Instead we chose to use the results from other studies that have performed the same measurements.

7.2.3 Results

We used the results from a study of *Chlorella vulgaris* as a possible feedstock for biodiesel. The researchers found that biodiesel produced from *Chlorella vulgaris* has a calorific value of 38,4 MJ kg⁻¹, similar to that of petrodiesel, which is listed at 42,2 MJ kg⁻¹ [103].

7.2.4 Experiment Discussion

It can be seen that the calorific value of biodiesel from *Chlorella vulgaris* is similar to that of petrodiesel, although slightly lower.

7.2.5 Cetane Numbers

The cetane number of a diesel type is of critical importance for its combustion performance.

When the piston in a diesel engine compresses to high pressures, it generates enough heat to ignite the diesel fuel. Due to this it is important to time the injection of diesel into the combustion chamber. Diesel does not ignite instantaneously, which means that there is a delay between the injection and the start of combustion. This ignition delay depends on the cetane number of the diesel used [104].

The effect of a high cetane number is to reduce the diesel “knock”. The “knock” occurs as fuel is injected into the combustion chamber and ignites after a delay causing a late shock wave. If this delay is minimized it results in less unburned hydrocarbons and less intense knocking. A higher cetane number therefore will make the diesel engine run more smoothly [105].

Modern diesel engines run best with a cetane rating of 45-55. Biodiesel possess a higher cetane number than petrodiesel [105], which is due to its allocation of fatty acids. The longer the fatty acid carbon chains and the more saturated the molecule, the higher the cetane number [106]. Biodiesel made from *Chlorella vulgaris* has a cetane number of 54,7 [107] while biodiesel from soybeans can have a cetane number as high as 62,7 [105]. Biodiesel made from *Chlorella vulgaris* may not be the most efficient in regards to cetane number compared to other biodiesels, but it still holds a considerable advantage over petrodiesel which has a cetane number of 48 [108].

Biodiesel and petrodiesel can also co-exist as blends. A very common blend is the B20²² where 20 % of the fuel is biodiesel. This fuel has a cetane number of 50 [108]. With a blend of both diesels it is possible to increase the cetane number while only slightly lowering other important fuel qualities such as calorific value. There exists an inverse relationship between cetane number and calorific value, so any increase in cetane number will be at the cost of calorific value [109].

²² The number represents the percentage of biodiesel in the fuel.

7.2.5.1 Additives

To optimize certain aspects of diesel fuel additives such as CaO, MgO, MgO₂ and CuO can be used. All these additives have different effects when combined with the diesel. Mn is powerful at reducing the freezing point, while the organic compounds Cu, Mg and Ca are less effective in this regard. Mn will also increase the cetane number by a slight amount.

Exhaust emissions with additives can reduce O₂, CO and SO₂, but increases CO₂ with a net effect of an increase in emissions of 0,8 %. It is also possible to reduce NO_x and SO₂ emissions by 20 % with the consequence of increasing the cetane number of petrodiesel from 46 to 54. [110]

7.2.6 Viscosity and Gel point

Both petrodiesel and biodiesel gel when cooled below a certain temperature depending on the type of diesel. Since there is no specific freezing point but instead a transition that takes place over a range of temperatures, different terms can be used to describe and compare different types of diesels. *“The cloud point is the temperature of the fuel at which small, solid crystals can be observed as the fuel cools. The cold filter plugging point is the temperature at which a fuel filter plugs due to fuel components that have crystallized or gelled. The pour point refers to the lowest temperature at which there is movement of the fuel when the container is tipped.”* [111]

While the cloud point of biodiesels depends on the plants used to derive it [112], the cloud point of biodiesel derived from algae is about -11 °C to -8 °C [113]. In comparison No. 1 petrodiesel has a cloud point of -40 °C while No. 2 petrodiesel has a cloud point between -28 °C and -7 °C. When temperatures approach the cloud point of the diesel used, changes must be made to ensure that the engine can run without problems. This can be achieved through certain additives that lower the cloud point, or by switching to No. 1 petrodiesel. When using mixtures up to B20, temperatures will generally not be low enough to reach the cloud point [114].

Another characteristic of biodiesel that affects engine performance is its viscosity. The kinematic viscosities of fuels have been shown to increase as the fraction of biodiesel increases [115]. Studies have found that the fuel consumption of engines using biodiesel and biodiesel blends as well as their emissions of pollutants such as CO, CO₂ and NO_x increases as the viscosity increases [116]. Like most substances, the viscosity of biodiesels and their blends is reduced as the temperature increases. Because of this, the effects of biodiesel's higher viscosity can be reduced by preheating the fuel before injection into the engine [117].

7.3 Cost

We have chosen to analyze the price of biodiesel compared to petrodiesel. In this analysis we will look at the major costs associated with biodiesel, but not that of petrodiesel since it only serves as a back drop for comparison. We have chosen this approach since we wanted to focus primarily on the unique cost considerations related to biodiesel produced from algae.

7.3.1 Methods

Our experiments were not related to a cost analysis. Instead we have chosen to rely on market data, consumer surveys, governmental policies and production cost estimates.

7.3.2 Production Cost

The price of petrodiesel and biodiesel is in part dependent on two scaling effects related to production. The first scaling effect is the scaling cost, which is the cost associated with scaling production capacity. Biodiesel production as for many other industrialized productions can be made less costly by realizing economies of scale. In other words, you need a rather large production facility to realize any significant output. This falls in line with our laboratory experience where two 5 liter jugs of *Chlorella vulgaris* and *Dunaliella tertiolecta* produced less than 0,1 g. Another important scaling aspect of the price is what is called learning effects. Learning effects can be defined as technological process improvements. These improvements range from knowledge of beneficial growth mediums to specific optimization of work routines and cultivation methods. There is a compounding aspect to both effects, where one improvement in production affects another outcome. For instance building algae production facilities next to a power plant will result in cheaper production based on the constant CO₂ and on the other end may lower the distribution costs since the power plant's distribution system can be utilized. [118]

The driving production costs of biodiesel from micro algae can be divided up into these subcategories:

- Size and type of equipment
- Depreciation
- Labor cost

Raceway ponds are associated with lower construction and material cost, while photobioreactors yield higher biomass and are easier to control [119]. Algae link estimated the construction cost of different size photobioreactors. The table below displays the cost associated with equipment and size.

Capacity (Tons of dry weight biomass per day)	Length (Meters)	Carbon dioxide (Kgs per day)	Area (Acres)	Electricity (Kilowatts)	Cost (Euros)
Demonstration	36	10	0.01	12	69,000
1	1,068	2,881	0.4	55	580,000
10	10,692	28,805	4.3	545	2.5 million
50	53,466	144,027	22	2,727	6 million
100	106,932	288,053	44	5,455	10 million

Figure 43 - Scaling cost of photobioreactor. [120]



Based on the data in figure 43 there is an economic advantage realized by expanding production as the cost per tons of dry weight biomass per day decreases when production capacity is expanded.

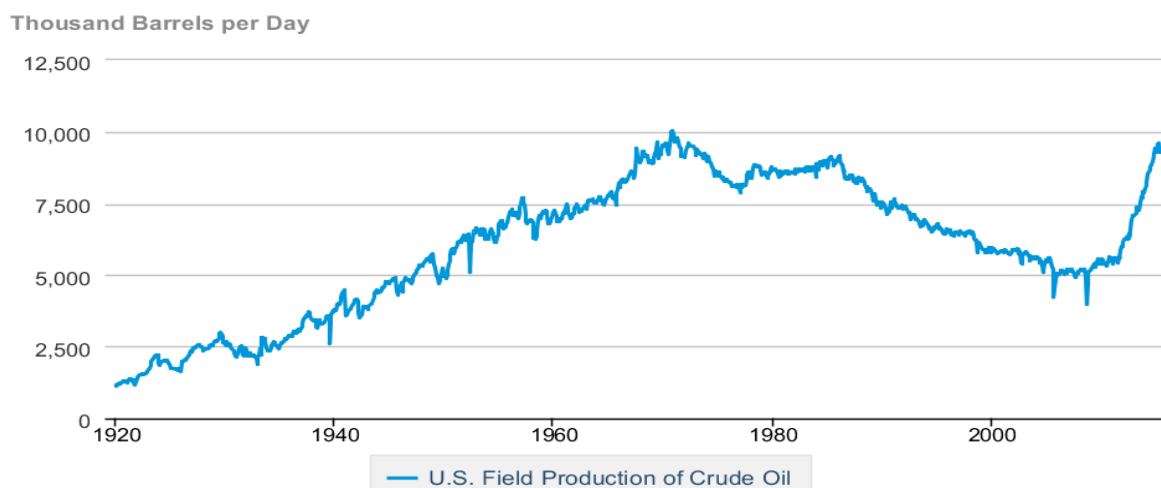
Depreciation cost is calculated based on the purchase price of capital equipment with an annual reduction in value based on a cumulative depreciation value. This cost is significant when calculating taxes on capital equipment and on the expected lifespan of a production equipment. A study analyzing cost of algae produced in Greenhouse found that among the highest cost of running such a facility was the depreciation of equipment. [121]

Labor cost is also a large component of production costs [121]. Due to collective bargaining agreements the minimum wage in Denmark is 110 kr. [122], which is very high compared to countries like Mexico where the minimum wage is 14 kr. [123] Open pond systems which require a considerable amount of man hours will therefore be far more expensive in Denmark compared with other less industrialized countries. If algae production facilities were to be constructed in Denmark we would estimate that most of the process would be fully automated to reduce labor cost.

These factors express themselves in the cost of biodiesel from algae grown in open pond systems varying between \$240 and \$332 per barrel (see section 2.4.3). Petrodiesel, in comparison, was priced by the American petroleum institute at \$2,64 per gallon in August of 2015.

This stark difference in price may be a combination of insufficient capital investment in the biodiesel industry sector and the relatively higher yield of petrodiesel per dollar invested. As the availability of crude oil decreases with use we may see biodiesel become a substitute product, however the surge of new oil extraction technology such as hydrolytic fracturing that allow previously unexplored reservoirs to be exploited, have caused petrodiesel prices to drastically fall.

U.S. Field Production of Crude Oil



 Source: U.S. Energy Information Administration

Figure 44 - Crude oil production of the U.S by time. [119]

The figure above shows how oil is currently as available as it was during the peak of the 1970's, which will decrease the price of petrodiesel significantly and reduce the purchase of alternate forms of energy.

7.3.3 Interest Groups

CO₂Star conducted a consumer survey in Germany during the summer of 2007 in order to investigate the buying behavior of German fuel consumers. The most important questions included:

- What are the most important motives when buying fuels?
- How aware are consumers about Climate Protection linked to their individual transport?
- How do consumers assess the product biodiesel?
- Are consumers willing to contribute to Climate Protection by using climate friendly fuels?
- Would they pay a higher price for those fuels?

CO₂Star concluded on their findings that the most important aspect in the consumers' purchasing decisions were fuel pricing. Biodiesel consumers are more price sensitive than fossil fuel consumers. A majority of consumers responded positively when asked about their willingness to contribute to climate protection, but only a minority would pay a surcharge for climate friendly fuels. CO₂Star found similar results in their surveys conducted in the UK. [124]

We assume that the Danish consumers share this general sentiment since both Germany and the UK possess similar cultural values as Denmark. Based on the findings of CO₂Star we can infer that biodiesel would have to be competitive with petrodiesel pricewise in order to be considered by the consumers as a variable alternative. This goal of competitive pricing seems to be unachievable in the current economic environment as discussed earlier. If a heavier commitment to the use of biodiesel had to be enforced it would be against and to the detriment of the consumers economic interest.

7.3.3.1 Producers

Producers drives the supply side of the equation and their willingness to adapt their industry to biodiesel production will be crucial for its availability and price.

Biodiesel producers will respond to market demand and new technology that makes biodiesel profitable. The biodiesel 2020: Global market survey predicts a rising demand for biodiesel due to two factors:

- Increasing governmental concern for sustainable economic growth and economic support for renewable diesel, biomass to liquid projects, algae, and cellulosic diesel.
- Further technological developments in production yields, which will push the price of biodiesel down.

[125]

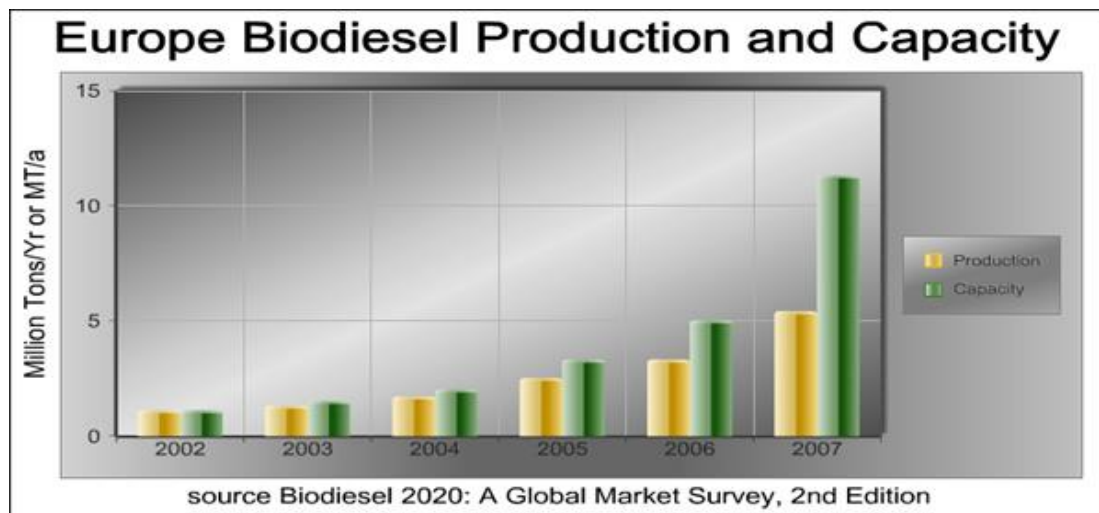


Figure 45 - Production and capacity of biodiesel in Europe. [125]

The graph in figure 45 above showcases how the European production of biodiesel does not match the capacity to produce. This indicates that producers can, with relative low cost, switch to algae production at a much higher volume than presently.

7.3.3.2 Governmental Institutions

Another factor that affects price is the regulatory burden on the energy sector and the prevailing attitude and goals of tax agencies. The specific impact resides with the laws regulating the oil and biodiesel industries.

Many foreign and domestic politicians and governmental agencies have, for the past 10 years, pursued an environmental policy that favours a reduction in CO₂ and other emissions. The Nordic Council of Ministers launched a study in February 2015 of the climate impact and commercial potential of using biofuels for aircraft [126]. The Danish Climate Minister, Rasmus Helveg Petersen, remarked “I hope that a Nordic approach will identify that there is potential for green growth in biofuels for aircraft. Denmark cannot propel this by itself, but the prospects may change dramatically if the Nordic countries join forces.” [126] This announcement falls in line with the general environmental strategy pursued by the Danish government that Denmark will be 100 % driven by renewable energy in 2050 [127].

A strategy that the Danish government have employed to reduce the CO₂ emissions of the Danish population is through a carbon tax.

A carbon tax is a pricing mechanism that requires a payment to be made for every ton of carbon emitted.

Denmark has since the 1980s been pursuing a carbon tax which was passed for private citizens in 1991 and for businesses in 1992. The stated goals of the tax are:



1. Reduce carbon emissions from 61,1 million tons (1988 levels) to 48,9 million tons before 2005 (a 20 % reduction in emissions)
2. Add incentives to Danish industry to shift from fossil fuel to carbon neutral forms of energy
3. Encourage both private households and firms to reduce carbon emissions on a large scale [128]

As a result of the carbon tax households has been charged 52 kr. per ton of carbon emitted while businesses has been charged 99 kr. per ton of carbon emitted. 60 % of the tax revenue is returned to the energy industry while 40 % is used for environmental subsidies. Furthermore, firms have the opportunity to sign an energy saving agreement with the ministry of transport and energy and receive a 25 % tax cut. [128]

The carbon tax has been effective in reducing per capita carbon emissions with as much as 25 % in the period 1990 to 2005. Industry emissions have been reduced by 23 % in the same period. Annual revenue generated from the policy was 3.370 billion kr. in 1994, 4.063 billion kr. in 1995 and 6.280 billion kr. in 2008 [129]. The tax policy has been effective in reaching its stated goals and been profitable for the Danish state.

The Danish tax agency “SKAT” held in marts 2014 a conference where they discussed the long term prospects of the carbon tax. In the report it was concluded that subsidies to industries that develop CO₂ neutral fuels should be pursued, along with a focus on a taxation strategy called “tax what you burn, not what you earn”, which gives rebates to lower income household in order to offset the negative impact on their disposable income that a carbon tax necessitates. The hope is to gradually change the Danish consumption of energy towards a lower level of carbon emissions. SKAT does recognize however that the global impact of such initiatives are in Denmark will have little to no observable effect, which is why they also think it is important for the European union to adopt a carbon tax model. [129] From our perspective it is clear that SKAT sees a benefit in the current carbon tax model and wishes for its continuation in a similar form to the present law. Due to this fuel combustion and fuel production that favor lower amounts of carbon are subsidized while industries that fail to stir towards lower carbon emissions are punished by increased taxation and biodiesel from algae receives a considerable bump in its rentability on the Danish energy market.

As previously mentioned algae consumes CO_x during growth and production, they release the same amount of CO₂ and less CO once burned, but the net effect from burning biodiesel compared with petrodiesel is a reduction in CO_x emissions of 48 %. [2] Through this carbon tax and the subsidy to production of renewable fuels the cost of biodiesel from algae may decrease. If this will push biodiesel from algae to be a variable competitor to petrodiesel is doubtful, but it is a step towards that goal.

7.3.3.3 Environmental Non-Governmental Organizations

Non-Governmental Organizations (NGOs) influences the public perception of and governmental decisions. These organizations may play a role in the future pricing of biodiesel and petrodiesel depending on how effective they are at influencing public opinion.

Environmental NGOs have a vested interest in promoting biodiesel due to the positive environmental effects a switch away from fossil fuels would have. The Danish Organization for Renewable Energy (VE) is one of the largest environmental NGOs in Denmark. Their stated goal is to reach 100 % renewable energy usage in Denmark by 2030. They want to achieve this aim through political lobbying, spreading public awareness and cooperation with private and public institutions that promote similar goals [130]. It is unlikely that they VE will be successful in achieving their stated goal considering the goal of the Danish government aim to reach this goal 20 years later. It is also uncertain what measurable effect NGOs have in both changing public perception and legal structures.

8 Discussion

How does the biodiesel made from algae compare to petroleum diesel, in terms of fuel efficiency, cost and environmental impact?

We went into this project with the impression that biodiesel had substantial advantages in terms of environmental impact, with the possibility of high yields using simple production methods. We furthermore thought that algae were the most promising of the different types of feedstocks that can be used to produce biodiesel.

Our expectations were only partially validated. In our experiments, where we followed the process of biodiesel from cradle to grave, we found that 5 liters of *Chlorella vulgaris* and 5 liters of *Dunaliella tertiolecta* only yielded less than 0,1 grams of biodiesel, which indicates to us that producing sufficient amount of biodiesel from algae to substitute our current petrodiesel usage would require an enormous water surface, making biodiesel less attractive than petrodiesel.

Through our analysis of environmental impact of both biodiesel and petrodiesel we found that biodiesel's primary advantage is that it is CO₂ neutral and emits a lower overall amount of pollutants when burned. The only aspect that does not favor biodiesel is the NO_x emissions, where petrodiesel releases a slightly lower amount. NO_x is an important pollutant since it contributes significantly to the death toll caused by pollution. Biodiesel produced from algae, however, releases the lowest amount of NO_x compared to other forms of biodiesel. Another factor to consider is filters and catalysts that can reduce upwards of 98% of all emissions. Because of this we do not think that the difference in emissions between the two fuels is the most substantial aspect; however biodiesel being CO₂ neutral is of importance, particularly in respects to legal subsidies and production. Algae production facilities can with benefit be constructed near power plants to consume some of the CO₂ they release. When we consider that the Danish energy laws require fuel producers to have 5.75% of their production be renewable energy sources, this solution seems beneficial for many fuel pro-



ducers. Another environmental aspect that we considered was bio- and petrodiesel's biodegradability. We found that biodiesel readily decomposes into its component parts while petrodiesel does not. Petrodiesel's biodegradability coupled with its toxicity makes it dangerous to humans and wildlife if unforeseen spills happen during extraction or production.

In our fuel efficiency analysis we found that there is a similarity between the calorific values of the two fuels, meaning that biodiesel can be used to the same extent as petrodiesel, but larger differences in the cetane number and viscosity. Biodiesel from *Chlorella vulgaris* possesses a cetane number of 54,7 while that of petrodiesel is 48. The cetane number is associated with lower emissions, higher fuel performance and easier engine startup, which are all preferable traits. Overall viscosity, especially at low temperatures, is higher for biodiesel making it less attractive at low temperatures; this difference becomes insignificant once heated. Biodiesel also reaches its gel point at higher temperatures than petrodiesel, making it more difficult to use in engines in cold climates. These drawbacks can be overcome by using a blend of the two fuels. The common blend of bio- and petrodiesel, B20, can be used in the same engines as petrodiesel without making modifications. It is important to point out that the more biodiesel which is added the higher the cetane number becomes and the higher the viscosity and gel point become. This trade-off can be remedied by additives such as CaO, MgO, MgO₂ and CuO which will increase the cetane number of the diesel.

Our cost analysis was the most one-sided. It was clear that the price difference between biodiesel and petrodiesel is immense. This difference springs from the low yield of algae production relative to petrodiesel, the costly construction cost of photobioreactors, and the high labor cost of open ponds, particularly in Denmark. Even with a favorable legal environment and public perception of biodiesel we found that this will not affect the price so significantly that the Danish consumers will change their fuel consumption patterns. A possible solution for the low yield of microalgae would be to increase effort in bioprospecting new strains of algae with higher yields and growth rates as well as less restrictive growth requirements. Another way of making biodiesel from microalgae a stronger market competitor would be to develop an inexpensive method of producing more algal biomass from each liter of water. What some algae producers have done to address these limitations is to focus their algae production primarily on higher value products such as cosmetics, medicines, food ingredients and carbon compounds for industrial non-food production. The leftover biomass after extracting the materials for these products is then used for biodiesel. This approach will, in all likelihood, be the strategy for future algae producers, however, the high labor costs and high technology costs associated with automation will be a difficult hurdle to overcome.

9 Conclusion

Biodiesel and petrodiesel have been compared on the parameters: Environmental impact, fuel efficiency and cost.

In terms of environmental impact we found that biodiesel is superior in terms of emissions such as CO₂ and unburned hydrocarbons, but not that of NO_x. Since NO_x is one of the most harmful types of emissions from diesels, it is one of the few environmental drawbacks for biodiesel. We also found that biodiesel is biodegradable, giving it an additional advantage over petrodiesel.

In respect to fuel efficiency biodiesel has been found to have a similar calorific value to that of petrodiesel, while the Cetane number is significantly higher. The fact that the gel point of biodiesel is higher than that of petrodiesel and that the viscosity is higher especially at low temperatures can present problems when used in winter times and in colder climates.

Looking at cost we have observed that the production cost of biodiesel from algae is much higher than that of petrodiesel due to labor costs, technology costs and low production yields rendering it a more expensive alternative to petrodiesel.

Overall, biodiesel is superior to petrodiesel on many parameters, especially in relation to environmental aspects. However, the major drawback that biodiesel experiences is the immense cost disparity between it and petrodiesel. Because of this we do not believe that biodiesel can be a complete substitute to petrodiesel, but may instead be used as a supplement fuel, perhaps in the form of B20 since this would not require any modifications to the engine.

10 Perspective

Further investigation could focus on finding the optimal ratio between biodiesel and petrodiesel. It could also focus on optimizing the production process such as finding better algae strains or genetically engineering currently known strains to optimize desired physical properties. The growth process could also be optimized through regulation in temperature, nutrients, pH value, CO₂ level and concentration of algae. Each of the three topics of environmental impact, fuel efficiency and cost could also be explored on their own in order to increase the competitive abilities of biodiesel.

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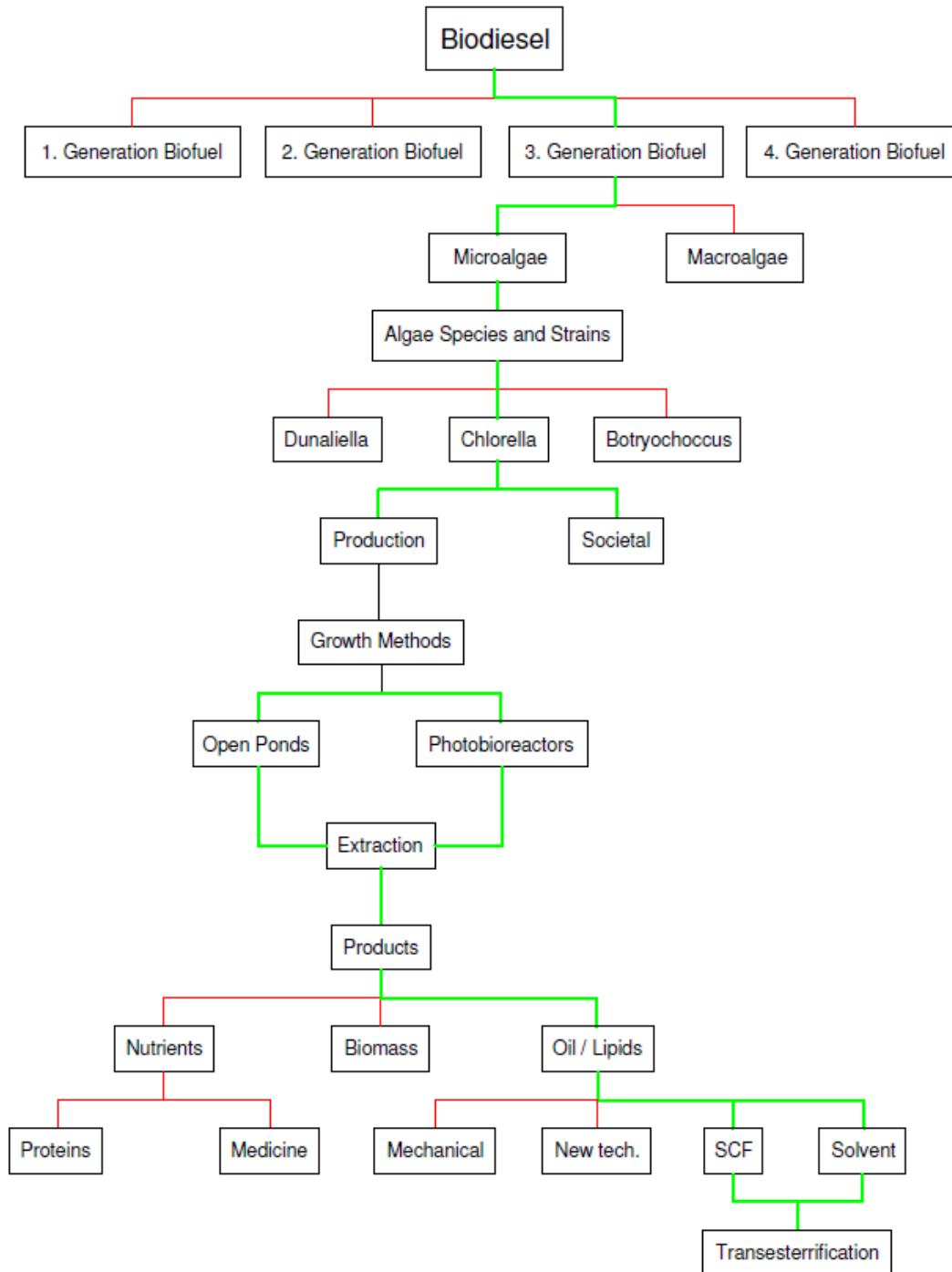


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12 Appendix List

12.1 Appendix A - Breakdown chart



12.2 Appendix B - Guidelines used for extraction and transesterification.

Fremstilling af bio-diesel fra (chlorella) alger

Eksperimentet er opdelt i 3 steps. Først ekstraheres olien fra algen og derefter produceres biodiesel og til sidst oprenses biodieselen

1. Ekstraktion af olie

- Algen findeles så meget som muligt, og der afvejes 10 g i en 100 ml rundbundet kolbe. Tilsæt 40 ml hexan. Blandingen koges med tilbagesval i 30 minutter.
- Filtrer blandingen gennem en tragt med filter eller ved sugfiltrering og efterskyl kolben og filterkage med 15 ml hexan.
- Vej → ➤ Vej en rundbundet 100 ml kolbe med bred hals.
- Overfør filtratet til kolben.
- Hexanen afdampes i rotationsfordamper.
- Vej kolben og bestem udbyttet. (Olie)

2. Biodiesel-produktion

- Oliens tilsættes 10 ml NaOH 0,5% i MeOH, og blandingen koges med tilbagesval 1 time.
- Overfør blandingen til et FAME-rør (25 ml) Lad blandingen stå og dele sig i 15 minutter.
- Det øverste lag er biodiesel (methylestre af fedtsyrerne) Glycerol er i det nederste lag.
- **Hvis der kun er én homogen fase fortsættes med "Oprensning af biodieselen"**
- Fortsæt med det øverste lag i "Oprensning af biodiesel"

3. Oprensning af biodiesel

- Overfør "biodiesel" eller den homogene blanding til en 100 ml rundbundet kolbe og inddamp til "olie - tørhed"
- Tilsæt 10 ml hexan og filtrer indholdet over i en ny rundbundet kolbe. Biodiesel er opløselig i hexan. Resterne fælder ud.
- Inddamp hexanblandingen.
- Rest i kolben er biodiesel.
- Biodieselens sammensætning kan ses på GC-MS ved at opløse en dråbe i 1,5 ml ~~hexan~~ ^{iso-octane} i en Vial. Indsprøjt prøven i GC-MS

