

Haute Ecole
Groupe ICHEC – ECAM – ISFSC



Enseignement supérieur de type long de niveau universitaire

To what extent is biofuel production from microalgae cost effective in europe. Analysis of the process and limitations of large-scale production.

Mémoire présenté par :
Samuel Bonet

Pour l'obtention du diplôme de:
Master en gestion de l'entreprise Année académique 2022-2023

Promoteur :
Jacques Spelkens

Boulevard Brand Whitlock 2 - 1150 Bruxelles

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Context and presentation of the thesis

This thesis aims to understand whether achieving profitability by creating synthetic oil from algae is possible, in the short or long term. We would like to place this dissertation in a comprehensive context, encompassing economic, geopolitical, scientific, and ecological aspects.

Hundreds of millions of years ago, algae were the first living organisms to produce oxygen from water molecules, today they are responsible for approximately 40% of the oxygen we breathe.

Millions of years ago, the oil we use today was formed from plant residues that grew by photosynthesis, using solar energy and absorbing the abundant CO₂ present in the atmosphere at that time. They eventually died and slowly degraded in the soil to become today's oil. Now, we are burning the CO₂ they stored over millions of years, which warms the planet and inevitably disrupts our fragile ecosystem.

However, for over 15 years now, we have known that oil, gas, or other energy derivatives can be produced from marine algae, while reducing the amount of CO₂ in the atmosphere. In fact, algae absorb twice as much CO₂ as they emit when burned, which could be beneficial for air purification.

But beyond the ecological benefits, there are also economic advantages. We have experienced turbulent years with the emergence of COVID-19 and the war in Ukraine. COVID-19 reminded us of how interdependent major global powers were and how interconnected the world was. This globalization was made possible by access to energy resources such as oil and gas at very affordable prices."

Subsequently, Europe experienced an energy crisis due to the war in Ukraine and the embargo of Russian oil by Europe. Oil and gas prices skyrocketed, and we faced some shortages. This was followed by a minor economic crisis with a record inflation rate that had to be curbed by raising interest rates, leading to an economic slowdown. This crisis reminded us of how tightly our economy was linked to energy prices and, most importantly, how dependent we were on them.

However, there are very few oil and gas deposits in Europe, and we are unable to meet our demand with what is present in our soils. It would be interesting, therefore, to explore a way to produce synthetic oil or gas.

There are companies and laboratories working on biofuels, such as AlgaEnergy (Spain), Algafuel (Spain), Algae Oil Services (UK), Algae Parc (Netherlands), Fermentalg (France), Biofuel System (Italy), Algae Cytes Limited (UK), Neomerys (France), etc. Even the company Total boasts of producing biofuels for over 20 years and aims to become a leader in this sector.

However, given all the advantages of energy production from algae, where do we stand currently? Why isn't this solution being implemented on a large industrial scale? Why aren't all our cars running on algae-derived fuel? Why does our economy still rely on oil prices and OPEC (Organization of the Petroleum Exporting Countries)?

There are several tracks to address these questions. First and foremost, from a biological standpoint, what is the process? Which type of algae is the most optimized among the 30,000 species? Is it possible to cultivate them in large quantities at sea without altering the current ecosystem?

In addition to the aforementioned points, this dissertation will primarily focus on the economic profitability aspect of energy production from algae. Is it possible to produce algae-based oil at a cost equal to or lower than current oil? What are the costly production or conversion steps? How can algae be cultivated profitably? What is the production cost per liter of oil for biofuel-producing companies today? And more broadly, what are the limitations of industrial production?

In this document, I will concentrate on the question of economic profitability based on scientific studies and analyze the biofuel production steps. The production costs of biofuel are currently very high because many steps are required to transform algae into fuel. They need to be produced, harvested, and processed. Throughout this thesis, I will analyze these steps to determine which ones are the most costly and assess whether economically and ecologically viable fuel is achievable.

There are primarily three costly stages in algae-based biofuel production:

1. **Biomass Production:** The first part of this dissertation will be dedicated to the existing types of algae and their production methods. We will explore the various ways they can be produced, either in their natural marine environment or in photobioreactors on land. We will also attempt to understand which method is the most economical.

2. **Biomass Harvesting:** Next, we will delve into how microalgae are harvested and why this represents a significant cost. Given their microscopic size, specific means must be employed to extract them from their growth medium, water.

3. **Lipid Extraction:** The third step involves extracting the oils known as lipids, which will form the fuel (biodiesel). This stage is equally important, as the extraction methods vary widely and are crucial for producing economically viable fuel.

I will conclude with a SWOT analysis explaining the outcomes and limitations encountered during this research.

Our motivations :

We are personally deeply concerned about climate change as well as geopolitics and its implications. We live in a world that is now bipolar, with the G7 on one side and the BRICS on the other, several superpowers engaged in economic competition, of which we are a part. The stakes, whether economic, climatic, or political, are enormous. We stand on the brink of a financial and climate system implosion, yet we prefer to look away.

According to the latest report from the IPCC (Intergovernmental Panel on Climate Change) in 2023, human-caused climate change is on the verge of reaching a point of no return. To recap, climate change has led to decreased food security. Roughly half of the global population currently experiences severe water shortages, which compromises the harvests. Approximately 3.3 to 3.6 billion people reside in regions highly vulnerable to climate change – Africa, Asia, Central and South America, LDCs, small islands, and the Arctic.

However, states have struggled to reach compromises to stem the warming, and their efforts fall short. They remain focused on economic growth, which is crucial to maintain their superpower status and financial equilibrium (a reference to countries' debt). Decreasing energy consumption is seen by many as a sign of economic downturn, as was evident during the energy crisis in 2022. Thus, we find ourselves in a paradoxical situation: the world as a whole should be concentrating on the climate issue due to its serious global ramifications, yet economies are orienting towards growth, accelerating climate change in the process.

This dissertation deeply motivates us, as it would help determine whether this potential solution could be scaled up while reducing Europe's energy dependence.

Therefore, by the end of this dissertation, we aim to address the following question: Is it feasible in Europe to create energy such as oil and gas from algae, in a cost-effective manner?

Algae :

A Brief History of Algae:

Firstly, microalgae are among the oldest life forms on Earth. While it is challenging to precisely determine their exact appearance on Earth due to the lack of well-preserved fossils, we estimate that they emerged around 3.8 billion years ago, well before the dinosaurs. The earliest photosynthetic life forms were cyanobacteria, also known as 'blue-green algae' (not true algae but photosynthetic bacteria). These cyanobacteria were capable of capturing solar energy and producing oxygen through photosynthesis. (Schopf, J. William)

Photosynthesis is the process by which photosynthetic organisms, such as plants, algae, and certain bacteria, convert light energy into chemical energy in the form of glucose and oxygen molecules.

The first step of photosynthesis involves capturing light energy from the sun. Photosynthetic organisms contain pigments like chlorophyll, which absorb photons of light. These pigments are found in cell organelles called chloroplasts in plants and algae, and in the cell membranes of photosynthetic bacteria. (Blankenship, R. E. 2010).

The captured light energy is then utilized to initiate a series of chemical reactions within chlorophyll and other molecules. These reactions lead to the separation of water molecules into oxygen and protons, as well as the release of highly energetic electrons in a process known as water photolysis. (Barber, J. 2003)

The high-energy electrons generated by water photolysis are used to reduce carbon dioxide (CO₂) from the air, forming glucose molecules. This process is also called CO₂ fixation and takes place in a series of complex reactions known as the Calvin cycle. (Bassham, J. A., Benson, A. A., & Calvin, M. 1950)

When carbon dioxide is reduced to form glucose molecules, oxygen (O₂) is also produced as a byproduct. The oxygen released during photosynthesis is vital for life on Earth, as it enables aerobic organisms (including animals and humans) to breathe and produce energy through the combustion of carbohydrates. (Govindjee, J. T. Beatty, & H. Gest. (Eds.). (2005)

In summary, photosynthesis is a crucial process that enables photosynthetic organisms to capture solar energy to produce glucose and oxygen from carbon dioxide and water. It is essential for maintaining life on Earth by providing oxygen and forming the foundation of the food chain through the production of carbohydrates used as an energy source by other organisms.

Evolution of Algae: Cyanobacteria have played a pivotal role in the evolution of algae and life on Earth. Over time, these organisms evolved by developing new features, such as the emergence of chloroplasts, cell organelles responsible for photosynthesis. Chloroplasts result from endosymbiosis, where a primitive photosynthetic cell was engulfed by another cell, establishing a symbiotic relationship. This evolution gave rise to true eukaryotic algae. (Raven, J. A. 1997)

Role of Algae in the Creation of Life on Earth :

Algae have had a significant impact on the creation of life on Earth through their ability to perform photosynthesis.

One of the major consequences of photosynthesis is oxygen production. Over time, the massive photosynthetic activity of cyanobacteria and algae led to the accumulation of oxygen in the Earth's atmosphere, triggering a major event known as the "Great Oxidation Event" or "Oxygen Catastrophe" around 2.4 billion years ago. (Holland, H. D. 2006)

The increase in oxygen in the atmosphere created favorable conditions for the evolution of new, more complex forms of life. Oxygen allowed for the emergence and development of multicellular life forms, paving the way for the subsequent evolution of terrestrial plants, animals, and other living organisms that we know today. (Bekker, A., Holland, H. D., Wang, P. L., Rumble, D., Stein, H. J., Hannah, J. L., ... & Coetzee, L. L. (2004)

The rise in atmospheric oxygen also had a significant impact on Earth's climate and environment. It played a role in the formation of ozone (O₃) in the stratosphere, which shielded the Earth's surface from harmful ultraviolet rays from the sun, thus enabling the colonization of terrestrial organisms. (Catling, D. C., Glein, C. R., Zahnle, K. J., & Mckay, C. P. (2005)

In summary;

The emergence and proliferation of algae were crucial for the increase in oxygen in the Earth's atmosphere, which facilitated the evolution of aerobic life and more complex life forms on Earth. This process has shaped our planet's environment and climate and played an essential role in the creation of the ecosystems as we know them today.

Types of algae :

There are two types of algae: macroalgae (also known as seaweed or multicellular algae) and microalgae, which are unicellular algae. Microalgae, depending on the species, range in size from a few micrometers (μm) to a few hundred μm , while macroalgae can reach several meters in size. Macroalgae and microalgae exhibit significant differences in terms of size, structure, habitat, chemical composition, and roles within ecosystems.

Within these two types of algae, there are over 30,000 species, each with specific differences.

Size and Structure:

Macroalgae are large algae visible to the naked eye. They are multicellular and come in various forms, such as kelps (brown algae), ulva (green algae), or rhodophytes (red algae). On the other hand, microalgae are much smaller, often unicellular, and can only be seen under a microscope. (Leliaert, F., Smith, D. R., Moreau, H., Herron, M. D., Verbruggen, H., Delwiche, C. F., ... & Bhattacharya, D. (2012))

Lipid Content:

Microalgae: Microalgae are generally rich in lipids, including polyunsaturated fatty acids (PUFAs) like omega-3s. The lipid content varies among microalgae species, but some can contain up to 50% or more lipids in their biomass. (Liang, Y., Sarkany, N., & Cui, Y. 2009).

According to Saleh Mobin's "Some promising microalgal species for commercial applications: A review" (2017), the microalga *Phaeodactylum tricornutum* is particularly suitable for biofuel production due to its high lipid concentration.

Macroalgae: Macroalgae generally have lower lipid content than microalgae. Their lipid content varies depending on species and environmental conditions, but it is generally lower than that of microalgae. (Stengel, D. B., & Connan, S. 2015)

Protein concentration :

"Microalgae: Microalgae are also rich in proteins, with protein content that can reach up to 70% of their dry weight. These proteins are of high nutritional value and contain all essential amino acids required for human nutrition. (Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. 2006)

Macroalgae: Macroalgae generally have lower protein content compared to microalgae. Their protein content varies among species and environmental conditions, but it is generally lower than that of microalgae. (Plaza, M., & Herrero, M. 2010).

According to a study by Mariam Al Hattab and Abdel Ghaly, "Microalgae Oil Extraction Pre-treatment Methods: Critical Review and Comparative Analysis" (Canadian Journal of Chemical Engineering), here is a table containing protein and lipid quantities of certain types of microalgae

Species of sample	Proteins	Carbohydrates	Lipids	Nucleic acid
<i>Scenedesmus obliquus</i>	50–56	10–17	12–14	3–6
<i>Scenedesmus quadricauda</i>	47	-	1.9	-
<i>Scenedesmus dimorphus</i>	8–18	21–52	16–40	-
<i>Chlamydomonas reinhardtii</i>	48	17	21	-
<i>Chlorella vulgaris</i>	51–58	12–17	14–22	4–5
<i>Chlorella pyrenoidosa</i>	57	26	2	-
<i>Spirogyra sp.</i>	6–20	33–64	11–21	-
<i>Dunaliella bioculata</i>	49	4	8	-
<i>Dunaliella salina</i>	57	32	6	-
<i>Euglena gracilis</i>	39–61	14–18	14–20	-
<i>Prymnesium parvum</i>	28–45	25–33	22–38	1–2
<i>Tetraselmis maculata</i>	52	15	3	-
<i>Porphyridium cruentum</i>	28–39	40–57	9–14	-
<i>Spirulina platensis</i>	46–63	8–14	4–9	2–5
<i>Spirulina maxima</i>	60–71	13–16	6–7	3–4.5
<i>Synechococcus sp.</i>	63	15	11	5
<i>Anabaena cylindrica</i>	43–56	25–30	4–7	-

Table 1: Microalgae composition [15].

Types of Algae for Biodiesel Production :

Identifying the optimal algae species for biofuel production is challenging as it depends on various factors. Production productivity will rely on growth rate, population density, the ease of lipid extraction, and of course, the lipid content.

According to the study by Kuan Shiong Khoo ^{a,b} , Imran Ahmad ^c , Kit Wayne Chew ^d , Koji Iwamoto ^c , Amit Bhatnagar ^{e,*} , Pau Loke Show, 'Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review' (2023), green algae (Chlorophyta) are particularly interesting for biodiesel production due to their high population density and rapid growth rate. Green algae possess an efficient photosynthesis system due to the presence of chlorophyll b, enabling them to accumulate a significant amount of lipids, primarily in the form of TAGs and DAGs, which are preferred neutral lipids for biodiesel production.

Certain species of microalgae, such as *Phaeodactylum*, *Nannochloropsis*, *Chlorella*, and *Dunaliella*, have been extensively studied over the last decade due to their ability to accumulate neutral lipids, primarily in the form of triacylglycerols (TAGs), when subjected to nutrient deprivation. These neutral lipids can then be converted into biofuels through transesterification.

In summary : the ideal type of algae would be one with rapid growth, high lipid content (primarily in the form of TAGs and DAGs), resistance to bacteria, and high population density. Among the 30,000 species, it is necessary to identify the most optimized one for biofuel production.

Absorption of Co2

According to the study by Kuan Shiong Khoo a,b , Imran Ahmad c , Kit Wayne Chew d , Koji Iwamoto c , Amit Bhatnagar e,* , Pau Loke Show, 'Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review' (2023), to produce 1 kg of dry algal biomass, at least 1.83 kg of CO₂ is required, assuming the algae contains 50% carbon. The CO₂ concentration needs to be several times higher than the given value. The potential efficiency of CO₂ absorption ranges from 20% to 90% depending on operational conditions. This confirms the effectiveness of algae in reducing greenhouse gas emissions, provided they require less CO₂ for conversion into fuel.

Biofuels :

According to the website [connaissancesdesenergies.org](https://www.connaissancesdesenergies.org) (<https://www.connaissancesdesenergies.org/fiche-pedagogique/biocarburant>), Biofuels are fuels produced from renewable organic materials such as plants, algae, and agricultural waste. They are considered an alternative to fossil fuels as they generally emit fewer greenhouse gases during combustion. Biofuels are classified into different generations:

First Generation: First-generation biofuels are produced from food crops such as corn, sugarcane, rapeseed, and palm oil. These biofuels are relatively simple to produce but are controversial as they can compete with food crops, leading to increased food prices and excessive use of agricultural land.

Second Generation: Second-generation biofuels are made from non-food materials like lignocellulose (wood, straw, etc.) and agricultural waste. These characteristics offer advantages of greater availability and non-food competition compared to first-generation biofuels. This reduces the risk of competition between food crops and biofuels.

Third Generation: Third-generation biofuels are produced from microorganisms such as algae. These microorganisms are cultivated and accumulate fatty acids as oils or energy-rich lipids, which can then be converted into fuels. Algae-based biofuels have high yield potential (up to 30 times higher per hectare than terrestrial oilseed species) and do not require agricultural land. This third-generation fuel is the focus of this thesis."

Biofuels from Algae:

According to the study by Kuan Shiong Khoo a,b, Imran Ahmad c, Kit Wayne Chew d, Koji Iwamoto c, Amit Bhatnagar e,* , Pau Loke Show, 'Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review' (2023), producing biofuel from microalgae offers several environmental advantages, including high growth rates, high energy yield, and reduced land use. Unlike other biomass sources, microalgae have a high yield per unit of surface area and require less freshwater as they can utilize nutrients present in wastewater.

First-generation biofuels, primarily produced from food crops that can be used in animal or human food chains (such as corn, wheat, or sugar beet), pose sustainability issues related to food resources, increasing agricultural product prices, and leading to intensive use of fertilizers and agrochemicals that can degrade water and soil quality.

In contrast, third-generation biofuel production (based on microalgae) can occur in closed photobioreactors or open ponds, without the need for arable and productive land or freshwater. This avoids conflicts between land use for food production and biofuels.

Furthermore, microalgae-based biofuels can be produced locally, reducing a country's dependence on foreign oil, creating employment opportunities, and stimulating the local economy.

Microalgae have a rapid growth rate, doubling their biomass concentration every 2 to 5 days, which is a significant advantage compared to other biomass sources that take longer to grow. Their production can occur almost continuously throughout the year, unlike first and second-generation crops that can only be harvested once or twice a year.

The fatty acids produced by microalgae can be converted into biodiesel, which is a renewable, biodegradable, non-toxic, and environmentally friendly fuel. Biodiesel has the advantage of emitting 78% less carbon dioxide when burned, 98% less sulfur, and 50% fewer particulate matter emissions (Brown and Zeiler, 1993).

Thus, the continuous production of microalgal biomass would be more optimal than first-generation biofuel production, as it does not require arable land, freshwater, pesticides, and does not compete with food consumption."

Table 3

Significance of microalgae over other feedstocks in producing biodiesel. (The data was adapted with permission from Elsevier [55] for comparison purpose).

Plant source	Oil composition by wt. in biomass (%)	Oil productivity (L oil/ha/year)	Land use (m ² year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ha/year)
Corn/Maize (<i>Zea mays</i>)	44	172	66	152
Soybean (<i>Glycine max</i>)	18	636	18	562
Jatropha	28	741	15	656
Canola/Rapeseed	41	974	12	862
Sunflower (<i>Helianthus annuus</i>)	40	1070	11	946
Castor (<i>Ricinus communis</i>)	48	1307	9	1156
Palm oil (<i>Elaeis guineensis</i>)	36	5366	2	4747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

source: Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review' (2023)

Characteristics of Algae-Based Biofuel:

According to the study by Kuan Shiong Khoo a,b, Imran Ahmad c, Kit Wayne Chew d, Koji Iwamoto c, Amit Bhatnagar e,* , Pau Loke Show, 'Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review' (2023),

A quality biodiesel: The best biodiesel would be composed of lipids rich in unsaturated fatty acids, particularly polyunsaturated fatty acids (PUFAs). Unsaturated fatty acids have a carbon-carbon double bond in their structure, making them more reactive and enhancing the fuel properties of biodiesel.

Among lipids, triacylglycerols (TAGs) rich in PUFAs are particularly desirable for high-quality biodiesel production. TAGs are esters of fatty acids with glycerol and are the main storage form of lipids in microalgal cells.

A biodiesel primarily composed of unsaturated fatty acids offers several advantages. It has better fluidity at low temperatures (improved cold flow), making it easier to use in cold climates. Biodiesel based on unsaturated fatty acids also has better oxidation stability, meaning it is more resistant to chemical degradation and aging. This reduces the formation of deposits and sediments that could disrupt engine operation.

Algae production:

The first step in producing biofuel from algae is the large-scale cultivation of algae.

How to cultivate microalgae:

According to the article by Muhammad Imran Khan¹, Jin Hyuk Shin¹, and Jong Deog Kim^{1,2*}, here are important elements of micro and macroalgae cultivation:

Carbon supply:

Carbon is essential for the growth of microalgae, as they use it in photosynthesis. Carbon sources can vary, such as carbon dioxide, methanol, acetate, glucose, or other organic compounds.

When carbon is consumed as a direct substrate, for example using CO₂ emitted by power plants, cement factories, etc., microalgae contribute significantly to air depollution. Microalgae absorb twice as much CO₂ as they emit.

Lipid production:

The proteins of microalgae are broken down and converted into energy-rich products, such as lipids.

To induce microalgae to produce lipids, they need to be stressed. This can be done in various ways, such as subjecting them to nutrient deficiency, altering the pH of the water, inducing nitrogen deficiency, or even infecting them with a virus. According to Muhammad Imran Khan, Jin Hyuk Shin, and Jong Deog Kim, intentionally infecting them with a virus could be a cost-effective way to stress microalgae and significantly increase their lipid production.

The nitrogen deficiency method is also widely used and low-cost, but it drastically reduces the growth rate of microalgae.

Light:

Microalgae need light for photosynthesis. The longer the light cycles, the faster the growth. However, they are sensitive to heat and should not be overexposed to it, as it can lead to burns.

Interestingly, microalgae don't necessarily need light for photosynthesis. They can obtain carbon through glucose (sugars), a process known as heterotrophy.

Carbon dioxide fixation:

Microalgae can use various sources of CO₂, such as the atmosphere, industrial flue gases, or soluble carbonates. About 1.83 kg of CO₂ is needed to produce 1 kilogram of dry algae.

However, the presence of an adequate amount of CO₂ in the atmosphere is necessary for good production and stable pH. This can be achieved by directly injecting flue gases from power plants, cement factories, and refineries. It's also important to control pH to enhance CO₂ absorption by microalgae.

Production structure:

There are different ways to cultivate algae. Microalgae can grow in natural environments, such as oceans or lakes, but harvesting them in these conditions is difficult due to their small size (1 cm = 10,000 micrometers). Studying them in their natural environment is also challenging as we cannot modify parameters like water pH, nutrient content, light exposure, etc.

That's why studies focus on closed-system microalgae production, which can be open ponds (similar to outdoor pools) and photobioreactors. We will delve into production methods later."

Production methods:

The method of algae production is a determining factor in analyzing the profitability of biofuel production. This step can require significant resources such as electricity, water, land, or fertilizers. These resources are more or less exploited depending on the production method, which influences the overall cost of biofuel production.

Algae can be produced in various ways: open ocean production, photobioreactors, and open ponds. We will discuss these three main methods for industrial use.

1. Open ocean production:

Open ocean production is only feasible for macroalgae. It involves placing structures like ropes with weighted strains of macroalgae. These strains will develop at varying rates depending on weather conditions and seasons and will need to be hand-harvested. They absorb nutrients present in the sea to grow. They have the advantage of depolluting the ocean, particularly from fertilizers that are discharged into it (as seen with the abundance of algae in Brittany, France). However, this production method is not suitable for microalgae, and the quantity produced fluctuates based on seasons and sunlight. Producing in the open ocean also requires accessible areas and permits from public institutions, as the cultivation must occur in a specific environment, unlike fishing.

A very interesting case of open ocean algae production is the Belgian company "Wier en Wind." In collaboration with Ghent University, in Belgium, it focuses on large-scale offshore algae production. Algae initially grow on ropes in a nursery for 6 weeks until they reach a size of about 6 mm. Once this size is achieved, the ropes are placed in offshore structures and grow for 6 months (from October to April) to reach a size of about 2 meters. Yields are 5 kg of wet algae per meter of rope or 10 kg of wet algae per square meter. The company currently produces brown kelp, but in the future, they intend to focus on algae types with much higher yields. The harvest period is between April and June; at that time, a boat with

appropriate equipment comes to harvest the kelp and transport it to land for processing in a dedicated facility.

Productivity : a typical open ocean algae farm could have a footprint of about 10 km² and an annual production volume of around 10,000 tons in wet weight. The company is also heavily focused on automating the farm, including placing cameras to monitor production at lower costs. They aim to achieve a production of 100,000 tons of wet algae per year by 2030."



Source : project brochure Wier en Wind

"According to the article 'Kelp (*Ascophyllum nodosum*), the Iodine-Rich Algae' from <https://www.mesbienfaits.com/kelp/>, kelp has a lipid content of about 27.8%, resulting in a production of 2,780,000 kg of lipids per 10 km² without significant energy requirements. This figure is quite interesting since we need 1 kg of lipids to produce 1 liter of biofuel, the calculation is straightforward. We could produce 2.78 million liters of biofuel annually with a 10 km² sea area.

However, production in a natural environment is not consistent, as factors such as fertilizers, water pH, and sunlight cannot be controlled."

Other Projects of production in open ocean :

We would also like to mention biologist Sylvain Huchette, who is a producer of macroalgae, particularly Royal Kombu, in Brittany. This seaweed is a food algae containing up to 15% protein. Unfortunately, he did not respond to my messages.

There is also the SeaCrops study conducted in Belgium by De Clerck and Cobe De Meester. The aim of this study is to produce algae in a natural environment, but unfortunately, I could not find any further information.

Another ongoing project is the SølKelp project, which is developing and implementing innovative cultivation strategies with mutual relevance for two geographical boundaries (Portugal and Norway). Activities include expanding a Kelp nursery (brown macroalgae) in Portugal, growth trials in the Portuguese sea (to study technical challenges with full wave exposure), and transferring cultivation techniques of *Palmaria palmata* ("Søl") from Portugal to Norway, where conditions are better for large-scale production of this valuable species. The results of this study have not yet been revealed." (theportugalnews, 2022)

2. Open ponds and photobioreactors production:

The main characteristic of this production method is that it takes place on land, within specific facilities. The production in open ponds and photobioreactors shares several similarities, particularly in the aspect that these methods are specially suited for the cultivation of microalgae. Water, nutrients, and CO₂ need to be provided to ensure proper growth, and the algae must be continuously mixed to ensure uniform exposure to light and nutrients.

This type of production is intriguing as it allows the monitoring of all parameters that could affect production, enabling their adjustment to understand how to optimize output.

Open ponds are like large pools exposed to sunlight, while photobioreactors are transparent structures in which microalgae grow in a completely closed circuit, often using artificial lights."



Figure 6.1 Photobioreactors for algae cultivation. Left: High Rate Algal Ponds, Earth Rise Farms, USA. Right: Tubular photobioreactor developed by IGV, Potsdam, Germany.

According to the article by Peter Styring, "Carbon Capture and Utilisation in the green economy" (2011), open ponds are the most common production method for commercial cultivation. They can be built at relatively low costs (around \$10 per square meter). Additionally, expansion is easy, and energy consumption is moderate.

However, this form of cultivation does not allow strict control, which limits productivity. Therefore, open ponds have lower productivity compared to photobioreactors. Moreover, contamination is more likely in open ponds due to the exposure of algae to the external environment. To conclude, the construction cost depends on the location of the pond, particularly the country or region where the construction takes place.

As for photobioreactors, the nearly complete control of the environment leads to much higher productivity, but energy costs are also significantly higher. Furthermore, initial investments are much higher for all infrastructures; according to the article, they are around 10 times higher than for open ponds. Lastly, a highlighted issue is the difficulty in expanding the system, which will be costly and more complex to implement, considering the equipment's need to handle a larger quantity.

A study by Abdul Hai Alami, Shamma Alasad, Mennatalah Ali, Maitha Alshamsi, "Investigating algae for CO₂ capture and accumulation and simultaneous production of biomass for biodiesel production" (2020), evaluated the productivity of microalgae in open ponds and estimated that the production ranged from 0.05 to 0.10 grams of dry biomass per liter of water per day, with a concentration of less than 1 gram per liter.

According to the same source, circular ponds, which are the oldest method of microalgae cultivation, produce about 15 grams of dry microalgae per square meter per day. These ponds typically have a depth of 30 to 70 cm.

Concerning the more commonly used open ponds, their productivity is around 22 grams of dry microalgae per square meter per day. These ponds have a depth between 15 and 30 cm. (Alami, A. H., Alasad, S., Ali, M., & Alshamsi, M. 2020).

According to the book "Biofuel from Algae," open ponds are the most cost-effective means of microalgae production. Energy consumption is lower compared to closed photobioreactors, cleaning is simpler, and the ponds can be protected from weather conditions using greenhouses.

However, an adequate amount of CO₂ in the atmosphere is necessary to ensure good production and stable pH. This can be achieved by directly injecting combustion gases from

power plants, cement factories, and refineries. Additionally, it is important to control pH to enhance the absorption of CO₂ by microalgae.

Closed tanks (photobioreactors):

According to a study by Alami, A. H., Alasad, S., Ali, M., & Alshamsi, M. (2020), the productivity in closed tanks is much higher than in open ponds. The production of dry microalgae would range between 0.8 and 1.3 grams per liter of water per day with a cellular concentration exceeding 1 gram per liter.

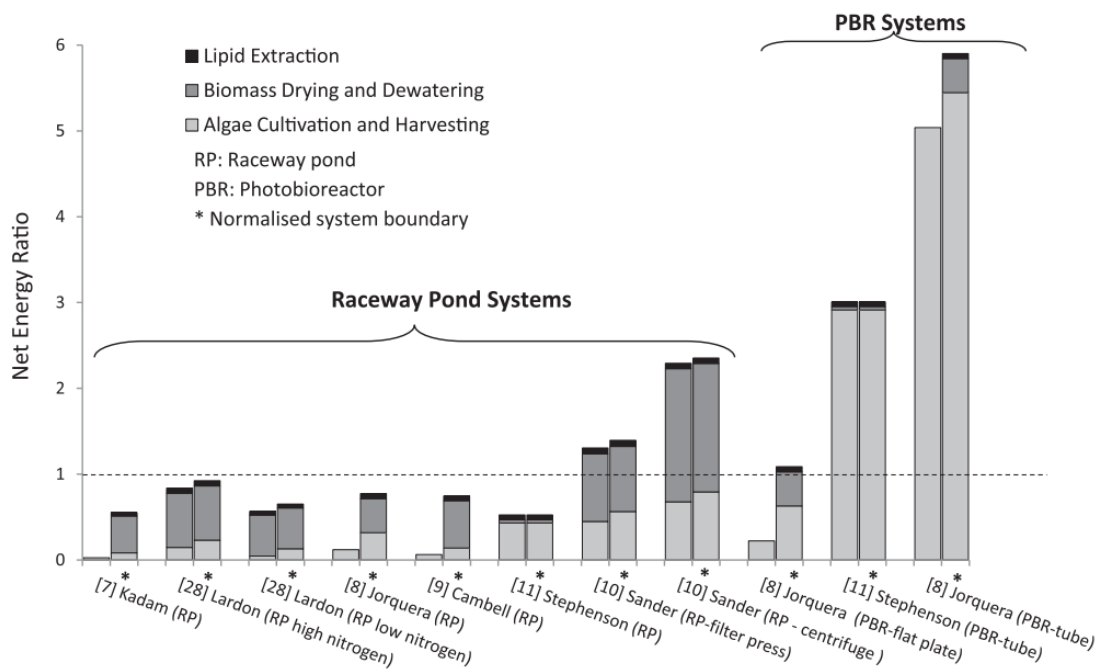
According to another article by El Shenawy, E. A., Elkelawy, M., Bastawissi, H. A.-E., Taha, M., Panchal, H., Sadasivuni, K. Kumar, & Thakar, N. (2019), which analyzes variations in productivity based on parameters such as temperature, tank depth, and others, the rate of microalgae productivity in closed tanks can vary between 0.41 and 1.12 grams of dry microalgae per day per liter. However, it is important to note that productivity is highly sensitive to the environment and the type of microalgae.

Energy Ratio Based on Different Tanks:

According to a study by Raphael Slade*, Ausilio Bauen titled "Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects" in 2012, which compares different studies on energy costs and environmental impacts between biomass production in open ponds or photobioreactors, production in open ponds is much more cost-effective than in photobioreactors. The energy ratio is negative for 6 out of 8 open pond productions, while it is positive for all photobioreactors.

We can also observe that in the case of production in photobioreactors, the energy required for harvesting and drying the biomass is relatively insignificant compared to the enormous energy demand for the production of that biomass.

A significant difference can also be noticed between "flat" photobioreactors, which consume much less energy than tube photobioreactors.



According to the same study, the productivity in grams per square meter per day would range from 10 grams to 20 grams for open pond production and from 20 grams to 40 grams for photobioreactor production.

This study also conducts an analysis of the cost per kilogram. In the case of photobioreactor production, the estimated baseline costs would be 9 to 10 euros per kilogram, but the projected costs would be 3.8 euros per kilogram.

In the case of open pond production, the estimated baseline costs would be 1.6 to 1.8 euros per kilogram, but the projected costs would be 0.3 to 0.4 euros per kilogram.

However, it is important to note that this study dates back to 2013, which is quite old considering the rapid advancements in this field. Furthermore, the projected costs only account for production and harvesting; thus, the costs of lipid recovery and conversion into fuel are not included.

In summary:

We have been able to compare three methods of algae production, whether microalgae or macroalgae.

Regarding microalgae, we can observe that open pond production is much more cost-effective. Even though it is about ten times less productive, it requires much less investment in infrastructure and significantly lower management and production expenses. The production volume is lower, but the cost of production is much more advantageous. This method also appears to have the best energy ratio, confirming its more attractive price. According to the article by Raphael Slade*, Ausilio Bauen, 6 out of 8 open pond productions have a profitable energy ratio, implying their potential economic viability. However, it's important to remember that the production stage is the initial step in biofuel production and constitutes only 10% to 40% of the total biofuel cost.

Photobioreactors are therefore much less profitable, but they are nevertheless valuable for research purposes, particularly in understanding how to optimize microalgae production by adjusting parameters.

The third production method, which seems economically viable, is offshore production. Although we don't have precise information about production costs, we can estimate that they are significantly lower since the production takes place in a natural environment without additional inputs.

This production method offers numerous advantages. Firstly, it contributes to ocean cleanup as the algae absorb nutrients and CO₂ from the sea while creating an ecosystem.

Furthermore, this approach can be easily scaled up, as demonstrated by our investigation; the company "Wier en Wind" plans to increase its production tenfold within ten years.

Lastly, it enables the production of lipid-rich algae at a very low cost without utilizing land space.

Microalgae harvesting:

Harvesting is the process that involves extracting microalgae from their cultivation environment. The harvesting process is crucial to consider in the profitability of biofuel production, as the small size and density of microalgae make them challenging and expensive to harvest. Microalgae have a size of only a few micrometers (1 cm = 10,000 micrometers), making traditional methods of harvesting not feasible. In this section, we will explore the methods and their costs.

According to the study by Xiaotong Zou, Kaiwei Xu, Wenjuan Chang, Yanhui Qu, Yanpeng Li, "Rapid extraction of lipid from wet microalgae biomass by a novel buoyant beads and ultrasound-assisted solvent extraction method" (2021), the harvesting step is crucial to consider in profitability studies, as it accounts for between 20% and 30% of the total manufacturing cost.

In this chapter, we will analyze various harvesting methods and attempt to determine which ones are most relevant, based on time, energy consumption, and environmental impact.

The type of microalgae also plays a role, as certain species are denser or larger. According to the article by A.H. Alami, S. Alasad, M. Ali, et al., microalgae have a concentration ranging from 0.3 to 1 gram per liter of water. To be industrially viable, the concentration must be in the range of 300 to 400 grams per liter of water, which means the process needs to allow for concentration up to 100 times higher.

There are different methods, varying in duration, cost, and environmental impact. In this section, we will explain these methods and highlight their advantages and disadvantages.

Gravity Sedimentation:

Microalgae cells tend to remain suspended in their production liquid. However, in this process, microalgae particles in a fluid are concentrated under the influence of gravity, allowing them to condense for harvesting. This phenomenon occurs due to a difference in density between the microalgae and the fluid. In the case of microalgae harvesting, they will be concentrated at the bottom of the container due to their higher density compared to the fluid.

Gravity sedimentation is a relatively simple and cost-effective method for microalgae harvesting, especially in small-scale operations or research environments. However, it may require longer settling times, and the process efficiency can vary depending on factors such as the size and shape of microalgae cells, cell concentration in the culture, and medium viscosity.

In some cases, coagulants can be added to the culture to enhance the sedimentation process.

According to the article by Abdul Hai Alami, Shamma Alasad, Mennatalah Ali, Maitha Alshamsi, gravity sedimentation is one of the most popular harvesting techniques. However, this process is very sensitive to microalgae density; if the density is too low, the process is not very efficient. Although this method is simple and applicable to all types of microalgae, it is also low in energy cost but requires a significant amount of time.

Filtration Method:

According to the article by Abdul Hai Alami, Shamma Alasad, Mennatalah Ali, Maitha Alshamsi, microalgae harvesting involves separating microalgae cells from the growth medium or culture using different types of filters. This technique is widely used for both small- and large-scale microalgae production due to its efficiency, low energy requirements, and ability to achieve high biomass recovery.

Filtration can be performed using various types of filters, such as microscreens, rotary drum filters, disk filters, and membrane filters. Microscreens are woven metal meshes with specific pore sizes that capture microalgae cells as they pass through. Rotary drum filters

consist of a rotating drum covered with filtering media, and as the liquid flows through the drum, microalgae are retained on its surface.

Disk filters operate similarly to microscreens but are circular in shape, and the liquid is forced through the disk, capturing microalgae. Membrane filters use membranes with controlled pore sizes to separate microalgae based on their size and charge.

The advantage of this method is that it allows the filtration of liquids with low microalgae concentrations and is easily scalable to larger operations. However, this method requires significant maintenance costs for unclogging, replacing, or cleaning the filters, making the overall cost less advantageous.

centrifugation :

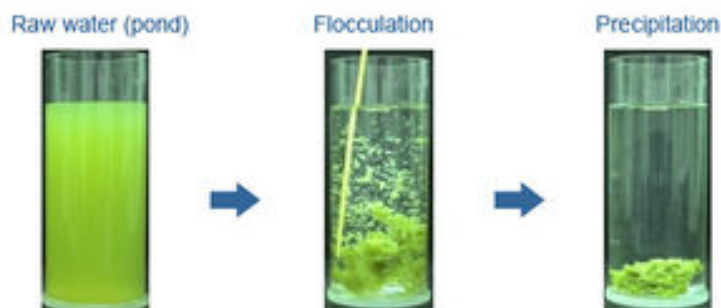
Centrifugation is a commonly used and effective process for harvesting microalgae from the culture medium. It involves spinning the microalgae suspension at high speed to create a centrifugal force, causing the denser microalgae cells to sediment at the bottom of the centrifuge tube. The clear supernatant, containing the growth medium, can then be carefully removed, leaving behind a concentrated microalgae biomass.

According to the article by Abdul Hai Alami, Shamma Alasad, Mennatalah Ali, Maitha Alshamsi, this is a widely used method and many studies are ongoing for this type of harvesting. The efficiency often depends on the type of algae, particularly their size. This method is rapid but requires a significant amount of energy, as studies show that 80 to 90% of microalgae can be harvested within 2 to 5 minutes. While this method is highly effective, it is also costly.

Chemical coagulation flocculation :

This process is widely used to harvest microalgae by inducing the aggregation of individual cells into larger clusters or flocs. The addition of flocculants or coagulants to the microalgae culture promotes the neutralization of surface charges on the cells, causing them to come together and form larger, settleable aggregates. These flocs can then be easily separated from the growth medium through sedimentation or other methods.

This is also a very popular and practical method for harvesting many types of algae. However, this technique is not suitable for large-scale operations because the chemicals added to coagulate the microalgae are difficult to extract afterward. These chemicals are also quite costly and render the algae unsuitable for human consumption, especially if the proteins are also intended for use.



In summary:

Our study did not conclusively determine the most effective method for microalgae harvesting. Flocculation and coagulation methods are not suitable for large-scale operations, while filtration and centrifugation methods can be applied at a larger scale but come with a relatively high cost.

The gravity sedimentation method appears to be the most promising, as it is energy-efficient and straightforward. However, there is limited information about its large-scale application, and the outcome heavily relies on the concentration of microalgae in the solution. Lastly, this method also tends to be time-consuming.

It's important to note that in the case of macroalgae production, this step would be significantly less expensive, as they do not require specific treatment.

Lipid extraction :

In the previous chapter, we discussed the methods for harvesting microalgae. This step resulted in the collection of a concentrated quantity of microalgae, which is referred to as biomass. From this biomass, the oils (lipids) need to be extracted for the creation of biofuel. There are two approaches to accomplish this: mechanical methods and chemical methods using solvents.

Lipid extraction is a crucial step in biofuel production. According to the study by Xiaotong Zou, Kaiwei Xu, Wenjuan Chang, Yanhui Qu, and Yanpeng Li, titled "Rapid extraction of lipid from wet microalgae biomass by a novel buoyant beads and ultrasound assisted solvent extraction method" (2021), it accounts for 30% to 40% of the total production costs.

As per the study by M. Farizal Kamaroddin, Ameera Rahaman, D. James Gilmour, and William B. Zimmerman, titled "Optimization and cost estimation of microalgal lipid extraction using ozone-rich microbubbles for biodiesel production" (2020), there are two major and energy-intensive steps to obtain lipids from the biomass: harvesting and lipid extraction.

The yield is calculated as follows:

$(\text{lipid mass extracted in grams} / \text{total mass of dried algae}) \times 100$

Mechanical Methods:

Ultrasonication: Ultrasonication involves exposing microalgae to high-frequency ultrasonic waves. This causes the rupture of microalgae cell walls and the release of lipids into the extraction medium.

Mechanical Pressing: This method entails applying mechanical force to crush the microalgae and release the lipids. Pressing can be done either at cold or hot temperatures, and various press configurations can be used. However, according to A.H. Alami, S. Alasad, M. Ali, et al., this method is considered more costly because the algae need to be dried before pressing.

Homogenization: The homogenization method involves passing microalgae through a high-pressure valve or orifice. This mechanical action leads to cell rupture and lipid release.

Microfluidics: Microfluidic methods involve the use of microscopic channels to process microalgae. These devices create shear forces that promote lipid release.

Mechanical Agitation: Mechanical agitation is performed by stirring the microalgae in an extraction medium using agitators or mixers, thus facilitating lipid release.

Chemical Method:

The chemical extraction method of oils is highly efficient and allows for the extraction of between 99.3% and 99.5% of lipids. This can be achieved using solvents, which are more effective than mechanical extraction.

According to the article by A.H. Alami, S. Alasad, M. Ali, et al., extraction with the solvent Hexane is quite popular due to its low cost and lower toxicity compared to solvents like benzene and diethyl ether. This method can also be combined with mechanical extraction, using the solvent only on the remaining biomass after mechanical extraction to extract the maximum amount of lipids. Ultimately, the lipids and the solvent are separated through distillation.

It's important to consider that the higher the solvent-to-biomass ratio and the longer the contact time between solvent and biomass (10 to 25 hours), the greater the yield.

According to a Canadian study by Mariam Al Hattab and Abdel Ghaly titled "Microalgae Oil Extraction Pre-treatment Methods: Critical Review and Comparative Analysis," there are three very relevant methods for lipid extraction:

1. Bath sonication
2. Steam explosion
3. Microwave radiation

This study is based on the comparison of nine different methods and eight selection criteria of varying importance. The criteria are referred to in the table below.

Criteria	Importance	Description
Cell wall disruption efficiency	15	The system should be able to effectively disrupt the cell wall of microalgae in order to increase the oil extraction efficiency
Cost	15	The operational costs of the pre-treatment process should be low, so that the additional incurred pre-treatment costs can be justified
Time	15	The rate of microalgae cell wall degradation should be quick to ensure the sustainability purposes
Suitability for Large Scale Use	15	The method should be effective in handling large volumes for industrial production
Toxicity and Health	10	The method should be non-toxic so that the retrieved algae biomass may be processed for a number of value added products for animal and/or human consumption
Environmental Impact	10	Method should be environmentally friendly with no toxic wastes produced
Reusability	10	The pre-treatment method should be reusable in order to reduce costs associated with equipment
Maintenance	10	Costs for maintaining the method should be low

Table 4: Criteria used for the comparative analysis of different oil extraction pre-treatment techniques.

Here are the results of their investigations. Thus, the most relevant methods are:

1. "Bath sonication" (81/100), a mechanical method.
2. "Steam explosion" (93/100), a thermal method.
3. "Microwave" (87/100), an electromagnetic method.

For this study, no solvent-based methods were considered.

Criteria	Mechanical				Thermal			Electromagnetic	Biological
	SBM	ABM	HS	BS	SE	FD	AC	M	EZ
Cell wall disruption efficiency (15)	10	12	15	15	12	12	10	13	15
Cost (15)	6	6	4	8	13	6	6	11	7
Time (15)	15	15	12	12	14	3	3	15	5
Suitability for Large Scale Use (15)	3	3	4	8	15	8	8	15	15
Toxicity and Health (10)	10	10	10	10	10	10	10	10	15
Environmental Impact (10)	10	10	10	10	10	10	10	10	6
Reusability (10)	10	10	10	10	10	10	10	10	5
Maintenance (10)	6	6	8	8	9	5	8	3	6
Total (100)	70	72	73	81	93	64	65	87	74

SBM= Shaking vessel bead mill SE= Steam explosion ABM= Agitated bead mill FD= Freeze-Drying HS= Horn sonication AC= Autoclave BS= Bath sonication EZ = Enzyme M= Microwave

Table 14: Comparative analysis of microalgae pre-treatment methods.

Ultrasonication bath:

In this method, algae are exposed to high-intensity ultrasonic waves, creating tiny cavitation bubbles around the cells. The bubbles implode and emit shockwaves that break the cell walls, releasing the lipids.

According to this method, the lipid extraction rate varies between 26% and 36% depending on the type of algae and processing time. Here is the energy consumption based on the type of algae:

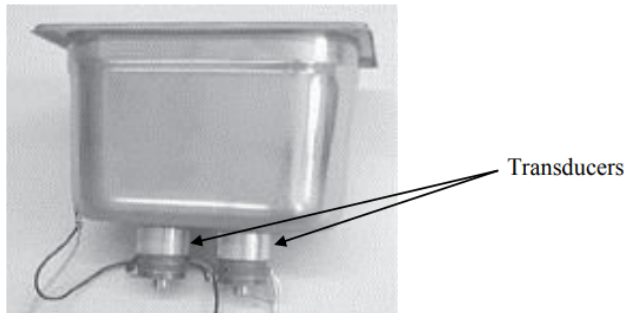


Figure 12: Ultrasonic bath transducers [68].

Scenedesmus : 119 Wh/g

Nannochloropsis oculata : 16.66 Wh/g

Chlorococcum sp : 36.66 Wh/g

Chlamydomonas reinhardtii : 36 Wh/g

The advantages of this method include its rapid processing, low solvent requirements, good efficiency, and the fact that the biomass doesn't need to be dry. However, it remains costly. In Belgium, 1 kWh costs about 45 cents. 1 kWh is equal to 1000 Wh. In the case of the microalgae *Nannochloropsis oculata* (the least costly option), it requires 16.66 Wh per gram extracted. Therefore, the cost per kilogram comes to $0.01666 \text{ kWh} \times 0.45 \text{ cents} \times 1000 = 7.49$ euros per kilogram.

Steam Explosion:

The goal of this method is to expose the biomass to high temperatures, ranging from 160 to 260 degrees Celsius, and high pressure. Upon depressurization, the cells rupture and release the lipids. With this method, lipid extraction is about 8%, which is considered quite good according to this article. However, with the species *Chlorella vulgaris*, if the temperature is raised to 210 degrees Celsius, lipid extraction reaches 77%. Nonetheless, this method varies significantly based on the algae type and temperature.

Electromagnetic Radiation (Microwaves):

This method involves rapidly heating the cells using electromagnetic waves to break the cells and release the lipids. The lipid recovery yield for this method ranges between 25% and 40%.

Energy Consumption:

Nannochloropsis gaditana consumed 1.6 Wh/g with a lipid yield of 40%, and 76-77% of the lipids in dry mass were extracted from the microalgae *Scenedesmus obliquus* using 60 Wh/g.

1 Wh corresponds to 0.001 kWh, so in the case of *Nannochloropsis gaditana* biomass, 0.0016 kWh is needed to extract one gram of lipids, which equates to 1.6 kWh for one kilogram of lipids. In Belgium, the average household electricity price was around 0.4 to 0.5 euro per kWh. This would result in a total cost of 0.72 cents per kilogram (1.6 x 0.45 cents).

However, it's important to note that this study was conducted in 2015 and that technologies are now much more energy-efficient, especially for microwaves.

According to the study by M. Farizal Kamaroddin, Ameera Rahaman, D. James Gilmour, William B. Zimmerman, "Optimization and cost estimation of microalgal lipid extraction using ozone-rich microbubbles for biodiesel production" (2020), ozonation-based extraction is more cost-effective. This method involves microflotation harvest followed by ozone rupture in methanol. According to them, this method consumes only 36% of the energy produced once converted to biodiesel, whereas other solvent-based methods consume more than 90% of the energy produced. The cost is also more attractive; they estimate the cost of ozone at 0.06 cents for extracting lipids from 1 kg of dry biomass.

However, their study was conducted at a small scale and focused on an algae type without cell walls (*D. salina*). They estimate that microalgae with cell walls would consume more energy to extract lipids. They also base their estimate on industrial ozone pricing, which is more challenging to obtain.

Another study by Alpesh Mehta, Dr. Nirvesh Mehta, "Algae Biodiesel: A Futuristic Fuel for Power Generation and for Automobile Industries" (2023), suggests using slow pyrolysis to extract lipids and directly obtain biofuel from dry biomass. However, they do not provide any results regarding the amount of energy consumed by this process. For this method, they dried the algae using solar heat.

Another study by Xiaotong Zou, Kaiwei Xu, Wenjuan Chang, Yanhui Qu, Yanpeng Li, "Rapid extraction of lipid from wet microalgae biomass by a novel buoyant beads and ultrasound-assisted solvent extraction method" (2021), conducted on lipid extraction from the microalgae *C. Vulgaris*, provides interesting information. The method used is the "buoyant beads and ultrasound-assisted solvent extraction" method, in which the biomass was first harvested using buoyant-bead flotation. The microbeads are mixed with the microalgae biomass, allowing lipids to bind to the microbeads due to their affinity for hydrophobic substances. Once lipids are bound to the microbeads, the mixture is introduced into a water container where the microbeads with lipids naturally float to the surface due to their lightness.

The maximum harvest efficiency for *C. vulgaris* and *S. obliquus* was 92.47% and 83.77%, respectively.

After harvesting, the biomass was further treated by ultrasound, and a hexane:isopropanol (HIP) solvent was used for lipid extraction. This solvent has low toxicity and good lipid extraction efficiency.

The goal of this study is to develop an economical and efficient method for lipid extraction from microalgae, addressing the challenges of biomass harvest, lipid extraction, and transesterification in industrial microalgae processing.

The results show the efficiency of their method compared to others:

Table 1
Different extraction techniques for lipid extraction from microalgal cells.

Techniques	Species	Solvents	Volume ratio	Solid concentration (w/v %)	Conditions	Lipid yield (wt%)	Refs
Bligh and dyer extraction	Dry <i>Chlorella vulgaris</i>	Chloroform: methanol	1:2	3.45	Shaken during 3 min, 25 °C; centrifugation at 3500 rpm, 8 min, 4 °C	6%	[16]
	Wet <i>Chlorella vulgaris</i> / <i>cyanobacteria</i>	Wet <i>Chlorella vulgaris</i> / <i>cyanobacteria</i>	2:2:1.8	0.16	Mixed vigorously for 6 min, centrifugation at 2000 rpm, 20 min	8.3%	[19]
Soxhlet extraction	Dry <i>Aceutodesmus obliquus</i>	Ethanol	–	8	30 min	9.48%	[27]
	Dry <i>Aceutodesmus obliquus</i>	Hexane	–	–	30 min	4%	[27]
	Dry <i>Aceutodesmus obliquus</i>	Ethanol: hexane	2:1	–	30 min	12.05%	[27]
	Dry <i>Scenedesmus obliquus</i>	Hexane	–	10	24 h, 80 °C	20.60%	[9]
Ultrasound-assisted	Dry <i>Chlorella vulgaris</i>	Chloroform: methanol	1:1	0.05%	5 min (10 kHz), 360 MJ kg ⁻¹ dry mass	<10%	[28]
	Dry <i>Scenedesmus obliquus</i>	Hexane: isopropanol	4:1	10	89.21 min (400 W, 100 mL, 24 kHz), 25–30 °C; 210 MJ kg ⁻¹ dry mass	26.63%	[9]
	Wet <i>Chlorella vulgaris</i> / <i>cyanobacteria</i>	Hexane: cell suspension	1:1	0.16	30 min (750 W, 232 mL, 20 kHz); 581 MJ kg ⁻¹ dry mass	7.6%	[19]
		Methanol: chloroform: cell suspension	2:2:1.8	0.78		16.9%	
	Wet <i>Chlorella vulgaris</i>	Hexane: isopropanol	4:1	1.24	13.05 min (247 W, 30 mL, 20–25 kHz), 520 MJ kg ⁻¹ dry mass	18.75%	this study

It can be observed that the lipid yield is better than other methods. For dry biomass of *Scenedesmus obliquus*, the lipid yield is 26.63%, and 210 MJ per kilogram of dry mass were required.

In the case of wet biomass *Chlorella vulgaris*, the yield is 18.75%, and 520 MJ per kilogram were required.

100 MJ correspond to 2.77 kWh, considering that in Belgium the price of one kWh is estimated between 0.40 and 0.5 euros, 100 MJ equate to 1.24 euros (2.77 x 0.45 cents).

For the case of dry biomass of *Scenedesmus obliquus*, 266.3 grams of lipids were extracted from one kilogram of biomass, at a cost of 2.60 euros. This amounts to 9.77 euros per kilogram of lipids. Additionally, the costs of biomass drying need to be added.

For the case of wet biomass *Chlorella vulgaris*, 187.75 grams were extracted at a cost of 6.44 euros. The cost of one kilogram of lipids is 34.3 euros in this scenario.

These figures only represent the energy costs of the ultrasonic lipid extraction step; the costs of added solvents and all other steps in biodiesel production, such as biomass production, harvest, and final processing, must also be taken into account.

According to M. Farizal Kamaroddin, Ameera Rahaman, D. James Gilmour, and William B. Zimmerman, the energy required for centrifugation-based extraction accounts for 90% of the energy gained in biodiesel production.

In the case of mechanical pressing, 79% of the total energy is consumed.

Ultrasonication also requires approximately 110% of the energy gained in biofuel production. With this in mind, it is evident that a more economical method of lipid extraction from biomass needs to be found.

In summary:

Lipid extraction is a significant step in biodiesel production. Indeed, according to the study by Xiaotong Zou, Kaiwei Xu, Wenjuan Chang, Yanhui Qu, and Yanpeng Li (2021), it accounts for between 30% and 40% of the total production costs.

Among the analyzed methods, microwave radiation appears to be the most economically viable. The lipid recovery rate for this method ranges between 25% and 40%, and it consumes minimal energy (1.6 Wh/g), resulting in a cost of 72 cents per kilogram of extracted lipids.

It's important to note that the results are highly dependent on the type of algae and its state (wet or dry). Comprehensive research on different algae types and extraction methods is crucial to optimize this process, as it is currently not economically feasible.

Comparison with conventional diesel:

A study by Alpesh Mehta, "Algae Biodiesel: A Futuristic Fuel for Power Generation and for Automobile Industries" (2023), compares a fuel derived from microalgae with conventional diesel.

The results show that biodiesel outperforms in many areas:

Flash Point: The flash point is the temperature at which fuel vapors ignite upon contact with an open flame. The flash point of biodiesel is at 165 degrees Celsius, whereas it is only at 60 degrees Celsius for conventional diesel, making it more hazardous to handle.

Metal Corrosion: Conventional diesel forms sediments upon contact with copper and bronze. Biodiesel is much less corrosive to these metals.

Cetane Number: The cetane number is a measure of how easily the fuel will ignite in the engine. The cetane number for algae biodiesel is 58, which is significantly higher than diesel (46). This indicates that algae biodiesel could ignite readily and present minimal risk of engine knocking in a compression-ignition engine.

Ash Content: It's a measure of the amount of suspended solids and soluble organometallic compounds present in the fuel, which could potentially damage the fuel injection system and lead to abrasive wear of engine components (piston rings). The ash content of the obtained biodiesel is considerably lower, suggesting it could be a better substitute for conventional fuel.

In the following table, they compare the exhaust gas analysis of the obtained algae biodiesel and its compliance with the Indian emission standards (BS-IV) in effect since 2011.

Parameter	Obtained biodiesel (gram/kilometer)	Diesel emission norms BS-IV (gram/kilometer) w.e.f. 2011
Carbon Monoxide	0.14	0.5
Nitrogen Oxides	0.046	0.25
HC + NOx	0.046	0.30
Particulate Matter	0.022	0.025
HC	0.00	0.08

Table 4: Algae biodiesel- Exhaust gas analysis and comparison with BS-IV^[18-25]

It can be observed that the biofuel produced in this study has a significantly better composition than conventional diesel and can therefore be used in the automotive industry without issues.

Genetic modification :

According to several studies, including Raphael Slade*, Ausilio Bauen's research titled "Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects" (2012), genetic modification could be a potential option to increase lipid productivity in algae. It might also be relevant to make them more resistant to diseases or overexposure to light. However, these processes also come with risks, such as the possibility of these genetically modified microalgae interacting with other ecosystems. Leaks are inevitable in open pond production, while they are somewhat less likely in closed systems like photobioreactors.

According to the study by Kuan Shiong Khoo a,b, Imran Ahmad c, Kit Wayne Chew d, Koji Iwamoto c, Amit Bhatnagar e,* , Pau Loke Show, titled "Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review" (2023), genetic modification of microalgae aims to enhance both yield and quality of products, particularly lipids, to offset production costs. This approach primarily focuses on modifying metabolic pathways to overexpress or repress certain genes in order to achieve higher biomass and desired product yields. Genetic modification involves inserting a gene of interest from a donor organism into the genome of the recipient organism using vectors such as plasmids. DNA delivery techniques include electroporation, agitation with glass beads, the use of *Agrobacterium tumefaciens*, and particle bombardment.

Nevertheless, large-scale production of microalgae-derived products remains challenging due to upstream and downstream processing costs. Ongoing advancements and breakthroughs in research, such as genetic engineering and synthetic biology, could contribute to making the process/system economically viable. Genetic modification is a recommended approach for improving strains to enhance growth and lipid productivity of microalgae strains of interest, whether through direct or indirect modification.

Genetic modification can enable the development of strains that accumulate lipids without affecting biomass productivity and microalgae growth.

In summary:

Genetic modification of microalgae is being explored as an option to increase lipid production and enhance product quality, aiming to offset high production costs. This involves inserting genes of interest into the microalgae genome to overexpress or repress specific metabolic pathways, potentially leading to higher lipid yields. However, this approach comes with potential risks, particularly concerning its impact on ecosystems.

Despite these advancements, large-scale production remains a challenge, and further research is needed to make the process economically viable. Genetic modification may help develop lipid-rich microalgae strains without compromising growth and productivity.

Estimated costs from different sources:

During my research, I have come across relatively precise cost estimates in certain studies. Here are the findings:

According to the study by Kuan Shiong Khoo a,b, Imran Ahmad c, Kit Wayne Chew d, Koji Iwamoto c, Amit Bhatnagar e,* , Pau Loke Show, titled "Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review" (2023),

Table 11
The estimated cost of biodiesel produced by different sources.

Source	Cost (USD/L)
Open ponds	2.65
Open ponds	2.75
Closed photobioreactor	5.5
Closed photobioreactor	4.5
Petroleum diesel	0.7

According to the thesis titled "Biofuel from microalgae," assuming a rapid microalgae growth rate of about 10 days and considering that it would contain approximately 30% lipids, the cost of lipid recovery would account for 50% of the total production costs. The total production cost would amount to \$2.8 per liter. However, if algae containing 70% lipids could be produced, the price would drop to \$0.72 per liter of biodiesel.

According to the article "Carbon Capture and Utilisation in the green economy" by Peter Styring, the company HR BioPetroleum, now known as Cellana (<http://cellana.com/>), developed a low-cost hybrid system consisting of photobioreactors and open ponds. The results from the large-scale pilot project show that costs can decrease and were estimated at \$84 per barrel (in 2006). Considering that a barrel contains 159 liters of oil, this corresponds to a cost per liter of 52 cents.

European projects for microalgae-based biofuel production :

FUEL4ME :

The FUEL4ME project, lasting for 4 years, aimed to establish a sustainable continuous production chain for biofuels using microalgae as a production platform, enabling second-generation biofuels to compete with fossil fuels. The project, started on January 1, 2013, and completed on December 31, 2016, had a total budget of €5,369,514.10, with €4,014,981.50 coming from the European Union.

The main objectives of this project were as follows:

- 1) Convert the two-stage biomass production process into a single-stage continuous process with high lipid content (production process).
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- 2) Develop a continuous downstream process using all components of the algal biomass (conversion process).
- 3) Combine the production and conversion processes.

After establishing and proving the concept in controlled indoor environments, the continuous process was tested outdoors in four distinct locations under real production conditions (Netherlands, Israel, Italy, Spain). A continuous downstream process was developed, as well as research on biomass production. Finally, the entire process (biomass production and conversion to biofuel) was integrated and subjected to economic and life cycle analysis.

Results : Further improvements, however, need to be made to make the process of biofuel production with microalgae fully economic and environmentally sustainable. Currently the process is best suited for the production of high value products such as polyunsaturated fatty acids , while a promising biorefinery approach has been shown to strongly improve economic stability.

GIAVAP :

The GIAVAP project (Genetic Improvement of Algae for Value-Added Products) commenced in January 2011 and concluded on December 31, 2013. The project was allocated a budget of €7,184,970.60. Researchers employed genetic engineering approaches on various economically significant algae strains, with a focus on carotenoid and PUFA (polyunsaturated fatty acids) synthesis. Existing model algae strains were used to explore different cultivation technologies, along with lipid, carotenoid, and protein harvesting and extraction procedures. Additionally, the products were intended for investigation in energy, pharmaceutical, nutritional, or medical applications to assess the economic viability of manufacturing techniques and their commercialization.

Results: Seven species of microalgae were successfully genetically modified to enhance the production of polyunsaturated fatty acids, carotenoids, and high-value medical proteins. Large-scale cultivation, harvesting, and extraction techniques were developed and applied, showcasing the potential of these biomaterials in various fields such as health, agriculture, and aquaculture.

The project resulted in five patents related to algae genetic engineering and the publication of 32 scientific articles. The findings contributed to a better understanding of microalgae genetic engineering and biotechnology. These advancements offer prospects for sustainable large-scale commercial production of high-value-added products at competitive costs.

BIOFAT :

The BIOFAT project (Biofuel from Algae Technologies), which took place between 2011 and 2015, aimed to develop technologies for converting microalgae into biofuels. The project consisted of two phases: firstly, process optimization was carried out in two pilot facilities of 0.5 hectares each located in Portugal and Italy. Secondly, economic modeling and scaling-up to a 10-hectare demonstration facility were performed.

The pilot facilities (BPPP and BCPP) were equipped with cutting-edge technologies for microalgae cultivation and biomass production, such as tubular photobioreactors, vertical cultivation panels, algae ponds, and cascade ponds. The microalgae species used were *Nannochloropsis oceanica* and *Tetraselmis suecica*. The primary goal of BIOFAT was to maximize the benefits of algae while minimizing their environmental impact. The four-year project aimed to achieve an annual production of 900 tonnes.

The EU FP7 Energy program supported three projects assessing the technical viability of algae-based biofuels. These projects covered the entire production chain, from strain selection, cultivation, biomass production, oil extraction, biofuel production to testing in different modes of transport. These three projects required an estimated budget of \$42 million, with \$27 million contributed by the European Union.

Additionally, the All-gas project evaluated large-scale biofuel production by cultivating low-cost microalgae strains with municipal wastewater. InteSusAl focused on innovative technologies to produce biofuels by optimizing algae production through phototrophic and heterotrophic pathways.

Results: This techno-economic analysis (TEA) demonstrates that, with a productivity of 36 tonnes per hectare per year, *T. suecica* biomass can be produced at a cost of €12.4 per kg (dry weight). Using conservative assumptions, it was estimated that at a scale of 100 hectares, the cost would be €5.1 per kg. By locating the facility in more favorable climatic conditions (e.g., Tunisia), an annual productivity of 54 tonnes per hectare could be achieved, reducing the cost to €6.2 per kg at a 1-hectare scale and €3.2 per kg at a 100-hectare scale. The main cost factors are labor at the 1-hectare scale in Tuscany and capital expenses in all other cases. This TEA confirms that microalgae technologies have strong potential not only for high-value-added products but also for medium and low-value products, while biofuel, protein, food, and animal feed production currently seem out of reach. However, the global agricultural product scenario is rapidly evolving, and other factors (such as sustainability) in addition to pure economic evaluation will gain greater importance in the future.

SWOT analysis :

Throughout this thesis, we have observed the promising potential of algae, particularly due to their chemical composition rich in lipids and proteins. Algae have numerous applications, including cosmetics, food, biofuel production, and more.

In the context of this thesis, we are examining the process of biofuel production from algae and attempting to determine its economic viability.

SWOT Analysis:

STRENGTHS	WEAKNESSES
<ul style="list-style-type: none">- ease of Production- Abundant Availability of Required Resources- Environmentally Friendly (Reduction of CO2 Emissions)- Doesn't Require Arable Land- Ease of Implementation of this New Fuel- Job Creation	<ul style="list-style-type: none">- Production price- Energy Efficiency- Little Known to the General Public
OPPORTUNITIES	THREATS

<ul style="list-style-type: none"> - Advancements in Algae Genetic Modification - Advancements in Algae Production Methods - Advancements in Microalgae Harvesting Process Optimization - Advancements in Algae Lipid Extraction Optimization - Country's Self-Sufficiency in Fuel Production - Fuel Price Stability - Significant Contribution to Energy Transition 	<ul style="list-style-type: none"> - Price of Conventional Fuel - Counter lobbying - Dangerous reputation of algae - Legal ambiguity regarding the taxation of alternative fuels
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STRENGTHS :

Ease of Production: Algae are relatively primitive organisms that do not require demanding conditions for their production. As we have seen in this thesis, production can occur in the sea, open ponds, or photobioreactors, each with its advantages and disadvantages. They do not need "clean" water or cultivable land.

Abundant Availability of Necessary Resources: To grow properly, algae simply need nutrients naturally present in water, sunlight, CO₂, and water. Of course, productivity can be optimized by adjusting certain parameters and using fertilizers, but no "rare" resources are needed for production. Production is possible in all territories.

Ecological (Reduction of CO₂ Emissions): Algae have tremendous potential to decrease the concentration of CO₂ in the atmosphere. In fact, to grow, algae need about twice as much CO₂ as they emit once used. This means they can cleanse the atmosphere.

Does Not Require Cultivable Land: Unlike first-generation biofuels, algae-based biofuels do not require the use of cultivable land to grow. Moreover, maritime areas in Belgium and France would be more than sufficient for large-scale production in the case of macroalgae production.

Ease of Implementation of this New Fuel: Algae-based fuel is almost identical to traditional fuels in all aspects. Vehicles do not require modifications to adapt to this fuel.

Job Creation: Domestic biofuel production would create jobs for operating businesses and fuel transport. It would also stimulate the economy of photobioreactor and open pond production, as well as all the maintenance that entails.

WEAKNESSES:

Production Costs:

As we have seen throughout this thesis, currently, biofuel production is not profitable and is the central challenge in large-scale biofuel production.

The production cost of algae-based biofuel depends on many factors. There are primarily three crucial stages in biofuel production:

1. Production of (micro)algae
2. Harvesting of microalgae
3. Lipid extraction

Algae production :

Algae production remains relatively expensive. Both open pond and photobioreactor methods consume energy and entail maintenance and infrastructure costs.

Regarding productivity in photobioreactors, it is estimated between 0.41 and 1.12 grams of dry microalgae per liter per day. For open ponds, productivity is evaluated between 0.05 to 0.10 grams of dry biomass per liter of water per day, with a concentration of less than 1 gram per liter.

However, it's important to note that productivity varies based on the environment and the type of microalgae.

According to our research, the most cost-effective production method would be in open ponds, estimated to be between 1.60 and 1.80 euros per kilogram of produced biomass.

As for production in a natural environment, we lack precise information about overall costs. Nonetheless, we can assume they are significantly lower than closed production, given that algae grow in their natural habitat without additional energy needs. Moreover, large-scale

production is easily achievable. For these reasons, production at sea is, in our view, the most viable production method currently.

Harvesting Microalgae:

Due to their microscopic size, a specific method is required to extract microalgae from their growth substrates.

The gravity sedimentation method seems the most promising—it's energy-efficient and straightforward. However, there's limited information on large-scale application, and outcomes depend heavily on microalgae concentration in the solution. Lastly, this method is relatively time-consuming.

It's important to note that in the case of macroalgae production, this step would be significantly cheaper, as they don't require specific treatment (refer to lipid extraction from macroalgae document).

Lipid extraction :

Lipid Extraction is the final significant step in biofuel production.

Among the analyzed methods, microwave radiation appears to be the most economically viable. The lipid recovery yield rate for this method ranges from 25% to 40%, and it consumes minimal energy (1.6 Wh/g), resulting in a cost of 72 cents per kilogram of extracted lipids.

It's crucial to note that results heavily depend on algae type and condition (wet or dry). Comprehensive research on different algae types and extraction methods is necessary to optimize this process, as it is currently not economically viable.

In summary:

According to our research, studies are solely focused on the production of biofuel from microalgae. That's why we will prioritize this approach throughout the rest of the paper.

Cost calculations for microalgae-based biofuel production in the best-case scenario:

Microalgae production costs: 1.80 euros / kilogram

Microalgae harvesting costs: Not disclosed, but we were able to estimate that it represents 20% to 30% of the total production cost.

Lipid extraction costs: 0.72 euros / kilogram

In total, the production cost of one liter of biofuel from algae could, according to our best estimations, approach the price of 3.51 euros.

Calculations: $((1.80 + 0.72) / 70) \times 30 = 0.99$ (estimated costs of microalgae harvesting)

Total costs: $1.80 + 0.72 + 0.99 = \mathbf{3.51 \text{ euros}}$.

Even in the best-case scenario of our research, biofuel production is not currently profitable. Much research needs to be conducted, especially in the areas of production, harvesting, and lipid recovery.

There are also other very promising avenues to explore, such as genetic modification, which could significantly increase algae lipid production. It could also be utilized to weaken algae cell walls, thus making the lipid extraction process much easier and less costly.

Energy Efficiency:

Energy efficiency is generally positively correlated with economic profitability. If the production cost is so high, it's primarily due to the energy costs required by various production phases. If these phases were optimized to require less energy, both economic and energy profitability would be achieved simultaneously.

Little Known to the General Public:

The creation of biofuel is relatively unfamiliar to the general public, which can hinder its development.

OPPORTUNITIES :

As we have seen throughout this thesis, there are numerous limitations to biofuel production, but there is still a lot of research to be done that could greatly contribute to the profitability of production. These advancements can be made in production methods, harvesting and lipid extraction, as well as in genetic modification, which is still a relatively unexplored solution.

Energy Independence of a Country in Fuel Production: Another interesting opportunity would be to enable a country to achieve greater energy independence by producing its own diesel, or at least a portion of it.

Fuel Price Stability : Producing a portion of the fuel domestically would reduce the reliance on external fuel sources and could, in the long run, lead to a decrease in fuel prices, both at the national and European levels.

Strong Contribution to Energy Transition: Biofuel could serve as a solution to climate change. Algae, in particular, absorb a significant amount of CO₂ to grow, and the ocean is often referred to as Earth's "lung" thanks to algae. Algae absorb approximately twice as much CO₂ as they emit when used as fuel, making them a potential contributor to combating global warming.

THREATS :

Conventional Fuel Price:

Currently, according to the website GoCar.be, in Belgium, the price of a liter of gasoline or diesel breaks down as follows:

- 29.3% for crude oil
- 12.3% for margin and distribution costs
- 0.5% Apetra contribution (additional tax)
- 40.7% excise duties
- 17.3% VAT

As a result, compared to the final cost of 1.79 euros per liter, the cost of oil and its distribution amounts to only 0.7471 euros per liter. This price poses a significant threat to our environmentally-friendly fuel, as the government collects a substantial amount of money from this product.

Counter-Lobbying:

Even though due to climate urgency and international commitments like the Paris Agreement, European oil lobbies are facing increasing pressure to align with greenhouse gas emission reduction goals and transition to cleaner energies, they still exert considerable influence on decision-making institutions. Some NGOs accuse them of having spent over 250 million euros on lobbying between 2014 and 2019 (<https://www.20minutes.fr/planete/2635463-20191024-geants-petrole-depense-250-millions-lobbying-europeen-selon-ong>).

They could hinder the development of biofuels, mainly because the petroleum market is enormous and the profits are much lower compared to conventional fuels.

Final conclusion :

Throughout this thesis, we have explored the opportunities that algae offer due to their rich chemical composition of proteins and lipids (oils), making them highly relevant for fuel production, dietary supplements, cosmetics, and other products.

The production of fuel from algae is a well-known process today. Methods for cultivating, harvesting, extracting, and transforming algae into fuel have been studied for many years, and it is evident that this is a feasible process, both on a small and large scale.

Biofuel presents a perspective for combating climate change by harnessing the properties of algae, which absorb approximately double the amount of CO₂ during their growth compared to the emissions they release when used as fuel.

However, there are limitations to algae-based biofuel production, with the main challenge being the current production cost. In this thesis, we analyzed the various stages of biofuel production to identify the causes of high production costs. We identified three main steps:

1. Algae cultivation
2. Harvesting
3. Lipid extraction

Research conducted in the scope of this thesis has led us to the conclusion that currently, biofuel production from algae is not profitable. According to our estimates, in the best-case scenario, the price would be around 3 euros per liter of fuel. This is confirmed by other studies, which estimate the price to be between 2.65 and 2.85 dollars per liter (Kuan Shiong Khoo a,b , Imran Ahmad c , Kit Wayne Chew d , Koji Iwamoto c , Amit Bhatnagar e,* , Pau Loke Show, 2023).

The main challenge in microalgae biofuel production is that the price varies depending on numerous factors such as the type of algae, species, production location (different sunlight exposure, labor costs), production scale, production method, harvest and extraction method, etc.

Research at the European level has been conducted, including projects like FUEL4ME in 2016, GIAVAP in 2012, and BIOFAT in 2011. However, unfortunately, significant breakthroughs do not seem to have been achieved.

Another significant hindrance to the development of algae-based biofuel is the current price of petroleum. As we have seen, 58% of the pump price consists of taxes, thus generating revenue for the government. Therefore, the price of biofuel should be produced at less than 1 euro per liter to compete with conventional fuel.

Biofuel production is, however, very promising as a response to the ecological crisis, but it still requires extensive research.

Limits :

During the course of this thesis, I encountered several limitations:

First and foremost, the topic is incredibly vast, and research is far from complete. Many discoveries are yet to be made. Results vary significantly based on various factors, such as the type of algae or the methods employed. The possible combinations are countless, as one would need to experiment with all means of production, harvesting, and extraction, across different types of algae, and then assess the scalability of the system.

Numerous areas remain unexplored and require scientific exploration, which will take considerable time but holds great promise. For instance, advancements can be made in genetic modification. If we could produce an algae strain rich in lipids, with rapid growth and disease resistance, it could greatly enhance productivity. Additionally, algae species could be modified to have more fragile cell walls, optimizing lipid extraction. However, as we have seen, genetic modification has its limits, and it's crucial to prevent genetically modified species from entering our ecosystem.

The invention of new harvesting or lipid extraction techniques could also enhance profitability. For instance, we might discover an eco-friendly and cost-effective solvent in the coming years.

Furthermore, once promising discoveries are made, they must be adapted for large-scale production, which could yield unexpected outcomes, both positive and negative. This might lead to economies of scale but could also pose other challenges such as carbon supply.

Next, even if biofuel production becomes profitable, it needs to yield sufficient profits. Currently, fuel production is generally overshadowed by other avenues. Given its challenging profitability, companies often turn towards producing dietary supplements (such as omega-3, which can also be extracted from lipids) and food rather than fuel. I observed this trend in numerous companies, and it was also confirmed to me via email (Appendix 1).

Lastly, I faced significant difficulty in engaging with stakeholders and companies active in algae-based biofuel production. To be honest, I received very few responses, and the ones I did get were negative. People did not respond to numerous emails, and I also struggled to connect through LinkedIn.

In conclusion, creating a comprehensive thesis covering all aspects of microalgae-based biofuel production is challenging due to the vastness and current lack of exploration in the field. However, the limitations have been well-defined and can serve as a foundation for further research.

Recommendations :

I believe that biofuel production could become profitable in the long term and on a large scale. However, as explained in the previous chapter "limitations," this remains highly complex, and extensive research is still required in this field.

It would be interesting to explore further avenues in lipid extraction, particularly from macroalgae. As we have seen, the production of macroalgae in open sea conditions is particularly straightforward and productive. Therefore, conducting more research into lipid extraction from macroalgae could be valuable. Additionally, identifying macroalgae species rich in lipids would be intriguing.

Further research in the field of genetic modification of algae could also be worthwhile. This avenue could lead to the creation of an algae species that is highly lipid productive and grows rapidly.

Moreover, during our research, we observed that production in photobioreactors can be expensive due to the energy required for lighting in certain cases. However, microalgae can also grow in complete darkness, provided they receive glucose (sugar) supplementation. This approach could be explored to reduce the production costs of microalgae.

Next, studying potential state funding would be interesting. Even if production became profitable in the long run, generating substantial funds would remain challenging due to the very low cost of conventional oil (0.80 euros/liter before taxes and charges).

However, developing biofuel is, in terms of state revenue, contradictory. Financing the development of eco-friendly algae-based fuel would cost the state significantly, and afterward, it would deprive them of revenue from conventional oil (which accounts for 47% of the selling price). To match the tax revenue from biofuel to that of conventional oil, biofuel would need to be produced at 80 cents per liter, which is a considerable challenge at the moment.

Biofuel production is highly appealing from a sustainability and ecological standpoint, but in my opinion, it will never generate sufficient profits to be relevant in a capitalist context.

To conclude, biofuel production is technically feasible but currently not profitable. However, I believe that with the research and scientific advancements that will be made in the coming years, we will be able to produce an ecological and economical biofuel. This new fuel will have the ability to reverse the trend of climate change, as algae will absorb more CO₂ than they emit once transformed into fuels.

References :

Alami, A. H., Alasad, S., Ali, M., & Alshamsi, M. (2021). Investigating algae for CO₂ capture and accumulation and simultaneous production of biomass for biodiesel production. *Science of the Total Environment*, 759, 143529. <https://doi.org/10.1016/j.scitotenv.2020.143529>

Al hattab MA, Ghaly AE (2015) Microalgae Oil Extraction Pre-treatment Methods: Critical Review and Comparative Analysis. *J Fundam Renewable Energy Appl* 5: 172. doi:10.4172/20904541.1000172

Barber, J. (2003). Photosynthetic water splitting. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 358(1429), 245-260. <https://doi.org/10.1098/rstb.2002.1197>

Bassham, J. A., Benson, A. A., & Calvin, M. (1950). The path of carbon in photosynthesis. *The Journal of Biological Chemistry*, 185(2), 781-787.

Bekker, A., Holland, H. D., Wang, P. L., Rumble, D., Stein, H. J., Hannah, J. L., ... & Coetzee, L. L. (2004). Dating the rise of atmospheric oxygen. *Nature*, 427(6970), 117-120. <https://doi.org/10.1038/nature02260>

Blankenship, R. E. (2010). Early evolution of photosynthesis. *Plant Physiology*, 154(2), 434-438. <https://doi.org/10.1104/pp.110.161687>

Catling, D. C., Glein, C. R., Zahnle, K. J., & McKay, C. P. (2005). Why O₂ is required by complex life on habitable planets and the concept of planetary "oxygenation time". *Astrobiology*, 5(3), 415-438. <https://doi.org/10.1089/ast.2005.5.415>

Cuéllar-Franca, R. M., & Azapagic, A. (2015). Carbon capture, storage and utilisation technologies: A critical analysis and comparison of their life cycle environmental impacts. *Journal of CO₂ Utilization*, 9, 82–102. <https://doi.org/10.1016/j.jcou.2014.12.001>

El Shenawy, E. A., Elkelawy, M., Bastawissi, H. A.-E., Taha, M., Panchal, H., Sadasivuni, K. kumar, & Thakar, N. (2019). Effect of cultivation parameters and heat management on the algae species growth conditions and biomass production in a continuous feedstock photobioreactor. *Renewable Energy*. doi:10.1016/j.renene.2019.10.166

Govindjee, J. T. Beatty, & H. Gest. (Eds.). (2005). *Discoveries in photosynthesis (Advances in photosynthesis and respiration, Vol. 20)*. Springer.

Holland, H. D. (2006). The oxygenation of the atmosphere and oceans. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361(1470), 903-915. <https://doi.org/10.1098/rstb.2006.1838>

Kamaroddin, M. F., Rahaman, A., Gilmour, D. J., & Zimmerman, W. B. (2020). Optimization and cost estimation of microalgal lipid extraction using ozone-rich microbubbles for biodiesel production. *Biocatalysis and Agricultural Biotechnology*, 23, 101462. <https://doi.org/10.1016/j.bcab.2019.101462>

Khan, M. I., & Shin, J. H. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, 17(1). <https://doi.org/10.1186/s12934-018-0879-x>

Khoo, K. S., Ahmad, I., Chew, K. W., Iwamoto, K., Bhatnagar, A., & Show, P. L. (2023). Enhanced microalgal lipid production for biofuel using different strategies including genetic modification of microalgae: A review. *Progress in Energy and Combustion Science*, 96, 101071. <https://doi.org/10.1016/j.pecs.2023.101071>

Leliaert, F., Smith, D. R., Moreau, H., Herron, M. D., Verbruggen, H., Delwiche, C. F., ... & Bhattacharya, D. (2012). Phylogeny and molecular evolution of the green algae. *Critical Reviews in Plant Sciences*, 31(1), 1-46. <https://doi.org/10.1080/07352689.2011.615705>

Li, W., Yu, H., & He, Z. (2013). Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies. *Energy and Environmental Science*, 7(3), 911–924. <https://doi.org/10.1039/c3ee43106a>

Liang, Y., Sarkany, N., & Cui, Y. (2009). Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnology Letters*, 31(7), 1043-1049. <https://doi.org/10.1007/s10529-009-9975-4>

Mehta, A., & Mehta, N. (2018). Algae biodiesel: a futuristic fuel for power generation and for automobile industries. *Kalpa Publications in Engineering*. <https://doi.org/10.29007/dwkk>

Plaza, M., & Herrero, M. (2010). Seaweed proteins: biochemical, nutritional and functional aspects. *Trends in Food Science & Technology*, 21(10), 48-57. <https://doi.org/10.1016/j.tifs.2010.06.007>

Raven, J. A. (1997). Phagotrophy in phototrophs. *Limnology and Oceanography*, 42(7), 1299-1310. <https://doi.org/10.4319/lo.1997.42.7.1299>

Reinhardt, G. A. (2018). Algae based biofuels: Boon or bane? *Journal of Fundamentals of Renewable Energy and Applications*. <https://doi.org/10.4172/2090-4541-c1-053>

Schopf, J. William. (2006). Fossil evidence of Archaean life. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361(1470), 869-885. <https://doi.org/10.1098/rstb.2006.1834>

Shenawy, E. E., Elkelawy, M., Bastawissi, H. A., Taha, M., Panchal, H., Sadasivuni, K. K., & Thakar, N. (2020). Effect of cultivation parameters and heat management on the algae species growth conditions and biomass production in a continuous feedstock photobioreactor. *Renewable Energy*, 148, 807–815. <https://doi.org/10.1016/>

Slade, R., & Bauen, A. (2013). Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. *Biomass & Bioenergy*, 53, 29–38. <https://doi.org/10.1016/j.biombioe.2012.12.019>

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, 101(2), 87-96. <https://doi.org/10.1263/jbb.101.87>

Stengel, D. B., & Connan, S. (2015). Temporal variations in the phenolic composition of the brown seaweed *Ascophyllum nodosum* (Fucales, Phaeophyceae). *Botanica Marina*, 58(1), 47-58. <https://doi.org/10.1515/bot-2014-0025>

Styring, P., Jansen, D. (2011). Carbon Capture and Utilisation in the green economy (ECN) ISBN: 978-0-9572588-1-5

