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Microalgae production with rotating algal biofilm reactors for water treatment in land-based fish farms

Bachelor's project in Renewable Energy
Supervisor: Kristian M. Lien & Jacob J. Lamb
May 2020

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Preface

This thesis is written as the final part of the study program Renewable Energy under the Department of Energy and Process Engineering at the Norwegian University of Science and Technology (NTNU). The thesis leads to 20 credits in the subject TFNE3001 and the degree of bachelor, engineer.

The purpose of this report is to investigate whether a microalgae production system can replace a denitrification filter in land-based fish farms. Important points of evaluation have been area usage, energy demand and emissions, and the profitability of the implementation of a microalgae system.

We wish to thank our supervisors, Kristian M. Lien and Jacob J. Lamb, for always being available for guidance and advice throughout the semester. We are also grateful for the support from our external contacts, Kari Attramadal from Nofitech and Olivier Bernard from Inalve, for their valuable contributions. Also thanks to Sayed Ebrahim for sharing his simulation skills with great patience. Finally, many thanks to our friends and families for their feedback, and especially to Vilde Revold Olberg for her illustrations.

Through the time spent on this thesis we have evolved our research and problem solving skills, also in fields previously unexplored. In addition to interesting theoretical insights, we have had useful experiences regarding teamwork and project management. We hope the reader of our work will find it interesting and educational.

Vanja R Olberg — Vanja Revold Olberg

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Abstract

Denitrification filters are considered an unsustainable option for water treatment in land-based fish farms as they do not allow for the reuse of organic nitrogen compounds which are valuable in for example fertilizer production. They are, however, necessary when reducing the release of excess nitrogen into water bodies, thus mitigating the risk of harmful algal blooming and eutrophication. This paper evaluates microalgae production in Norway as an alternative to denitrification filters and whether it offers a more sustainable and profitable option.

Two different alternatives are considered in the analysis based on the cultivation method; Scenario 1 (S1) with artificial light and Scenario 2 (S2) with natural light. For cultivation, two different designs of the rotating algal biofilm (RAB) reactor are evaluated. A vertical and a triangular construction is used for S1 and S2, respectively.

To remove nitrogen corresponding to 70% of production from a module, the microalgae system must produce 166.8 tonnes of dry weight microalgae biomass, which can be sold as a paste or for further processing, generating profits of over 40 MNOK for both scenarios. The system also reduces the emission of CO_2-eq by 710 tonnes when excluding construction and electricity consumption. To achieve this, S1 demands an area of 6,178 m^2 while S2 requires 8.537 hectares. When considering the impact of a large footprint area on ecology and the increasing demand for land areas for biofuel production, S1 is the preferred alternative. However, S1 consumes more electricity due to the cultivation lights, which also lead to larger costs compared to S2. According to the literature and the results in this thesis, both scenarios are potentially profitable, and when considering the client's goal of a compact system which, if possible, breaks even economically, Scenario 1 with artificial light is the optimal choice.

Sammendrag

Et denitrifiseringsfilter kan ansees som et lite bærekraftig alternativ til vannbehandling i landbaserte fiskeoppdrettsanlegg siden det ikke tar vare på organiske nitrogenforbindelser som er verdifulle i for eksempel gjødselproduksjon. De er likevel nødvendige for å redusere utslipp av overflødig nitrogen i naturen, noe som reduserer risikoen for skadelig algeoppblomstring og eutrofiering. Denne rapporten evaluerer mikroalgeproduksjon i Norge som et alternativ til denitrifiseringsfiltre og om det kan være et mer bærekraftig og lønnsomt alternativ.

To forskjellige alternativer evalueres i analysen basert på kultiveringsmetode, nemlig Scenario 1 (S1) med kunstig lys og Scenario 2 (S2) med naturlig lys. For kultiveringen blir to forskjellige design av en reaktor med roterende biofilm evaluert. En vertikal og triangulær konstruksjon blir brukt for S1 og S2, henholdsvis.

For å fjerne nitrogen tilsvarende 70 % av produksjon fra en modul må mikroalgesystemet produsere 166,8 tonn med tørrvekt mikroalge biomasse som kan bli solgt som pasta eller til videre prosessering, noe som fører til lønnsomhet på over 40 MNOK for begge scenarioer. Systemet reduserer også utslipp av CO_2-ekv på 710 tonn, ekskludert konstruksjon og elektrisitetsbruk. For å oppnå dette krever S1 et areal på 6178 m^2, mens S2 behøver 8537 hektar. Når en tar i betraktning effekten av et stort fotavtrykk på økologi og det økende behovet for landareal til biobrenselproduksjon er S1 det foretrukne valget. Derimot krever S1 mer elektrisitet på grunn av kultiveringslysene, noe som også fører til høyere kostnader sammenlignet med S2. Ifølge litteraturen og resultatene i denne rapporten er begge scenarioene potensielt lønnsomme, og når man tar i betraktning oppdragsgivers mål om et kompakt system som, om mulig, går i null økonomisk sett, er Scenario 1 med kunstig lys det optimale valget.

Contents

List of Abbreviations

Abbreviation	Description
CAPEX	Capital Expenditures
DW	Dry Weight
GHG	Greenhouse Gases
HAB	Harmful Algal Blooming
OPEX	Operational Expenditures
PE	Photosynthetic Efficiency
RAB	Rotating Algal Biofilm
RAS	Recirculating Aquaculture System
TAN	Total Ammonia Nitrogen
TN	Total Nitrogen
WW	Wet Weight
WWT	Wastewater Treatment

Glossary

Word	Meaning
Aerobic	Organisms that require the presence of oxygen to live.
Anaerobic	Under anaerobic condition, no molecular oxygen, and no oxidized nitrogen species (e.g. nitrite, nitrate) are present.
Anoxic	Under anoxic conditions, no molecular oxygen is present, but nitrite/nitrate is.
Biofilm	A thick layer of prokaryotic organisms in a colony.
Denitrification	The process of reducing nitrates/nitrites that usually results in the escape of nitrogen into the air. Commonly performed by bacteria
Eutrophication	When a body of water becomes enriched in dissolved nutrients that stimulate the growth of algae usually resulting in the depletion of dissolved oxygen.
Heterotroph	An organism that cannot produce its own food, and will instead take nutrition from other sources of organic carbon, mainly plant or animal matter.
Mixotroph	An organism that can act as both a heterotroph and autotroph.
Nitrification	Oxidation of ammonia to nitrite and of nitrite to nitrate.
Nitrogen fixation	When nitrogen gas from the atmosphere is converted to nitrogenous compounds which can be stored in biomass.
Oxygen cone	Oxygen cones are used to enter and mix oxygen in the water for the fish.
Photoautotroph	Organisms that can make their own energy using light and carbon dioxide via the process of photosynthesis
Photobioreactor	A photobioreactor (PBR) is a bioreactor that utilizes a light source to cultivate phototrophic microorganisms.
Photoinhibition	Light-induced reduction in the photosynthetic capacity plant, alga, of a or cyanobacterium
Photosynthetic	Relating to or involved in the process by which green plants and some other organisms use sunlight to synthesize nutrients from carbon dioxide and water.

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1 Introduction

1.1 Thesis Statement

The number of land-based fish farms globally is increasing and thus also the release of wastewater into nature [1]. This creates the need for a sustainable treatment option. Environmental challenges today include a continued increase of greenhouse gas (GHG) emissions, a rising demand for food production and an enrichment with nutrients to marine habitats, causing a disrupting effect on ecosystems [2]. In recent years, microalgae has attracted attention due to their diverse uses in different sectors. Through photosynthesis, microalgae fixate CO_2 from the atmosphere, while it consumes potentially harmful chemicals [3]. As a sustainable and renewable resource, microalgae can help solve global challenges both by exploiting available nutrients, and providing microalgae biomass that can be utilized for energy production or in food or the health sector. This thesis investigates the possibility of using microalgae for water treatment.

1.2 Nofitech and Inalve

Norwegian Fishfarming Technologies AS (Nofitech) designs modules for land-based fish farming. Like all industries, fish farms produce emissions, but Nofitech wishes to reduce these following the increasingly pressing situation regarding environmental challenges.

Nitrogen removal from wastewater by denitrification filters might be considered unsustainable as it converts reactive nitrate to non-reactive nitrogen gas, rather than exploiting the valuable nitrate. Wastewater is too high in salinity to be used directly as fertilizer for plants, however, many microalgae species thrive in high salinity environments. Thus, growing microalgae using nutrients from wastewater will create a circular economy by combining microalgae production and wastewater treatment (WWT).

For the microalgae cultivation, technology and data provided by the French innovation company Inalve will be used as it has proved efficient in production. The solution for this thesis case will therefore be based on a case provided by Nofitech and technology provided by Inalve.

1.3 Scope of Thesis

This thesis intends to answer the following research question: In what way can microalgae production act as an alternative to denitrification filters for water treatment in land-based fish farms? This also raises the following question: Does the solution offer a more profitable and sustainable option than the denitrification filter?

The perspective when answering the research questions will be regarding the functionality of the microalgae system. Nofitech ultimately wants a solution which is compact, sustainable, and preferably, breaks even or profits economically. A precondition for this thesis was to use Inalve's microalgae cultivation technology to reach such a solution. These factors will be kept in mind when evaluating the microalgae species, design and technology,

implementation of the system in the existing module, light and water provision, energy consumption, and economic aspects.

1.4 Outline of Thesis

The thesis consists of eight sections. This first section introduces the background for the research question. Section 2 provides a theoretical basis on eutrophication and regulations linked to emissions, Nofitech and their current fish farm modules, microalgae and culturing systems for microalgae growth, and some theoretical background on commercial production and sale of microalgae. The 3rd Section presents the different scenarios where the microalgae system is implemented into the Nofitech fish farm and also shows how laboratory work can complement the report with its results. In Section 4 all results from the study are presented. Section 5 consists of an economic analysis of the current fish farms and compares the two scenarios. In Section 6, both the results and economic analysis are discussed. A conclusion of the discussion is presented in Section 7. Further work, and expectations on this are in Section 8. The appendices in an attachment at the end of the thesis provide supporting information on the subjects evaluated.

2 Theory and Literature

To answer the research question presented in Section 1.3, relevant literature must be collected and evaluated with the most important findings presented in this section. This includes an introduction of regulations on water contamination, land-based fish farm modules in Norway with corresponding water treatment options. Finally, the section presents microalgae cultivation, production, and market potential.

2.1 Eutrophication and Regulations

Municipal wastewater and wastewater associated with food production typically contain high levels of phosphorus, and nitrogen in the form of ammonia, nitrites, and nitrates, which can be harmful when released into nature. Eutrophication is when a water body is overly enriched with nutrients, and it leads to harmful algal blooming (HAB) [4]. Algae is an important basis in a balanced ecosystem, and growth is managed by the organisms consuming it. However, during HAB the food chain cannot keep up with the growth. Algae trapped under the new growth are depleted of nutrients and sunlight, die, and sink to the bottom of the water body. Here, bacteria break them down while consuming oxygen, depleting the water body to such a degree that it can kill other organisms like fish or amphibians, leading to aquatic dead-zones. Although eutrophication can occur naturally, cultural eutrophication is when humans speed up the process by introducing large discharges of nutritious wastewater. Due to both climate change and cultural eutrophication, HABs have been appearing more frequently in the last few decades [5–7]. Some HABs also produce toxins that can be harmful to humans and aquatic organisms.harm human health and other organisms such as fish and molluscs [8, 9]. Regulations of wastewater treatment are therefore necessary when protecting the environment, especially during summer when the risk of algal blooming is higher [10].

Fish farms are the biggest contributor to anthropogenic discharge of nutrients in waters in Norway, as of 2017 [11]. In addition to the fish farms, high nutrient content in the coastal waters of Norway is partly caused by ocean currents, transporting nutrients from other European countries. International efforts, including stricter regulations in Europe as a whole, could ,therefore, be beneficial for Norwegian waters. Regarding nitrogen removal requirements in Norway, each land-based fish farm is evaluated individually based on nitrogen production and location and recipient sensitivity. For example, the land-based fish farm Sørsmolt AS, which was required to remove 20% of their produced nitrogen, removed only 8% [12]. Regulations from environmental authorities regarding WWT in fish farms are becoming stricter [13]. This is especially true for farms applying for renewed concessions or expansions, as the requirements seem to follow those for municipal wastewater by Norwegian law. It specifies a nitrogen removal rate of 70% and phosphorous removal of 90% from municipal wastewater in densely populated areas [14]. With potentially stricter regulatory framework being introduced in the near future affecting treatment requirements, the necessity of a system dedicated to nitrogen removal is becoming increasingly apparent. Through filtrating larger particles, it might be possible to remove from 7 to 32% of nitrogen, but more extensive treatment is achieved through chemical and biological processes,

with the most common option being denitrification filters [13, 15].

2.2 Nofitech

The number of land-based fish farms globally is growing as the demand for salmon increases [1, 16]. An economic analysis done on a fish farm producing large smolt estimated a total investment of 500 MNOK for a fish farm with 3,000 metric tonnes (t) production capacity [17]. A Nofitech fish farm module has a production capacity of around a third of this [18]. Based on this, an estimate for the investments required for a Nofitech fish farm, is approximately 100-150 MNOK, based on produced fish. As in all industries, there is a pressure to develop new approaches that lead to reductions in costs and allow for increases in efficiency, product quality, and, especially in recent years, sustainability. The Recirculating Aquaculture System (RAS) is a land-based module which reuses its water to a varying degree, depending on the number of treatment steps involved, as opposed to more traditional flow-through modules that do not recirculate water [19]. The new "ModulRAS" designed and produced by Nofitech is supposed to yield a more efficient and sustainable production with better conditions for both fish and employees. About 99.2% of the RAS water is recycled within the system, minimizing the influent and effluent water volumes [18]. This percentage is based on the total nitrogen (TN) level in the water, which can be found in Table 1, among other limiting factors for the living conditions of the fish. Some common values are presented in Table 2. [18, 20]

Table 1: Constraints of different parameters for water in a fish tank. [18]

Parameter	Constraints
CO_2 [mg L^{-1}]	< 15
TN [mg L^{-1}]	40-100
TAN [mg L^{-1}]	< 2
Alkalinity [mg L^{-1}]	60-90
Oxygen saturation [%]	80-100
Salinity [ppt]	< 15

Table 2: Typical values for different parameters for water in a fish tank. [18]

Parameter	Average value
TN [mg L^{-1}]	70
TAN [mg L^{-1}]	0.3
pH	7.2
Temperature [°C]	14

To achieve a high recycling percentage in the RAS, the module water is treated in several steps, as illustrated in Figure 1 [18]. First, the water from the fish tanks is lead to the drum filter. The drum filter extracts large particles and sludge, consisting primarily of feces and uneaten fish feed from the tank. In the biofilter nitrifying bacteria convert ammonia to nitrate, which is less harmful to the fish and is therefore accepted in larger concentrations.

The hydraulic retention time in the fish tanks is one hour, meaning that the full 4,600 m^3 in the tanks are exchanged every hour, and is decided by the CO_2 concentration. An important step in the treatment process is therefore the removal of CO_2 in the aeration filters. Then the percentage of water that is not recycled is lead out of the system as wastewater and typically dumped in the ocean. Finally, the water is put back into the tank after being saturated with O_2 through the oxygen cones.

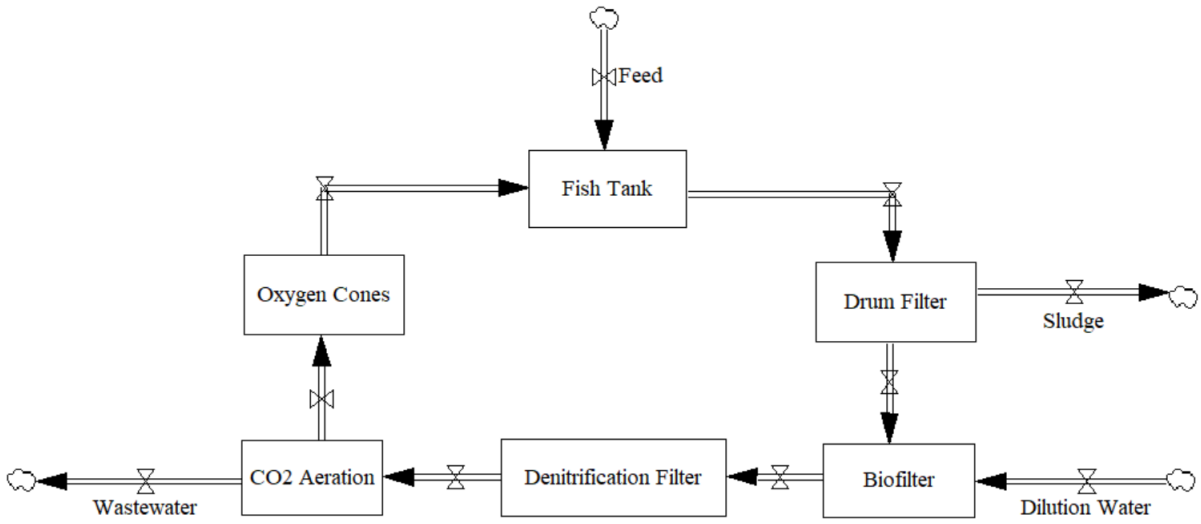

Figure 1: Simplified illustration of the RAS module system. [18]

As mentioned, the recycling percentage of the RAS module is regulated based on the nitrogen concentration in the water. Nitrogen accumulates indirectly through the fish feed. A certain percentage of nitrogen is stored in the fish biomass, while the rest is excreted through the gills or as feces. The feed not consumed by the fish is broken down by bacteria on the module floor. An estimated 3-4% of the feed is released as nitrogen in the form of TAN to the water, meaning that the dilution water amount is based on feed added to the modules. For Nofitech, the amount needed to keep nitrogen levels below the constraint is 300 L kg^{-1} fish feed, which reaches 3,000 kg d^{-1} at maximum capacity [18]. Most Norwegian fish farms keep their recycling percentage low enough, for example below 99.7%, to avoid concentration issues with nitrogen, but with a higher recycling percentage, the nitrogen must be removed from the water [15]. The most common solution is a denitrification filter.

2.3 Denitrification Filter

In a denitrification filter, bacteria reduce nitrate to nitrogen gas, which can be released from the water and into the atmosphere. The denitrification process may produce nitrous oxide (N_2O) as a by-product, but in wastewater engineering it is common to assume that all nitrate becomes nitrogen gas. Organic forms of nitrogen are required for the production of artificial fertilizer, a process that consumes over 1% of the world's energy demand [21]. In this process, nitrogen fixation is performed to convert nitrogen gas to ammonia by adding hydrogen. Ammonia is available in the fish tanks, but instead of using this directly

for fertilization, it is converted to nitrate through nitrification in the biofilm and then to nitrogen gas in the denitrification tank. The fish tank water cannot be used directly on crops due to the high salinity [18, 21].

The processing steps connected to a denitrification filter as opposed to using the ammonia directly can be separated into three steps; conversion of ammonia to nitrate in the biofilter, nitrate to nitrogen gas in the denitrification filter, and nitrogen gas to ammonia in fertilization production. The emissions associated with the biofilter is estimated at 5.93 kg CO_2-eq per kg of ammonia converted to nitrate [18]. The emissions associated with the denitrification process, i.e. converting nitrate to nitrogen gas, is approximately 1.4 kg CO_2-eq per kg of methanol used [22]. Finally, the emissions associated with ammonia production is approximately 2.867 kg CO_2-eq per kg of ammonia produced [23]. All these figures are presented in Table 5 in Section 3.3.

The energy consumption of the denitrification filter consists of the methanol catalyst and a small amount of electricity. Due to its favorable chemical properties, methanol is the most common catalyst where the required amount is approximately 2.9 kg methanol per kg nitrate converted to nitrogen gas[22, 24]. Based on the last three years, the average methanol price in Europe is 402 USD t^{-1}, or 3,537 NOK [25]. The electricity required is approximately 0.197 kwh per kg of nitrate converted to nitrogen gas for a filter with a capacity of 37.85 million liters per day [22, 26]. This is about a third of the water stream in Nofitech's module, but since the correlation between electricity demand and filter capacity is unclear, it is assumed that the electricity demand applies for the Nofitech module. The energy demand of a denitrification is summarized in Table 3.

Table 3: Energy consumption by a denitrification filter. [22, 25]

	Ratio
Methanol demand [kg kg^{-1} NO_3^--N]	2.9
Methanol cost [NOK t^{-1}]	3,537
Electricity demand [kWh kg^{-1} NO_3^-]	0.197

Due to the potential reduction in energy consumption and environmental benefits, there is a general interest in finding a solution that makes use of valuable nutrients like ammonia and nitrogen instead of releasing them into the air or nature. The Swedish company EasyMining received 19 million Swedish kroner from EU's LIFE program for their pilot project which extracts nitrogen from wastewater which then can be used for fertilization [27]. Another interesting option is using microalgae production, as the algae can thrive in saline conditions and consume nitrogen, storing it in their biomass which can later be used for other purposes. This would reduce the CO_2 emissions associated with the denitrification filter.

2.4 Microalgae

Microalgae are oxygenic photosynthetic microorganisms, meaning they produce oxygen gas by consuming light photons and carbon dioxide. They thrive in both saline and freshwater

environments and have a potential for rapid growth in the right cultivation culture. Consequently, different microalgae species, as well as strains of one specie, can show diverse behaviors. The optimal growth conditions can, however, be challenging to establish, as they depend on a variety of factors like availability of illumination, nutrients, temperature, pH, and salinity. These factors will be discussed individually in this section.

As the sole energy source for photoautotrophic microalgae, the light irradiance has a great impact on productivity. The photosynthetic efficiency (PE) is a measure of how much of the energy from solar irradiance is converted and stored as chemical energy in the microalgae biomass. It is commonly assumed to be 4-6% but can be increased by altering the growth conditions [28, 29]. Hetero- and mixotrophs generally show a higher PE than autotrophs [30]. PE can also drastically decrease due to photoinhibition, a phenomenon occurring when the microalgae are overexposed to irradiance and the excess of photons damages the cells and halts production [28]. The risk of photoinhibition can be mitigated by avoiding constant and high-intensity irradiance. Due to photoinhibition, the microalgae growth rate is only linearly correlated to irradiance up to a certain point [31]. In addition to light intensity, color can also affect productivity. Microalgae show the highest productivity when exposed to wavelengths on the red and blue side of the spectrum [32], and pink light is considered to increase growth rates [33]. By only including wavelengths absorbed by the microalgae, the amount of irradiance needed can be reduced.

When it comes to microalgae cultivation with artificial lighting, photosynthetically active radiation (PAR) efficiency is important. It is commonly used when estimating the electricity demand as this represents how much energy from the outlet is available to the microalgae for storage in the biomass. The PAR depends on the wavelength of the light and is higher for blue light and lower for red. Microalgae grow more efficiently in red light because they absorbs photons with this wavelength. For high power red LEDs the PAR efficiency is assumed to be 2.6 µmol-ph s^{-1} W^{-1}. For white light it is currently about 1.9, while it is estimated to reach 3.0 in the future. [29]

The chemical composition of microalgae depends on the microalgae species and cultivation conditions, meaning that the proportion of for example lipids, proteins or carbohydrates in the biomass can vary widely [34]. The calorific value, or energy density, depends on these values, but for microalgae with low oil contents, the energy density can be estimated to 17 MJ kg^{-1} of dry weight (DW) biomass [35]. The nitrogen content of microalgae does vary a bit but is assumed to be 10% on average in this thesis [36–38]. It is assumed that all nitrogen fixed by the microalgae is stored in the biomass, meaning that the fixation rate of nitrogen is estimated to be 10% of the growth rate.

Microalgae primarily need the nutrients carbon (C), nitrogen (N), and phosphorus (P) to grow, in addition to various trace elements like metals [39]. Autotrophic microalgae can grow without an organic carbon source, needing only CO_2, as opposed to heterotrophs. Mixotrophic organisms can grow with both organic and inorganic carbon sources. Typical N sources are nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), or ammonium (NH_4^+), while P can be found for instance in phosphate (PO_4^{3-}). As mentioned, the amount of nitrogen fixed

by the microalgae can be predicted as 10% of growth. The corresponding amount of carbon and phosphorus can be predicted with the Redfield ratio C:N:P, which is approximately 112:16:1, although this can vary greatly on growth conditions and microalgae species [35, 40].

Nutrient availability can alter productivity greatly and has been explored in several studies [41, 42]. A study in Florence, Italy, showed promising results on increased productivity and photosynthetic efficiency of outdoor cultures of the microalgae species *Tetraselmis Suecica* in annular columns [41]. They report an overall footprint area production of 38.2 g m^{-2} d^{-1} and a PE of 9.4% on average. This was achieved by adding CO_2 as an additional carbon source for the microalgae. The addition of CO_2 also helped regulate the pH. The optimal temperature determined through testing was 27°C, and the pH level in the water was approximately 8.0. Another study, in Wageningen, the Netherlands, also use *T. Suecica* for testing, here grown in tubular photobioreactors on wastewater from a sh farm [42]. Both productivity and nitrogen and phosphorous fixation rate was improved by adding phosphate ions (PO_4^{3-}). The reason was to make the ratio of C, N, and P closer to the Redfield ratio, which is supposed to increase growth.

The optimal temperature and pH for microalgae cultivation depends on the species, as microalgae grow in all parts of the world, but for the species popular for production the optimal temperature usually is between 15 and 30°C and the pH level is normally between 7 and 9 [43–45]. *T. Suecica* can survive in temperatures between 2 and 34°C [46], but the optimal temperature seems to be close to 20°C, although this depends on conditions like pH level, salinity, and type of reactor [47–49]. Another species, *Phaeodactylum Tricornutum*, also has an optimal growth temperature of 20°C, based on a general consensus [50–53]. Research on *T. Suecica* and *P. Tricornutum* claims an optimal pH level of 7.5 and 7.8, respectively [54, 55]. If the pH level deviates too far from the optimal value, the productivity will decrease and the microalgae might even die [56].

In addition to research on all the parameters discussed above, a very important factor when it comes to microalgae growth is the cultivation system. Since the 1950's, two types of microalgae culture designs have dominated the sector [57]. One of them is the closed photobioreactors, growing the microalgae in cylindrical reactors providing a sterile environment while obtaining a maximum surface to volume ratio to absorb sunlight [58]. The other type is called an open pond system, which is more exposed to contamination, but often preferred to the closed photobioreactor because it is cheaper [59]. They are both still used today all around the world, but during the last decade, a new way of growing microalgae has been introduced, the Rotating Algal Biofilm (RAB).

2.5 Rotating Algal Biofilm

The RAB is supposed to maximize microalgae productivity through its innovative design. It consists of a conveyor belt which is placed in a pool of cultivation water, letting it move through both water and air, as shown in Figure 2. The belt is made of a specific type of cotton where a biofilm of bacteria can grow [60]. A biofilm is defined as a thick layer of

microorganisms that have aggregated to form a colony [61]. The microalgae can grow on the biofilm, and be sequentially exposed to air and water as the conveyor belt moves, as illustrated in Figure 2. By scraping off the microalgae biomass frequently, the film can be kept thin and thus ensure better access to light and CO_2 in the air and to the nutrients from the water. The light and dark cycle created by the movement also mitigates the photoinhibition risk. Most likely due to these factors, the RAB has shown to be more productive than other algae production methods [62]. It also allows for easier harvesting by scraping off the excess algae produced about once a week, which can be done manually or with machines. The biomass harvested has a water content of about 80-90%, which is similar to microalgae harvested from traditional production after centrifuging treatment, meaning the de-watering step of biomass growth is not required [62, 63].

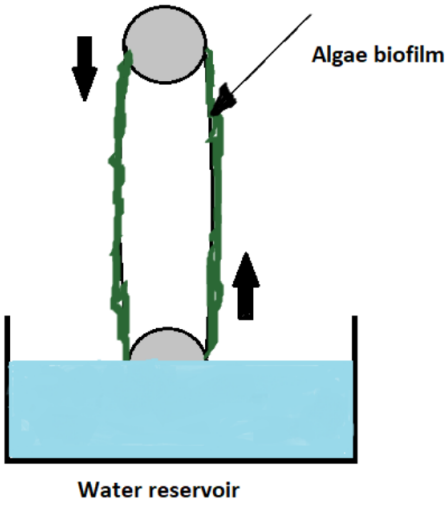

Figure 2: Concept of rotating algae biofilm. Designed by Simon Bjerkan Steinvoll, 2020.

The RAB design of the French innovation company Inalve is presented in Figure 3. With its 45° angled surface it is optimized for natural light exploitation. The biofilm surface area to footprint area ratio is about 3. For outside testing with their own strain of *Tetraselmis Suecica* in natural light Inalve has achieved an annual production of approximately 20-30 t of DW algae biomass per hectare (ha). In controlled laboratory conditions with an approximate temperature of 20°C and irradiance of 400µmol m^{-2} s^{-1} the results were 54-72 t ha^{-1} per year (y^{-1}), or about 60 as an average, although they are expecting to reach a production of 100 t ha^{-1} y^{-1}. The microalgae cultivation water used for this laboratory testing is based on the recipe for F/2 medium [64]. F/2 medium is a common and widely used general enriched seawater medium considered optimal for microalgal growth. In Inalve's laboratory testing, the water temperature is kept at an average of approximately 20°C [63, 65].

The RAB design used for microalgae production by Gross Wen Technologies is built vertically as illustrated in Figure 4. This comprises a large area of biofilm on a relatively small footprint area, making it space-efficient, but if built too tall there will be mutual shading by the adjacent belts. Using artificial lighting whose intensity can be freely altered

based on the biofilm area will avoid this issue as a conveyor belt ensures illumination on all surfaces. From the first pilot testing, the RAB productivity was 300% more efficient than the traditional raceway pond [60]. [62, 66]

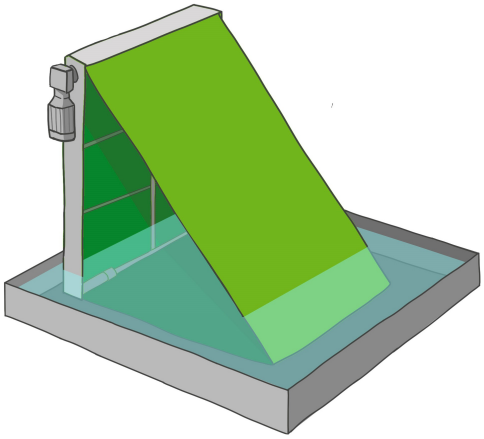

Figure 3: Triangular RAB design. Designed by Vilde Revold Olberg, 2020, based on [63].

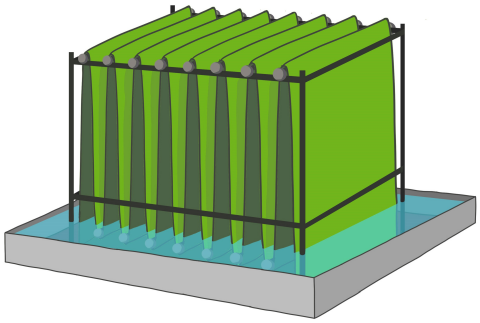

Figure 4: Vertical RAB design. Designed by Vilde Revold Olberg, 2020, based on [62, 66].

2.6 Commercial Production and Market Potential

When it comes to commercialized microalgae production, there are several examples of successful projects. They have to produce either very high-value products for nutraceutical purposes, or the production costs have to be very low, if used as a low-value product (e.g. fish larva feed or biofuel) [67]. It is commonly viewed as more profitable to use natural lighting for production instead of artificial lighting due to a high electricity consumption that makes the production costs increase [28, 29]. Producing high-value products requires extensive processing and treatment, available area for cultivation, and a product that can compete on the global market.

Astaxanthin and β-carotene are examples of high-value caretenoids, that has been commercially produced from the microalgae *Haematococcus Pluvialis* and *Dunaliella Salina* respectively, since the 1980s [68]. Due to high production costs, only a few of the producers have continued [68]. These two caretenoids can serve as important ingredients in the nutraceuticals, cosmetics, and food industry [69]. Depending on purity, the market value of astaxanthin can vary between 22,000 and 132,000 NOK per kg DW[69]. One of the reasons both β-carotene and astaxanthin from microalgae can compete on the global market, is that they are natural, which gives them an advantage over the synthetic alternatives in relation to the increasing demand and the higher selling price [70, 71]. *H. Pluvialis* and *D. Salina*, each has over 90%, astaxanthin and β-carotene respectably from their total caretenoid content, while other microalgae have a content of at best 70% of total caretenoids, which requires extensive processing if they are to be used in production [68]. A third microalgae species that also shows promise in connection with a high-value caretenoid, is *P. Tricornutum* [72]. It is a naturally rich source of fucoxanthin, a caretenoid with anti-oxidant effects [72, 73]. Studies conducted in France and Australia show encouraging results for *P.*

tricornutum as a natural source of fucoxanthin in nutraceutical applications [74]. Algatech, a company based in Israel, recently produced the first and only microalgae-derived fucoxanthin product on the market [75]. Normally, extractions from harvested seaweed is the way to obtain fucoxanthin. Seaweed contains approximately 0.01% fucoxanthin, compared to *P. tricornutum* that contains more than 1% [73]. The market value for 1 kg of pure fucoxanthin is estimated to be 352,000-704,000 NOK [76].

It is common to combine microalgae production with municipal or agricultural wastewater treatment as this provides "free" nutrients like carbon dioxide, nitrogen and phosphorus. Gross-Wen Technologies has replaced the municipal WWT plants in several American cities and claims to reduce costs with this solution [66, 77]. Similar interest has been shown by the Norwegian biofuel production company Biokraft, who owns the world's largest liquid biogas production factory [78]. In 2019 they cooperated with students from NTNU on microalgae production on the by-products from their production [35]. A similar option is to combine microalgae production with WWT for land-based fish farms like the ones Nofitech produces, as this serves two purposes and thus should decrease total costs.

After using the microalgae production for WWT, the harvested biomass can be used in a range of products. As a more environmentally friendly alternative to fossil fuels, microalgae can be used for biofuels. The International Energy Agency (IEA) states that by 2030, biofuels should make up 10% of the total global fuel consumption as part of the goal to reduce GHG emissions [79]. To achieve this, biofuel production must increase with 10% every year, which would require subsidies as production costs are too high for biofuels to compete with the low market price of the alternatives [80]. One problem with biofuel production is the requirement for fertile fields which could be used for food production of planting forests [81]. Microalgae production would have a similar effect as planting trees as it fixates CO_2 from the atmosphere in biomass.

Selling the microalgae as a paste without any further processes other than drying is also a possibility, like the recently developed product Juan Algae Paste, made by the University of the Philippines-Visayas College of Fisheries and Ocean Sciences (UPV-CFOS) and the UPV Museum of Natural Sciences [82]. It is a product primarily meant as feed in early stages of fish larvae and crustaceans. However, it can also be sold to actors wishing to further process it for fertilizers or livestock feed [83, 84]. The microalgae are sold at a price of 600 PHP (Philippine pesos) per kg of wet weight (WW) paste, or 102 NOK kg^{-1} WW. Microalgal biomass harvested and centrifuged normally has a water content between 80% and 90% [60, 85]. Due to unavailable information on the water contents on the Juan Algae Paste, it is therefore assumed to be 85%. Consequently, the corresponding value for dry weight microalgae would be 680 NOK kg^{-1} DW. This price concurs with other research estimating the market price for unprocessed microalgae to be 50-150 USD, or 440-1,320 NOK, per kg of DW biomass [86]. The currency exchanges are based on rates presented in Table 11, Section 5.

3 Methodology

As an answer to the research question, two different scenarios are considered, which will be considered in this Section. All general calculations required are presented first before the area utilization and energy demand are analyzed. Finally, the laboratory methodology is presented. All relevant economic analyses are based on results from this section and can be found in Section 5.

3.1 Scenario Description

Before making decisions on the microalgae system itself, the placement in relation to the other Nofitech module components must be decided. Several options were evaluated and discarded, like placing the system by the effluent stream for minimal interruption of the current module. Although this would allow for easier adaptation to other WWT systems, one of the main benefits of the system would be to increase the recycling degree of the module, which can only be done if the treated microalgae water is kept in circulation. Another option is placing the system between the drum filter and the biofilter from Figure 1 in Section 2.2. If the system fails to meet the expectations, it can simply be shut off without interrupting the rest of the treatment steps in the module. Letting the water flow go through the biofilter after the microalgae system also ensures that all TAN is treated, which is important as the TAN concentrations cannot surpass the limits in place regarding fish environment. Another benefit of this placement is that most microalgae species fixate ammonia quicker than nitrate, making the system more efficient [63]. However, the main benefit of placing the system within the treatment process in the module is that the treated water can be reused and, therefore, increase the recycling degree, thus decreasing the need for dilution water.

When evaluating the design of the system itself, two different scenarios have been developed; Scenario 1 (S1) and Scenario 2 (S2). Table 4 presents the difference in design choices for the scenarios. Although cultivation with natural light is considered more economically profitable, in addition to reducing energy consumption, the option with artificial light was still considered since it would reduce the required area. Early on it was clear that the scale of this case would leave a massive footprint area, which would have a negative impact on the surrounding ecosystem, something Nofitech is interested in avoiding [18]. Both the goal of space efficiency and economical viability should be considered as options. As explained in Section 2.5, the triangular RAB design of is more optimal for natural light, while the vertical design is more space efficient when using artificial lighting. Therefore, the triangular shape is considered for Scenario 2, while the vertical design is used in Scenario 1.

Table 4: The differences between Scenario 1 and 2.

	Scenario 1	Scenario 2
Min goal	Space efficiency	Economic profitability
Cultivation light	Artificial	Natural
RAB design	Vertical	Triangular

The RAB designs of the scenarios can be altered for optimal production. The up-facing slope of the triangular RAB should be placed at a degree which allows for optimal solar irradiance. Inalve uses 45° for their RAB, which also fits for Norwegian conditions in the summer. The cultivation area to footprint area of the triangular RAB is approximately 3 [63]. A useful function of the vertical RAB is that the area efficiency can be further improved by increasing the height. In previous testing the height has been restricted, most likely based on the risk of mutual shading when using natural light which does not provide sufficient light for the microalgae, as well as practical restrictions in regard to the moving conveyor belt [62]. However, the intensity of artificial light can be regulated based on the amount of surface area that must be illuminated, and a taller structure should ,therefore, be possible. The cultivation area to footprint area is also challenging to determine. An estimation is based on numbers from Gross et al. [62] where a 1.88 m tall RAB showed productivity of 5.5 g per surface area and 46.8 g per footprint area, making the surface to footprint area ratio 8.5. As most data from GWT on the vertical RAB is unavailable, a possible height for testing is assumed to be 3 m, which makes the surface to footprint ratio 13.5.

3.2 Area Utilization

One of the most important results regarding the microalgae system is the footprint area required for each scenario. Before determining this, the production scale and growth rate, or the productivity, must be calculated.

The production scale is determined by the amount of nitrogen that must be removed in order to achieve a given total removal percentage. Using the requirements for municipal WWT as a basis, a goal of 70% removal of produced nitrogen is chosen. Nitrogen production depends on the annual feed use, which is 661,963 kg. With a nitrogen production rate of 3.6% this yields a nitrogen production of 23,831 kg. Assuming the nitrogen removal rate is 70% and the nitrogen fixation rate of microalgae is estimated at 10%, the corresponding microalgae biomass production can be calculated. The CO_2 production from the fish and bacteria in the tank is 317,742 kg. [18]

The microalgae footprint-productivity will be based on numbers provided by Inalve for both artificial and natural lighting. In laboratory testing, they achieve approximately 60 t ha^{-1} y^{-1} using irradiation of 400 µmol m^{-2} s^{-1}. Assuming the LED lights for S1 will provide the same amount of light as for Inalve, the productivity is thus assumed to be 60 t ha^{-1} y^{-1} for Scenario 1.

The microalgae growth rate in Scenario 2 is more challenging to predict as it will not perfectly correlate with Inalve's numbers due to a lower solar irradiance in Norway compared to France. Microalgae productivity is not directly proportional to irradiance due to photoinhibition and other cultivation parameters, and thus cannot be calculated directly based on solar irradiance in Norway. Instead, the production achieved in Nice in addition to theoretical values calculated by Inalve will act as a basis for the productivity of S2. Inalve has achieved annual production of 20-30 t ha^{-1} y^{-1} in Nice. Theoretical productivity

values for Nice and Trondheim, calculated by Inalve, are 144.7 and 89.4 t ha^{-1} y^{-1}, when the production time is 100.0% for Nice and 58.5% for Trondheim, or 7 months, due to the low irradiance during the winter months in Norway. The ratio between theoretical and actual productivity for Nice can be applied to the productivity in Trondheim to determine the actual values. As the ratio is 0.174, the predicted productivity in Trondheim would be 15.5 t ha^{-1} over the 7 months with most solar irradiation. As the placement of the system is based on data from Levanger, the numbers for Trondheim is assumed to be applicable due to the similarity of latitude of the two cities.

The annual productivity in Trondheim cannot be scaled directly based on time because most of the irradiation is during the summer months, with relatively little daylight during the other part of the year. Appendix A shows the irradiance for the years 2011 to 2016 for Levanger at a 45° angle, corresponding to the triangular RAB angle. Here it can be found that the 7 months with most irradiation, March to September, makes up 82% of the annual irradiation, leaving only about 18% for the months October to February. A realistic assumption would then be to increase the productivity over 7 months with 18% for an estimation of annual production. With the 15.5 t ha^{-1}, this results in 18.3 t ha^{-1} y^{-1}.

The sludge produced from the fish farm can be fed to the microalgae to further increase production for Scenario 2, as well as reduce electricity demand for Scenario 1. Since the microalgae can grow mixotrophically, consuming energy from both photons and other energy sources, feeding sludge to them will reduce the total energy demand. It is assumed that the energy content of the sludge is 20 MJ kg^{-1} DW and that 10% of this energy can be stored in the biomass. The sludge production is approximately 1.5 kg per kg of fish feed added to the module, and about 10% of this is dry weight [18]. Using this and the fish feed amount found earlier, the WW sludge production is 992,945 kg while the DW is 99,294 kg. For Scenario 1, the electricity demand can be reduced according to the sludge energy. For Scenario 2, the productivity can be increased based on the ratio of sludge energy to required energy.

The footprint areas, A, of the scenarios are determined by the footprint productivity, p, and the production required to achieve 70% nitrogen removal, or capacity, C. The calculation can be done with Equation (1).

$$A = \frac{C}{P} \tag{1}$$

3.3 Energy Analysis

An energy analysis should be conducted for the scenarios as it is important for both the environmental and economic perspectives. The primary energy demand of the system is heating, pumping, and light. For comparative reasons, energy consumption by a typical denitrification filter will also be estimated.

For the artificial light source for Scenario 1, red LED lights are considered the most effi-

cient option with PAR of 2.6 µmol-ph s^{-1} W^{-1}. Assuming Inalve uses red light for their microalgae laboratory cultivation, the power needed from a LED lamp, P, can be calculating using their irradiation, I, of 400 µmol-ph s^{-1}, the area illuminated, A, and the PAR of red LED lights, as presented in Equation 2.

$$P = \frac{I \times A}{PAR} \tag{2}$$

The footprint for Scenario 1 can be exploited further by installing photovoltaic (PV) solar panels on the roof. This would make up for some of the electricity demand for microalgae growth without using more space. The possible electricity produced from the solar panels can be calculated with equation 3 using the available solar irradiation, I, PV efficiency, η, and roof area, A, of the Nofitech module.

$$P = I \times A \times \eta \tag{3}$$

The flow rate of the microalgae water depends on the nitrogen available in the water, $N_{available}$, and how quickly the microalgae system can reduce it, which corresponds to the reduction capacity, C. A buffer of for example 10% can also be introduced so that only 90% of the nitrogen is fixed from the water as the microalgae productivity might decrease when there is barely enough available nitrogen. Thus, the flow rate $\dot{m}$ can be determined with Equation 4.

$$\dot{m} = \frac{C}{90\% \times N_{available}} \tag{4}$$

As the temperature of the water from the fish tanks is 14°C on average, it should be heated to achieve optimal growth conditions for the microalgae. A more careful evaluation of the trade-off between heating costs and higher microalgae growth should be conducted, but for this thesis it is assumed that a temperature of 20°C will greatly increase the productivity and corresponding heating costs will be calculated. The required heating, Q, can be found with Equation 5 when knowing the mass flow $\dot{m}$, specific heat capacity C_P, and the temperature difference δT. Since the water is heated from 14 to 20°C, the temperature difference is 6, while the C_P for water is 4.186 J g^{-1} $°C^{-1}$ [87].

$$Q = \dot{m} \times C_p \times \delta T \tag{5}$$

When it comes to pumping-need for the microalgae system, it is assumed to be negligible. The water must be led from the fish tanks with a height of 4.9 m, through the microalgae system, and then to a pumping area of 1.6 m, which all the water flows through regardless of the system. This means that if the microalgae system has a water height between 1.6-4.9, then the only additional pumping required will be due to head loss, or friction loss, in the pipes. To avoid this, the water height in the system should be higher than 1.6 m with a small margin. [18]

The dilution water for the module must be heated to the 14°C in the fish tank. The amount is based on the nitrogen concentration in the water, meaning that with the microalgae system, less dilution water is needed. An assumption is made that a nitrogen reduction of

70% means a corresponding reduction of dilution water, meaning that the implementation of the microalgae system would reduce dilution water heating demand with 70%. The heating need can be determined by finding the amount and the start temperature of the water. The average monthly temperatures for the years 1998 to 2020 in a river near Levanger are presented in Appendix B. From this, one can find the temperature difference between the river and the fish tank water for every month of the year, and thus the heating required.

The energy consumption of a denitrification filter consists of methanol and a small amount of electricity. These quantities are based on the amount of nitrate-N and nitrate, respectively, that is converted into nitrogen gas, as presented in Section 2.3. After finding the amount of nitrogen that must be removed by the filter, the corresponding amount of nitrate can be found using the molar mass ratio between them as illustrated in Equation 6, where m_X and m_Y are the mass of two compounds and M_X and M_Y are the molar mass of the same compounds. The same principle can be applied to the biofilter and ammonia production to find the amount converted based on the nitrogen. Combined with the numbers from Table 5, found from Section 2.3, the emissions associated with the biofilter, denitrification filter, and ammonia production are calculated based on the amount of nitrogen that is converted from one compound to another. The molar mass is 14 g mol^{-1} for nitrogen, 62 g mol^{-1} for nitrate, and 17 g mol^{-1} for ammonia.

$$m_X = m_Y \times \frac{M_X}{M_Y} \tag{6}$$

Table 5: Emissions associated with biofilter, denitrification filter, and ammonia production. [22, 25]

	Ratio of CO_2-eq emitted
Denitrification [kg CO_2 kg^{-1} NO_3^-]	1.4
Biofilter [kg CO_2 kg^{-1} NH_4^+]	5.93
Ammonia production [kg CO_2 kg^{-1} NH_4^+]	2.867

Another relevant feature of the microalgae system is the amount of CO_2 it can capture. This can be calculated with the Redfield ratio, as presented in 2.4, which shows that the microalgae need 7 times more carbon than nitrogen for growth. To find the amount of CO_2, the molar mass ratio between C and CO_2 must be used as in Equation 6, and then multiplied with the amount of nitrogen and then by 7 due to the Redfield ratio. The molar mass is 12 g mol^{-1} for carbon and 44 g mol^{-1} for CO_2.

3.4 Laboratory Testing

Laboratory testing has been included as a part of the research for this thesis as it can provide valuable information regarding algal growth. Due to the limited resources and time, the testing is restricted to include only a few variables, which in turn can be tested more thoroughly and thus yield more reliable results. The most important aspect of this laboratory work is to evaluate the productivity and nitrogen fixation of different species of microalgae to find out which is more preferable for fish farm water treatment. These

results could be used to estimate which algae is better for water treatment, and also how the microalgae grown in fish farm water compares to the microalgae grown in F/2 medium. The purpose of this section is to describe the process of planning, preparing, and growing microalgae. First, the planning for what to test is described, then the required practical preparations for this, then an explanation of how testing will be conducted, before ending with a description of the risk assessment required.

3.4.1 Planning and Preparation

Two different species of microalgae will be used for testing, namely *T. Suecica* and *P. Tricornutum*. The former is the same species used by Inalve in their RAB, although the strain is different as Inalve has developed one that cannot be obtained due to patent issues. The latter species was easily accessible for the laboratory work and has shown rapid growth characteristics, as presented in Section 2.4. Each condition for growth will be evaluated in the following paragraphs and consist of bioreactor container, water culture, light, and temperature and pH.

As a growth basis, both container and water must be evaluated. The preferred container would be a RAB prototype from Inalve, as was first the plan, as this is what would be used in the case solution. However, due to some unforeseen issues regarding patent and time management, the backup plan of using petri dishes is put in place. To achieve the same dark/light lapse effect as a RAB provides, the flashing effect is considered. Varying the light provision has proved beneficial for algal growth as described in Section 2.5. Due to the restrictions of light management in the lab, this was discharged.

Regarding the water culture, two different options will be tested: F/2 medium and wastewater from the land-based fish farm Hardingsmolt. The fish farm wastewater will provide similar conditions as the thesis case, and comparing the productivity for these two growth conditions will, therefore, provide valuable information of what to expect for growth comparing Inalve's results with the actual case.

A parameter thoroughly considered testing was the color of the light. As discussed in Section 2.4, several studies show that microalgae grow quicker in red and blue light. Depending on available time, tests with both red and blue light, and a combination of the two, will be conducted for each species and water culture.

Both temperature and pH affect algae growth. The pH in Nofitech's fish tank water is close to neutral, which is considered optimal for both microalgae species that will be tested, meaning that varying the pH would yield minimal difference when trying to optimize growth. The temperature however is approximately 14°C in fish tanks, which is most likely too low for optimal growth. It would provide interesting information if comparing algae growth in 14°C and for example 20°C to see whether heating the fish tank water would be worth it in terms of heating costs versus increased algal growth. Finding the optimal trade-off between temperature and growth would be valuable for the case calculations. However, this was deemed too comprehensive for this type of experiment as it probably would require

both heating and cooling elements, or different tanks and rooms. Nonetheless, the water and air temperature will be measured on at least a daily basis for future reference.

3.4.2 Laboratory Methodology

The required materials and equipment required for the laboratory work are listed in Table 6 and 7, respectively. These are used for preparatory work and work-space setup. The different measuring devices that will be used during testing are listed here:

- Colorimetric test kit
- TN test kit
- pH meter
- Thermometer
- Irradiation meter

The irradiation meter will be used to measure how much light intensity the algae are exposed to. Other than that, the other four parameter-measuring devices are for the water conditions. The Colorimetric and TN test kits are for measuring levels of nitrate/nitrite and the total nitrogen, including organic nitrogen and ammonia, nitrate/nitrite levels in the water. How to use them is described in their respectable user manuals [88, 89]. The pH meter and the thermometer is for measuring the pH levels and the temperature in the water during growth.

Table 6: Materials required for laboratory work described.

Materials	Description
Phaeodactulum Tricornutum	Provided by the Biology department at the NTNU
Tetraselmis Suecica	Shipped from Blackpool, England
F/2 medium	Description in Section 2.5
Fish farm wastewater	Shipped from Hardanger (Hardingsmolt)
Ethanol	Used for sterilization of surfaces

The work-space for the microalgae cultivation was prepared with containers and lighting. An area of 50x50 cm^2 was filled with 20 petri dishes with 9 cm in diameter, which each contain a 6x6 cm^2 cotton patches. The LED light was propped with the steel stand over the center of the work-space. It is height adjustable and should provide sufficient light for all petri dishes. To shield the microalgae from light in the laboratory, apart from the LED work light, a sheet can be propped over the space. This ensures a more trustworthy measurements of irradiation, which is measured with an irradiation meter. The ethanol is used to sterilize the space both before and during testing. Laboratory coats and protective gloves should be used at all times during measurements and handling.

Preparation of the F/2 medium was performed in four steps; filtering seawater, mixing this with filtered freshwater, autoclaving it and then adding a chemical medium. The seawater was filtered with a vacuum filter and mixed at a 50:50 ratio with the freshwater to achieve

Table 7: Equipment required for laboratory work described.

Equipment	Description
Organic cotton x20	Squares of 6x6 cm
Petri dishes x20	9 cm diameter
LED light	Cotech LED 1270 lm
Steel stand	For rigging the light to obtain an adjustable height
Plastic folders	A particular set of colors; including red and blue
Sheet	To regulate light intensity
Plastic tube	For extracting samples for measurements
Coat and gloves	For safety purposes
Vacuum filter	To filtrate the seawater
Autoclave	Sterilizing the seawater/freshwater mix

a fitting salinity level of 30 ppt. Then the mixed water was autoclaved at 5 bar, 121°C, for 30 minutes. The cotton pieces was autoclaved concurrent with the mixed water, wrapped in aluminum foil, to prepare it for algae growth. The chemical medium was cooled at -20°C before use to avoid bacteria contamination, and after thawing it was added at a rate of 1:50 to the sterilized test water. With the test water measuring 1.6 liters, 32 ml of chemical medium was added with pipettes before mixing the mediums by shaking the container, then stored coolly. When starting the testing both microalgae are mixed with the test water, and the water from Hardingsmolt.

Finally, before any testing commenced, a risk assessment was completed and finalized as a report which is attached in Appendix C. This was a mandatory step for being allowed to start the experiment, as measures to keep the risk at a minimum for any kind of laboratory work. As part of the risk assessment, both a "unit card" and an "experiment in progress" document must be printed and displayed by the work station. The unit card is an instruction for the laboratory setup and includes emergency instructions and safety evaluations. The experiment in progress document describes the experiment, as well as operational times.

Just as the risk assessment report was approved, the required documents were hung up, and the clearance from the laboratory administration had been given, the administration announced campus lock-down due to the Covid-19 pandemic. As all laboratories were closed to students, a back-up plan consisting of extended literature reviews was put in place, making the thesis project purely theoretical. Laboratory results are, therefore, not included in the remainder of this thesis.

4 Results

In this section all relevant results form calculations are presented. This includes all aspects of the microalgae system in general and specifically for the two scenarios presented, apart from economic calculations which are presented in Section 5. Finally, the results from the sensitivity analysis are presented.

4.1 Scenario Description

The annual nitrogen production of the module is 23.83 t, meaning 16.68 t nitrogen must be fixed by the system annually to achieve 70% removal rate. Assuming a 10% nitrogen fixation rate, this corresponds to a microalgae biomass production of 166.8 t y^{-1}, or 457.0 kg d^{-1}. With an average water content of 85%, the biomass produced in WW is 1,112 t y^{-1}. The annual CO_2 production by the fish and bacteria in the tank is 317.7 t.

The sludge production is 993.0 t y^{-1} in wet weight (WW). This is 89% of the WW microalgae biomass produced. With a DW ratio of 10%, energy content of 20 MJ kg^{-1} DW, and a microalgae energy storage ratio of 10%, the resulting annual usable sludge energy is 198.6 GJ. The required energy for the microalgae in one year, based on an energy content of 17 MJ kg^{-1}, is 2,836 GJ. The sludge energy reduces this amount to 2,637 GJ, or 732.6 MWh.

4.2 Area Utilization

Based on the microalgae footprint productivity of 60 t ha^{-1} y^{-1} and a 3 m tall RAB, the footprint area required for Scenario 1 is 6,178 m^2. This is 4.2 times larger than the Nofitech module of 1,466 m^2. For Scenario 2, with a productivity of 18.26 t ha^{-1} y^{-1}, the footprint area needed is 8,537 ha. If the RAB height for S1 was increased to 5 m, the footprint area would be 3,707 m^2.

The ratio between usable sludge energy and energy required by the microalgae is 7.00 %. Increasing the productivity of Scenario 2 with this ratio results in a productivity of 19.54 t ha^{-1} y^{-1}, and a footprint of 8.538 ha. Based on these results, the footprint area of S2 is 58 times the size of the Nofitech module and almost 14 times larger than the footprint of S1.

4.3 Energy Analysis

The water flow through the microalgae system is approximately 30 m^3 h^{-1}, calculated from Equation 4. The heating required to increase the temperature from 14 to 20°C in the water flowing through the system is 1.83 GWh. This is the same for both scenarios as the production capacity is equal between them.

Without a nitrogen removal system, the dilution water need is 300 L of dilution water per kg of fish feed. The required annual amount of dilution water can thus is found knowing the total amount of fish feed used in a year whish is 662.0 t. The temperature difference

is found from Appendix B, averaging at 5.07°C. The required heating to increase this to the 14°C in the fish tank is 2.26 GWh y^{-1}. By assuming 70% reduction of dilution water in the microalgae system, only 30% of the original heating demand still applies, which is 678 MWh.
However, if the remaining 30 % of dilution water is mixed with the microalgae water which has a temperature of 20°C, excluding losses, it results in a temperature above 14°C. To calculate this, the combined stream of microalgae and dilution water would consist of 81.52% of microalgae water at 20°C and 18.48% dilution water at 5.07°C. The combined temperature is approximately 17.24°C. This means that the saved heating demand for the microalgae system is equal to the total heating need for the module without the removal system.

Based on Equation 2 the power needed to grow 60 t ha^{-1} y^{-1} can be calculated for Scenario 1. The Inalve irradiation of 400 µmol-ph s^{-1} W^{-1} is for a RAB with a surface to footprint area ratio of 3, while the vertical RAB of S1 has a ratio of about 13.5. After scaling the irradiance for surface area, the power needed for cultivation LEDs in S1 was found with the footprint area of 6,178 m^2 and PAR efficiency of 2.6 µmol-ph s^{-1}, resulting in 37.47 GWh y^{-1}. When including the energy from sludge, the final power needed for one year of cultivation is 36.37 GWh y^{-1}.

Electricity demand for the Inalve RAB is estimated at 1.0 W m^{-2} of biofilm surface [63, 90]. The biofilm area for Scenario 1 and 2 is 83,409 m^2 and 256,134 m^2. This is found with the footprint area and the biofilm to footprint ratio of 13.5 for S1 and 3 for S2. As the information on the electricity demand for GWT's RABs is not available, it is assumed that biofilm reactor electricity demand for Scenario 1 is the same as used for Inalve's RAB, scaled for the surface area in S1.

All electricity consumption for the microalgae system, including biofilm reactors, microalgae water heating, and LED lights are presented in Table 8. The electricity consumption of S1 is 5.8 times larger than for S2.

Table 8: Annual electricity demand, not including LED.

	Scenario 1 [kWh]	Scenario 2 [kWh]
Biofilm reactors	730,663	2,243,590
Water heating	1,833,468	1,833,468
LED light	37,469,878	–
Total	40,034,009	4,077,058

The electricity provided by the potential solar panels on the roof of the Scenario 1 is not included in the total energy analysis. Using Equation 3 and PV panel efficiency of 20%, the roof area of 1,466 m^2, and annual solar irradiance in Levanger from Appendix A, the electricity production by the PV panel is 1.308 GWh y^{-1}. This would reduce the LED electricity consumption for S1 with 3.60%. This is assuming the PV panels have an angle of 45 degrees as this is the angle of measurement for the irradiance in Levanger.

The energy consumption of a denitrification filter is based on the amount of nitrate-nitrogen that is converted by the filter. Assuming the same removal rate of the microalgae system, the amount of nitrogen, and nitrate and ammonia when using Equation 6, is presented in Table 9. The resulting amount of methanol, electricity, and CO_2-eq emissions are also included.

Table 9: Energy consumption by denitrification filter.

	Amount
Nitrogen [kg]	48,372
Nitrate [kg]	73,877
Ammonia [kg]	20,256
Methanol demanded [kg]	48,372
Electricity demand [kWh]	14,552
CO_2-eq [kg]	103,427

The CO_2-eq emissions saved by avoiding the denitrification filter are presented in Table 5. They include emissions from the biofilter, denitrification filter, and ammonia production. In addition, the CO2 fixed by the microalgae system is included. All emissions associated with the construction and operation of the microalgae are excluded. The result is presented in Table 10.

Table 10: CO_2-eq emissions reduced by the microalgae system.

Process	CO_2-eq emitted [kg]
Biofilter	120,118
Denitrification	103,428
Ammonia production	58,074
Total emissions	281,620
Fixed by system	428,166
Total	709,786 kg

5 Economical Analysis

This section presents the economic analysis of all relevant aspects of the thesis. It evaluates capital expenditures (CAPEX), which consists of major equipment costs and other investments, and operational expenditures (OPEX). Combining the OPEX with the depreciation on CAPEX results in the total annual costs of the microalgae system for both Scenario 1 and 2. Furthermore, the denitrification filter costs are evaluated, before comparing it with the microalgae systems, including production costs per kg of biomass. Finally, sales price is used to calculate income, and the resulting profits are presented. The final section is a sensitivity analysis that evaluates the most relevant uncertainties regarding economy, including calculations that show the best and worst-case scenarios for S1 and S2.

Most of the calculations in this section are partly based on the results from Section 4, the most important one being the footprints for S1 and S2 of 6,178 m^2 and 85,373 m^2, respectively. All conversions between currencies will follow the exchange rates presented in Table 11. The table shows conversions from American dollars (USD) and Philippine pesos (PHP) to Norwegian kroner (NOK) as all cost calculations will be presented in NOK.

Table 11: Average currency exchange rates from 2019 for PHP and USD to NOK.

Currency	NOK	Reference
1 PHP	0.17	[91]
1 USD	8.7996	[92]

5.1 Capital Expenditures

The estimated cost for Inalve's biofilm reactors, based on footprint area, is about 960 NOK m^{-2} [63]. Due to unavailable information on investment cost for GWT RABs this will be used as a basis also for S1, but will be scaled based on biofilm area per footprint area for the different RAB designs. The biofilm area per footprint area is 3 for Inalve and 13.5 for the GWT design, with height of 3 meter. The conversion ratio from Inalve's to GWT's design is, therefore, 4.5 for biofilm surface area, resulting in RAB costs for S1 of 4,320 NOK m^{-2} for S1.

The cost of a greenhouse fitted for an area of 100 m^2 is estimated at 80,000 NOK [35]. A fitting district heating system is estimated at 1.5 MNOK for 1 ha [35]. Both these costs can be scaled to fit each scenario based on the area with the volume up-scaling ratio shown in Equation 7 [35, 93]. Here X and Y represent two different areas and their corresponding costs. Scenario 1 would most likely not be using a greenhouse as there is no need for solar irradiance. The building would also be higher as the RAB structures are taller than the ones in Scenario 2. For simplicity, the construction and district heating costs for S1 is assumed to be equal to those of S2 [93].

$$Cost_{Xm^2} = Cost_{Ym^2} \times \left(\frac{Xm^2}{Ym^2}\right)^{\frac{2}{3}} \tag{7}$$

The LED light investment costs are evaluated assuming 5% PE [29]. The source assumes an expected lifetime of a LED lamp to be 50,000 hours, which corresponds to 5.7 years of 100% running time. To achieve about 10 years of lifetime, the investment costs have therefore been doubled. The result is presented in Table 12. As LED prices are expected to decrease greatly in the future the report also includes predicted prices. All costs are given per kg of DW microalgae biomass produced, and the total costs can therefore easily be calculated knowing that the total annual DW production is 166.8 t.

Table 12: Current and predicted investment costs of LED lights. [29]

	Current	Future
Specific LED cost [NOK kg^{-1}]	130.23	65.12
Total LED cost [NOK]	21,724,708	10,863,188

Table 13 shows total major equipment costs for Scenario 1 and 2, consisting of the biofilm reactors, greenhouse, district heating system, and LED lights included for S1.

Table 13: Major equipment investment costs. [29, 35]

	Scenario 1 [NOK]	Scenario 2 [NOK]
Biofilm reactors	26,690,872	81,957,643
Greenhouse	7,199,497	7,199,497
District heating	6,265,707	6,265,707
LED light	21,724,708	-
Total	61,880,784	95,422,846

Lang factors are used to estimate investment costs for industrial production based on major equipment costs. The results for each scenario are presented in Table 14 [94]. Finally, the CAPEX is found by adding all investment costs. To find the annual costs based on CAPEX, the depreciation rate of 10% is used [95].

Table 14: Total investment costs based on major equipment and their corresponding Lang factors [94].

	Lang factor	Scenario 1 [NOK]	Scenario 2 [NOK]
Major equipment costs	1	61,880,784	95,422,846
Installation	0.20	12,376,157	19,084,569
Instrumental and control	0.15	9,282,118	14,313,427
Piping	0.20	12,376,157	19,084,569
Electrical	0.10	6,188,078	9,542,285
Buildings	0.23	14,232,580	21,947,255
Yard improvement	0.12	7,425,694	11,450,742
Service facilities	0.20	12,376,157	19,084,569
Engineering and supervision	0.30	18,564,235	28,626,854
Constriction	0.05	3,094,039	4,771,142
Contractor's fee	0.03	1,856,424	2,862,685
Contigency	0.08	4,950,463	7,633,828
CAPEX		164,602,885	253,824,771
Depreciation		16,460,289	25,382,477

5.2 Operational Expenditures

Labor costs are estimated at 628,500 NOK for 1 ha and 6 months of running time [35, 63]. Assuming labor costs are constant for both size and productivity, only operation time is used for scaling [93]. As both scenarios operate 12 months of the year, the labor costs are multiplied with 2. The land rent is estimated at 20 NOK m^2 y^{-1} and can be used to calculate the land rent costs for 6,178 m^2 for S1 and 85,373 m^2 for S2 [93]. Electricity costs are based on the results in Table 8 and the industrial electricity price of 0.40 NOK kWh^{-1} [93]. The results for OPEX are presented in Table 15.

Table 15: OPEX for Scenario 1 and 2.

	Scenario 1 [NOK]	Scenario 2 [NOK]
Labor	1,257,000	1,257,000
Land	123,569	1,707,451
Electricity	16,013,604	1,630,823
OPEX	17,394,172	4,595,274

5.3 Denitrification Filter Costs

The costs associated with a denitrification filter are primarily investment and methanol consumption, as well as a small amount of electricity. The investment costs depend on the capacity needed, but for a Nofitech module it is estimated at 5 MNOK [18]. With a depreciation ratio of 10% the annual investment cost is 0.5 MNOK. Using the price of methanol of 3,537 NOK t^{-1}, the energy cost of 0.40 NOK kg^{-1}, and the values found in Table 9, the resulting annual costs are calculated and presented in Table 16.

If a denitrification filter was to be implemented instead of the microalgae system, there would be a reduction in dilution water heating demand of about 70%. The costs saved through this is also presented in Table 16, together with the total costs included those saved on dilution water heating.

Table 16: Annual denitrification filter costs.

	Cost [NOK]
Depreciation	500,000
Methanol	171,131
Electricity	5,821
Total	676,953
Costs saved	632,800
Total incl. costs saved	45,301

5.4 Cost Comparisons

The total annual costs associated with the microalgae system are presented in Table 17. For comparison, the costs are evaluated both including and excluding the saved costs

from dilution water reduciton. The costs excluding saved costs are found by adding the depreciation of CAPEX to the OPEX. The saved costs is based on the reduction in heating demand, which was found to be about 2.26 GWh in Section 4, and the corresponding costs of 0.904 MNOK is subtracted from the microalgae system costs. Finally, the microalgae system is compared to the denitrification filter, and the total annual cost difference is also included in Table 17.

In addition to the total annual costs, the production cost per kg of DW microalgae biomass produced is also included in Table 17, both for S1 and S2.

Table 17: Comparisons between the microalgae system and denitrification filter costs as opposed to no nitrogen removal option.

	S1 [NOK]	S2 [NOK]	S1 [NOK kg^{-1}]	S2 [NOK kg^{-1}]
Microalgae excl. saved costs	33,854,461	29,977,751	202.9	179.7
Microalgae incl. saved costs	32,950,461	29,073,751	197.5	174.3
Microalgae vs. denitrification	32,906,308	29,029,599	197.3	174.0

5.5 Microalgae sales

In order to break even with a microalgae system, the price per kg of DW microalgae biomass must be 197.3 NOK for S1 and 174.0 NOK for S2 when comparing it to a denitrification filter, as presented in Table 17. The lowest estimated microalgae biomass price from Section 2.6 of 440 NOK kg^{-1} DW is used for calculations to leave room for uncertainties. This is 2.23 and 2.53 times more than the production cost for S1 and S2, respectively, when comparing the scenarios to a denitrification filter. Based on the annual biomass production and the assumption that all biomass is sold at the price mentioned, the total annual profits of each scenario are presented in Table 18. If the biomass is sold at the same price as Juan Algae Paste, the sales income would be approximately 113.4 MNOK.

Table 18: Profits of the microalgae system for Scenario 1 and 2.

	Scenario 1 [NOK]	Scenario 2 [NOK]
Costs	32,906,308	29,029,599
Income	73,399,920	73,399,920
Profits	40,493,612	44,370,321

5.6 Sensitivity Analysis

Some aspects of the economic analysis are relatively uncertain due to limited resources and time spent on research after the change in resource allocation during the project. The sensitivity analysis will evaluate some of the parameters and the effect they have on the resulting production cost per kg of microalgae biomass.

Maybe the most important factor in creating the cost difference between the scenarios is productivity. First of all, Inalve expects to increase productivity from 60 to 100 t ha^{-1}

y^{-1}, which is an increase of 66.67%. When applying the same increase to Scenario 2 with 19.54 t ha^{-1} y^{-1}, the predicted productivity is approximately 32.58 t ha^{-1} y^{-1}. With these productivities, S1 is about 25% more expensive than S2. If the productivity achieved in Nice of 25 t ha^{-1} y^{-1} is applied to Scenario 2, S1 ends up being 40% more expensive than S2.

For the remaining of the sensitivity analysis, certain chosen parameters will be evaluated and plotted. For both scenarios, productivity, CAPEX in general, and land rent cost will be evaluated. For S1, which contains more uncertainties than S2, PAR efficiency, LED investment, and RAB investment costs will also be evaluated. The resulting best and worst case, as well as the base numbers used, are presented in Table 19 and 20 for S1 and S2, respectively. The resulting costs for each parameter are all based on production costs per kg of biomass produced for the microalgae system, excluding costs saved and comparisons with a denitrification filter. These results are displayed in the tornado plots in Figures 5 and 6 for S1 and S2, respectively. A total of all parameters combined is also included.

The productivity for both scenarios are based on the measurements from Inalve. They claim annual productions in the range of 54-72 t ha^{-1}, which will then make up the worst and best case for S2. As the productivity for S1 is linearly based on that of S2, the same ratio is used with resulting productivity of 17.59 and 23.45 t ha^{-1} y^{-1} for worst and best case, respectively. The CAPEX uncertainty includes all investment costs and it is realistic to assume that it can increase with 50% or decrease with 10% [35]. For the land rent, the increase and decrease are assumed to be 40% and 20%, respectively. This is applied to both scenarios.

The LED lights are based on the approximate prices today, and 10% is added for worst case. The prices are assumed to decrease drastically in the future as indicated in Table 12, and for best case they are therefore assumed to decrease with 50%. The PAR efficiency of the LED is also expected to increase to about 3.0, while the PAR for white light is 1.9 and will therefore be assumed to be worst case for S1 [29]. A large source of uncertainty is the lacking information on RAB costs for the Gross-Wen Technologies design. Therefore the cost is assumed to be 30% higher in worst case and 10% lower for best case.

Table 19: Parameters for sensitivity analysis of Scenario 1.

	Worst	Base	Best
Productivity [t ha^{-1} y^{-1}]	54	60	72
LED PAR efficiency [µmol s^{-1} W^{-1}]	1.9	2.6	3.0
LED investment [NOK kg^{-1}]	+10%	130.23	-50%
RAB investment [NOK m^{-2}]	+30%	960	-10%
CAPEX [MNOK]	+50%	165	-20%
Land rent [NOK m^{-2} y^{-1}]	+40%	20	-20%

Table 20: Parameters for sensitivity analysis of Scenario 2.

	Worst	Base	Best
Productivity [t ha^{-1} y^{-1}]	17.59	19.54	23.45
CAPEX [MNOK]	+50%	254	-20%
Land rent [NOK m^{-2} y^{-1}]	+40%	20	-20%

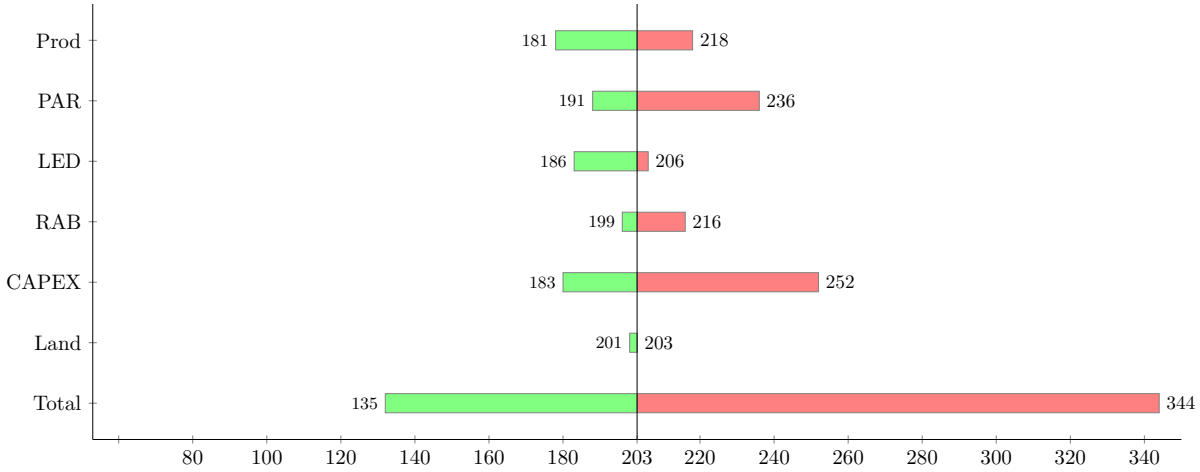

Figure 5: Tornado plot of the sensitivity of four different parameter values for Scenario 1.

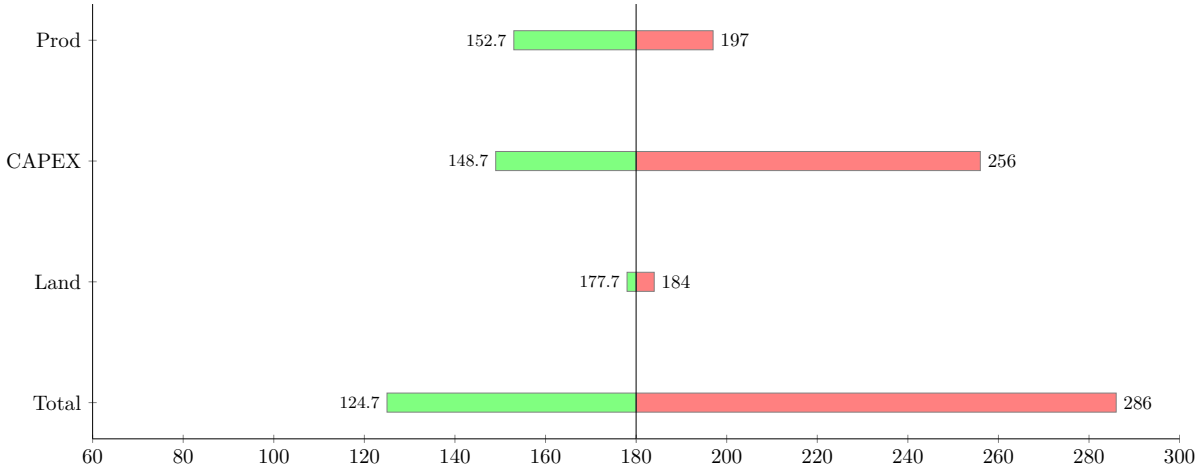

Figure 6: Tornado plot of the sensitivity of four different parameter values for Scenario 2.

6 Discussion

This section discusses both theoretical and practical aspects connected to a microalgae production system for nitrogen removal. It also compares Scenario 1 and Scenario 2 in terms of biological footprint, energy consumption, type of lighting, and profitability. The sensitivity of the most significant parameters and their possible effect on the results will also be evaluated.

6.1 Research question

This thesis has presented two options which answer the research question on how microalgae production can be used for water treatment in a land-based fish farm. Both Scenario 1 and 2 are possible alternatives to a denitrification filter in theory, with results claiming they can both be profitable. However, the preferred solution must be based on the practical aspects of each scenario and the goals of the user. Apart from providing a more sustainable solution than a denitrification filter, Nofitech wants the solution to require a minimal footprint and preferably, to break even economically.

Implementing a microalgae system in a land-based fish farm will contribute positively in mainly three ways. Firstly, the recycling percentage of the module will go up, thereby reducing the need for dilution water and wastewater treatment. Secondly, less nutrients will be released into nature through wastewater, reducing the fish farms' impact on cultural eutrophication in water bodies. Lastly, the need for unsustainable denitrification is reduced, thus removing steps in an energy consumption chain. In addition, the microalgae biomass harvested from the production can be sold as algae paste directly, or processed and sold as biofuels, fertilizers, animal feed, or food supplements. This creates a circular economy where little energy goes to "waste", also creating an additional income.

6.2 Scenario Description

The system solution presented regarding microalgae species, cultivation design, implementation in the Nofitech module, and water provision seems to be a realistic option in terms of available technology. The large scale of the biomass production can become an issue in terms of storage, further processing, and sales, making it necessary for cooperation with a partner. As Nofitech already sells their produced sludge for fertilization, and the biomass production is on the same scale, this seems like a realistic achievement. This especially when feeding the sludge to the microalgae, which the results prove is beneficial. For microalgae sales to other purposes than fertilizer production, an arrangement should first be established. For the biomass that is not further processed, it can be sold to for example the closely located Biokraft factory as biofuels.

6.3 Area Utilization

Although the product from the microalgae system is in the same scale as the fish farm, the area-utilization of both scenarios surpasses that of the module. This was expected as one of the main issues with microalgae production, apart from low-profit margins, is space

utilization. However, Scenario 2 demands an enormous area, both relative to Scenario 1 and to the Nofitech module. This is due both to the S2 productivity being about a third of the S1 productivity and the less space-efficient bioreactors, which is necessary when using natural light for cultivation. The area of S1 will be even smaller if higher vertical bioreactors are achievable in the future.

Available areas might be in short supply in the future due to increased biofuel production and planting of forests as part of the IPCC's goal to halt the global temperature increase. A microalgae system with a large footprint will therefore most likely cause issues when searching for a fitting location, in addition to the negative impact it has on the ecosystem. Since the main justification for implementing a treatment system is the positive biological effect of reduced HAB, this should be a big contributor to the evaluation of the scenarios.

6.4 Energy Analysis

When comparing the microalgae system to a denitrification filter, the main benefit would be the utilization of organic nitrogen compared to converting it to nitrogen gas, and then back again in later stages, thus reducing the overall energy consumption and emissions. Therefore the energy consumption of the microalgae system is important to discuss. Unexpectedly, the energy consumption of S1 is larger than for S2 due to the electricity demand for the LED cultivation lights. Due to the energy consumption of the RABs, which S2 need a lot more of due to lower productivity, the difference is not as high as expected. If the RAB power consumption is reduced or the productivity of S2 is increased, the difference would be more significant. The emissions connected to electricity consumption depend on how the electricity was produced, and the corresponding environmental impact will therefore vary. The energy demand would also be reduced by small percentage if PV solar panels were installed on the roof of the S1 building.

The total CO_2 emissions saved by avoiding a denitrification filter is the combination of emissions from nitrification, denitrification, and nitrogen fixation, as well as the amount fixed by the microalgae. When excluding emissions from building the system, it reduces a significant amount of emission. With ammonia production being such a significant contributor to global CO_2 emissions, this reduction is important when evaluating the microalgae system.

6.5 Economic Analysis

Although the economic analysis in this thesis potentially provides uncertain results, it still gives interesting and realistic perspectives. First of all, according to the analysis, S2 with natural lighting would be more profitable than when using artificial lighting in S1, something that concurs with the reviewed literature. This is due to the much larger operational costs for S1, caused primarily by the electricity needed.

The most important contribution from the economy results is the realization of how important productivity is for the cost difference between cultivation with natural and artificial light. The literature reviewed presents microalgae production as profitable in general and

with larger profit margins when using natural lighting. It seems that a microalgae system can only be profitable in Norway when productivity meets a certain standard. For natural lighting with limited irradiance, additional measures like more efficient light exploitation, for example by using reflection, can contribute to the necessary increase in productivity. It can also be reached through technology advancements as expected by Inalve.

As the economic figures show, a profitable microalgae system can be achieved. When basing the selling price on the lowest market price, a profit of over 40 MNOK annually is estimated. This leaves some room for uncertainty regarding the market potential in Norway and the production costs. Due to the shift in resources during the course of this project following the restrictions on the planned laboratory work, an extensive analysis of costs and market potential could not be conducted. This is necessary before drawing more definite conclusions for the economic aspect of a microalgae production system.

A microalgae system is meant to be an addition to a fish farm, providing solutions regarding water treatment. If the microalgae system is profitable in itself or provides the fish farm with benefits such as reduced dilution water, CO_2-emission, and harmful effluents, it can be a desired investment. The algae system is more than a small addition to the fish farm. This is not necessarily a bad thing, but it is worth noticing that the investment costs for the algae systems are up to about double the cost as a fish farm. However, economically viable options for the algae systems do exist, and if they are profitable, then the main challenge with implementing such algae systems, are the investments, and not the operating costs.

6.6 Laboratory Work

Even though the laboratory work yielded no testing results due to the unexpected governmental restrictions, the work performed still yields an important perspective. The extensive planning revealed which parameters can be tested even with limited resources while still providing a valuable contribution to the calculations performed. The most important contribution would probably have been the comparison of the microalgae productivity in F/2 medium and fish farm wastewater. A different production in the wastewater would mean that the productivity values used in the calculations, which are based on production by Inalve in a medium similar to F/2, should be adjusted accordingly. Also different microalgae species, light intensity and color, and nitrogen fixation rate would provide interesting new perspectives on the results and conclusion of this thesis.

6.7 Recommendation

Even though microalgae cultivation with wastewater from land-based fish farms is relatively common, the implementation of production as part of a module has to the authors' knowledge not been attempted in practice. For the former alternative, the focus would be on the product itself, including quality and profitability. Natural light might be a better choice for this option as it increased profit margins and reduces energy demand. However, for a system implemented in the fish farm, it would be more important with a compact, stable, and reliable production. Scenario 1 of this thesis leaves a much smaller footprint,

promoting a more environmentally friendly solution both in terms of biodiversity and leaving available areas for biofuel production, although the additional electricity demand is a drawback.

7 Conclusion

This thesis has proved that it is theoretically possible to replace a denitrification filter for water treatment in land-based fish farms by producing microalgae, both with artificial and natural lighting. The practicality of the solutions, however, poses challenges due to the large production scale and required land area. Both scenarios presented in this thesis require larger areas and investment costs than a denitrification filter. The production scale of the systems is also significant, with 166.8 t DW biomass produced annually, which might pose challenges in regard to the market potential. This can be avoided if the microalgae are sold in the rapidly increasing biofuel production field. The recommendation to Nofitech is based on these factors as well as their goals as presented in the case. The main goal of Nofitech regarding the replacement of a denitrification filter is a more sustainable option that utilizes the available nutrients while leaving a relatively low biological footprint, and, preferably, breaks even economically.

Sustainability, biological footprint, and emissions are all important when evaluating environmental impact. A microalgae system would without doubt make better use of the available nitrogen, as it contributes to the growth and can be re-used through whichever product is created from the microalgae biomass. Because of this, CO_2 emissions are reduced by about 710 t per year. With artificial lighting, the energy demand increases significantly compared to solar illumination, meaning that the associated emissions are larger. However, the biological footprint is also significant when discussing environmental impact, and with natural lighting, the system would leave a footprint of almost 9 hectares, over 58 times larger than the Nofitech module.

When it comes to profitability, the results in this thesis show that production with artificial and natural light would cost approximately the same, mainly due to the location providing lower solar irradiance compared to other parts of the world. Even though production with artificial light leaves lower profit margins than with natural light, according to research and the results of this thesis, with the goal being to break even economically, artificial light is still relevant for production.

Based on the goal of leaving a small footprint while breaking even economically, the recommendation to Nofitech is to choose microalgae production with artificial lighting. Scenario 1 has a much smaller area usage, especially when increasing the height of the RABs. Although Scenario 2 will be more economically profitable, it can never compete on area usage. To profit from the system, it is probably a requirement that the biomass produced is sold as a high-value product, either by further processing by Nofitech themselves, or in cooperation with other actors in the market. Even though a microalgae production system would benefit the environment by exploiting available nutrients and reducing water contamination, it would also demand large investments of both time and money, and should, therefore, be considered carefully. As this type of system is in the earliest stages of development, the possibilities for future research and innovations are many and could likely alter the conclusion of this thesis.

8 Future Work

For this thesis the primary function of the microalgae production is nitrogen fixation. The focus of Inalve's microalgae production is to create a pure, high-value product with specific nutrient and vitamin contents that can be sold at a reasonable price, and the productivity is therefore compromised, the microalgae demanding specific growth conditions regarding temperature and light. However, if the biomass is used for bulk products like biofuels or feed replacement it is less important that specific content criteria are fulfilled, and consequently, pure microalgae cultures during production is not vital. When reducing the importance of the quality of the biomass, a high productivity can be maintained at less energy and area demand. This can be done by choosing different microalgae species, or not microalgae at all, or letting a biofilm form more freely and fit the surroundings through natural selection. This could reduce heating and lighting demand and thus lower production costs and area utilization.

As the idea in this thesis of using microalgae production for water treatment in fish farm module is very new and has not been tried, there is still room for alternative superior ideas. There are a multitude of options when considering further development or adaptions, like the choice of microalgae species, RAB design, and nutrient provision, which are factors only briefly explored in this thesis. The further treatment of the microalgae biomass produced is a thesis in itself, and will increase costs, but also profits considerably. The solution proposed in the report can also be adapted to other industries, like dairy production or municipality wastewater for the purpose of WWT only. The benefit of dilution water reduction is lost, but one can still get "free" nutrients for the microalgae production while also solving a treatment issue. Production outside of Norway is also important to consider, as it can mean stricter regulations, more valuable freshwater, and more solar irradiance, all factors that make the use of microalgae in WWT more valuable, both in terms of profitability and the environment.

References

[1] Aqua Nor. These exhibitors will make the growth of land-based farming possible, August 8th 2019. URL https://www.aqua-nor.no/these-exhibitors-will-make-the-growth-of-land-based-farming-possible/?lang=en.

[2] United Nations (UN). Global issues overview. URL https://www.un.org/en/sections/issues-depth/global-issues-overview/. Accessed: 19.05.2020.

[3] Boliden. Boliden grows algae to reduce emissions. URL https://www.boliden.com/news/microalgae-to-reduce-emissions#. Accessed: 19.05.2020.

[4] National Oceanic and Atmospheric Administration. What is a harmful algal bloom? - sometimes tiny algae can cause big problems. April 27th 2016. URL https://www.noaa.gov/what-is-harmful-algal-bloom.

[5] F. X. Fu, A. O. Tatters, and D. A. Hutchins. Global change and the future of harmful algal blooms in the ocean. *Mar Ecol Prog*, (470:207-233), 2012.

[6] P.M. Visser, J.M.H. Verspagen, G. Sandrini, L.J. Stal, H.C.P. Matthijs, T.W. Davis, H.W. Paerl, and J. Huisman. How rising co2 and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae*, 54:145–159, April 2016.

[7] Z. Ma, T. Fang, R.W. Thring, Y. Li, H. Yu, Q. Zhou, and M. Zhao. Toxic and non-toxic strains of microcystis aeruginosa induce temperature dependent allelopathy toward growth and photosynthesis of chlorella vulgaris. *Harmful Algae*, 48(1):21–29, 2015.

[8] I. Dundas, O.M. Johannessen, G. Berge, and B. Heimdal. Toxic algal bloom in scandinavian waters. *Oceanography*, 2(1):9–14, , May-June 1988.

[9] E. Testai, S. Scardala, S. Vichi, F. M. Buratti, and E. Funari. Risk to human health associated with the environmental occurrence of cyanobacterial neurotoxic alkaloids anatoxins and saxitoxins. *Critical Reviews in Toxicology*, 46(5), February 2016.

[10] Michael F. Chislock, Enrique Doster, Rachel A. Zitomer, and Alan E. Wilson. Eutrophication: Causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge*, 4(4):10, 2013.

[11] Norwegian Environment Agency. Eutrophication, May 22nd 2017. URL https://www.environment.no/topics/marine-and-coastal-waters/eutrophication/.

[12] G. H. Johnsen. Miljørapport 2010. sørsmolt as, kragerø kommune. (1456), August 15th 2011. URL https://www.radgivende-biologer.no/wp-content/uploads/2019/06/1456.pdf.

[13] T. W. Rosten. Karakterisering av avløpsvann fra norske landbaserte settefiskanlegg. 50(3), 2015.

[14] Lovdata. Kapittel 14. krav til utslipp av kommunalt avløpsvann fra større tettbebyggelser, § 14-2. December 15th 2005. URL https://lovdata.no/dokument/SF/forskrift/2004-06-01-931/KAPITTEL_4-4#%C2%A714-2.

[15] T. W. Rosten, K. Azrague, and B. Toldnes. Primærrensing og aktuelle løsninger for slambehandling i norske settefiskanlegg. May 24th 2013.

[16] Food and Agriculture Organization of the United Nations. Profitable growth continues in the global salmon sector despite price volatility, January 10th 2019. URL http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/1176223/. Market report.

[17] T. Bjørndal and A. Tusvik. Økonomisk analyse av alternative produksjonsformer innan oppdrett. *SNF-rapport*, 07/18(5730), October 2018.

[18] Kari Attramadal. Personal communication, 2020.

[19] International Salmon Farmers Association. The evolution of land based atlantic salmon farms. November 2016. URL https://www.cermaq.com/wps/wcm/connect/9144716c-37a5-4f53-8f4a-44c73396c414/ISFA_LandFarmingreport_web.pdf?MOD=AJPERES&CONVERT_TO=url&CACHEID=ROOTWORKSPACE-9144716c-37a5-4f53-8f4a-44c73396c414-lZL5WJR.

[20] Nofitech. Vi utvikler framtidas ras-løsninger. URL https://nofitech.com/om-oss/. Accessed: 30.04.2020.

[21] T. K. Jenssen and G. Kongshaug. Energy consumption and greenhouse gas emissions in fertiliser production. *The International Fertiliser Society (IFS)*, 2003.

[22] L. T. Thomas and A. Hicks. White paper, methanol use in wastewater denitrification. *Methanol Institute*, July 2012.

[23] Trevor Brown. Ammonia production causes 1% of total global ghg emissions, April 26th 2016. URL https://ammoniaindustry.com/ammonia-production-causes-1-percent-of-total-global-ghg-emissions/. Accessed: 15.05.2020.

[24] Methanol Institute. Methanol use in denitrification - importance of denitrification. September 2015.

[25] Methanol Institute. Methanol price. URL https://www.methanol.org/methanol-price-supply-demand/. Accessed: 13.04.2020.

[26] L.K. Wang, N.K. Shammas, and Y. Hung. *Advanced Biological Treatment Processes*, volume 9 of *Handbook of Environmental Engineering*. Humana Press, 2009.

[27] EasyMining. We are recycling nitrogen from waste water. URL https://www.easymining.se/article-startpage/n-project/. Accessed: 29.04.2020.

[28] M. D. Ooms, C. T. Dinh, E. H. Sargent, and D. Sinton. Photon management for augmented photosynthesis. *Nature Communication*, 7(12699), September 2016.

[29] W. Blanken, M. Cuaresma, R.H. Wijffels, and M. Janssen. Cultivation of microalgae on artificial light comes at a cost. *Algal Research*, 2(4):333–340, 2013.

[30] J. Masoj idek, G. Torzillo, and M. Koblizek. Photosynthesis in microalgae. *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*, 2013.

[31] M. G. J. Janssen. Cultivation of microalgae: effect of light/dark cycles on biomass yield. March 2002.

[32] T. de Mooij, G. de Vries, C. Latsos, R. H. Wijffels, and M. Janssen. Impact of light color on photobioreactor productivity. *Algal Research*, 15:32–42, April 2016. URL `https://doi.org/10.1016/j.algal.2016.01.015`.

[33] Håvard Zeiner. Rosa led-lys skal øke avlingen med 25 prosent. *Teknisk ukeblad*, June 3rd 2018. URL `https://www.tu.no/artikler/rosa-led-lys-skal-oke-avlingen-med-25-prosent/438877`.

[34] R. Dineshkumar, R. Narendran, P. Jayasingam, and P. Sampathkumar. Cultivation and chemical composition of microalgae chlorella vulgaris and its antibacterial activity against human pathogens. *Aquaculture & Marine Biology*, 5(3), March 2017.

[35] S. R. Rue, S. M. Søgnen, and M. Tengesdal. Economic analysis of microalgae production in by-product from biogas plant by installation of rotating biofilm reactors. Bachelor thesis, NTNU, Norway, May 2019.

[36] A. M. Åkerström, L. M. Mortensen, B. Rusten, and H. R. Gislerød. Biomass production and nutrient removal by chlorella sp. as affected by sludge liquor concentration. *Journal of Environmental Management*, 144:118–124, November 2014.

[37] A. Beuckels, E. Smolders, and K. Muylaert. Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. *Water Research*, 77:98–106, June 2015.

[38] R. Whitton, A. Le Mével, M. Pidou, F. Ometto, R. Villa, and B. Jefferson. Influence of microalgal n and p composition on wastewater nutrient remediation. *Water Research*, 91:371–378, March 2016.

[39] G. Markou, D. Vandamme, and K. Muylaert. Microalgal and cyanobacterial cultivation: The supply of nutrients. *Water Research*, 65:186–202, November 2014.

[40] R. Geider and J. La Roche. Redfield revisited: variability of c:n:p in marine microalgae and its biochemical basis. *European Journal of Phycology*, 37:1:1–17, 2002. URL `https://doi.org/10.1017/S0967026201003456`.

[41] G. C. Zittelli, L. Rodolfi, N. Biondi, and M.R. Tredici. Productivity and photosynthetic efficiency of outdoor cultures of tetraselmis suecica in annular columns. *Aquaculture*, 261(3):932–943, December 2006.

[42] M. H.A. Michels, M. Vaskoska, M.H. Vermuë, and R. H. Wijffels. Growth of tetraselmis suecica in a tubular photobioreactor on wastewater from a fish farm. *Water Research*, 65:290–296, November 2014.

[43] E. V. Esbroeck. Temperature control of microalgae cultivation under variable conditions. Master thesis, Wageningen University, The Netherlands, May 2018.

[44] P. Lavens and P. Sorgeloos. Manual on the production and use of live food for aquaculture. Chapter 2.3, 1996. URL http://www.fao.org/3/w3732e/w3732e00.htm#Contents.

[45] Center for Earth and Environmental Science. What causes algal blooms? URL https://cees.iupui.edu/research/algal-toxicology/bloomfactors. Accessed: 07.05.2020.

[46] E. Molina, E. Martinez, S. Saticheze, F. Garciaa, and A. Contrera. The influence of temperature and the initial n:p ratio on the growth of microalgae tetrasehis sp. *Elsevier Science Publishers Ltd*, pages 183–187, July, 1991.

[47] T. S. Abu-Rezq, L. Al-Musallam, J. Al-Shimmari, and P. Dias. Optimum production conditions for different high-quality marine algae. *Hydrobiologia*, 403:97–107, May 1999.

[48] L. Pane, E. Franceschi, L. De Nuccio, and A. Carli. Applications of thermal analysis on the marine phytoplankton, tetraselmis suecica. *Journal of Thermal Analysis and Calorimetry*, 66:145–154, 2001.

[49] V. Weiss, Z. Gromet-Elhanan, and M. Halmann. Batch and continuous culture experiments on nutrient limitations and temperature effects in the marine alga tetraselmis suecica. *Water Research*, 19(2):185–190, 1985.

[50] S. Buono, A. Colucci, A. Angelini, A. L. Langellotti, M. Massa, A. Martello, V. Fogliano, and A. Dibenedetto. Productivity and biochemical composition of tetradesmus obliquus and phaeodactylum tricornutum: effects of different cultivation approaches. *Journal of Applied Phycology*, 28(6):3179–3192, December 2016.

[51] A. M. S. Benavides, G. Torzillo, J. Kopecky, and J. Masojídekde. Productivity and biochemical composition of phaeodactylum tricornutum (bacillariophyceae) cultures grown outdoors in tubular photobioreactors and open ponds. *Biomass and Bioenergy*, 54:115–122, July 2013.

[52] I. Kudo, M. Miyamoto, Y. Noiri, and Yoshiaki Maita. Combined effects of temperature and iron on the growth and physiology of the marine diatom phaeodactylum tricornutum (bacillariophyceae). July 2008. URL https://doi.org/10.1046/j.1529-8817.2000.99042.x.

[53] H. Jiang and K. Gao. Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom phaeodactylum tricornutum (bacillariophyceae). June 2004. URL https://doi.org/10.1111/j.1529-8817.2004.03112.x.

[54] N.R. Moheimani. Inorganic carbon and ph effect on growth and lipid productivity of tetraselmis suecica and chlorella sp (chlorophyta) grown outdoors in bag photobioreactors. *Journal of Applied Phycology*, 25(2), April 2012.

[55] E. B. Pérez, I. C. Pina, and L.P. Rodríguez. Kinetic model for growth of phaeodactylum tricornutum in intensive culture photobioreactor. *Biochemical Engineering Journal*, 40(3):520–525, July 2008.

[56] C. Bennouna. The ph, a major parameter for your microalgae cultures. URL `https://www.synoxis-algae.com/the-ph-a-major-parameter-for-your-microalgae-cultures`. Accessed: 30.04.2020.

[57] M. Paddock. Microalgae wastewater treatment: A brief history. URL `10.20944/preprints201912.0377.v1`.

[58] B. Wang, C. Q. Lan, and M. Horsman. Closed photobioreactors for production of microalgal biomasses. *Biotechnology Advances*, Volume 30(4):904–912, July-August 2012.

[59] J. A. V. Costa and M. G. de Morais. Chapter 1 - an open pond system for microalgal cultivation. *Biofuels from Algae*, pages 1–22, 2014.

[60] M. Gross, W. Henry, C. Michael, and Z. Wen. Development of a rotating algal biofilm growth system for attached microalgae growth with in situ biomass harvest. *Bioresource Technology*, 150:195–201, 2013.

[61] Biofilm definition. URL `https://biologydictionary.net/biofilm/`. Last Updated: August 6, 2017.

[62] M. Gross, V. Mascarenhas, and Z. Wen. Evaluating algal growth performance and water use ef ciency of pilot-scale revolving algal biofilm (rab) culture systems. *Biotechnology and Bioengineering*, 112(10), October 2015.

[63] Olivier Bernard. Personal communication, 2020.

[64] R. R. L. Guillard and J. H. Ryther. Studies of marine planktonic diatoms: I. cyclotella nana hustedt, and detonula confervacea (cleve) gran. *Canadian Journal of Microbiology*, 8(2):229–239, 1962.

[65] Jacob Joseph Lamb. Personal communication, 2020.

[66] Gross-Wen Technologies. Algae treatment technology. URL `https://algae.com/algae-treatment-technology`. Accessed: 28.04.2020.

[67] V. G. Gude. *Green Chemistry for Sustainable Biofuel Production*. Apple Academic Press, Inc., 2018.

[68] M. A. Borowitzka. High-value products from microalgae- their development and commercialisation. *Journal of Applied Phycology*, 25:743–756, January 2013.

[69] Md. M. R. Shah, Y. Liang, J. J. Cheng, and M. Daroch. Astaxanthin-producing green microalga haematococcus pluvialis: From single cell to high value commercial products. April 2016. URL `https://www.frontiersin.org/articles/10.3389/fpls.2016.00531/full`.

[70] Deinove. Carotenoids market, natural carotenoids meeting consumer needs. URL https://www.deinove.com/en/profile/strategy-and-markets/carotenoids-market. Accessed: 07.05.2020.

[71] L. Novoveská, M. E. Ross, M. S. Stanley, R. Pradelles, V. Wasiolek, and J-F. Sassi. Microalgal carotenoids: A review of production, current markets, regulations, and future direction. *Marine Drugs*, 17(11), November 2019.

[72] Algatech. Our microalgae, 2019. URL https://www.algatech.com/our-microalgae/?microalgae=79. Accessed: 05.05.2020.

[73] U. Neumann, F. Derwenskus, V. F. Flister, U. Schmid-Staiger, T. Hirth, and S.C. Bischoff. Fucoxanthin, a carotenoid derived from phaeodactylum tricornutum exerts antiproliferative and antioxidant activities in vitro. *Marine Algal Antioxidants*, 2019.

[74] D. D. McClure, Audrey Luiz, B. Gerber, G.W. Barton, and J. M. Kavanagh. An investigation into the effect of culture conditions on fucoxanthin production using the marine microalgae phaeodactylum tricornutum. *Algal Research*, 29:41–48, January 2018.

[75] Nutraceutical Business Review. Algatech triples fucovital production capacity, October 2019. URL https://www.nutraceuticalbusinessreview.com/news/article_page/Algatech_triples_FucoVital_production_capacity/158620/cn99085. Accessed: 21.05.2020.

[76] Trendlines. Background and market. URL https://www.trendlines.com/company/algahealth/. Accessed: 04.05.2020.

[77] SPN Team. Gross-wen technologies continues strong 2019. *SPN*, September 11th 2019. URL https://siliconprairienews.com/2019/09/gross-wen-technologies-continues-strong-2019/.

[78] Biokraft. I 2017 ferdigstiller biokraft verdens største produksjonsanlegg for flytende biogass drivstoff (lbg) på skogn i nord-trøndelag. URL https://www.biokraft.no/biokraft-skogn/.

[79] International Energy Agency. Tracking transport, transport biofuels. URL https://www.iea.org/reports/tracking-transport-2019/transport-biofuels. Tracking report 2019.

[80] Status of biofuels in norway and worldwide 2018. URL https://blog.sintef.com/sintefenergy/bioenergy/status-of-biofuels-in-norway-and-worldwide-2018/. November 15th, 2018.

[81] Intergovernmental Panel on Climate Change (IPCC). Ipcc special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. August 7th 2019.

[82] Z. Trinidad. Innovative, cost effective feed for aquaculture. March, 2019. URL https://www.pna.gov.ph/articles/1063375.

[83] Bureau of Agricultural Research. Juan algae paste. URL https://www.slideshare.net/bardotgov/juan-algae-paste. Accessed: 03.04.2020.

[84] R. A. M. Aya. Juan algae, a microalgal paste, is a cost-effective feed for milkfish hatcheries. February 27th, 2019. URL http://www.pcaarrd.dost.gov.ph/home/portal/index.php/quick-information-dispatch/3394-juan-algae-a-microalgal-paste-is-a-cost-effective-feed-for-milkfish-hatcheries.

[85] M. B. Johnson and Z. Wen. Development of an attached microalgal growth system for biofuel production. *Applied Microbiology and Biotechnology*, 85:525–534, 2010.

[86] K. de Rosbo and O. Bernard. Évaluation du gisement potentiel de ressources algales pour l'énergie et la chimie en france à horizon 2030. 2014.

[87] HyperPhysics. Specific heat, 2020. URL http://hyperphysics.phy-astr.gsu.edu/hbase/thermo/spht.html. Accessed: 19.03.2020.

[88] Nitrite/nitrate, colorimetric method. https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Roche/Bulletin/1/11746081001bul.pdf, April 2016. User manual for colorimetric test kit, version 6.

[89] Merck KGaA. Photometric test kit, 2020. URL https://www.merckmillipore.com/NO/en/product/Nitrogen-total-Cell-Test,MDA_CHEM-100613?bd=1#anchor_PI. User manual for total nitrogen cell test kit for a NOVA 30 spectroquant.

[90] M. Morales, H. Bonnefond, and O. Bernard. Rotating algal biofilm versus planktonic cultivation: Lca perspective. *Journal of Cleaner Production*, 257(120547), February 2020.

[91] Philippine peso to norwegian krone spot exchange rates for 2019, Accessed: 14.04.2020. URL https://www.exchangerates.org.uk/PHP-NOK-spot-exchange-rates-history-2019.html.

[92] Us dollar to norwegian krone spot exchange rates for 2019, Accessed: 14.04.2020. URL https://www.exchangerates.org.uk/USD-NOK-spot-exchange-rates-history-2019.html.

[93] Kristian Myklebust Lien. Personal communication, 2020.

[94] F. G. Acién, J. M. Fernández, J. J. Magán, and E. Molina. Production cost of a real microalgae production plant and strategies to reduce it author links open overlay. *Biotechnology Advances*, 30(6):1344–1353, 2012.

[95] Skatteetaten. Avskrivningssatser, 2019. URL https://www.skatteetaten.no/satser/avskrivningssatser/?year=2019#rateShowYear. Accessed: 17.04.2020.

[96] European Commission PVGIS. Solar irradiation tool. URL https://re.jrc.ec.europa.eu/pvg_tools/en/tools.html#MR. Accessed: 17.04.2020.

[97] Ånund Sigurd Kvambekk. Personal communication, 2020.

Appendices

A Solar Irradiation in Levanger

The irradiance for each months from the years 2011-2016 are displayed in Table 21 for Levanger in Norway. The latitude is 63.696 degrees and the longitude is 11.224 degrees. The irradiance is measured at a 45 degree angle.

Table 21: Solar irradiance in kWh m^{-2} in Levanger, Norway. [96]

Month/Year	2011	2012	2013	2014	2015	2016
January	8.41	16.78	19.18	24.85	17.6	18.97
February	62.61	34.30	51.41	49.32	43.21	46.94
March	98.70	65.75	141.75	98.43	106.95	88.31
April	124.75	132.67	153.59	126.36	107.37	141.46
May	164.54	161.18	164.03	154.95	138.57	162.01
June	128.00	149.45	134.67	143.2	119.21	163.78
July	133.28	117.51	127.64	198.37	114.36	135.41
August	122.17	122.99	134.40	135.42	167.65	118.44
September	86.49	79.34	113.61	102.53	98.13	95.89
October	62.00	63.81	59.04	74.72	61.59	103.09
November	23.81	19.82	15.06	29.2	19.71	23.43
December	4.18	9.01	4.68	4.96	4.04	3.3

B Temperatures at Floan Bridge

The average temperatures for each month from 1998 to 2020 at Floan bridge in Levanger are displayed in Table 22 [97]. The average temperature for all years and months, excluding July, is 5.072°C. N.A. is for months without data.

Table 22: Average temperatures in the years 1998-2020 at Floan bridge, Levanger. [97]

	Jan	Feb	March	April	May	June	July	Aug	Sep	Oct	Nov	Dec
1998	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	10.038	4.816	0.368	0.280
1999	0.096	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	3.207	3.065	-0.089
2000	-0.110	-0.142	0.052	1.836	7.070	10.749	12.958	11.862	9.306	7.131	2.821	1.458
2001	0.854	0.551	0.513	1.807	5.411	9.950	12.734	11.614	9.984	6.461	2.600	1.742
2002	0.833	0.656	0.685	3.128	8.980	13.881	14.399	15.690	10.814	4.314	2.114	1.319
2003	0.930	0.818	0.716	2.400	6.263	10.938	15.176	13.751	10.063	5.418	2.365	1.392
2004	1.020	0.772	1.141	3.420	7.698	10.177	11.718	13.683	10.264	6.211	3.627	2.093
2005	1.619	1.251	1.117	2.852	5.252	8.986	13.113	12.174	9.830	6.928	4.724	2.061
2006	1.295	1.141	0.885	1.749	6.193	9.931	12.996	14.540	11.663	8.089	3.866	3.297
2007	2.044	1.282	1.178	2.522	5.939	11.596	13.460	12.759	9.046	6.754	4.461	N.A.
2013	N.A.	N.A.	N.A.	N.A.	N.A.	12.325	14.193	13.778	10.273	5.155	1.356	0.885
2014	0.306	0.091	0.587	3.180	9.066	13.187	18.914	14.913	10.876	5.719	1.294	0.283
2015	0.165	0.362	1.524	3.429	7.356	10.131	13.418	14.568	10.789	6.046	3.019	1.166
2016	0.064	0.062	0.206	2.982	8.299	14.744	15.713	13.126	11.432	3.427	0.534	0.891
2017	0.622	0.084	0.562	2.779	7.112	12.139	14.020	13.414	10.491	5.573	1.627	0.060
2018	0.060	0.060	0.060	0.733	10.096	12.857	17.603	13.813	10.305	5.489	1.689	0.295
2019	0.595	0.264	0.338	3.613	7.983	13.073	15.140	14.921	9.713	3.810	0.319	0.189
2020	0.816	0.636	0.983	2.730								

C Risk Assessment Report with Attachment

Risk Assessment Report

Bioreactor for microalgae growth

Project name	Microalgae growth
Facility name	Bioreactor for microalgae growth
Building and room number	Heat technical, room C162
Project leader	Jacob Lamb
Rigg responsible	Jacob Lamb
HES coordinator	Morten Grønli
HES responsible	Jacob Lamb
Risk assessment performed by	Simon Steinvoll

Approval:

Apparatur kort (UNIT CARD) valid for:	
Forsøk pågår kort (EXPERIMENT IN PROGRESS) valid for:	

Role	Name	Date	Signature
Project leader	Jacob Lamb		
HES coordinator	Morten Grønli		
HES responsible	Jacob Lamb		

TABLE OF CONTENTS

1 INTRODUCTION

The experiment is planned to start in March. Prior to that, preparations and risk analysis will be done. On the 2nd of March, construction of testing equipment will start. The plan is to finish it the same week, before 8th of March. Then testing with test water will start. Different containers for different tests will be done simultaneously. After a couple of weeks, some testing will be done on wastewater from Hardingsmolt in Hardanger. The aim is to grow algae in the water tank on a cotton based material. The testing are planned to be finished before April, for the purpose of our Bachelor project. However, we plan to continue the testing for our summer project, as student interns, working for Enersense until the middle of July.

2 DESCRIPTIONS OF EXPERIMENTAL SETUP

Drawing/sketch/Photo of the test device with a description of the setup, location of the setup:

Principal sketch of the test setup in C162, Heat technical building, Gløshaugen, NTNU.

Process and Instrumentation Diagram w/list of components:

Apparatus	Description of the component
Petri dishes	The biggest part of the test-setup. Volume: 100 ml each.
Working light	Cotech LED 1270 lm

"F/2-medium"	A chemical mixture with NaNO3 75, NaH2PO4, Na2SiO3, a trace metal solution and a vitamin solution. Mixed with seawater and some freshwater becomes our testwater.
Ammonia	In addition to the F-medium into the test-water mix.
Test water	This will contain one part seawater and 2-3 parts freshwater. It will also contain the "F/2-medium" and ammonia.
Cotton film	Organic cotton (Organic Isoli white, brushed).
Clothespins	This is for the dark blue sheet. For regulation of the lighting.

3 EVACUATION FROM THE EXPERIMENTAL AREA

Evacuate at signal from the alarm system or local gas alarms with its own local alert with sound and light outside the room in question, see 6.2

Evacuation from the rigging area takes place through the marked emergency exits to the assembly point, (corner of Old Chemistry Kjelhuset or parking 1a-b.)

Action on rig before evacuation:

1. Closing of chemical containers.
2. Turn of the light (in the setup).
3. Turn of the oven/drying cabinet if it is in use.
4. Immediate evacuation.

4 WARNING

4.1 Before experiments

Experiments will only be run if approval from the laboratory management has been received. To obtain the approval, an email including information such as:

- Name of responsible person:
- Experimental setup/rig:
- Start Experiments: (date and time)
- Stop Experiments: (date and time)

Will be sent to: **iept-experiments@ivt.ntnu.no**

All running experiments are displayed in the activity calendar for the lab to be sure they are coordinated with other activity.

4.2 Abnormal situation

FIRE

If you are NOT able to extinguish the fire, activate the nearest fire alarm and evacuate area. Be then available for fire brigade and building caretaker to detect fire place.
If possible, notify:

NTNU
Morten Grønli, Mob: 918 97 515
Jacob Lamb: Mob: 902 38 329
NTNU – SINTEF Beredskapstelefon

GAS ALARM

If a gas alarm occurs, evacuate the lab.

PERSONAL INJURY

- First aid kit in the fire / first aid stations
- Shout for help
- Start life-saving first aid
- **CALL 113** if there is any doubt whether there is a serious injury

OTHER ABNORMAL SITUATIONS

NTNU:
You will find the reporting form for non-conformance on:
https://innsida.ntnu.no/wiki/-/wiki/Norsk/Melde+avvik

5 ASSESSMENT OF TECHNICAL SAFETY

5.1 HAZOP

The experiment set up is divided into the following nodes:

Node 1	Algae production containers with following side components

Attachments:
Conclusion: (Safety taken care of)

5.2 Flammable, reactive and pressurized substances and gas

Are any flammable, reactive and pressurized substances and gases in use?

YES	Ammonia is in dilute (1 mg / L) concentrations
NO	

Conclusion: Gloves have to be used when handling reactive substances.

5.3 Pressurized equipment

Is any pressurized equipment in use?

YES	
NO	There is no pressurised equipment in use

Attachments: Certificate for pressurized equipment (see Attachment to Risk Assessment)
Conclusion:

5.4 Effects on the environment (emissions, noise, temperature, vibration, smell)

YES	

NO	There will be no effects on the environment

5.5 Radiation

The experiment will not release any kind of radiation or radioactive material.

5.6 Chemicals

A low concentration of ammonia (mg / L) may be used. In case of use it will be stored and handled appropriately.

5.7 Electricity safety (deviations from the norms/standards)

YES	The light source used will be been placed in a safe location with the cable avoiding any potential hazards (e.g. away from water or any potential water spills)
NO	

6 ASSESSMENT OF OPERATIONAL SAFETY

In order to ensure that the procedure cover and manage all the risk factors identified in the previous section; this numeral sets specific guidelines for the use of the equipment in the experimentation set-up, as well as it set protocols that have to be followed by the operators of the bioreactor.

6.1 Procedure HAZOP

Attachments:: HAZOP_MAL_Prosedyre

6.2 Operation procedure and emergency shutdown procedure

The operating procedure is a checklist that must be filled out for each experiment.
Emergency procedure should attempt to set the experiment set up in a harmless state by unforeseen events. Operational procedure is detailed in the attachment, while the emergency shutdown of the system has already been described in Section 3 of this report.

Attachments: Procedure for running experiments

6.3 Training of operators

Operators of the bioreactor must follow a training plan that involves:
1. Completed the HSE course provided by the HSE-coordinator.
2. Must have received a guided tour from the staff of laboratory.
3. Be familiar with the equipment and its operation.
4. Have received the approval from the Project Leader.

6.4 Technical modifications

If modifying the system is required for the experiment, the possible technical or operational risk emerging from such variation have to be assessed. According to the risk involved, modification will be authorized and assigned to proper staff. Changes with low risk, as is more likely, can be carried out by the operator of the system, while risky tasks have to be handled by Project Leader.

6.5 Personal protective equipment

Use gloves, lab coat and safety goggles when there is opportunity for contact with chemicals.

6.6 General Safety

The experiment is in general low risk. When there are potential hazards such as chemicals in use, the HSE responsible/Project leader will be involved to ensure safety.

6.7 Safety equipment & Special predations

Experiments involving the use of the bioreactor setup require neither additional safety precautions nor safety equipment.

7 QUANTIFYING OF RISK - RISK MATRIX

The risk matrix will provide visualization and an overview of activity risks so that management and users get the most complete picture of risk factors.

IDnr	Activity	Consequence	Probability	RV
xx	*Water spill when filling/emptying the containers.*	B	2	B2
	Chemicals in contact with skin/eyes	C	2	C2

Conclusion: The *Participants has to make a comprehensive assessment to determine whether the remaining risks of the activity/process is acceptable.*

RISK MATRIX

CONSEQUENSE	(E) Catastrophic	**E1**	**E2**	**E3**	**E4**	**E5**
	(D) Extensive	**D1**	**D2**	**D3**	**D4**	**D5**
	(C) Moderate	**C1**	**C2**	**C3**	**C4**	**C5**
	(B) Negligible	**B1**	**B2**	**B3**	**B4**	**B5**
	(A) Insignificant	**A1**	**A2**	**A3**	**A4**	**A5**
		(1) Rare	(2) Unlikely	(3) Possible	(4) Likely	(5) Almost certain
		PROBABILITY				

COLOUR		DESCRIPTION
Red		Unacceptable risk Action has to be taken to reduce risk
Yellow		Assessment area. Actions has to be considered
Green		Acceptable risk. Action can be taken based on other criteria

The principle of the acceptance criterion. Explanation of the colors used in the matrix

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Attachment to Risk Assessment report

Bioreactor for microalgae growth

Project name	Microalgae growth
Facility name	Bioreactor for microalgae growth
Building and room number	Heat technical, room C162
Project leader	Jacob Lamb
Rigg responsible	Jacob Lamb
HES coordinator	Morten Grønli
HES responsible	Jacob Lamb
Risk assessement performed by	Simon Steinvoll

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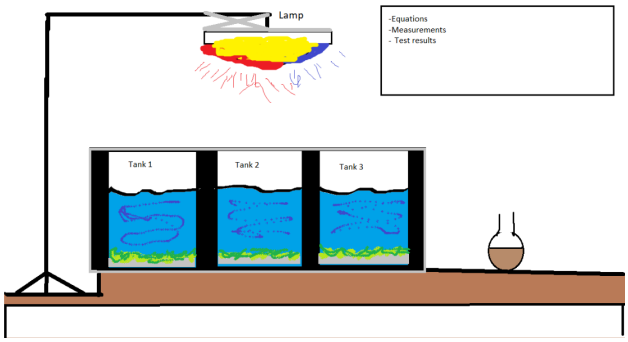
ATTACHMENT A: PROCESS AND INSTRUMENTATION DIAGRAM (PID)

Simplified sketch of the bioreactor setup with lighting.



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ATTACHMENT E: PROCEDURE FOR RUNNING EXPERIMENTS

Project Microalgae growth		
Facility Bioreactor for microalgae growth	**Date**	**Signature**
Project leader Jacob Lamb		

	Conditions for the experiment:	**Completed**
	The experiment will run continuously. Every week measurements will be done on the water, on a daily basis. This will be done mainly during normal working hours, 08:00-16:00, and sometimes in the afternoon or in the weekend.	
	Be sure that everyone taking part of the experiment is wearing the necessary protecting equipment and is aware of the shut down procedure and escape routes.	
	Preparations	**Carried out**
	Post the “Experiment in progress” sign.	
	Start up procedure	
	During the experiment	
	Measure temperature	
	Measure PH levels	
	Measure algae growth	
	Measure nitrate and ammonia levels	
	End of experiment	
	Shut down procedure	
	Remove all obstructions/barriers/signs around the experiment.	
	Tidy up and return all tools and equipment.	
	Tidy and clean up work areas.	
	To reflect on before the next experiment and experience useful for others	
	Was the competence which was needed for security and completion of the experiment available to you?	
	Do you have any information/ knowledge from the experiment that you should document and share with fellow colleagues?	

Operator(s):

Navn	Dato	Signatur
Jacob Lamb		
Vanja Olberg		

Simon Steinvoll		

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ATTACHMENT F: TRAINING OF OPERATORS

Project Microalgae growth		
Facility Bioreactor for microalgae growth	**Date**	**Signature**
Project leader Jacob Lamb		

	Knowledge about EPT LAB in general	
	Lab • Access • routines and rules • working hour	
	Knowledge about the evacuation procedures.	
	Activity calendar for the Lab	
	Early warning, experiments@ept.ntnu.no	
	Knowledge about the experiments	
	Procedures for the experiments	
	Emergency shutdown.	
	Nearest fire and first aid station.	

I hereby declare that I have read and understood the regulatory requirements and has received appropriate training to run this experiment and are aware of my personal responsibility by working in EPT laboratories.

Operator(s):

Navn	Dato	Signatur
Jacob Lamb		
Vanja Olberg		
Simon Steinvoll		

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APPARATURKORT / UNITCARD

Dette kortet SKAL henges godt synlig på apparaturen!
This card MUST be posted on a visible place on the unit!

Apparatur (Unit) Bioreactor for microalgae growth	
Prosjektleder (Project Leader) Jacob Lamb	**Telefon mobil/privat** (Phone no. mobile/private) 902 38 329
Apparaturansvarlig (Unit Responsible) Jacob Lamb	**Telefon mobil/privat** (Phone no. mobile/private) 902 38 329
Sikkerhetsrisikoer (Safety hazards) **Chemicals, and water spillage.**	
Sikkerhetsregler (Safety rules) 1. Use protective googles, lab coat and gloves. 2. Handle water containers with care.	
Nødstopp prosedyre (Emergency shutdown) 1. Turn of the light. 2. Turn of the oven/drying cabinet. 3. Close containers for chemicals. 4. Immediate evacuation.	

Her finner du (Here you will find):

Prosedyrer (Procedures)	Next to the bioreactor
Bruksanvisning (Users manual)	Next to the bioreactor

Nærmeste (Nearest)

Brannslukningsapparat (fire extinguisher)	At the door between C114 and C162
Førstehjelpsskap (first aid cabinet)	At the door between C114 and C162

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Institutt for energi og prosessteknikk

Dato

_____

Signert

_____

NTNU

FORSØK PÅGÅR /EXPERIMENT IN PROGRESS

Dette kortet SKAL henges opp før forsøk kan starte!
This card MUST be posted on the unit before the experiment startup!

Apparatur (Unit) Bioreactor for microalgae growth	
Prosjektleder (Project Leader) Jacob Lamb	**Telefon mobil/privat** (Phone no. mobile/private) 90238329
Apparaturansvarlig (Unit Responsible) Jacob Lamb	**Telefon mobil/privat** (Phone no. mobile/private) 90238329
Godkjente operatører (Approved Operators) Simon Steinvoll Vanja Olberg Jacob Lamb	**Telefon mobil/privat** (Phone no. mobile/private) 40638389 93601849 90238329
Prosjekt (Project) Bioreactor for microalgae growth	
Forsøkstid / Experimental time (start - stop)	
Kort beskrivelse av forsøket og relaterte farer (Short description of the experiment and related hazards**)** **In this experiment, microalgae is grown on cotton biofilm in water containers.**	

NTNU
Institutt for energi og prosessteknikk

Dato

Signert

NTNU

Norwegian University of
Science and Technology