Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Engineering
Department of Energy and Process Engineering

Henriette Skaret Kjos-Hanssen

Recirculating aquacultural system (RAS) wastewater treatment using microalgae

Master's thesis in Energy and Environmental Engineering
Supervisor: Kristian Myklebust Lien
Co-supervisor: Jacob Joseph Lamb
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Abstract

The wastewater from land-based fish farming facilities contains nutrients (e.g., nitrogen and phosphorus) originating from uneaten fish feed and feces. If this waste is released into ecosystems, the nutrients in the water can lead to eutrophication which is a process that can create extensive damage (e.g., increasing harmful algae growth). When the nutrients are removed from the water, the water becomes reusable for the fish farming facility or can safely be released to ecosystems.

Removing nutrients from wastewater can be done using microalgae by letting the water run through a microalgae cultivation facility. In the case addressed here, the facility will be a rotating algal biomass (RAB) facility. This is one of the most optimal cultivation facilities because of the rotating cultivation belts. These belts provide a high growth rate because of their continuous rotation from a gas stage in a room filled with light to a dark cultivation liquid medium full of nutrients. In addition to this, the cultivation belts are built vertically to save footprint area. This process will also create a biomass byproduct, which can be sold for profit. Therefore, this process looks at turning a waste product into a commodity, resulting in reduced waste into ecosystems and the growth of bio-commodities.

In this thesis, two microalgae species *Phaeodactylum Tricornutum* and *Synechocystis* SP. PCC 6803 and a mixed culture from both the species are being tested for their nutrient absorption rate. The nutrient absorption rate can be used to see how well these species could remove nutrients from wastewater to be a treatment facility for a land-based recirculating aquacultural system (RAS). From the laboratory experiments, it was found that all three cultures absorbed about 75 $\mathrm{mg} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ of nitrogen and 4.411 $\mathrm{mg} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ of phosphor. The *Phaeodactylum Tricornutum* biomass had trouble attaching to the surface and had, therefore, low biomass growth. As a result of this *Synechocystis* SP. PCC 6803 has been the chosen microalgae, as it had a steady growth rate.

The laboratory experiments and calculations performed in this thesis further indicated that for a RAS plant producing 219 tons of salmon per year, a microalgae wastewater treatment facility would require a RAB surface area of approximately 10,000 m^2 and a footprint area between 900 to 1,200 m^2 with cultivation belt heights of 2.50 to 1.83 m respectively. Calculations show that the microalgae *Synechocystis* SP. PCC 6803 would produce up to 14 tons of microalgae biomass a year. This differs from information found in a literature search that the same RAB reactor could produce up to 111 tons of microalgae biomass.

There are several reasons why the calculated biomass production from the laboratory experiments in this thesis is around 15 % of the RAB biomass production from literature. Initially, this thesis was going to do a pilot test of microalgae growth in an actual RAB reactor. Because of Covid-19, it was impossible to transfer the reactor to Norway in time, so the laboratory testing was a backup plan. The microalgae grown in the lab were cultivated in bottles and supplied nutritious water every ten days. This is far from the optimal environment in a RAB reactor and presented with shallow values for biomass production, as expected from bottle cultivation.

Sammendrag

Avløpsvannet fra landbaserte oppdrettsanlegg inneholder næringsstoffer (f.eks. nitrogen og fosfor) som stammer fra uspist fiskefôr og avføring. Hvis dette avfallet slippes ut i økosystemer, kan næringsstoffene i vannet føre til eutrofiering, som er en prosess som kan skape omfattende skader (f.eks. øke skadelig algevekst). Når næringsstoffene blir fjernet fra vannet, kan vannet bli gjenbrukbart for oppdrettsanlegget eller kan trygt slippes ut i økosystemer.

Fjerning av næringsstoffer fra avløpsvann kan gjøres ved bruk av mikroalger ved å la vannet renne gjennom et mikroalgedyrkningsanlegg. Dette anlegget vil være et roterende alge-biomasse (RAB) anlegg. Dette er et av de mest optimale dyrkingsanleggene på grunn av de roterende dyrkingsbeltene. Disse beltene gir høy vekstrate på grunn av deres kontinuerlige rotasjon fra et gassstadium i et rom fylt med lys til et mørkt dyrkningsmedium fullt av næringsstoffer. I tillegg til dette er dyrkingsbeltene bygget vertikalt for å spare fotavtrykk. Denne prosessen vil også skape et biprodukt fra biomasse, som kan selges for profitt. Derfor ser denne prosessen på å gjøre et avfallsprodukt om til en vare, noe som resulterer i redusert avfall til økosystemer og vekst av biovarer.

I denne oppgaven blir to mikroalger *Phaeodactylum Tricornutum* og *Synechocystis* SP. PCC 6803 og en blandet kultur fra begge artene testet for absorpsjonshastighet for næringsstoffer. Næringsopptakshastigheten kan brukes til å se hvor godt disse artene er egnet til å fjerne næringsstoffer fra avløpsvannet til et behandlingsanlegg for et landbasert resirkulerende akvakulturelt system (RAS). Fra laboratorieeksperimentene ble det funnet at alle tre kulturene absorberte ca 75 $\mathrm{mg} \cdot \mathrm{m}^{-2} \cdot \mathrm{døgn}^{-1}$ nitrogen og 4.411 $\mathrm{mg} \cdot \mathrm{m}^{-2} \cdot \mathrm{døgn}^{-1}$ fosfor. Biomassen til *Phaeodactylum Tricornutum* hadde problemer med å feste seg til overflaten og hadde derfor lav biomassevekst. Som et resultat av dette ble *Synechocystis* SP. PCC 6803 valgt som den beste mikroalgen.

Laboratorieeksperimentene og beregningene som ble utført i denne avhandlingen indikerte videre at for et RAS-anlegg som produserer 219 tonn laks per år, vil et renseanlegg for mikroalger kreve et RAB-overflateareal på ca. 10,000 m^2 et fotavtrykkareal mellom 900 til 1200 m^2 med dyrkningsbeltehøyder henholdsvis mellom 1,83 og 2,50 m høye. Beregninger viser at mikroalgene *Synechocystis* SP. PCC 6803 ville produsere opptil 14 tonn mikroalger biomasse i året. Dette skiller seg fra informasjonen som ble funnet i et litteratursøk at den samme RAB-reaktoren kunne produsere opptil 111 tonn mikroalgerbiomasse.

Det er flere grunner til at den beregnede produksjonen av biomasse fra laboratorieforsøkene i denne oppgaven er rundt 15 % av den faktiske beregnede produksjonen av biomasse fra et RAB anlegg. Opprinnelig skulle denne oppgaven gjennomføre en pilottest av mikroalgevekst i et RAB anlegg. På grunn av Covid-19 var det umulig å transportere reaktoren til Norge i tide, så laboratorietestingen var en reserveplan. Mikroalgene dyrket i laboratoriet ble dyrket i flasker og tilført næringsrikt vann hver tiende dag. Dette er langt fra det optimale miljøet i en RAB-reaktor og presenteres med grunne verdier for produksjon av biomasse, som forventet fra dyrking i flaske.

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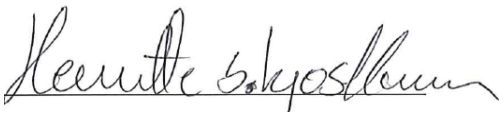
First and foremost I would like to thank my supervisors Associate Professor Jacob J. Lamb and Professor Kristian M. Lien for all their help and good conversations. Secondly I would like to thank fellow students, especially Simon Lorentzen and Martin Sande for for being there in bad times and celebrating together in the good times. I want to thank my roommates; Pia, Ida, Malin and Ingrid for always putting a smile on my face after a long day at school. And lastly I would like to thank family for always supporting me and standing by my side.

Trondheim, June 11, 2021



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Abbreviations

BDO Biochemical oxygen demand

BNR Biological nutrient removal

C Carbon

CNP carbon, nitrogen, and phosphorus

DBR Drum biofilm reactor

HRAP High rate algal ponds

LEA Lipid-extracted algae

N Nitrogen

NTNU Norges Tekniske-Naturvitenskaplige Universitet

OPR Open raceway ponds

P Phosphorus

PBR Photobioreactor

ppt Parts per million

RAB Rotating algal biofilm

RAS Recirculating aquaculture systems

TAN Total ammonia nitrogen

TN Total nitrogen

TOC Total organic carbon

TP Total phosphorus

TSS Total suspended solids

WWT Wastewater treatment

1 Introduction

In this Chapter, the background and motivation for this thesis is presented. In Section 1.2, the objectives and scope, as well as the research question of this thesis can be found. The last Section 1.3, contains the outline of the entire thesis.

1.1 Background and motivation

During the last 70 years, there has been an enormous growth in the fish farming industry globally due to the steadily increasing demand for fish. This demand has caused growth from a global production of approx. Twenty million tons in 1950, to approx. 171 million tons in 2016. This includes fish and shellfish from seawater and freshwater and wild fishing and farmed fish [1, 2]. While the global harvest of wild fish has stagnated around 90 million tons a year, the fish farming industry keeps growing. Aquaculture is still the fastest growing food-producing sector globally; to keep up with the increasing demand and is based mainly on farmed fish. In 2014 the human consumption of farmed fish outweighed the consumption of wild-caught fish and had continued to grow since. Because of the impact on the environment, it is of utmost importance that the environmental damage often related to traditional fish farming is avoided in this expansion [3, 4].

Aquaculture is a term that embraces farming and cultivation of all kinds of organisms in the water. Fish farming is a form of aquaculture and is a process where fish are cultivated in captivity throughout their lifespan. One of the biggest problems with offshore fish farming is the waste that accumulates from uneaten fish feed, feces and the nutrient build up in the water from this, the water is emitted straight into the marine environment from the wastewater. Alternatively, there are also land-based fish farming facilities, and these can control their production and emissions much better, as the wastewater is regulated and flows out through one pipe. Land-based facilities often have wastewater treatments to clean the nutrients before the water is either emitted out to the surrounding environment or transferred back into the fish farming facility.

Several different technologies can be used for wastewater treatment. Some land-based facilities have integrated wastewater treatment in their process, the treated water can then be recirculated back into the fish tank. The most common facility is called recirculating aquaculture systems (RAS). This facility uses conventional methods that include denitrification and biofilters to remove nutrients. Microalgae is another way to clean nutrients from the wastewater, the microalgae absorb the nutrients and use the photosynthesis to grow biomass. This method has been studied for several years but has never been tested beyond the pilot scale. There has yet to be any large corporation that has utilized this technology. Traditional microalgae growth facilities are predominantly based on growing the microalgae directly in water. To remove the microalgae biomass in the harvesting face is therefor challenging, time demanding and expen-

sive, this is one of the reasons why microalgae cultivation is not utilized at a large scale yet.

There are several types of microalgae cultivation facilities, one of the most efficient ones is the rotating algal biofilm (RAB) reactor. This reactor has rotating belts that are built vertically, that rotate between nutrient-rich water medium and a room filled with O_2 and light; this gives the microalgae get optimal photosynthetic conditions. This microalgal biomass is grown on rotating belts, which makes it easy to harvest the biomass by scraping, therefor eliminating a large obstacle in microalgae cultivation. Wastewater treatment using microalgae can be pretty spacious, as there is a large amount of microalgae needed to remove nutrients from an entire fish farm facility. RAB facilities utilize the footprint area better by having the cultivation belts built vertically. The area utilization will be focused on in this thesis to research how large a microalgae cultivation facility needs to be as there is little knowledge of the area needed for large-scale production.

Microalgae have been demonstrated to be an environmentally friendly and sustainable alternative to energy-intensive and conventional biological treatment processes that are widely used today. Microalgae is both a renewable source for biomass, and wastewater treatment is cost-effective and a feasible method for bio-fixation of CO_2. The rationale behind the use of mixotrophic microalgae to treat wastewater lies in their ability to utilize organic and inorganic carbon and inorganic nitrogen (N) and phosphorus (P) for their growth. This results in a reduction in the concentration of these substances in the wastewater. Achieving improved ecological status of water sources is growing focus for many developed and developing nations, in particular with removing N and P, therefor microalgae treatment can help with this [5]. The microalgae species that are being tested in this thesis are *Phaeodactylum Tricornutum* and *Synechocystis* SP. PCC 6803, they were chosen because they were the only available species for use at NTNU.

Nofitech is a Trondheim-based company that delivers recirculating aquaculture systems (RAS) facilities; they work towards delivering environmentally friendly, compact, and cost-efficient facilities for land-based fish farming. As a distributor of these facilities, they are looking into sustainable ways to treat their wastewater. They have been considering microalgae, but since this is a technology with limited information and testing performed, they need to know how large a facility like this would be. This thesis will focus on the values of nutrient levels from their ModulRAS reactors wastewater to estimate the scale of the microalgae cultivation facility.

1.2 Objectives and scope

The objective of this thesis is to assess how large does a microalgae cultivation facility has to be to absorb the wastewater nutrients (e.g., nitrogen (N) and phosphorus (P)) from a land-based fish farming facility. The microalgae nutrient absorption rate from wastewater can indicate their ability to grow in a large-scale production.

Initially, this thesis was going to be a pilot test to see the growth rate and production of the two microalgae *Phaeodactylum Tricornutum* and *Synechocystis* SP. PCC 6803 in a physical rotating algal biomass (RAB) reactor. As a consequence of the Covid-19 pandemic, the transportation of the RAB reactor was delayed and therefore was not in Norway at the time of the thesis. As a backup solution to find the nutrient absorption rate, a laboratory test was completed instead. Microalgae have been grown in a laboratory; while the microalgae have grown, water and biofilm samples have been collected. These samples have then been tested for their nutrient absorption rate and growth values, using the test results and process parameters from a RAS facility provided by Nofitech. It was assumed that the biomass production from the laboratory experiments would not be as high as biomass production in a RAB reactor from the literature.

The nutrient absorption values can be used to indicate the surface and footprint area needed for large-scale production of microalgae in a RAB facility. The water medium used in the laboratory tests will be artificial wastewater, to portray as the wastewater from a RAB facility. In addition to this, the growth values found in from the tests can give an estimation of the microalgal growth rate. The surface area needed and the growth rate can give an indication for how much microalgae biomass could be produced in a year. The research question for this thesis is: *How well does the two microalgae species; Phaeodactylum Tricornutum and Synechocystis SP. PCC 6803 absorb nutrients from wastewater, and how does this affect the size of a cultivation facility?*

This thesis does not have an economic analysis of how much it would cost to build a microalgae facility. However, suppose an economic analysis is needed, there is a bachelor's thesis written by Rue et al. [6], they did an economic analysis of microalgae production as a by-product from biogas plant by installation of rotating biofilm.

1.3 Outline

This thesis is divided into seven Chapters. Chapter 1 contains the introduction, which includes motivation, a literature review, the objectives, scope, and an outline of the thesis. Chapter 2 is the Theory Chapter; here, there is a thorough review of the relevant theory of this thesis. Chapter 3 is the methodology Section; which explains and defines the execution of the laboratory experiment. There is also an explanation of how the nutrient tests were executed and how the needed cultivation area was calculated. The results of the lab experiments and the calculations are presented in Chapter 4. Chapter 5 contains the discussion of the validation of the results. Chapter 7 concludes the most important aspects from this thesis, and Chapter 6 focuses on what further work can be done to increase the knowledge of the topics of this thesis.

2 Theory

To answer the research question presented in Section 1.2 relevant literature must be collected and evaluated with the most important findings presented in this Chapter. The first four Sections in this Chapter are there to get a background overview of the topic. The first Section 2.1, nutrients and eutrophication, contains information on why wastewater should be treated and the effects it would cause if extensive amounts of nitrogen and phosphor would be let out into ecosystems. Section 2.2 - fish farming is about the basis of fish farming and the theory of recirculating aquaculture systems (RAS), as that is the facility the thesis calculations are being based off. Section 2.3, contains information about aqua-cultural wastewater and different ways to treat it, both conventional and with the use of microalgae. Section 2.4 gives a background for how microalgae grow and its perks of it being utilized in the industry.

The two following Sections will overview how the microalgae can be grown in large-scale cultivation facilities. Section 2.5 gives an introduction to different cultivation technologies and closer information about rotating algal biofilm (RAB) reactor, which is the facility in focus for this thesis. The last Section 2.6 explains different process parameters that need to be accounted for when modeling a cultivation facility.

2.1 Nutrients and eutrophication

Humans strongly influence almost every significant aquatic ecosystem, and their activities have dramatically altered the fluxes of growth-limiting nutrients from the landscape to receiving waters. Over the last half-century, the use of nitrogen- and phosphorus-based synthetic fertilizer in agriculture has increased rapidly [7, 8]. Also, aquaculture has changed its behavior over the same half-century. Previously the fish feed mainly consisted of raw material from marine environments. The composition of feed today is drastically different, with up to 80 % of the feed consisting of vegetable content [9]. The change of fish feed ingredient sources from 1990 - 2019 is presented in Figure 2.1.1 [10, 11].

The feed with the higher vegetable content has led to higher emissions of nitrogen (N) and phosphorus (P) because the fish fails to digest the plant-based feed at the same rate as marine-based feed [12, 13]. Table 2.1.1 shows the nutrient release of nitrogen and phosphorus from fish feed from Skretting Norway [11].

Table 2.1.1: *Nutrient release of nitrogen and phosphorus from fish feed from Skretting Norway [$\mathrm{g} \cdot (\mathrm{kgfeed})^{-1}$] [11]*

	Nitrogen [$\mathrm{g} \cdot (\mathrm{kgfeed})^{-1}$]	**Phosphorus [$\mathrm{g} \cdot (\mathrm{kgfeed})^{-1}$]**
In feces	7.6	5.5
Dissolved in water	29.6	1.0
Total discharge	37.1	6.5

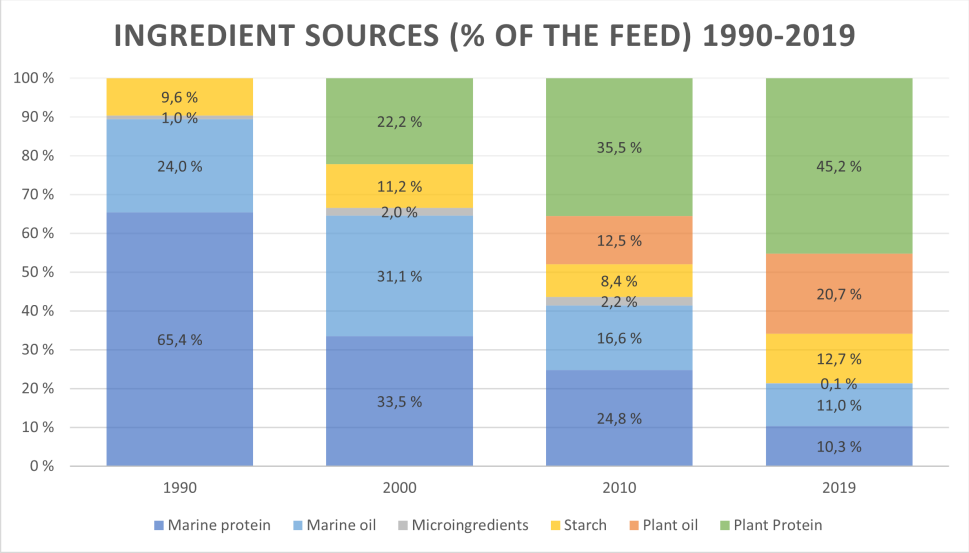


Figure 2.1.1: *The distribution of fish feed ingredient sources from 1990 to 2019. [10, 11]*

Combining the changes in agriculture and aquaculture has led to profound effects upon the quality of the receiving waters. The reason why fertilizer is added to agriculture at all is to enhance plant growth. However, the N and P can also cause eutrophication if it gets transferred into different aquatic ecosystems. Eutrophication can often occur because the rain will wash large parts the fertilizer away and into rivers and streams that lead to larger aquatic ecosystems [7].

Eutrophication is a disruption in the ecosystem caused by N and P (e.g., toxic algal blooms, loss of oxygen, dead fish, loss of aquatic plant beds and coral reefs, or loss of biodiversity). All these disruptions end up degrading the aquatic ecosystem and damage it for further use (e.g., drinking water, industry, agriculture, and recreation) [14, 15]. Figure 2.1.2 shows some aspects of how aquatic ecosystems are negatively influenced by eutrophication [16]. The most destructive effect of eutrophication is the explosive growth of problematic algae. These algae can be harmful to livestock, humans, and other organisms. Blooms in algal growth will lead to the spread of toxins. As dead algal decomposes, it consumes all oxygen in the area, leading to an anaerobic environment that can cause other organisms also to die [14, 13]. Another effect of eutrophication is that the increase in nutrients would decrease the heterogeneity of food quality for invertebrates, which leads to lower biodiversity. In addition, there can arise symptoms directly or indirectly related to nuisance growth of aquatic plants [7].

Oxygen is fundamental for many organisms on the planet, both for the survival of individual animals and regulating the global cycles of significant nutrients and carbon. The ocean's and coastal water's oxygen content has decreased at the same rate as nitrogen (N), and phosphorus (P) levels have increased. This has led to worse living conditions for the organisms that exist there [17]. As it is also shown in Figure 2.1.2, whereas the nutrient levels rise, there are more algal bloom appearing, which then leads to lower O_2 levels. When the O_2 levels decrease, the

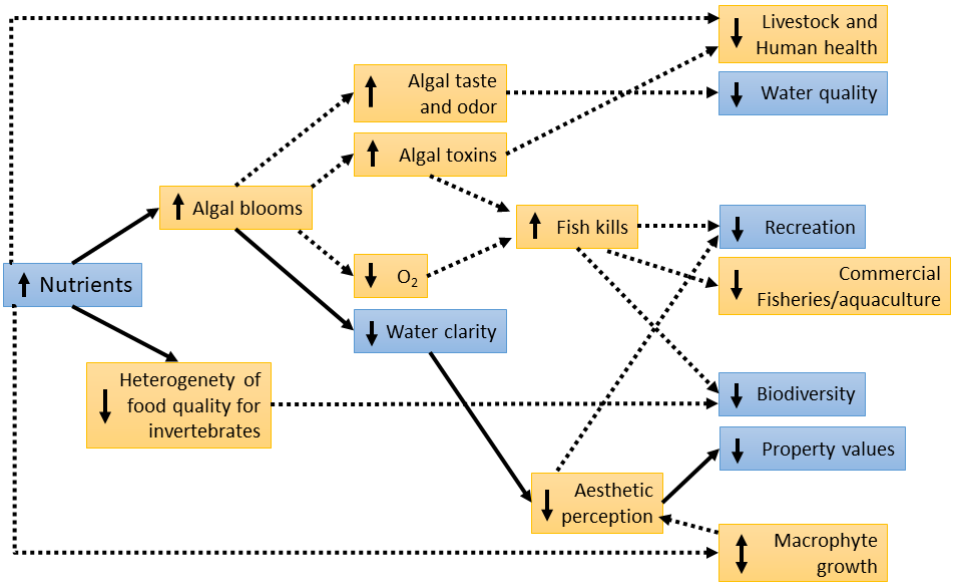


Figure 2.1.2: *Effects of increased nutrients that could influence the value of ecosystem goods and services. The values that can be appointed are marked in blue, the solid lines indicate the chain of influence that could be used to calculate the values [16]*

rate of fish mortality rises, as explained previously. A higher rate of fish mortality leads to lower recreation, less commercial fisheries/aquaculture, and reduced biodiversity. These are just a few of many detriments of higher nutrient levels in the ocean [16].

These changes have accelerated oxygen consumption by microbial respiration, reduced solubility of oxygen in water, and reduced the rate of oxygen resupply from the atmosphere to the ocean interior, with a wide range of biological and ecological consequences. Some biological consequences are to constrain productivity, biodiversity, and biogeochemical cycles. Several extinction events in the earth's history have been associated with warm climates and oxygen deficits within the oceans. Under current trajectories, anthropogenic activities could drive the ocean toward widespread oxygen deficiency within the next thousand years [17]. Because of this, nutrient removal is essential for wastewater treatment, to protect receiving waters from eutrophication and for potential reuse of treated water [18].

The Norwegian government has started to evaluate the impact of emissions from nutrients in the environment. Over the last couple of years, they have started to set demands for environmental monitoring around fish farming facilities. These demands give the breeders and management more knowledge of its impact on the environment and what measures to implement before irreversible damage has been inflicted on local environments [12]. The government has also been working on reducing emissions related to agriculture; they have primarily aimed at reducing pollution and run-off of nutrients to watercourses [19].

Nutrient recovery technologies are rapidly expanding due to the need to recycle critical elements from waste resources to move towards a genuinely sustainable modern society based on a

circular economy. Nutrient recycling is a promising strategy for reducing the depletion of non-renewable resources and the environmental impact linked to their extraction and manufacture [20].

2.1.1 Nitrogen (N)

All organisms in the world depend on nitrogen (N). On average, it accounts for 6.25 % of the dry mass of all organisms. Figure 2.1.3 shows the nitrogen cycle [21] from a agricultural perspective.

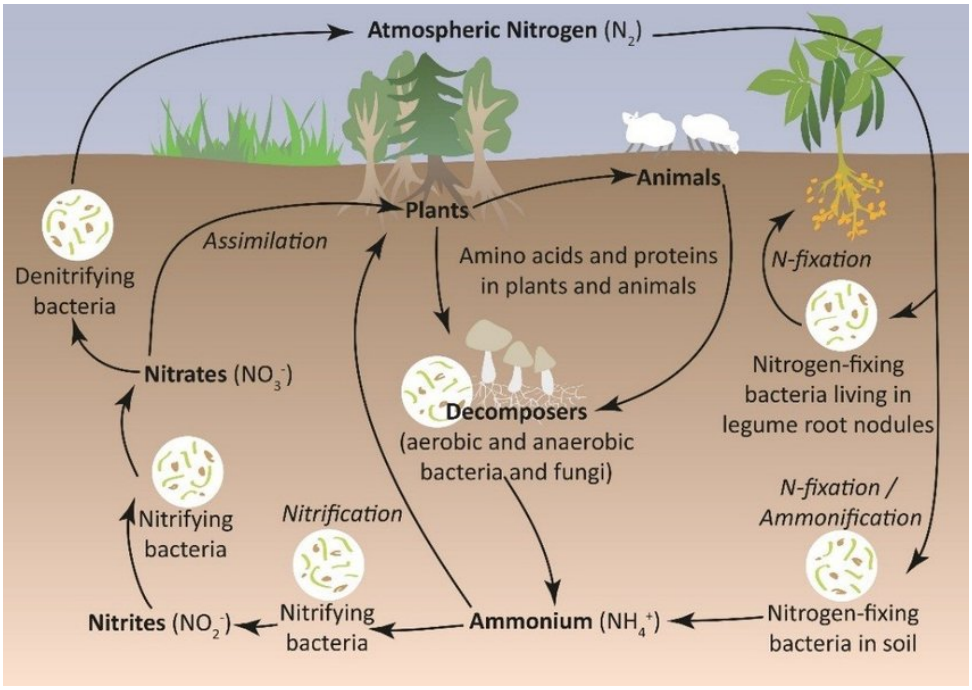


Figure 2.1.3: *The natural nitrogen cycle, this shows how nitrogen is recirculated in different forms in a natural environment, not disrupted of humans [21].*

In biology, N undergoes a variety of oxidation and reduction steps that produce compounds ranging from -3 in reduction state (i.e., ammonia; $\mathrm{NH_3}$) to +5 in oxidation state (i.e., nitrate; $\mathrm{NO_3^-}$), this is shown in Equation (2.1.1). These nitrogen cycle redox reactions are performed differently by different organisms, (e.g., bacteria, archaea, and some special fungi). The reactions in total make up the biological N-cycle [22, 23].

$$NH_4^+ \longrightarrow NO_2^- \longrightarrow NO_3^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \tag{2.1.1}$$

The nitrogen cycle can also be found in fish farming facilities, as shown in Figure 2.1.4. The fish feces and uneaten feed accumulate as ammonia ($\mathrm{NH_3}$). The ammonia-oxidizing bacteria, *Nitrosomonas*, convert the ammonia into nitrite ($\mathrm{NO_2^-}$), and the bacteria, *Nitrobacter*, convert the nitrite to nitrate ($\mathrm{NO_3^-}$). This is also shown in Equation (2.1.1). During both these chemical

conversions, H^+ is released and causes a pH-level reduction in the water. If the pH gets too low, the ammonia will convert to ammonium, which the bacteria cannot convert. Ammonia and nitrite are toxic to the fish, but nitrate is far more toxic to the fish [22]. Because of this, the wastewater must be cleaned before further use, and this can be done by denitrification as mentioned in Section 2.3. The denitrification process can start from both nitrite, and nitrate [24]

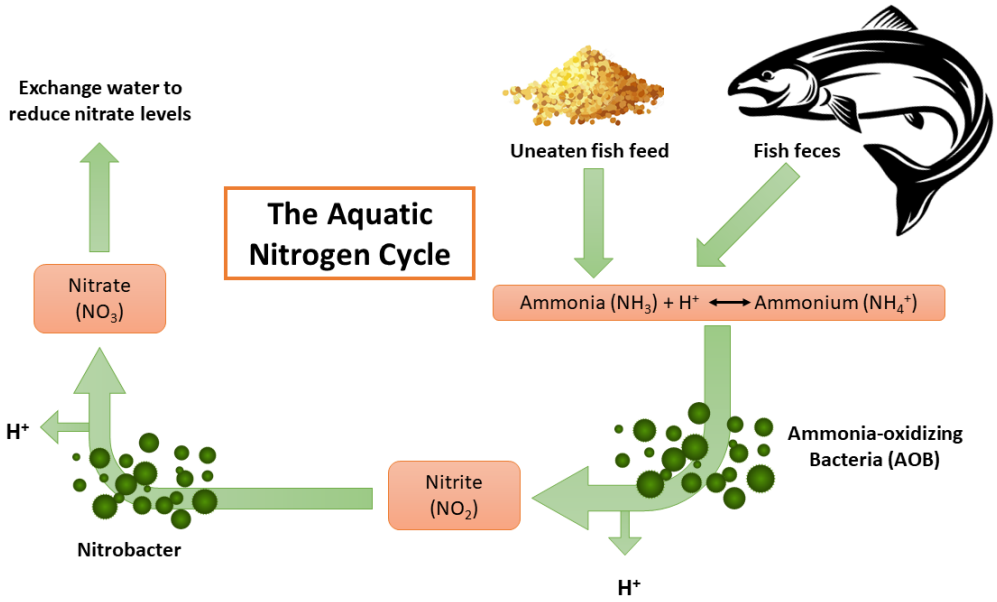


Figure 2.1.4: *The nitrogen cycle in aquaculture systems and aquarium tanks. Ammonia is built up from fish excretion and uneaten food. Nitrosomonas, a bacterium, converts ammonia into nitrite, converted into nitrate by Nitrobacter, another bacterium. During both chemical conversions by the bacteria, H + is released, causing a reduction in pH. A reduced pH will change ammonia into [24]*

2.1.2 Phosphorus (P)

Phosphorus (P), the 11th most common element on earth, is a resource that are being used at a much faster pace than they can be replenished, it is a fundamental to all living organisms. It is essential for creating DNA, cell membranes, and bone and teeth formation in humans. Phosphorus cannot be manufactured or destroyed, and there is no substitute or synthetic version of it available. P is mined from fossil phosphate resources, around 22×10^6 metric tons are mined every year, adding it to the world economy [25].

The size of the remaining fossil phosphate resources is uncertain, but as it cannot be created and fossil reserves are heavily relied upon, it is a finite resource in terms of fossil P usage. Because of this, the fossil P may become depleted by ongoing mining [8]. P is vital element for biodiversity. It is also one of the three nutrients (nitrogen, potassium, and phosphorus) used in commercial fertilizer. Traditionally, fertilization in agriculture has thoroughly dominated the usage of P resources; more than 90 % of the current usage of P resources goes to fertilization

of agriculture, whereas >80 % of this comes from fossil P resources [25].

Limiting the consumption of P to essential uses, increased efficiency of agricultural use, and increased recycling of P may substantially contribute to the reduction of demand for fossil P resources. Recycling of P has to face concerns regarding the efficiency of P recovery, pathogenic organisms, and contaminating substances. Much work remains to be done to address these concerns [25] effectively. Secondary phosphate resources such as human and animal excrement, harvest residues, organic wastes, ashes, and crushed bones have traditionally been used to maintain P stocks available to crop plants [26, 27].

Figure 2.1.5 shows the natural phosphorus (P) cycle [21, 28], and shows how rocks release phosphate ions and other minerals due to weathering. These are then distributed into soil and water. In soils, phosphate is absorbed by plants and subsequently taken up by animals consuming the latter. Phosphate returns to the soil through the process of animal excretion (and decomposition of dead animals and plants). Sedimented oceanic phosphorus may form phosphate rocks on the ocean floor during the process of diagenesis. This process has a time scale of the order $\times 10^8 - 10^9$ years [29].

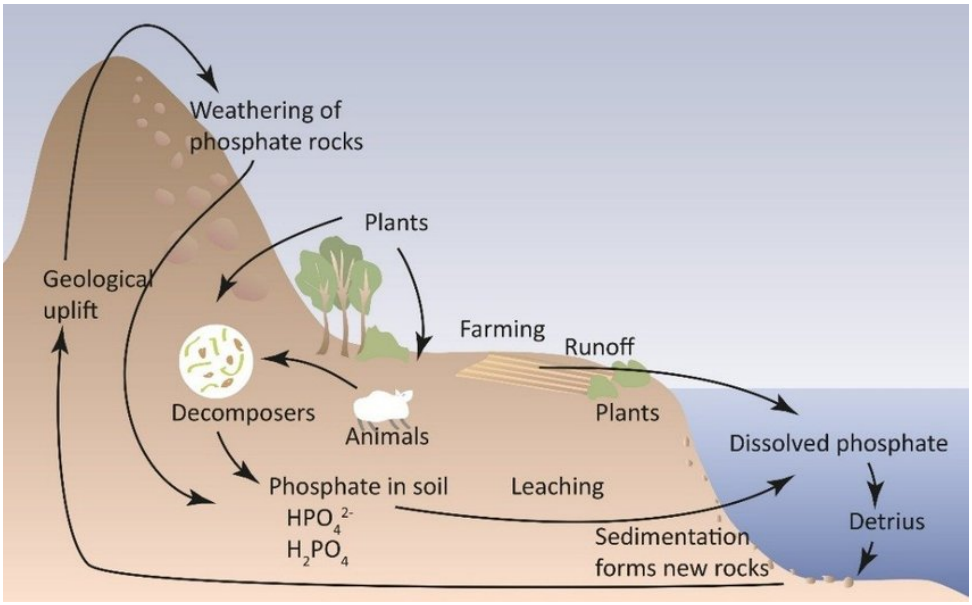


Figure 2.1.5: *The natural phosphorus cycle, showing how theoretically phosphorus can be recirculated naturally, through deterioration, sedimentation and geological uplift [21].*

In addition to the natural recycling of phosphor being extremely slow, industrial agriculture disrupts this natural cycle. Large quantities of fertilizer are continually applied to enrich the agricultural soil.Because of the last century's intensive use of fertilizers and phosphate-based feed and food additives, the geological formation of P from run-offs can now be seen as negligible. The phosphorus cycle is no longer a cycle; it is instead an unequivocally linear process [30].

Phosphorus is not directly toxic to humans or animals; therefore, there are minor restrictions

on how much P should be in water bodies (e.g., drinking water). The toxicity from P is indirect through the toxic algal blooms that can result in eutrophication [14]. The real problem regarding P is that the world is running out of fossil reserves, as mentioned before. It is vital to recycle P through all kinds of wastewater treatment, to utilize the P in use.

2.2 Fish farming

Aquaculture is a term that embraces farming and cultivation of all kinds of organisms in the water. This industry represents a large and growing part of Norwegian exports and economy, and it is an essential source for both income and jobs in the major industry. Fish farming is a form of aquaculture, as it is a process where fish are raised in captivity. It can be done commercially in tanks on land filled with seawater or enclosed marine cultures in open offshore facilities. The fish is in captivity during its entire life course, where the farmer has full control over the fish breeding, genetics, and production of fish roe for optimum growth.

The fish farming process is relatively simple and consists of five steps related to the life cycle of the fish, the steps are shown in Figure 2.2.1. During the month the fertilized eggs are incubated in egg trays in freshwater hatcheries. When the eggs hatch and Alevins emerge, Alevins carry a large, orange-colored yolk sack which contains all the nutrients they need, they stay like this from month 1-3. The fish reach fry when they lose the yolk sack and are transferred from the egg trays to tanks, and are able to feed themselves. The fry are in the freshwater hatchery for about four months, where they grow to the Parr stage. At Parr stage, the fish can quickly double their weight in a month, at the end of the Parr stage they change color to a silvery blue as they turn into Smolts. Smolts are young salmon which are ready to migrate from the freshwater to marine environment, they have spent between 10-16 months in freshwater and are now ready to mature to marine environment. Once they reach sea water, the Salmon continue their development until they become adult salmon, this step takes between 14-24 months, fed up to the preferred size. Salmon can be harvested when they reach 1-2 kilos, but it is most common to harvest the fish at around 3-5 kilos. [31, 32, 33].

Fish farming has three main production methods; extensive, semi-intensive, or intensive. Extensive is when the fish fry is set out in dams, lakes, fjords- or sea areas and needs to feed itself with the nutrients in the area. Semi-intensive fish farming is similar to extensive, but the smolt is placed closer together and is given additional feed. Intensive fish farming is when the entire life cycle is under human control, and the fish are placed even closer together than the extensive method, and all feed is provided for [31].

In semi-intensive or intensive production, the fish are dependent on the feed that is provided. The different kinds are live, wet, soft, or dry feed. The last three mentioned are vegetable products, raw marine materials, and slaughterhouse waste from livestock. In addition, minerals, vitamins, and possibly medication to prevent certain fish diseases and parasites are added. For

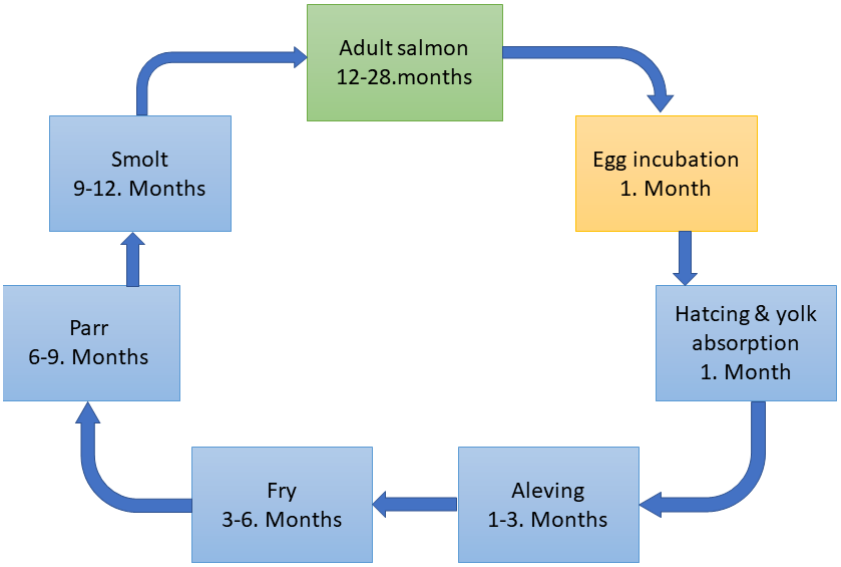


Figure 2.2.1: *The fish process cycle, from eggs to adult salmon. It shows the process of the life span of a salmon, and how long the specimen stay in each cycle [33].*

salmon, the carotenoid astaxanthin is added to get the famous red color of the salmon meat, as the color is not a natural component in salmon. Most of the Norwegian fish farming is intensive, and they use dry feed exclusively. [31]

All types of fish farming have emissions that can be damaging to the surrounding environment. These emissions can come from wastewater from the facility. This wastewater consists of a combination of uneaten fish feed, feces, and other nutrients emitted from the process. These components contain large amounts of nutrients like nitrogen (N) and phosphorus (P), disrupting the natural aquatic environment if emitted. The difference between sea- and land-based fish farming is how well they can control and account for the emissions. All the emissions that come with the fish waste (i.e., fish meal, fish feces, drugs, and chemicals) are released directly into the ocean, disrupting the natural ecosystem. This can lead to algal blooms and the death of natural organisms. In addition to this, diseases or parasites among the fish will be exposed to the wild population in the open water. Other problematic scenarios with open water fish farming are escaping fish from the farm and attack from wild predators.

The most extensive issues with offshore fish farming are the use of fish meal and oil as ingredients, escapees of the fish cages into the wild, and discharge of waste into the environment [34].

2.2.1 Land-based fish farming

Land-based facilities are a new type of fish farming for the aquacultural industry. Land-based fish facilities consist of large tanks on land that are filled with seawater. With this kind of facility, better tracking of the process parameters for production can be achieved. As it is in an

enclosed environment, everything can be tracked and optimized. The inlet and outlet flow of water is managed from the control room. The waste made in the tanks that come from feces and uneaten food can be monitored, which will also decrease fish feed use compared with offshore fish farming. There is no room for fish escaping, and as the quality of the water can be regulated, fish mortality can be reduced. The wastewater from the facility goes through treatment before it is reused in the tanks or let out in the environment, which can positively impact compared to offshore fish farming.

As space availability, water utilization, and nutrient discharge in wastewater are significant challenges facing sustainable development in aquaculture, recirculating aquaculture systems (RAS) offer potential ways to handle the issues mentioned before [35]. RAS facilities are explained further in Section 2.2.2.

2.2.2 Recirculating aquaculture systems (RAS)

Recirculating aquaculture systems (RAS) are land-based fish farming facilities that (partially) reuse water after it has been treated. RAS systems provide the opportunity to produce fish at a large scale ecologically and sustainably and have been developed to aid nations with limited access to land and water [36]. This is because a RAS facility significantly reduces water usage to improve waste management and nutrient recycling [3, 34].

In RAS, water from fish culture tanks is recirculated after removing the toxic nutrients through bio-reactors. Removal of nitrogenous waste products forms the core activity in ensuring the optimally functioning of the RAS [35]. The water which is transferred back into the RAS facility must maintain values of nutrients (e.g., CO_2, Nitrogen, pH, temperature alkalinity, oxygen, salinity, and total ammonia nitrogen (TAN)) that are within that of the fishes own healthy limits, these boundary conditions can be found in Table 2.2.1.

Table 2.2.1: *The boundary limitations of properties for the fish to survive.*

Property	**Limit**
CO_2	15 $\mathrm{mg} \cdot \mathrm{L}^{-1}$
pH	6,8-7,6 (use 7,2)
Temperature	14 °C
Alkalinity ($CaCO_3$)	60-90 $\mathrm{mg} \cdot \mathrm{L}^{-1}$
Oxygen	80-100 % saturation
TAN	2 $\mathrm{mg} \cdot \mathrm{L}^{-1}$
Salinity	15 ppt

The definition of re-circulation is the water exchange rate. Water exchange is the relative amount of freshwater (i.e., makeup water) replaced in the system per kg feed used. The makeup water is added at a rate that does not allow N and P to build up too high in the pool due to feeding. Therefore, if the makeup water is entering the RAS system decreases, the system

flushing is reduced, and then the water quality within the system is consequently degraded. Equation (2.2.1) shows the straightforward way of calculating the water exchange rate [37, 38]. Martins et al. (2010; [34]) stated that conventional RASs operated at a variable water exchange rate of (0.1 - 1 $\mathrm{m}^3 \cdot (\mathrm{kgfeed})^{-1}$).

$$\text{Water exchange rate } [\mathrm{m}^3 \cdot (\mathrm{kgfeed})^{-1}] = \frac{\text{Water exchange/day } [\mathrm{m}^3 \cdot \mathrm{day}^{-1}]}{\text{Feeding/day } [\mathrm{kg} \cdot \mathrm{day}^{-1}]} \tag{2.2.1}$$

Different water treatment steps reduce the system water exchange to the needs of the next limiting waste component. Based on different system water exchange rates, it is suggested the following classification of water exchange rates for the different farming facilities: flow-through (> 50 $\mathrm{m}^3 \cdot \mathrm{day}^{-1}$), re-use (1-50 $\mathrm{m}^3 \cdot \mathrm{day}^{-1}$), conventional re-circulation (0.1-1 $\mathrm{m}^3 \cdot \mathrm{day}^{-1}$) and RAS (<0.1 $\mathrm{m}^3 \cdot \mathrm{day}^{-1}$) [34]. This shows a clear decrease in water consumption needed in a traditional flow-through fish farming facility compared to a RAS facility.

Figure 2.2.2 shows a schematic Figure of a RAS facility. Feed enters the tank for the fishes well being; after the water has circulated through the tank, it exits the tank and enters a treatment facility. The water first enters a mechanical filter, separating the water from the suspended solids made from feces and uneaten fish feed. The separated solids are exited as sludge. Next, there is a biofilter that removes the excess nutrients, which among other things, turns ammonia into nitrate. It removes the excess N, and it is released into the surrounding environment. Furthermore, there is a CO_2 stripper, which filters out the excess CO_2; this can be reused for further growth requirements (e.g., to grow plants). Both the biofilter and the CO_2 degasser have an input of air for their processes to work. After this, the water goes through a UV filter, which disinfects the water from impurities. Here, extra water is supplied for the water that is lost throughout the process. In the last process, before the water is supplied back to the tank, oxygen and ozone are added.

RASs offer advantages in terms of reduced water consumption [39], improved opportunities for waste management and nutrient recycling [40], and for better hygiene and disease management and biological pollution control (e.g., no escapees) [36]. RAS reduces the risk of diseases because all incoming water can be filtrated, and the surroundings can be closely monitored. Incoming water can be further treated to achieve the desired quality [34, 36]. RAS has these advantages because of the different wastewater treatment steps enforced (e.g., denitrification reactors, sludge thickening technologies, and ozone treatments). In addition, the discharged waste is more concentrated, facilitating waste reuse options as fertilizers or in integrated partially or wholly closed systems [34]. Further information on these wastewater treatment steps can be found in Section 2.3.1. To make the most out of the advantages of RAS, the water exchange should be as small as possible. This implies high demands for wastewater treatment (e.g., the maintenance of efficient nitrification, denitrification, and organic removal) [37].

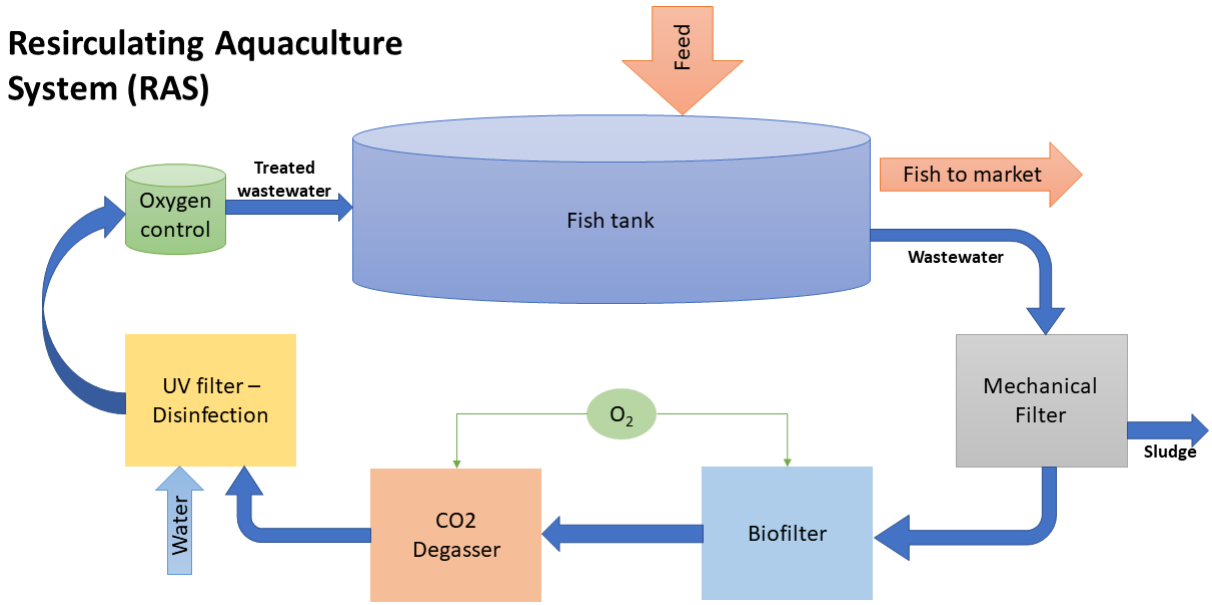


Figure 2.2.2: *Schematic representation of a RAS facility, showing that the wastewater goes through at least five water treatment processes before reentering the facility. First a mechanical filter, then a biofilter, CO_2 degasser, a UV filter disinfection and at last the water goes through oxygen control.*

The system can achieve temperatures that enable optimal and stable production all year round, independent of seasonal variation. This makes the production predictable for 365 days. This advantage is one of the critical aspects that will surpass the current solutions of conventional fish farming. The critical area for given production in RAS is relatively tiny because a very high density and a high growth rate are possible in the controlled environment [37, 36].

There are also some disadvantages with RAS. First and foremost, it is an advanced system that requires skilled staff and a security system around the clock. It is a sizeable advanced system, and it requires high energy consumption. As the environmental conditions are as closely controlled as in RAS, a power shutdown would have fatal consequences, and a backup power supply is essential. One of the most significant disadvantages is that it is costly to establish; therefore, there is need of a minimum production capacity for achieving positive economical operation [37].

2.2.3 Post-smolt modulRAS facility, Nofitech

According to Nofitech's website, their reactor ModulRAS is the market's most standardized, compact, proven, and cost-effective RAS concept designed for operation with seawater or freshwater. They design and deliver facilities for all parts of the production process, from smolt, post-smolt, food fish, and broodstock [41]. The values given in this Section have all been provided from the R&D department of Nofitech.

The current treatment method the wastewater is going through is approximately the same as explained in Chapter 2.2.2. There are three output streams and two input streams into the reactor, as shown in Figure 2.2.3.

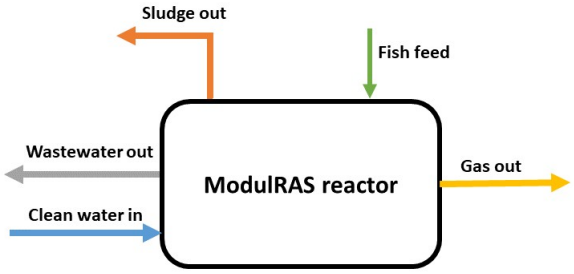


Figure 2.2.3: *Schematic overview of the inputs and outputs streams from the modulRAS facility from Nofitech.*

The two input streams to the reactor are fish feed and water to ensure the water exchange rate; in addition to this, oxygen is also added to the biofilter and CO_2 - degasser. The values of these input streams are given in Table 2.2.2.

Table 2.2.2: *The input stream values of the Nofitech modulRAS reactor, which include the amount of feed, the water echange rate and oxygen input.*

Input stream	Value	Unit
Maximum feed	3,000	$\mathrm{kg} \cdot \mathrm{day}^{-1}$
Water exchange rate	300	$\mathrm{L} \cdot \mathrm{min}^{-1} \cdot \mathrm{kg}^{-1}$
Oxygen	0.32	$\mathrm{kg} \cdot (\mathrm{kgfeed})^{-1}$

The three output streams from the reactor are sludge, wastewater, and gas out. *Sludge* is a bi-product made up of all the suspended solids that are filtered out from the wastewater. The wastewater stream has been through treatment, and this is the stream that is not qualified to reenter the fish tank. The last output stream is the gas out, which is the gas stream made up of the CO_2 stripped in the CO_2 degasser. All the values for these output streams can be found in Table 2.2.3.

Table 2.2.3: *The output stream values of the Nofitech modulRAS reactor, there are three different streams; sludge out, wastewater out and gas out.*

Output stream	**Value**	**Unit**
Sludge out		
Total max flow	18-22	$\mathrm{m}^3 \cdot \mathrm{h}^{-1}$
Total suspended solid (TSS)	8-12	% DW
Phosphor concentration in TSS	11	$\mathrm{g} \cdot \mathrm{kg}^{-1}$ TSS
Wastewater out		
Total organic carbon (TOC)	3	$\mathrm{mg} \cdot \mathrm{L}^{-1}$
Total ammonia nitrogen (TAN)	0.7	$\mathrm{mg} \cdot \mathrm{L}^{-1}$
Nitrate - N	50	$\mathrm{mg} \cdot \mathrm{L}^{-1}$
Gas out		
Flow rate, O_2	550	$\mathrm{m}^3 \cdot \mathrm{h}^{-1}$
CO_2	9.6	$\mathrm{mg} \cdot \mathrm{L}^{-1}$

2.3 Aqua-cultural Wastewater

Typical aqua-cultural wastewater combines uneaten fish feed, fish feces, and other inorganic and organic compounds. Wastewater is mainly treated by aerobic or anaerobic biological degradation; however, the treated water still contains inorganic compounds such as nitrate, ammonium, and phosphate ions. These nutrients can cause eutrophication if released into lakes, which can lead to harmful algal blooms [42].

Nutrients found in waste streams are mostly compounds of carbon, nitrogen, and phosphorus (CNP). All of them are important for the sustenance of various life forms. Nitrogen and phosphorus are essential components of a cell's DNA, amino acids, and chlorophyll. In eukaryotic cells, phosphorus is the energy currency of the cells in the form of adenosine triphosphate (ATP). Nitrogen and phosphorus play critical roles in plant growth and food supply. While nitrogen abundantly exists in the atmosphere (78 %) in a highly stable and nonreactive form N2 gas, its content is limited in soils. Therefore, in order to make it usable and increase its availability in soils, nitrogen is fixed in reactive forms such as amino-acids, nitrate, and ammonia [8].

2.3.1 Conventional wastewater treatments

As mentioned previously, there are many different ways to treat wastewater of its effluents. The main goal of any treatment is to remove the biochemical oxygen demand (BOD), suspended solids, nutrients (e.g., nitrogen (N) and phosphorus (P)), coliform bacteria, and other toxic compounds [42]. Some wastewater treatments used are aeration, degassing of carbon dioxide (CO_2), the addition of pure oxygen, mechanical filtration, biological filtration, UV disinfection, temperature control, nitrate removal, phosphor removal, and sludge thickening [37]. Some of

these treatments are being addressed in this Section.

Biochemical oxygen demand (BOD) represents the amount of oxygen consumed by bacteria and other microorganisms while decomposing organic matter under aerobic (oxygen is present) conditions at a specified temperature. BOD can be a good indicator at wastewater treatment plants as an index of the degree of organic pollution in water [43]. BOD can indicate a microorganism's ability to oxidize material to $\mathrm{CO_2}$ and water using molecular oxygen as an oxidizing agent. Low BOD leads to depletion of the dissolved oxygen of receiving water, leading to fish kills and anaerobiosis [42]. BOD is a measure of the amount of oxygen required to remove waste organic matter from water in the process of decomposition by aerobic bacteria (those bacteria that live only in an environment containing oxygen) [43]. If there is a depletion of dissolved oxygen, it can be added by aeration or oxygenation, while $\mathrm{CO_2}$ is removed by degassing.

Nutrient removal requirements for water resource recovery facilities are nearing the limit of current technologies (e.g., the limit of biological nutrient removal (BNR) is roughly 3 mg N $\mathrm{L^{-1}}$ for total nitrogen and 0.1mg P $\mathrm{L^{-1}}$ for total phosphorus [44]. The nutrients that need to be removed are mainly dissolved N and P; this wastewater treatment step is vital for both water that is let out into the aquacultural environment and water reused in the RAS facilities. When substantial amounts of nutrients are discharged into sensitive water bodies, it leads to eutrophication. Eutrophication can lead to extensive blooms of unwanted plants and organisms such as harmful microalgae. Further information on eutrophication can be found in Section 2.1.

Suppose there is a significant amount of N in wastewater. In that case, this can lead to consequences (e.g., the toxicity of non-ionized ammonia to fish and other aquatic organisms, interference with disinfection where a free chlorine residual is required, and methemoglobinemia in influences due to excessive nitrate concentration (above 45 $\mathrm{g \cdot m^{-3}}$) in drinking water [42]. It is also essential to remove from water being reused because there is a total nitrogen (TN) and total phosphor (TP) limit for the water that the fish cannot exceed.

Nitrogen is present in the wastewater in the form of ammonia ($\mathrm{NH_3}$), nitrate ($\mathrm{NO_3^-}$), and nitrite ($\mathrm{NO_2^-}$). Ammonia is mostly converted into nitrate through nitrification in aerobic biological filters. In a conventional RAS, the maximum allowed concentration of $\mathrm{NO_3^-}$ steers the external water exchange rate [34]. High nitrate concentration can be counteracted by denitrification [45]

Through denitrification, oxidized inorganic nitrogen compounds (such as nitrite and nitrate) are reduced to elemental nitrogen ($\mathrm{N_2}$). This is done using facultative anaerobic microorganisms with electron donors derived from either organic (heterotrophic denitrification) or inorganic sources (autotrophic denitrification). In addition to nitrate removal, denitrifying organisms are associated with other processes relevant to water quality control in aquaculture systems. Denitrification raises the alkalinity and, hence, replenishes some of the inorganic carbon lost through nitrification. Organic carbon discharge from recirculating systems is reduced when endogenous carbon sources originating from the fish waste fuel denitrification. In addition to the carbon

cycle, denitrifiers also are associated with sulfur and phosphorus cycles in recirculating systems [46].

Elemental nitrogen (N_2) is not considered a greenhouse gas, and releasing it into the atmosphere does not contribute to global warming. Nevertheless, the release of nitrogen into the atmosphere means that it would need to be re-fixed to be used as a fertilizer, an energy-intensive process.

Figure 2.3.1 shows an example of a RAS facility with an up-flow sludge denitrification reactor. The reactor is fed with dissolved and particulate fecal organic waste, bacterial flocs, and inorganic compounds trapped by the solids removal unit. The waste flow enters the reactor at the bottom center. The up-flow velocity in the reactor is smaller than the settling velocity of the significant fraction of the particulate waste to create a sludge bed at the bottom. In the sludge bed, the fecal particulate waste is digested by the denitrifying bacteria [34].

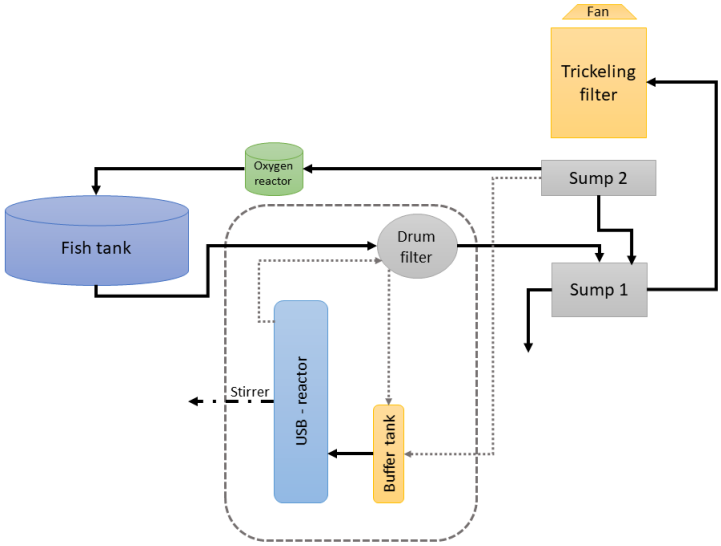


Figure 2.3.1: *Schematic drawing of a RAS facility using a denitrification (USB) reactor. Water flows from the fish tanks - drum filter - sump 1 - trickling filter - sump; 2- rearing tanks. One parallel flow across the denitrification reactor, using only fecal carbon as energy source, flows from the drum filter - buffer tank - denitrifying reactor - drum filter [34].*

P is one of the nutrients contributing most to the eutrophication of waters receiving effluents from intensive aquaculture. Because of this, any reduction of P levels in aquaculture effluents will improve the environmental sustainability of RAS. As most P in wastewater are placed in suspended solids, removing suspended solids more efficiently is a significant step to improve RAS's sustainability. There are many suspended solids in aquacultural wastewater in the form of uneaten fish feed and fish feces. Suspended solids reduce water clarity and clog waterways, and many solids are also biodegradable organic pollutants. This is why suspended solids need to be removed from the wastewater. Suspended solids are principally removed by physical sedimentation before or during biological treatment [42, 47, 34].

Other waste can be found in wastewater, like pathogens that are infectious microorganisms or agents (e.g., virus, bacterium, fungus). This would be treated with disinfection (e.g., UV irradiation, chlorine, ozone) to remove these from the wastewater [47]. Another waste that can be present in wastewater is heavy metals, there is seldom a lot of it, but high amounts can lead to acute and chronic toxicity and bioaccumulation. Conventional treatment methods to remove this could be chemical precipitation (lime), electrochemical precipitation, or various biomaterials (e.g., waste wood chips, bacteria, or yeasts from industrial fermentation [47].

2.3.2 Microalgae-based wastewater treatment (WWT)

Many of the pollutants present in aquacultural wastewater can be removed by microalgal wastewater treatment (WWT). Microalgae use energy derived from photosynthesis to remove nutrients from the wastewater and CO_2 and convert it into biomass that can be used for several different applications [47]. Figure 2.3.2 shows a schematic drawing of an overall microalgae WWT facility, focusing on the fact that the algal slurry can be used further as either whole cell algae or Lipid-extracted algae (LEA). Whole-cell algae can be utilized further in biodiesel, cosmetics, aquaculture, human food, and pharmaceuticals, while LEA can be utilized as fertilizer, feed/-food, and energy [48].

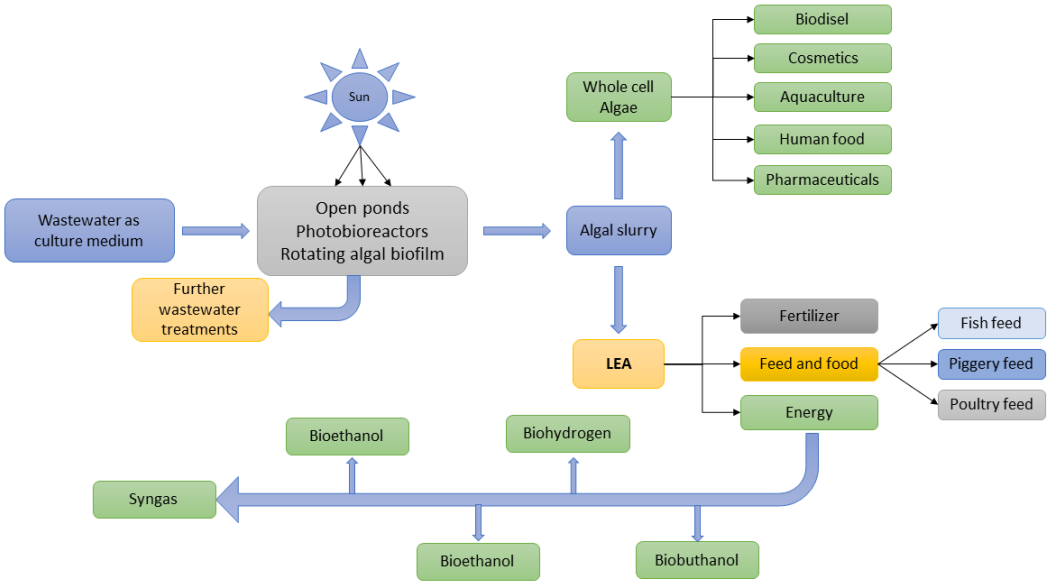


Figure 2.3.2: *Schematic overview of generalized schematic representation of algae cultivation and how the biproduct of biomass can be used for many different areas (e.g., Biofuel, feed, pharmaceuticals, etc.) [48].*

Other emissions in the water that need removing are among other suspended solids, and the microalgae-based WWT is not designed for removing inert of nonbiodegradable solids. To avoid operational issues and improve light penetration in the influent, suspended solids should be removed before entering the microalgae cultivation facility [49]. For biodegradable organic

pollutants, the microalgae provide an additional metabolic ability and support to aerobic heterotrophic degrades via exchange and substrates [49]. Microalgae photosynthesis can promote pathogen disinfection by contributing to raising the pH and dissolved oxygen concentration. The high illuminated surface/volume ratio also factors disinfection via UV radiation [49]. There might be some heavy metals in the wastewater, microalgae-based WWT has the potential to generate significant amounts of cost-effective biosorbent. Algae photosynthesis can also favor heavy metal precipitation at high pH [47].

Wastewater treated by microalgae does not need to transition between different operating environments to facilitate inorganic nitrogen (N) and phosphorus (P) removal, requiring only a single-step treatment process. This is because microalgae assimilate ammonia/nitrate (NH_3/NO_3^-) and phosphate (PO_4) directly and in concert for cell growth and metabolic function. As a result of this, microalgae treatment processes have a lower greenhouse gas emission rate; for instance, most N is assimilated by the microalgae instead of being converted to oxides of nitrogen [5]. Multiple studies have reported negligible emissions of N_2O caused by microalgae in conjunction with associated microorganisms in wastewater treatment. In a study performed by Alcántara et. al [49], a microalgae wastewater process is estimated to have an emission factor of 0.0047 % g N_2O-N g^{-1} N-input. In order to get significant savings in energy demand and reductions in associated greenhouse gas emissions, furnishing wastewater with dissolved O_2 through microalgal photosynthesis is a good way to go [5].

On the contrary to all advantages that are being portrayed for using microalgae in wastewater, several practical and economic challenges stand in implementing large-scale wastewater treatment using microalgae for the industry. The first challenge is the energy consumption in the cultivation process. To keep up an optimal environment for its performance, there is a need for aeration and pumping systems. This is used to generate a turbulent flow that can hold the perfect exchange of O_2 and CO_2 [5].

The price is also something that has been widely studied, the energy consumption used per m^3 has been proven to be 100-fold higher compared to mechanical and aerated mixing in conventional wastewater treatment processes. The energy consumption for microalgae was 15 kWh m^{-3} compared to conventional 0.15 - 0.62 kWh m^{-3} [5].

2.4 Microalgae

Microalgae are the leading primary producer on the planet and have more than 20 times higher growth rates than conventional crops [50, 51]. Microalgae, the most uncomplicated and tiniest form of organisms, hold fantastic potential for the extraction of various nutrients from water to carbon dioxide (CO_2) from the air. Some influential bioactive products that are produced by microalgae are polymers, peptides, fatty acids, carotenoids, toxins, and sterols [52]. These are bioactive compounds that are required (e.g., in fish and animal feed) but can also be used

in bioremediation, biofuels, and several specialty chemicals [52, 50]. These organisms hold great potential desperately required for sustainable and renewable management of food, fodder, and fuels, if managed in an appropriate manner [50]. The two fundamental characteristics of microalgae are that they have high efficiency of converting solar energy into cellular biomass that contains high proportions of protein and fatty acids [53].

2.4.1 Fundamental microalgae biology

Microalgae are microorganisms that use photosynthesis to produce biological energy equivalents to convert light, carbon dioxide (CO_2), nitrogen (N), and phosphorus (P) into oxygen and organic matter [54, 55, 5]. They contribute to half of the global photosynthetic activity and are found in marine environments like lakes, fjords, ponds, and the ocean. In addition to this, they are a fundamental part of the primary biological production of a wide variety of organisms on the planet that are not in aquaculture, and this is because they contribute to the food source of 70 % of the world's biomass [56]. They use significantly less area to grow than other crops due to their high photosynthetic efficiency per area. Microalgae can be cultivated even in non-arable lands; therefore, they do not impact agricultural land availability [57]. When microalgae are exposed to high light frequencies, that can lead to photoinhibition. Photoinhibition is when microorganisms absorb excess excitation energy, which causes photodamage to cells [58].

Microalgae are a group of microorganisms that have independently acquired chloroplasts, which are intracellular structures with their photosynthesis mechanism [56]. Additionally, microalgae are a potential source of energy generation. They can utilize both organic and inorganic nitrogen (in the form of ammonium/ammonia), as well as nitrite and nitrates [5].

Figure 2.4.1 [59] show schematically how wastewater is treated with the use of microalgae in the cleaning process for the product to be reused. Light and CO_2 are added to the water that already contains N, P, and other necessary vitamins. This produces biomass that can be utilized for many different applications and recirculated water that can either be put back in the processor used for another process [59].

Microalgae produce long-chain polyunsaturated fatty acids (LC-PUFA) such as those in the omega-3 and omega-6 families. These unsaturated fatty acids are essential in the diets of humans and animals. It is not possible to produce a synthetic alternative to these fatty acids; therefore, microalgae are well suited for this production [60].

The distribution of microalgae in the biosphere is nearly ubiquitous [56]. As microalgae can be found almost everywhere, they grow in rich humus soil, desert sands, rocks, snowfields, and in some more unusual sites (e.g., the fur of sloths and polar bears) [56]. Microalgae have a high CO_2 fixation efficiency that is 10-50 times greater than in plants and can fix CO_2 from various sources. Microalgae can grow 10-50 times more protein at the same amount of area as peak soybean yields. This can reach a production of 50-110 $\mathrm{tons} \cdot \mathrm{ha}^{-1} \cdot \mathrm{year}^{-1}$ and a potential max-

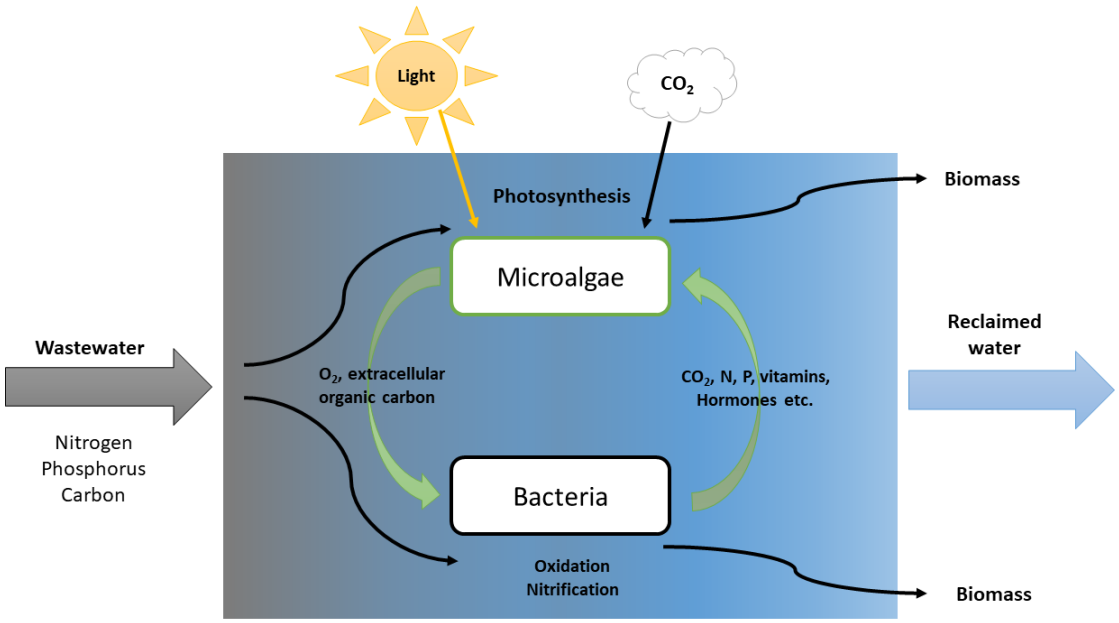


Figure 2.4.1: *The photosynthetic process of wastewater treatment using microalgae, showing how the contaminated wastewater is inserted to the cultivation and the microalgae and bacteria absorb the nutrients, and reclaimed water is excited as well as biomass separately [59].*

imum of 500 $\text{tons} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ [60, 53]. This includes gaseous CO_2 from the atmosphere, industrial flue gas, and soluble carbonates [60]. What kind of carbon source is added depends if the microalgae is an autotrophic or heterotrophic organism [61], these terms are described further down in this Section.

Microalgae is qualified as an aquatic microorganism because the specific nutrients needed for their metabolic activities are primarily found in aquatic environments. The environmental conditions of the microalgal surroundings like light, temperature, and pH affect the production of lipids, proteins and carbohydrates [61]. In the environment, there are also stresses that can be inflicted on the microalgae and can affect both the lipid synthesis and accumulation and composition of n-3 l LC-PUFAs. These stresses are either nitrate (NO_3^-) starvation [62], increased salinity, changes in light intensity, or changes in the amount and composition of carbon [54]. Microalgae also require non-mineral nutrients like carbon (C), hydrogen (H), and oxygen. As C is not in the immediate surroundings of the process, it needs to be added and is the limiting factor.

Microalgae are divided into two groups depending on what the C source is; autotrophic and heterotrophic [61]. Autotrophic algae obtain C by the use of solar energy to transform inorganic sources of C; CO_2, carbonate, or bicarbonate. Heterotrophic algae use chemical energy to transform organic forms of C, acetate, or glucose; into biological energy equivalents. C is essential in growth reproduction because the dry weight of an algae consists of around 50 % carbon. It is used as an energy source and raw material for cell division. Other nutrients

that have positive effects on the production of microalgae are; nitrogen, phosphorus, sulfur, potassium, and magnesium [61].

Nitrogen is a significant source for algae when it comes to the production of proteins and nucleic acids. An algae cell consists of 7-20 % N of the dry cell weight. N can be supplied in different forms like nitrate, nitrite, ammonia, and urea. If the microalgae are deficient in nitrogen, it can account for this by accumulating lipids and reducing protein content. P is also important for microalgae production. It accounts for about 1.0 % of the dry cell weight. P is important in some essential molecules like adenosine triphosphate (ATP; a biological energy equivalent), DNA, RNA, and a key component of phospholipids (a major component of the total lipid content and membranes). These nutrients are supplied from an external medium. Absorption of these nutrients is categorized as recirculating the nutrients. Nitrogen and phosphorus are often the limited source of nutrients for microalgae in natural environments [61]. Laboratory studies have demonstrated that microalgal biofilms are able to remove N and P from wastewater effluent at removal rates of 0.1–1.3 g N $\mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ and 0.006–0.19 g P $\mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ [63, 64].

2.4.2 Nutrition from algae

The biochemical composition of microalgae biomass provides an insight into the organism's behavior and its adaptation response to changes in its environment. To understand and optimize the large-scale production of microalgal biomass, it is essential to know the microalgae ecophysiology [65]. The components of microalgae vary for the different species, but they are primarily made up of proteins, carbohydrates, fats, and nucleic acids. This is also influenced by environmental conditions, including light intensity, temperature, pH, and available nutrients. The values of these components vary as follows: proteins (10-50 %), carbohydrates (10-40 %), and lipids (20-80 %). This makes some ideal microalgae sources of nutrients for both animals and humans [65, 53].

As mentioned in Section 2.1, the current fish feed consists largely of vegetable ingredients. To make up for the missing nutrients, there are often synthetic protein supplements added to the feed. The protein from the synthetic supplements can not measure up to the protein from the organic microalgae [53]. When looking at the effects of studies where biomass from microalgae was added to the fish feed, it showed several positive effects compared to the vegetable-based feed. Some of these positive effects in fish are listed below [60]:

- Weight gain
- Improved resistance to disease
- Increased triglyceride and protein deposition in muscle
- Improved taste of consistency of the flesh

- Decreased nitrogen output to the environment
- Increased omega-3 LC-PUFA
- Physiological activity
- Starvation tolerance
- Increase in rate of growth in aquatic species due to better digestibility.

As algae are the natural feed source for fish in the wild, it is not surprising that they are well suited to also be ingredients in the fish feed. A vegetable diet is harder to digest for fish, but with a diet based on microalgae, the digestibility will increase, which leads to faster growth [60].

Proteins are the major primary metabolites in living organisms, including microalgae. Amino acids are the fundamental constituents of proteins that define protein's nutritional quality or value based on essential amino acid content, proportion, and availability. Most of the microalgal proteins are rich in essential amino acids. The proteins and amino acid profile of microalgae have been compared to different sources of food proteins and their proportion in which algal protein composition is nutritionally more favorable. High protein yield directly depends upon cultivation conditions and rich nitrogen source medium. In nitrogen limitation/starvation, fixed carbon produced by photosynthesis switches the metabolic pathway from protein to lipids or carbohydrates, subsequently decreasing the protein yield [48].

The lipids produced in microalgae can be divided into two categories;

1. Storage lipids (neutral or nonpolar lipids)
2. Structural lipids (membrane or polar lipids)

Storage lipids mostly include triacylglycerol (TAG), predominantly saturated fatty acids, and some unsaturated fatty acids. Structural lipids contain the full content of omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) are also vital nutrition to have in both human and animal feed [65]. Many essential health benefits have been reported associated with the consumption of LC-PUFA. In research performed by Cuellar-Bermudez et al. [66] Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are highlighted as essential LC-PUFAs. Some of their benefits include reduced heart disease risk, reduced the risk of early preterm births, preventing inflammation and lowered blood pressure, and supports circulation.

Among the three prominent metabolites, carbohydrates are the least rich in energy (15.7 $\mathrm{kJ} \cdot \mathrm{g}^{-1}$). The carbohydrates such as starch, cellulose, and other polysaccharides are found in the form of storage products or the structural components of the cell. Although lower in energy content, microalgal carbohydrates have the potential to become preferable feedstock for the

production of biohydrogen, bioethanol, biobutanol, and biomethane through integrating with biotechnological conversion technologies [48].

Some microalgae species contain various high-value compounds that can be extracted from the biomass. Carotenoids such as Betacarotene and astaxanthin are some of the high-value pigments that can be extracted from microalgae. These two can be used as antioxidant supplements in human and animal feed, as well as food colorants [60]. Astaxanthin is known to be one of the most potent antioxidants in nature. It is also known for producing the pink-ish color in salmon, shrimp, lobster, and crayfish and contains vitamin A. Astaxanthin can also be produced synthetically. The synthetic alternative has the majority of the market because it costs less to produce. However, it is reported that synthetic astaxanthin has 20 times lower antioxidant capacity than the natural sources [66, 67]. The value of astaxanthin varies between \$2500-7000 kg^{-1}; in 2014, the global market potential was estimated at 280 metric tons and was valued at \$447 million [67].

2.4.3 Microalgal growth

Microalgae are essential in the marine food chain; this is shown in Figure 2.4.2, where algae are at the bottom of the chain while large predators are at the top [68, 61]. The main requirements for their growth are water, light, salinity, and the medium's optimal composition. To achieve the most optimal commercial microalgae cultivation, one of the most important measures to consider in selecting the suitable species that are best suited for growth in that particular environment. Microalgae are suitable to live in most environments, depending on the species. This is primarily a consideration in open cultivation affected by the local geographical, climatic, and ecological conditions. How the algae react and the size in a cultivation reactor is a significant factor for how it will be harvested [61].

For microalgae to grow, it depends on the right amount of light, the water temperature, nutrient concentration, salinity, and pH [54, 52]. Many parameters must be accounted for in microalgae biomass production to be successful at a commercial scale. For example, the type of culture, the production rate, and the isolation of the cultivation in an enclosed reactor are essential. Growing high-quality microalgae requires optimization of collection, sampling and preservation techniques. Some of the nutrients required to be present while the algae grow are nitrate, urea, ammonium, vitamins, phosphorus, nitrogen, iron, manganese, selenium, cobalt, nickel, and zink [52].

There are mainly two aspects to be considered; the culture media and culture condition. The cultivation happens in mainly two types of systems; open cultivation or enclosed photobioreactor. Open cultivation can be a natural source of microalgae, like a lake or a pond. While an enclosed photobioreactor is a more advanced system that has fewer disturbances from natural sources; therefore, it has less contamination, and environmental disturbances [52].

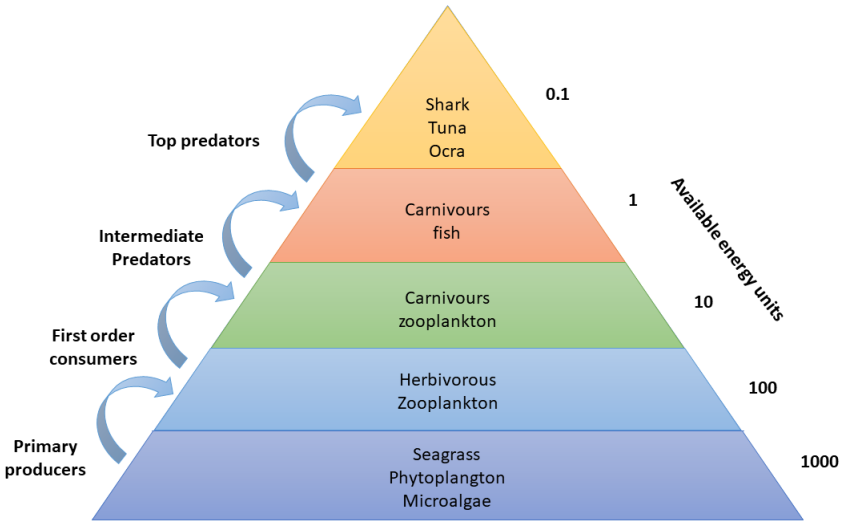


Figure 2.4.2: *The marine food chain, it shows how microalgae and plankton is on the bottom and the upwards the fish gets larger towards the top predators as shark, tuna and ocra [68].*

Much research done on microalgae cultivation is done by monoculture, where only one species are living and growing. The downside of monoculture is that it is not easy to maintain; it is easily contaminated with bacteria or fungi, or something else. This contamination leads to a culture crash, loss of bio-resources, low metabolites productivity, and the low quality of biomass [69]. When looking at nature, microorganisms usually exist as a part of organized communities, gaining benefits from co-habitation. There is an interest to transfer this into microalgae cultivation to co-cultivate for better production rates, contamination, and product diversity [70]. Co-cultivation can mean several microalgae species that grow together, or microalgae species grow together with bacteria and fungi. The different organisms can exchange nutrients and metabolites, transfer genes, and interact with each other through the complex metabolic mechanism. According to Rashid et al. [71], an optimized synergetic relationship is reported to have enormous potential to treat wastewater per effluent standards.

2.4.4 *Phaeodactylum Tricornutum* - Potential microalgae candidate

Phaeodactylum Tricornutum, (*P. Tricornutum*), is a microalga that can be found in brackish and marine waters worldwide. It is easy to cultivate, has a high growth rate, and is rich in Eicosapentaenoic acid (EPA) content, an omega-3 fatty acid. This is a microalga that has been studied a lot for large-scale production ([72, 53]).

The optimum growth temperature for *P. Tricornutum* is 20 °C [73]. Physiological variables can be adjusted to induce lipid accumulation and change the composition [74]. For instance, it has been reported that the EPA + DHA content could increase by 120 % when the temperature gets lowered from 25 °C to 10 °C [73].

Using *P. Tricornutum* as feed has been successfully tested by adding it as an unprocessed,

raw ingredient in the feed for Atlantic salmon. The protein digestibility reached 80 %, and the lipid digestibility was around 96 % [74]. The carbohydrate content in marine and freshwater microalgae varies significantly, where the microalgae contained 19.7% carbohydrates [48].

The dry biomass of *P. Tricornutum* contains 36.4 % protein, 26.1 % available carbohydrates, 18.0 % lipids and 15.9 % ash. When the biomass is compared to that of soybean flour, it is shown that its functional properties are approximately equal. Because of the high carbohydrate and protein content of these microalgae, it works very well as feed for both humans and animals [53].

2.4.5 *Synechocystis* SP. PCC 6803 - Potential microalgae candidate

Synechocystis sp. PCC 6803 (*Synechocystis*) is a cyanobacteria (blue-green algae) that is present in ecosystems around the world [75]. It is a robust species, surviving in a wide range of temperatures, salinities, and pH conditions, and is less susceptible to invasive species. It also has high values of lipid, protein and fatty acid content [76].

The optimal growth of this species is around 30-35 $^{\circ}$C at a pH = 7-8. If the temperature deviates from these temperatures, the photosynthetic effect decreases, which leads to a decrease in growth rate [77, 58]. *Synechocystis* can store ammonium (NH_4^+) inside of the cell by producing a polypeptide. It is also capable of accumulating phosphorus (P) inside the cell [75]. This microalgae is sensitive to light exposure, and the lipid, protein, and fatty acids contents vary depending on the available light [58].

Unlike most microalgae, which achieve their peak lipid levels via nitrogen starvation in the stationary phase [78], *Synechocystis* produces its highest lipid content during the exponential phase [79], thus the overall lipid productivity is not offset by increased growth rate. It is also an ideal genus for metabolic engineering to improve its performance; this is because its genomic data has been extensively studied [76]. *Synechocystis* has been reported to have a relatively high biomass productivity of about 200 mg $\mathrm{L}^{-1} \cdot \mathrm{day}^{-1}$ [79].

2.5 Microalgae cultivation technologies

Cultivation reactors are required when microalgae are going to be grown at a large commercial scale. The reactor provides an environment that can be designed for optimal growth. There are several types of different cultivation reactors; the different reactors are based on various technologies and techniques to achieve optimal growth while having high effectiveness in nutrient and contaminant removal as well as accommodate large volumes of wastewater [5].

The cultivation of microalgae can occur both open or enclosed to the environment. For enclosed cultivation rectors, all environmental parameters are closely monitored and controlled, where some of the most important parameters for optimal growth are illumination, temperature, pH, nutrient levels, and contamination. While for open cultivation rectors, these parameters

are presented with high variability. This is because open cultivation reactors are open to the surroundings and are not protected against the variations in the local environment [61, 5].

The cultivation methods that are primarily used today are suspension reactors [80], and these are called tubular photobioreactors (PBR) and open raceway ponds (ORP) [81]. Another cultivation rector technology has recently been developed known as the rotating algal biofilm (RAB) reactor. Figure 2.5.1 shows a schematic of the three cultivation reactors [6]. The ORP is a reactor that is open to the environment, while PBR and RAB are often in an enclosed environment.

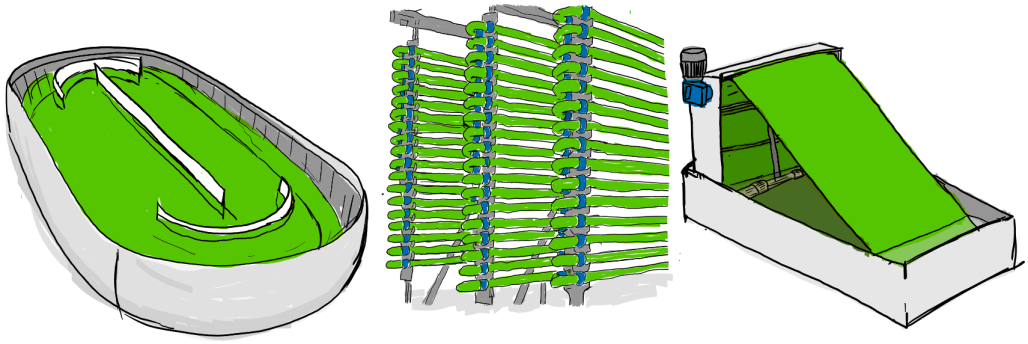


Figure 2.5.1: *Illustrations of three different microalgae cultivation methods. From left: Open raceway pond (ORP), tubular photobioreactors (PBR) and rotating algal biofilm (RAB) reactor.[6]*

For ORP and PBR reactors, three parameters are mainly used to evaluate the performance. These are productivity per unit reactor volume ($\mathrm{g} \cdot \mathrm{L}^{-1} \cdot \mathrm{day}^{-1}$), productivity per unit ground area occupied by the reactor ($\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$) and productivity per unit of reactor illuminated surface area ($\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$) [60]. ORP and PBR are used more frequently because they are easy and relatively inexpensive, but the poor illumination and loss of water due to evaporation give them a disadvantage. In addition to this, the ORP has a high risk of contamination because of the open environment, this can directly affect production cost, and annual productivity [57].

The efficiency of wastewater treatment and biomass productivity varies considerably based on the reactor types, the surrounding environment, and the type of microalgae. In addition to the efficiency of the reactor, the economic aspect is always important when choosing a reactor [5].

Poonam Choudhary et al. [82] has stated that most documented studies on microalgal biofilms are focused on nutrient removal at the lab- and pilot-scale levels. At the same time, there is limited research done on the utilization of microalgal biofilm systems for biomass production and further conversion into biofuels. They further state that the biomass production efficiency of advanced reactors is essential for successful application. For the long-term operation of a microalgal biofilm, the choice of attachment material and suitable harvesting frequency

is the most critical design parameters to consider. Table 2.5.1 shows an overview of seven different cultivation systems and their growth productivity. When comparing these systems, two stand out: the rotating algal biofilm (RAB) reactor and drum biofilm reactor (DBR). Where the biomass productivity per day is as high as 29.58 ($\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$) and 54.46 ($\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$), respectively. This thesis will further focus on the RAB reactor to cultivate microalgae on a large-scale basis.

Table 2.5.1: *Categorization of different biofilm systems, which type of attachment material is used, what species is optimal and the productivity rate [82].*

Biofilm cultivation systems with types	**Attachment material**	**Species**	**Productivity** ($\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$)	**References**
Twin-layer PBR	Polycarbonate membranes	*Halochlorella rubescens*	1.7–6.6	[83]
Pilot-scale phototrophic biofilm reactor	Polyethylene woven geotextile	Wastewater consortium	2.7-4.5	[84]
Algal biofilm reactor (ABR)	Nonwoven spun bond fabric	Mixed culture (*Chlorella* and *Phormidium*)	3.1-4.0	[85]
Algal Turf Scrubbers (Horizontal/inclined)	Polyethen	Wastewater consortium	25	[86]
Drum biofilm reactor (DBR)	Canvas	*Chlorella vulgaris*	54.46	[87]
Rotating algal biofilm (RAB) growth system	Cotton duct canvas	*Chlorella vulgaris*	29.58 (through based) 15.2 (race-way-based)	[88]

Harvesting microalgae is removing the biomass from the culture medium. This is one of the most critical challenges of biomass production at the industrial scale. The process can be done in one or more steps, with chemical, physical or biological methods. Conventional methods such as centrifugation, flocculation, filtration, or in some cases gravity sedimentation, can be used individually or in combination with each other [61, 81]. Since the specific gravity of biomass and water are about the same, dewatering will be a challenge that is both energy and capital cost intensive [61].

The harvesting frequency is essential for any cultivation of microalgae when the biofilm develops on the surface of the attachment material. The biofilm grows in layers of cells; the initial biofilm must stick to the attachment material. As the biofilm grows, eventually, the appropriate thickness can be formed. This is at a thickness where the biofilm keeps growing fast, but not think that the top layer will overshadow the underlying cells. It will lead to smaller eutrophication, as explained in Section 2.1, where the bottom cells do not get access to any light or air and start to decompose. Therefore an appropriate thickness must be achieved to

avoid loss of productivity and, worst case, mortality over the microalgae. This is the reason why the harvest time is one of the most critical parameters in optimal cultivation [82].

After the biomass is harvested, it needs to be dried. It is required to decrease moisture content to 12 % or less; this makes up a significant fraction of the total production cost. Here, spray drying is the most commonly used method and gives a product with good quality but is a costly method which is not feasible for low-value products [61]. Harvesting can account for 20-30 % of the operational cost of a traditional photobioreactor, and 21 % of the capital cost of an open pond reactor [81, 88].

The capital and operational costs of large-scale microalgae production can be very high. When comparing microalgae-based biofuel with crude oil at \$100 barrel^{-1}, the cost of algal biodiesel production cost must drop 10-folds [51]. When algal biomass is a byproduct of wastewater treatment (WWT), the production cost will decrease as the algal biomass is produced and harvested from WWT systems. This is because the biomass can be converted through various pathways to biofuels, for example, anaerobic digestion to biogas, transesterification of lipids to biodiesel, fermentation of carbohydrate to bioethanol, and high-temperature conversion to bio-crude oil as mentioned before in Section 2.4.

2.5.1 Rotating algal biofilm (RAB)

A rotating algal biofilm (RAB) is generally a conveyor belt rotated by a plurality of drive shafts, where the biofilm is rotated between air and liquid medium. The belt can be made out of different materials. Some algae species can attach faster to some specific materials than others (e.g., cotton is a good material for microalgae growth). It makes the biomass production from microalgae much more manageable, and it is possible to have a continuous flow of oxygen, light, and CO_2 from the air and nutrients from the wastewater medium. This ensures that the photosynthesis can operate continuous [89, 90, 91].

Figure 2.5.2 shows a schematic drawing of how two RAB reactors could be set up [90]. The one on the left is vertical, and the one on the right is horizontal. The total ground area that is needed for a cultivation reactor is called the footprint area. Because of the large surface area of the biofilm and that the liquid water is not required to be moved with the microalgae, a RAB reactor uses a smaller footprint area than a conventional open raceway pond (ORP). RABs have a much higher growth efficiency and because they can utilize the footprint area better. Of the two RAB reactors shown in Figure 2.5.2, it is the vertical that has the best footprint efficiency as it grows in vertical conditions, which significantly enhances the efficiency of space regulations. By increasing the area vertically, the cells are more efficient at capturing light and conducting $\mathrm{CO}_2/\mathrm{O}_2$ gas exchange [88].

As stated earlier in Section 2.4.1, photoinhibition is damage caused to photosynthetic cells due to excess light energy. It is when too many photons are absorbed, and the organism cannot

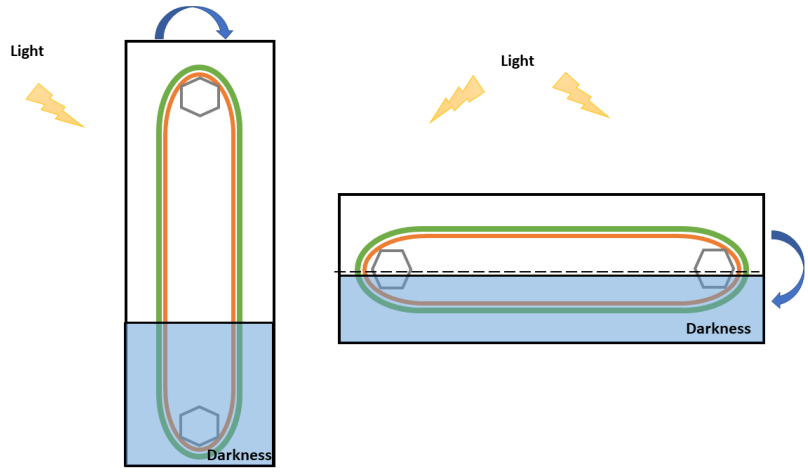


Figure 2.5.2: *Schematic drawing of rotating algal biofilm (RAB) cultivation belts. Left: vertically, right: horizontally.*

utilize them quickly enough. This results in the production of radical molecules within the organism that can cause extensive damage to essential components of the cell. It is one of the main factors affecting the photosynthetic dynamics in microalgae, and therefore their growth. Because the microalgae biofilm on the RAB reactor constantly rotates between air and water, the biofilm is also only exposed to light periodically. This extensively reduces photoinhibition of the microalgae, as it allows them time to maximize the utilization of photons. This allows the RAB system to obtain high growth productivity of microalgae cells [90].

The RAB ensures a very high growth rate, which can be towards 20 g of biomass [82, 92, 90], per m^2 of biofilm surface, per day. For a RAB, the biomass productivity is based on two criteria: 1) surface biomass productivity $\mathrm{P}_{\mathrm{Surface}}$, which is based on the surface area of the attachment material. (2) footprint biomass productivity $\mathrm{P}_{\mathrm{Footprint}}$, which is based on the footprint area of the biofilm reactor [92]. When comparing a RAB to a conventional ORP, the biomass productivity can increase over 302 % [90]. The RAB reactor maximizes the microalgae production in the WWT process while reducing nitrogen and phosphorus concentration.

Table 2.5.1, shows the productivity of a two different RAB reactors, one raceway based which had a biomass production rate of 15.2 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ and a through based which had a biomass production rate of 29.58 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$. Figure 2.5.3 shows simply how the raceway reactor is set up, a regular open raceway pond (ORP) is combined with RAB cultivation belts. The belts rotate in and out of the water as the wastewater flows through the tank. The belts are modeled as the vertical belt in Figure 2.5.2. In the research this was abstracted from [88], there were room for more belts in this area, Figure 2.5.3 is only a simplification to understand the set up.

Figure 2.5.4 shows a simple schematic drawing of a through based RAB from the same study. The wastewater flows through a pipe, where the pipe is cut in half at some areas. In

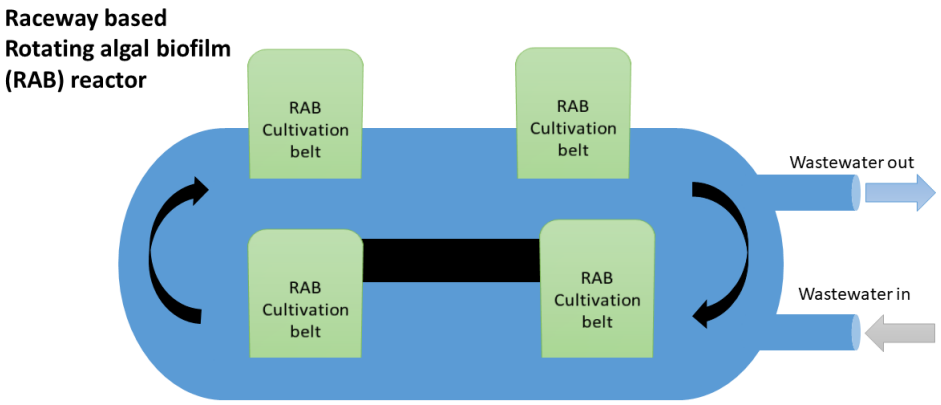


Figure 2.5.3: *Schematic drawing of a raceway based rotating algal biofilm (RAB) reactor, the wastewater flows through the raceway reactor while cultivation belts are placed in the raceway to absorb the nutrients, the belts rotate.*

these openings, vertical RAB cultivation belts are placed, rotating in and out of the wastewater medium as they absorb the nutrients. This was the cultivation reactor with the second to best biomass production rate from Table 2.5.1.

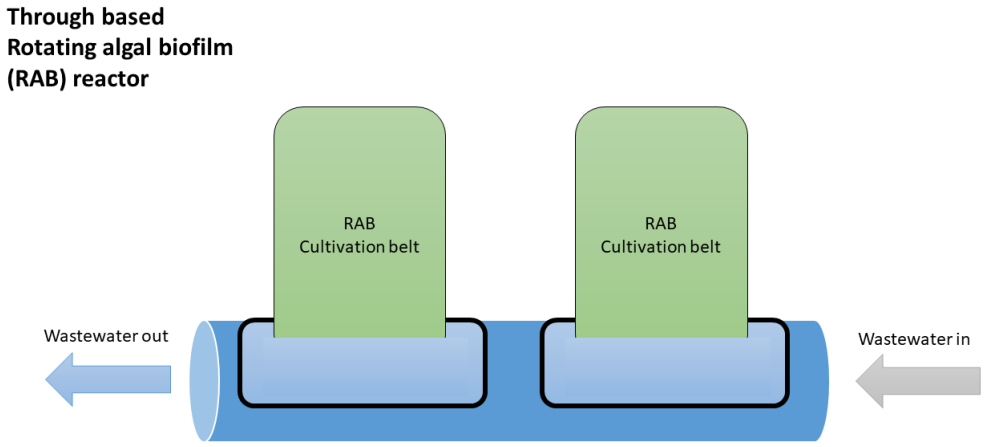


Figure 2.5.4: *Schematic drawing of a through based rotating algal biofilm (RAB) reactor, the wastewater flows though a pipe, that has openings in it. In these openings, RAB cultivation belts are placed for the absorption of nutrients, the belts rotate.*

In a RAB, the microalgal biomass is harvested through scraping the biofilm in the air phase [89]. When harvested, the microalgal paste already has a water content similar to post-centrifuged algal biomass (80-90 %) from a regular photobioreactor (PBR) [88]. Scraping is less complicating than using expensive sedimentation, and centrifugal approaches [80]. The harvest process is more straightforward, and it can harvest at a significantly lower cost. Despite this, it often has a higher workforce, as there has not been discovered a mechanized harvest

device, it is still a manual operation, which can be very labor-intensive if the surface area of the biofilm is large [92]. However, it is possible to automate this process to reduce the labor requirements.

The essential benefits to highlight from this cultivation reactor are its efficient use of the light distribution, enhancement of CO_2 mass transfer, extended biomass retention time, smaller footprint, and better water utilization efficiency [92].

The most important aspect of modeling a microalgae cultivation reactor for large-scale microalgae production is to look at the process parameters. These are the parameters that are going to ensure optimal photosynthetic growth of biomass (e.g., light, temperature, nutrients, oxygen accumulation, salinity, pH and carbon) [57]. As mentioned in Section 2.4.1, microalgae use significantly less area to grow than crops, and it does not depend on arable land. This means that they will not compete for land availability [57].

Mathematical modeling is an essential step for optimal growth and to get efficient operation and process control variables. The modeling will show the effect of each process condition (e.g., temperature and light) related to the critical production parameters (e.g., growth rate and productivity). The monitoring of this will show the effect of every change in the process conditions without the necessity to experimentally test the effects separately [57].

2.6 Modeling a Rotating algal biofilm (RAB) reactor

There are many requirements for modeling a rotating algal biofilm (RAB) reactor, it is one of the most optimal cultivation rectors - but needs extensive surrounding control to achieve this optimization. In this Section, there are five parameters that are being explained; light, temperature, velocity of the harvesting belts, harvesting frequency and cultivation area. All these parameters are essential for a good facility, but this thesis is focused on the cultivation area.

2.6.1 Light

One of the essential parameters of the microalgal system is the quantum requirement of the photosynthetic process. This is the efficiency with which the microalgae take up light energy and convert it to chemical energy (i.e., new biomass) while releasing O_2. As mentioned previously in Sections 2.4.1 and 2.5.1, light is the source of energy in photosynthesis and is the most crucial parameter in the modeling of microalgae growth. Section 2.5.1 discussed how too much light could damage the proteins in microalgae, and too little light would not ensure optimal growth. Because microalgae is a media that grows thicker, it is known that the light intensity decreases as it goes deeper into the media due to the absorption of light by the cells [93]. The Beer-Lambert Law is usually used to describe this, and the law suggests that light intensity exponentially decreases with depth [94]. This law is explained by Equation (2.6.1),

where I is light intensity, I_0 is light intensity entering the media perpendicular to the surface, z is depth, and k is attenuation rate.

$$I = I_0 e^{-kz} \tag{2.6.1}$$

k is defined differently according to different scientists. However, the focus in this thesis will be on Grima et al. [95] who defined k as a linear function of biomass concentration. The Beer-Lambert law is extensively used; it is based on the assumption that light is not scattered in the media, light scattering is a process when incident light of energy is absorbed by a system (sample) and subsequently light of energy is emitted. This is not correct when it comes to microalgae cultures, and it is why some researchers are hesitant of using the law and would instead take light scattering into account [57].

2.6.2 Temperature

The influence of temperature on process dynamics can be equivalent to that of light [96]. The two microalgae species *Phaeodactylum Tricornutum*, (*P. Tricornutum*) and *Synechocystis* sp. PCC 6803 (*Synechocystis*) are described in Sections 2.4.4 and 2.4.5, respectively. There it states that *P. Tricornutum* has optimal growth at temperatures of 20 °C [73], while *Synechocystis* has optimal growth temperatures of 30-35 °C [77, 58]. The good thing about microalgae is that they will keep on growing even though the temperature drops below the optimal temperature, but it will lead to a slower growth rate. However, if the temperature goes above their optimum, their growth rate rapidly drops.

2.6.3 Velocity of the belt

The circulating belt needs a velocity to maintain optimal biomass growth from getting the right amount of light to maximize growth while preventing photoinhibition. P-O Lamare et al. [91] studied the gradient-based optimization of the RAB process through a partial differential Equation (PFE) model of the RAB, which represented local microalgal growth submitted to the time-varying light. They concluded that for every initial condition they tested, the optimal velocity computed is constantly equal to $\mathrm{U}_{\max} = 0.3\mathrm{m} \cdot \mathrm{s}^{-}1$ [91].

The greater the maximum light intensity is, the greater the optimized length of the biofilm belt. A high maximum light intensity implies much more inhibition of photosynthesis than at low maximum light intensity. Therefore, much more time in the dark is needed for the damage to recover, meaning a longer belt should be considered. P-O Lamare et al. [91] also observed that for microalgae permanently exposed to PFD larger than 168 $\mu\mathrm{mol} \cdot \mathrm{m}^{-2} \cdot \mathrm{s}^{-1}$, the growth rate μ decreases as the value of incident light (I) increases [91].

2.6.4 Harvesting frequency

As mentioned in Section 2.5, the harvesting frequency is one of the most critical parameters to obtain optimal growth from actively growing cells. When applying microalgal biofilms for wastewater treatment, the biofilm should be continuously maintained in the exponential or linear growth phase. This ensures a high biomass production and, thereby, a high nutrient removal rate. It is essential when harvesting biofilm that there is a fraction of biofilm left, so the biomass can continue to grow and remove nutrients without needing to establish that first growth again [97].

Boelee et al. [97] has studied "The effect of harvesting on biomass production and nutrient removal in phototrophic biofilm reactors (PBR) for effluent polishing." The study was based on a vertical PBR in a room, where the average temperature of the liquid medium acting as wastewater was 21 °C. They tried different harvest frequencies to gather the optimum time; they harvested every 2, 4, and 7 days. The average areal biomass production rate achieved was 7 g DW $\mathrm{m}^{-2} \cdot \mathrm{day}{-}1$ for all three frequencies. The biomass productivity is similar for a wide range of thicknesses. They also tried to wait two weeks to harvest; after this amount of time, the biomass spontaneously detached from the carrier material [98].

When it comes to the thickness of the biofilm at the different stages, the biomass production doubled as the biofilm thickness was increased from 130 $\mu\mathrm{m}$ to 2 mm. This increased production was explained by the lower density and looser structure of the 2 mm biofilm. The study concluded that during biomass production, the optimum harvest frequency is once per week because of the production and labor requirement [97]. A pilot study was done by Gross et al. [88], also concluded to harvest every 5 - 7 days.

2.6.5 Cultivation area

Olberg et al. [99] wrote a bachelor thesis at NTNU in 2020 about producing microalgae in RAB reactors. They looked at two different scenarios. Scenario 1 was based on space efficiency, had artificial cultivation light and a vertical RAB design. Scenario 2's main goal was economic profitability with natural cultivation light and a triangular RAB design. Both scenarios would lead to a large footprint area, but scenario 1 had a better outcome because the artificial light provides a more optimal growth requirement. They concluded for scenario 1 they could produce 60 t $\mathrm{ha}^{-1} \cdot \mathrm{y}^{-1}$, with 3 m tall RAB and a footprint area of 6.178 m^2. If the height of the RAB increased to 5 m, the footprint area would end up at 3.707 m^2, which is a significant decrease. The space between each RAB module must increase as the height increases because they will cover each other from the sun. In comparison, scenario 2 with natural light required 8.537 ha to produce 18.26 $\mathrm{tha}^{-1} \cdot \mathrm{y}^{-1}$ [99].

Gross et al. [88] has evaluated the microalgae growth performance and water use efficiency of pilot-scale RAB culture systems. They evaluated a pilot-scale through based RAB system of 150 L liquid and a footprint area of 3.5 m^2; the through based pilot was made with a pipe

(20 cm), was cut in half, and then the wastewater ran through the pipe as the belt was also placed in the pipe. Hence, it absorbed the nutrients from the water. They varied the surface area throughout the experiment, with each belt having a width of 1 m and the height and surface area given in Table 2.6.1. There was room for eight belts in the 3.5 m^2 footprint area. It started by establishing a thick biofilm for 14 days, then harvested by scraping. After this, the cycle repeated with harvesting every seven days, and half the working volume of water was replaced every seven days. The surface biomass productivity for the through-based RAB and the raceway-based RAB is given in Table 2.5.1. They found a slight decrease in surface biomass productivity as the height of the RAB increased, but it was so small that it was insignificant. The decrease in productivity could come from a mutual shadowing from the adjacent belt. They also compared the through-based pilot with a raceway-based RAB, where the belt was placed in the raceway pool. The through based performed 388 % better than the raceway based RAB [88].

Table 2.6.1: *Values of needed area and productivity based on the size of the RAB cultivation belts [88].*

Height of belt [m]	**Surface area [m^2]**	**Total surface area [m^{-2}]**
0.91	1.85	14.8
1.22	2.42	19.36
1.52	3.04	24.32
1.83	3.64	29.12

In an analysis made by Boelee et al. [98], three different scenarios of nutrient removal from municipal wastewater by microalgal biofilm were assessed. The three scenarios used microalgae biofilm in a different part of the process: (1) as a post-treatment; (2) as the second stage of wastewater treatment, after a first stage in which COD is removed by activated sludge; and (3) in a symbiotic microalgal/heterotrophic system. It is the third scenario that is most relevant for this thesis. Figure 2.6.1 shows how scenario three would look like, it can be compared to the cultivation of RAB as it is a symbiotic area where the microalgae access CO_2 and O_2 easily. The study was located in the Netherlands, looking at municipal wastewater (MWW) from 100,000 inhabitants producing 130 L per person equivalent (PE) per day [98].

The biomass is assumed to be present in the biofilm is kept at optimal thickness through regular harvesting of the biofilm. By keeping the biofilm at this thickness, it will reduce respiration losses and ensure an optimal nutrient uptake capacity [98].

If the amount of biomass produced is known, and the fraction of N and P present in the wastewater is known, the N and P uptake from the wastewater can be calculated. Equation (2.6.2) shows the uptake of N, while Equation (2.6.3) shows the uptake of P, these two equations are equivalent [98].

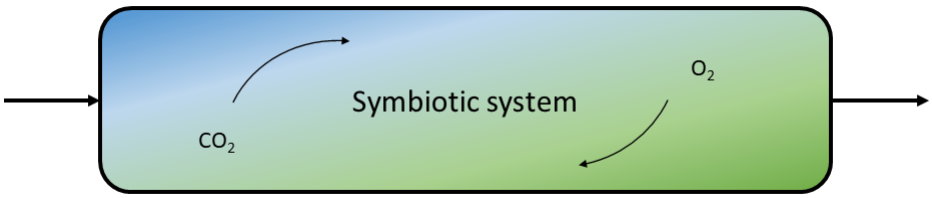


Figure 2.6.1: *Schematic drawing of a microalgal symbiotic system, where the microalgae have access to CO_2 and O_2 easily.*

$$R_{N,A,algae} = P_{x,A,algae} \cdot f_{N,algae} \tag{2.6.2}$$

$$R_{P,A,algae} = P_{x,A,algae} \cdot f_{P,algae} \tag{2.6.3}$$

$\mathrm{R_{N,A,algae}}$ and $\mathrm{R_{P,A,algae}}$ are the areal N and P uptake rates by microalgae [$\mathrm{g \cdot m^{-2} \cdot day^{-1}}$]. $\mathrm{P_{x,A,algae}}$ is the areal microalgal biomass production rate [$\mathrm{g \cdot m^{-2} \cdot day^{-1}}$], and $\mathrm{f_{N,algae}}$ and $\mathrm{f_{P,algae}}$ is the fraction of N/P in the microalgal biomass [g/g]. The desired amount of N or P removed and the area needed for biomass production can be calculated with Equation (2.6.4), and (2.6.5) [98].

$$A = \frac{Q \cdot (N_{in} - N_{out})}{R_{N,A,algae}} \tag{2.6.4}$$

$$A = \frac{Q \cdot (P_{in} - P_{out})}{R_{P,A,algae}} \tag{2.6.5}$$

Where A is the area [$\mathrm{m^2}$], Q is the flow rate [$\mathrm{L \cdot day^{-1}}$], and $\mathrm{N_{in}/P_{in}}$ and $\mathrm{N_{out}/P_{out}}$ is the nutrient concentration in the wastewater on the way in to the microalgae cultivation reactor and desired nutrient concentration for the water stream out of the reactor [$\mathrm{mg \cdot L^{-1}}$] [98].

3 Methodology

This Chapter presents the methodology used to address the research question posed. The first Section 3.1; Laboratory testing, explains how the laboratory testing was completed. The second Section 3.2; Nutrient tests, explains how the three different nutrient tests were executed. The last Section 3.3 focuses on calculating the surface and footprint area of a microalgae facility based on the results from the nutrient tests, these calculation are based on three different scenarios of the nutrient states of wastewater presented that will be evaluated. Lastly in this Section 3.3.3, it is explained how the biomass production can be calculated.

3.1 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. The most important aspect of this laboratory work is to evaluate the productivity and the nitrogen (N) and phosphorus (P) fixation of different species of microalgae to find out which is preferable for aquacultural wastewater treatment (WWT). These results can be used to estimate which algae is better for WWT. The purpose of this Section is to describe the process of planning, preparing, and growing microalgae. First, the pre-experimental planning is described, then the actual growth period for the microalgae.

3.1.1 Pre-experimental growth

The microalgae used were *Phaeodactylum Tricornutum* (*P. Tricornutum*) and *Synechocystis* sp. PCC 6803 (*Synechocystis*). These two microalgae species, along with a mixed culture of both the species, have been tested for their ability to absorb nitrogen (N) and phosphorus (P). They are also being tested for their growth rate. The lab experiments went on for 20 days for each cycle. As mentioned in Section 1, these microalgae are chosen because they were the only species available for research at NTNU. After the first experiment, a third cultivation bottle was added to test the co-cultivation concept explained in Section 2.4.3, where microalgae have a better growth production with other species of microalgae. Co-culture can also include growth with other organisms like bacteria or fungi, but this was not tried in this lab experiment.

The water culture is used as artificial marine wastewater. The water contained half part pure water and half part filtered seawater. To make this water mixture comparable to actual wastewater, it was infused with Gulliard's (F/2) Marine water Enrichment solution [100]. This is a suitable marine nutrient supplement for plant cell culture. Since the nutrient mixture is maintained in a 50x concentrate, only 20 mL of this nutrition mixture was added per liter of water. When fully diluted, this nutrient solution contained 75 $(\mathrm{mgnitrate}) \cdot \mathrm{L}^{-1}$, and 4.411 $(\mathrm{mgphosphate}) \cdot \mathrm{L}^{-1}$.

The preferred growth area for this test would be an actual RAB reactor; however, due to

unforeseen problems resulting in the reactor being unavailable in Norway, the testing was done in flat culture bottles. The bottles were placed in the Varmeteknisk laboratory at NTNU. There was surrounding light during work hours at this lab from 08:00 - 16:00, Monday through Friday. In addition to this, a lamp was placed above the cultures to get continuous light, even though it was less light at night and on weekends. The microalgae needed to grow a thin layer of biofilm before the actual experiment could begin. The experiment's goal was to measure the nutrient absorption rate of the microalgae for N and P and calculate the microalgae growth during the same period.

The average temperature of this lab set up was 20.8 ± 0.1 °C, and the average light the microalgae were exposed to during work hours was 0,180mW, while during nights and weekends, the light was 0,055mW. When the actual growing conditions are compared to the optimal growing conditions, they vary from each other. The microalgae in this experiment did not have an optimal growth rate, meaning that they could not grow as fast as they could in more controlled conditions.

The needed materials and equipment for the growth and testing of microalgae are listed in tables 3.1.1 and 3.1.2, respectively.

Table 3.1.1: *Material required for laboratory work*

Materials	**Description**
Phaeodactylum tricornutum	Microalgae for testing, provided by the biology department at NTNU
Synechocystis sp. PCC 6803	Microalgae for testing, provided by the biology department at NTNU
F/2 Guilliard's medium	Marine nutrient suppliment
Seawater	Local seawater from the fjord in Trondheim
Pure water	

Table 3.1.2: *Equipment required for laboratory work*

Equipment	**Description**
Square culture bottles	68 m^2 and 172 m^2 bottles for growth
LED light	Extra light for the microalgae to have continuous growth
Vacuum filter	To filter the seawater
Autoclave	Sterilizing the seawater/freshwater mix
Centrifuge	To separate microalgae from the sample water
Colorimetric tests	Different tests to measure the nitrate/nitrite and phosphate content of water
Tubes and pipette	For the implementation of the colorimetric tests
Spectrometer	Measure the absorbance of the nutrient colorimetric tests

3.1.2 Growth period

Once the biofilm had developed, a new experiment could be started. The experiment started with changing the artificial wastewater for new media to continuously measure nitrogen (N) and phosphorus (P) from the artificial wastewater. Before adding the new water to the culture bottle, a 2 mL sample of the new water was set aside to be frozen, and this was the sample with the initial N and P concentration. The culture bottle was filled to the recommended max with this water.

Once the experiments had started, the following sampling regime was completed throughout the experiments:

- Every day:
  - Take a 2 mL sample from each culture flask, put it in a plastic tube, and store at -20 °C. These samples would measure the N and P at the specific time the sample was taken. The tubes were labeled with name, date, and culture sample, and the time and date were also noted in a notebook.
- Every third day:
  - Take a sample of the biofilm where the wet weight was measured then and then after 24 - 72 hours in a drying cabinet, the dry weight was measured.
- Every ninth day:
  - Refill the culture flasks with new artificial wastewater for a new infusion of nutrients for the microalgae.
- After the experiment was complete:
  - Take the frozen water samples where the N and P concentrations were then measured with different colorimetric methods explained in Section 3.2.

Method for getting the wet and dry weight measurements:

- Get a clean 2 mL plastic tube and weigh it. Make a note of the weight.
- Take about a 2 cm^2 patch of the biofilm and remove it from the flask by scraping it off by a clean small spatula. The amount of biofilm area taken was noted, but the accuracy of the exact area could not be guaranteed.
- The removed biomass was deposited in small 2 mL plastic tubes. The tube was then measured with the biomass within. The initial tube weight was subtracted to this weight, and the resulting weight was the wet weight of the biomass.

- The water in the biofilm samples needed to evaporate to obtain the dry weight. This was done by adding the tubes in a warm cabinet at 40 °C for 24-72 hours. A hole was also made in the tubes for the water to escape the tube. When the biofilm was dry, it was weighed again, and the initial tube weight was subtracted to get the dry weight of the biofilm sample.

- It was now possible to calculate the wet and dry weight of the biofilm per cm^2.

The biomass samples were taken every third day, but the experiments started with a thick biofilm already. The biofilm did not grow that much over the parts of the experimental period. To get an imprecise estimate of the growth rate of these microalgae, the biomass weight given in these tables could be divided by ten because the biofilm that was established when started had grown for about ten days. This could be an estimated growth rate, but not very precise.

3.2 Nutrient test

Section 3.1.1 addresses the first part of this project, where microalgae were grown in the lab while monitoring them and taking water and biofilm samples. This Section focuses on the process of measuring the microalgae nitrogen (N) and phosphorus (P) uptake. This nutrient absorption rate of the two microalgae species and the mixed culture were found using different colorimetric methods. N and P require different tests to calculate the absorption rate. This is done by adding different color reagents to the microalgae samples. The color changes are dependent on how high the nitrogen or phosphorus concentration is in the water sample. All the samples were going to measured compared to a standard in a spectrophotometer.

Before any of the tests could be carried out, the water samples needed to be prepared. As these water samples were extracted from microalgae cultures using a pipette, there could be some algae residue in the water samples. It should be as little as possible microalgae residue in the water because it can affect the results. The microalgae have absorbed the N and P from the water and can affect the test results. The residue was removed by putting the water samples in a centrifuge for 2 min at 15 000 rpm. The microalgae residue is forced to the bottom of the tubes, so it does not affect the water samples. When removing water from the small tube, it is essential not to get the algae sample.

There were four rounds of the experiment where the microalgae water samples were going to be tested for their nitrogen uptake, and two different nitrite/nitrate NO_2^-/NO_3^-) tests were used because of the availability of the tests.

After the absorption rate is found with the nutrient tests, the concentrations have to be found with the equations needed for the respective tests. For these concentration calculations, the parameters from Table 3.2.1 were used. The parameters were the sample volume used in tests and the molar weight [$\mathrm{g} \cdot \mathrm{mol}^{-1}$] of nitrate, nitrite, and phosphate.

Table 3.2.1: *Parameters of cultivation setup for calculation nutrient concentration*

Parameter	**Value**	**Unit**
Sample volume	500	μL
Nitrate molar weight	62.01	$\mathrm{g} \cdot \mathrm{mol}^{-1}$
Nitrite molar weight	46.01	$\mathrm{g} \cdot \mathrm{mol}^{-1}$
Phosphate molar weight	94.97	$\mathrm{g} \cdot \mathrm{mol}^{-1}$

When tests are going to find measurements using a colorimetric test, the absorption rate is measured through the light absorption in a spectrometer. The maximum value one can get from the spectrometer is 3.000, but if the value is of this magnitude, there may be some inaccuracy in the measurements. The calculated concentration with these high values has a chance of being wrong. The artificial wastewater contains high amounts of N and P. Thus, the first values of any of the experiments will have high absorption values, leading to incorrect concentration measurements. In this case, the nutrient values of the artificial wastewater are known to be 75 $\mathrm{mg} \cdot \mathrm{N} \cdot \mathrm{L}^{-1}$, and 4.411 $\mathrm{mg} \cdot \mathrm{P} \cdot \mathrm{L}^{-1}$, as it is stated in Section 3.1.1. The first water samples of all experiments will have this value, and if the calculation of concentration is wrong, this will be overridden to the known values [101].

3.2.1 Nitrite/nitrate test method 1

The first experiment was tested with a nitrite/nitrate colorimetric method and Photometric end-point determination. It tested the nitrate concentration and the nitrate + nitrite concentration. When both concentrations were measured, it was possible to calculate the nitrate concentration by taking (nitrate + nitrite) - nitrite.

When the water samples were prepared and ready, the test could start. It was completed through the instructions given with the test. The test kit contained:

1. **Bottle 1** with 22 ml solution, consisting of potassium phosphate buffer, pH 7.5 and stabilizers.

2. **Bottle 2** with 7 tablets, each tablet contains 0.5 mg NADPH, 0.01 mg FAD and stabilizers.

3. **Two bottles 3** each containing four units of nitrate reductase enzyme that was lyophilized.

4. **Two bottles 4** each containing 8 ml color reagent I, consisting of sulfanilamide and stabilizers.

5. **Two bottles 5** each containing 8 ml color reagent II, consisting of N-(1-naphthyl)-ethylenediamine dihydrochloride and stabilizers.

Bottles 1, 4, and 5 could be used undiluted, while bottles 2 and 3 needed to be prepared. The process needed to be done is described in the list below. The prepared bottles needed to be stored in a fridge with a temperature between +2 and +8 °C until use.

- **Bottle 1, 4 and 5** use undiluted
- **Bottle 2** place one tablet from bottle 2 in a beaker and dissolve in 3 ml of the solution from bottle 1. The tablet should be removed from bottle 2 using tweezers. The resulting solution is reaction mixture 2 and is sufficient for 12 nitrate determinations.
- **Bottle 3** dissolve content of bottle 3 in 0.7 ml of milliQ deionized water to give solution 3.

The parameters for the measurements are given in Table 3.2.2.

Table 3.2.2: *Parameters for wavelength measurements of nitrate/nitrite uptake, test method 1*

Wavelength	540 nm (Hg 546 nm)
Disposiable cuvette	1.00 cm, semimicro
Temperature	+20 to +25° C
Volume	1.27 ml
Measurement against blank	
Sample solution	0.05 mg - 5.00 mg nitrite or nitrate L^{-1}

Before measuring the water samples from the microalgae cultivation, the last step was to add the different solutions from the bottles in the test kit to the sample tubes and the two tubes used for the standard solutions. The standard solutions consisted of one tube for a blank sample of nitrite and one tube for a blank sample of nitrate + nitrite.

The final preparations of the blank and water solutions are shown in Table 3.2.3. First, the water samples were added to small 2 ml tubes, then Milli-Q deionized water was added for the blank samples. After this, reaction mixture 2 and solution 3 were added to the blanks and water samples. The sample bottles were then mixed and incubated between the temperature of +15 to +25 °C for 30 min. After this, the first absorption measurements (A1) were taken from a spectrophotometer at 540 nm.

The sample must be transferred into a disposable cuvette with a 1 cm long light path for the sample to be measured in the spectrophotometer. Water must be set as the reference for the machine before assessing the samples and blanks. After this, all the samples were inserted to measure the absorption for each sample and blank.

Once completed, 0.250 ml of both color reagent I and II were added to blank and all water samples. The two color reagents are substances that will show different colors depending on the nitrate/nitrite + nitrate content of the sample. After adding the color reagents, the samples were mixed and incubated in the dark between a temperature of +15 to +25 °C for 10 to 15 minutes.

The second absorption measurements (A2) could now be read off the spectrophotometer the same way A1 was measured.

Table 3.2.3: *Final preparations of water samples and blank samples before measurements, nitrate/nitrite test method 1*

Pipette into cuvettes	**Blank nitrite**	**Sample nitrite**	**Blank nitrite + nitrate**	**Sample nitrite + nitrate**
Sample	-	0.500 ml	-	0.500 ml
Redist. water	0.770 ml	0.270 ml	0.500 ml	-
Reaction mixture 2	-	-	0.250 ml	0.250 ml
Solution 3	-	-	0.020 ml	0.020 ml
Mix, incubate for 30 min at +15 to +25° C, read off A1 and then add				
Color reagent I	0.250 ml	0.250 ml	0.250 ml	0.250 ml
Color reagent II	0.250 ml	0.250 ml	0.250 ml	0.250 ml
Mix, allow to stand in dark at +15 to +25° C for 10 to 15 min, read off A2				

All the measurements were written down in a notebook and inserted into an excel sheet. The absorption measurements for the first experiment can be found in Section 4.2.2 in Chapter 4.

To calculate each water sample's nitrate and nitrite concentration, the absorption measurements from the nitrite/nitrate test needed to be found. First, the total absorption of the nitrite and nitrate needs to be calculated. This is done by Equation (3.2.1) which shows the calculation of the $\Delta A_{nitrite}$, Equation (3.2.2) which shows the calculation of $\Delta A_{nitrite+nitrate}$ and Equation (3.2.3) calculating $\Delta A_{nitrate}$ using the other two equations.

$$\Delta A_{nitrite} = (A_2 - A_1)_{nitrite} - (A_2 - A_1)_{Blanknitrite} \tag{3.2.1}$$

$$\Delta A_{nitrite+nitrate} = (A_2 - A_1)_{nitrite+nitrate} - (A_2 - A_1)_{Blanknitrite+nitrate} \tag{3.2.2}$$

$$\Delta A_{nitrate} = A_{nitrite+nitrate} - A_{nitrite} \tag{3.2.3}$$

The total nitrite and nitrate concentrations (C) were found by Equation (3.2.4) and (3.2.5), respectively. The unit of measurement for the $C_{nitrite}$ is $\mathrm{mg} \cdot \mathrm{nitrite} \cdot (\mathrm{kgsample})^{-1}$, and for $C_{nitrate}$ it is $\mathrm{mg} \cdot \mathrm{nitrate} \cdot (\mathrm{kgsample})^{-1}$. The final concentration of nitrite and nitrate is given in Section 4.2.2 in Chapter 4.

$$C_{nitrite} = \frac{C_{nitrite} x 1000}{Mass_{sample} \text{in g/l sample solution}} \tag{3.2.4}$$

$$C_{nitrate} = \frac{C_{nitrate} x 1000}{Mass_{sample} \text{in g/l sample solution}} \tag{3.2.5}$$

3.2.2 Nitrite/nitrate test method 2

Nitrite/nitrate colorimetric Assay kit from Sigma-Aldrich it is used for detection of nitric oxide metabolites, catalog number 23479. This kit was sufficient for 100 assays in a 96 well plate that needed to be provided. In this experiment a 96 well plate was not used, instead 2 ml centrifuge bottles were used. As the previous nitrite/nitrate test, the spectrophotometric multiwell plate reader was used to measure the nitrogen (N) absorption of the water samples. The test kit needed to be stored in a fridge between +2 and +8 °C, and the kit contained these components:

1. **1 bottle** $\mathrm{NaNO_2}$ Standard Solution (100 μM)
2. **1 bottle** $\mathrm{NaNO_3}$ Standard solution (100 μM)
3. **1 bottle** Buffer solution (20 mM, pH 7.6)
4. **1 vial** Nitrate Reductase
5. **1 vial** Enzyme Co-factors
6. **1 bottle** Griess Reagent A
7. **1 bottle** Griess Reagent B

Before the test could start, there were preparations for the kit components. $\mathrm{NaNO_2}$ Standard Solution, $\mathrm{NaNO_3}$ Standard solution, Buffer solution, Griess Reagent A, and Griess Reagent B needed to reach room temperature before use. While Nitrate Reductase and Enzyme Co-factors needed to be reconstituted in 1.2 ml of the Buffer solution before use, it was necessary to mix well after this was done. The Nitrate Reductase should not mix with the Enzyme Co-factors before use.

The first step in the procedure was to make standards for the colorimetric detection of $\mathrm{NO_2^-}$ and $\mathrm{NO_3^-} + \mathrm{NO_2^-}$. Table 3.2.4 and 3.2.5 shows how much standard solution, and therefore the concentration of the standards of $\mathrm{NO_2^-}$ and $\mathrm{NO_3^-} + \mathrm{NO_2^-}$, respectively. All the standards have a total volume of 1 ml comprised of the standard and buffer solutions.

Table 3.2.4: *Standard preparations for colorimetric detection of* $\mathrm{NO_2^-}$*, nitrate/nitrite test method 2*

Bottle name	NO2-S0	NO2-S20	NO2-S40	NO2-S80
$\mathrm{NaNO_2}$ **standard solution added [μ M]**	0	100	200	400
Buffer solution added [ml]	1	0.9	0.8	0.6
$\mathrm{NaNO_2}$ **concentration [nmole/bottle standard]**	0	2	4	8

Table 3.2.5: *Preparations to make standards for colorimetric detection of* $\mathrm{NO_3^- + NO_2^-}$

Bottle name	NO3-S0	NO3-S20	NO3-S40	NO3-S80
$\mathrm{NaNO_3}$ **standard solution added [μ M]**	0	100	200	400
Buffer solution added [ml]	1	0.9	0.8	0.6
$\mathrm{NaNO_3}$ **concentration [nmole/bottle standard]**	0	2	4	8

The standard and water samples are now prepared and ready for the assay reaction. The reaction procedure goes as follows:

1. Add 10 μl of the Nitrate Reductase solution and 10 μl of the Enzyme Co-factors solution to the sample and standard wells for ($\mathrm{NO_3^- + NO_2^-}$) detection.

2. Mix well using a horizontal shaker or pipetting and incubate the tubes at 25 °C for two hours.

3. Add 50 μl of Griess Reagent A to all tubes. Mix well using a horizontal shaker or pipetting and incubate the tubes at 25 °C for five minutes.

4. Add 50 μl of Griess Reagent B to all tubes. Mix well using a horizontal shaker or pipetting and incubate the tubes at 25 °C for ten minutes.

5. Measure the absorbance at 540 nm (A540) in a spectrophotometer.

The absorption was measured the same way as the first nitrite/nitrate test, with the same wavelength; 540 nm. All the measurements were written down in a notebook and inserted into an excel sheet for further analysis. The absorption measurements for the first experiment can be found in Section 4.2.3 in Chapter 4.

The background for the $\mathrm{NO_2^-}$ determination was the value obtained for the 0 nmol / tube (blank) $\mathrm{NaNO_2}$ Standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate $\mathrm{NaNO_2}$ Standards to plot a standard curve. The same procedure was followed to plot a standard curve for $\mathrm{NO_3^- + NO_2^-}$, using the blank value for $\mathrm{NaNO_3}$ Standard.

The $\mathrm{NO_2^-}$ blank value was subtracted from the $\mathrm{NO_2^-}$ sample readings, and $\mathrm{NO_3^- + NO_2^-}$ blank value was subtracted from the $\mathrm{NO_3^- + NO_2^-}$ sample readings. The correct absorption measurements have now been found for both tests. Using these measurements, the amount of $\mathrm{NO_2^-}$ or $\mathrm{NO_3^- + NO_2^-}$ was determined from the respective standard curves.

The correct absorption measurements were used to calculate the concentration of $\mathrm{NO_2^-}$ and $\mathrm{NO_3^- + NO_2^-}$ in the different water samples. Equation (3.2.6) shows the calculation performed

to find the concentration of nitrite. Where $\mathrm{A}_{\mathrm{NO}_2^-}$ = amount of nitrite in sample tube (nmole) from nitrite standard curve, $\mathrm{V}_{\mathrm{NO}_2^-}$ = sample volume (μl) added into the tube, $\mathrm{C}_{\mathrm{NO}_2^-}$ = concentration of nitrite in sample and the nitrite molecular weight = 46.01 $\mathrm{g} \cdot \mathrm{mole}^{-1}$.

$$A_{NO_2^-}/V_{NO_2^-} = C_{NO_2^-} \tag{3.2.6}$$

Equation (3.2.7) shows the calculation performed to find the concentration of nitrate + nitrite. Where $\mathrm{A}_{\mathrm{NO}_3^-+\mathrm{NO}_2^-}$ = amount of nitrate + nitrite in sample tube (nmole) from nitrate + nitrite standard curve, $\mathrm{V}_{\mathrm{NO}_3^-+\mathrm{NO}_2^-}$ = sample volume (μl) added into the tube and $\mathrm{C}_{\mathrm{NO}_3^-+\mathrm{NO}_2^-}$ = concentration of nitrate + nitrite in sample.

$$A_{NO_3^-+NO_2^-}/V_{NO_3^-+NO_2^-} = C_{NO_3^-+NO_2^-} \tag{3.2.7}$$

Equation (3.2.8) shows the calculation done to find the concentration of nitrate where the nitrate molecular weight is 62.01 $\mathrm{g} \cdot \mathrm{mole}^{-1}$.

$$C_{NO_3^-} = C_{NO_3^-+NO_2^-} - C_{NO_2^-} \tag{3.2.8}$$

All concentrations of the water samples from the second experiment can be found in Section 4.2.3 in Chapter 4.

3.2.3 Phosphorus test

The third test was to test for the microalgae absorption rate of phosphorus (P). All four experiments used the same test to measure/calculate this.

The test was a Phosphate colorimetric Assay kit, catalog number MAK030 from Sigma - Aldrich. The test needed to be stored at room temperature during the entire process. The test was sufficient for 100 assays in 1 ml cuvettes. 2 ml disposable centrifuge tubes were used to make the sample reactions. The spectrophotometer was also used in this test. The kit contained these components:

1. **1 bottle** Phosphate Reagent (15 ml), Catalog number MAK030A
2. **1 bottle** Phosphate standard 10 mM (0.5 ml), Catalog number MAK030B

The reagents that were supplied in the kit needed no preparation. For dilutions in the assay, MilliQ deionized water was used. Because many laboratory detergents contain high levels of phosphates, which can adhere to cleaned glassware, it was recommended to use disposable plastic lab-ware for all samples, standards, and reagents to avoid contamination. This was done to the degree it was possible.

For the procedure, the first thing that was done was to make phosphate standards for colorimetric detection. 10 μl of the 10 mM phosphate standard was diluted with 990 μl of water

to prepare a 0.1 mM phosphate standard solution. Table 3.2.6 shows how much standard solution, and therefore the concentration of the phosphate standards. All the standards have a total volume of 1 ml combined of standard solution and water.

Table 3.2.6: *Standard preparations for colorimetric detection of phosphate*

Name of bottle	SP0	SP10	SP20	SP30	SP40	SP50
0.1 mM phosphate standard solution added [μl]	0	50	100	150	200	250
Water added [μl]	850	800	750	700	650	600
Phosphate standards [nmole/bottle standard]	0	1	2	3	4	5

There were 500 μl from all the individual water samples for the sample preparation and were put into new 2 ml centrifuge tubes.

For the assay reaction, there were four steps:

1. Add 150 μl of the phosphate reagent to each tube, both water samples, and the standard tubes.

2. The samples were brought to a final volume of 1 ml as 350 μl of water was added to the tubes. Both the samples and standards had a final volume of 1 ml.

3. Mix well and incubate reaction for 30 minutes at room temperature. All the tubes were covered and protected from the light.

4. Measure absorbance at a wavelength of 650 nm (A_{650}).

The measured absorbance can be found in Section 4.2 in Chapter 4. The background for the assay was the value obtained for the 0 (blank) Phosphate Standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and were be subtracted from all readings. Use the values obtained from the appropriate Phosphate Standards to plot a standard curve. The amount of phosphate can be determined by comparing the value in the samples to the standard curve.

Equation (3.2.9) shows the calculation of the concentration of Phosphate. Where $\mathrm{S_a}$ = amount of phosphate in unknown sample tube (nmole) from the standard curve, $\mathrm{S_a}$ = sample volume (μl added to the reaction tube and C = the concentration of phosphate in sample.

$$S_a / S_v = C \tag{3.2.9}$$

The concentration of phosphate for all experiments can be found in Section 4.2 in Chapter 4.

3.3 Calculation of cultivation area

Section 3.1 describes how to take samples from growing microalgae to measure the growth rate and absorption rate. Section 3.2 shows how these water samples can be tested for their nutrient absorption rate and nutrient concentration. This Section explains how to use the nutrient concentration and absorption rate to calculate the total cultivation area needed to absorb the number of nutrients in the wastewater from a RAS.

3.3.1 Surface area

The calculations shown in this Section are based on the theory given by Boelee et. al [98] in Section 2.6.5. The cultivation surface area ($\mathrm{A_{surface}}$) is calculated with Equation (2.6.4) and (2.6.5) for nitrogen (N) and phosphor (P), respectfully.

In order to calculate $\mathrm{A_{surface}}$, the nutrient uptake rate ($\mathrm{R_{N,A,algae}}$/$\mathrm{R_{P,A,algae}}$) needs to be found first. This is done based on Equation (2.6.2) for N and (2.6.3) for P, but has been altered to fit the values that were available, this is shown in Equation (3.3.1) and (3.3.2)

$$R_{N,A,algae} = (C_N/A_{CB}) \cdot Q_{WW} \tag{3.3.1}$$

$$R_{P,A,algae} = (C_P/A_{CB}) \cdot Q_{WW} \tag{3.3.2}$$

Taking the nutrient concentration ($\mathrm{C_N}/\mathrm{C_P}$) and dividing it by the area of the cultivation tube ($\mathrm{A_{CB}}$) it grew in, this will equal the areal N, P uptake rate by microalgae ($\mathrm{P_{N,A,algae}}$/$\mathrm{P_{P,A,algae}}$). After this, the value can be multiplied by the total flow rate/day of wastewater medium from the modulRAS facility ($\mathrm{Q_{WW}}$). The flow rate and cultivation area for the bottle are shown in Table 3.3.1. The flow rate is given in $\mathrm{m^3 \cdot day^{-1}}$, so the following calculations also have to be given daily.

Table 3.3.1: *Areal parameter for laboratory growth, and flow and feed rate values from the Nofitech ModulRAS facility for areal calculation.*

Parameter	Value	Unit
Cultivation Area, bottle	0.0172	$\mathrm{m^2}$
Flow rate	900	$\mathrm{m^3 \cdot day^{-1}}$
Flow rate	900,000	$\mathrm{L \cdot day^{-1}}$
Max feed to fish	3000	$\mathrm{kg \cdot day^{-1}}$

Now that the nutrient uptake rate has been found, the total ($\mathrm{A_{surface}}$) can be calculated with the use of Equation (2.6.4) and (2.6.5) for area for N and P, respectfully. For the equation, the flow rate is needed again. This time it is given in [$\mathrm{L \cdot day^{-1}}$] as the nutrient concentration ($\mathrm{N_{in}}/\mathrm{P_{in}}$) and ($\mathrm{N_{out}}/\mathrm{P_{out}}$) is given in [$\mathrm{mg \cdot day^{-1}}$], and the nutrient uptake has already been

found ($R_{N,A,algae}/R_{P,A,algae}$). The nutrient concentration depends on the amount of nutrients in the wastewater and the desired quantity of nutrients out of the stream. The desired nutrient concentration is to be as low as possible, and the optimal target is 0 $\mathrm{mg} \cdot \mathrm{day}^{-1}$; this should be put as the out stream value. Through the research for the input levels, three different scenarios were interesting to look closer at. The three scenarios A, B, and C are:

A. Total nutrient release to wastewater from Skretting Norway's fish feed, taken from Skretting's sustainability rapport, values shown in Table 2.1.1.

   - Nitrogen concentration
     - 37.1 $\mathrm{g} \cdot (\mathrm{kgfeed})^{-1}$
     - 125 $\mathrm{mg} \cdot \mathrm{L}^{-1}$
   - Phosphor concentration
     - 6.5 $\mathrm{g} \cdot (\mathrm{kgfeed})^{-1}$
     - 21.67 $\mathrm{mg} \cdot \mathrm{L}^{-1}$

B. Nutrient release that dissolved in the wastewater from Skretting Norway's fish feed, taken from Skretting's sustainability rapport, values shown in Table 2.1.1.

   - Nitrogen concentration
     - 29.5 $\mathrm{g} \cdot (\mathrm{kgfeed})^{-1}$
     - 98.33 $\mathrm{mg} \cdot \mathrm{L}^{-1}$
   - Phosphor concentration
     - 1 $\mathrm{g} \cdot (\mathrm{kgfeed})^{-1}$
     - 3.33 $\mathrm{mg} \cdot \mathrm{L}^{-1}$

C. Nutrient in wastewater after bio treatment in ModulRAS facility, N concentration taken from rapport from Nofitech's information about their RAS facility, values shown in Table 2.2.3. P concentration calculated from the relationship between the nitrogen concentration given in scenario scenario C divided by the nitrogen concentration given from Nofitech. This is the relationship between the filtered water and the water treated in the RAS facility, the P concentration given in scenario C is divided by this relationship to get the P value below.

   - Nitrogen concentration
     - 50 $\mathrm{mg} \cdot \mathrm{L}^{-1}$
   - Phosphor concentration
     - 1,69 $\mathrm{mg} \cdot \mathrm{L}^{-1}$

$A_{surface}$ was calculated for all three scenarios. As seen in the equations (2.6.4) and (2.6.5), the area required to absorb the nutrients are calculated separately for nitrogen and phosphorus. Therefore there were six calculations for $A_{surface}$.

100 % removal of nutrients is the goal, but not always the case. In some studies, they have accounted for between 70 - 80 % total removal percentage. To outline a more extensive scope, the surface area was also calculated for 70 % removal percentage. Adding these surfaces with the total cultivation surface areas comes out as 12 calculated cultivation areas.

As the surface area is found for the three scenarios, finding the number of belts needed for the calculated surface area is possible. This is done by taking the total surface area and divide it by the individual different belt areas, depending on the belt height.

3.3.2 Footprint area

The area for the cultivation of microalgae is often very spacious, so the RAB system is a good choice as the reactor type. There reactors can operate with large surface areas ($A_{surface}$) in a smaller footprint area ($A_{footprint}$) by growing vertically. In Section 2.6.5 in the theory, it is explained how Gross et al. [88] tested a pilot RAB reactor. They researched how the different sizes of $A_{surface}$ can affect the total $A_{footprint}$ needed to cultivate microalgae at a large scale, and this is the basis of the calculation of $A_{footprint}$ in this thesis. The values of ($A_{footprint}$) as well as the biomass productivity given in Table 2.6.1 will be the foundation of the calculations. In addition to these four heights of the belt from Gross et al. research, three extra heights were looked into with inspiration from the thesis written by Olberg et al. [99]. They looked at RAB reactors with belts in height between 3-5m; because of this, heights 2, 2.5, 3, and 5 m were also used.

The setup is eight cultivation belts with a width of 1 m and alternating height, and these belts are set up at a 3.5 m^2 footprint area. With this information, the total footprint area can easily be calculated from Equation (3.3.3).

$$A_{Totalfootprint} = (A_{Totalsurface}/A_{Beltsurface}) \cdot A_{Beltfootprint} \tag{3.3.3}$$

In this equation, $A_{Beltsurface}$ is the surface area of the eight belts in the pilot, varying in size because of the alternating height. $A_{Beltfootprint}$ is the footprint area that the eight belts are placed on.

3.3.3 Biomass produced

The biomass productivity is the weight of biomass produced per area per day $g \cdot m^{-2} \cdot day^{-1}$. Now that the surface area can be found from the methodology in Section 3.3.1, the total amount of biomass can be found. As this thesis has grown microalgae in the lab, at the end of Section 3.1.2, it is explained that from the biomass samples, there can be an estimate of biomass

productivity of the three different cultivation. Although the productivity found is not 100 % dependable, they can estimate biomass production for a simpler cultivation reactor. To find total biomass produced in a day, the biomass productivity must be multiplied by the total surface area. The biomass produced will only be found from scenario C - nitrogen, as this is the most likely scenario to happen; the area is 10,320 m^2. To find the total biomass produced in a year, the total biomass productivity must be multiplied by 365 days.

In addition to using the estimated biomass productivity from the lab experiment, it is also interesting to look at other scenarios that are more equivalent to an actual rotating algal biomass (RAB) reactor. From Section 2.6.4, Boelee et al. [97], found out that the average optimal biomass production for a phototrophic biofilm reactor (PBR) with a harvest cycle of seven days was 7 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$. This will also be used to calculate the total biomass produced.

The last biomass productivity being used to calculate the amount of biomass produced is from Gross et al. [88], the biomass productivity given in Table 2.5.1. They found out that the biomass productivity for a through based RAB reactor could be up to 29.58 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ and for a raceway based it could be 15.2 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$. As these are RAB reactors, it is interesting to calculate the total biomass production compared to a PBR and in cultivation bottles.

4 Results

The results from the analysis done with the methodology from Chapter 3 are presented in this Chapter. Section 4.1 presents the result of the dry weight of the biomass samples from the cultivation bottles, en estimated growth rate is also calculated in this Section. Section 4.2; Nutrient absorption tests, shows the results from the lab experiments. The nutrient absorption and concentration of the medium water are presented with graphs. Section 4.3 contains the calculated result of the surface area (section 4.3.1), footprint area (section 4.3.2), and biomass production (section 4.3.3) for the rotating algal biofilm facility. Section 4.4 has an overview of all the lab experiments, and in Section 4.3 the result of the calculation of the surface and footprint area needed for large-scale cultivation.

4.1 Biomass samples

This Section contains the results of the biomass samples taken from the cultivation bottles during the lab experiments. The results is given in dry weight [$\mathrm{g} \cdot \mathrm{m}^{-2}$], and there are samples from the three different cultivation cultures; *Phaeodactylum Tricornutum*, (*P. Tricornutum*, *Synechocystis* SP. PCC 6803 (*Synechocystis*) and the mixed culture.

Table 4.1.1 shows the dry weight [$\mathrm{g} \cdot \mathrm{m}^{-2}$] of the biomass samples from experiment 1. In this experiment there there were two cultures growing with species; *P. Tricornutum* and *Synechocystis*. The average dry weight throughout the experiment was 43.93 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *Synechocystis* and 36.07 $\mathrm{g} \cdot \mathrm{m}^{-1}$ for *P. Tricornutum*.

Table 4.1.1: *Dry mass weight of the biomass samples from experiment 1*

Date collected	***Synechocystis*** **Dry weight [$\mathrm{g} \cdot \mathrm{m}^{-2}$]**	***P. Tricornutum*** **Dry weight [$\mathrm{g} \cdot \mathrm{m}^{-2}$]**
19.01.21	37.50	72.50
22.01.21	62.50	10.00
25.01.21	25.00	30.00
28.01.21	32.50	5.000
31.01.21	47.50	20.00
03.02.21	45.00	45.00
06.02.21	47.50	70.00
Average	**43.93**	**36.07**

Table 4.1.2 shows the dry weight [$\mathrm{g} \cdot \mathrm{m}^{-2}$]of the biomass samples from experiment 2. In this experiment there there were two cultures growing with species; *P. Tricornutum*, *Synechocystis* and the mixed culture between them. The average dry weight throughout the experiment was 30.00 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *Synechocystis*, 26.67 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *P. Tricornutum* and 33.75 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for the mixed culture.

Table 4.1.2: *Dry mass weight of the biomass samples from experiment 2*

Date collected	***Synechocystis*** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]	***P. Tricornutum*** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]	**Mix** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]
09.02.21	45.00	11.25	31.25
16.02.21	30.00	31.25	33.75
21.02.21	15.00	37.50	36.25
Average	**30.00**	**26.67**	**33.75**

Table 4.1.3 shows the dry weight [$\mathrm{g\ m}^{-2}$] of the biomass samples from experiment 3. In this experiment there were two cultures growing with species *P. Tricornutum*, *Synechocystis* and the mixed culture between them. The average dry weight throughout the experiment was 30.94 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *Synechocystis*, 49.38 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *P. Tricornutum* and 46.88 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for the mixed culture.

Table 4.1.3: *Dry mass weight of the biomass samples from experiment 3*

Date collected	***Synechocystis*** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]	***P. Tricornutum*** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]	**Mix** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]
15.03.21	42.50	56.25	52.50
18.03.21	40.00	40.00	52.50
22.03.21	30.00	60.00	42.50
27.03.21	11.25	41.25	40.00
Average	**30.94**	**49.38**	**46.88**

Table 4.1.2 shows the dry weight [$\mathrm{g} \cdot \mathrm{m}^{-2}$] of the biomass samples from experiment 5. In this experiment there there were two cultures growing with species *P. Tricornutum*, *Synechocystis* and the mixed culture between them. The average dry weight throughout the experiment was 42.00 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *Synechocystis*, 60.00 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *P. Tricornutum* and 32.25 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for the mixed culture.

Table 4.1.4: *Dry mass weight of the biomass samples from experiment 5*

Date collected	***Synechocystis*** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]	***P. Tricornutum*** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]	**Mix** **Dry weight** [$\mathrm{g} \cdot \mathrm{m}^{-2}$]
15.03.21	42.50	45.00	26.25
18.03.21	43.75	57.50	30.00
22.03.21	42.50	52.50	45.00
27.03.21	40.00	85.00	26.25
30.03.21	41.25	60.00	33.75
Average	**42.00**	**60.00**	**32.25**

Tables 4.1.1, 4.1.2, 4.1.3 and 4.1.4 shows the biomass dry weight. This is not the biomass growth rate for these microalgae, an estimated biomass growth rate would be:

1. *Synechocystis* - 4.393 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$, *P. Tricornutum* - 3.607 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$.
2. *Synechocystis* - 3.000 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$, *P. Tricornutum* - 2.667 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$, Mix - 3.375 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$.
3. *Synechocystis* - 3.094 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$, *P. Tricornutum* - 4.938 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$, Mix - 4.688 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$.
4. –
5. *Synechocystis* - 4.200 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$, *P. Tricornutum* - 6.000 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$, Mix - 3.225 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$.

Table 4.1.5: *Average estimated growth rate from lab experiments.*

Cultivation microalgae	**Estimated average growth rate [$\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$]**
Synechocystis	3.672
P. Tricornutum	4.303
Mix	3.763

4.2 Nutrient absorption tests

The results of the nutrient tests performed in this thesis are presented in this Section. All the experiments except for number two were cultivating for around 20 days. Halfway through this period, the water medium was exchanged for a new artificial wastewater medium. This was done because of an assumption made that the microalgae had absorbed all the nutrients after ten days. This is why the graphs show intervals of ten days. The x-axis shows the days of the water samples were tested, as not all water samples are tested; which samples that are tested are described in each subsection.

4.2.1 Damage

When experiment 2 needed a refill of synthetic wastewater and experiment 3 was about to start, new synthetic wastewater would be added to both these cultures. The synthetic wastewater had accidentally frozen; this led to a minor setback as the water needed to thaw to be reused. When the water was liquid again, there were small visible particles. Not knowing the effect, this water was used to refill ex. 2 and 3 and start experiment 4. The name of this water sample was "5W, 23.4, HK".

The three microalgae cultures were placed alone for a week, with no new growth. An assumption was made that the water was too contaminated to use for further growth and that the

microalgae had either died or gone into hibernation. The experiment was put on hold while new synthetic wastewater was made. The new water was added back into the cultures, and a new culture (experiment 5). A week after the new water was added to the four cultures, experiments 3 and 5 were growing well. Experiment 2 had less activity, and experiment 4 had no further activity. The decision was made to continue with experiments 3 and 5. This is why experiment 2 only has values for ten days.

4.2.2 Experiment 1

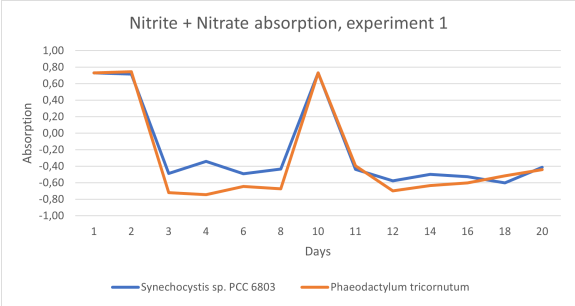
The duration of this experiment was 20 days. There were originally 42 water samples from the first round of the experiment, 20 samples of *P. Tricornutum*, 20 samples of *Synechocystis* as well as two samples of water before it entered the cultivation bottle. Out of these, 11 samples of the water from each cultivation were chosen to be tested and the two samples of the original water. Sample names were days after initial sample; 1W was the original water, and 2S/Y was day two of water for *Synechocystis*/(*P. Tricornutum*), respectively. The tested samples were: 1W, 2W, 2S/Y, 3S/Y, 4S/Y, 6S/Y, 8S/Y, 10S/Y, 11S/Y, 12S/Y, 14S/Y, 16S/Y, 18S/Y, and 20S/Y. In total, 26 water samples were tested.

Initially, the *Synechocystis* SP. PCC 6803 (*Synechocystis*) had a thick layer at the bottom of the cultivation bottle, and this made the removal of biofilm easy. While *Phaeodactylum Tricornutum* (*P. Tricornutum*) only had small biofilm fragmentation in the water, this made it impossible to get a clean biofilm sample. Instead of scraping the biofilm, 1 mL of (*P. Tricornutum*) was collected to see how much biofilm fragmentation was in that area of water.

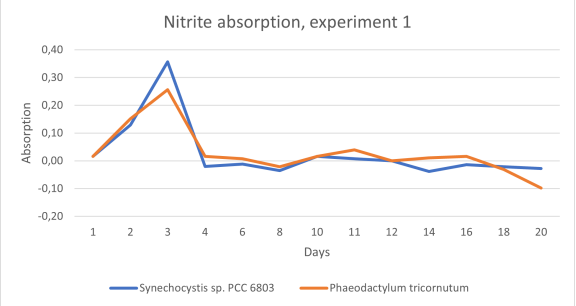
After ten days, the culture bottles were refilled with synthetic wastewater, as it is assumed that all the nitrogen (N) and phosphorus (P) present had been absorbed by then. Figure 4.2.1a shows that both the algae species had absorbed all the nitrate within day three and that the nitrate absorption of nitrate was high again at day 10 when new water was added. Figure 4.2.1b shows that nitrite has a high presence at day three when the nitrate had converted into nitrite, as is explained in Section 2.1.1. The two graphs have negative values because absorption measurements are not the most accurate measurements. The new water added was named "2W, 28.1, HK".

Figure 4.2.2 shows the concentration of nitrate in the synthetic wastewater. These are calculated from the absorption measurements for nitrate with the equations given in Section 3.2.2. The initial synthetic wastewater had a nitrate value of 75 $\mathrm{mg} \cdot \mathrm{L}^{-1}$, this was all gone by day three for both *P.Tricornutum* and *Synechocystis*.

Figure 4.2.3a and 4.2.3b shows the phosphor absorption and concentration, respectively. The synthetic wastewater also had 4.411 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ of phosphate. Both microalgae species used two days to absorb all the phosphate in the water the first two days, as shown in Figure 4.2.3b. After the refill of water, the phosphate was absorbed completely in one day.



(a) Nitrate absorption



(b) Nitrite absorption

Figure 4.2.1: *Absorption of nutrients per day for microalgae* Phaeodactylum Tricornutum *and* Synechocystis *SP. PCC 6803 in experiment 1*

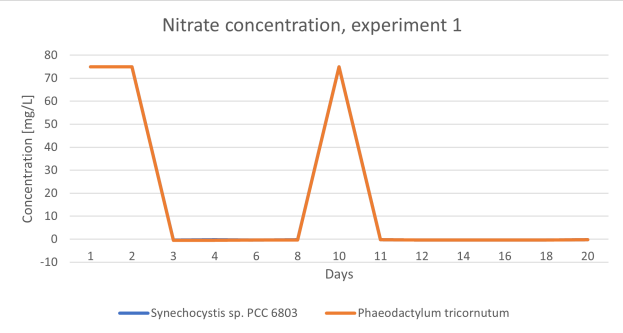
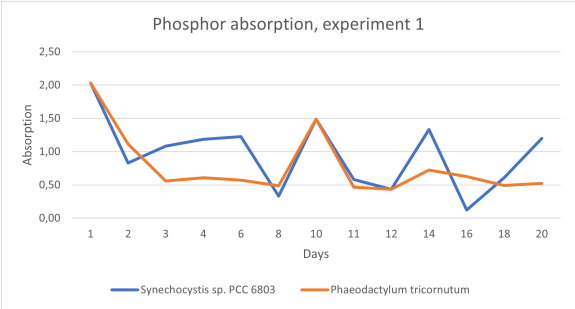
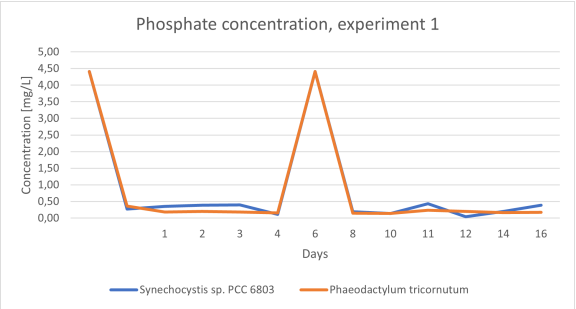


Figure 4.2.2: *Nitrate concentration of microalgae* Phaeodactylum Tricornutum *and* Synechocystis *SP. PCC 6803 for experiment 1*



(a) Phosphor absorption



(b) Phosphor concentration

Figure 4.2.3: *(a) Absorption and (b) concentration of nutrients per day for microalgae* Phaeodactylum Tricornutum *and* Synechocystis *SP. PCC 6803 in experiment 1*

4.2.3 Experiment 2

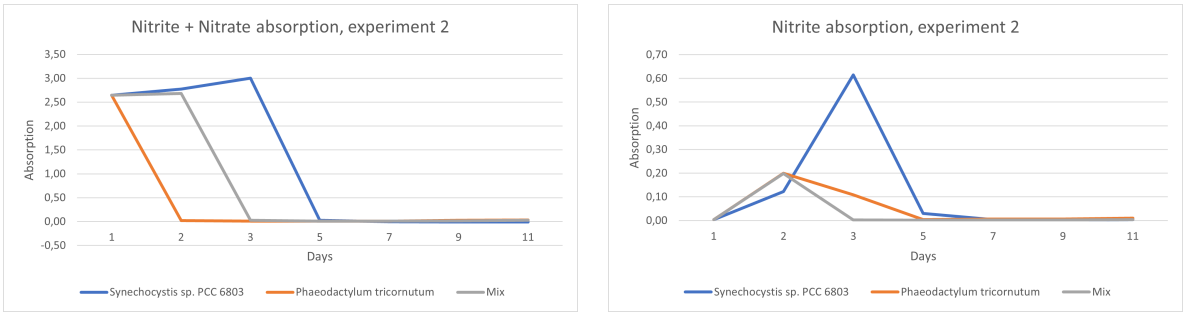
The duration of this experiment was initially 21 days, but as explained in Section 4.2.1, the microalgae cultures were damaged by the frozen water. It had some growth when it received new freshwater, but the experiment was terminated. Because of this, this experiment only had 11 viable samples per culture. It was almost the same experiment as previous, in addition to the two culture species *P. Tricornutum* and *Synechocystis*, a third culture, was made; this culture

was a mix of the two species.

There were 34 water samples from the three cultures and the original water sample (4W, 10.2, HK). As learned from testing experiment one, a water sample from every other day would be measured, but the three first water samples would be tested. This is to get a better overview of the first days after new water is added. Names of water sample bottles were tested from experiment two; 4W, 2S/Y/M, 3S/Y/M, 5S/Y/M, 7S/Y/M, 9S/Y/M, 11S/Y/M, 19 water samples in total.

All three cultures had a steady growth of biofilm attached to the bottom surface, the first ten days of the experimental period. *Synechocystis* had continuous regrowth of the biofilm patches that were taken as biofilm samples. In comparison, the two other cultures did not grow back the patches that were taken. All three cultures kept their color throughout the ten days, *Synechocystis* and the mix in vibrant green color and *P. Tricornutum* in a yellow/orange color.

Figure 4.2.4a shows the graph of the nutrient absorption of the microalgae in experiment 2. In this experiment, *Synechocystis* uses three days to absorb the nitrate, but Figure 4.2.4b shows that *Synechocystis* also has a large amount of nitrite the third day. *P. Tricornutum* absorbed all its nitrate on day one and all its nitrite on day two. The mixed culture absorbed all the nitrate within day three and all the nitrite within day three.

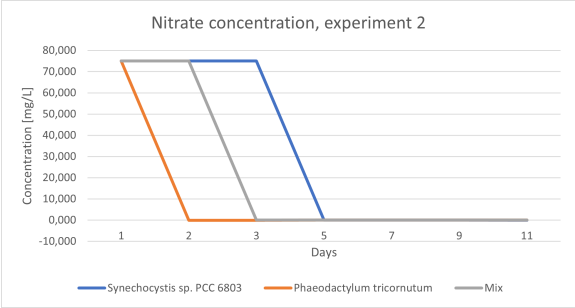


(a) *Nitrate absorption* ***(b)*** *Nitrite absorption*

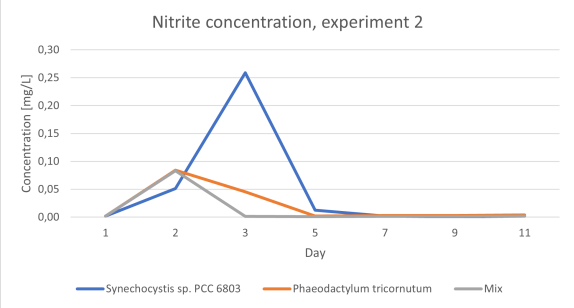
Figure 4.2.4: *Absorption of nutrients per day for microalgae* Phaeodactylum Tricornutum*,* Synechocystis *SP. PCC 6803 and the mixed culture in experiment 2*

Figure 4.2.5a and 4.2.5b shows the concentration of nitrate and nitrite in the water for the same time period. This was calculated from the absorption measurements, with equations shown in Section 3.2.2. All three cultures stared with 75 $(\mathrm{mg\ nitrate}) \cdot \mathrm{L}^{-1}$ and ended up with 0 $(\mathrm{mg} \cdot \mathrm{nitrate}) \cdot \mathrm{L}^{-1}$ after one, two and three days for *P. Tricornutum*, the mix, and *Synechocystis* respectively.

Figure 4.2.6a and 4.2.6b shows the phosphor absorption and concentration respectively. The synthetic wastewater also had 4.411 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ of phosphate. Both microalgae species only used one day to absorb all the phosphate in the water, as shown in Figure 4.2.6b.

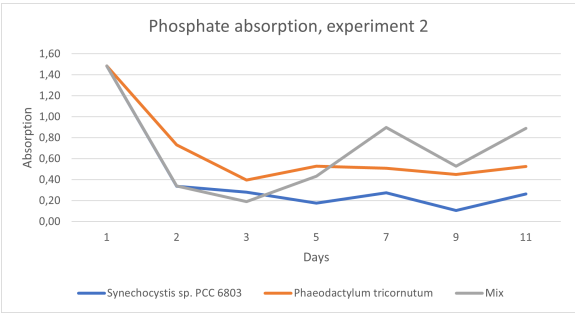


(a) Nitrate concentration

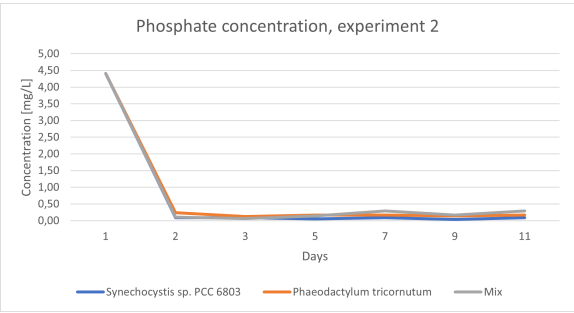


(b) Nitrite concentration

Figure 4.2.5: *Concentration of nutrients per day for microalgae* Phaeodactylum Tricornutum, Synechocystis *SP. PCC 6803 and the mixed culture in experiment 2*



(a) Phosphor absorption



(b) Phosphor concentration

Figure 4.2.6: *(a) Absorption and (b) concentration of nutrients per day for microalgae* Phaeodactylum Tricornutum, Synechocystis *SP. PCC 6803, and the mixed culture in experiment 2*

4.2.4 Experiment 3

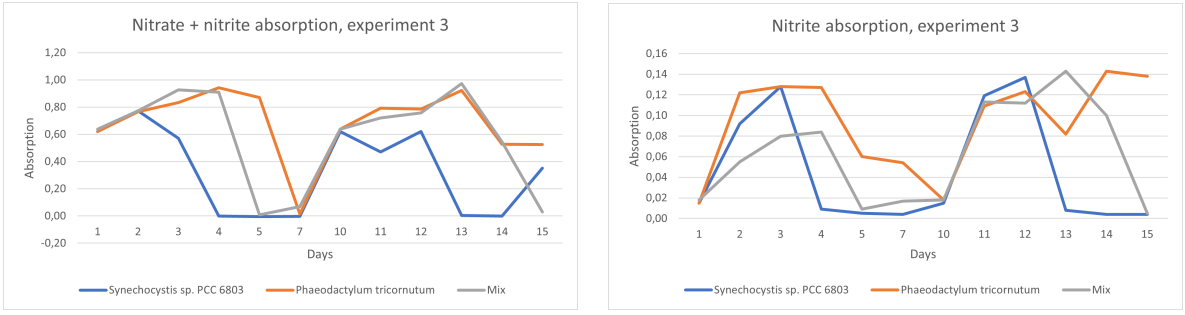
This experimental round lasted for 35 days because of the delay (section 4.2.1). Initially, this round was started one week too late. Because the biofilm had already grown pretty thick, and as stated in Section 2.4.3; too thick a layer would result in the bottom algae not getting enough light to continue to grow and then die.

The initial water refill that would have started the experiment was the contaminated water as mentioned in 4.2.1. It had the same reaction as exp. 2, where the algae did not grow further, and the already faded green color of both *Synechocystis* and mix culture got brown. The new water named "11W, 5.3, HK" was added on March 5th. A week later, on March 13th, the microalgae looked well and recovered; both *Synechocystis* and the mix had restored their original vibrant green color. While *P. Tricornutum* had not attached really to the surface, instead of scraping the biofilm, 1 mL of (*P. Tricornutum*) was collected to see how much biofilm fragmentation was in that area of water.

The effective duration of the experiment was 18 days, which led to 18 viable water samples from each culture. 10 water samples from each culture were tested for nutrients as well as the original water sample "11W, 5.3, HK". In total, there were 31 samples to be tested. The

names of the samples were; 11W, 9S/Y/M, 10S/Y/M, 11S/Y/M, 12S/Y/M, 14S/Y/M, 18S/Y/M, 19S/Y/M, 20S/Y/M, 21S/Y/M, 22S/Y/M.

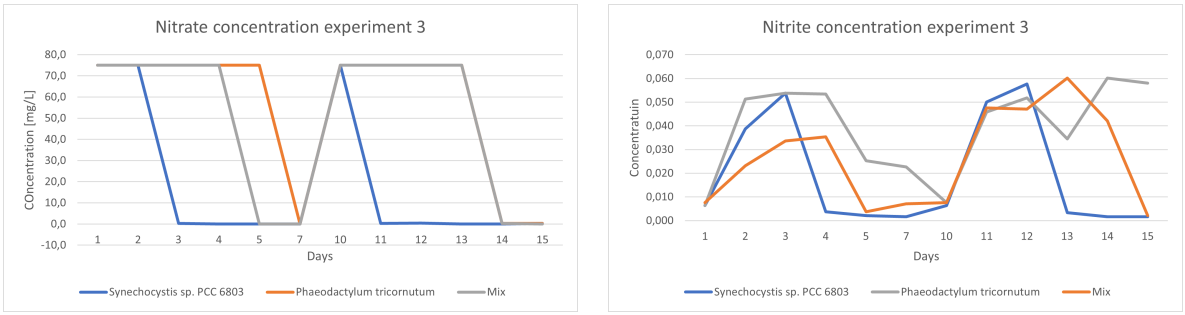
Figure 4.2.7a shows the graph of the nitrate absorption of the microalgae in experiment 3. In this experiment, *Synechocystis* the shortest amount of time to absorb the nitrate, but all three cultures have a more unstable lifespan than the previous experiments. The same goes for the nitrite absorption in Figure 4.2.7b.



(a) *Nitrate absorption* ***(b)*** *Nitrite absorption*

Figure 4.2.7: *Absorption of nutrients per day for microalgae* Phaeodactylum Tricornutum*,* Synechocystis *SP. PCC 6803 and the mixed culture in experiment 3*

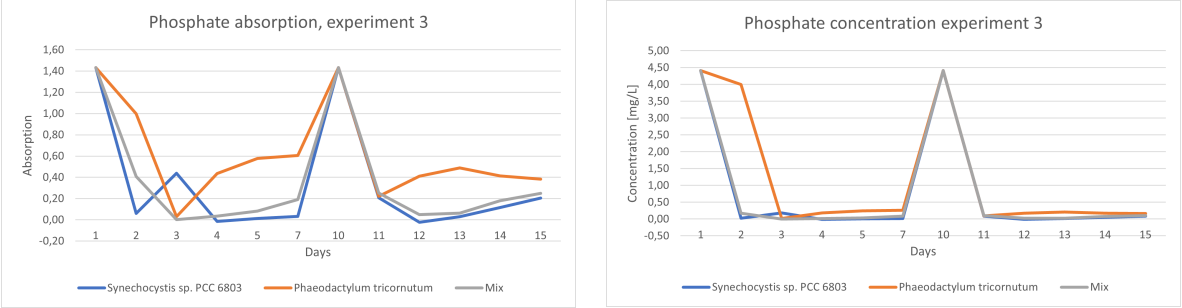
Figure 4.2.5a and 4.2.5b show the concentration of nitrate and nitrite in the water for the same period. The concentration was calculated from the absorption measurements, with equations shown in Section 3.2.2. All three cultures stared with 75 $(\mathrm{mg} \cdot \mathrm{nitrate}) \cdot \mathrm{L}^{-1}$ and ended up with 0 $(\mathrm{mg} \cdot \mathrm{nitrate}) \cdot \mathrm{L}^{-1}$ after two to seven days. The nitrite concentration is a bit more unstable than previous ones, but the high nitrate comes up after the nitrate is gone/partly gone.



(a) *Nitrate concentration* ***(b)*** *Nitrite concentration*

Figure 4.2.8: *Concentration of nutrients per day for microalgae* Phaeodactylum Tricornutum*,* Synechocystis *SP. PCC 6803 and the mixed culture in experiment 3*

Figure 4.2.9a and 4.2.9b shows the phosphor absorption and concentration respectively. The synthetic wastewater also had 4.411 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ of phosphate. *P. Tricornutum* and *Synechocystis* both use only one day to absorb all the phosphate in the water both initially and for the refill. The mixed culture uses one more day to absorb the phosphate initially, but at the refill, it only uses a day.



(a) *Phosphor absorption* ***(b)*** *Phosphor concentration*

Figure 4.2.9: *(a) Absorption and (b) concentration of nutrients per day for microalgae* Phaeodactylum Tricornutum*,* Synechocystis *SP. PCC 6803, and the mixed culture in experiment 3*

4.2.5 Experiment 4

A fourth-round was made because of concern that the delay of the third round would not give good enough results. No viable water samples for experiment 4, as it never grew a biofilm layer after receiving the contaminated water mentioned in Section 4.2.1.

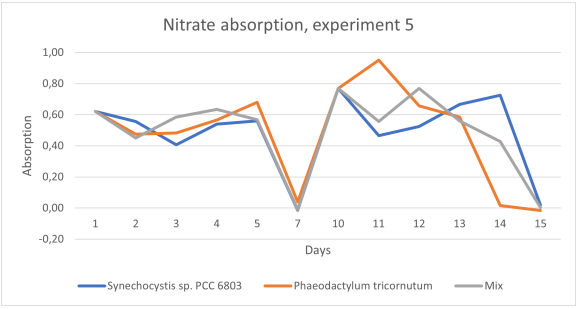
4.2.6 Experiment 5

Since experiment 4 as a backup did not survive, the last experiment was done with the new water "11W, 5.3,HK". This experiment grew simultaneously as experiment 3, for 18 days, with 18 viable water samples. Ten samples were used from each culture, and water "11W, 5.3,HK" as initial water and "12W, 5.3,HK" were used as refill water were tested. In total, 32 water samples ready to be tested. The names of the samples were; 11W, 1S/Y/M, 2S/Y/M, 3S/Y/M, 4S/Y/M, 6S/Y/M, 10S/Y/M, 11S/Y/M, 12S/Y/M, 13S/Y/M, 14S/Y/M.

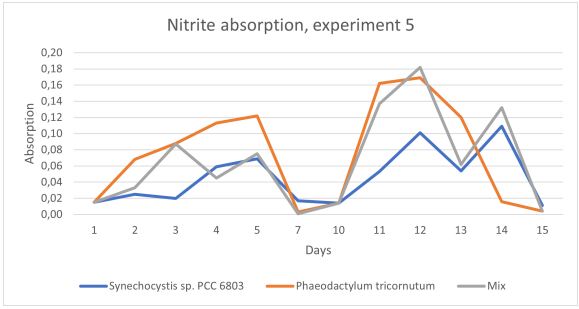
The growth in these cultivation bottles were about the same as in experiment 3 (Section 4.2.4), the *Synechocystis* and mixed culture had high growth rate and vibrant green colors. While *P. Tricornutum* had hardly any biofilm attached to the surface bottom, instead of scraping the biofilm, 1 mL of (*P. Tricornutum*) was collected to see how much biofilm fragmentation was in that area of water.

Figure 4.2.10a and 4.2.10b shows the graphs of the nitrate and nitrite absorption of the microalgae respectively in experiment 3. These absorption graphs vary a lot from the absorption graphs from experiments 1, 2, and 3. They are the opposite, high until the last day before refilling.

Figure 4.2.11a and 4.2.11b show the concentration of nitrate and nitrite in the water for the same period. This was calculated from the absorption measurements, with equations shown in Section 3.2.2. All three cultures stared with 75 $(\mathrm{mg} \cdot \mathrm{nitrate}) \cdot \mathrm{L}^{-1}$ and ended up with 0 $(\mathrm{mg} \cdot \mathrm{nitrate}) \cdot \mathrm{L}^{-1}$ after one day. Even though the nitrate and nitrite absorption measurements were unstable, the calculations worked out.

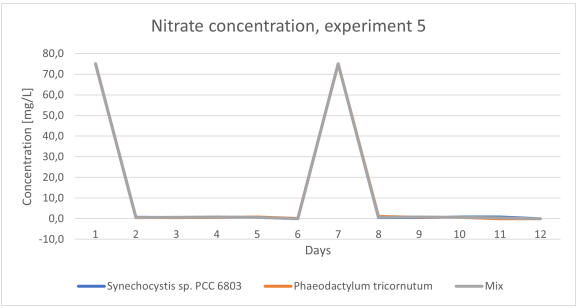


(a) *Nitrate absorption*

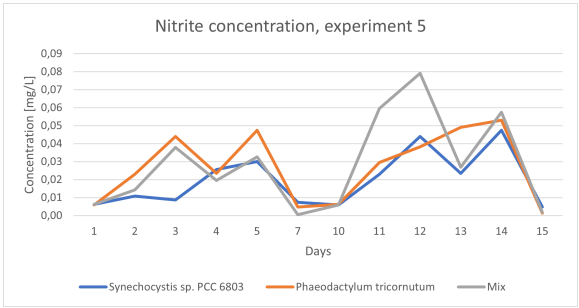


(b) *Nitrite absorption*

Figure 4.2.10: *Absorption of nutrients per day for microalgae* Phaeodactylum Tricornutum, Synechocystis *SP. PCC 6803 and the mixed culture in experiment 5*



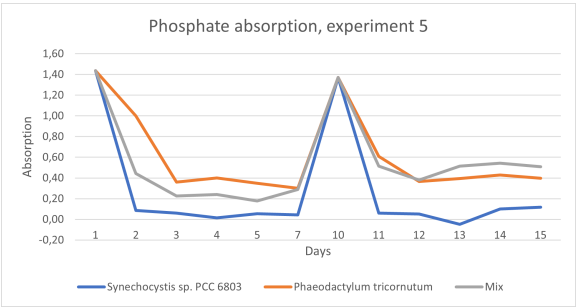
(a) *Nitrate concentration*



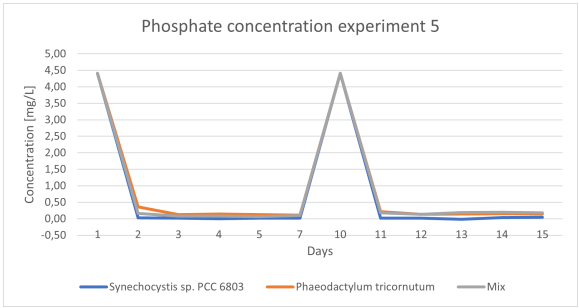
(b) *Nitrite concentration*

Figure 4.2.11: *Concentration of nutrients per day for microalgae* Phaeodactylum Tricornutum, Synechocystis *SP. PCC 6803 and the mixed culture in experiment 5*

Figure 4.2.9a and 4.2.9b shows the phosphor absorption and concentration respectively. The synthetic wastewater also had 4.411 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ of phosphate. *P. Tricornutum*, *Synechocystis* uses one day to practically absorb all the phosphate, while the mixed culture uses two days initially. After the refill, all three only use one day to absorb all the phosphate.



(a) *Phosphor absorption*



(b) *Phosphor concentration*

Figure 4.2.12: *(a) Absorption and (b) concentration of nutrients per day for microalgae* Phaeodactylum Tricornutum, Synechocystis *SP. PCC 6803, and the mixed culture in experiment 5*

4.3 Calculation of cultivation area

Now that the nutrient absorption has been found, and it is known from Section 4.2, it can be said that the microalgae from the laboratory tests absorb 75 $\mathrm{mg} \cdot \mathrm{L}^{-1} \cdot \mathrm{day}^{-1}$ of N and 4.411 $\mathrm{mg} \cdot \mathrm{L}^{-1} \cdot \mathrm{day}^{-1}$ of P. From this it is possible to calculate the surface area (section 4.3.1 and footprint area (section 4.3.2 needed to absorb nutrient in wastewater.

4.3.1 Surface area

Section 3.3.1 in the methodology Chapter explains three scenarios of nutrient concentration from wastewater. The three scenarios are based on the treatment it has gone through, from no treatment (scenario A), after filtering out solid sedimentation (scenario B), and after treatment in a recirculation aquaculture systems (RAS) facility (scenario C).

Table 4.3.1 shows the surface area needed to absorb all nutrients available in the wastewater from the three scenarios, and it shows both for both 100 % absorption rate and 70 % absorption. The highest surface area needed is scenario A from phosphor with the need for 76,039 m^2 to remove 21.67 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ phosphors. In comparison, the smallest area needed is from phosphor in scenario C with a needed area of 5,948 m^2.

Table 4.3.1: *The surface area microalgae needed to absorb all the nutrients from different wastewater scenarios. Scenario A; nutrient in form of solid sediments and dissolved water from untreated wastewater, Scenario B; the nutrient dissolved in water from untreated wastewater, Scenario C; nutrient left after wastewater treatment in a RAS facility.*

Nutrient	**Nutrtient concentration [**$\mathrm{mg} \cdot \mathrm{L}^{-1}$**]**	**Surface Area** [m^2]	**70 % absorption area [**m^2**]**
Nitrogen A	125.0	25,800	12,060
Phosphor A	21.67	76,038	53,226
Nitrogen B	98.33	20,296	14,207
Phosphor B	3.333	11,698	8,189
Nitrogen C	50.00	10,320	7,224
Phosphor C	1.695	5,948	4,163

4.3.2 Footprint area

In this Section, the results of the footprint area calculated from the surface area for the three scenarios are presented; the various calculations are based on the height of the circulation belt that is different. The footprint area calculations are only based on the surface area for 100 % absorption. Table 4.3.2 shows the footprint area needed for all scenario A based on the eight different heights that are being evaluated here.

Table 4.3.2: *The footprint area required for scenario A, absorbing all nutrient from wastewater that come in the form of solid sediments and dissolved in water from fish feed, this water is untreated.*

	Scenario A - Nitrogen	**Scenario A - Phosphor**
	$A_{TotalSurface}$ [m^2]	$A_{TotalSurface}$ [m^2]
	25,800	76,037
Height of belt	$A_{TotalFootprint}$ [m^2]	$A_{TotalFootprint}$ [m^2]
0.91	6,101	17,982
1.22	4,664	13,746
1.52	3,713	10,943
1.83	3,101	9,139
2.00	2,822	8,317
2.50	2,258	6,653
3.00	1,881	5,544
5.00	1,129	3,327

Table 4.3.3 shows the footprint area needed for all scenario B based on the eight different heights that are being evaluated here.

Table 4.3.3: *The footprint area required for scenario B, absorbing all nutrient let from wastewater that is dissolved in water from fish feed, this water has only filtered away its solid sediments.*

	Scenario B - Nitrogen	**Scenario B - Phosphor**
	$A_{TotalSurface}$ [m^2]	$A_{TotalSurface}$ [m^2]
	20,296	11,698
Height of belt	$A_{TotalFootprint}$ [m^2]	$A_{TotalFootprint}$ [m^2]
0.91	4,800	2,766
1.22	3,669	2,115
1.52	2,921	1,684
1.83	2,439	1,406
2.00	2,220	1,279
2.50	1,776	1,024
3.00	1480	853
5.00	888	512

Table 4.3.4 shows the footprint area needed for all scenario C based on the eight different heights that are being evaluated here.

Table 4.3.4: *The footprint area required for scenario C, absorbing all nutrient let from wastewater that has already been treated in a RAS facility.*

	Scenario C - Nitrogen	**Scenario C - Phosphor**
	$A_{TotalSurface}$ [m^2]	$A_{TotalSurface}$ [m^2]
	10,320	5,948
Height of belt	$A_{TotalFootprint}$ [m^2]	$A_{TotalFootprint}$ [m^2]
0.91	2,441	1,407
1.22	1,866	1,075
1.52	1,485	856
1.83	1,240	715
2.00	1,129	651
2.50	903	520
3.00	753	434
5.00	452	260

The amount of cultivation belts needed to cover all the surface area from scenario C - nitrogen, is given in Table 4.3.5. The amount of belts decreases as the height increases.

Table 4.3.5: *Number of belts needed for the different cultivation belt heights using the surface area for scenario C - nitrogen, 10,320* m^2.

Height belt [m]	**Total area belt** [m^2]	**Amount of belts**
0.91	1.85	5,578
1.22	2.42	4,264
1.52	3.04	3,395
1.83	3.64	2,835
2.00	4.00	2,580
2.50	5.00	2,064
3.00	6.00	1,720
5.00	10.0	1,032

4.3.3 Biomass production

The biomass produced from at surface area for scenario C - nitrogen as shown in Table 4.3.4 in Section 4.3.1, it was 10,320 m^2. The calculated biomass production for this area is shown in Table 4.3.6, the biomass production is calculated for six different scenarios where the biomass production is different.

Table 4.3.6: *Biomass produced both per day and per year, based on different biomass productivity rates from several studies. Calculated based on the surface area of scenario C - N, 10,320* m^2.

Origin of biomass productivity	**Biomass productivity** ($\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$)	**Biomass produced a day** [$\mathrm{g} \cdot \mathrm{day}^{-1}$]	**Biomass produced a year** [$\mathrm{tons} \cdot \mathrm{year}^{-1}$]
Lab experiment *Synechocystis*	3.672	37.89	13.83
Lab experiment *P. Tricornutum*	4.303	44.41	16.21
Lab experiment Mix	3.763	38.83	14.17
Boelee et al. [97]	7.000	72.24	26.37
Gross et al. [88] RAB raceway based	15.20	156.9	57.26
Gross et al. [88] RAB through based	29.58	305.3	111.4

4.4 Overview results

This Section is an overview of the results that comes from the laboratory experiments in Section 4.1 and 4.2 and the calculations of surface area from Section 4.3.1, footprint area from Section 4.3.2 and at last the calculation of biomass production in Section 4.3.3.

Section 4.1 had the results of the dry weight of the microgalgal biomass samples taken about every third day (could vary some because of human error) during the experimental period. The dry weight of *Synechocystis* SP. PCC 6803 (*Synechocystis*) varied some from the different experiments, the average weight was between 30.00 and 43.93 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for the four experiments. The average dry weight of *Phaeodactylum Tricornutum* (*P.Tricornutum*) varied more, and was between 26.67 and 60.00 $\mathrm{g} \cdot \mathrm{m}^{-2}$. The mixed culture varied even less than *Synechocystis* and kept between 32.25 and 46.88 $\mathrm{g} \cdot \mathrm{m}^{-2}$. The estimated average growth rate from the same lab experiments are; for *Synechocystis* it was 3.672 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$, for *P.Tricornutum* it was 4.303 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ and for the mixed culture the growth rate was 3.763 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$.

In Section 4.2 the results from the microalgae nutrient absorption, from Section 3.1.1 it is mentioned that the artificial wastewater contains contained 75 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ of N, and 4.411 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ of P. According to the absorption tests that were completed of the water samples,

the results are stated in Section 4.2, the average time for absorbing the nitrogen was one day for nitrate and another day for nitrite for experiment 1 and 2 (section 4.2.2 and 4.2.3). While for experiment 3 and 5 this was not the case, these are very variable. Basing the proceeding calculations on experiment 1 and 2. The nitrate absorption rate 75 $\mathrm{mg} \cdot \mathrm{L}^{-1} \cdot \mathrm{day}^{-1}$ and the phosphate absorption rate 4.411 $\mathrm{mg} \cdot \mathrm{L}^{-1} \cdot \mathrm{day}^{-1}$ for these experiments.

When calculating the surface area for the three scenarios, scenario C for nitrogen came out as most reliable. As it would remove all the nutrients from the values given from Nofitech. All the surface areas can be seen in Table 4.3.1, where the chosen one is 10,320 m^2.

After the surface area was found, the footprint area could be calculated, and this is the needed area that will occupy the ground. These calculations were done for all 6 scenarios as seen in tables 4.3.2, 4.3.3 and 4.3.4. The calculations of footprint area are based on how tall the vertical belts are; the taller the belts, the less footprint area is needed. Table 4.3.5 shows how many belts are needed for scenario C - nitrogen for the different belt heights.

The last Section 4.3.3, in the results, contains the calculations of potential biomass production from the lab experiment and the biomass production from two literature studies. Table 4.3.6 shows the biomass produced per day and year for all of the different scenarios. The biomass production from lab experiments would lead to 13-16 tons biomass a year, while for the best RAB reactor, the production can be up to 111 tons a year.

5 Discussion

The discussion in this Chapter centers around what microalgae should be used for cultivation and which scenario gives the best areal outcome as well as are the most likely to be used forward. It is necessary to keep in mind that results from the laboratory experiments and calculations thereafter have uncertainties about them. This is because it is laboratory testing done in cultivation bottles, which are then scaled up to a large scale. Not only can the upscaling affect the results, but the cultivation of the biomass was done in an environment far from the environments presented in the literature. As the laboratory experiment was done in bottles, while the RAB environment is explained in Section 2.5.1.

5.1 Laboratory experiment

In this Section of the discussion, the laboratory experiments will be discussed. The productivity of each microalgae cultivation will be compared against each other and compared against other biomass cultivation technologies that can be found in the literature Section 2.5, where the rotating algal biofilm (RAB) is the one focused on in this thesis. Table 2.5.1, shows different cultivation methods that are being used.

The microalgae being used in this thesis is *Synechocystis* and *P. Tricornutum*, these are not the species that are the most used in wastewater treatment situations, but these were the species that were available to test. In a different scenario, it would be interesting to test, for example, *Chlorella vulgaris* as this is a known species in the cultivation world. In Table 2.5.1, three cultivation reactors have put this species as their preferable, including the RAB. In addition to the microalgae species *Synechocystis* and *P. Tricornutum*, there is third cultivation that has been tested which is the mixed cultivation where there is 50/50 *Synechocystis*/*P. Tricornutum* added to it. This was done to test the theory explained in Section 3.1.1, where it is stated that biomass production increase among other microorganisms.

Section 2.1 and 2.3, explains why is it important to remove the nutrients nitrogen (N) and phosphorus (P) from the wastewater. In Section 5.1.2, the absorption rate of the microalgae from the laboratory tests will be discussed.

5.1.1 Biomass samples

When gathering the biomass samples, some complications made the results uncertain. As written in Section 3.1.2 in the method, when gathering the biomass, the area and amount of biomass taken could vary from time to time. The opening of the bottle was small and hard to navigate through as well as when scraping the biofilm of the bottom it often spread throughout the culture medium, which made it hard to gather it all. In addition to this, the specific area taken was hard

to get exact as measuring it was to take a ruler and measure the area outside the bottle. This led to the biomass weight samples varying from day to day and not getting very accurate results.

These uncertainties have led to the dry weight of biomass not being consistent. The average dry weight of the biomass samples were approximately between 30.0 - 44.0 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *Synechocystis*, 27.00- 60.00 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for *P. Tricornutum* and 32.00 - 47.00 $\mathrm{g} \cdot \mathrm{m}^{-2}$ for the mixed culture. These numbers cannot be compared to, for example, the biomass productivity per day from different biofilm cultivation systems in Table 2.5.1 because the biomass samples taken from the lab experiment were taken every three days. When the biomass samples were collected, the biomass had grown for ten days, and there was seldom much growth in the duration of the experiment, as they did not have a continuous flow of nutrients. When looking at the nutrient absorption graphs in Section 4.2, the microalgae absorbed all the nutrients by one or two days.

An estimation of the biomass productivity for the three cultivation's were made. In Table 4.1.5 from Section 4.1, the average estimated growth rate for the cultivation's are shown. These are the average dry weight measurements found in tables 4.1.1, 4.1.2, 4.1.3 and 4.1.4, divided by 10. As explained in Section 3.1.2, it is divided by 10 because it took about 10 days for the biomass layer to grow, and after the experiments started it did not really grow that much. It is very imprecise, but the values were all around 4.00 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ give or take.

From the biomass weight found, it shows that *P. Tricornutum* has the broadest range of weight and also has the highest and lowest values. As mentioned in several of the Sections about the experiments in Section 4.2, is that the *P. Tricornutum* biofilm did not attach to the material as fast or much as *Synechocystis*. During experiments 2 and 3, there was hardly any biomass attached to the ground, only some biomass particle flowing around the water. The biomass samples consisted then of 1 mL of sample water, trying to gather as much biomass as possible, but this again made it hard to estimate the area growth rate and is probably why the weight varied that much. From this laboratory experience, *P. Tricornutum* is not reasonably fit or dependable to be producing a steady amount of biofilm.

The mixed culture had an averagely higher growth rate than the *Synechocystis*. Both the cultivations had steady growth throughout the process. This could be a consequence of the uncertainties that came with the biofilm samples, or it can prove that co-culturing microalgae (several species grow together) increase biomass production. In that case, either *Synechocystis* or the mixed culture should be chosen as the cultivation microalgae. The benefit of choosing the mix is that the productivity level increases, becoming a more diverse bio-product. It might be even better if it grew together with other bacteria or fungus, but this is not tested in this thesis. However, it is easier to manage just one species at the time, so *Synechocystis* will be the microalgae of choice. Co-culturing could be tested more in the future, as the microalgae growth of one type is more established.

Table 2.5.1 in Section 2.5 shows the biomass productivity of some biofilm cultivation sys-

tems (e.g., Twin layer phototropic biofilm reactor (PBR), Pilot-scale PBR). These systems do not have the same continuous flow of nutrients and gas with O_2 and CO_2 present, as the rotating algal biofilm (RAB) has. Because of this, they have a lower biomass production rate, and this is easily comparable in Table 2.5.1. When comparing the estimated growth rate for the microalgae grown in the lab and the values from this table, the lab results were more like the PBR reactors than the RAB reactor. The microalgae are grown in the lab, and the PBR reactors have similar growing conditions that are different from RAB. If the lab had a RAB reactor that could rotate in and out of water and air, this would increase the growth rate largely based on the theory behind RAB, which is explained closely in Section 2.5.1.

5.1.2 Nutrient tests

In Section 4.2, it is explained that the growth periods are split into ten weeks intervals. The intervals were this long because there was little knowledge of how long it would take for microalgae to absorb all the nutrients from the artificial wastewater. In hindsight, a nutrient test of the first experiment should have been done after it was finished to prepare and adapt the following three experiments. All experiments were done with the same assumptions and only added water after ten days. As the graphs show in Chapter 4.2, that the nitrate is absorbed on an average by day three, nitrite by day five, and phosphate by day three. Had this been noticed sooner, the intervals could have been for seven days instead of 10.

The absorption values from experiment 1 and 2 (section 4.2.2 and 4.2.3) are more steadily and consistent, the absorption values from experiment 3 and 5 (section 4.2.4 and 4.2.6) which were more variable. When looking at the experimental absorption values from 3 and 5, while looking at the damaged the contaminated water from Section 4.2.1 did. An assumption was made that the damage had a bigger effect on the microalgae than just putting them into hibernation. It looks like the nutrient absorption ability of the microalgae was corrupted. Another reason why experiments 3 and 5 were had less accurate results is that the tubes used in the testing equipment were reused from the tests done to experiment 1 and 2. Even though the tubes were cleaned well, some nitrogen (N) and phosphate (P) could be left.

Originally there were going to be done three experiments, but after the setback explained in Section 4.2.1 happened, there was added more experiments. In total, there are nutrient test results from four experiments; 1, 2, 3, and 5. Experiment 4 never recovered after the setback. However, because of the variable results and possible damage to experiments 3 and 5 shown in Section 4.2.4 and 4.2.6, further calculations will be based on the continuous values from experiments 1 and 2, showin in Section 4.2.2 and 4.2.3. Experiments 1 and 2, have an average absorption rate of nitrate 75 $\mathrm{mg} \cdot \mathrm{L}^{-1} \cdot \mathrm{day}^{-1}$ and of phosphate 4.411 $\mathrm{mg} \cdot \mathrm{L}^{-1} \cdot \mathrm{day}^{-1}$. Nitrite comes after a denitrification process of nitrate as explained in Section 2.1.1.

There were several flaws when it came to the nutrient tests. The first nitrate/nitrite test that

was explained in Section 3.2.1 had a max nitrate test value of 5 $\mathrm{mg} \cdot \mathrm{L}^{-1}$, which is 70 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ less than the max value of the artificial wastewater. This was not noticed until after all the testing was completed. Since the nitrate value of the artificial wastewater was known to be 75 $\mathrm{mg} \cdot \mathrm{L}^{-1}$, the last assumption from Section 3.2 was put into place. The assigned maximum nitrate concentration was set to 75 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ for the high absorption values. When the absorption values went below a certain amount, the calculations were done with the absorption values found. The first nitrate/nitrite test was only done on experiment 1; another human flaw was done to this test. A standard curve was not made for this test round, which resulted in it not being possible to calculate the concentration. However, the nitrate and nitrite absorption curves in Figure 4.2.1a were so accurate, and it is possible to see that all the nutrients are gone by one day. For the phosphate test, it was essential to not use reusable glass containers for the testing, and only plastic containers should be used. This is because the detergent used to clean equipment in laboratories often contains much P, disturbing the tests. The test was completed to the best ability without glass equipment, but the purified water was contained in a glass bottle, disturbing the results.

When looking at the results from experiments 1 and 2, the water's nutrient levels decrease fast, with the duration of one day, making them all viable for use as wastewater facilities. All three cultivation are possible to use for the treatment. However, as concluded in Section 5.1.1, the *Synechocystis* or the mixed cultivation would be the best fit because of its biofilm production.

5.2 Calculation area

In this Section, the calculation of area based on the results from the laboratory tests is being discussed. The calculation of surface area needed for different scenarios is being compared. There are three scenarios; the first scenario is based on no pre-treatment of the wastewater. The second scenario had a mechanical filtration of suspended solid sediments. The facility that the area is based on is a through based RAB facility, as shown in Figure 2.5.4. The last scenario is wastewater after it has gone through a reticulation aquaculture system (RAS). The footprint area can be calculated from the surface area based on the height of the vertical belts used in the cultivation reactor. The footprint area for all surface areas is calculated based on eight different belt heights. The number of belts needed for the surface area is also based on how high the cultivation belts are. After finding the footprint area and the number of belts needed, the biomass production was found from the surface area and the biomass productivity of the laboratory results and biomass productivity from the literature study from Section 2.5 and Section 2.6.

5.2.1 Surface area

The nitrogen:phosphorus content ratio in the wastewater scenarios will result that either the surface area calculated for the nitrogen (N) content is higher than for the phosphorus (P) content or the opposite. This is because there is a difference in how much nutrient that needs to be removed; it is essential to choose the larger area that will take up the nutrients of both N and P. The different scenarios are explained more closely in Section 3.3.1. Scenario C has the nutrient levels that Nofitech has provided for and are the nutrient levels that were the baseline for the rest of the calculations, like footprint area and biomass production. This is because scenario C has already gone through wastewater treatment from the RAS facility. It is interesting to compare it to untreated and partly untreated wastewater.

The nutrient levels from scenario A comes from the total nutrient emissions from Skretting Norway's feed, this was given in their sustainability rapport [11]. Table 2.1.1 shows the total nutrient concentrations are for N 125 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ and of P 21.67 $\mathrm{mg} \cdot \mathrm{L}^{-1}$, this is converted from the values that are given in [$\mathrm{g} \cdot (\mathrm{kgfeed})^{-1}$].

The nutrient levels from scenario B come from the same Table 2.1.1, these are the nutrient levels after the wastewater has gone through a mechanical filtration of the suspended solid sediments that come from uneaten fish feed and feces. The nutrient concentrations are for N is 98.33 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ and for P 3.33 $\mathrm{mg} \cdot \mathrm{L}^{-1}$. Comparing these nutrient levels to scenario A, the N concentration has decreased 21.3 % and the P concentration has decreased 85 %. This makes sense when looking at Section 2.3.1 in the background Chapter, where it is explained how most of the N is dissolved in water while most of the P is concentrated in the suspended solids. When the suspended solids are filtered away between scenarios A and B, the P concentration drops 85 %. It also shows some N in the suspended solids but that most of the N dissolves in water and needs further treatment.

The nutrient levels from scenario C comes from Nofitech and are the actual values that the microalgae cultivation facility is going to base off, the nutrient concentrations of this wastewater are for N 50 $\mathrm{mg} \cdot \mathrm{L}^{-1}$ and for P 1.69 $\mathrm{mg} \cdot \mathrm{L}^{-1}$. Both the N and P concentration has decreased about 50 %, but the amount of N is way higher than the amount of P.

It is assumed that the more nutrients there are in the wastewater, the bigger surface area is needed to absorb all the nutrients. When looking at the calculated surface areas for all three scenarios in Table 4.3.1 from Section 4.3.1. It is also interesting to see that to remove 22.67 $\mathrm{mg} \cdot \mathrm{P} \cdot \mathrm{L}^{-1}$ in scenario A, 76,000 m^2 is needed; this is the largest surface area calculated. While for the same scenario, only 25,800 m^2 is needed to remove 125 $\mathrm{mg} \cdot \mathrm{N} \cdot \mathrm{L}^{-1}$. This proves that it requires large quantities of microalgae to remove that large amount of P from wastewater and that mechanical filtration is necessary before a cultivation facility. However, that speaks for itself; if the solid sediments were to be in the cultivation facility, there would arise many complications regarding clogging, disrupting, and contamination as it is explained

in Section 2.3.2, this completely cancels scenario A out.

Scenario B has a much higher concentration of N than P. The surface area needed to remove all the N is around 20,000 m^2 while to remove all the P is around 11,700 m^2 is needed. These areas are not as high and might be more feasible to carry out. 20,000 m^2 is still a lot; the next Section 5.2.2 will mention the footprint required. Scenario C has the lowest surface area requirement; this is also because it has the lowest nutrient levels. The area requirement here is even more feasible to carry out; the surface area requirement is approximately 10,000 m^2 for N and 6,000 m^2 for P. To model the cultivation facility to remove 100 % of the N in scenario C, approximately 10,000 m^2 is required.

The reason why the calculations of the 70 % absorption area included in Table 4.3.1 were to see the difference it would make to only calculate for 70 %. After some consideration, and seeing that it did not have an extensive impact on the areas, it seemed wrong to model a cultivation reactor not to absorb 100 %. Instead, the reactor should be modeled for 100 % so it can afford to have some deficiency.

5.2.2 Footprint area

The footprint area was calculated for all 100 % absorption surface areas; depending on the height of the belts, eight different heights were considered. The footprint area for scenario A was still calculated even though it was concluded in Section 5.2.1 that this scenario was canceled. The footprint area for this scenario can be seen in Table 4.3.2 in Section 4.3.2, but will not be considered further in this discussion.

When choosing how tall the cultivation belts in the rotating algal biofilm reactor (RAB), the mutual shadowing from the other cultivation belts should be in mind. Gross et al. [88] have considered this as it says in Section 2.6.5, the biomass productivity of their RAB reactor decreased a little bit as the height of the cultivation belts increased. They concluded that this came from mutual shading from the adjacent belts, where they looked at the belt height of 0.91, 1.22, 1.52, and 1.83 m. The mutual shading from these heights was so subtle that they could be dismissed. However, in the footprint calculations in this thesis, taller heights have been considered, including the four that Gross et al. tried. The four following heights are 2.00, 2.50, 3.00, and 5.00 m, exponentially taller than the other four. An assumption is made that the mutual shading will significantly affect the biomass production from these taller heights. With this in mind and considering that the footprint area should be as limited as possible, the three lowest and two tallest heights are discarded. This leaves the heights 1.83, 2.00, and 2.50 m; these heights might affect the biomass production some, but an unreasonable amount. A closer look at how much the height of cultivation belts would affect biomass production was not included in this thesis, but would be very interesting to look into in the future.

The footprint area for scenario B is shown in Table 4.3.3 in Section 4.3.2. The total surface

area for nitrogen in scenario B is around 20,000 m^2. According to the decision about the height of the cultivation belt made in the last paragraph, to cover this surface area with a belt height of 1.83, 2.00, and 2.50 m would require a footprint area between 2,400 and 1,800 m^2.

The footprint area for scenario C is shown in Table 4.3.4 in Section 4.3.2. The total surface area for nitrogen in scenario C is approximately 10,000 m^2. According to the decision about the height of the cultivation belt made in the last paragraph, covering this surface area with a belt height of 1.83, 2.00, and 2.50 m would require a footprint area between 1,200 and 900 m^2. How to decide how tall the belts should also depend on the room, and light is hard to decide without more knowledge, in addition to this, the values used in these calculations are very uncertain, however, these numbers give an indication of the size needed.

For perspective, the footprint area can be compared to a UEFA-certified football field, which needs to have a range of 105 x 68 meters, which is a total area of 7,140 m^2 [102]. For scenario B - nitrogen with a cultivation belt height of 2.00 m, the footprint area would be 2,220 m^2, this is 31 % of the size of a UEFA football field. For scenario A - nitrogen with a cultivation belt height of 2.00 m, the footprint area would be 1,129 m^2, this is 16 % of the UEFA size football field. In addition to this, the cultivation facility could, in theory, be covered over several floors in a building, but this will not be considered here.

When looking at the footprint area required by both scenarios B and C, they could be built and used to cultivate facilities. This thesis focuses on the values from Nofitech, which is scenario C - nitrogen at a surface area around 10,000 m^2, and therefor has chosen this for the subsequent calculations. Nevertheless, it was interesting to see that it is possible to build a cultivation facility without pre-treatment.

Table 4.3.5 in Section 4.3.2, shows the area of each cultivation belt would have for the different heights of the belts; in addition to this, it shows the number of belts needed for each belt for a surface area of 10,320 m^2. The number of belts needed for heights 1.83, 2.00, and 2.50 m with the belt area would be 3.64, 4.00, and 5.00, respectively, and the number of belts needed would be between 2,800 and 2,000.

5.2.3 Biomass production

The calculated biomass production shown in Table 4.3.6 in Section 4.3.3, are from six different scenarios. Different scenarios are needed because the biomass productivity area is relative to specific cultivation areas, surroundings, and species. This makes it hard to find a productivity rate for one set of microalgae in one facility. This Section comes with the arguments of why these productivity rates were chosen.

First of all, the three biomass production rates estimated from the lab experiments for this thesis were added to see how far off the biomass production would be compared to the other values that have come from other studies. The way these were found are explained in Section

3.1.2, and the calculated results of the estimated growth rate are in Table 4.1.5. As mentioned in Section 5.1.1, the estimated values that were calculated are more comparable to the other cultivation methods in Table 2.5.1 (e.g., Twin layer phototropic biofilm reactor (PBR), pilot-scale PBR, algal biofilm reactor) rather than the RAB reactor, which makes sense as they are less optimal construction which leads to slower growth.

As mentioned, the biomass production from the laboratory experiments is a little low compared to the biomass production of the theoretically found productions from Boelee et al.[97], and Gross et al. [88]. The expected production based on the laboratory productivity is 14, 16, and 14 tons for the *Synechocystis*, *P. Tricornutum* and mixed culture respectfully. While from the literature it the biomass produced can be 26, 57, and 111 tons for Boelee et al., RAB raceway based and RAB through based from Gross et al. [88] respectively. As explained several times previously, the laboratory cultivation environment is of such different quality than a RAB environment. The laboratory experiments were cultivated in bottles and only given nutrition's water every ten days, and the RAB conditions are presented in Section 2.5.1. This is probably why the laboratory biomass production is only 15 % of the highest possible RAB production. It can be expected that an actual RAB facility would have a higher biomass production than from the laboratory experiments in this thesis.

6 Further work

In this Chapter, the author's suggestions for further work and thoughts on development are presented. The suggestions are beyond the scope of this thesis. Based on the work, several things come to mind that can be researched froward. As mentioned in the Conclusion, this thesis was based on laboratory testing, and the next natural step would be to test the microalgae *Synechocystis* SP. PCC 6803 in an actual rotating algal biofilm (RAB) facility. Because of the uncertainties from laboratory research, a pilot test is needed before scaling it up to a large-scale facility. If it is possible to test microalgae growth in an existing RAB facility, it would be interesting to test some other species as well, like the more known *Chlorella Vulgaris*.

In Section 2.6.5, it is mentioned that mutual shading from tall cultivation belts could decrease the biomass growth rate. Another thing that could be looked into is how tall the cultivation belts have to be before the mutual shading is too large. This could screen how high the belts could be before it affects production, and with that, optimize footprint area after the best height for cultivation and the surrounding area.

In addition to this, when modeling a RAB facility it is important to map the microalgae productivity rate changes when other process parameter is changed. Section 2.6 shows other essential parameters for a RAB facility like light, temperature, belt velocity, and harvesting frequency. It would be interesting to look closer at how much these parameters affect the production rate: especially belt velocity and the frequency of change between light and darkness. There can also be experiments of the growth rate in different colors as it is known that microalgae react to different wavelengths.

7 Conclusion

This thesis was initially going to be based on a pilot test of microalgae growth done on an actual rotating algal biofilm (RAB) reactor. However, because of COVID-19, it was not possible to transport the RAB to Norway in time. Instead, this thesis turned into a laboratory test. Therefore the microalgae in question were grown in cultivation bottles, which led to a production rate that was, as expected, much lower than what is found in the literature.

In addition to this, the experiments performed had several other uncertainties based on the growth conditions. The calculation of growth rate was affected by this, and as the estimated growth rate from the lab experiments was as low as 3.00 to 4.50 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$. Literature shows that the growth rate of rotating algal biomass (RAB) reactor could be up to 29.59 $\mathrm{g} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$. As mentioned, the microalgae were grown in cultivation bottles. They had only an infusion of new nutrients every ten days when comparing this to a RAB reactor, which has a continuous flow of nutrients enriched medium, O_2 and light to get an optimal photosynthetic effect.

From the laboratory results, it was also found that both microalgae species *Phaeodactylum Tricornutum*, *Synechocystis* SP. PCC 6803 and the mixed culture absorb about 75 $\mathrm{mg} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ of nitrogen and 4.411 $\mathrm{mg} \cdot \mathrm{m}^{-2} \cdot \mathrm{day}^{-1}$ of phosphor. The *Phaeodactylum Tricornutum* biomass had trouble attaching to the surface at several of the experiments, therefore *Synechocystis* SP. PCC 6803 was chosen as the optimal microalgae from this testing.

From this, it was concluded that the RAB facility needs to have a surface area of approximately 10,000 m^2, where the footprint area differs between 900 and 1,200 m^2 for cultivation belt heights between 2.50 to 1.83 m, respectively. It is important to note that these areas and biomass production are scaled up from laboratory tests; scaling up to this extent from laboratory experiments can lead to vast uncertainties. Therefore, a pilot experiment of an RAB system with actual wastewater from a RAS should be performed before full operational up-scaling.

With the absorption rate and growth rate calculated, it can be assumed that the facility will produce around 14 tons of microalgal biomass per year with the *Synechocystis* SP. PCC 6803 from the lab experiment. This is only 15 % of the RAB biomass production found in literature, which says that a RAB facility can produce up to 111 tons of microalgae a year. This is because of the factors previously explained; the lab experiment completed has an entirely different growth environment. Consequently, the biomass growth in the lab is low and cannot be compared on the same basis.

Overall, both microalgae species show promise that they would work well as a cleaning process for wastewater, and *Synechocystis* SP. PCC 6803 is a steady candidate, which have a good growth rate and produces nutrient rich biomass. From all the uncertainties in the experiments, it can be concluded that the difference between the the laboratory tests and literature are much higher than the difference between the two microalgae species.

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