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# Cultivation of microalgae on fishery effluent in industrial environment

## Scoping the challenges of sustainable aquaculture

Pavle Estrajher

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60 ECTS thesis submitted in partial fulfilment of a *Magister Scientiarum* degree in Environmental Sciences

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Örþörungaræktun á afrennsli frá fiskeldi undir iðnaðaraðstæðum
Cultivation of microalgae on fishery effluent in industrial environment
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## Yfirlýsing

*Hér með lýsi ég því yfir að verkefni þetta er byggt á mínum eigin athugunum, er samið af mér og að það hefur hvorki að hluta né í heild verið lagt fram áður til hærri prófgráðu.*

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Pavle Estrajher

## Abstract

Aquaculture industry is facing a resource limitation due to its rapid expansion – wild fish used as aquaculture feed is a finite resource. Terrestrial plant feed emerged as a substitute for wild fish feed comes with its disadvantages, e.g., competing for land and water with plants intended for human consumption. Additionally, farmed fish needs feed supplements of aquatic origin as its Omega-3 content decreases without them. After the feed cycle is finished in the fisheries, a bi-product is created in the form of run-off effluent. If released to the nature without treatment, this effluent causes negative impacts - its high nitrogen (N) and phosphorus (P) content can destabilize and pollute even well-balanced ecosystems. The effluent is at the same time an untapped resource that can be utilized for the cultivation of microalgae. Microalgae can turn as high as 88% and 99% of released N and P, respectively, into a multipurpose biomass. However, intensive microalgal cultivation carries a high demand for electricity making the carbon footprint high – up to 80% of biomass production footprint in the operational stage attributes to electricity. In Iceland, most electricity is produced sustainably, either by hydropower or geothermal power. Theoretically, a low-tech setup can keep the carbon footprint of microalgal cultivation low, avoiding the need for carbon offsetting, and producing sufficient biomass to be commercially viable. To investigate this concept, a semi-intensive microalgal cultivation was set up with low energy input using atmospheric $CO_2$. The setup has conceptually proved that the aquaculture effluent can be partially remediated and re-used. The re-use of effluent minimizes its impacts on the wild fish populations and lowers the carbon footprint of fishery. A more extensive trial would be necessary to find the cultivation methods suitable for higher scale production and prove the profitability of such production. The possible environmental incentives and other benefits which may come with the advance of aquaculture can help develop a better infrastructure for recycling and reuse of aquacultural bi-products.
Keywords: aquaculture expansion, microalgae as fish feed, fishery effluent, fishmeal substitute

# Ágrip


## [Örþörungaræktun á afrennsli frá fiskeldi undir iðnaðaraðstæðum]

Eftir mikinn vöxt á síðustu árum stendur fiskeldi nú frami fyrir því að auðlindir geti orðið takmarkandi fyrir frekari vöxt - viltur fiskur sem notaður er í fóður er ekki óendanleg auðlind. Plöntufóður ræktað á landi gæti komið í stað fisks, en það hefur þó sína ókosti s.s. samkeppni um land við matvælaframleiðslu til manneldis og þá þyrfti eldisfiskur á fæðubótarefnum að halda til að ekki dragi úr Omega-3 innihaldi hans. Úrgangur frá fiskeldi hefur neikvæð umhverfisáhrif ef hann fer ómeðhöndlaður út í náttúruna. Hátt köfnunarefnis- (N) og fosfór- (P) innihald úrgangs getur valdið óstöðugleika og mengað vistkerfi jafnvel þó þau séu í góðu jafnvægi. Hins vegar, er úrgangurinn vannýtt auðlind sem hægt væri að nýta til ræktunar á örþörungum. Fræðilega séð geta örþörungar breytt 88% og 99% af losuðum N og P í fjölnota lífmassa. Hins vegar er áköf smáþörungarækt orkufrek (hefur mikið rafmagnsspor) - allt að 80% af fótspori af framleiðslu lífmassa á rekstrarstigi má rekja til notkunar á raforku. Lágtækniuppsetning getur haldið kolefnisfótspori lágu eða jafnvel neikvæðu og komið í veg fyrir þörf á mótvægis aðgerðum en þó framleitt nægjanlegan lífmassa. Til þess að prófa þessa hugmynd var sett upp örþörungarækt í hálf-stríðeldi með minni ræktunarákefð þar sem byggt var á nýtingu CO2 úr andrúmslofti og minna orkuframlagi. Athugunin hefur sýnt fram á að hægt er að nýta og endurnýta frárennsli frá fiskeldi ásamt því að lágmarka áhrif þess á villta fiskstofna og draga úr kolefnisspori þess. Strærri framleiðslu er þörf til að sanna efnahagslega ávinningu, samhliða því að svara henni verður að hafa í huga mögulega umhverfishvata ásamt öðrum ávinningi sem getur fylgt þróun fiskeldis.


Lykilorð: Stækkun fiskeldis, örþörungar sem fiskifóður, fiskeldisfrárennsli

***Þakkir***

Ég vil þakka leiðbeinanda mínum, René Groben fyrir allan hans tíma og orku, fróðlega ráðgjöf, og innsæi í örverufræði. Stefaníu Karlsdóttur fyrir aðgang að tækjabúnaði hjá Matorku, ráðlagningu við uppsetningu og framkvæmd verkefnis. Einnig vil ég sérstaklega þakka Ragnhildi Helgu Jónsdóttur fyrir aðstoð við uppsetningu á náminu mínu og ómetanlegan stuðning á öllum tímum. Bjarna Diðrik umsjónarmanni meistaranáms fyrir að vera alltaf tilbúinn að hlusta á endalaus vandamál sem þetta verkefni hefur lent í, finna bestu lausnir fyrir mig án vandræða og gera mér kleift að halda áfram í námi. Þessi stuðningur hefur komið sér sérstaklega í erfiðleikum vegna Covid 19 faraldursins, og það var ómetanlegt að hafa þau með mér í liði, skilningur og hjálpsemi beggja þeirra er endalaus. Kennsluskrifstofa LBHÍ fær sérstakar þakkir fyrir fagmennskuna sína, fljót viðbrögð og metnað. Börnin mín þrjú Dagmar, Emil og Kristín fá þakkir fyrir að vera svo stillt og góð og konan mín Sonja líka fyrir alla þolinmæðina sem þetta verkefni krafðist. Einnig vil ég þakka Framleiðnissjóðnum landbúnaðarins fyrir fjármálastyrk.

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# 1. Introduction

## *1.1 The challenge of sustainable aquaculture feed*

Aquaculture, or farming of fish and other aquatic organisms, has become more extensive with the increase of world population. In many countries, fish farms provide larger quantities of fish then any other food source (FAO, 2020). However, because of its rapid expansion, aquaculture is facing a resource limitation. Extensive aquaculture has heavy impacts on the aquatic ecosystems and the sustainability of exploited resources (Froehlich, Gentry, & Halpern, 2017). In Iceland, the production of farmed fish has increased by almost tenfold in the last two decades (Icelandic Food and Veterinary Authority, 2020), and the Icelandic authorities have additionally approved substantial growth in the coming years (Hafrannsóknastofnun, 2020).

Fish oil is one of the most important resources needed for aquaculture feed, as it supplements the nutrients necessary for the healthy growth of farmed fish and its final quality. Most fish oil used in aquaculture comes from wild fish populations, which are exclusively exploited for that purpose. One species, the Peruvian anchoveta, stands for 80% of fish oil supplement used for farmed fish - mostly for the rapidly growing salmonid cultivation (Gíslason, 2020). The anchoveta, just like all other wild fish stocks, is a limited, finite resource. This is especially true when having in mind the former fishery collapses in the anchoveta population (Arias Schreiber, 2012), and the already mentioned rapid growth of aquaculture. Fishing bans caused by the population collapses in anchoveta have created its shortages and price fluctuations in its market value (Gíslason, 2020). The demand has, however, prevailed, and the anchoveta is still one of the most fished species in the world (Di Dario, 2021). A substitute in form of terrestrial plant oil has been used and accepted in aquaculture, but the different nutrient content in the plant-based oil tends to decrease Omega-3 acid levels in farmed fish, especially salmon. To make up for the nutrient deficiency, the farmed fish still receives (some) supplement of anchoveta oil. Nevertheless, the Omega-3 levels in salmon, according to some researchers, have decreased by up to 50 percent recently, and use of vegetable oils as a supplement has increased the amount of terrestrial fatty acids in the farmed fish (Sprague, Dick, & Tocher, 2016).

### *1.2 The challenge of land-based aquaculture waste management*

One of the main challenges of land-based aquaculture is the control of the effluent coming from fish tanks. Although this effluent may contain 70% to 80% of unused leftover nutrients from fish feed (mainly N and P) it is often released into the surrounding environment without treatment (Hawrot-Paw, Koniuszy, Gałczyńska, Zając, & Szyszlak-Bargłowicz, 2020). The amount of the released effluent grows reciprocally with the expansion of aquaculture (Hawrot-Paw et al., 2020), while its high N and P content continually destabilizes and pollutes even well-balanced ecosystems. In specific, the aquaculture company Matorka releases 2300 L/s of effluent, which can translate to a daily release of 25kg of nitrate ($NO_3^-$) (Darrason, 2016) potentially causing problems like eutrophication. Eutrophication causes nutrient (N and P) surplus, which can lead to high phytoplankton densities and toxic algal and cyanobacterial blooms (Desmit et al., 2018; Le Moal et al., 2019). Eutrophication caused by human activities is the main reason for coastal pollution and one of the most common problems of aquatic systems today (Desmit et al., 2018). Treatment and usage of fishery effluent is necessary and beneficial to the environment. The fisheries may also benefit economically by turning the effluent into fish feed to lower their purchase costs. Developing safety and regulatory measures may limit the use of effluent as they are being changed with the advance of aquaculture. Nutrient recycling is, however, seen positively, especially if the substances like the fishery effluent are used. The use of waste bi-products is valuable recycling practice and contributes to solving environmental problems.

### *1.3 Cultivating microalgae on fishery effluent*

Apart from light and carbon dioxide, photosynthetic microalgae need the already mentioned nutrients (N and P), like terrestrial plants do, for their growth. This makes them able to grow on fishery effluent and substantially reduce the total N and P content in it - *Chlorella minutissima* was recorded of capturing up to 88% of N and 99% of P when grown on effluent (Hawrot-Paw et al., 2020). Other species from *Chlorella, Scenedesmus* and *Nannochloropsis* genera have been successfully grown on effluent as a eutrophication remedy (Aslan & Kapdan, 2006; Saiu et al., 2016; Urrutia, Serra, & Llama, 1995). Yet other microalgae were also successfully cultivated on fishery effluent from salmonids and tilapia (Enwereuzoh, Harding, & Low, 2021; Hawrot-Paw et al., 2020). The algae grown on effluent were also incorporated in the fish tanks creating the complete artificial biosystem (Tossavainen et al., 2019).

Recycling can also be extended to other fishery bi-products, like the sludge in form of gutted fish insides and bones. Removing fishery sludge alongside effluent remediation additionally increases the environmental value of microalgal cultivation. In the mentioned experiment by Tossavainen et al. (2019) the algae *Euglena gracilis* and *Selenastrum sp.* grown with added sludge from pikeperch and catfish cultivation had higher biomass production than the algae grown without the addition of fishery sludge. The algae with added sludge also showed better growth under nutrient depletion, producing sufficient amounts of EPA, DHA and tocopherol needed for fish feed. There are obviously many ways to improve the management of different types of waste in aquaculture. Additional research will show the best ways of salmonid effluent treatment as most experiments have been done in laboratories and not in practical industrial environment (Hawrot-Paw et al., 2020).

After cultivation, microalgal biomass is either fed to juvenile fish (smolt), the adult fish, or to a variety of other cultivated animals. It is more often further processed and implemented as a feed supplement (Dineshbabu, Goswami, Kumar, Sinha, & Das, 2019). Microalgae are grown for many other purposes, regardless to whether fishery effluent is used in their cultivation or not - their usage is not limited to one product nor to a same group of products or not even to the same industrial branch. Apart from fish feed supplement microalgal biomass can be used as fuel, (like biodiesel or biogas), agricultural fertilizer, pharmaceutical or nutraceutical products, and nutritional supplement in human diet (Mata, Martins, & Caetano, 2010; Raja et al., 2018). Biogas and agricultural fertilizers of microalgal origin are valuable replacements for fossil fuel based products. Pharmaceutical, nutraceutical and nutritional microalgal supplements have recently created a new market of niche products increasingly gaining popularity.

Because large scale fisheries combined with microalgal cultivation facilites are relatively new, the legislative aspect around them is still developing. Many countries have yet to decide in which category to classify microalgal cultivation in general. In some cases, microalgae may classify as „traditional“ agricultural products and undergo the same agricultural legislature. However, the already mentioned products like biodiesel or biogas, farmaceuticals, and nutraceuticals produced from microalgae (and, additionally, microalgal cultivation combined with wastewater treatment) complicate the classification and call for improvement (Trentacoste, Martinez, & Zenk, 2015).

### *1.4 The ecological footprint of aquaculture feed and algal cultivation*

Fish feed produced by terrestrial sources carries a high carbon footprint because it occupies large arable land areas, depends on fossil-based fertilizers, requires the use of chemical pesticides, and needs fresh water for irrigation, competing with other products intended for human consumption at the same time (Tibbetts, 2018). Most aquaculture feed is produced in agricultural sector with high greenhouse gas (GHG) emissions, due to heavy release of methane and nitrous oxides. The high emissions are primarily related to artificial fertilizer production, sewage sludge release, and soil & atmospheric nitrogen contamination, while emissions from fossil fuel used for running agricultural and fishing equipment come next (Ziegler et al., 2013).

Electricity, which is essential in most cultivation setups, makes the carbon footprint of microalgal cultivation in photobioreactors exceedingly high. In some cases, 80% of the total $CO_2$ footprint goes to production of electricity used for biomass cultivation (Mata et al., 2018). The reason for the high footprint of electricity are the non-renewable fossil fuels used in its production. This high footprint may be significantly different depending on geolocation and the way the electricity is produced (Dziuba, 2020).

The production of nutrients used for biomass cultivation is the second largest factor after electricity, contributing to over 20% of the total carbon footprint (Mata et al., 2018). Main nutrients are carbon in form of $CO_2$, N, and P. $CO_2$ is essential, among else, for microalgal growth and pH control, and apart from N and P a steady $CO_2$ supply is necessary. The $CO_2$ expense is also one of the highest expenses during the production period and can contribute to a third of the total cost of cultivation (Acién, Fernández, Magán, & Molina, 2012). N and P are the main ingredients for the nutrient medium production and carry the highest footprint after $CO_2$ itself. Nitrogen is supplied in form of nitrate, which is industrially produced and has a high footprint due to the complicated synthesis process (Dziuba et al., 2016). Phosphorus is essential for microalgae but unlike nitrogen, phosphorus, or phosphates, the form of phosphorus that algae can absorb, can not be artificially prepared and is thus a finite nutrient source, making its proper management even more important (Scholz, Ulrich, Eilittä, & Roy, 2013).

By combining sustainable geothermal/hydropower sourced electricity, efluent reuse and lowering the use of industrial $CO_2$, the total footprint of microalgal cultivation would be substantially lowered. The setup would combine recycling and sustainability with low carbon footprint.

## *1.5 Matorka and microalgae cultivation efforts: waste-to-value circular economy as a solution*

Matorka is a land-based aquaculture company growing arctic char and steelhead trout without the use of antibiotics or hormones. Using lava-filtered water they create ideal environment for fish cultivation, naturally free of parasites and pathogens, without impact on the wild fish populations and with high sustainability. The company offsets its carbon footprint completely by using sustainable geothermal energy and planting trees to make up for the possible difference (Matorka, 2020). Matorka also releases around 2300 L/s of fishery effluent into the environment, an amount which can translate to a daily release of 25kg of nitrate ($NO_3^-$) (Darrason, 2016). There are no legislative measures (at present) for the fishery effluent disposal in Iceland. The regulations will soon change, especially if the fisheries continue growing at the current rate. In relation, land-based fisheries like Matorka offer almost full waste control, and high possibilities to recycle the effluent which can, besides being an environmental threat, also be seen as an unused resource. Photosynthetic microalgae can use this effluent (N, P) and turn it into high lipid content biomass with multipurpose application (Hawrot-Paw et al., 2020). The biomass can be, in some cases, used immediately without treatment, or processed into a variety of already mentioned products. Since microalgal cultivation on fishery effluent is known and researched, the practice could be applied at Matorka's fishery. Instead of highly intensive microalgal cultivation, which is energy demanding and can often be costly, a simpler, low energy input design is also possible – Matorka's infrastructure could be adapted for low or semi-intensive cultivation. Using atmospheric $CO_2$ from air instead of pressurized $CO_2$ from bottles or surplus industrial flue gas could lower the purchase and/or operational costs. With the application of such setup, Matorka could research the possibility of growing microalgae on the effluent by running it from its fish tanks, opposed to releasing it unused into the environment.

Most electricity produced in Iceland is considered sustainable and its carbon footprint is among lower, if not the lowest in the world (Carbon Footprint Ltd, 2020). Combining sustainable electricity and the use of fishery effluent to cultivate carbon neutral microalgae could be a solution to several aquacultural problems (feed deficiency, dangers of eutrophication, and high carbon footprint) through recycling and sustainable energy use.

The use of microalgae as fish feed could also improve Omega-3 fatty acid levels in farmed salmon which have dropped with the introduction of terrestrial plant feed. Higher levels

of Omega-3 oils would give the fish better supply of protein, lipids, and vitamins, as well as natural lice protection (Gíslason, 2020). It would allow aquacultural companies to produce sustainable microalgal extract ready to be used as Omega-3 source for all aquafeed.

Restrictions for the usage of effluent could be applied in the EU for microalgae intended for human food production, as the EU requires the recycled water used in food industry to be the same quality as the potable water (*Who on the hygiene of foodstuffs*, 2004). On the other hand, WHO approves the use of the effluent as recycling and gives it additional value as it interconnects disciplines (aquaculture and waste management) (WHO, 2006). In Iceland, the legal body responsible for implementing government propositions, the Icelandic Environment Agency, sees the remedation of wastewater as beneficial and the state protocols encourage projects which apply it (Umhverfisstofnun, 2020). If microalgae are produced for aquacultural use, their cultivation on effluent is not specifically restricted, but regulatory restrictions follow the EU regulations and apply for human food products, according to M. Þorsteinsdóttir (personal communication, 2021).

The use of fishery effluent without limitations to produce fish feed would be a good step further towards waste recycling and circular economy. Many scenarios for the design of such cultivation systems exist - the systems can be intensive, but they may need countermeasures and carbon offsetting if the company's footprint is to remain low. As the electricity in Iceland is produced in a sustainable way, the carbon footprint is kept low and may not even need offsetting. A simple cultivation system with low carbon footprint using recycling to produce microalgal biomass intended for fish feed supplement is what this research tried to apply.

# 2. Materials and methods

## *2.1 Algal strains*

*Nannochloropsis sp.* (N1) and *Chlorella vulgaris* (CV) were obtained from University of Akureyri (UNAK). *Nannochloropsis oculata*, (N2) was obtained from the Scottish Marine Institute‘s Culture Collection of Algae and Protozoa (CCAP). N2 from CCAP was isolated from Skate Point, Isle of Cumbrae, Scotland. UNAK obtained the CV from an unconfirmed source in Germany. UNAK isolated *Nannochloropsis sp.* from the sea around Akureyri. According to UNAK, the Icelandic Marine Research Institute (MRI) observed the *Nannochloropsis sp.* culture under a microscope and narrowed the possible genera either to *Nannochloropsis* or *Nannochloris*. In lack of better information, N1 species will be referred to as *Nannochloropsis sp.*

## *2.2 Maintenance and upscaling of algal cultures*

For the cultivation medium, three different water sources were used - a run-off fishery effluent (Eff) from land-based aquaculture tank with salinity of 5-7ppt, borehole sea (30+ ppt) (BHS), fresh water (3-5 ppt) (FW) and artificial sea water with adjusted salinity (Art). No content analysis of the growth medium water or effluent was conducted. Conway nutrient mixture (Appendix 1) was used as nutrient additive. Artificial sea water was prepared according to the recipe in Appendix 2.

Cultures from UNAK were obtained in two-litre plastic bottles, two at the same time. Upscaling in trials 1-8 was done immediately after the cultures arrived from UNAK. For upscaling, four generic 5-litre transparent glass bottles were used. The bottles had polyurethane taps with incisions which allowed for the insertion of pipes. The cultures were divided in half - one litre of medium was poured into each 5-litre upscaling bottle along the rest of the cultivation medium. Aeration was enabled by two Marina 200 aquarium pumps. The air ran through ca. 35 cm long stainless-steel pipes inserted through the taps connected by standard 3 mm diameter aquarium hoses. The culture from CCAP, used in trials 9-12, arrived in a 50 ml container which was upscaled to 1 litre in artificial sea medium and the addition of Conway nutrient mixture. After that it was upscaled from 1 litre to 4 litres, and then divided to four upscaling bottles in the same way as the cultures from UNAK. The contents of all trials (1-12) are listed in Appendix 3.

The cultivation was divided in two parts, where in the first part the cultures were upscaled to five litres and in the second part to fifteen litres, each part taking 7 days. This way the

chance was higher that the growth curve will be faster than if the cultures were at once put into fifteen litre containers.

Borehole sea water and fresh water were pumped out of two different nearby boreholes in the coastal area near the aquaculture facility. Aquaculture effluent from arctic char cultivation was taken straight from a fish tank outlet. For trial 9 the borehole sea water was obtained from the MRI at their nearby facility as the borehole sea water was not available at Matorka. Fresh water with adjusted salinity was used in trials 10, 11 and 12 for the same reason, the unavailability of borehole sea water. The water and the effluent were filtered through a cloth to remove contaminants and added to the cultures in the cultivation bottles. The bottles were placed on shelves with lights behind for the time of cultivation (Figure 1).

For lighting, a double fluorescent bulb light (2x18w/840) with Osram Cool White bulbs was placed at 10 cm distance from the bottles which were about 10 cm apart from each other. One bulb had a nominal 4000K light, and approximately 3.6W PAR (7.2W PAR combined). The upscaling room had two sets of double fluorescent ceiling lights (4 bulbs total) of the same specifications at approximately 2.5 metres height. There were no windows in the room.

***Figure 1*** *Upscaling set-up. Bottles are bubbled to upscale the algae from one litre to five litres*

To avoid temperature stress, the growth medium was for a brief time (an hour approximately) warmed to a temperature higher than 10°C before mixing. Temperature measurements were taken by a digital thermometer inserted directly into the culture. Levels of pH were measured by a generic no-name pH meter. Nutrients were added to the culture using a measurement pipette. Aeration pipes were inserted through rubber taps into the necks of the bottles. Air was fed to the bottles by aquarium pumps through 3 mm air hoses controlled by valves, each pump supplying two bottles. Aeration was adjusted by valves to cause moderate turbulence and movement of medium, while still having an airflow of approximately ten 2-3 cm diameter bubbles per second with steady pace. The amount of fluctuated air was not measured. Sometimes, it was not possible to adjust the flow equally in all the bottles, and the bottles with slower airflow showed lesser growth when compared to the ones with good aeration (Appendix 3). Temperatures in the upscaling bottles are listed in the Appendix 3. Salinity was measured by a Keg King refractometer with Brix scale. All measurements were taken once weekly, at the beginning and the end of the trials. Photos were taken either daily (trials 1-4), twice a week (trial 5) or at the beginning and the end of the trials (trials 6-12).

### *2.3 Main cultivation*

In the main cultivation the medium from the upscaling bottles was placed in 15-litre bags with the rest of the contents (water, effluent, and nutrient mixture). The contents were poured into transparent round plastic bags, 150 cm long and 30 cm wide. The bags were tied at the bottom end while the top end was used to secure the bags to a metal frame (Figure 2). There were two ropes for the lights behind the bags. The horizontal bar for the bags was at height of around 2.5 metres, with the ropes for the lights stretched and tied between the two vertical metal bars in the back.

Lights behind the bags were 4 Osram 18w/840 bulbs placed at ca 50 cm distance from the bags and emitting approximately 14.4W PAR. The lights were attached to the bars behind the bags and hanged vertically to illuminate the largest surface possible in the

bags. The room was illuminated by four ceiling lights of the same type as mentioned (8 bulbs total) at approximately 4 metres height. There were no windows in the room.


***Figure 2*** *Main cultivation set-up. Contents from bottles from upscaling are put in a bag with added 10 litres of medium*

To avoid temperature stress, the growth medium was warmed to at least 10°C before mixing, like for the upscaling. The contents of the bags in individual trials are listed in Appendix 3.

An industrial electric air pump of Hurricane brand was used to supply the air to the bags through plastic piping. The air was fed through four 3mm diameter hoses with bubblers with added weight, as the hoses would float due to the airlifting and the weight of bubblers alone could not hold them down. The hoses were inserted approximately ¾ deep into the bags through an incision which was taped to reduce outside contamination. There were no valves connected to individual hose/bag, and amount of pumped air and its speed were constant. If one hose were lifted the airflow speed up in that bag would and slow-down in other bags. All the variables were measured in the same way as in the upscaling, and the photos taken at the same time. The temperatures measured in the bags are listed in Appendix 3.

### *2.4 Biomass separation and analysis*

After seven days in bottles and seven days in bags, the contents from the cultivation were transferred to 20L plastic buckets and slowly poured through a strainer cloth. Biomass of trials 1-4 was not separated, but the contents from a 250 ml sample from trial 4 was examined under a microscope, centrifuged, and weighed (data available). Cultures from trials 5 to 8 were separated by filtration through a cloth made of cotton sheet (one layer). In trials 9-12 a filter made of polyester microfiber REPREVE material (Buff) was used (one layer). The exact porosity of neither of these materials is known. The results of these experiments are only approximate and serve to prove the concept, not to put an exact standard to it. After filtration, the cloth (filter) was placed on a table and the algal sludge was scooped, scraped off and collected to a container. The remaining algae which settled in the bottom and the sides of the bags were also scraped off. When all the algae were collected off the filtration cloth, they were centrifuged by MSA Centaur 2 centrifuge in several steps as the capacity of the centrifuge was insufficient for all the content (4 x 50 ml PP). The samples were poured into those tubes, 50 ml from each container initially, and centrifuged at 3600 rpm for 15 minutes or longer, until the supernatant water was clear and algal biomass pellets firmly separated in the bottom of the container. After that, the supernatant water was discarded to keep the algal pellet at the bottom. The tube was then topped with remaining algae from the container. The desired condition was obtained when the algae were firmly stuck together in a pellet and the supernatant water clear and transparent. This was repeated until the content from all the containers was centrifuged. The supernatant water was discarded, and the biomass was pulled out of the bottom and set aside. Then the container and the biomass were weighed. After weighing, the pellets were placed on a wire mesh and air-dried until by using an air ventilator and weighed again.

In trial 4, after the biomass was filtrated and separated, a 50 ml sample was taken from the filtrated medium which was normally discarded. The sample was taken for centrifugation to show the efficiency of the filtration by finding the approximate algal content in the discarded cultivation medium. The results are approximately calculated in subchapter 3.5.5

Air-dried pellets from trial 5 were additionally dried for 12 hours at 60°C to eliminate the atmospheric humidity and then immediately weighed. After that, the organic content was burned at 600°C for two hours and the remaining ash content weighed (Figure 3).

Untreated centrifugated biomass (UCB) pellets from trial 6 were taken to Efnagreining hf where they were analysed for protein content, ash, and dry matter.

# 3. Results

## *3.1 Growth observations*

All three species showed growth in the cultivation period, which was initially visible by the change of colour in the cultivation medium. The darker colour in the end of cultivation cycles was identical to the colour of the medium sent from UNAK/CCAP, as opposed to the much lighter colour in the beginning of the inoculation periods. Cultures grown in lower salinity from which less biomass was retrieved had lighter colour.

## *3.2 Nannochloropsis sp. (N1)*

Growth of sp. N1 with effluent was compared to its growth without effluent in trials 2-6. Lower salinity levels were used as control (cultures A, Appendix 3). The final weight of dehumidified biomass and its ash content from trial 5 are shown in Figure 3. Culture A was grown in low salinity (14 ppt) with Conway nutrients added but no effluent. Cultures B and C with Conway nutrient mixture and effluent were grown in more saline medium (31 ppt and 26 ppt respectively), while culture D was grown with effluent but without Conway in 29 ppt salinity. Culture D showed better growth than culture A. When looking

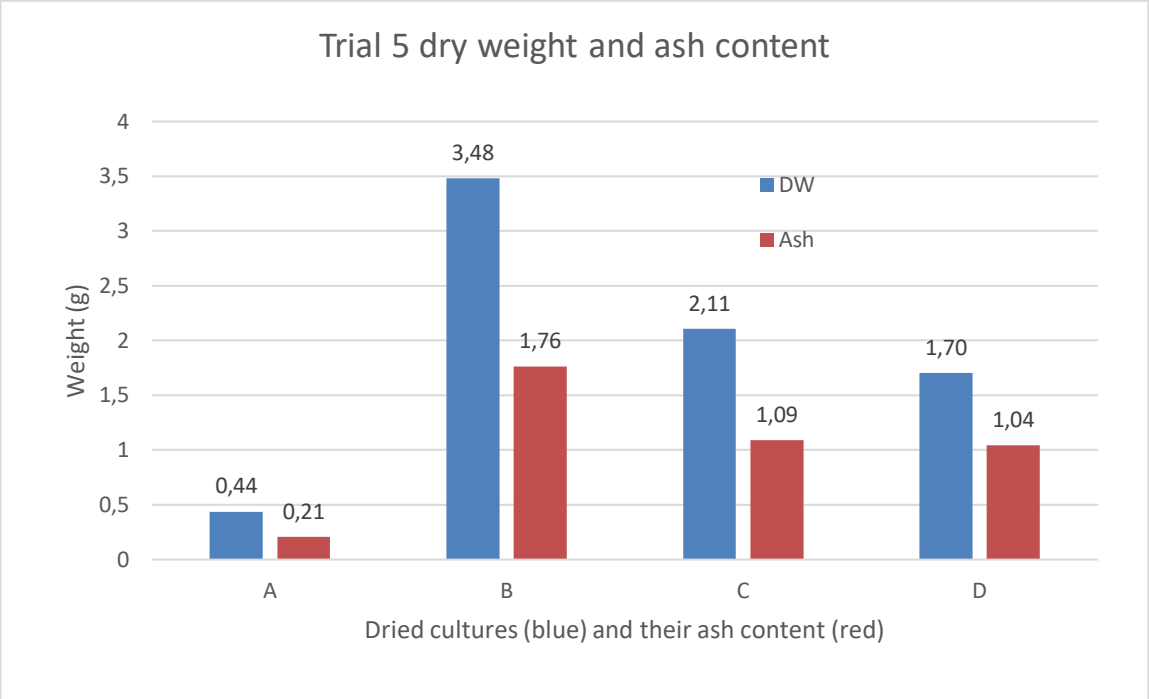


***Figure 3*** *Results from trial 5 after biomass separation, centrifugation, drying and burning*

only at the centrifugated and air-dried biomass data (Figure 5), the growth on effluent without Conway nutrient mixture is not significantly lesser.


***Figure 4*** *Air dried cultures from trial 5. Culture D grown on effluent without Conway nutrient mixture is lighter in colour than other cultures which may point to nutrient imbalance.*

Two-tailed t-test for equal variances was done to calculate the differences in biomass of UCB. The difference of *Nannochloropsis sp.* accumulated biomass if cultivated with added nutrient mixture and effluent (M = 14.36, SD = 2.55, n = 7) and only with effluent (M = 11.11, SD = 3.28, n = 3) (Figure 5) was found not significant, $t$ (8) = 1.71. Highest UCB quantity after centrifugation for this species was recovered from trial 5 (18.76 g) for algae grown with nutrients (Appendix 3). Highest UCB recovery for algae without nutrients was also in trial 5 (13.94 g) (Appendix 3). However, cultures D in trial 5 to 8 grown on effluent without Conway nutrient mixture showed heavy discoloration when compared to those with higher nutrient in the medium, as seen in Figure 4.

Results from a protein and fat content analysis for trial 6 can be seen in Table 1. Ash, protein, and fatty acid levels were measured from the biomass samples while dry matter

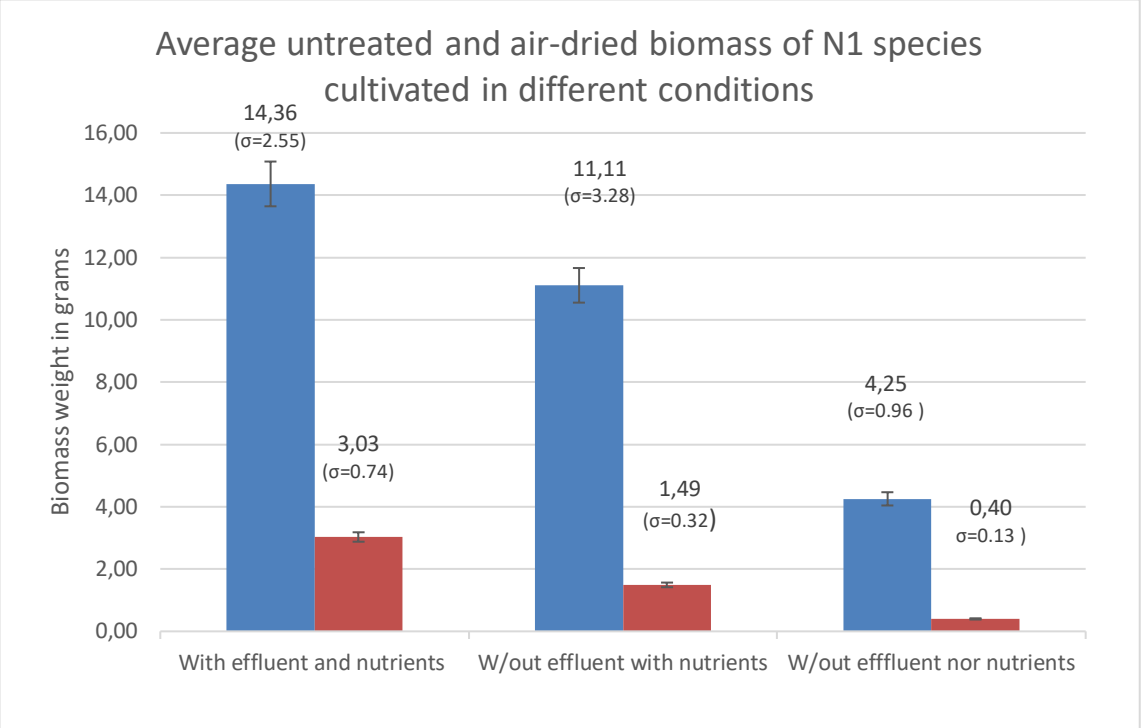


***Figure 5*** *Cultivation of N1 species, data for UCB (blue) and air-dried cultures (red)*

was then extracted by heat, exposing the biomass first to 103°C for dehumidification and to 600°C to remove all organic matter and find the ash content.

These measurements give a good approximation, but more detailed analysis of all compounds is needed to show how the nutrient and organic matter compositions compare between the different algal species. The comparison would also show whether the growth environment should be changed to improve the biomass quality.

***Table 1*** *Soxhlet-analysed extracted biomass from cultures in trial 6. The analysis show percentages of dried matter, ash, protein, and fat extracted from each culture in the same trial*

| Compound (expressed in grams) | Dry matter | Ash | Protein | Fatty acids |
|---|---|---|---|---|
| *Culture A* | 9.8 | 5.8 | 2.1 | 0.07 |
| *Culture B* | 11.7 | 6.9 | 2.2 | 0.11 |
| *Culture C* | 14.6 | 8.1 | 2.9 | 0.34 |
| *Culture D* | 10.7 | 6.1 | 2.1 | 0.08 |

Appendix 3 contains all biomass extraction results and monitored cultivation conditions from all 12 trials.

### *3.3 Nannochloropsis oculata 849/1*

Growth of species N2 with and without the effluent was compared in trials 9-12. Average values for trials with species N2 are shown in Figure 6. Highest UCB of N2 cultures grown with effluent was 9.65 g and 6.20 g without effluent (Appendix 3). After a t-test assuming equal variance, a significant difference, $t$ (10) = 1.84 was shown in growth of cultures without effluent (M = 6.45, SD = 2.21, n = 8) and without effluent (M = 4.02, SD = 2.06, n = 4).

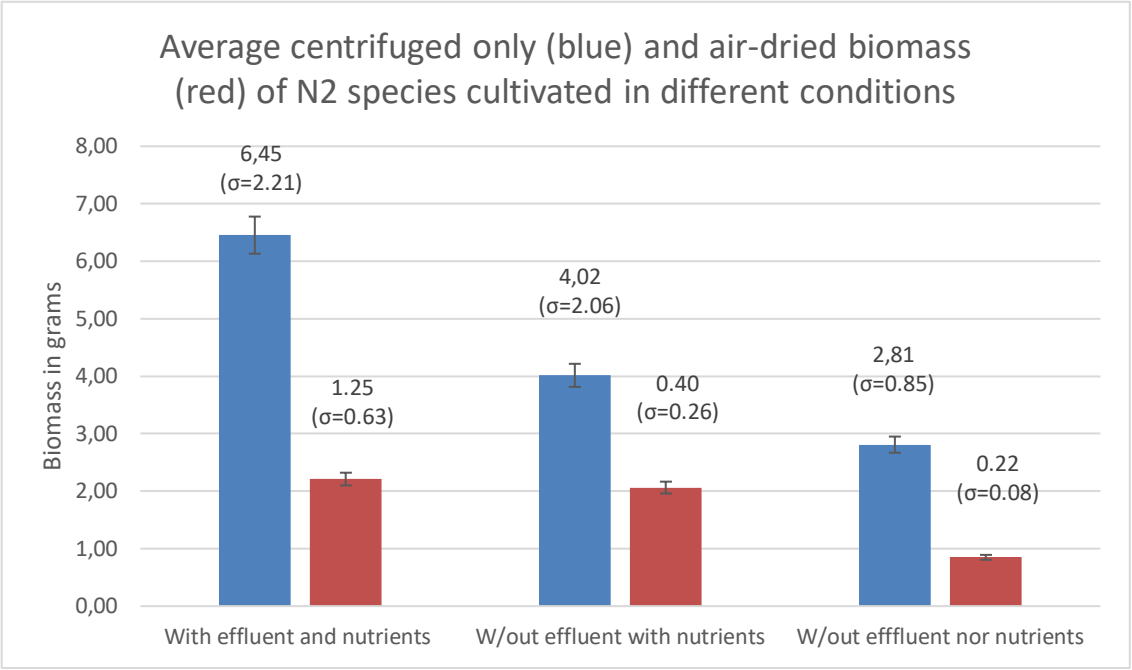


***Figure 6*** *N. oculata 849/1 cultivation. Bars represent standard deviation of 8 samples (with effluent and nutrients), 4 samples (without effluent with nutrients) and 4 samples (without effluent nor nutrients) for both UCB (blue) and air-dried (red) cultures*

The filtration losses may explain the lower biomass recovery from N2, however there may be more factors influencing the lower biomass recovery as in trial 9 (which had the desired higher salinity) this species had lower biomass production then trials 10, 11 and 12 which had lower salinity. It may also mean that the N1 species can grow in less saline conditions, which is positive.

### *3.4 Chlorella vulgaris*

In trials 7 and 8 the growth of species CV was compared to the growth of species N1 and N2. All four CV cultures were grown in the same environmental conditions. Salinity ranged from 27-31 ppt. Temperatures in the beginning of cultivation ranged from 15 °C to 21°C. Nutrient mixture and effluent were added to all cultures in these trials. Average values for these trials are shown in Figure 7. *C. vulgaris'* highest UCB was 16.08 g and lowest 13.49 g (Appendix 3). CV species had highest biomass production per trial of all three species cultivated in this research.

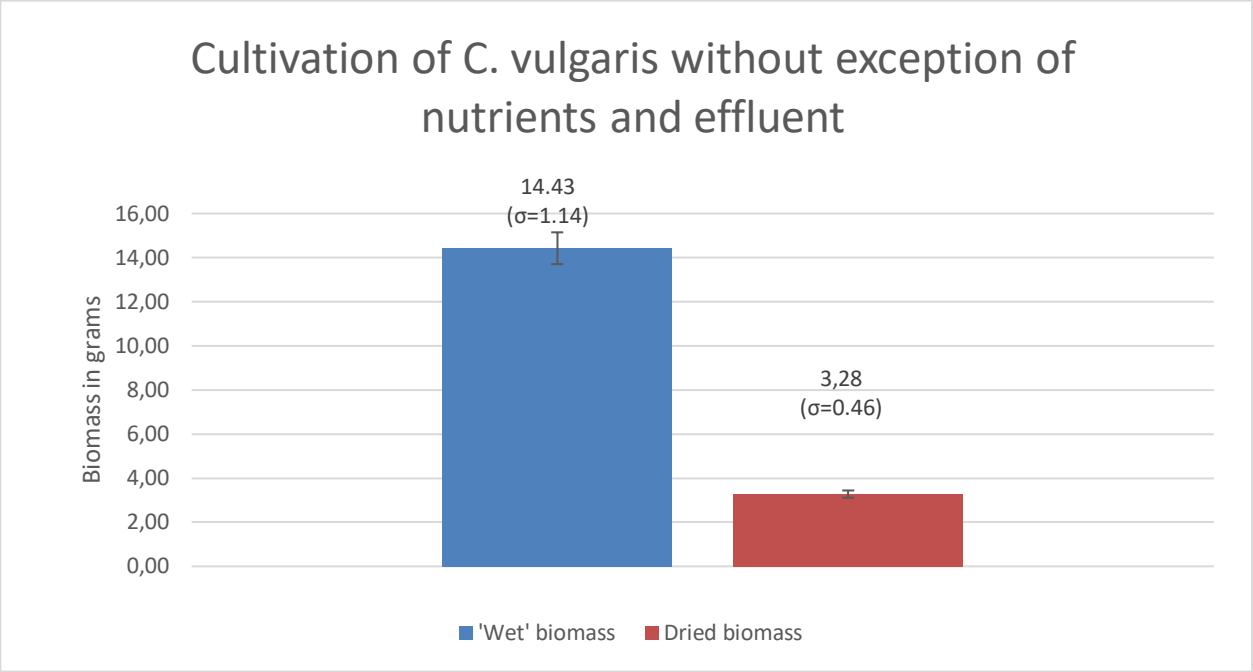


***Figure 7*** *C. vulgaris was grown in two trials with both effluent and nutrients added. Average values of the four cultures are shown*

## *3.5 Growth conditions*

### 3.5.1 Aeration

Aeration is important for growth because it supplies $CO_2$ and creates movement in growth medium which exposes the algal cells to light needed for photosynthesis. Constant efforts were needed to adjust the airflow in both bottles and bags. The airflow would often completely stop regardless to the efforts, and sometimes individual cultures had too slow or too fast air flow (Appendix 3). These obstructions likely affected the final biomass quantities, as too slow aeration inhibits growth (no $CO_2$ supply or medium movement - no light for algal cells). Problems also occurred with bottles if the metal tube was too tight in the PU tap causing the rise of air pressure in a bottle. The airflow would then become slower or stop completely in that bottle, making the airflow in other bottles also change. This was addressed by widening the hole in which an individual tube was inserted in the tap, allowing the air to escape the bottle. After that the problems occurred when the end opening of the tube fell flat to the bottom of the bottle. As the pressure of the air pumped by the small aquarium pump was low, the weight of the tube was enough to stop the airflow in the tube. The tubes were then fastened in the taps which held them in place and

away from the bottom, while still allowing the air to escape and not create high pressure in the bottle. Similar problems occurred during the cultivation in bags - imbalance in the airflow would occur if an air hose surfaced the cultivation medium allowing most air from the system to escape through its bubbler. The air flow in the other bags would then drop (all bags were connected to a pump through a central pipeline system). This was partially solved by attaching aquarium bubblers and adding weight to the hoses, but the bubblers then started sinking to the bottom of the bags causing increase of air flow in other bags. The hoses were then taped to the bag to solve this, but the tape would usually fall off due to dampness in the environment. All these problems were managed through constant monitoring and adjustment efforts.

### 3.5.2 Acidity levels (pH)

The pH levels were not in any specific way adjusted. They ranged from 6.3 to 7.3 pH just after inoculation in bottles and bags, and 8.1 to 9.2 after 7 day cultivation in bottles and

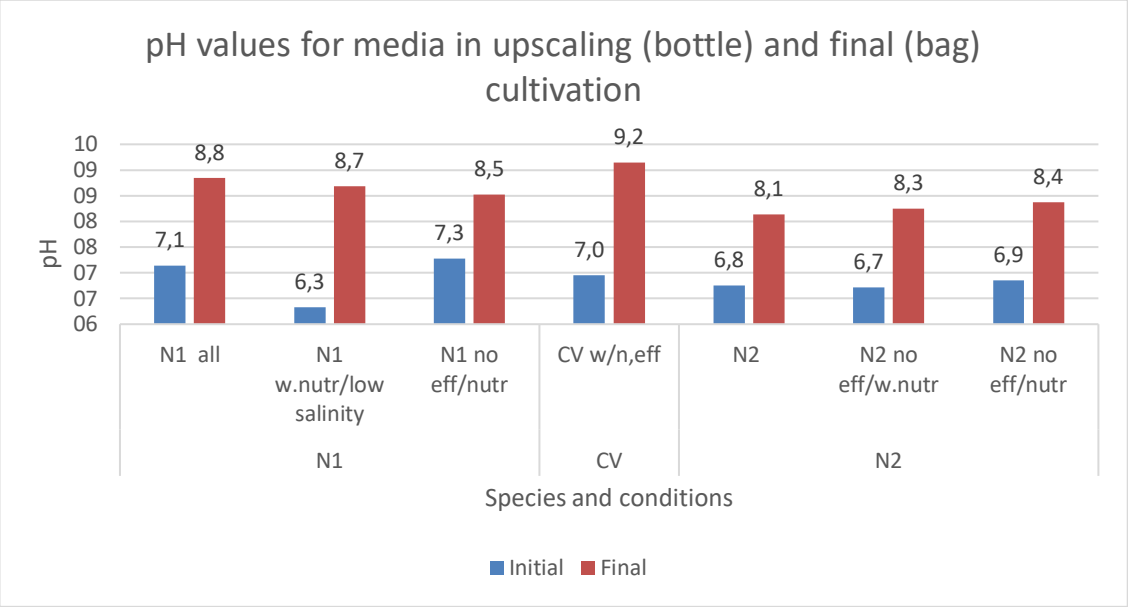


***Figure 8*** *Average pH levels in the beginning (blue) and in the end (red) of cultivation.*

bags (Figure 8). The inoculation in bottles should have theoretically reached the highest density after one week, and by mixing these cultures from bottles to bags for new inoculation the growth curve was kept close to the ideal exponential. The cultivation medium became more basic towards the end of the trials in most cultures regardless of the medium content, salinity, or nutrient/effluent addition. In trial 5 (species N1), when highest biomass quantity from a culture grown without nutrients was retrieved (culture

D) (Appendix 3), the pH before harvesting was lower than average (6.8), compared to 8.5 average for cultures without nutrients in the same trial (Figure 8).

### 3.5.3 Salinity

The average salinity levels ranged from 22 ppt to 31 ppt and remained stable during trials. The initial salinity was measured when bottles and bags were ready for cultivation. The final salinity in the bottles was measured before the medium from them was mixed into the bags. The final salinity from the bags was measured when the medium from them was taken for dewatering.

### 3.5.4 Temperature

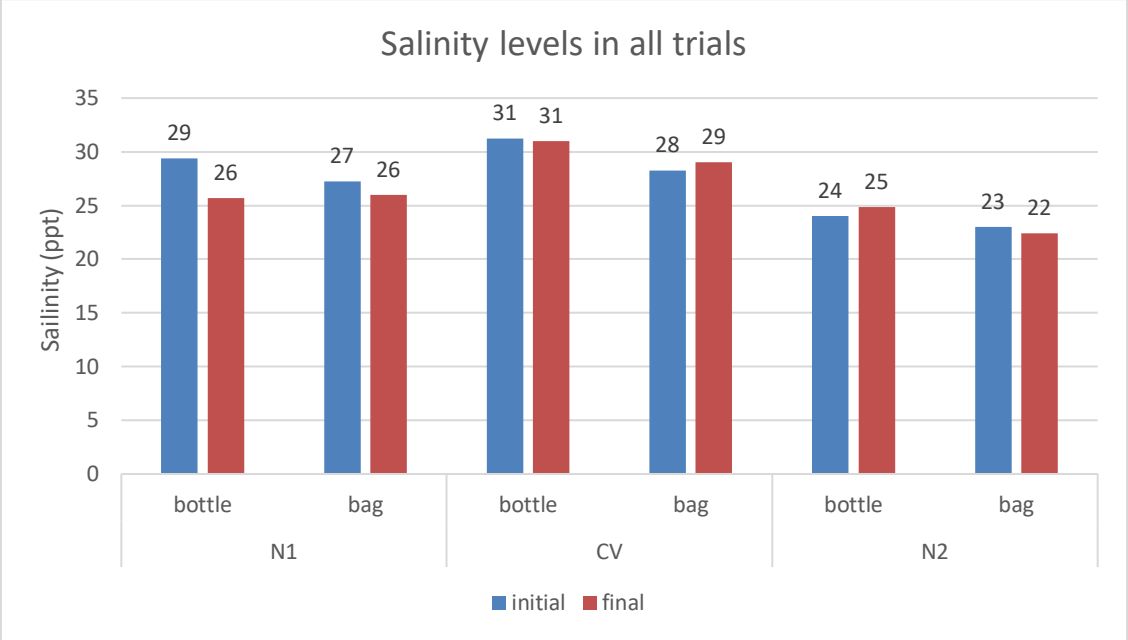


***Figure 9*** *Average salinity levels for all trials, recorded in the beginning and the end of cultivation in both bottles and bags*

The temperature shortly after inoculation ranged from 12°C to 20°C in bottles and bags. The temperatures of the medium reached room temperatures within several hours after the inoculation. The final temperature after cultivation periods in bottles in bags ranged from 19°C to 32°C. Room temperature was controlled by heat blowers in the rooms and fluctuated sometimes due to the routine working activities of Matorka's employees.

### 3.5.5 Filtration process

A 50 ml sample taken from the already filtrated cultivation medium (which was normally discarded) from trial 4 retrieved 8.4 mg of algal biomass after it was centrifugated. Translated to the full size 15 L medium, the amount of the lost biomass would be 2.5

grams. The highest extracted amount in one trial was 16.08 g. The extraction model obviously needs improvement as the amount of un-filtrated microalgae is potentially as high as 15%. In trials 9 – 12, when a synthetic polyester material was used for filtration, the losses were likely much higher because of the higher porosity of that material, which may explain why the N2 species had much lower biomass.

# 4. Discussion

## *4.1 Summary*

Successful growth of all three cultures (N1, CV, N2) proves that microalgal cultivation is a potential solution to at least partially solving the challenges of aquaculture. After additional research it can become clear how much microalgae can contribute to aquaculture sustainability. Sustainability, stability, and quality of fish feed resources are the biggest challenges aquaculture is facing, as the main resource providing necessary nutrients is one fish, the Peruvian anchoveta. Terrestrial plant substitutes for the anchoveta are used today, but they need alteration in form of Omega-3 acids to make up for nutrient deficiency. Since many microalgae are aquatic primary producers, they may belong to the same food chain as the fish, which means they (microalgae) may also have the ideal nutrient composition for the fish. Microalgae cultivated on fishery effluent could help solving the environmental challenges caused by the effluent's release and lower the chances of eutrophication. There are, however, difficulties with algal cultivation itself - it has a high carbon footprint, caused by the electricity, produced in unsustainable way. A solution may be in using sustainable sources of electricity, where available. This would lower the carbon footprint of algal cultivation depending on the way electricity is produced.

Another problem of microalgal cultivation is the industrial pumped-in carbon dioxide which contributes both to the complexity of cultivation and its higher costs, and often carbon footprint. It was known to be theoretically possible to use atmospheric $CO_2$ instead, and looking at the experimental results, this research proves that concept - all three microalgal species grew successfully without the $CO_2$ sourced from the atmospheric air. Omitting the addition of pure $CO_2$ completely by introducing semi-intensive systems, microalgal cultivation with fishery effluent would be made less energy demanding and less expensive. This kind of semi-intensive cultivation would, like all other types of cultivation on effluent, create a circular economy by turning waste (effluent) into value (microalgal biomass). It would also additionally avoid the demanding energetic and production setups, reducing the need for carbon offsetting.

The last considered challenge of microalgal cultivation lies in legislation – as microalgae are new in the industrial sector, the legal aspect for microalgal cultivation is in most countries still unregulated or undefined, which means the regulations set by authorities may in future limit or obstruct the usage of microalgae.

### *4.2 Challenges of sustainable aquaculture feed*

As already mentioned, because of their high Omega-3 fatty acid content (which can account for as high as 50% of their total mass) (Breuer et al., 2013) microalgae have the potential to supplement the fish feed and be more suitable for it than the terrestrial plant products. Research has shown that plant-based feed coated with 10% extracted microalgal oil was beneficial for the growth of farmed salmon and resulted in much higher Omega-3 oil content (Gíslason, 2020). In fact, not only did the Omega-3 content of the tested fish significantly increase, but so did the amount of palmitoleic acids, resulting in the increased natural pathogen protection, most importantly against the salmon louse (*Lepeophtheirus salmonis*) (Gíslason, 2020). The salmon louse is a parasite threatening farmed salmon populations, infesting the farmed fish, and transmitting itself to wild fish populations, causing substantial economic and ecological losses to aquaculture and other industries (Elghafghuf, Vanderstichel, Hammell, & Stryhn, 2020; Torrissen et al., 2013). The development of salmon´s natural resistance to the louse alone would be worth the necessary research for inclusion of microalgal oil in the farmed fish diet.

The fatty acid levels from trial 6 this of research are seen in Table 1. The fatty acid levels in the N1 culture grown with effluent as the only nutrient source (culture D) were similarly low as the fatty acid levels in the control culture (culture A), grown in low salinity. Cultures grown with effluent and nutrient mixture had a higher fatty acid content (Table 1, cultures B and C). This may be caused by nutrient deficiency in the effluent. Further content analysis of the effluent can help understand these differences and similarities, as the effluent content may be different depending on the time it is obtained - nutrient fluctuations in fish tanks may be related to the fish feeding time. Content analysis could show these differences in the effluent and point to what they could be related.

Feeding farmed fish by growing terrestrial plants, which already directly or indirectly feed the entire human population, means competing for arable land and water in an already burdened industry. In addition, the alternative fuel production efforts (biodiesel, methanol) have entered the terrestrial plant market and are often boosting the prices of plant-based products up by competing for the mentioned resources (land and water). Microalgae do not enter that competition when it comes to land usage, as they can be grown on non-arable lands, using much less space per unit of area than terrestrial plants. Lastly, they can be grown in industrial areas, close to the production facilities, which lowers the need for their transport (Dębowski, Zieliński, Kazimierowicz, Kujawska, &

Talbierz, 2020). At Matorka's facility, the water for the trials was pumped in at the same premises as the algal cultivation took place, while the effluent had to be transported from the fish tank outlet some 200 meters away. If the algal cultivation were to take place at the same facility, all the contents would be well within reach for implementing a more convenient system through pipelines which would connect directly to the microalgal inoculation area.

Problems of introducing algal oil as fish feed supplement could also be in growth lags related to the changes of the diet the fish receive, as it can take time for the fish to get used to the new feed (if the feed is different from the one the fish are used to). This is especially important if the hatcheries that supplied the fish use standard plant oil based feed with addition of fish oil, instead of microalgal oil enriched one (Gíslason, 2020). The oil and fatty acid contents from the trials at Matorka prove that it is possible, with adjustments in cultivation medium content, to grow the microalgae suitable as additive for fish feed. These microalgae could make an ideal additive to the plant-based fish feed, after the extraction of oil. The oil would be then incorporated to the fish feed. Alternatively, it would be possible to grow species which the fish could digest directly, without compound extraction. For this a careful selection of microalgae would be necessary, as adult fish is not likely to accept microalgae as the only feed ingredient. In some hatcheries, microalgae are cultivated as feed for juvenile fish (Oostlander, van Houcke, Wijffels, & Barbosa, 2020). Adopting the same practice would facilitate easier introduction of the algae-based feed to the adult fish by lowering or voiding the lag period due to the feed change. In other words, the adult fish would not experience extensive changes in diet, as opposed to jumping from plant-based diet in the hatchery to microalgal oil enriched in adult stage.

### *4.3 Cultivating microalgae on fishery effluent as means of mitigation for land-based aquaculture challenges*

Despite being present for a long time, aquaculture is still a developing industrial branch, as it has not been practiced as intensive until recently. Legislature is following its development, and the additional regulations still need to be applied. The European Union proposes the recycled water quality to be equal as potable water, while Icelandic authorities limits the growth of human intended food on fishery effluent (*EU Regulation on the hygiene of foodstuffs*, 2004, M. Þorsteinsdóttir, personal communication, 2021). However, if the effluent is used for fish feed production, the process is seen as environmentally friendly, and recycling/reuse of waste materials is encouraged (M.

Þorsteinsdóttir, personal communication, 2021). The laws yet must address the specific subject of microalgal cultivation and set the rules for it.

Eutrophication caused by the release of effluent is one of environmental challenges aquaculture is facing today. Eutrophication is estimated by measuring the primary production of phytoplankton caused by excessive nutrient availability, mainly nitrogen in the form of nitrates and nitrites, and phosphorus in the form of ortho-phosphates (Ærtebjerg et al., 2001). Nutrient surplus causes phytoplankton blooms which can lead to organic matter settlement in the bottom. The accumulation of organic matter changes the species composition and depletes oxygen levels by facilitating growth of oxygen consuming species and seafood resource depletion (Ærtebjerg et al., 2001). This destabilizes ecosystems and the businesses connected to them. The amount of nutrients we dispose into the nature should ideally be zero, which gives us our goal – the quantity of nutrients released into the nature from the aquaculture is the quantity which we need to eradicate.

The elements present in the effluent are nitrates, nitrites, and phosphates - the amount Matorka has is 25 kg of nitrate ($NO_3^-$) per day and 5 kg orthophosphates/day (Darrason, 2016). Microalgae can successfully capture most nitrogen and phosphates from effluent and their productivity ranges from low to exceedingly high amounts, depending on the cultivation methods, which need to be chosen carefully (Oostlander et al., 2020). Dangers of eutrophication can be lowered or avoided if Matorka lowers or ceases the release of nutrients into the ecosystem. Allowing microalgae to absorb these nutrients is proven to be possible and does not require any demanding changes to the infrastructure, especially if their cultivation remains as a low-impact, semi-intensive type one. Based on the experimental cultivation results presented here, it is difficult to say how much microalgae Matorka would have to produce to completely clear the effluent from nutrients, but the limiting factors may be calculated using the Redfield ratio of C:N:P which is 106:16:1. If we compare the data from Matorka and assume that the effluent has similar composition and nutrient values as the effluent from Silfurstjarna (Darrason, 2016), the N:P ratio is somewhat disturbed and a high surplus of phosphates is clear compared to nitrates and nitrites (0.20 mg/L of orthophosphate and 0.13mg/L nitrates) while nitrites were found in very low amounts.

Light (together with carbon dioxide) is most crucial factors that influencing the scale and quantity of microalgal cultivation. Artificial lighting is more efficient than natural light, but it also costs (more) as it needs electric energy. A compromising solution would be the

use of greenhouses for microalgal cultivation. Greenhouses use natural light, but their productivity is lower in dark winter months. To compensate, they are normally equipped with artificial lighting. Combining the natural and artificial lighting could lower the expenses if the premises are properly designed. The shape of cultivation vessels should allow the light to penetrate deep enough for all algal cells to get enough photons, without lag zones in the middle which can cause light deficiency (Huang et al., 2016). The width of the tubes of around 40 to 50 cm in diameter can achieve good results if separate columns are used (Chini Zittelli, Rodolfi, Biondi, & Tredici, 2006). Bags used in this research had a slightly lower diameter, but the size may still be comparable. The pumped in air needs to create enough turbulence for all the algal cells to get exposed to light.

### *4.4 Carbon footprint of microalgal cultivation*

The already mentioned excessive $CO_2$ cost may possibly be lowered to some extent. $CO_2$ needs to be supplied to increase the favourable growth factors for the algal cells in the water. Constant turbulence in the medium is necessary if the algae are suspended/confined within tubes in photobioreactors. The supply of bubbled gas improves the growth and at the same time increases the exposure of algae to the light by creating motion in the suspension. One option of reducing the high $CO_2$ cost is using industrial flue gas if available, which highly reduces the carbon footprint and consequently lowers the production costs (Doucha, Straka, & Lívanský, 2005). As there was no available surplus of $CO_2$ in these trials, atmospheric air was bubbled through the medium instead. Regardless to the low $CO_2$ content in the atmospheric air, it was possible to successfully grow microalgae, which is in accordance with some previous research (Ataeian et al., 2019). If the atmospheric $CO_2$ is used, the air still needs to be pumped through the system. The air supply is done by air pumps, so the electricity cost remains, but the expense of having to supply the $CO_2$ externally is cut.

Electricity is, however, the highest issue in microalgal cultivation, both financially because the cultivation itself is energy (electricity) demanding, and environmentally because of the unsustainability in the way the electricity is produced. In the operational part of the cultivation, electricity is by far the largest part of the energy pie chart, as it is used for heating and pumping of the medium through the setup (Mata et al., 2018). Electrical lighting is often also a large and mainly inefficient expense, especially when it comes to energy conversion (only 4% to 6% of energy is converted) (Blanken, Cuaresma, Wijffels, & Janssen, 2013). To avoid these expenses and contribute to sustainability, it is

possible to turn to more sustainable electricity sources, which are available if a particular area is rich in natural power like geothermal and hydropower. In Iceland, electricity is produced almost exclusively by hydropower (72%) and geothermal sources (26%), with recent introduction of windmills (Orkustofnun, 2020). By using clean energy, the carbon footprint for the operational part of the cultivation would be lowered, and in Matorka's case no heavy carbon offsetting would be needed. If the algae were produced and used at the (same) fishery, their transport as a ready product (algal biomass) would also be minimal, and the only footprint would thus be in the production of the cultivation equipment.

### *4.5 Confirmation of a semi-intensive microalgal cultivation concept combined with the use of aquaculture effluent*

As shown, there are numerous problems aquaculture is facing to achieve sustainability. Terrestrial plants used for fish feed require a marine supplement for the farmed fish to achieve the nutrient content it needs as the final product. The aquaculture effluent released into the nature causes environmental problems, but it can also be an ideal source of nutrients for microalgae. Microalgae could be the link for the solution of these two tightly connected problems, but their cultivation is burdened with a separate set of problems, such as high $CO_2$ footprint and energy demands. Addressing these problems by applying cultivation with lower intensity could help, but the line is thin when it comes to profitability of such systems. Lower energy input could be solved by minimizing use of lights, pumps, and centrifugation. Avoiding the additional use of $CO_2$ could make the cultivation even less extensive, however these cut downs also come with the expense of less productive cultivation. On the other hand, lower productivity may be justified by the environmental factor, as the algae would be used as an effluent remedy and carbon sink.

In this research, photobioreactors with uncomplicated design were used with fluorescent lighting and stationary cultivation medium mixed by air movement. This way the impact of several most intensive (and expensive) cultivation processes was lowered – firstly, the cultivation medium was stationary and not pumped through the entire cultivation system, secondly, less energy intensive lights were used (fluorescent instead of LED), and finally, no $CO_2$ other than from atmospheric air was pumped into the system. Also, no pH or salinity control was applied, except from initial and final measurements. When combined with the re-use of the effluent, this system can be best described as semi-intensive with low energy input.

Growth was shown in all trials and no significant difference was recorded when algae were grown on effluent only compared to the ones grown on both effluent and with nutrient mixture added. The total dried biomass from culture D in trial 5 was 1.70g and the amount of water was 15L. The electricity used in the upscaling trials was drawn by two 18 Watt flourescent bulbs (36W total) and had total consumption of 10.5 kWh in the seven day period. The main cultivation used four 18W light bulbs (72W total), consuming 21 kWh during the seven day cultivation, or 31.5 kWh in total to produce the four cultures in trial 5, or 7.88 kWh for the biomass of culture D (1.70g). This means it would take 4635 kWh to make one kilo of dried biomass. Taking the highest industrial price for the production and delivery of electricity, which is around 15 ISK/kWh (Verðskrá fyrir dreifingu og flutning raforku, 2020), and calculating the price of electricity used per kilo of algae, the amount of their production would be 69529 ISK. Hot water used for heating costs at HS Orka around 150 ISK/$m^3$ (HSveitur, 2020). Supposing that the area needed for the cultivation equipment and its management was approximately 10 $m^2$ this would add another 1500 ISK, using the highest ratio of 1:1 (cubic water/square meter) for the usage. The approximate price of electricity and heating would thus be around 71000 ISK, apart from the costs of housing and labour which come on top of this. This final price is likely within the range of small-scale productions (Oostlander et al., 2020). When compared to the price of industrial feed (Fóðurblandan, 2020) it is not competitive, if introduced in the ten percent magnitude as suggested by Gíslason (Gíslason, 2020). However, the price is expected to become very soon more competitive, with the change of regulations and intoduction of penalties for the release of effluent, and improved biomass separation techniques.

This research proved the conceptual possibility of growing microalgae by using effluent from land-based fishery, and a low-tech semi-extensive cultivation setup. To fully combine the effluent disposal and $CO_2$ mitigation before the next cultivation the effluent content should be analyzed. After analysis of effluent the necessary improvements to the cultivation based on this research could be made. Improvements in filtration/biomass separation and cultivation setup are clearly necessary, and can only positively contribute to all the addressed questions. Algal strains for future cultivation should be carefully selected, and either experimentally fed directly to the farmed juvenile and adult fish or supplemented as extracted fish oil. Introducing microalgal feed or supplement would supply the necessary nutrients and improve the acceptance of microalgal oil enriched feed in the transition from juvenile to adult feed.

## 5. Conclusion

Fish feed supplement in form of microalgae could make an ideal replacement for the overfished anchoveta and improve the terrestrial plant feed. Its high Omega-3 fatty acid contents increase the fatty acids in the fed fish, giving it more market value and natural pathogen resistance. However, microalgae grown at high rates producing large biomass quantities come with a high energy bill and carbon footprint. High energy inputs are attributed to electricity, which is used for lighting, medium circulation, CO2/air bubbling, biomass separation and processing. Electricity is also responsible for a high environmental footprint, due to carbon emissions of its production. The second largest carbon footprint of microalgal cultivation comes from nutrients - $CO_2$, N and P. High footprint of $CO_2$ contributes to its production and transportation. Nitrogen needs to be synthesized and phosphorus is only found in nature, thus finite. Avoiding the extensive use of electricity by changing and adapting the microalgal cultivation systems lowers this footprint. The carbon footprint is additionally lowered if cultivation recycles fishery effluent rich in N and P. Finally, by using atmospheric $CO_2$ instead of injecting it alone, the eco-footprint of microalgal cultivation is minimized. With the constant improvement of industry, these environmental aspects are increasingly achieving value, and may soon be obligatory standardized.

While the overall proof-of-concept in this combination of semi intensive microalgal cultivation showed promising results, the setup could be improved on multiple levels. The nutrient contents of microalgae should be examined to find the species most suitable for cultivation, and the best cultivation methods and conditions. The use of daylight instead of artificial lighting should also be considered, especially for utilizing the Icelandic polar day conditions with 24-hour sunlight. Cultivation located in a greenhouse may allow for the best usage of daylight, combined with artificial lights for the winter periods. Species accustomed to growth in freshwater and brackish conditions such as CV would be best for the use of effluent without the addition of water. Plastic columns of the same or adjusted proportions would be better instead of plastic bags, which were discarded at the end of each trial, as the columns could be re-used. The columns would also be easier to work with and shorten the whole harvesting process, allowing for the cultures to be poured out for processing. Inoculation could be done by leaving a part of the medium in the column and filling the column with new effluent. Centrifugation as a separation method is very efficient but also energy demanding and expensive. Combining centrifugation with other separation methods could lower the costs. A two-step separation

with centrifugation as the last step would be possible. Low-intensity methods like gravitational settling, microwave separation or electro-flocculation should be considered as the first step. When most water is separated from the biomass, the centrifugation could finally remove the remaining remediated supernatant which does not contain any nutrients and can be used as recycled water.

In theory, the re-use of fishery effluent in low energy cultivation combined with using atmospheric $CO_2$ is possible and has lots of positive aspects. In practice, these ideas need adaptations and may turn out that intensive cultivation types are economically more viable. In Iceland, where the electricity carries a low carbon footprint, more intensive cultivation may be a better choice. But the environmental footprint, the introduction of carbon offsetting legislation, and simplicity of low-tech setups like this could justify their application.

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# 7. Appendix

## *7.1 Appendix 1: Conway mixture*

30g NaCl

0.3g KCl

6.6g MgS

0.5g $NaHCO_3$

1.3g CaCl

## *7.2 Appendix 2: Artificial sea water mixture*

45.0g $Na_2$ EDTA

100.0g $NaNO_3$

34.0g $H_3BO_3$

20.0g $NaH_2PO_4$

0.4g $MnCl_2$ $4H_2O$

1.3g $FeCl_3$ $H_2O$

## *7.3 Appendix 3*

Contents and data from measurements for all trials; results of trials (weight of algal biomass obtained). Shortcuts (explanation): BHS = Borehole sea water, Eff = effluent, FW = fresh water

| | | | | | initial data | | | | | | final data | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Species | Culture | Water | Conway ml | °C | pH | Salinity ppt | airation | °C | pH | Salinity ppt | WW (g) | DW (g) | Recovery % wet/dried |
| 2017 | Trial1 | Flask | N1 | A | FW/Eff 1/1 | 5.0 | n/a | n/a | 20 | average | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N1 | B | FW/Eff 1/1 | 5.0 | n/a | n/a | 20 | average | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 5.0 | n/a | n/a | 27 | average | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N1 | D | FW | 5.0 | n/a | n/a | 8 | average | n/a | n/a | n/a | n/a | n/a | n/a |
| | | Bag | N1 | A | FW/Eff 1/1 | 10.0 | n/a | n/a | 20 | average | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N1 | B | FW/Eff 1/1 | 10.0 | n/a | n/a | 20 | average | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 10.0 | n/a | n/a | 27 | average | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N1 | D | FW | 10.0 | n/a | n/a | 8 | average | n/a | n/a | n/a | n/a | n/a | n/a |
| 2018 | Trial 2 30.1.-14.2. | Flask | N1 | A | FW/Eff 1/1 | 5.0 | n/a | n/a | n/a | average | 23.1 | 9.3 | 7 | n/a | n/a | n/a |
| | | | N1 | B | BHS/Eff 3/1 | 5.0 | n/a | n/a | n/a | average | 23.1 | 9.8 | 25 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 5.0 | n/a | n/a | n/a | average | 23.1 | 9.8 | 28 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | 5.0 | n/a | n/a | n/a | average | 23.1 | 9.8 | 27 | n/a | n/a | n/a |
| | | Bag | N1 | A | FW/Eff 1/1 | 10.0 | 15.6 | 5.3 | 4.0 | average | 20.2 | 10.2 | 7 | n/a | n/a | n/a |
| | | | N1 | B | BHS/Eff 3/1 | 10.0 | 15.5 | 7.6 | 26.0 | average | 19.7 | 10.2 | 25 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 10.0 | 15.5 | 7.0 | 24.0 | average | 19.4 | 10.2 | 25 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | none | 15.5 | 7.8 | 23.0 | average | 19.1 | 9.8 | 25 | n/a | n/a | n/a |
| | Trial 3 6.2.-20.2. | Flask | N1 | A | FW/Eff 1/1 | 5.0 | 15.8 | 6.0 | 8.0 | average | 31.0 | 9.7 | 3 | n/a | n/a | n/a |
| | | | N1 | B | BHS/Eff 3/1 | 5.0 | 15.3 | 7.2 | 26.0 | average | 32.1 | 9.9 | 24 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 5.0 | 15.3 | 7.8 | 25.0 | slow/poor | 32.6 | 9.8 | 24 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | none | 15.3 | 6.8 | 24.0 | average | 31.9 | 8.5 | 25 | n/a | n/a | n/a |
| | | Bag | N1 | A | FW/Eff 1/1 | 10.0 | 16.0 | 6.6 | 7.0 | slow/poor | 18.9 | 6.3 | 7 | n/a | n/a | n/a |
| | | | N1 | B | BHS/Eff 3/1 | 10.0 | 17.4 | 6.2 | 27 | slow/poor | 18.9 | 8.6 | 26 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 10.0 | 18.3 | 6.5 | 27 | slow/poor | 19.1 | 8.5 | 25 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | none | 17.3 | 7.7 | 13 | slow/poor | 18.6 | 7.1 | 13 | n/a | n/a | n/a |
| | Trial 4 14.2.-27.2. | Flask | N1 | A | FW/Eff 1/1 | 5.0 | 16.4 | 6.7 | 15.0 | average | 25.4 | 8.5 | 14 | n/a | n/a | n/a |
| | | | N1 | B | BHS/Eff 3/1 | 5.0 | 16.8 | 7.4 | 26.0 | average | 26.3 | 8.5 | 31 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 5.0 | 16.7 | 7.4 | 30.0 | average | 26.1 | 8.4 | 31 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | none | 15.8 | 7.9 | 25.0 | average | 24.8 | 8.6 | 24 | n/a | n/a | n/a |
| | | Bag | N1 | A | FW/Eff 1/1 | 10.0 | 18.0 | 5.9 | 20 | average | 23.5 | 8.2 | 8 | 4.03 | n/a | n/a |
| | | | N1 | B | BHS/Eff 3/1 | 10.0 | 18.0 | 6.3 | 29 | average | 23.5 | 9.5 | 25 | 12.87 | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 10.0 | 17.5 | 6.4 | 29 | average | 23.5 | 9.4 | 25 | 11.54 | n/a | n/a |
| | | | N1 | D | FW/Eff 1/1 | none | 17.4 | 6.4 | 28 | average | 23.5 | 8.1 | 25 | 6.33 | n/a | n/a |
| | Trial 5 20.2.-6.3. | Flask | N1 | A | FW/Eff 1/1 | 5 | 22.8 | 6.2 | 14.0 | average | 29.2 | 9.2 | 13 | n/a | n/a | n/a |
| | | | N1 | B | BHS/Eff 3/1 | 5 | 14.5 | 6.4 | 31.0 | average | 29.4 | 9.5 | 26 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 5 | 14.5 | 6.3 | 26.0 | average | 31.6 | 8.9 | 26 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | none | 14.3 | 6.5 | 29.0 | average | 31.6 | 8.4 | 26 | n/a | n/a | n/a |
| | | Bag | N1 | A | FW/Eff 1/1 | 10 | 20.5 | 6.4 | 11 | fast | 21.4 | 9.5 | 8 | 4.93 | 0.49 | 9.89 |
| | | | N1 | B | BHS/Eff 3/1 | 10 | 19.5 | 7.2 | 26 | fast | 21.1 | 8.8 | 26 | 18.76 | 3.93 | 20.94 |
| | | | N1 | C | BHS/Eff 3/1 | 10 | 19.8 | 7.7 | 26 | fast | 21.1 | 8.6 | 25 | 12.84 | 2.35 | 18.33 |
| | | | N1 | D | BHS/Eff 3/1 | none | 18.3 | 6.6 | 29 | fast | 20.8 | 6.8 | 26 | 13.94 | 1.84 | 13.20 |
| | Trial 6 27.2.-13.3. | Flask | N1 | A | FW/Eff 1/1 | 5.0 | 14.6 | 6.8 | 14.0 | average | 25.3 | 8.9 | 12 | n/a | n/a | n/a |
| | | | N1 | B | BHS/Eff 3/1 | 5.0 | 16.1 | 6.7 | 31.0 | average | 25.5 | 9.4 | 28 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 5.0 | 16.2 | 6.7 | 31.0 | average | 25.4 | 9.4 | 28 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | none | 15.9 | 7.2 | 31.0 | average | 25.4 | 8.9 | 28 | n/a | n/a | n/a |
| | | Bag | N1 | A | FW/Eff 1/1 | 10.0 | 20.8 | 7.1 | 9 | average | 19.4 | 9.2 | 14 | 3.58 | 0.31 | 8.66 |
| | | | N1 | B | BHS/Eff 3/1 | 10.0 | 18.1 | 7.8 | 25 | average | 18.9 | 9.1 | 27 | 11.74 | 1.99 | 16.96 |
| | | | N1 | C | BHS/Eff 3/1 | 10.0 | 18.3 | 8.0 | 28 | average | 19.3 | 8.5 | 26 | 13.15 | 2.74 | 20.83 |
| | | | N1 | D | BHS/Eff 3/1 | none | 19.6 | 7.5 | 28 | average | 19.9 | 9 | 27 | 7.51 | 1.20 | 16.01 |
| | Trial 7 20.3.-3.4. | Flask | CV | A | BHS/Eff 3/1 | 5.0 | 14.9 | 6.9 | 31 | average | 25.4 | 9.2 | 31 | n/a | n/a | n/a |
| | | | CV | B | BHS/Eff 3/1 | 5.0 | 14.8 | 7.0 | 31 | average | 25.4 | 9.5 | 31 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 5.0 | 14.8 | 7 | 32 | average | 24.9 | 9.3 | 31 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | 5.0 | 14.6 | 7.2 | 32 | average | 24.3 | 9.4 | 28 | n/a | n/a | n/a |
| | | Bag | CV | A | BHS/Eff 3/1 | 10.0 | 15.8 | 7.1 | 31 | average | 25.0 | 9 | 31 | 16.08 | 3.75 | 23.33 |
| | | | CV | B | BHS/Eff 3/1 | 10.0 | 15.8 | 7.5 | 28 | average | 25.9 | 9.2 | 27 | 13.49 | 2.65 | 19.68 |
| | | | N1 | C | BHS/Eff 3/1 | 10.0 | 15.9 | 7.5 | 31 | average | 25.9 | 9.4 | 27 | 15.70 | 3.39 | 21.57 |
| | | | N1 | D | BHS/Eff 3/1 | 10.0 | 15.9 | 7.3 | 29 | average | 25.9 | 8.8 | 27 | 16.07 | 3.89 | 24.22 |
| | Trial 8 27.3.-10.4. | Flask | CV | A | BHS/Eff 3/1 | 5.0 | 15.6 | 6.8 | 31 | average | 29.7 | 9.4 | 31 | n/a | n/a | n/a |
| | | | CV | B | BHS/Eff 3/1 | 5.0 | 15.6 | 6.6 | 32 | average | 30.5 | 9.1 | 31 | n/a | n/a | n/a |
| | | | N1 | C | BHS/Eff 3/1 | 5.0 | 14.8 | 7.2 | 32 | average | 30.7 | 9.1 | 0 | n/a | n/a | n/a |
| | | | N1 | D | BHS/Eff 3/1 | none | 14.6 | 7.1 | 32 | average | 29.5 | 9.1 | 30 | n/a | n/a | n/a |
| | | Bag | CV | A | BHS/Eff 3/1 | 10.0 | 21.1 | 6.8 | 27 | poor | 22.4 | 8.4 | 28 | 13.90 | 3.29 | 23.67 |
| | | | CV | B | BHS/Eff 3/1 | 10.0 | 21.4 | 6.9 | 27 | poor | 21.9 | 9.4 | 30 | 14.23 | 3.41 | 23.96 |
| | | | N1 | C | BHS/Eff 3/1 | 10.0 | 20 | 7.9 | 27 | poor to none | 21.6 | 9.2 | 28 | 12.28 | 2.91 | 23.70 |
| | | | N1 | D | BHS/Eff 3/1 | none | 20 | 7.9 | 27 | poor | 21.4 | 9.4 | 28 | 11.87 | 1.43 | 12.05 |
| 2019 | Trial 9 18.2.-4.3. | Flask | N2 | A | BHS/Eff 3/1 | 5.0 | 13.3 | 6.5 | 27 | average | 21.4 | 9 | 26 | n/a | n/a | n/a |
| | | | N2 | B | BHS | 5.0 | 13.1 | 5.7 | 31 | average | 21.6 | 8.6 | 31 | n/a | n/a | n/a |
| | | | N2 | C | BHS/Eff 3/1 | 5.0 | 13.3 | 6.8 | 29 | average | 21.5 | 9 | 27 | n/a | n/a | n/a |
| | | | N2 | D | BHS | none | 12.7 | 6.6 | 32 | average | 21.4 | 8.5 | 31 | n/a | n/a | n/a |
| | | Bag | N2 | A | BHS/Eff 3/1 | 10.0 | 14.6 | 6.1 | 27 | average | 21.1 | 7.5 | 28 | 3.18 | 0.57 | 17.81 |
| | | | N2 | B | BHS/Eff 3/1 | 10.0 | 14.3 | 5.6 | 27 | average | 21.3 | 8.4 | 26 | 3.80 | 0.71 | 18.68 |
| | | | N2 | C | BHS | 10.0 | 14.5 | 6.3 | 31 | average | 21.2 | 8.4 | 31 | 1.22 | 0.07 | 5.98 |
| | | | N2 | D | BHS | none | 14.2 | 6.2 | 31 | average | 20.9 | 8.3 | 29 | 1.69 | 0.22 | 13.02 |
| | Trial 10 17.5-3 6. | Flask | N2 | A | Art/Eff 1/1 | 5.0 | 19.3 | 7.4 | 20 | average | 23.1 | 7.8 | 21 | n/a | n/a | n/a |
| | | | N2 | B | Art/Eff 1/1 | 5.0 | 19.5 | 7.2 | 20 | average | 22.9 | 8.3 | 19 | n/a | n/a | n/a |
| | | | N2 | C | Artificial | 5.0 | 19.6 | 7.9 | 21 | average | 23.4 | 7.9 | 19 | n/a | n/a | n/a |
| | | | N2 | D | Artificial | none | 19.4 | 7.3 | 20 | average | 23.2 | 8.6 | 20 | n/a | n/a | n/a |
| | | Bag | N2 | A | Art/Eff 1/1 | 10.0 | 18.9 | 6.5 | 23 | average | 24.3 | 7.7 | 20 | 6.85 | 1.00 | 14.63 |
| | | | N2 | B | Art/Eff 1/1 | 10.0 | 19.3 | 7 | 22 | average | 24.5 | 7.9 | 19 | 5.82 | 0.80 | 13.73 |
| | | | N2 | C | Artificial | 10.0 | 19.7 | 6.7 | 22 | average | 24 | 8 | 22 | 4.42 | 0.43 | 9.75 |
| | | | N2 | D | Artificial | none | 18.6 | 6.8 | 21 | average | 23.9 | 8.5 | 20 | 2.89 | 0.17 | 5.72 |
| | Trial 11 3.6-11 6. | | N2 | A | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N2 | B | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N2 | C | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N2 | D | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | | Bag | N2 | A | Art/Eff 1/1 | 10.0 | 19.8 | 6.9 | 22 | average | 24.9 | 7.9 | 19 | 9.65 | 2.32 | 24.07 |
| | | | N2 | B | Art/Eff 1/1 | 10.0 | 19.6 | 7.2 | 21 | average | 24.1 | 8.4 | 21 | 7.20 | 1.35 | 18.78 |
| | | | N2 | C | Artificial | 10.0 | 18.5 | 6.8 | 19 | average | 24.6 | 8.1 | 21 | 6.20 | 0.70 | 11.27 |
| | | | N2 | D | Artificial | none | 18.4 | 7 | 21 | average | 24.9 | 8.2 | 18 | 3.75 | 0.33 | 8.90 |
| | Trial 12 11 6.-24.6. | | N2 | A | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N2 | B | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N2 | C | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | | | N2 | D | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| | | Bag | N2 | A | Art/Eff 1/1 | 10.0 | 19.9 | 6.6 | 22 | average | 24.7 | 8.2 | 21 | 8.72 | 2.03 | 23.27 |
| | | | N2 | B | Art/Eff 1/1 | 10.0 | 19.3 | 7.3 | 23 | average | 24.6 | 7.6 | 18 | 6.41 | 1.24 | 19.32 |
| | | | N2 | C | Artificial | 10.0 | 20.1 | 6.9 | 21 | average | 24 | 8.5 | 23 | 4.22 | 0.39 | 9.27 |
| | | | N2 | D | Artificial | none | 18.9 | 7.2 | 23 | average | 23.7 | 8.1 | 19 | 2.90 | 0.15 | 5.14 |