IMPACTS OF ADVANCED OXIDATION PROCESSES TO MICROALGAE CULTIVATION IN RAS WATER

Lappeenranta–Lahti University of Technology LUT

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ABSTRACT

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Impacts of advanced oxidation processes to microalgae cultivation in RAS-water.

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Keywords: water treatment, AOPs, microalgae, recirculating aquaculture systems, RAS.

Land-based aquaculture is a growing industry driven by increasing food demand and changes in marine environment. Recirculating aquaculture systems (RAS) are one option to produce aquatic species in closed and controlled environment with minimal impacts on surroundings. Key element in these recirculation systems is the water treatment unit which maintains the water quality of the system. To increase the economical feasibility of the RAS-based production, development of the water treatment unit is desired.

For the development of the water treatment unit, the implementation of advanced oxidation processes (AOPs) and microalgae cultivation have been suggested to improve removal of recalcitrant compounds and nitrogen removal together with value adding with microalgae production. In this thesis, co-application of these methods is studied to determine how the implementation of AOP-treatment would impact to latter microalgae cultivation.

While using marine microalgae species *Nannochloropsis oculate*, pretreatment of RAS-water with ozone, ozone/UV and photocatalysis did overall decrease the microalgae growth over 14 days cultivation period compared to untreated samples. Higher doses of ozone did completely prevent the microalgae growth whereas best results were achieved with photocatalysis. Even though the microalgae growth was hindered in AOP-treated samples, the pretreated samples produced more consistent growth between the replicates compared to untreated samples, and achieved comparable nutrient removal.

TIIVISTELMÄ

Lappeenrannan–Lahden teknillinen yliopisto LUT

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Kemiantekniikka

Mikko-Jussi Laine

Kehittyneiden hapetusmenetelmien vaikutus mikroleväviljelyyn kiertovesilaitoksen vedenkäsittelyssä.

Kemiantekniikan diplomityö

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58 sivua, 23 kuvaa, 15 taulukkoa

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Kalankasvatus on kasvava ruuantuotannon muoto, joka on myös siirtymässä meristä maalle. Yksi maalla tapahtuvan kalankasvatuksen mahdollistavista tekniikoista on kiertovesiviljely (recirculating aquaculture systems, RAS). RAS-laitoksissa kasvatus tapahtuu suljetussa ja kontrolloidussa ympäristössä minimoidulla ympäristökuormituksella. Näissä laitoksissa vedenpuhdistus on tärkeä osa järjestelmää sen ylläpitäessä systeemissä kiertävän veden laatua. Vedenpuhdistusjärjestelmän kehittämisellä voitaisiin vaikuttaa laitosten taloudelliseen kannattavuuteen, joka on tunnistettu ongelma alalla.

RAS-laitosten vedenkäsittelyn kehittämiseksi kehittyneiden hapetusmenetelmien (advanced oxidation processes, AOPs) ja mikroleväviljelyn hyödyntämistä on tutkittu. Näillä menetelmillä voitaisiin tehostaa haitta-aineiden ja typen poistoa sekä tuottaa lisäarvoa levänkasvatuksella. Tässä työssä tutkitaan näiden kahden menetelmän yhteensopivuutta RAS-veden käsittelyssä ja pyritään selvittämään miten AOP-käsittely vaikuttaa sitä seuraavaan mikroleväviljelyyn.

Työssä käytetyn mikrolevän, *Nannochloropsis oculate*, kasvu oli heikompaa vesinäytteissä, jotka oli esikäsitelty käyttäen otsoni-, otsoni/UV- ja fotokatalyysimenetelmiä verrattuna käsittelemättömiin näytteisiin 14 päivän viljelyjaksolla. Korkeimmat otsoni annokset estivät levän kasvun täysin, kun taas parhaimmat tulokset saavutettiin fotokatalyysilla. Vaikka esikäsittelyt heikensivätkin mikrolevän kasvua, vaihtelu toisintonäytteiden välillä oli huomattavasti pienempää käsitellyissä näytteissä ja ravinteiden poisto onnistui myös heikennetyn kasvun tapauksissa.

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SYMBOLS AND ABBREVIATIONS

Abbreviations

AOP	Advanced Oxidation Process
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EEM	Excitation-Emission Matrix
IC	Inorganic Carbon
ORP	Oxidation-Reduction Potential
RAS	Recirculating Aquaculture System
TAN	Total Ammonia Nitrogen
TC	Total Carbon
TSS	Total Suspended Solids

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1 Introduction

Recirculating aquaculture systems (RAS) have been proposed to counter the concerns related to conventional aquaculture systems. RAS are wholly constructed systems where water is circulated between fish tanks and water treatment unit. As a closed system RAS minimize water consumption and nutrient release, enables production in new geographical locations and can ensure production in changing environment. (Ahmed et al. 2019; Ebeling & Timmons 2012). Currently RAS based fish production is hindered by poor economic feasibility related to high capital and operational costs, and quality issues in the product (Liu et al. 2016). As a major contributor to these issues, development of water treatment in the system is required. Considering the economic aspects, microalgae-based water treatment has been proposed as an alternative or supplement for conventional biological treatments (Li et al. 2019). Cultivation of microalgae in RAS-water could result in more comprehensive nutrient removal and produced microalgae could create value to operations (Milhazes-Cunha & Otero 2017). Simultaneously, application of Advanced Oxidation Processes (AOPs) has gained interest as method to enhance water treatment regarding minor constituents in the water (Rodriguez-Gonzalez et al. 2019).

Microalgae has been cultivated successfully in RAS-water and has presented capability to remove nutrients efficiently (Halfhide et al. 2014). Additionally resulting algae biomass has potential to be used as a source for biofuels, fish feed or other valuables (Ansari et. al 2017; Villar-Navarro et al. 2021a). Resulting, that potential lies in application of microalgae cultivation in aquaculture systems in the future. Considering the quality issues in RAS-systems, AOPs have been studied for removal of pathogens and taste and odor compounds (Aalto et al. 2022; Rodriguez-Gonzalez et al. 2019). However, considering the taste and odor compounds, removal requires high dosing, which could negatively impact on the overall system (Schrader et al. 2010).

As presented, application of both microalgae cultivation and AOPs has been studied to some extent in RAS. However, the co-application of these methods has not been presented. Since both methods possess positive attributes considering RAS-water treatment, their combined impact and interactions should be studied. Therefore, in this thesis the aim is to study the impacts of different AOPs (ozonation and photocatalysis) to RAS-water quality, and most importantly how application of AOPs affects to following microalgae cultivation. In the literature review, principles of RAS, microalgae-based water treatment and AOPs are presented. According to literature, a hypothesis about the impacts of different pre-treatments to microalgae cultivation is stated. In the experimental section, hypothesis is examined with laboratory experiments including the AOP treatments and microalgae cultivation.

2 Recirculating Aquaculture System (RAS)

Aquaculture provides an increasing share of rising food production in the world. In 2018 global fish production was estimated to be 179 million tons, from which aquaculture provided 45% (FAO 2020). As production from capture fisheries stagnates, the importance of aquacultural activities is expected to rise also in the future along with the population growth (Han et al. 2019). Together with rising demand for produced fish, environmental concerns related to conventional culturing systems as ponds and net-pens have risen. These systems consume significant volume of water and are a source for eutrophication and biological pollution (Ahmed et al. 2019; Ebeling & Timmons 2012).

Most of the aquaculture fish production is performed in open or semi-open systems (Waite & Beveridge 2014). In open systems, the fish is reared caged in natural aquatic environment, with direct contact with surroundings. Important functions as temperature, oxygen concentration and waste control are handled by the surrounding waters. In semi-closed systems, water is extracted from natural source and conducted into pond or raceway where fish is reared. Used water is then conducted to receiving waters, optionally after treatment. (Tidwell 2012).

The simplicity of these systems allows cheap fish production but on the other hand they are noted to have considerable impacts on surroundings. Environmental concerns related to aquaculture today include large water and land demand, nutrient release and eutrophication, biotic depletion, and greenhouse gas emissions (Ahmed et al. 2019), of which nutrient release and resulting eutrophication are highly related to uncontrolled or poor waste management in open and semi-closed systems. Additionally, these systems may be vulnerable for impacts of climate change as droughts, floods, salinity, and sea level changes (Ahmed et al. 2019). These issues have and may in the future grow interest towards closed systems as RAS, which in principle is disconnected from surrounding environment, decreasing direct impacts to environment and securing the operations.

RAS is a land-based rearing system in which the rearing conditions are created and maintained artificially. Recirculating system consists of rearing tank or tanks, and water treatment system which aims to maintain desired conditions in the tank (Ebeling & Timmons 2012). RAS can recirculate up to 99% of the water which compared to flow through systems decreases the water consumption per fish significantly (Bregnballe 2015). RAS can be used to grow full-size fish or just to rear juveniles which are later moved to open systems (Dalsgaard 2013), which can be described as most traditional application of RAS (Vielma et al. 2022). With little requirements for external water resources, RAS can enable fish production in novel locations, possibly bringing production closer to consumers, which decreases cost and environmental impact of logistics (Good 2020).

2.1 RAS water composition

In rearing tanks, the water quality is constantly degraded by fish, which consume the dissolved oxygen and provided feed. At the same time water is enrichened with carbon dioxide (CO_2) and excreta. Excreta i.e., urine and feces, most importantly increase nitrogen concentration in forms of ammonia (NH_3) and ammonium (NH_4^+), which may be converted to nitrite (NO_2) and nitrate (NO_3) by bacteria (Crab et al. 2007). Feces and surplus feed in cultivation water increase turbidity and can enhance uncontrolled microbiota growth, which can have a negative impact to production and welfare of the fish (Rurangwa & Verdegem 2015; Burr et al. 2012). In operation, water quality in RAS is often controlled with a focus on dissolved oxygen (DO), ammonia (NH_3/NH_4^+), biosolids, CO_2, total gas pressure (TGP), nitrate (NO_3) and alkalinity (Espinal & Matulić 2019), and these parameters are most importantly responsible for maintaining habitable environment in rearing tanks. Since different fish species have different preferred conditions (temperature, DO, salinity etc.) and tolerance towards constituents, the operational parameters are species specific (Dalsgaard 2013). In Table 1, guidelines for safe concentrations considering these parameters in RAS are presented.

Table 1 Common values for RAS-water parameters (Bregnballe 2015).

Parameter	Normal level	Unfavorable level
CO_2 (mg/L)	10-15	> 15
NH_4^+ (mg/L)	0-2.5	> 2.5
NH_3 (mg/L)	< 0.01	> 0.025
NO_2^- (mg/L)	0-0.5	> 0.5
NO_3^- (mg/L)	100-200	> 300
PO_4^{3-} (mg/L)	1-20	-
Suspended solids (mg/L)	25	> 25
COD (mg/L)	25-100	-
pH	6.5-7.5	< 6.2 and > 8.0

The conditions in RAS are maintained initially to make the fish population flourish. But together with the cultivation of fish, a population of microorganisms is sustained in the water. The microbiota present in RAS contributes to biodegradation of waste as they feed on the surplus of the fish food and excreta in suspension, and the microbiota in biofilter is responsible for desired nitrification. On the negative side, microbiota can be responsible for secretion of pathogens and off-flavor-causing compounds. (Rurangwa & Verdegem 2015). Off-flavor compounds, recognized as geosmin and 2-methylisoborneol (MIB) are produced by certain cyanobacteria species which may be present in RAS-water. When accumulated to fish, the flesh gains an undesired “earthy” or “musty” flavor. Consequently, fish must go through additional depuration process, which increases the costs. (Burr et al. 2012). To prevent such incidents, treatment methods specifically targeted for removal of these compounds has been presented (Rodriguez-Gonzalez et al. 2019), which could be added to RAS water treatment system in future.

2.2 RAS water treatment

The water treatment system (Figure 1) in RAS conventionally includes unit operations for solids removal, biofiltration, CO_2-stripping, oxygenation, and disinfection (Ebeling & Timmons 2012).

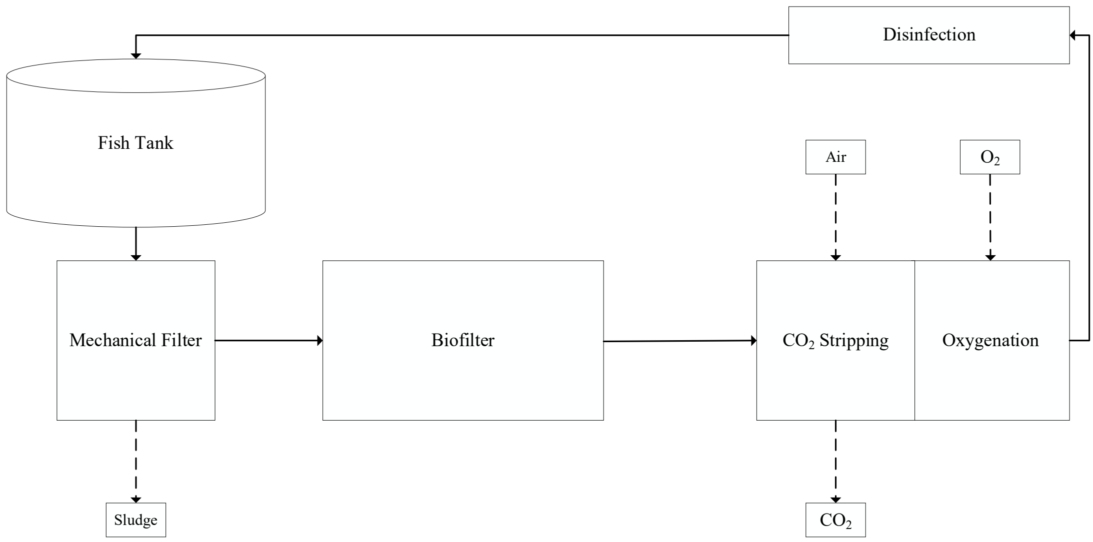


Figure 1 Schematic process scheme of RAS as described by Ebeling & Timmons (2012).

Removal of solids as feces, feed and biomass can be carried out with settling or filtration-based methods. By removing solids, the BOD and nutrient load is effectively withdrawn from the system. After solids removal, water is introduced to biofiltration, where bacterial consortium most importantly oxidizes toxic ammonium and nitrite to significantly less toxic nitrate. (Ebeling & Timmons 2012). Even though nitrate toxicity is minimal in comparison to ammonium and nitrite (Bregnballe 2015), with high stocking densities the nitrate concentration may reach the level of toxicity. Thus, removal of nitrate could be additionally implemented to process. (Espinal & Matulić 2019). Impacts of increased nitrate concentration differ between species, but overall lower nitrate concentrations increase fish welfare (Davidson et al. 2017). After the treatment of solids and nutrients, the gas content of water is restored with CO_2 stripping and oxygenation. Additionally, it is possible to control the microbiota in RAS-system for example with UV-irradiation or ozonation, which also decomposes organic matter remaining in the system (Bregnballe 2015).

2.3 Current state, environmental impact, and economic feasibility of RAS

As expected, the capital and operational costs of RAS are higher compared to conventional systems. Liu et al. (2016) have compared the economic performance together with carbon footprint between RAS and conventional open net pen (ONP) for Atlantic salmon. They presented that the capital costs of RAS would be 80% higher than ONP system. However, the difference in operational costs is not as high (Liu et al. 2016), as RAS can be operated with much higher stocking density, which results in greater production (Martins et al. 2010). High stocking densities on another hand has raised concerns about the welfare of the fish (Espinal & Matulić 2019). Overall, the costs of RAS production may be covered with higher pricing of fish, in the case of Atlantic salmon the price premium of 30% could be required to achieve profitability (Liu et al. 2016; Good 2020).

Related especially to higher capital costs recirculating systems are currently in minority considering fish production. In European Union RAS-produced fish accounts only 1.5-2% of the total aquaculture production, with majority (90%) of the RAS-production in freshwater systems, which have longer history of expertise. Saline water possess more operational difficulties as corrosion in structures, which must be accounted in material choices. (European Commission 2020, 18-22). However, the number of RAS projects has increased in recent years indicating growing interest (Vielma et al. 2022). Motivations for this growing interest have been already pointed out in this thesis and are summarized in Table 2.

Table 2 Summary of the benefits and current issues in recirculating systems.

<table>
<tr><th>Issues in traditional aquaculture</th><th>RAS solution</th></tr>
<tr><td>High water and land usage</td><td>Minimal water usage, high stocking density enables lesser land area requirements.</td></tr>
<tr><td>Direct environmental impacts</td><td>Closed system has minimal nutrient emissions. Risk of fish escape and disease transfer is decreased.</td></tr>
<tr><td>Limited area availability</td><td>Does not require access to sea or vast water resources.</td></tr>
<tr><td>Impacts of global warming</td><td>Controlled environment is not affected.</td></tr>
<tr><th colspan="2">Issues or development fields in RAS</th></tr>
<tr><td colspan="2">High capital costs due to completely constructed system.
Higher operational costs due to energy consumption of pumps and water treatment
Accumulation of taste and odor compounds
Intensive systems are vulnerable to pathogens</td></tr>
</table>

High capital costs in RAS systems are somewhat unavoidable. To counter, higher profitability should be achieved during operation, by either reducing the operational costs or by increasing produced value. In the field of water treatment microalgae-based water treatment has been proposed as alternative for conventional biological treatment to enhance forementioned aspects (Li et al. 2019). In the following chapter the fundamentals of microalgae and implementation to RAS water treatment has been discussed.

3 Microalgae

Rather comprehensively defined, microalgae are primarily unicellular microorganisms capable of photosynthesis. Abundant in nature, microalgae are responsible for half of the photosynthetic activity on earth. Consequently, they provide a major share of oxygen and biomass on earth. Number of species under the term microalgae is vast, including over 200 000 different microalgae species. (Singh & Saxena 2015). Different microalgae species are found in variety of environments including fresh and marine waters in nature, and also in anthropogenic wastewaters (Li et al. 2019).

Cultivation of microalgae in wastewater at industrial scale has been studied for decades, and several different wastewaters and their fractions have been examined, together with a variety of different microalgae species. (Li et al. 2019; Cavalcanti Pessôa et al. 2022). Overall, efficient application of microalgae in wastewater requires matching of wastewater characteristics with suitable algae species. Although the variation in wastewaters composition outruns the diversity in algae living conditions. In many cases the cultivation of microalgae wastewater is hindered by the presence of toxic compounds e.g., chemicals and heavy metals together with unbalanced nutrient concentrations and high turbidity (Cavalcanti Pessôa et al. 2022). Considering the microalgae cultivation, aquacultural wastewater has gained interest with positive attributes (Egloff et al. 2018), which include adequate nutrient concentrations and relative absence of toxic compounds (Egloff et al. 2018; Han et al. 2019).

3.1 Physiology

Precise definition and taxonomy of microalgae and algae is discussed topic in the field of biology (Andersen 2013; Sing & Saxena 2015). From engineering perspective, it is feasible to determine microalgae merely by its main functionality, which is the capability to photosynthesize. As described by Andersen (2013) *"Microalgae are a diverse collection of microorganisms that conduct oxygen-evolving photosynthesis".* This definition comprehends eukaryotic photoautotrophic protists as well as cyanobacteria, which are prokaryotic species (Singh & Saxena 2015). In literature, when addressing the application or cultivation of microalgae, microalgae often refers solely to eukaryotic organisms and cyanobacteria are considered separately, which is the approach also used in this thesis.

As mentioned, microalgae are vastly diverse group of organisms and comprehensive morphology is hard to describe (Andersen 2013). Like plant cells, they contain chloroplasts enabling photosynthesis and often possess a cell wall with varying biochemical composition (Andersen 2013). In contrast to plants, microalgae do not possess roots, leaves, or stems, but there are similarities in overall structure of the cells (Sing & Saxena 2015). In addition to chloroplasts microalgae cells possess the usual organelles found in eukaryotes. Nucleus, possessing the DNA and controlling the cell. Endoplasmic reticulum, ribosomes, and Golgi apparatus, which take part in synthesis and excretion of proteins and cell-building blocks, and mitochondria, often referred as the powerhouse of the cell, which provides chemical energy for cell operations. (Sing & Saxena 2015). Microalgae cells can also contain vacuoles, which are used as storage for metabolic by-products, and they control pressure in the cell. Some microalgae species are equipped with flagella which enables movement in water. Associated with flagella, moving algae species can also possess an organelle called eyespot, which detects light and is used for navigation. (Andersen 2013).

3.2 Growth and nutrient requirements

Photosynthesizing microalgae are capable to absorb light energy for redox reactions, converting the radiant energy to chemical energy. These autotrophic species elementary reduce carbon dioxide to carbohydrates and oxidize water to oxygen in chloroplasts. (Masojídek et al. 2013). Produced carbohydrates then form the chemical energy source for cell operations. Microalgae can also utilize external organic carbon as energy source, which is referred as heterotrophy, and the co-existence of both pathways for energy supply is referred as mixotrophy. (Grobberaal 2013). The capability to utilize both organic and inorganic carbon sources can be seen as an advantage in microalgae cultivation as mixotrophic nutrition has presented higher biomass yields compared to strictly autotrophic (Wan et al. 2011).

For photosynthesizing microalgae, the microalgae growth most importantly requires light and inorganic carbon for energy production. In chloroplasts, the photosynthetic pigment chlorophyll absorbs light in wavelengths of 450-475 nm and 630-675 nm, which correspond to blue and red color ranges on visible spectrum (Masojídek et al. 2013).

For efficient photosynthesis and autotrophic growth, adequate amount of inorganic carbon CO_2 and HCO_3^- must be present in culture media. (Grobberaal 2013). Apart from carbon, microalgae nutrition consists of around 30 different elements required for living (Grobberaal 2013). From which, few essentials i.e., nitrogen, phosphorus and micronutrients are briefly represented in following.

3.2.1 Nitrogen

Second to carbon, nitrogen contributes significantly to production of biomass and the nitrogen content may exceed 10% in microalgae biomass (Grobberaal 2013). Nitrogen is required for the formation of proteins and nucleic acids, which are essential and abundant macromolecules in cells. (Procházková et al. 2014). With variance between species, microalgae can utilize nitrogen in variety of forms including nitrate, nitrite, ammonia, urea etc. although there are preferences (Grobberaal 2013). In general, ammonia is most preferred form for microalgae as assimilation is most energy efficient. However, in cultivation of microalgae in constructed media, nitrogen is most often supplied in the form of nitrate. This is due to toxicity of ammonia in surplus concentrations. (Grobberaal 2013; Procházková et al. 2014).

In scarcity of nitrogen, metabolism of microalgae changes. When energy, i.e. carbohydrates, is available but there is a lack of nitrogen for protein synthesis and production of new cell structures, the carbohydrates are stored. (Procházková et al. 2014). As a result, the cell or biomass growth is reduced but there is an increase in starch and lipid content in cells. However, this strategy can be used when microalgae is cultivated e.g., for biodiesel production, where high lipid content is desired. (Procházková et al. 2014; Yaakob et al. 2014).

3.2.2 Phosphorus

Together with carbon and nitrogen, phosphorus is found in several cellular components including phospholipids, ATP and DNA, which are crucial for the life and reproduction of microalgae (Yaakob et al. 2014). Phosphorus is utilized by microalgae in forms of PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, again with some variation between species (Procházková et al. 2014; Yaakob et al. 2014). In aqueous media phosphorus is easily precipitated with ions as CO_3^{2-} and Fe^{3+}, and it is often growth-limiting factor in natural environments. As a counteract, when phosphorus is abundant microalgae store phosphorus as polyphosphate, this phenomenon is referred as "luxury uptake". In regular conditions phosphorus accounts for less than 1% of biomass, but after luxury uptake the share may rise as high as 3%. (Grobberaal 2013; Procházková et al. 2014). Because of the significant storage ability, microalgae can withstand some irregularities in phosphorus supply in cultivation.

Similarly to nitrogen deprivation, the lack of phosphorus has also noted to move the metabolisms from cell growth towards starch and lipids production (Grobberaal 2013; Yaakob et al. 2014). However, regarding the intentional lipids production, nitrogen deprivation may be more effective strategy (Procházková et al. 2014).

As the macronutrients carbon, nitrogen and phosphorus are used to build cell structures they are acquired by microalgae in similar relations as they are found in structures. A classic approximation of sufficient nutrient content in media is 106:16:1 for C:N:P, which is referred as Redfield ratio. Through which it can be assumed that in media with N:P ratio different from 16:1 discussed impacts of nutrient limited growth are seen. (Grobberaal 2013).

3.2.3 Micronutrients

In addition to discussed main macronutrients C, N, and P, microalgae utilize several minerals and trace elements, referred as micronutrients (e.g., Fe, S, K, Na, Mg, Mn, Se, Mo, Na). These nutrients are minor reactants in metabolic reactions or promote the reactions as cofactors. (Grobbelaar 2013; Procházková et al. 2014). In constructed cultivation medias, micronutrients are added in concentrations of mg/L or even µg/L, even though these elements are essential for microalgae, they can be toxic even in moderate concentrations (Procházková et al. 2014). Addition of micronutrients could also be required in wastewater cultivation as it is possible that ideal assortment is not present.

3.3 Microalgae cultivation in RAS water

Microalgae has been seen as a potential biomass raw material for various purposes including biofuels, food supplements, cosmeceuticals, pharmaceuticals, and livestock feed (Venkatesan et al. 2015). Compared to terrestrial plants conventionally applied for forementioned purposes, microalgae present faster growth rate and require less arable land. Most importantly, as aquatic organisms, the cultivation could be carried out in water-based waste streams enhancing the utilization of waste streams and resource recycling. (Ansari et al. 2017).

Growth of algae biomass results from assimilation of nutrients from water (Li et al. 2019). For RAS-water, microalgae cultivation could provide an alternative or support for conventional biofilter treatment (Milhazes-Cunha & Otero 2017). In comparison to nitrifying bacteria, which merely convert ammonia to less harmful nitrate (Ebeling & Timmons 2012), microalgae would assimilate nitrogen to biomass irretrievably removing nitrogen from the system. Simultaneously, microalgae could contribute to CO_2 removal and oxygenation, reducing the need for energy consuming unit operations (Han et al. 2019). Apart from wastewater treatment, the produced microalgae biomass could bring value to process, enhancing economic feasibility of RAS, as schematically presented in Figure 2.

As mentioned, wastewater cultivated microalgae is proposed to be utilized as a raw material for several biobased products. Considering aquaculture, one proposed application which could be implemented directly back to main process is the production of fish feed (Villar-Navarro et al 2021a; Michels et al. 2014). Conventionally, fish feed is produced from caught wild fish and by-products of fish processing e.g., waste fractions. In 2018, approximately 10% of the fish production was used as fish feed. (FAO 2020). As stagnating catch volumes and ecological concerns related to fishery can impact on availability of conventional feed, alternative protein and lipid sources could become more important (Guedes et al. 2015). Currently the feed for fish is responsible for a major share of the operational costs of RAS (Liu et al. 2016), and thus the increase of autonomy in this field could enhance the viability of system. With some variance between species microalgae contains adequate number of amino acids, lipids, and minor nutrients to be utilized as fish feed (Villar-Navarro et al. 2021a) and could be utilized in the diet especially for fish larvae (Guedes et al. 2015; Michels et al. 2014).

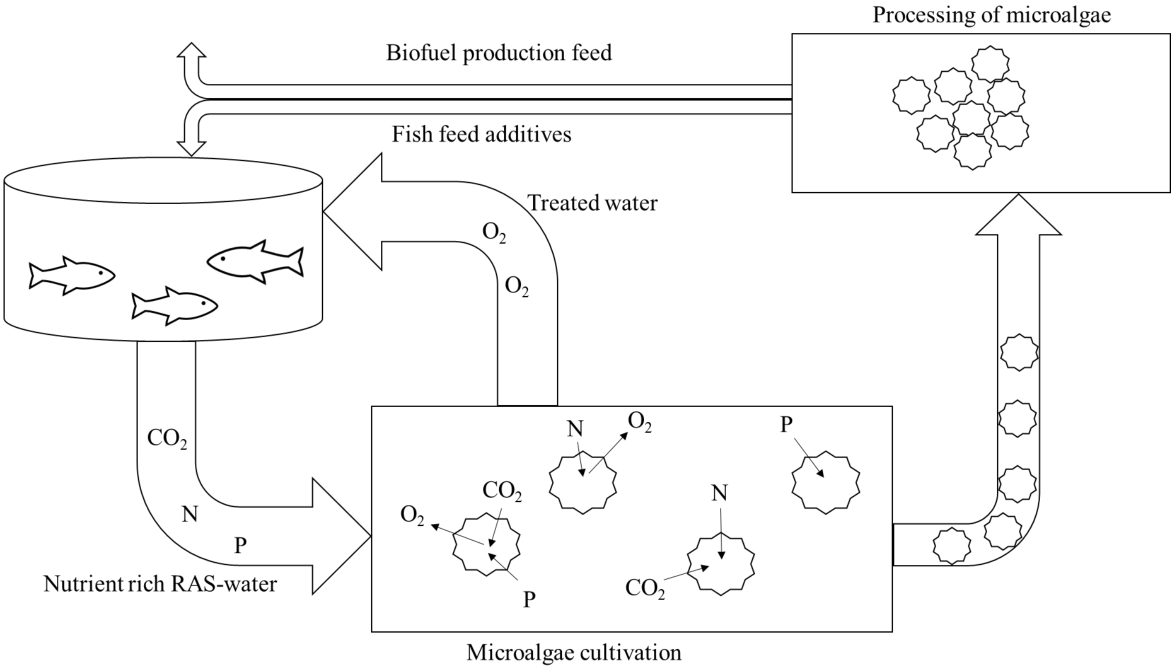


Figure 2 Implementation of microalgae cultivation to RAS-system.

As was presented, the nutritional properties of cultivation media impact on the growth of algae and also on the composition of microalgae. Therefore, if the microalgae are cultivated in aquaculture water for value products, achieving desired growth and composition may require adjustment of the media with supporting supplementation of nutrients. For example, addition of macronutrients N and P together with trace metals and vitamins could be necessary to ensure desired metabolism and growth. (Michels et al. 2014; Ansari et al. 2017; Villar-Navarro et al. 2021a). However, it would be convenient if the sufficient cultivation required minimal number of additional inputs. Together with imperfect nutrient supply, feasible microalgae cultivation can be threatened by competing or grazing microorganisms, which may hinder the growth of desired species or ravage the culture (Day et al. 2017).

In consequence, efficient cultivation of microalgae in wastewaters could require preliminary treatments. Considering the whole system, in this case RAS, ideally these preliminary treatments would make a positive impact also to main process. One possibility for achieving the sort of synergy could be the application of Advanced Oxidation Processes (AOPs).

4 Advanced oxidation processes and application as pre-treatment for microalgae cultivation

Advanced oxidation processes (AOPs) are water treatment methods based on the generation of highly oxidative species, most primarily hydroxyl radicals, in treated water. Through oxidation, hydroxyl radicals are aimed to degrade and decompose pollutants and undesired compounds into less harmful form. (Ameta & Ameta, 2018 6-8). In many cases AOPs are studied for treatment of recalcitrant compounds (pharmaceuticals, PPCPs, dyes etc.), which cannot be degraded by conventional biological treatments. Together with degradation of chemical compounds powerful oxidants can destroy cell structures of microbes, resulting in disinfection of water (Aalto et al. 2022). AOPs include a set of methods including use of O_3, H_2O_2, Fenton-process, photocatalysis and their combinations. However, in this thesis we merely focus on the processes which are used in the experimental part. Which include ozonation and photocatalysis. Additionally to AOPs, application of UV-irradiation is presented, as it is used in the experimental section of the thesis.

Considering RAS-water, UV-irradiation and ozonation are utilized in disinfection (UV) and polishing (ozonation) (Attramadal et al. 2012). Whereas photocatalytic methods have recently gained attention in removal of minor recalcitrant substances as off-flavor compounds (Rodriguez-Gonzales et al. 2019), and could be implemented to RAS-systems in the future. This represents the common field of application for AOPs, which may not be economically feasible in treatment of high pollutant concentrations but are effective in removal recalcitrant substances. In industrial water treatment, AOPs are mostly used as finishing treatment after biological treatment, which is mainly responsible for more comprehensive removal of pollutants. (Amor et al. 2019). As AOPs are non-specific in degradation of pollutants, they are most efficiently applied after the easily degradable compounds are removed, leaving the recalcitrant ones more exposed. In the RAS, where water is continuously treated and circulated to maintain certain water quality, application of AOPs would not necessarily require such pre-treatment as the amount of oxidizable matter is moderate to begin with.

4.1 Ozonation

Oxidation with ozone i.e., ozonation, is water treatment method widely used for production of potable water and for treatment of wastewater streams. Ozone is a powerful oxidizer and in most basic manner, ozonation is a process where ozone is added to water where it may interact with pollutant species. When absorbed into water, ozone may oxidize species directly (Reaction 1) or additionally produce alternative oxidative species, as OH°, through radical chain reactions (Overall reaction 2) which can oxidize pollutants. These reaction routes may be referred as direct and indirect ozonation. (Gottschalk et al. 2010,13-24). Since OH° possess higher oxidation potential and reactivity in comparison to ozone, indirect ozonation may be pursued by applying supporting agents as H_2O_2 or UV-irradiation, which enhance the decomposition of ozone (Gorito et al. 2021). For decomposition of ozone UV-light wavelength should be between 250 and 260 nm. With these wavelengths doses of 60-75 mJ/cm^2 can decompose ozone residuals up to 0.5 mg/L. (Summerfelt 2003).

$$O_3 + M \rightarrow M_{oxid} \tag{1}$$

$$3O_3 + OH^- + H^+ \rightarrow 2OH^{\circ} + 4O_2 \tag{2}$$

Because of the high reactivity of OH° it does not remain in the system for long, which is a characteristic of AOPs. However, if the decomposition of ozone (Reaction 2) does not occur and direct oxidation (Reaction 1) is dominant, lifetime of ozone increases, which must be accounted in applications.

As mentioned, ozone treatment is applied in RAS to some extent in final steps of the water treatment to provide disinfection and polishing of water. When ozone is applied right before the return of water to fish tank, the dose is restricted by impacts of residual ozone to fish, thus surplus dosage is not feasible. Since the ozone is consumed by organic matter in RAS-water, ozone dose is often preliminary determined in relation to fish feed input, which correlates with NOM in water. Additionally, safe ozone dosage can be determined on-site by monitoring the oxidation reduction potential (ORP) in water, which increases in respect to residual ozone in water. Safe ORP value is case determined but as estimate ORP values above 350 mV could harm the fish culture. (Tango & Gagnon 2003). However higher ozone doses, resulting in higher ORP, would provide more complete disinfection and could degrade low-concentration pollutants as geosmin and MIB (Li et al. 2014; Schrader et al. 2010).

4.2 Photocatalysis TiO_2

Photocatalysis is an AOP based on the exposure of photo-active semiconductors to light which activates the material to function as photocatalyst. The catalyst is then responsible for formation of radicals and other reactive species performing the oxidation in treated water. (Dionysiou 2016, 2-4).

Semiconductors are materials which have a relatively small energy difference between the valence band and conduction band, referred as band gap. One of the most utilized semiconductors for photocatalysis is TiO_2, which is also used in experimental part of this work. Depending on the polymorphic form, TiO_2 possess band gap around 3 eV making it responsive to UV light (Dionysiou 2016, 2-4). As light with adequate wavelength is radiated on semiconductor, the electrons initially at the valence band are excited to the conduction band, resulting an electron hole at the valence band. In this situation the semiconductor is capable to donate and receive electrons making it active for redox reactions. (Ameta & Ameta 2018, 135-138). Schematic illustration of photocatalysis is presented in Figure 3. In aquatic environment, where dominantly water is adsorbed on the surface of the catalyst, one probable redox reaction is the oxidation of water to hydroxyl radical, together with reduction of dissolved oxygen.

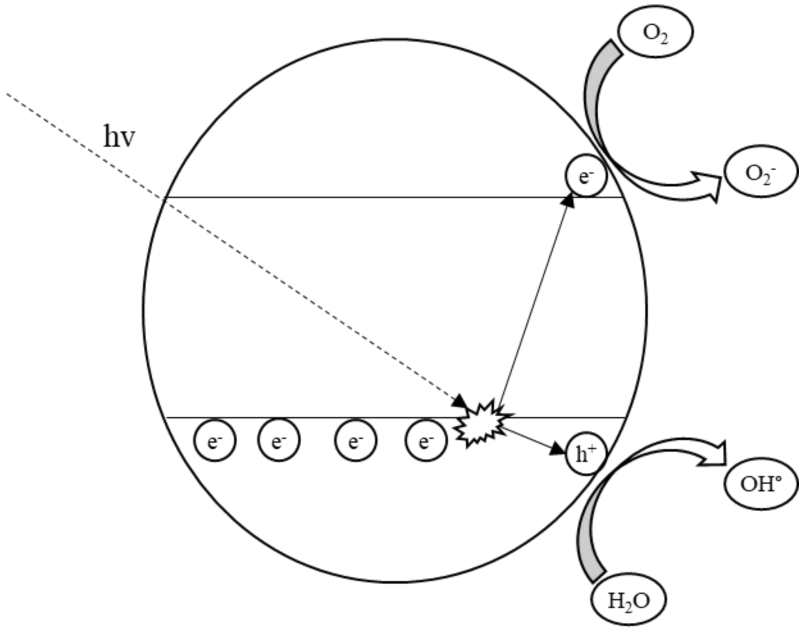


Figure 3 Principle of photocatalysis.

As the radical producing reactions occur on the surface of the catalyst, the surface area of catalyst increases radical production. Even though highest area would be achieved with dispersed powder-like implementation of photocatalyst, practical issues are experienced when catalyst should be collected from media. Therefore, immobilized catalyst must be used, unfortunately immobilized photocatalyst tends to present lower activity due to lower surface area. (Marugan et al. 2011). To overcome decreasing activity, polymer nanostructures have been presented to increase surface area in immobilized catalyst (Ameta & Ameta 2018, 162-163).

Treatment of RAS water with UV-TiO_2 has been examined by Rodriguez-Gonzales et al. (2019), presenting that UV-TiO_2 is capable to remove GSM and MIB from RAS-water in batch system. In contrary to ozonation, photocatalysis oxidation would rely most importantly on OH° species. Due to higher reactivity OH° would result in oxidation without the residual impacts, which is a concern in ozone application.

4.3 UV-irradiation

Additionally to oxidation processes, UV-irradiation is also used as a pre-treatment to represent the impact of disinfection alone in this thesis. Also, UV-irradiation could be applied together with chemical AOPs as O_3 and H_2O_2 to enhance formation of desired radicals.

UV-irradiation is a conventional method used in disinfection of waste and drinking water. Water is irradiated with high energy radiation which damages living cells resulting in destruction or inactivation of biota in water (Linden & Mamane 2015). Most efficient germicidal impact is seen with wavelengths in the range of 220-320 nm, corresponding to UV-C (200-280 nm) and UV-B (280-315 nm) regions (Verlicchi et al. 2011). In cellular level, UV-radiation most importantly effects to nucleic acids in DNA and RNA, which control the reproduction and metabolism of organism. UV-radiation within the range 240-280 nm dimerizes adjacent nucleotide bases in DNA or RNA structure, inhibiting the transcription. (Linden & Mamane 2015). UV-irradiation has an impact merely on living cells and does not result in chemical conversion in system. Suspended solids are not degraded by radiation and can hinder the impact of radiation by shading targeted microorganisms. (Verlicchi et al. 2011).

The "dose" of UV-irradiation is conventionally measured as fluence which is calculated as,

$$D = E_{avg} \cdot t \cdot F \tag{3}$$

where,	D	Fluence, mJ/cm^2
	E_{avg}	Irradiance, mW/cm^2
	t	Time of exposure, s
	F	Correction factors.

Received dose of UV-irradiation is impacted by several factors in the system, one of which is the absorption of UV light by water (Linden & Mamane 2015). This loss of irradiance in water can be taken account with water factor, calculated as,

$$Water\ Factor = \frac{1 - 10^{-al}}{al \cdot \ln(10)} \tag{4}$$

where a Absorption coefficient, cm^{-1}

l Path length in water, cm.

Irradiance of UV-lamp can be measured with radiometer and used for calculation of fluence. Required fluence for inactivation varies between microorganisms and higher fluence results in more complete reduction. Fluence applied in RAS can range from 2 mJ/cm^2 to 200 mJ/cm^2 (Bregnballe 2015).

4.4 Application as pre-treatment for microalgae cultivation

In the following sections, presented and possible effects of ozonation, UV/TiO_2 and UV-irradiation on RAS-water are discussed together with hypothesized impacts on following microalgae cultivation. As ozonation and UV-irradiation are utilized in the field, their impact on aquacultural wastewater is studied to some extent (Aalto et al. 2021; Attramadal et al. 2021; de Jesus Gregersen et al. 2021; Davidson et al. 2011), whereas photocatalytic treatment of aquaculture water has been studied quite exclusively in relation to removal of specific substances as off-flavor compounds, pharmaceuticals, and pathogens (Rodriguez-Gonzales et al. 2019; Silva et al. 2022). Therefore, there is a limited amount of information available about the effect on comprehensive quality of the aquaculture water.

4.4.1 Physiochemical impacts

CARBON AND LIGHT

With sufficiently high dose of strong oxidants produced by AOPs, organic species in water are eventually degraded to mineralization products as water, CO_2 and inorganic salts (Ameta & Ameta 2018, 9). Thus, in theory oxidation would increase the presence and availability of nutrients and carbon in inorganic form in water. In practice the effect of oxidation, most often ozonation, to RAS-water has been studied to some extent. Ozonation most importantly has noted to reduce BOD, COD, TSS and turbidity (de Jesus Gregersen et al. 2021; Davidson et al. 2011), which implies the efficient oxidation of organic carbon to carbon dioxide. An increase in light penetration and in inorganic carbon concentration would most presumably improve the microalgae photosynthesis and growth. Even if the oxidation would not completely mineralize carbon, it could provide assistance for microbiota in production of CO_2.

NUTRIENTS

As was noted earlier, microalgae species are capable to utilize a variety of nitrogen sources including NO_3^-, NO_2^-, NH_4^+ and urea. Although, between the sources, NO_3^- is least toxic, why it is often used in cultivation. Also, the differences in available nitrogen species are noted to effect on growth and fatty acid content of microalgae (Xu et al. 2001).

Considering different nitrogen species in RAS-water, ozone oxidizes nitrite to nitrate effectively (Schroeder et al. 2011), which could be beneficial for microalgae cultivation. Ammonia however is not significantly reactive with oxidative species O_3 and OH° in neutral pH, even though presence of ions as bromide and chlorine may enhance the oxidation (Yang & Liu 2022; Schroeder et al. 2011; Tanaka & Matsumura 2002). In practice significant oxidation of ammonia with ozone is not to be expected in RAS water (Park 2015), even though forementioned salts can be present when saline waters are used. Similarly photocatalytic oxidation of ammonia to nitrate is possible but in practice requires high pH and suspended catalyst (Altomare et al. 2012).

In theory the degradation and decomposing of larger organic molecules as amino acids, humic acids and fulvic acids could increase the bioavailability of nutrients which these molecules contain. Impact of oxidation processes to nutrient biodegradability in wastewater has been studied by Tooker et al. (2021), who applied H_2O_2 with UV. Results indicate that oxidation treatment, which decomposes organic matter, can increase the bioavailability of nutrients N and P, even though results were not entirely consistent.

In conclusion, there is theoretical possibility that oxidation could impact on the nutritional properties in water, even though the impact might not be significant. The behavior of non-specific oxidative species is not easy to predict, and thus experimental information is needed to determine the interactions in specific water matrix.

BY-PRODUCTS and RESIDUAL

As discussed before, AOPs producing OH-radicals do not fundamentally result in residual dose to water. However especially when ozone is applied, treated water may not be immediately safe for living organisms as the decomposition of ozone may take tens of minutes (Spiliotopoulou et al. 2018). Tolerance of microalgae species to ozone or other oxidative species is not widely presented but it can be assumed that high ozone concentration in water can harm microalgae cells and prevent cultivation (Ng et al. 2023).

Application of ozone to marine waters can also result in toxic by-products. Formation of these intermediates and products of ozonation can restrain the application to some extent (Schroeder et al. 2011). One example of these by-products, related to marine environments, is the formation of bromate (BrO_3^-), a carcinogenic compound, which is formed by reactions with bromide and ozone. In drinking water, permission limits for BrO_3^- is 10 μg/l, however there is no clear limits for bromate in terms of either fish or microalgae cultivation (Tango & Gagnon 2003). It has been noted that the presence of ammonia in water hinders the formation of bromate in ozonation, by reacting with intermediates in bromate formation pathway (Tanaka & Matsumura 2002). Considering this, the application of ozone could be safer in ammonia containing water. In RAS, the ammonia is intentionally converted to nitrite/nitrate during biofiltration. Resulting, that if ozone is applied as a finishing treatment, it is applied to water with minimized ammonia concentration, where formation of bromate is more probable. From bromate formation aspect, higher ozone doses could be applied to ammonia containing non-biofiltered RAS-water.

4.4.2 Biological impacts

As previously mentioned, additionally to physiochemical properties RAS-water contains a selection of microbes, some of which are seen beneficial, some neutral, and some damaging for fish production. Controlling the microbiota in RAS is a delicate task, as the disinfection methods used for the purpose are not highly selective between desired and undesired microbes. (Rurangwa & Verdegem 2014). Also, the demolition of existing microbiota may result in blooming of some harmful microbe as the ecological niche opens (Attramadal et al. 2021). But despite the challenges in controlled modification of microbiota in RAS-water, oxidation methods and UV-irradiation can be used to redefine the microbiota in terms of overall abundance and composition of different species (Aalto et al. 2021; Attramadal et al. 2021). As well as photocatalytic methods have shown to inactivate bacteria in aquaculture water (Villar-Navarro et al. 2021b).

Considering the cultivation of microalgae in wastewater, removal of existing microbiota could enhance the growth of microalgae, as one threat for successful microalgae cultivation in wastewater streams are competitive or grazing microorganisms, which can destroy the algae culture (Day et al. 2017). Sterilization by filtration or autoclave has been utilized as pre-treatment for wastewater, even though the impact of pre-treatment on growth is not always examined (Cavalcanti Pessôa et al. 2022). For RAS-water, the impact of sterilization has been studied by Halfhide et al. (2014) and Tejido-Nuñez et al. (2019). In both studies cultivation of different algae species (*Chlorella vulgaris*, *Tetreadesmus obliquus, Chlorella* sp., *Scenedesmus*) was studied in filtration sterilized RAS-water in comparison to untreated. According to both studies it seems that the performance of microalgae in these environments is dependent on the species. As other species of algae are more vulnerable to algae grazing protozoa, the removal or reduction of grazer populations can enhance the survival of algae culture (Tejido-Nuñez et al. 2019). On the other hand, presence of bacteria in water can enhance and support algae growth by converting substances to bioavailable form and by secreting growth factors and micronutrients. (Halfhide et al. 2014).

4.5 Summary

According to literature review it could be possible that oxidative pre-treatment of RAS-water would support microalgae cultivation as summarized in Table 3.

Table 3 Possible impacts of AOP treatments to RAS-water with respect to latter microalgae cultivation.

Impact of AOP to media		Impact on microalgae culture
Physiochemical	Decreased turbidity. Increased light penetration	Enhancing photosynthesis
	Decomposition of organics. Increase biodegradability of carbon and amount of inorganic carbon.	More available carbon increases photosynthesis and growth.
	Increase nutrient availability, oxidize nitrite to nitrate	More available nutrients and less toxic nitrite increase growth and survival of microalgae.
	Formation of toxic by-products and residual impacts	Could hinder the growth and decrease survival. Residual oxidation could kill microalgae.
Biological	Elimination of grazing protozoa	More secure environment for microalgae
	Elimination of competitive species	More available resources for microalgae
	Elimination of heterotrophic bacteria	Inhibit the formation of bacteria-algae consortium, which can be beneficial for microalgae culture.

Considering microalgae, in best case scenario the algae cultivation could replace the current biofilter unit as more comprehensive nitrogen removing unit. Meaning that the microalgae cultivation would be carried out with water straight from tank or after solids removal. When AOP-treatment would be performed for this minimally treated RAS-water, high doses would be necessary to achieve impact on minor pollutants as MIB and geosmin.

As was presented considering ozone, in current RAS-systems the application of high dosage is restricted by oxidative residual which can affect the fish. Similarly negative impacts of residual ozone in water can be expected to restrict cultivation algae (Ng et al. 2023). Rise of ozone concentration in water caused by ozonation decreases over time as the oxidative species go through decomposition and reduction. It could be possible that intensive ozonation before algae cultivation would require actions to restrict ozone and oxidative species entering the cultivation unit. In a most simple manner, retention time between ozonation and cultivation could be optimized. Another option could be application of UV-irradiation which would accelerate the ozone decomposition. With AOPs primarily producing hydroxyl radicals, residual impacts could most likely be neglected.

Experimental section

In the experimental part of this thesis, the impacts of different pre-treatment methods for RAS-water in relation to microalgae cultivation were studied. In total, two cultivation experiments were performed including a set of different pre-treatments. For the experiments two different RAS-water samples were received from two different facilities, from which one sample was used in first cultivation experiment and another in the second.

In the first cultivation experiment, pre-treatments included ozonation, photocatalysis and UV-irradiation in different doses (Table 4). The impacts of pre-treatment on microalgae cultivation were studied in relation to growth and nutrient removal.

Table 4 Applied treatments in experiment 1

Treatment	**Dose**	**Name**
Ozonation	7 mg/L (0.20 O_3/DOC)	O_3-7 mg/L
Ozonation	10 mg/L (0.27 O_3/DOC)	O_3-10 mg/L
Ozonation	20 mg/L (0.55 O_3/DOC)	O_3-20 mg/L
Ozonation	40 mg/L (1.1 O_3/DOC)	O_3-40 mg/L
Photocatalysis	4 h	UV/TiO_2-4h
Photocatalysis	8 h	UV/TiO_2-8h
UV-irradiation	260 mJ/cm^2	UV

Ozonation and photocatalysis have been previously reported to remove geosmin and MIB efficiently from DI-water with same setups as in this thesis, by applying doses of 40 mg/L and 8 h, with initial geosmin and MIB concentrations of 500 and 1000 ng/L (Wahlberg 2022). UV-disinfection was applied to present the impacts of disinfection strictly and chosen dose aimed for complete elimination of initial microbiota.

In the second experiment, pre-treatment and cultivation was repeated for another RAS-water sample. Additionally, to ozone and photocatalysis, the ozone treatment was coupled with UV to enhance the formation of hydroxyl radicals. The impact of sheer UV was also tested with a dose similar to one used in Ozone/UV treatments. List of treatments and doses is presented in Table 5.

Table 5 Applied treatments in experiment 2

Treatment	**Dose**	**Name**
Ozone/UV	7 mg/L (0.33 O_3/DOC)	UV/O_3-7 mg/L
Ozone/UV	20 mg/L (0.94 O_3/DOC)	UV/O_3-20 mg/L
Ozone	7 mg/L (0.33 O_3/DOC)	O_3-7 mg/L
Ozone	20 mg/L (0.94 O_3/DOC)	O_3-20 mg/L
UV	35 mJ/cm^2	UV
Photocatalysis	8 h	UV/TiO_2-8h

5 Materials and methods

In the following sections, used instruments and methods are presented together with applied treatment setups.

5.1 Water analyses

Composition and properties of RAS water were analyzed in relation to parameters presented in Table 6, together with applied instruments and methods.

Table 6 Applied instruments and methods for water analysis.

pH	pH110, VWR
Turbidity	Turb 355 IR, WTW
Salinity	CO310, VWR
NO_3^--N	DR3900, HACH, method 8039
PO_4^{3-}-P	DR3900, HACH, method 8048
DOC	Multi N/C 2100S, Analytik Jena, Filtered 0.45 nm
TC	Multi N/C 2100S, Analytik Jena, Filtered 0.45 nm
IC	Multi N/C 2100S, Analytik Jena, Filtered 0.45 nm
TN	Multi N/C 2100S, Analytik Jena, Filtered 0.45 nm

For C/N analysis samples were diluted as instrument could not cope with salinity. To compensate the impact of impurities in DI-water (the diluent), measured concentration in DI-water were subtracted from the analysis results as follows,

$$c_s = c_d * DF - c_w(DF - 1) \tag{5}$$

where, c_s concentration in sample, mg/L

c_d concentration in diluted sample, mg/L

DF	dilution factor
c_w	concentration in DI-water, mg/L.

Additionally, to water quality parameters, ozone concentration in water was analyzed with DR3900 (HACH, method 8311), and ORP with pH110 (VWR).

To provide additional information about the presence of dissolved organic matter, fluorescence analysis was performed with Cary Eclipse Fluorescence Spectrometer (Agilent). Fluorescence excitation-emission matrix (EEM) were constructed with excitation wavelengths from 200 to 400 nm while measuring the emission intensities in wavelengths 250-500 nm. In these ranges presence of common fluorescent dissolved organic matter can be observed as presented by Hudson et al. (2007) (Table 7). From the obtained intensity data, intensity peaks caused by Rayleigh-Tyndall effect were removed and contour plots were made using Matlab.

Table 7 Excitation-Emission peaks of common fluorescent DOM (Hudson et al. 2017)

Fluorophore type (Name)	**Ex/Em wavelength (nm)**
Humic-like (A)	237-260/400-500
Humic-like (C)	300-370/400-500
Marine Humic-like (M)	312/380-420
Protein-like (B)	225-237/309-321 and 275/310
Protein-like (T)	225-237/340-381 and 275/340

5.2 RAS-Water and storing

RAS-water sample used in the first experiments was received from RAS facility in Germany (latter referred as RAS-1), collected before the water treatment unit of the processes. Received water was brackish, with 20 ppt salinity and pH 8.15, and containing very little suspended solids having turbidity of 0.4 NTU.

For the second experiment set, RAS-water was received from another facility from Norway (latter referred as RAS-2). The water was brackish as before (18 ppt salinity) but had some suspended solids with turbidity of 2.0 NTU and had pH 6.41. As RAS-2 was received from facility applying nitrification-denitrification, the nitrogen levels were low. Therefore $NaNO_3$ (Sigma-Aldrich) was added, although the nitrogen levels remained low compared to RAS-1.

Received water samples were stored at 4 °C in plastic container. After the treatments, treated water was stored in glass bottles over the night at 4 °C before the algae cultivation. From the treated water, sample was taken to plastic bottle and frozen (-18 °C) for latter analysis.

5.3 Treatment processes

Specifics of used treatment processes, including setup-schemes and operation parameters are presented in following sections.

5.3.1 Ozonation setup

Ozone treatments in the first experiment were performed with setup presented in Figure 4. In operation, pure oxygen flow was connected to ozone generator (Modular 4HC Lab, Wedeco). Flow from generator continued to bubble diffusers in reactors and through valves V1 and V2 to ozone analyzer (BMT 964, BMT MESSTECHNIK) to measure ozone concentration in influent flow. After reactors the flow was conducted to scrubber and through valves V3 and V2 to analyzer, measuring concentration in effluent. Both reactors held 400 mL and after the treatment, contents were mixed.

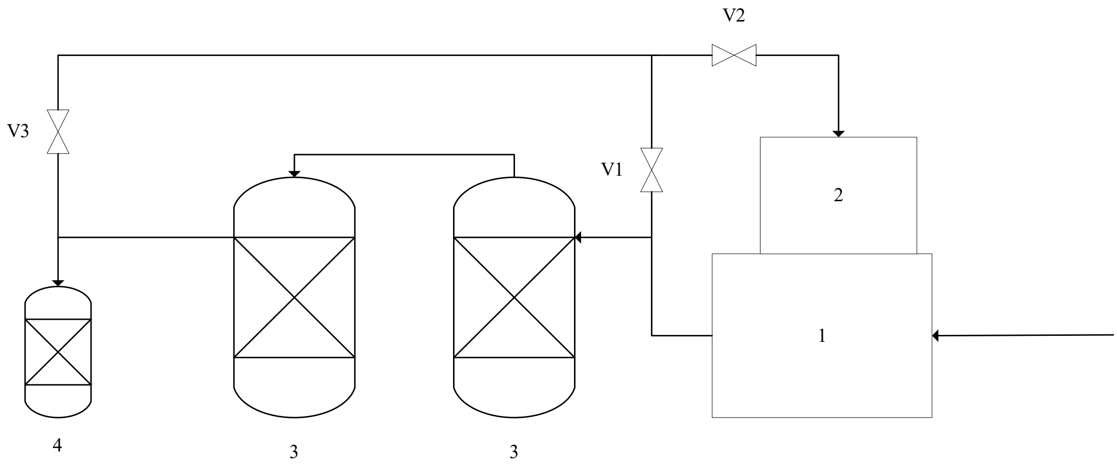


Figure 4 Notations: 1. Ozone generator, 2. Ozone analyzer, 3. Reactors, 4. Ozone scrubber.

The set-up did not enable continuous concentration measurement in both influent and effluent flow. Thus, the valves V1 and V3 were used to conduct flow to ozone analyzer from influent and effluent flows in turns. Average concentrations in both flows were determined for a time of measurement (30 s), from which cumulative absorbed ozone was determined according to Equation 4.

$$c_{abs} = \sum_{i=1}^{n} c_{abs,i} = \sum_{i=1}^{n} \frac{t_i \cdot Q \cdot (c_{in,i} - c_{out,i})}{V} \tag{4}$$

where,	t	time of measurement, min
	c_{in}	concentration in influent gas flow, mg/L
	c_{out}	concentration in effluent gas flow, mg/L
	Q	volumetric flow rate of gas, L/min
	V	volume of the water, L.

When destined ozone concentration was reached, ozone generator was switched off and content of reactors was collected to glass container. Immediately after collection, residual ozone concentration and ORP were measured from treated water.

In Figure 5 the absorption of ozone to RAS-water during treatments is presented. During the treatments the amount of absorption of ozone to water was quite constant. Approximately 1 mg/L/30s in 7 and 10 mg/L treatments, and 2 mg/L/30s in 20 and 40 mg/L treatments. In 7 mg/L and 10 mg/L treatments lower ozone generation setup was used, resulting in lower influent concentration and lower absorption rates.

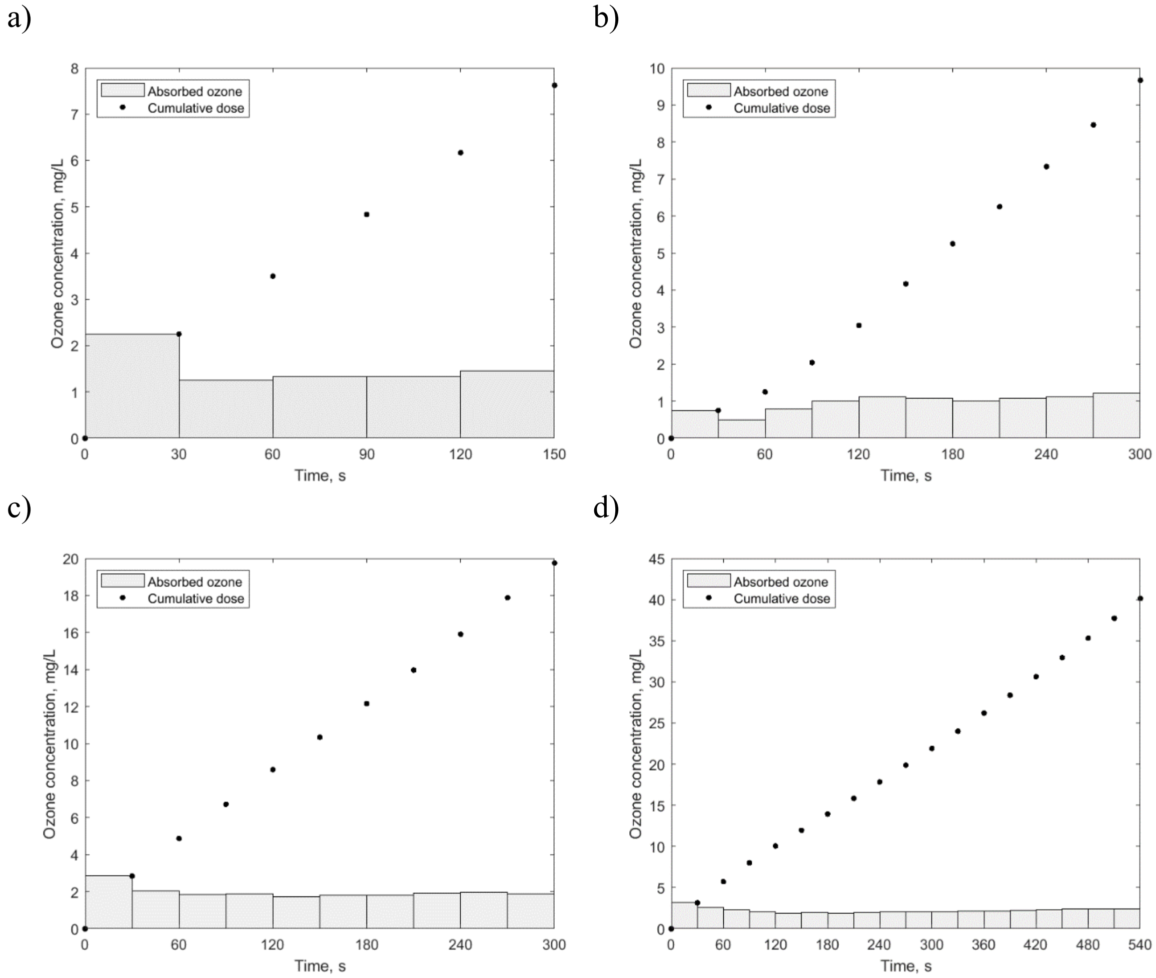


Figure 5 Ozone absorption during the ozonation experiments. Grey bars present the ozone doses in 30 s time spans with black dots presenting the development of cumulative dose. a) 7mg/L dose b) 10 mg/L dose c) 20 mg/L dose d) 40 mg/L dose.

Residual ozone concentration and ORP after the treatments are presented in Table 8.

Table 8

Treatment	**7 mg/L**	**10 mg/L**	**20 mg/L**	**40 mg/L**	**Untreated RAS**
O_3, mg/L	<0.01	0.7	0.6	0.64	0
ORP, mV	690	720	718	740	280

5.3.2 UV/TiO_2 setup

Photocatalysis reactor configuration is presented in Figure 6. Setup was inside a cabinet blocking external light. Solid TiO_2-coated catalyst inside the reactor possessed surface area of 50.15 cm^2.

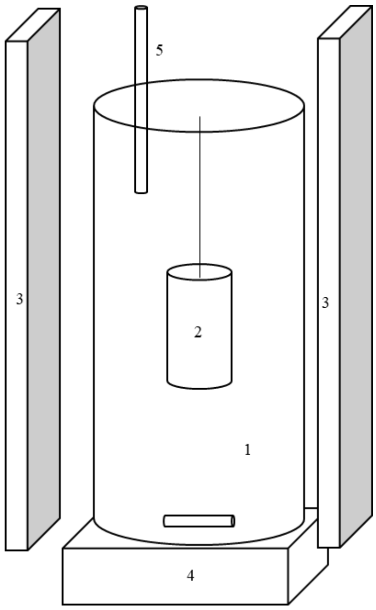


Figure 6 Notations: 1. Reactor, 2. TiO_2, 3. UV-lamp, 4. Magnetic mixer, 5. Thermometer.

Radiation spectrum and irradiance of light source (UVP 3UV, Analytik Jena) was determined with BLACK-Comet UV-VIS Spectrometer together with SpectraWiz-software. Irradiance was measured at different points along the lamp length and average value was calculated. Used light source emitted a peak in wavelength of 365 nm, as presented in Figure 7. Spectrum and irradiance were measured at the distance of 6 cm, equal to distance from lamp to reactor in set-up. Average irradiance in UV-C range (320-400 nm) was 18.12 W/m^2. Glass plate was observed to transmit approx. 94% from UV-C resulting that 16.97 W/m^2 would reach the media in reactor trough glass wall.

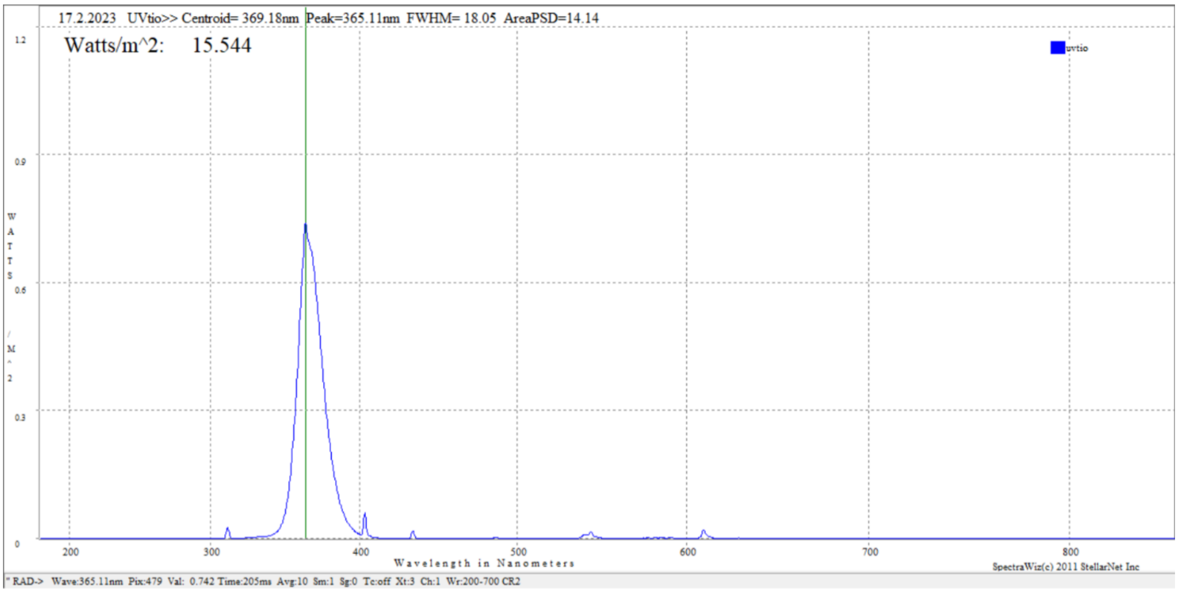


Figure 7 Spectrum of lamp used in photocatalysis setup.

Photocatalysis treatment of RAS water was performed with two different treatment times, 4 h and 8 h. In both treatments, 1 h dark adsorption time was used. During the treatments water temperature increased gradually from initial room temperature to approximately 30 °C.

5.3.3 UV-irradiation setup

In the first experiment, UV-irradiation was performed in following setup. Due to relatively small volume of the available reactor, irradiation was conducted with circulation. A schematic presentation of treatment unit is presented in Figure 8.

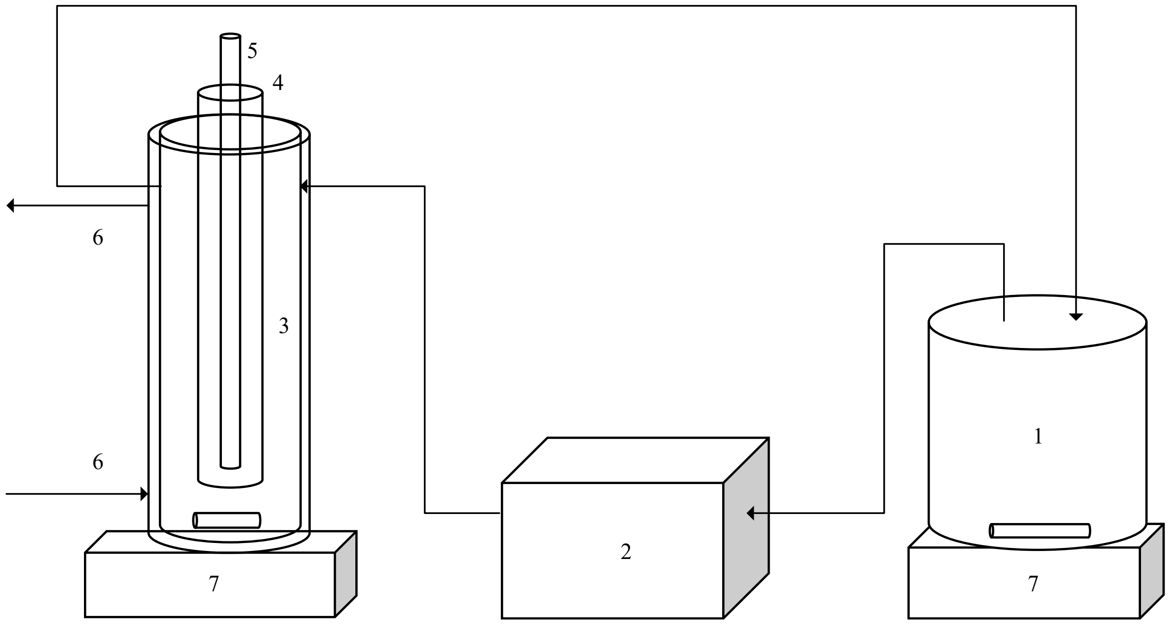


Figure 8 Notations: 1. Feed tank, 2. Pump, 3. UV-reactor, 4. Quartz sleeve, 5. UVC-lamp, 6. Cooling water circulation, 7. Magnetic mixer.

Volume of the reactor was 300 mL, and the thickness of water phase around the lamp was 1.5 cm.

Similar to UV-TiO_2, spectrum and irradiance was determined for used lamp (UVMax A, Trojan). Radiation spectrum (Figure 9) of used lamp was measured through the used quartz sleeve, directly presenting the spectrum which is applied to water in reactor. The lamp emitted a set of peaks from UV-C to visible light, with highest peak at 252.5 nm as desired. In the germicidal UV-C range, average intensity was 5.76 W/m^2.

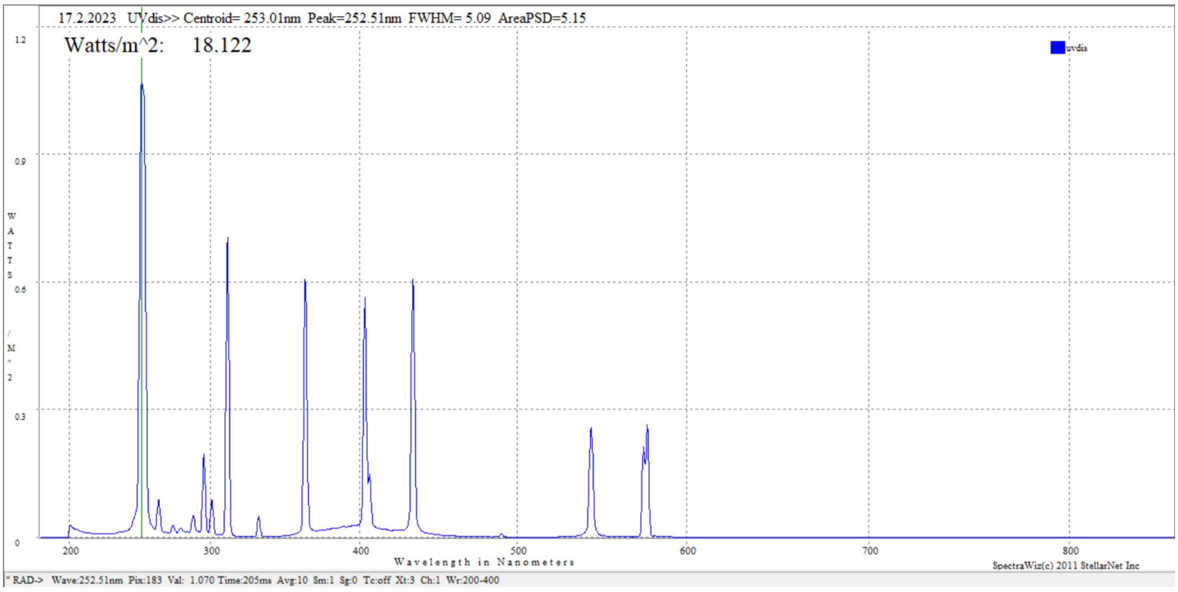


Figure 9 Spectrum of the lamp used in UV-treatment.

UV-treatment in the first experiment was performed aiming for high fluence (above 200 mJ/cm^2). System was loaded with 900 mL of RAS water which was circulated between storage tank and 300 mL reactor with volumetric flow of approximately 300 mL/min. Treatment time was 25 minutes, equivalent to 8.3 min total residence time in UV-reactor. With average intensity of 5.76 W/m^2 total fluence applied was then 288 mJ/cm^2. Accounting the water factor, with 0.049 cm^{-1} absorbance for 254 nm, the received fluence was 265 mJ/cm^2.

5.3.4 O_3/UV setup

In the second cultivation experiment the combination of ozone and UV was studied as pre-treatment. Applied treatment setup is presented in Figure 10. In practice, the setup was constructed by combining the previously presented ozonation process and UV-treatment. To compare the impacts of O_3/UV to O_3 and UV alone, all treatments were performed in same setup.

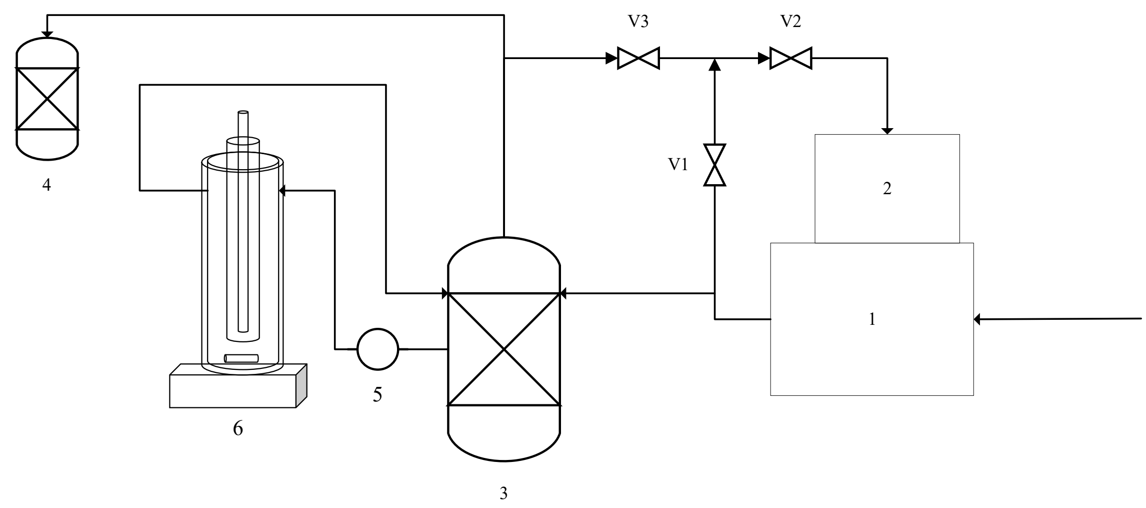
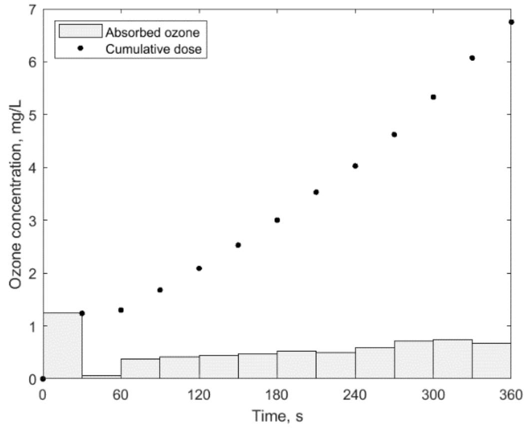


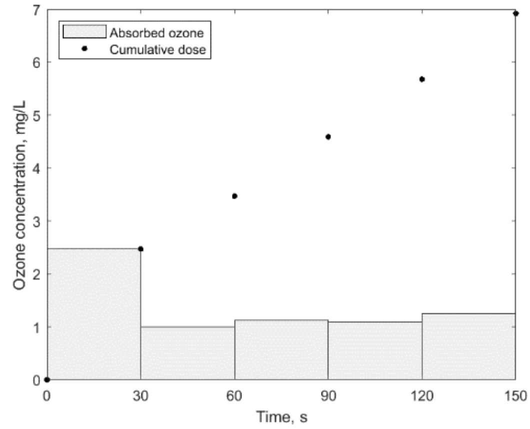
Figure 10 O_3/UV setup. Notations: 1. Ozone generator, 2. Ozone analyzer, 3. Ozone reactor, 4. Ozone scrubber, 5. Pump, 6. UV-reactor.

In the setup, ozone dose was measured alike in ozonation setup, and absorption of ozone in different treatments is presented in Figure 11.

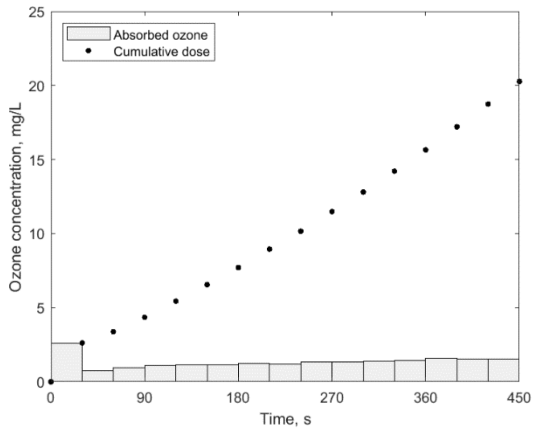
a)



b)



c)



d)

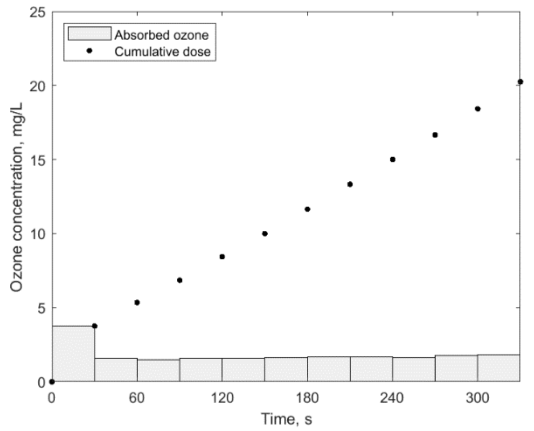


Figure 11 Ozone absorption during the ozonation experiments. Grey bars present the ozone doses in 30 s time spans with black dots presenting the development of cumulative dose. a) O_3-7 mg/L dose b) O_3/UV-7 mg/L dose c) O_3-20 mg/L dose d) O_3/UV-20 mg/L dose.

As seen in the figures above, with UV-treatment ozone was absorbed to sample faster. With 7 mg/L dose the treatment time was 360 sec without UV, compared to 150 sec with UV-treatment. This shows that UV-treatment did enhance the decomposition of ozone, allowing more ozone to absorb into sample.

For irradiation, same lamp as in UV-setup was used. Accounting the water factor counted from absorbance of 0.058 cm^{-1}, the received fluences during the treatments for O_3/UV-7, O_3/UV-20 and sheer UV were respectively: 29.4, 64.6 and 35.2 mJ/cm^2. Residual ozone concentration and ORP right after the treatments are presented in Table 9. Notably the measured residual ozone in UV-treated samples was lower while the ORP reached slightly higher levels.

Table 9 Residual ozone concentration and ORP after the treatments.

Treatment	O_3-7 mg/L	O_3/UV-7 mg/L	O_3-20mg/L	O_3/UV-20 mg/L	Untreated RAS
O_3, mg/L	0.02	<0.01	0.06	<0.01	0
ORP, mV	490	510	770	810	295

5.4 Microalgae cultivation

Microalgae cultivation was performed in glass bottles equipped with aeration and illumination with 12/12h light/dark cycle (white LED-light). Picture of the setup is presented in Figure 12. Volume used in cultivation was 150 mL in the first experiment and 200 mL in the second. Setup did not include separate mixer as aeration to the bottom would provide adequate mixing in the system. In both experiments the cultivation time was 14 days, and cultivation in water samples was performed in duplicates. Cultivated species was green marine microalgae *Nannochloropsis oculate*. *Nannochloropsis* species are recognized to have potential as aquaculture feed and in wastewater treatment. They are unicellular and compared to other algae species have high lipid content. (Paterson et al. 2023). Ideal pH for *Nannochloropsis* is between pH 7.5 and 8.5 (Khatoon et al. 2014), in lower pH as in RAS-2 (pH 6.41) lower growth rate can be presumed.

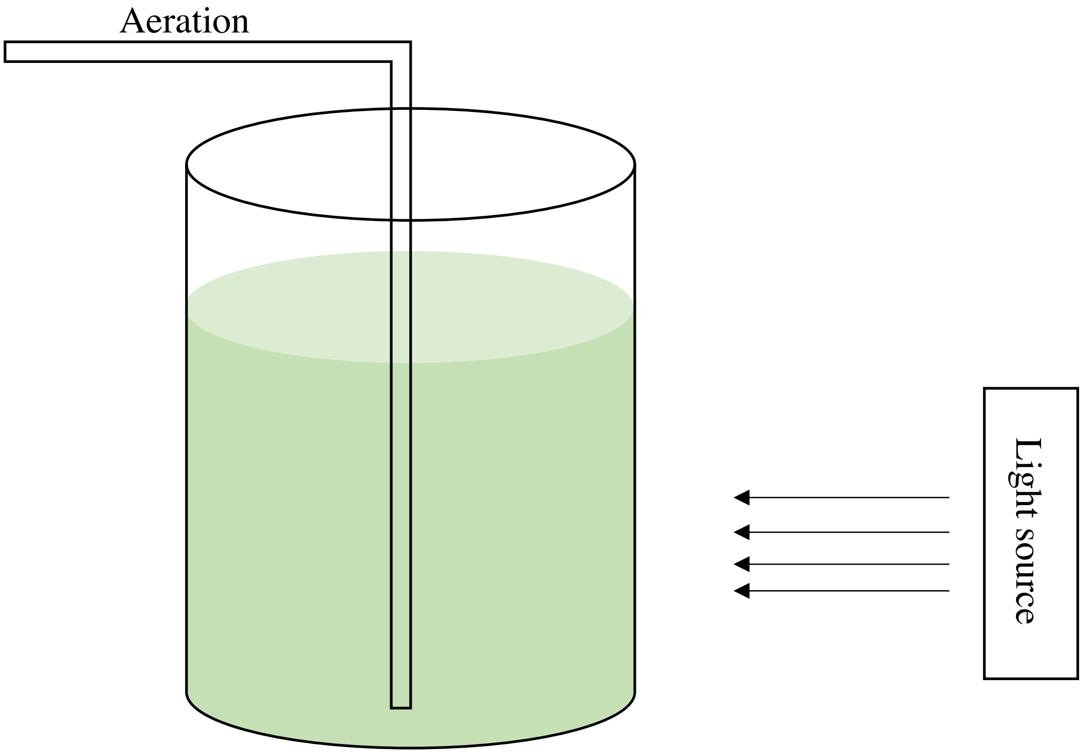


Figure 12 Microalgae cultivation setup.

In the first cultivation experiment biomass concentration in samples was determined by mass at the end of cultivation. First centrifuging the whole sample, where the supernatant was collected, and the pellet was washed into pre-weighed petri dish. Collected supernatant was then filtered through pre-weighed filter (0.45 µm). Filter and petri dish were dried (80 °C) and weighed, resulting in dry biomass per sample volume.

In the second experiment optical method was used to monitor the biomass during the cultivation. In optical method, absorbance of culture media was measured at the wavelength of 680 nm. Biomass concentration was then determined from calibration curve presented in Figure 13.

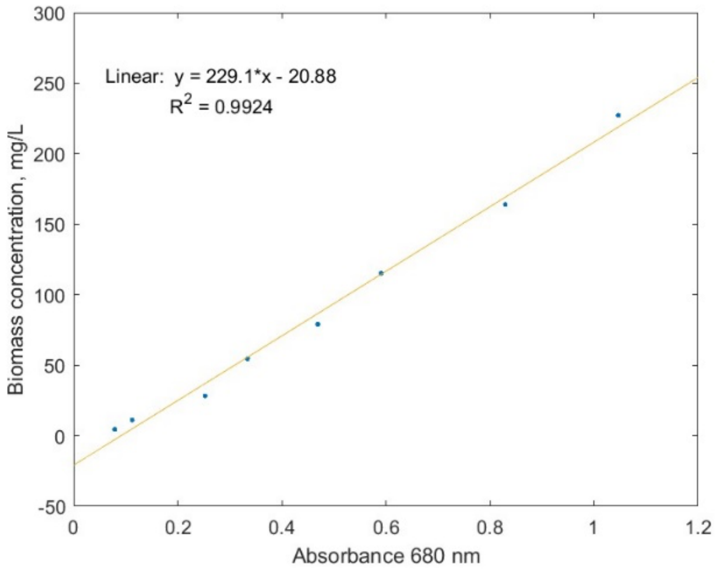


Figure 13 Calibration curve for optical biomass measurement.

During the cultivation, optical measurement was performed after 1, 4, 6, 8 12, and 14 days. Small sample (2 mL) was collected from the cultivation batch to cuvette from which absorbance was measured without further preparations.

6 Results and discussion

The results and discussion are divided for two performed experiments consisting of pretreatment and microalgae cultivation.

6.1 Experiment 1

For the first treatment-cultivation experiment, water sample RAS-1 was used. Measured properties and quality parameters of untreated RAS-1 are presented in Table 10

Table 10 Water quality parameters of untreated RAS-1.

Parameter	pH, -	Salinity, ppt	Turbidity, NTU	ORP, mV	DOC, mg/L	TC, mg/L	IC, mg/L	TN, mg/L	NO_3^--N, mg/L	PO_4^{3-}-P, mg/L
Value	8.15	19.10	0.43	266	36.64	58.02±2.44	21.38±0.18	142.06±0.30	101±15.56	8.80±0.37

Considering the microalgae cultivation, the nutrient content of the RAS-water was efficient with N:P ratio approximately 16:1 equal to Redfield ratio mentioned in chapter 3.2.3. Turbidity of RAS-water could be considered insignificant and hence the impact of pre-treatments to turbidity was not later studied.

6.1.1 Impact of treatments to water quality

Analyzed water quality parameters in treated and untreated (control) samples are presented in Figure 14.

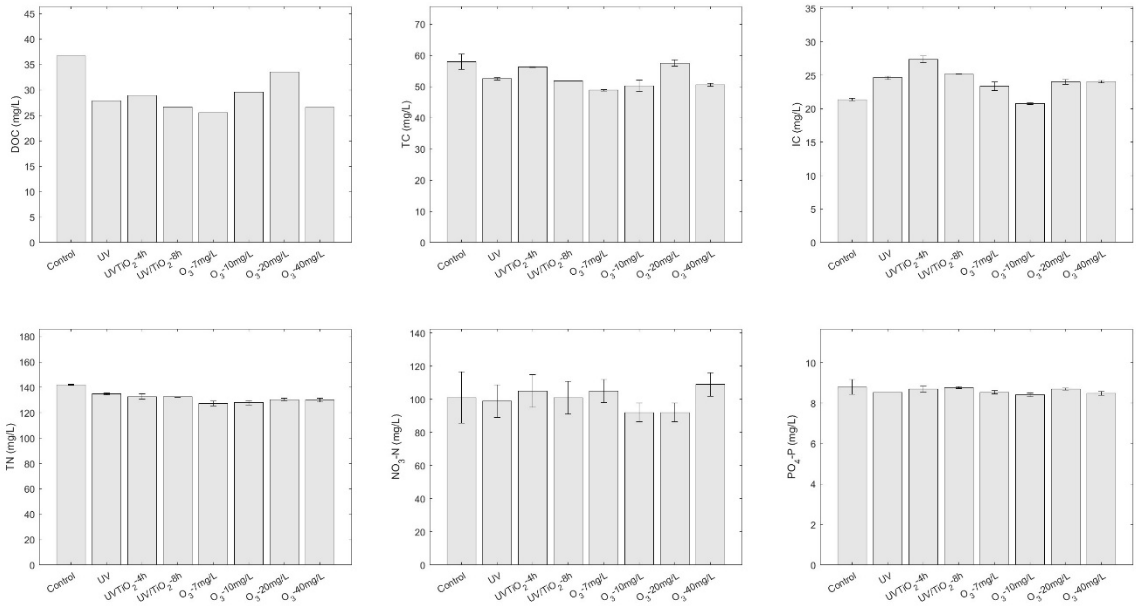


Figure 14 Impact of different treatments to carbon and nutrient content in RAS-1 water.

All treatments decreased the DOC concentration in water. Neglecting the results from O_3-20 mg/L the degree of DOC removal was similar in all cases and higher doses did not significantly differ from lower ones. As organic carbon concentration was decreased, slight increase in IC was seen, indicating that some mineralization occurred. Surprisingly also in UV-treated sample DOC was reduced, and IC increased in similar fashion as with oxidizing treatments. Share of inorganic carbon was highest in UV/TiO_2-8h and UV/TiO_2-4h with DOC/IC ratio of 1.06 in both. DOC/IC-ratio in UV-treated sample was 1.13 (1.71 in control), which is lower than in 10 mg/L and 20 mg/L ozone treated samples (1.42 and 1.40) and close to 7 mg/L and 40 mg/L samples (1.09 and 1.10). Even though UV-treatment can result in DOC reduction by assisting the biological mineralization (Paul et al. 2012) the results can be considered questionable. Because of the sensitivity of used analyzer for salinity, samples were highly diluted (40-times), which may have caused error in the analysis. Considering the ozonation treatments it must be noted that some of the carbon dioxide may have been stripped from water phase during the process, influencing the IC results.

Nitrogen and phosphorus concentrations were not impacted by the treatments as was presented by Park (2015) and considering that factor, oxidative treatments would not influence the microalgae cultivation.

In the Figure 15 fluorescence EEMs of samples are presented. Compared to control sample UV and UV/TiO_2 treatments did not drastically break down fluorescent DOM. In control sample and forementioned treated samples the presence of humic-like substances A and C can be observed. In comparison, samples treated with ozone do not show any major fluorescence. This is most likely due to selectivity of ozone towards aromatic structures (Gottschalk 2010, 18), which are found in fluorescent compounds.

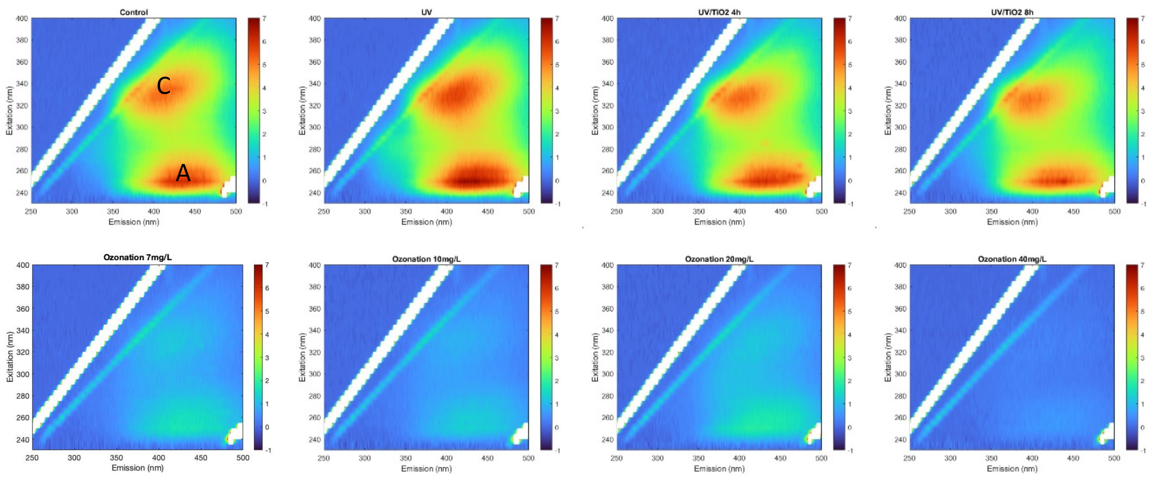


Figure 15 Fluorescence EEM after different treatments in experiment 1.

Looking more closely on change in intensity of specific peaks presented by Spiliotopoulou et al. (2017) (Table 11) it can be noted that ozonation did have a slightly higher impact on peak C than peak on A, which can be also seen in the study by Spiliotopoulou et al. (2017). In both peaks, a slight increase in intensity can be seen after UV-treatment, this could result from breakdown of larger molecules to smaller and more fluorescent ones (Rodríguez et al. 2014). In addition to the intensity increase in peaks A and C, in the EEM (Figure 15) we can see formation of novel peak at wavelengths Ex280/Em340 (peak T) which is connected to protein-like matter. This could result from death of microorganisms, which while breaking down would produce fluorescent matter.

Table 11 Reduction of intensity in treated samples compared to control in identified peaks.

Sample	UV	UV/TiO_2-4h	UV/TiO_2-8h	O_3-7 mg/L	O_3-10 mg/L	O_3-20 mg/L	O_3-40 mg/L
Ex335/Em450 (Peak C)	-5 %	7 %	17 %	79 %	81 %	72 %	95 %
Ex250/Em450 (Peak A)	-11 %	3 %	9 %	72 %	78 %	71 %	90 %

6.1.2 Microalgae cultivation

ORP and pH values at the beginning of cultivation in different samples are presented in Table 12. pH was proportionally decreased with increasing ozone dose.

Table 12 pH and ORP at the start of cultivation.

Sample	Control	UV	UV/TiO_2-4h	UV/TiO_2-8h	O_3-7 mg/L	O_3-10 mg/L	O_3-20 mg/L	O_3-40 mg/L
pH, -	8.15	7.84	8.26	7.92	7.8	7.79	7.7	7.53
ORP, mV	266	299	300	406	513	621	661	691

High ORP in ozonated samples indicates that in the RAS-water in case, ozone and its oxidative derivatives were not entirely decomposed or consumed even though samples were preserved overnight.

In the first cultivation experiment biomass and nutrient concentrations were only measured at the end of the cultivation (14 days). After the cultivation, samples were inspected with microscope and no contaminations were found. Final biomass concentrations in different samples are presented in Figure 16, together with relation between initial ORP-value and final biomass concentrations.

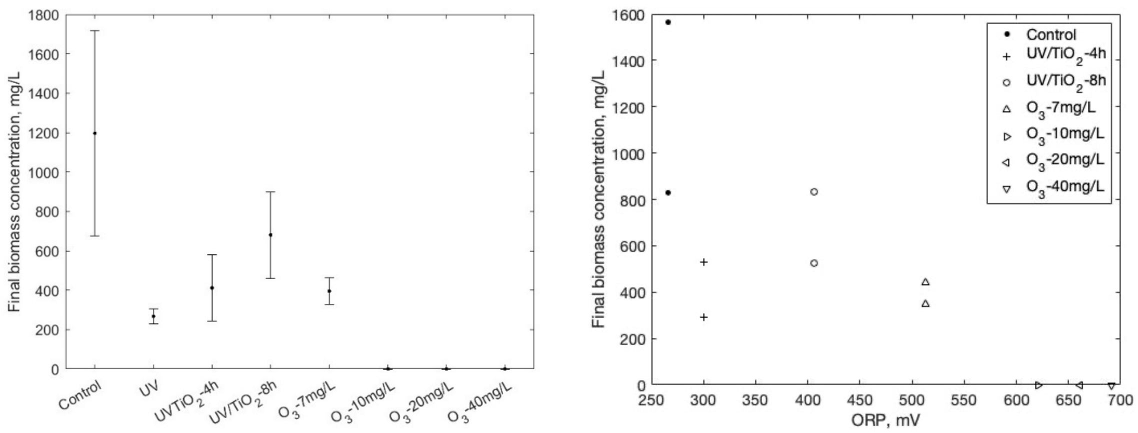


Figure 16 On the left, final biomass achieved with different pretreatments, standard errors are noted as error bars. On the right relation of biomass to ORP at the start of cultivation, with data points from both duplicates. Initial algae biomass concentration was 62.2 mg/L.

The microalgae cultivation was most successful in untreated control sample, where final biomass was on average 1.2 g/L. All pretreated samples performed significantly worse, with

UV/TiO_2-8h being the runner-up with 0.68 g/L final biomass. From ozone treated samples only the lowest dose enabled microalgae growth. Whereas with 10, 20 and 40 mg/L doses the culture did not survive. When observing the relation between ORP and final biomass, a clear pattern between these two variables can be observed, with increasing ORP reducing the success of algae culture and ORP above 550 mV completely restricting the growth. However, especially UV-treated sample differs from this pattern. This suggests, that in addition to ORP the high-rate disinfection hinders the growth of culture.

As presented in Figure 17 the nutrient removal increased together with increasing biomass. With phosphorus, complete removal (>99%) was reached even in samples with moderate final biomass. However, PO_4^{3}-P was reduced also in the samples where algae did not survive, indicating that some of the PO_4^{3}-P removal could be due to other than assimilation by algae. Compared to phosphorus removal nitrogen was withdrawn with lower rate. At highest, 47% removal was achieved in the sample with highest final biomass (control sample). Similar rates where however also witnessed in samples with lower biomass. In O_3-7 mg/L, with average final biomass of 395 mg/L, TN was removed with a rate of 43%. On the TN-removal graph samples treated with O_3-7 mg/L seem to point out from the trend on top left corner.

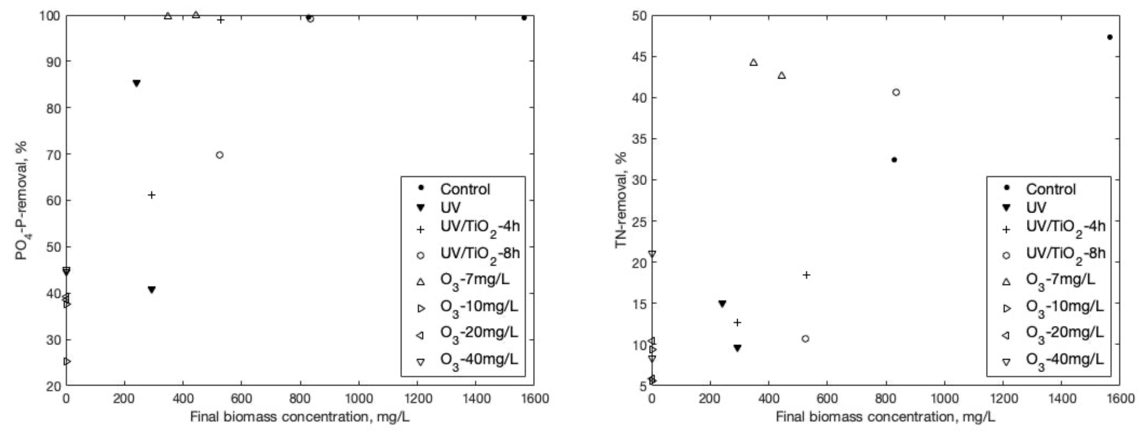


Figure 17 Relation between final biomass and nutrient removal in microalgae cultivation.

Calculating the ratio of removed nutrients and produced biomass, presented in Figure 18, show difference between the samples. The ratio between nitrogen removed and produced biomass in ozonated sample is close to four times higher than in control sample. This could result from the different metabolisms in different phases of growth (Anderson 2013). It could be possible that at the end of 14 days of cultivation ozonated sample was still in the rapid growth phase having high nitrogen uptake for protein formation, whereas other samples were closer to stagnant phase where biomass increase would be related to lipid and carbohydrate formation, with less nitrogen consumption.

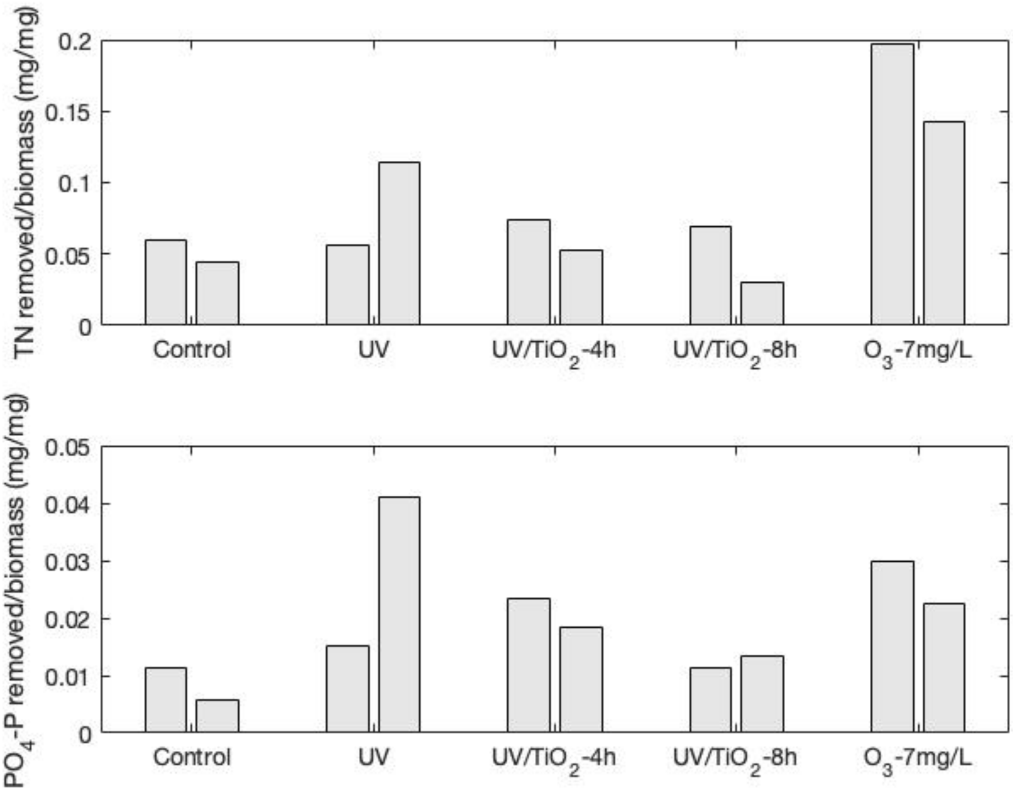


Figure 18 Ratio between removed nutrients and produced biomass in grown samples, both duplicates presented.

Overall, the results from the first experiment show that oxidation treatments primarily hinder the microalgae cultivation. In terms of biomass production, the untreated control sample performed significantly better than the treated samples. Most importantly high dose ozonation (10, 20 and 40 mg/L) did completely prevent the cultivation, likely due to the remaining oxidative species. Since the impacts of treatments to basic physiochemical properties were insignificant, the likely cause for hindered growth would be the changes in the microbiota. This theory would be also supported by the fact that from the samples with growth, the UV-disinfected one performed worst. On the positive side, also the samples with lower biomass growth (UV/TiO_2 and O_3-7 mg/L) could provide similar nutrient removal rates (41 and 43 % of TN) as the sample with highest growth (47 % of TN). However, it must be noted that towards the end of cultivation, most grown samples can start to decay and release the nutrients back to media. Also, nitrogen uptake is related to protein formation in algae cells, which is most active during the rapid growth phase and slows down when coming to stationary phase (Andersen 2013). Therefore, it is possible that cultures still in rapid growth phase can provide similar nitrogen accumulation even though they are lacking in biomass.

6.2 Experiment 2

For the second experiment, second water sample RAS-2 was used. Measured properties and quality parameters of untreated RAS-2 are presented in Table 13. Compared to the first sample, water used in the second experiment most importantly was slightly acidic, which is not ideal for the cultivation of *Nannochloropsis oculate*. Also nitrogen concentration was lower. RAS-2 had some turbidity and therefore the impacts of treatments to that parameter could be inspected.

Table 13 Water quality parameters of untreated RAS-2.

Parameter	**pH, -**	**Salinity, ppt**	**Turbidity, NTU**	**ORP, mV**	**DOC, mg/L**	**TC, mg/L**	**IC, mg/L**	**TN, mg/L**
Value	6.41	17.6	2.03	293	21.323	26.642	5.318	30.953

6.2.1 Impact of treatments to water quality

Similar to the first experiment all treatments decreased DOC and the differences between treatments were not drastic (Figure 19). As in the first experiment, the sheer UV treatment decreased DOC concentration in similar manner that oxidative treatments, which was not expected. Measured inorganic carbon did not differ much between the samples with exception of O_3-20 mg/L and the decrease in DOC compared to control sample is mostly explained by the decrease in measured TC. As in the first experiment the quality of data can be questioned as the measured carbon content varies between the samples, but no pattern can be observed. For the future different analysis method could be assessed for carbon analysis in saline water.

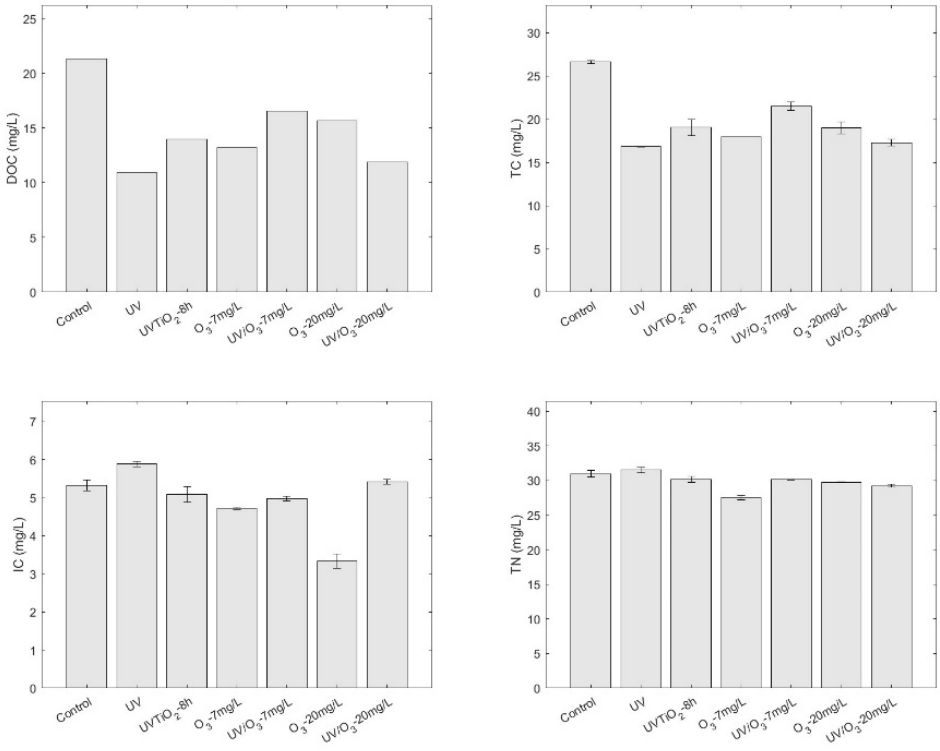


Figure 19 Impact of different treatments to carbon and nitrogen content in RAS-2 water.

Fluorescence EEMs (Figure 20) show that fluorescent compounds found in untreated water (humic-like A and C, and protein-like) were decomposed by O_3 and UV/O_3 entirely as before. With UV and especially with UV/TiO_2 some decrease in intensity can also be noted.

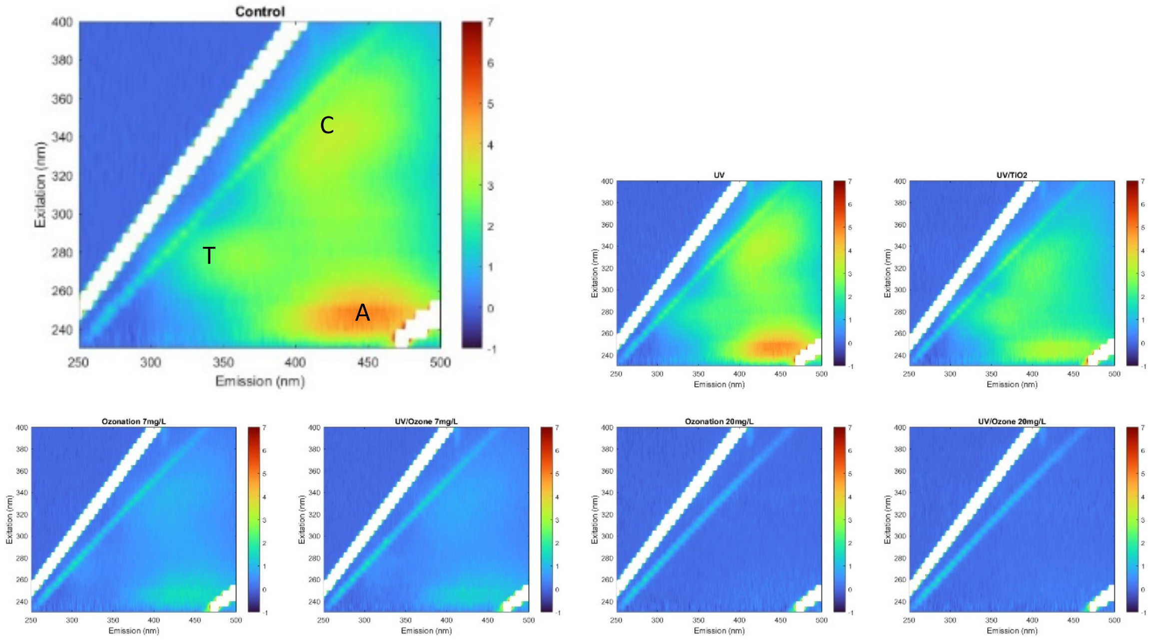


Figure 20 Fluorescence EEMs after different treatments in experiment 2.

As in experiment 1, the impact of treatments to peaks in specific wavelengths is presented in Table 14. Unlike in experiment 1 the UV-treatment did not have a promoting impact on fluorescence, perhaps due to lower dose of irradiation. Compared to experiment 1, photocatalysis produced higher decrease in intensity, indicating differences in the type of initial fluorescent matter. Ozonation with 7 mg/L dose (0.33 O_3/DOC) had slightly lower impact on intensity as 7 mg/L dose in the first experiment (0.2 O_3/DOC), whereas 20 mg/L (0.94 O_3/DOC) resulted in slightly higher reduction than 40 mg/L dose (1.1 O_3/DOC) in the first experiment. The addition of UV-irradiation increased the fluorescence removal in both ozonation treatments.

Table 14 Reduction of intensity in treated samples compared to control in identified peaks.

Sample	UV	UV/TiO_2-8h	O_3-7 mg/L	UV/O_3-7 mg/L	O_3-20 mg/L	UV/O_3-20 mg/L
Ex335/Em450 (C)	7 %	38 %	72 %	75 %	94 %	97 %
Ex250/Em450 (A)	1 %	38 %	69 %	73 %	94 %	98 %
Ex275/Em340 (T)	7 %	36 %	69 %	76 %	90 %	95 %

6.2.1 Microalgae cultivation

As presented in Table 15 oxidative treatments slightly increased pH. In samples with low dose ozonation the ORP was similar to control whereas with higher dose the high ORP maintained through the preservation overnight. Regarding the oxidative treatments, UV/TiO_2 did not decrease turbidity, whereas ozonation clearly had a positive impact. Turbidity however was not a limiting factor in the cultivation process as presented next.

Table 15 pH, ORP and Turbidity measured before the cultivation.

Sample	Control	UV	UV/TiO_2	UV/O_3-7 mg/L	O_3-7 mg/L	UV/O_3-20 mg/L	O_3-20 mg/L
pH, -	6.41	6.49	6.68	6.72	6.62	6.72	6.57
ORP, mV	293	295	297	290	300	780	805
Turbidity	2.03	3.11	2.63	1.63	1.33	0.76	1.22

Development of biomass in different samples is presented in Figure 21. During the 14-day cultivation UV/TiO_2 and UV-samples reached the stagnant phase of growth which was witnessed also by the color change from pure green to brownish. In these samples the average biomasses at the end were 437 mg/L (UV/TiO_2) and 434 mg/L (UV). Similar final biomass was also achieved in one of the control samples (450 mg/L), however the other control sample performed much worse, producing only 254 mg/L. Comparing the control samples to all treated samples one notable difference is the much greater variation between the duplicates. Similar pattern was also witnessed in the previous experiment, which would indicate that even though applied treatments hinder the biomass growth in mass, the growth is more consistent. Possible explanation could be that untreated RAS contained vaster microbiota with species with both positive and negative impacts on microalgae growth, resulting in variation between duplicates. In treated samples disinfection resulted in reduced and more uniform microbiota which did not have such significant impact on growth. As the sheer UV-treatment did not have negative impact on growth, similar low dose used in UV/O_3-treatments would not cause negative impacts as with high UV dose in the first experiment. After the cultivation samples were inspected with microscope and no contaminations were found.

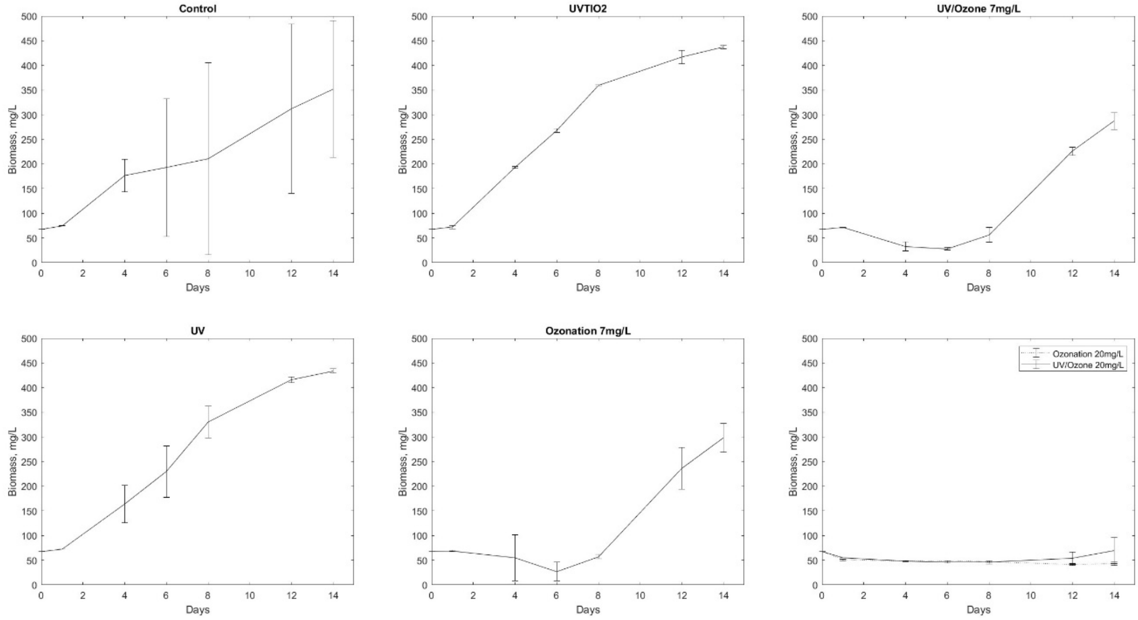


Figure 21 Biomass growth during the cultivation. Initial algae biomass concentration was 67.3 mg/L.

The samples treated with 20 mg/L ozone did not show algae growth as presented in bottom right. With lower ozone dose, no growth was observed during the first week but towards the end of the experiment algae begun to grow rapidly. Between days 8 and 12 these samples had average growth rates of 44.8 mg/L/d (O_3-7 mg/L) and 42.4 mg/L/d (UV/O_3-7 mg/L), which are comparable to growth rates between days 4 and 8 with UV (41.4 mg/L/d) and UV/TiO_2 (41.7 mg/L/d).

Comparing the two ozone doses, in the high dose samples with ORP above 700 mV the green color was completely lost during the first days whereas with low dose samples, greenish color was maintained even though notable growth was not witnessed during that period. The reason for this prolonged lag phase is not entirely clear. In both low ozone dose samples, there was a slight decrease in absorbance/biomass during the first week, gesturing that a portion of the algae culture died. Since the measured ORP in these samples did not differ from control sample, it is improbable that residual oxidative species would be responsible for the death of algae, which most likely is the case in high dose samples.

Nitrogen concentration was measured at the end of cultivation and in respect to initial concentrations, removal efficiency was overall higher than in first cultivation (Figure 22). This is most likely due to lower N:P ratio in the water, as in first cultivation P was the limiting factor. Phosphorus concentration was not measured in this experiment due to limited volume of sample.

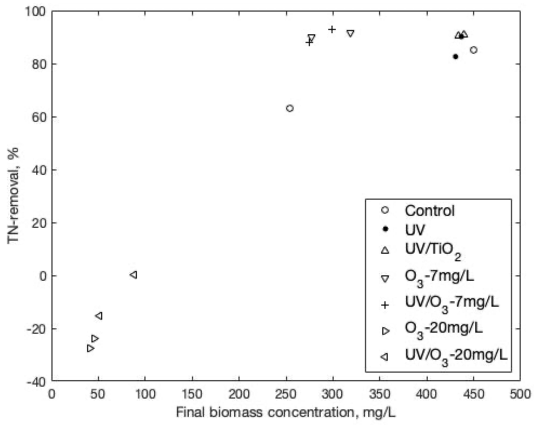


Figure 22 Nitrogen removal in relation to final biomass concentration in experiment 2.

Similarly to the first cultivation, efficient nitrogen removal was achieved also in samples with lower growth. In samples with hindered growth (O_3 and UV/O_3-7 mg/L) nitrogen was removed with average rates of 91 and 90 % respectfully. For comparison UV and UV/TiO_2 samples, which produced 1.5 times the biomass, average removal rates were 86 and 91 % respectfully. As concentrations were only measured at the end of cultivation, highest growing samples were approaching the stagnant phase as can be seen in Figure 21, where metabolism of algae changes (Anderson 2013). Calculating the ratio between nitrogen removed and biomass as in experiment 1 (Figure 23), shows a difference between O_3 and UV/O_3-7 mg/L and other samples. Similar higher ratio was seen in the first experiment with ozone treated sample (Figure 18). This difference could be connected to difference in the phase of growth and would support the idea that also in the first experiment ozone treated sample suffered from prolonged lag phase compared to samples with higher produced biomass.

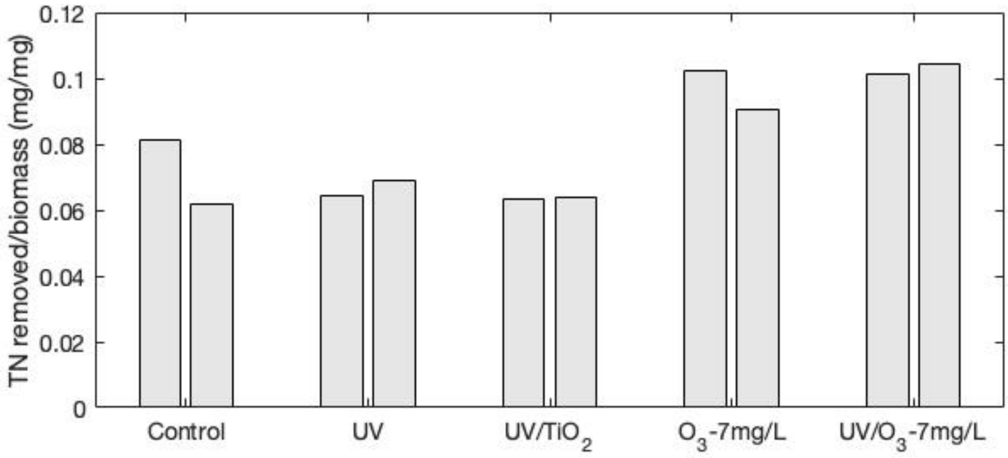


Figure 23 Ratio between removed nitrogen and produced biomass in grown samples, both duplicates presented.

6.3 Feasibility of co-application of AOPs and microalgae

Based on the two experiments oxidative pretreatment primarily hinders the cultivation, however treatments did reduce the variance between duplicates, which would provide consistency for microalgae application. Also, even with hindered growth microalgae was capable to remove nutrients from the wastewater. These preliminary results suggest that co-application of AOPs and microalgae cultivation in RAS could be applied. From the three examined AOPs (UV/TiO_2, O_3 and UV/O_3), photocatalysis provided the best result considering the microalgae cultivation. Most importantly, photocatalysis did not cause significant increase in measured ORP, indicating that it produced rapidly reactive oxidative species which did not remain in the media and interfered with algae process, which was a noted problem with ozone. In the second experiment the ORP level in ozone-treated sample was equal to ORP in photocatalysis and control sample. Also, in that case photocatalysis performed best and algae growth in ozone was severely hindered. Therefore, the residual oxidative species would not be the only difference between methods.

One option could be the differences in microbiota resulting from different treatments, which produce different oxidative species. For example, considering gram-positive and gram-negative bacteria, some studies suggest that ozone treatment is more lethal for gram-negative species (Moore et al. 2000). Whereas photocatalysis would exterminate more gram-positive species (He et al. 2021). Another possible growth inhibitor could be the absence of humic acids in ozone treated samples, presented in Figures 15 and 19. Yu et al. (2023) and Gim & Kim (2019) have presented that some humic-like substances could act as growth promoters in algae cultures. In that perspective, intense decomposition of these substances, which was presented by ozone, would not favor the latter algae cultivation. Additionally, the formation of toxic by-products was possible. In saline systems where bromide is present the formation of toxic bromate can occur and is also a factor when choosing the AOP. Ikehata et al. (2016) studied the bromate formation with different ozone based AOPs (O_3, O_3/H_2O_2, O_3/UV and O_3/H_2O_2/UV) and presented that in these processes most bromate was formed with O_3 in alkaline water (pH 10). Bromate formation was slightly hindered with addition of UV but best results were obtained with H_2O_2 addition and by applying ozone in lower pH (pH 6). Considering the two RAS-waters used in the experiments, RAS-2 with lower pH would be safer in terms of bromate formation in O_3-process.

Considering the different AOPs studied in this work, the photocatalysis treatment provided the best results in algae cultivation. Compared to O_3 and UV/O_3, photocatalysis does most strictly produce rapidly reactive hydroxyl radicals which could be a desired feature in this application. However, for the real-life application the O_3 and UV/O_3 can be considered more ready, whereas full-scale application of photocatalysis could have its challenges as was discussed in chapter 4.2. Also, as the RAS-operators are currently applying ozone in their processes, the enhancement of radical formation O_3 would be reasonable to study firstly. The UV/O_3 setup applied in this work cannot be described ideal and further experiments should be done with setups which have immediate UV-radiation with sufficient fluence together with diffusion of ozone. Considering the dosing of ozone, in the first experiment ozone dose of 0.2 O_3/DOC did only hinder the growth of algae, whereas with 0.27 O_3/DOC completely prevented the growth, whereas in experiment 2 algae growth was seen with slightly higher dose in relation to DOC (0.33 O_3/DOC). In experiment 2 the initial biomass concentration was slightly higher (67.3 mg/L) than in experiment 1 (62.2 mg/L) which could impact on the results. The growth curves presented for experiments 2 implies that in ozone treated water a portion of initial algae may die, after which the survived algae can grow with similar rate as in unozonized water. Naturally, the survival of algae would be related to initial amount of algae in the culture. Therefore, the impact of ozonated water could be less drastic when the receiving culture is already grown to some extent, which would be the case if continuous cultivation is applied.

7 Conclusions

According to experiments performed in this thesis, the pretreatment with AOPs has a neutral or negative impact on latter microalgae cultivation in RAS-water. In all cases, untreated control samples performed best in terms of biomass growth. However, the variation between these samples was great compared to treated samples. Therefore, pretreatment did provide positive impact in terms of consistency in microalgae cultivation. Best results were achieved with UV/TiO2 photocatalysis, which provided biomass growth similar to untreated sample. In ozone treated samples microalgae growth was hindered significantly, with highest doses completely preventing the algae growth. Hindered growth of biomass was observed as prolonged lag phase in culture, but the exact factors for the phenomena could not be determined. With certainty residual oxidative species measured as ORP in water restricted the algae thriving. Other possible factor could be the oxidation of growth supporting microbiota and DOM in AOP-treatment. Differences between photocatalysis and ozone-based treatments indicate that rapidly reactive hydroxyl radicals are safer for latter microalgae cultivation.

Considering the future research additionally to photocatalysis, experiments could be carried out with ozone-based processes in which radical formation is enhanced with UV. Implemented dosages of AOPs where in this work based on previous experiments with taste and odor compounds in DI-water, and the removal of those AOP-targeted compounds was not specifically studied now. Since the impact of AOPs to microalgae cultivation was neutral or negative, depending on method and dose, the removal of AOP-targeted compounds should be taken account in later research.

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