NTNU
Norwegian University of
Science and Technology

TMR4570 - Marine Resources and Aquaculture

PROJECT THESIS IN MARINE TECHNOLOGY

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Treatment of seawater to semi-closed aquaculture systems in sea

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Submission date: 16th December 2016

Preface

This report is a result of my work in the course TMR4575 Marine Resources and Aquaculture, Specialisation Project at the Department of Marine Technology, NTNU. The project work is a result of one semester, from late August until December 16th 2016, and gives 7.5 credits. This report will be a pre-study to the master thesis next semester. The pre-study should review relevant literature and build a foundation of understanding to the main study.

The combination of earlier courses like TVM4145 Unit Processes in Water and Wastewater Treatment, TMR4140 Design of Marine Production Plants, TMR4254 Marine Systems Design and TEP4265 Thermal and Process Engineering of Food, has given me motivation for writing this project thesis. Because this project is somewhat interdisciplinary it has been time consuming to get an understanding of every aspect related to the topic. The project thesis is a cooperation with the Centre for Research-based Innovations in Closed-Containment Aquaculture, CtrlAQUA. *INTAKE* is the name of a specific project in CtrlAQUA that this project thesis is linked to. The experiment was conducted at Nofima Research Station for Sustainable Aquaculture, Sunndalsøra. I spent 5-6 weeks in Sunndalsøra during the experimental period.

The report should give the reader a better understanding of water treatment of seawater in aquaculture with focus on disinfection by-products. Targeted reader of this report is other students at master level, and somewhat professionals with limited insight to the specific topic. My main goal this semester was to increase my understanding of water treatment technology related to the aquaculture industry.

December 16, 2016

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Summary

Norwegian aquaculture industry is evolving and new concepts such as semi-closed containment systems, offshore farming and landbased production are on the doorstep. These new production systems have different requirements than traditional production. This project focuses on the possibility of treating the seawater to semi-closed systems in sea, with a direct link to landbased production.

Even though treatment of freshwater and seawater differs it is the same water quality parameters we want to optimise. Physical and chemical measures of water quality covers parameters like oxygen, temperature, pH, total organic carbon, turbidity, salinity and total suspended solids. All of which affects the production of Atlantic salmon. Optimising water quality increases fish health and welfare and can increase production rate. Disinfection of water will also increase the safety of production, keeping pathogenic microorganisms out of the rearing environment.

Aquaculture industry is using the same theoretical basis and treatment technologies as in drinking water and wastewater treatment, but the treatment goals might differ. The influence of salinity and the precautions needed to avoid problems with disinfection by-products are presented in this task. Seawater contains bromide which reacts with oxidants, like ozone, to create disinfection by-products.

Results from the experiment show that the use of ozone increases the total residual oxidants (TRO) to reach possible toxic levels for fish. Compared to other treatment technologies like ultraviolet light (UV) and advanced oxidation process (AOP) the use of ozone has been shown to give the highest level of TRO. This is resulting in the need for treatment to remove some of the active bromine (TRO), e.g. use of activated carbon filter (GAC), to stay beneath toxic levels. Use of AOP did not change the level of TRO.

Results regarding viable bacteria, measured as Colony Forming Unit (CFU), show that the AOP reach the same disinfection level as ozone with UV. Only using UV also gives decent results, but is varying a bit more than the AOP, which delivers stable results for every test executed.

Still there are a lot of unknown results from the experiment needed before it is possible to conclude or at least propose a treatment system for semi-closed systems in sea. Analysis like amount of bacteria removed, types of bacteria removed, total organic carbon present, total suspended solids and turbidity are all of interest when narrowing down the number of treatment possibilities. The results must be verified statistically before reaching a final conclusion.

Furthermore, there are other aspects to look into, such as functionality of treatment system in sea and design of a system for sea operations. Some of these aspects will be covered in the master thesis next semester.

Sammendrag

Norsk havbruksnæring er i utvikling og nye konsepter som semi-lukkede anlegg, offshore havbruk og landbasert produksjon er like rundt hjørnet. Disse nye produksjonssystemene stiller andre krav til teknologi og utforming, enn tradisjonell produksjon. Denne oppgaven fokuserer på mulighetene for behandling av sjøvann til semi-lukkede anlegg i sjø, som også er gjeldende for landbasert produksjon.

Det er forskjell i behandling av ferskvann og sjøvann, men det er de samme vannkvalitetsparameterne vi ønsker å optimalisere. Fysiske og kjemiske målinger av vannkvalitet omfatter parametere som oksygen, temperatur, pH, totalt organisk karbon, turbiditet, saltholdighet og totalt suspenderte faststoffer. Alle påvirker produksjonen av Atlantisk laks. Optimalisering av vannkvaliteten kan øke fiskens helse og velferd, og vil kunne øke produksjonsraten. Desinfeksjon av inntaksvannet vil også øke sikkerheten i produksjonen ved å fjerne eller inaktivere patogene mikroorganismer før de kommer i oppdrettsenheten.

Oppdrettsnæringen bruker samme type renseteknologi som i drikkevann og avløpsrensing, men målet med behandling kan ofte være forskjellig. Påvirkningen av salinitet og hvordan man må unngå problemer med restprodukter fra desinfeksjon er presentert i denne oppgaven.

Resultater fra eksperimentet viser at bruk av ozon øker total rest oksidanter (TRO) opp til et mulig giftig nivå for fisken. Sammenlignet med andre behandlings metoder, som ultrafiolett lys (UV) og avanserte oksidasjonsprosess (AOP), gir ozon det høyeste nivå av TRO. Dette resulterer i behov for behandling for å fjerne noe av andelen aktivt brom (TRO), f.eks. ved bruk av aktivt kullfilter (GAC). Bruk av AOP øker ikke TRO verdiene.

Resultatene fra tellingen av aktive bakterier, målt som kolonidannende enheter (CFU), viser at AOP leverer tilsvarende desinfeksjons nivå som ozon med UV. Kun bruk av UV gir også resultat av interesse, men er litt mer varierende enn AOP, som leverer stabile resultater igjennom hele forsøket.

Likevel er det mange ukjente resultater fra eksperimentet som er nødvendige å få på plass før det er mulig å konkludere, eller i det minste foreslå et behandlingssystem for semi-lukkede anlegg i sjø. Gjenstående analyser, som mengden av bakterier fjernet, typer bakterier fjernet, totalt organisk karbon, totalt suspendert stoff og turbiditet er alle av interesse når systemene skal måles opp imot hverandre. Resultatene må sjekkes statistisk før en endelig konklusjon kan presenteres.

I tillegg til resultatene fra eksperimentet, er det andre aspekt det er viktig å tenke på, som hvordan man skal designe et funksjonelt system, kapabelt til å operere i sjø. Noen av disse aspektene vil bli dekket i masteroppgaven neste semester.

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Abbrevations

AOP   Advanced oxidation process

AOT   Advanced Oxidation Technology

BOD   Biochemical oxygen demand

CFU   Colony Forming Unit

CO_2 Carbon dioxide

COD   Chemical oxygen demand

DO   Dissolved oxygen

FNU   Formazin Nephelometric Units

FTU   Formazin Turbidity Units

HO•   Hydroxyl radicals

IPN   Infectious Pancreatic Necrosis

LC_{50} Lethal concentration that will kill half of sample population

NOM   Natural organic matter

NTU   Nephelometric Turbidity Units

ORP   Oxidation-reduction Potential

RAS   Recirculating Aquaculture System

THM   Trihalomethanes

TiO_2 Titanium dioxide

TOC   Total organic carbon

TRO   Total residual oxidants

TSS   Total suspended solids

TU   Turbidity Units

UVT   Ultraviolet transmittance

1 Introduction

Aquaculture production systems are in constant development to meet the demands of increasing production and at the same time maintain a sustainable production method. In Norway, the main aquaculture species is Salmon. Normally salmon smolts are produced on land to around 80-100 g before transport to open net cages in the Norwegian fjords, however we are looking at some immense developments. Some producers are developing huge structures for exposed aquaculture for the on-growing phase, while others are looking to increase the control of the cultivating environment by developing closed and semi-closed production systems in sea and on land, and to keep the smolts in such systems for a longer period before being transported to open net cages. Besides meeting the demands of increased production, the new designs and strategies are also developed to solve problems the industry are struggling with, like sea-lice and escapes. When describing production and production parameters, this report will focus on Atlantic Salmon since this is the main species in Norway.

1.1 Motivation for aquaculture in Norway

Since it's early days in the 1970s, fish farming in Norway has taken small incremental steps towards a more sustainable production with more control and higher standards. Yet, there are still a long way to go before we can be satisfied with respect to environmental impacts, sustainability, fish health and welfare, and control. There are still fish escaping, net cages collapsing in stormy weather and the sea-lice are negatively effecting the production costs as well as environment.

So why should we be interested in developing this industry? In 2015 Norway produced approximately 1.4 million tons seafood from aquaculture, with an estimated value of 47 billion NOK (SSB, 2016). Aquaculture industry is one of the fastest growing industries in Norway and it is estimated that the growth will be formidable towards 2050 (Olafsen, Winther, Olsen, & Skjermo, 2012). For Norwegian export and trade it is absolutely critical that we are leading the development of aquaculture, especially when it comes to Atlantic Salmon. We must continue to deliver top quality products and at the same time optimise the growth rate of fish sustainably.

1.2 System description

Semi-closed fish farming in sea means that you produce fish in closed tanks instead of open net pens. This gives you a totally different set of problems to handle. A closed structure floating in sea requires more attention to hydrodynamic forces and environmental loads like waves. Minimising these loads means to move inshore and in sheltered waters. But what happen then with the influent water quality? It may seem that there are some sort of compromise when it comes to how sheltered you can go before it will affect the water quality parameters which are extremely important for the production of fish. As an illustration of a semi-closed aquaculture system we can take a look at Marine Harvest and Hauge Aqua's system, called "The Egg".

Figure 1: A proposed semi-closed system from Marine Harvest and Hauge Aqua.

In semi-closed system you still rely on nature doing it's part in providing the right temperature, bringing enough oxygen into the tank and remove waste. Water entering a semi-closed system will only be used once before it will leave the rearing tank, bringing with it waste. Compared to an open system it will have a much higher production rate because of the increased control. Some of the advantages of semi-closed systems is a more efficient feed rate, water replacement, temperature control and detection of diseases. On the other side we have larger construction costs, operational costs and demand for monitoring.

Marine Harvest have tested a similar production system at Molnes in Skånevik and it has shown weakness related to parasites and diseases (Breck, 2014). These kind of systems need to have a safe supply of intake water, which could mean there is a need to have some kind of water treatment before the flow enters the rearing environment. According to Nilsen, Nielsen, Biering, and Bergheim (2016) sealice seems to be less of a contributor to the discussion regarding the need to treat intake water, but Handeland, Calabrese, Kolarevic, Breck, and Terjesen (2015) found that these parasites still finds their way into the rearing tanks, even though below the legal limits.

So far it seems the conceptual design of the production unit and location determines whether it will be affected by sealice or not. Handeland (2016) reported that Lerøy's concept, *Preline*, had no cases of sealice during their full scale testing.

Since the system will operate in sea and not on land, like smolt production systems where they have water treatment, it is essential that the components used are suited for operations in sea. Design and development of such systems will not be covered in this project report, but are important considerations to keep in mind and might be of interest for further work.

1.3 Problem description

This project focuses on the semi-closed aquaculture systems in sea. These systems are built as flow through systems. Pumping water from a certain depth is an effort to avoid contamination from top water levels, which has the highest concentration of sea-lice. In some cases it seems not to be sufficient since it has been discovered that parasites still have found their way into the production tanks (Breck, 2014; Handeland et al., 2015). Furthermore, pathogens like *Tenacibaculm* and *M. viscosa* causing winter ulcers (Takle et al., 2015), and amoeba and algae have also shown to cause problems for some closed systems, as for the Neptun system by Marine Harvest (Berge, 2014).

Water entering semi-closed systems in sea does not undergo any treatment (Rosten et al., 2011). For land-based systems the most often used method to disinfect the seawater is by using ultraviolet light (UV). This method alone has shown some weaknesses and is less effective against certain pathogens. It is however shown that filtration with pores sizes less than 50 µm increases the effectiveness of the treatment substantially (Liltved & Cripps, 1999). This is a combination of removal of particles associated with bacterias and reduction in particles shielding the bacteria. An alternative to UV could be the advanced oxidation process (AOP) (Oturan & Aaron, 2014). Radicals produced during AOP can oxidise and destroy all cells. AOP could however produce harmful by-products in seawater, e.g. bromates, depending on material used in the process (Gunten, 2003).

The SFI center CtrlAQUA had a project in 2015 called INTAKE. In this project information was collected from the R&D partners and technology suppliers outside the center on what kind of treatment technology that would be best suited for semi-closed systems in sea. This formed the basis for INTAKE 2016, and it is the treatment technology recommended in INTAKE 2015 that will be tested now in INTAKE 2016. The treatment system tested can also be seen suitable for landbased post-smolt production systems using seawater.

1.4 Objective

This project thesis will consist of a literature search to review state of art within treatment of seawater related to aquaculture. It will also contain a section where the most important water parameters will be presented and a section describing the different types of treatment technologies in use.

Besides the literature research I will participate in a small scale test at Sunndalsøra., In this test different water treatment technologies will be tested out on untreated seawater containing natural occurring micro-flora. Some of the results from this test will be included in this project thesis, and some will be used for proposal to further work.

The aim for the project thesis is to evaluate parts of the system tested in the experiment at Sunndalsøra. The main focus will be on the bromine formation related to the use of Ozone and UV, and also compare it to the Titanium Advanced Oxidation Process which claims not to produce any disinfection by-products.

The evaluation of bromine levels are important, due to the fact that bromine is acutely toxic to fish. Bromine can furthermore create bromate and bromoform which is difficult to remove and control.

1.5 Tasks

- Review state of the art within the topic of treatment of seawater related to aquaculture. That means to document what others have done and published previously.

- Get an understanding of what total residual oxidants mean to the cultivating environment and how it can be controlled/avoided. Describe the elements covered as total residual oxidants.

- Collect samples of the seawater intake at Sunndalsøra and measure total suspended solids (TSS), turbidity, dissolved organic carbon (DOC), UV transmittance (UVT). This is done to describe the water quality and parameters on the location. The methods used for these analysis are standards.

- Participate during the tests at Sunndalsøra in September, October and November.

- Measure and document trends regarding bromine levels measured as total residual oxidants, using the DPD colour-metric method. Relate the measured bromine levels to the different configuration of the system.

- State a set of recommended tasks to be covered in the master thesis.

1.6 Deviation from original problem description

In the original problem description I was a bit to direct when saying it was a need for water treatment. The version you are reading now is more subdued and says there might be need of water treatment. This was also confirmed by Ragnar Joensen, Group Manager Technology at Marine Harvest, when asked about possible improvements for the semi-closed system Neptun, where there has been a disease outbreak.

Besides the mentioned change, I have also included the newly presented fact that sealice is not necessarily a problem for all semi-closed systems in sea. The possible need for water treatment is directed towards pathogenic microorganisms and not sealice alone. Results presented from Sigurd Handeland, (Handeland, 2016), at the *Fremtidens smoltproduksjon* conference October this year, combined with the results of Nilsen et al. (2016), is basis for these changes.

An author of papers referred to in this project, commented that bromine levels should be presented as a biocidal compound and not as a disinfection by-product itself. The biocidal compound active bromine can further develop into disinfection by-products as bromate and bromoform. Since it is the bromine that is measured, this will be focus, and not bromate or bromoform formation as first stated in the task.

These changes show that writing a somewhat interdisciplinary project thesis can be difficult, especially in the beginning, when there is a lot of new information to adapt.

2 Physical and chemical measures of water quality

In this chapter the aim is to get a common ground of knowledge and understand what kind of water parameters that are important when it comes to aquaculture. In this section the book *Recirculating Aquaculture* (2nd edition, Cayuga Aqua Ventures, 2002) of Timmons and Ebeling, and *Aquaculture Engineering* (2nd edition, Wiley, 2013) by Odd Ivar Lekang are much of the basis. *Recirculating Aquaculture* is by many seen as the most influential book in modern times when it comes to water treatment in aquaculture systems, mainly on land, like smolt production.

Before we can start discussing water treatment, technology, the experiment, advantages and disadvantages of different system configuration, we must have a common understanding of the important water quality parameters for aquaculture production, and in this case farming of Atlantic Salmon. The review of these parameters does not cover all use, but is especially oriented towards farming. It is important to understand that different species, temperature regimes and production systems makes any list of water quality parameters only recommendations (Timmons, Ebeling, Wheaton, Summerfelt, and Vinci, 2002).

The focus on water quality is increasing as the aquaculture industry evolves into more intensive production. When trying to optimise the production, an increase in production density will require exquisite water quality, and quality of the water flowing through the rearing units will degrade throughout the system. Optimised production means to maximise the growth rate of the fish. In open net cages it is difficult to improve or control the water quality, but in semi-closed and closed system the improvement of water quality are possible and extremely important for production. Another important factor when it comes to controlling the rearing environment is the increased focus on environmental impacts from aquaculture industry. Controlling the water quality gives an opportunity to control the effluent from the facilities and reduce the impact on the environment (Lekang, 2007). It is also interesting to see if it is possible to use the effluent waste material in production of bio-fuel, fertilizer etc. Since we are looking at treatment of intake water to these systems this part will not be further discussed in this report, but will most certainly be of interest for further work. Now we will list and describe some important physical and chemical measures of water quality.

2.1 Oxygen

Of all the parameters listed here oxygen is definitely the most crucial one. Without enough oxygen in the water the fish will get negative physiological effects. The fish needs oxygen for basic metabolism and food conversion. To specify we are talking about dissolved oxygen (DO), meaning the available oxygen for the fish. At higher temperatures the fish needs higher oxygen concentration than when temperatures are low. This is opposite of what nature gives, because at low temperatures the oxygen concentration are high and at high temperatures the oxygen concentration are low (Timmons et al., 2002). Because of this it is even more important to monitor oxygen levels and temperature.

Oxygen is slightly soluble in water, which means the fish needs to use a lot of energy to remove

the dissolved oxygen from the water. The solubility will decrease as temperature and salinity increase. Oxygen demand can be calculated when you know the system flow and system daily feed. Timmons et al., 2002, use a Oxygen Demand Factor (ODF) of 0.6 [kg oxygen/kg feed] when doing the computations.

$$TSF \ [kg/day] * ODF \ [kg \ oxygen/kg \ feed] \ = \ TOD \ [kg/day] \tag{1}$$

TSF = Total system feed
ODF = Oxygen demand factor
TOD = Total oxygen demand

When we know the total oxygen demand in the system in [kg/day] it is straight forward to convert it to [mg/min] or [mg/L], given that you have the system flow. One important aspect in (1) is that the amount of feed are the deciding design factor. In real systems the dissolved oxygen will be monitored continuously to ensure an optimum oxygen level required for the specific specie.

2.2 Temperature

Next to dissolved oxygen, the temperature is decisive for production. The temperature does, besides affecting the fish directly, also impact the economic viability of a production system. You can have a perfect rearing environment, but if the temperature is out of optimum range the production will suffer. When it comes to fish farming, it is common to divide the fish into three classifications related to their temperature preferences.

- Cold-water (15°C)

- Cool-water (15-20°C)

- Warm-water (20+°C)

These temperatures are not exact definitions and there will be great variation between species. Atlantic salmon has optimum temperature at 15°C, while Brown trout prefers temperatures between 12 and 14°C (Aston, 1981). Atlantic salmon is classified as cold-blooded, meaning it's body temperature is more or less the same as the water temperature. Being able to control and maintain a stable temperature is important when optimising the growth rate in a production system. Too high temperatures, above optimum, will require more energy for food conversion and the food conversion ratio will decrease (Timmons et al., 2002).

2.3 pH and alkalinity

pH measures acidity or alkalinity in the solution and is represented as a number between 1 and 14, where the lower numbers state increase in acidity and the higher end of the scale state

increase in alkalinity. pH 7 is a neutral solution. In cases of low pH the gills, skin and eyes can get damaged. A too low pH value will in the end kill the fish, but it will first be registered a reduction in growth before it gets lethal.

Different water sources have different pH values and seawater normally has a pH between 7.8 and 8.3. There are normally no need for adjusting the pH in seawater since it has a good buffering capacity due to free bicarbonate (HCO_3^-) (Lekang, 2007).

The most important aspect regarding pH is that it affects a lot of other different water variables which can be crucial. Timmons et al., 2002, state that:

> ≪*pH controls a wide variety of solubility and equilibria reactions, the most important of which is the relationship between the un-ionised and the ionised form of ammonia and nitrate. pH also affects the toxicity of hydrogen sulfide and metals such as copper, cadmium, zinc and aluminium.*≫

Because of this link between pH and other variables it is ranked as an important parameter to consider in any aquaculture system. In seawater, since the buffering capacity is good, the pH is quite stable.

When talking about pH it can be practical to mention alkalinity as well. Alkalinity is basically a measure of the pH-buffering, the capacity the water has to neutralise acids. It is measured as [mg/L $CaCO_3$], where $CaCO_3$ is calcium carbonate. Alkalinity in seawater is normally 120 [mg/L $CaCO_3$]. Required alkalinity concentrations can be seen in direct relationship with the pH of the system and carbon dioxide (CO_2) concentrations. So to maintain a given carbon dioxide concentrations and pH value in the system we rely on the alkalinity (Timmons et al., 2002).

2.4   Carbon dioxide

Carbon dioxide in the water is mainly produced by fish respiration, in addition to decomposition of organic matter. High concentrations of carbon dioxide in the water column reduces respiration efficiency while the tolerance to low dissolved oxygen concentrations decreases. Besides affecting the water column, high carbon dioxide concentrations also affect the fish physiology, decreasing the carbon dioxide release through the fish gills and by that increase the carbon dioxide concentrations in the blood. The fish can then go into respiratory acidosis because of blood plasma pH is lowered. In this condition, the carrying oxygen capacity of hemoglobin is reduced, even if the dissolved oxygen concentration is high. Causing respiration distress (Timmons et al., 2002).

2.5   Total organic carbon

TOC is a measurement of organic carbon contaminants in the water. Sources of organic carbon contaminants varies and compositions are often humic-like substances, carbohydrates, protein

substances, fulvic acids, phenols and organic peroxides. Organic carbon can operate as a energy source for many microorganisms resulting in bad water quality for aquatic life. Because of this and the fact that treatment costs increases when TOC is high, it is desired to keep the TOC levels as low as possible. TOC can in some cases be related to biochemical oxygen demand (BOD) and chemical oxygen demand (COD), but TOC measurements are favoured because the test-procedure is much faster than BOD and COD. An advanced oxidation process (AOP) can reduce amount of TOC in the solution (Droste, 1997). For seawater it is not that easy because of formation of disinfection by-products as mentioned in section 3.2. Ozone in combination with UV treatment can also be categorised as an AOP, so this is also valid for use of ozone. Ultraviolet light is another effective method for reducing TOC, but because of maximum efficiency around 184 nm it needs special UV equipment to work satisfactory (UV Sciences Inc., n.d.). TOC measurements are used as a indicator on general water quality and equipment efficiency of water treatment systems.

2.6 Total suspended solids

Total suspended solids are usually a combination of feed, feces and algae. The common definition of TSS and the definition Lekang (2007) uses are:

> ≪Total suspended solids are defined as the amount of particles stopped
> by a special fibre-glass filter with pore size of 0.45 [μm].≫

It has been reported that fish produce 0.3-0.4 kg TSS for every 1 kg of feed fed. The amount of TSS will effect every aspect of the system downstream. In freshwater the limits for TSS are 25 [mg TSS/L], but 10 [mg TSS/L] are usually the recommended upper limit for maintaining a good operation (Timmons et al., 2002). A high TSS value in the intake water will make disinfection and treatment more difficult and require a highly functioning system. Particles in the water can hide harmful bacteria and increase the risk of disease in the rearing tank. TSS is an important parameter to monitor.

2.7 Salinity

The salinity of the water defines what type of water we are talking about. Usually we divide it into fresh, brackish and saltwater. It is important to know that we do not have any clear range regarding these terms. In Timmons et al., 2002, the definition of salinity is:

> ≪Salinity is defined as the total concentration of dissolved ions in
> water, and is usually reported as parts per thousand (ppt) or grams
> of salt per kilogram of water.≫

Calcium, sodium, bicarbonate, potassium, sulfate and chloride are the major contributors of dissolved ions. In some way, the salinity reflects the surroundings, as climate and geography (Timmons et al., 2002). Species farmed usually have a broad tolerance range when it comes to

salinity, but they all have an optimal salinity for growth and reproduction. Postsmolt-production tests done with salmon shows that it has an optimum salinity at 12 ppt (Terjesen, 2014).

Osmoregulation is the process where fish regulate the uptake of ions from the environment and restrict ion loss. If salinity is far away from the optimum range for a species, it will spend considerable more energy on osmoregulation at the expense of growth (Timmons et al., 2002).

2.8 Ultraviolet transmittance

UVT is a measure of the amount of UV light that goes through a water sample compared to a pure water sample, usually deionised water (Realtech, 2016). UVT is reported as percentage where a high value means a clean water without much material to absorb the light.

It is important to measure UVT when UV are used in a disinfection-process. The UVT measurements gives important inputs to the UV treatment systems so an effective UV dose can be determined. UV dose is dependent on intensity, exposure time and UVT (Tchobanoglous et al., 2014; Realtech, 2016).

UVT changes in time and differs from site to site. Amount of organics, colloidal solids and other particles in the water absorbs light, decreasing the UVT. If the UVT is too low a pre-treatment process is needed to ensure an efficient UV treatment (Lekang, 2013). Most disinfection systems have a lower limit, varying from 45% to 80% depending on type of system and manufacturer. High UVT, > 80%, will give good conditions for most disinfection process using UV light. UVT is usually measured at 254 [nm] because of the germicidal effect (Tchobanoglous et al., 2014; Realtech, 2016).

2.9 Turbidity

Turbidity also influences light penetration in water. It differs from UVT because turbidity is a measure of the cloudiness in the water, everything that is scattering light in the water column (Zweig, Morton, and Stewart, 1999). These colloids are floating in the water column, and this is why turbidity samples need to rest before analysing. It is important to know that a low turbidity measure, e.g. 0.2 NTU, is not the same as a high UVT. Yet, turbidity is still an important parameter to any disinfection processes because these fine particles can hide microbes and absorb or protect from disinfection. Units used to measure turbidity is nephelometric turbidity units (NTU), formazin turbidity units (FTU), formazin nephelometric units (FNU) or simply turbidity units (TU). A low turbidity value indicates a clear water sample. Solutions with high turbidity, e.g. 50 NTU, and high amount of colloids can damage system equipment and can be spotted as the water is unclear and looks cloudy (Nedre Romerike Vannverk, 2016). In Norwegian drinking water regulations turbidity of 0.5 NTU is the maximum limit (FOR-2001-12-04-1372, n.d.). In the Norwegian Aquaculture regulations it is not stated a maximum limit, but to ensure a efficient treatment of the water turbidity should be kept as low as possible.

3 Water treatment in aquaculture

Treatment of water to aquaculture purposes differ a bit from drinking water treatment and wastewater treatment, but uses the same theoretical basis and similar treatment technologies. The difference is mainly in the objective of treatment and what we want to achieve, and of course the difficulties related to salinity.

According to Norwegian regulation For-1997-02-20-192 (n.d.), land based facilities taking in seawater have to disinfect with a desired removal at 99.9% inactivation of both Aeromonas salmonicida and infectious salmon anemia virus (ISA virus). There is currently no regulatory requirements for disinfection neither the inlet or waste water from closed systems in the sea (Rosten et al., 2011). Except for very coarse inlet filters water entering semi-closed systems in sea does not undergo any treatment (Handeland et al., 2015; Rosten et al., 2011).

Now that we have some common understanding of water quality related to aquaculture we can discuss different technologies used and why. In *INTAKE* 2015 different treatment technologies were discussed and proposed for the semi-closed production system in sea. Ozone, drum filter, UV and AOP where chosen to be the main treatment technologies to test using seawater. The different technologies focus on different parts of a treatment process, such as disinfection and particle removal. These technologies will be focused on in this chapter, even though similar and equivalent technology is also mentioned.

3.1 Disinfection

Disinfection is a process where the goal is to inactivate bacteria, viruses, parasites and fungi in a solution. This is done to reduce the risk of fish getting infectious diseases. In this report we are focusing on disinfection of seawater. It is important to get the required inactivation of pathogenic microorganisms. These pathogenic microorganisms are defined as an organism that are capable of hurting its host by either devastate its cell or competing for metabolic resources (Medical Dictionary, 2009). In aquaculture production plants the water is usually disinfected in several steps (Lekang, 2007).

Commercial aquaculture facilities usually operates with a desired degree of removal of log 3, equal to 99.9% removal (Lekang, 2013). This will also be the desired level of disinfection in the experiments related to this project. A log disinfection of 3 is usual sufficient for the most commonly aquaculture bacterias (Lekang, 2013).

We can divide disinfection into four classifications, separated by type of disinfectant:

- Chemical agents
- Physical agents
- Mechanical agents
- Radiation

Chlorine, bromine, iodine, ozone and hydrogen peroxide are all chemical agents. They usually oxidize organic materials and destroying microorganisms. Oxidizing potential describes how effective the agent will be. Ozone is, in this context, the most effective agent with the highest oxidizing potential (Tchobanoglous et al., 2014). Physical agents include heating and use of light, where ultraviolet (UV) is the most common one. Particle separation is the main part of the third group. Separating out particles will remove microorganisms since they often attach to the organic particles. For some parasites, like *Gyrodactylus*, the particle separation will also remove the parasite alone when using a small mesh size ($20\mu m$) in the filter. Disinfection by radiation can be an expensive method to use, and includes acoustic and electromagnetic disinfection (Lekang, 2007).

The most crucial parameter when it comes to disinfection, regardless of method, is the quality of the water entering the disinfection system. Water with high concentration of organic matter and high TSS will be much more difficult to disinfect than pure inlet water. Usually this type of water needs to be pre-treated before disinfection (Lekang, 2013). Particle removal can be a type of pre-treatment in this case.

The degree of removal is an important aspect of disinfection since it represent the amount that are reduced from the starting concentration of microorganisms. Usually it is stated as log_{10} removal or inactivation and it defines the disinfection yield. Log_{10} removal of 2-4 corresponds to a 99-99.99% degree of removal (Lekang, 2007).

$$log(disinfection) \ = log\frac{N_1}{N_2} = logN_1 - logN_2 \qquad (2)$$

In equation 2 N_1 equals the starting concentration and N_2 is the end concentration. Concentration can, as an example, have units [10^7/ml]. When talking about disinfection and degree of removal it will be naturally to move on to Chick's law, Watson's law and the combined Chick-Watson law. Harriet Chick made an important discovery in 1908, the longer the contact time for a given concentration of disinfectant, the greater the effect (Chick, 1908).

Below is Chick's law in differential form

$$\frac{dN_t}{dt} = -kN_t \qquad (3)$$

$\frac{dN_t}{dt}$ = rate of change in the concentration of organisms with time
k = inactivation rate constant, T^{-1}
N_t = number of organisms at time t
t = time

If we integrate equation 3, and setting N_0, the amount of organisms when t = 0, we get

$$\frac{N_t}{N_0} = e^{-kt} \tag{4}$$

While Chick focused on the importance of contact time, Herbert Watson developed an expression where the inactivation rate constant were related to concentration. Watson's law is as follows (Watson, 1908):

$$k = k^{'}C^n \tag{5}$$

k = inactivation rate constant
k'= die-off constant, coefficient of specific toxicity
C = concentration of disinfectant
n = coefficient of dilution

When combining Chick and Watson's law we get the relationship between contact time and concentration. This product is the relation between active and inactive micro-organisms. Haas and Kara (1984), proposed the combination on differential form

$$\frac{N_t}{dt} = -k^{'}C^n N_t \tag{6}$$

Integrating equation 6 gives us

$$ln\frac{N_t}{N_0} = -k^{'}C^n t \tag{7}$$

In practice this gives a Ct-value. If we take the die-off constant to the left and state a wanted degree of removal, like 99.99%, we obtain the following

$$K = C^n t \tag{8}$$

K = constant, including die-off constant and degree of removal

The concept of Ct means we can create Ct data for different bacterias and disinfection agents. Ct values are useful when either time or concentration is a limitation in the process. pH and temperature will effect this Ct data. It is important to understand that this is empirical data.

3.1.1   Ultraviolet (UV) radiation disinfection

UV light has a wavelength between 1 to 400 [nm] and is used to inactivate bacterias and micro-pathogens (Lekang, 2013). DNA is damaged by absorption of irradiation. Between 190 to 280 [nm] the DNA absorbance is high resulting in an effective disinfection (Timmons et al., 2002). UV light from low pressure low intensity mercury vapour lamps has a wavelength of 253,7 [nm] making them suitable for disinfection purposes. Operating within wavelengths of 250 to 260 [nm] will also destroy ozone residuals. Inactivation is related to dose and exposure time, where dose is radiation per unit area and might be better described as intensity.

$$D = It \tag{9}$$

D = Inactivation, UV dose, $[mWs/cm^2]$ or $[mJ/cm^2]$
I = Intensity, $[mW/cm^2]$
t = time, [s]

Effective disinfection with UV depends on lamp intensity, cleanliness of lamp surface, distance between lamp and organisms, age of lamp, type of organism, purity of water and duration of exposure (Lekang, 2007). A rule of thumb is that UV bulbs must be changed at least once a year, sometimes every 6th month (Timmons et al., 2002). New systems monitor these things to ensure an effective treatment.

There are mainly three different UV systems used, low pressure low intensity UV lamps, low pressure high intensity and medium pressure high intensity UV system, where low pressure is most commonly used. This system gives a monochromatic irradiation specific to 254 [nm]. Medium pressure has a broader spectrum of UV and can operate between 200-415 [nm]. Normally it is required fewer medium pressure bulbs than low pressure bulbs (5-20% of the bulbs), to achieve a given UV dose. On the other hand, the medium pressure UV system will require 2-3 times more power (Summerfelt, 2003).

A low pressure low intensity system uses liquid mercury in the bulbs with an optimum temperature at 40°C which controls the mercury vapour pressure. It is important that the lamp remain near the optimum temperature so the mercury does not condense back to its liquid state, since this will decrease the number of mercury atoms available to release photons (Tchobanoglous et al., 2014).

Low pressure high intensity bulbs uses mercury-indium amalgam instead of liquid mercury, which gives a greater UV output (2 to 10 times). Low pressure high intensity lamps are highly efficient in converting input power into UV light and operates at temperatures between 100-150°C. The amalgam maintain a constant level of mercury atoms, making it more stable over a wider temperature range. UV output of low pressure high intensity lamps can be between 30-100% (Tchobanoglous et al., 2014).

Medium pressure high intensity lamps operate at temperatures between 600-800°C and generate

20-50 times as much UV output than low pressure high intensity lamps. Even so, because of their low efficiency related to power input, they are not so often preferred (Tchobanoglous et al., 2014).

Latest developments in UV disinfection systems are that medium pressure high intensity lamps seems to give better overall treatment as they almost eliminate the problem of microorganisms ≪repairing≫ themselves and continue to reproduce (Atlantium Technologies, 2016).

Radiation dose, retention time and UV transmittance are important parameters when designing a UV treatment system. Usually, in commercial systems, a UV dose of $30-40[\mathrm{mWs/cm^2}]$ is enough to get a log 3 disinfection for most of the aquaculture bacterias and viruses. But not all bacterias and viruses will get inactivated, such as Infectious Pancreatic Necrosis (IPN), a virus that demands a much higher dose to get rid off (Lekang, 2007).

3.1.2 Ozone

Ozone (O_3) has a high oxidizing potential and is an powerful agent to improve water quality. In aquaculture systems ozone is added to inactivate fish pathogens and destroy organic material (Summerfelt, 2003). Production of ozone appears when oxygen molecules separate into atomic oxygen. Ozone is an unstable gas and can be produced in different ways, by electrolysis, radio-chemical reaction by electrical discharge or photo-chemical reaction (Tchobanoglous et al., 2014). In equation 10 the transformation from oxygen to ozone is shown.

$$3O_2 + energy = 2O_3 \tag{10}$$

To make effective and stable disinfection using ozone in aquaculture it is important to have good control of the ozone generation, transfer into solution, contact time and residuals that can hurt the production downstream (Summerfelt, 2003). Generation is usually done using an electrical discharge and oxygen feed gas instead of air. Since ozone has a half-life of 15 minutes, and then will be converted to oxygen, it must be produced on site (Lekang, 2007).

After generation the ozone must be transferred into the water column. Because of the cost of producing ozone and the low concentration generated, it is important to optimise the transfer to the water. Transfer of ozone in aquaculture purposes is commonly being conducted by liquid phase diffusers that bubble ozone into the solution. If designed correctly the system should achieve a 90% transfer of ozone (Tchobanoglous et al., 2014).

Using ozone to kill microorganisms requires maintaining a certain level of dissolved ozone concentration in the water for the given contact time. The efficiency of the disinfection depends on the product contact time and the ozone residual concentration. Required contact time and ozone residual concentration is dependant on the desired degree of removal. When using ozone in seawater the ozone residual will more or less convert to active bromine, because of the ozone reaction with bromide. This is measured as total residual oxidants (TRO) and will be present in the solution after the ozone disinfection step. Oxidation-reduction potential (ORP) are used to

monitor the oxidizing power of ozone and its oxidants, given in millivolts [mV]. ORP provide a continuous monitoring for ozone and measures the oxidizing capacity in the solution using ORP probes. The relationship between ORP levels and total residual oxidants (TRO) are not linear, making it difficult to use ORP as an indicator for TRO concentration (Timmons et al., 2002). Organic matter or antioxidants in the water reduces the ORP (Lekang, 2013).

Ozone treatment can be placed in different stages of a treatment process depending on what kind of system the treatment is a part of. In a recirculating aquaculture system (RAS) there are at least 3 different ways of using ozone. If ozone is used before particle removal treatment it is probably meant that the ozone dose used should create a coagulating effect of the particles to increase removal further downstream (Droste, 1997)

Ozone is very unstable in water and the decomposition of ozone is affected by several parameters, like temperature and pH. In water with higher temperature the solubility of ozone decreases and is less stable. When the pH increases, so does the formation of hydroxyl radicals (HO$\bullet$). Increasing pH also makes the ozone decay much faster (Lenntech, n.d.).

3.2 Ozonation of seawater

Treatment of seawater with ozone gives a series of redox-reactions, compared to ozonating freshwater where ozone are decomposed to oxygen quickly. Bromide ions in seawater makes ozone treatment more complex because of the danger of making toxic byproducts like bromate and bromoform, a trihalomethanes (THM).

In seawater bromide (Br^-) is an ion which can react with oxidants to create bromine (Br_2). Bromine is an oxidizing agent and forms oxyacids as hypobromous ($HOBr$), bromous ($HBrO_2$) and bromic ($HBrO_3$) (World Health Organization, 2009). The concentration of bromide in seawater is in the range of 65-80 [mg/L]. In freshwater the bromide concentrations are about 0.5 [mg/L] (World Health Organization, 2009). Compared to other oxidizing agents, like chloride which is in the range of 18 980-23 000 [mg/L], the concentration is small, but since the ozone reacts much faster with bromide there will not be much chloride and ionide reacting with the ozone (Sørensen, Højgaard, and Liltved, 2002). Regardless of the concentration compared to other oxidizing agents, bromine is acute toxic to fish (Fisher, Burton, Yonkos, Turley, and Ziegler, 1999). The result is that bromine is very important regarding ozonation of seawater for fish production.

3.2.1 Bromine compounds

Bromine compounds can be divided into two sub-groups:

Inorganic:

- Hypobromous ($HOBr$)

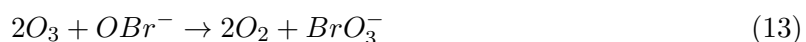- Hypobromite (OBr^-)

- Bromate (BrO_3^-)

Organic:

- Bromoform ($CHBr_3$)

The amount of hypobromous and hypobromite will depend on the pH of the seawater. Normally it will be 60% of hypobromous and 40% of hypobromite, when pH around 8. Hypobromous and hypobromite can be detected in the water hours after ozonation and is called active bromine (or biocidal bromine). Filtering through an activated carbon filter (GAC) or adding redox like sodium thiosulfate can be appropriate treatment methods to remove the active bromine (Liltved, Vogelsang, Modahl, and Dannevig, 2006). This aspect will not be covered in more depth in this project, but can be of interest for further work if a treatment with ozone is applicable.

The active bromine is the main part of the total residual oxidants, leading to disinfection by-products, in ozonated seawater. Active bromine is measured as TRO (total residual oxidants) which also could include other oxidants present, but because of the reaction rate between ozone and bromide most will be active bromine.

3.2.2   Chemical reactions

Haag and Hoigne (1983) proposed the following reaction kinetics for ozone and bromide:

$$O_3 + Br^- \rightarrow O_2 + OBr^- \tag{11}$$

$$O_3 + OBr^- \rightarrow 2O_2 + Br^- \tag{12}$$

$$2O_3 + OBr^- \rightarrow 2O_2 + BrO_3^- \tag{13}$$

In equation (11) ozone oxidize bromide ion into hypobromite ion. Hypobromite will hydrolyze into hypobromous acid ($HOBr$). Some of the hypobromite ion can be regenerated back to bromide as seen in equation (12).

Ozone can oxidize hypobromite further into bromate (BrO_3^-) (Westerhoff, Song, Amy, and Minear, 1998), as seen in equation (13). Bromate is not acute toxic to fish, but it has shown to be carcogenic in laboratory test and it is low biodegradable in water. Because of this it is important to keep the concentration as low as possible. Chum salmon have shown a high tolerance level of bromate and Crecelius, 1979, reported that chum salmon had a $LC_{50} = 512$ [mg/L]. This does not mean Atlantic Salmon will have the same tolerance so this should be studied further. In freshwater small amounts of bromate is easy to detect, but in seawater it is difficult to measure low bromate concentrations. Unlike hypobromous and hypobromite, there are no applied method of removing bromate from seawater (Kruithof and Schippers, 1993). Less bromate will be formed with decreasing pH values (Portjanskaja, 2011).

In addition there will be formed organic residuals, like bromoform ($CHBr_3$) and other halogenated organic compounds when ozonating seawater (Sørensen et al., 2002). Bromoform is not acute toxic for fish, but like bromate it has shown to be carcogenic, and fish can have 50 times as much in the body as measured in seawater (Gibson, 1980). Another important aspect is the fact that bromoform has half-life of 100 years (Harboe and Poleo, 1997).

3.2.3 Ammonia

When using ozone on seawater in aquaculture production systems it is also important to take into the account the ammonia produced by the fish. Ammonia is the result of the metabolism of protein catabolism and is being discharged as unionised ammonia across the gills of the fish. The concentration of ammonia is a function of pH, salinity and temperature (Timmons et al., 2002). Ammonia can react quickly with active bromine, which do not participate in bromate formation. As a result ozonated water without ammonia should yield a higher bromate concentration than water with ammonia (Hofmann and Andrews, 2000).

When un-ionised ammonia (NH_3) reacts with hypobromite (OBr^-) it forms bromamine (NH_2Br) (Portjanskaja, 2011).

$$NH_3 + OBr^- \rightarrow NH_2Br + OH^- \tag{14}$$
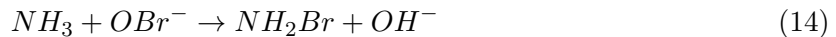
Bromamines are not well documented when it comes to ozonation of seawater and ammonia reactions in aquaculture productions, but it has disinfection attributes. Because the bromate formation from active bromine is slower than active bromine that reacts with ammonia, bromide concentrations are usually conserved while ammonia is consumed (Hofmann and Andrews, 2000).

3.2.4 Bromine formation using AOP

The advantages of using a Titanium Advanced Oxidation Process, as described in chapter 3.5, are that it should not contribute in making harmfull disinfection by-products, like bromine (Wallenius Water, 2016). Even so, we will measure TRO when testing the AOP as well.

3.3 Bromine formation related to the experiment

Since the tests at *INTAKE* 2016 are conducted without fish, focus will be on the bromine residuals, and not bromamines. When measuring active bromine it will basically be hypobromous ($HOBr$) and hypobromite (OBr^-) we are getting as [mg/L] of total TRO.

3.3.1 Method of measuring TRO

The method mostly used for measuring bromine and bromate after ozonation is the N,N-diethyl-p-phenylenediamine (DPD) colourimetric method. Primarily used to measure levels of chlorine,

but measures any oxidants present, as ozone and bromine. The kits are commercially available and it is a quick method for measuring. Sample of water and reagent (DPD) are added to the test-tube, causing reaction. The darker the colour, the higher oxidant concentration. This must be compared to a blank using a spectrophotometer or a colour wheel. Units are [mg/L] of total residual oxidants (TRO). Buchan, Martin-Robichaud, and Benfey, 2005, tested five different methods for measuring disinfection by-products in seawater and the conclusion was that the DPD method is the most convenient.

3.4 Removal of particles

Removal of particles are important when it comes to water treatment, since the particles can hide bacterias and micro-pathogens, as well reduce the general water quality. High concentrations of particles will reduce growth and can contribute to increased mortality. Removal of particles will also increase the treatment efficiency of other treatment processes, such as UV treatment.

Filter, dependant on the mesh size will stop, and remove particles. In that way it is possible to choose mesh size on the filter dependant on size of the particle you want to remove. The Norwegian regulations specify the required filtration needed for intake water to aquaculture systems (For-1997-02-20-192, n.d.).

> ### §9 Pretreatment of intake water and wastewater
> ≪Intake Water for aquaculture should be filtered through screening with pore-size ≤ 0.3 mm.≫

Bigger particles are easier to remove so it is important to handle the flow with it's particles gently before treatment to avoid particles reducing their size. $40 \mu m$ can be seen as a lower limit for separating and identifying single particles. There are different principles for removing particles, such as mechanical filtration, depth filtration and settling (Lekang, 2007).
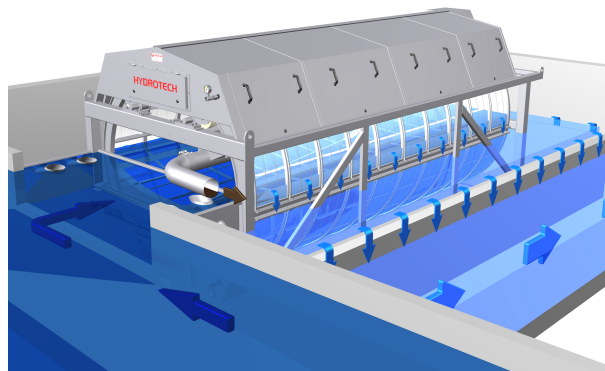

Figure 2: Hydrotech drum filter. (Veolia Water Technologies, n.d.)

Mechanical filtration means obstructing the water flow to collect particles. Drum-filter is a type of mechanical filter, like the one seen in figure 2. Particles bigger than the pore size will be

stopped at the surface of the filter while water will flow through. When particles are collected it is essential that they are removed from the surface to avoid clogging of the system. Clogging of the system will result in head loss and is decreasing the system efficiency. The most common way of cleaning the filter is by back-flushing as seen in figure 3.

In treatment of intake-water the mesh size can be as low as $20\mu m$, because this might also remove some parasites bigger than mesh size. Decreasing filter mesh size will increase the frequency for changing of filter cloth, exponentially when below $60\mu m$ (Lekang, 2007).
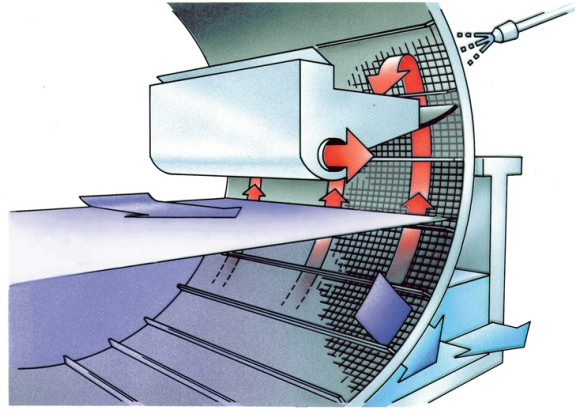

Figure 3: Automated cleaning of a drum filter. (Akva Group, n.d.)

Depth filtration can be described as a process where water flows through a granular medium, usually sand, to trap particles in the medium. Granular medium can have different size depending on what type and size of particles you want to remove. As for the mechanical filtration there is a need of back flushing to avoid clogging of the system. Depth filtration is not widely used in aquaculture, but mostly in treatment of potable water and wastewater (Lekang, 2013).

Treatment equipment and filter design are usually specified for a give volume flow to give optimal treatment efficiency, but mechanical filters are normally designed to handle a range of volume flow. Even though, if the flow gets to large and it contains high amounts of TSS the system may fail.

Efficiency of a particle removal system is measured by it's purification efficiency and can be described as follows (Lekang, 2013):

$$C_e = \frac{(C_{in} - C_{out})}{C_{in}} x 100 \tag{15}$$

C_e = efficiency (%)
C_{in} = concentration of the actual substance entering the filter
C_{out} = concentration of the actual substance exiting the filter

Efficiency can then be represented by TSS removed, or other substances. Phosphorus is a compound that can be removed with particle removal. Up to 80% of phosphorus will be attached

to particles, making it a source of interest when it comes to effluent water from aquaculture (Lekang, 2013).

3.5 AOP

Advanced oxidation processes generate hydroxyl radicals (HO●), which is highly capable of oxidising organic compounds. The dot tells us that there is an unpaired electron in the outer orbital of the molecules. It is because of this that HO● reacts so rapidly with electron-rich organic compounds. For many organic compounds the second order HO● rate constant are 10^8 to 10^10 [L/mole s], which is much greater than for a lot of other oxidants (Tchobanoglous et al., 2014).

In table 1 the oxidation potential for different oxidizing agent are stated. Here we can see that the HO● is one of the most powerful known agents available. Many combinations of treatment methods can be used to produce HO●, and the most common is the use of ozone and hydrogen peroxide. UV light and hydrogen peroxide is another method, as well as a combination of all three.

An advantage with using AOPs is the fact that they produce the hydroxyl radicals at ambient temperature and atmospheric pressure, making design of systems more cost-effective. When designing a new system it is important to consider the quantity of oxidants that are required to destroy the targeted organics (Crittenden et al., 2012).

Table 1: Oxidation potential for some selected oxidizing agents. (Lekang, 2013)

Oxidizing agent	Electrochemical oxidation potential (V)
Fluorine (F2)	3.06
Hydroxyl radical (HO●)	2.80
Oxygen (atomic) (O)	2.42
Ozone (O3)	2.08
Hydrogen peroxide (H2O2)	1.78
Hypochlorite (ClO-)	1.49
Chlorine (Cl)	1.36
Chlorine dioxide (ClO2)	1.27
Oxygen (molecular) (O2)	1.23

The rate law of HO● reacting with organic compounds are shown here (Crittenden et al., 2012):

$$\frac{dC_R}{dt} = r_R = -k_R C_{HO●} C_R \qquad (16)$$

r_R = destruction rate of R with HO$\bullet$ radicals, $[mol/L \times s]$

k_R = second-order rate constant for destruction of R with HO$\bullet$ radicals, $[L/mol \times s]$

$C_{HO\bullet}$ = concentration of hydroxyl radical, [mol/L]

C_R = concentration of target organic R, [mol/L]

If concentration of HO$\bullet$ is assumed constant it is possible to calculate the half-life of a targeted organic compound. Usually half-life values and HO$\bullet$ rate constant are given in tables for different organic and inorganic compounds. The half-life equation is obtained by replacing the rate expression with mass balance on a batch reactor completely mixed (Crittenden et al., 2012):

$$t_{\frac{1}{2}} = \frac{ln2}{k_R C_{HO\bullet}} \tag{17}$$

$t_{\frac{1}{2}}$ = half-life of organic compound, [s]

There are some major factors affecting an AOPs performance, by either absorbing UV light or scavenging HO$\bullet$ radicals. Carbonate species, pH, natural organic matter (NOM), reduced metal ions, reactivity of the parent component with HO$\bullet$ radicals and UV transmittance are all factors that affect the overall performance of the AOP system. Because of this it is important to asses parameters like alkalinity, pH, chemical oxygen demand (COD), TOC, UVT, iron (Fe) and manganese (Mn) to ensure a highly efficient system.

3.5.1 AOP by combining UV and Titanium dioxide

UV light and titanium dioxide (TiO_2) combined are defined as a photocatalytic process. This kind of photocatalytic process can either use TiO_2 in suspension or fixed to material surface (Lekang, 2013). If TiO_2 is in suspension it must be collected and pumped back into the system, which make this the less preferable way of using this method. When TiO_2 is fixed at a surface photons with energy from the UV light hits the TiO_2 surface which results in release of HO$\bullet$ radicals.

Brookman and Gagnon (2011) did an experiment where they wanted to look at disinfection by-products applying an advanced oxidation process of UV/TiO_2 in water with different salinity's and comparing it with the use of ozone and UV. The results from the study gave them a good understanding of the differences between the systems with respect to the by-products, bromate and bromoform.

- TiO_2 does not reduce bromide concentrations significantly

- UV-TiO_2 treatment does not increase ORP

- Not observed bromate formation post UV-TiO_2 treatment

- No formation of bromoform
  (Brookman and Gagnon, 2011)

These results give us a good indicator on the potential of this method and how applicable it might be in aquaculture systems. The combination of high disinfection efficiency and low risk of disinfection by-product sounds promising. One important question related to the *INTAKE* experiment is how well this system will perform with respect of removing the targeted bacterias and microorganisms.

4 Project Intake 2016

4.1 Introduction

Different treatment technologies recommended in *INTAKE* 2015 was tested in the *INTAKE* 2016 experiment. The experiment was a pilot scale treatment process executed under normal conditions, with equipment available and being used in commercially production facilities.

4.2 System description

Raw, untreated seawater was pumped into the mixing tank, see figure 4, so the total suspended solids can be adjusted (increased) using sludge from the waste water tank at the research station. After the mixing tank the flow was led to a tank for ozone injection. Hydraulic retention time in the mixing tank for ozone was approximately 1.5 minute.

From the mixing tank for ozone the flow will be pumped into the container where the drum filter is placed together with the UV and the AOP. The flow will go through the drum filter before entering either the UV or AOP treatment. The red points are sampling points.
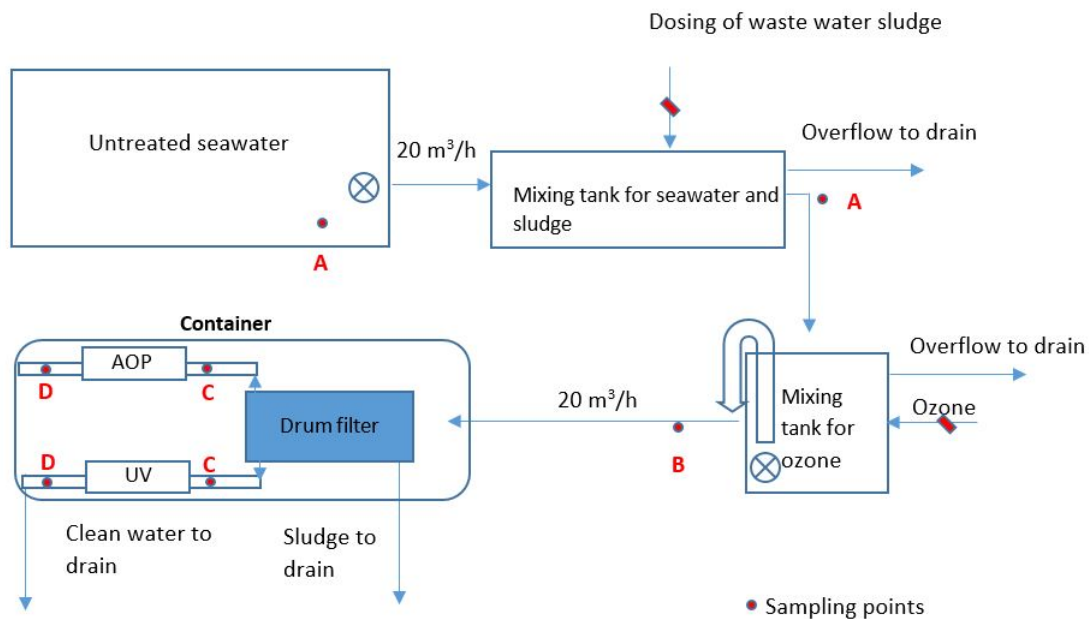

Figure 4: View of the system to be tested

4.3   Intake 2016 - experimental setup

Three tests will be conducted at low, 1-2 [mg TSS/L], and high, 20 [mg TSS/L], TSS level. Each test will take two working day, in total 12 working days. Two replicates per run, at start (0 min) and end (20 min), will be taken prior to and after each treatment. Test 4-6 are as test 1-3, except under high TSS levels. Besides this setup it was also carried out some runs with higher ozone doses, up to 700 [mV ORP]. This was done to see if we could estimate some sort of a threshold related to ozone dose and increase of TRO levels. The results from those runs will be discussed and evaluated later in the report.

Low TSS

- Test 1: 50-60 µm screen Hydrotech filter (Table 2)

- Test 2: 300 µm screen Hydrotech filter (Table 3)

- Test 3: Without the Hydrotech filter (Table 4)

High TSS

- Test 4: 50-60 µm screen Hydrotech filter (as table 2)

- Test 5: 300 µm screen Hydrotech filter (as table 3)

- Test 6: Without the Hydrotech filter (as table 4)

Table 2: Test 1 and 4, with 50-60 µm screen Hydrotech filter.

Total suspended solids (TSS)	Run	1.disinfection Ozone	Filter	2.disinfection UV/AOP	Number of samples
	1	-	50-60 µm	UV 40 mJ/cm2	6
	2	-	50-60 µm	UV 60 mJ/cm2	6
Low/high	3	300-350 mV	50-60 µm	-	6
	4	400-500 mV	50-60 µm	-	6
	5	400-500 mV	50-60 µm	UV 60 mJ/cm2	8
	6	-	50-60 µm	AOP	6
Sum					38

Table 3: Test 2 and 5, with 300 µm screen Hydrotech filter.

Total suspended solids (TSS)	Run	1.disinfection Ozone	Filter	2.disinfection UV/AOP	Number of samples
	1	-	300 µm	UV 40 mJ/cm2	6
	2	-	300 µm	UV 60 mJ/cm2	6
Low/high	3	300-350 mV	300 µm	-	6
	4	400-500 mV	300 µm	-	6
	5	400-500 mV	300 µm	UV 60 mJ/cm2	8
	6	-	300 µm	AOP	6
Sum					38

Table 4: Test 3 and 6, without filter.

Total suspended solids (TSS)	Run	1.disinfection Ozone	2.disinfection UV/AOP	Number of samples
	1	-	UV 40 mJ/cm2	4
	2	-	UV 60 mJ/cm2	4
Low/high	3	300-350 mV	-	4
	4	400-500 mV	-	4
	5	400-500 mV	UV 60 mJ/cm2	6
	6	-	AOP	4
Sum				26

4.4 Responses

Listed below are the responses analysed in the experiment. Through these analysis the efficiency of the different treatment system configurations will be determined. All analysis were performed immediately after sampling. This report will not go in detail on how the microbial analysis was conducted, but they where all following protocol in the laboratory at the research station.

4.4.1 Microbial aspect - disinfection efficiency

- A wide spectre of natural occurring microorganisms was measured by filtering water (1L) through electro positive filters. The filter was then to be analyzed by Pharmaq for *Tenacibaculum sp, Flavobacterium, Yersinia ruckeri, Aeromonas salmonicida, Moritella viscosa*, Poxvirus and IPNV, *Paramoebae perurans* (AGD). Common DNA preparation, performed real time and deep sequencing.

- General microbiota in water (for deep sequencing). 1 L of water sample was filtered in several steps through 80, 3 and 0.2 µm.

- DAPI for bacterial counting (Fonnes, 1999).

- Flow cytometry for bacterial counting (Andersen, 2005).

- Live bacteria: Plating of water samples on marine agar (for heterotrophic bacteria) and blood agar (pathogens). Made a dilution series when found necessary. Counting CFU after two days.

4.4.2 Formation of TRO from Ozone/AOP

When oxidating seawater most of the total residual oxidants formed will be bromine because of the high reaction rate of ozone with bromide. It it is important to measure the TRO levels in the process because it gives us an indication on the formation of bromate and bromoform further down in the reaction series. As mentioned earlier bromine is not classified as disinfection by-product, but a biocidal effect of the treatment process, which could lead to formation of disinfection by-products (bromate and bromoform). Ozone was produced in the ozone generator at the research station and injected into seawater using a diffuser. Ozonation reduction potential (ORP) was measured in the mixing tank using portable analyser (WTW).

Total residual oxidants was analysed by the DPD method using PhotoLab 6100 VIS (WTW). 10 [ml] from the water sample was mixed together with reagents before analysing. Since the research station had no chromatography, the samples for analysing bromoform and bromate was frozen to be analysed later on a different location.

4.4.3 Organic material

Measuring the organic material in the solution was done to ensure desired water quality. TSS and TOC was measured before and after UV, ozone and AOP. They where also measured to

monitor the effect the different treatment methods had on the specific parameters.

4.5 Sampling

The different sampling points are marked as red dots on figure 4. Dependent on the type of run, samples was taken at the outlet of the seawater tank, outlet of the mixing tank for seawater and sludge, outlet of the mixing tank for seawater and ozone, outlet of drum filter (permeate, clean side), and after the UV/AOP. Samples was taken prior to and after each treatment. Each run was ran for 20 min, samples taken at the start and after full time. The lamps in the UV and AOP needed approximately 10 min to reach stable effect. Equipment was stable before starting of each run.

4.6 Technical equipment

The AOP used in the experiment was of the type AquaWorker from Wallenius Water. It has a maximum flow limitation on intake water of 80 [m^3/h] and uses three UV lamps. The reactor is made of titanium (Grade 2) and can tolerate a water temperature of maximum 40°C. In figure 6 the UV treatment unit is presented.

Figure 5: The advanced oxidation treatment, Aquaworker, using UV and Titanium Dioxide reactions. Water entering at the bottom and being exposed to the UV and titanium dioxide.

Figure 6: Atlantium Technologies UV treatment equipment which uses medium pressure lamps.

The drum-filter used in the experiment was of the type Hydrotech drum-filter from Kruger Kaldnes. It was mounted inside the container and used a automatic filter cleaning-system. It is similar to the one shown in figure 2.

4.7   Seawater description

Seawater tested at Sunndalsøra was pumped from Tingvollfjorden at 40 m depth. The seawater quality was stable during the experiment and showed low values of TSS and turbidity. In table 5 some of the measured physicochemical and biological parameters are listed.

Table 5: Raw untreated seawater description

Analysis	Unit	Average value $\pm$ standard deviation
pH		8.1 ± 0.03
Temperature	°C	13 ± 1.49
TOC	ppm C	1.3 ± 0.42
O_2	mg/L	8.2 ± 0.73
Salinity	‰	32 ± 0.30
UVT	%	96.77 ± 0.57
Turbidity	NTU	0.23 ± 0.05
Total Suspended solids	mg/L	3.3 ± 3.93

4.8   Limitations

This experiment was performed without fish, so the parameters monitored shall be seen in light of this. To conduct an experiment like this with fish would be extremely much more extensive and demanding, with respect to surveillance and planning. The aim of the project, at this stage, was not primarily to test a system that could be directly implemented in commercial aquaculture, but rather to look at efficiency and potential side effects. Results of this experiment will hopefully be the foundation for next year's project.

5 Results and discussion

This chapter presents discussion and presentation of some of the results from the experiment at Sunndalsøra with the focus on disinfection and measurements of total residual oxidants. Because of the fast reaction rate between ozone and bromide, most of the measured TRO will be the biocidal compound active bromine. High levels of TRO, meaning bromine, can result in high levels of disinfection by-products, like bromate and bromoform (THM). A comparison between using ozone/UV and AOP will be presented, as well as other aspects that seemed to have an effect on the measurement of TRO.

The experiment was performed during September, October and November. Before the experiment could take place, the system had to be tested for a period to stabilise and ensure optimal operation. Over 2300 samples where taken during the experiment. Most of the results and analysis of bacteria and microorganisms inactivation will be ready during spring 2017, and will also be part of the foundation of a possible master thesis. However some of these results, such as the CFU, are ready and is mentioned in this thesis. A lot of the results from the experiment will not be presented, but some might be mentioned if appropriate.

The fact that there are no clear lethal limits regarding TRO specific for atlantic salmon makes it difficult to derive claims related to the measured TRO levels, but since higher levels can lead to increase in bromate (DBP) and bromoform (DBP) it is important to minimise the TRO levels. Fisher et al. (1999), found that trout had a $LC_{50}4 = 0.068$ [mg/L]. This does not mean we can assume similar levels for Atlantic salmon, but it might give an indication of the range we are talking about.

5.1 Overview of different system configurations

The tests show that there are differences in the TRO results dependent on system configuration, and the treatment units specification. Therefore the first results presented are results across the whole system, sorted by test number. The different runs are named related to the treatment units being used. In figure 7 we see the change in TRO [mg/L] developing throughout the system in test 1. The increase is especially noticeable in the run with ozone dose of 400-500 [mV]. Seawater, in figure 7, is before any treatment and was measured at every run.
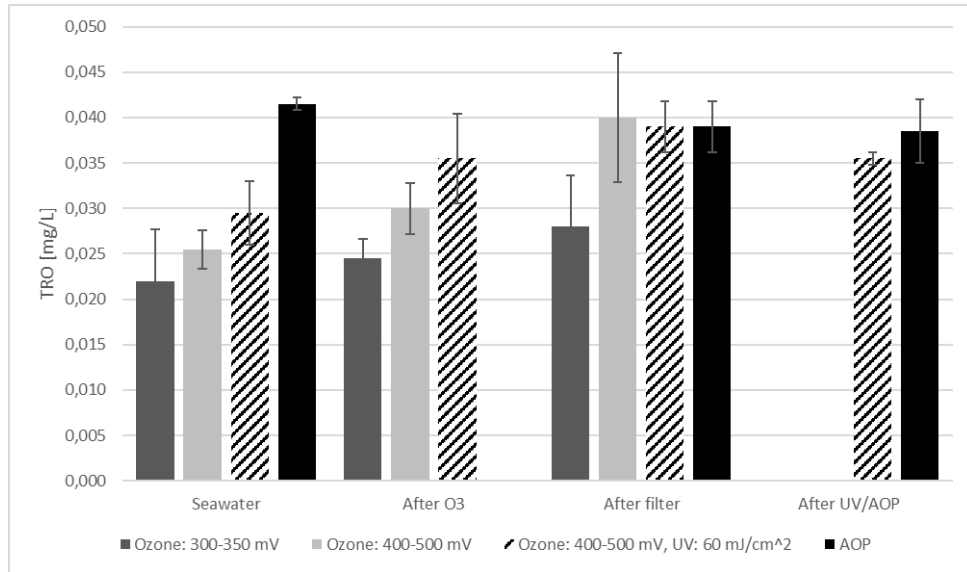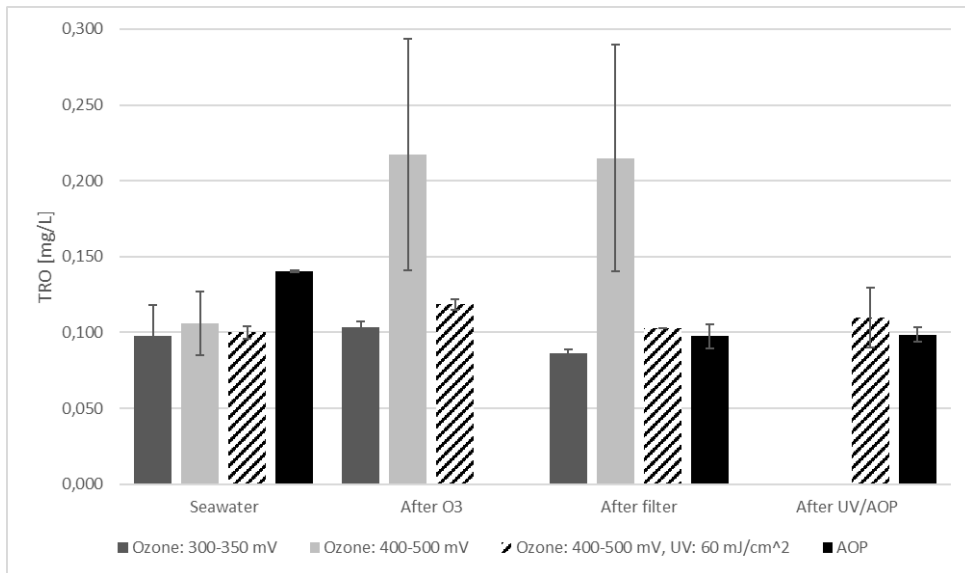
Figure 7: Change in TRO [mg/L] throughout test 1, with low TSS and filter mesh size of 40 [μm]. Results are average of two samplings, at start (0 min) and end (20 min) at each sampling point.

A more interesting way of looking at the development throughout the system is the change in percentage in each run. In figure 8 it is also seen that the run with ozone dose of 400-500 [mV] has greatest change. The difference between high ozone with and without UV treatment is also interesting. As expected the AOP using titanium dioxide and UV does not affect the TRO level .
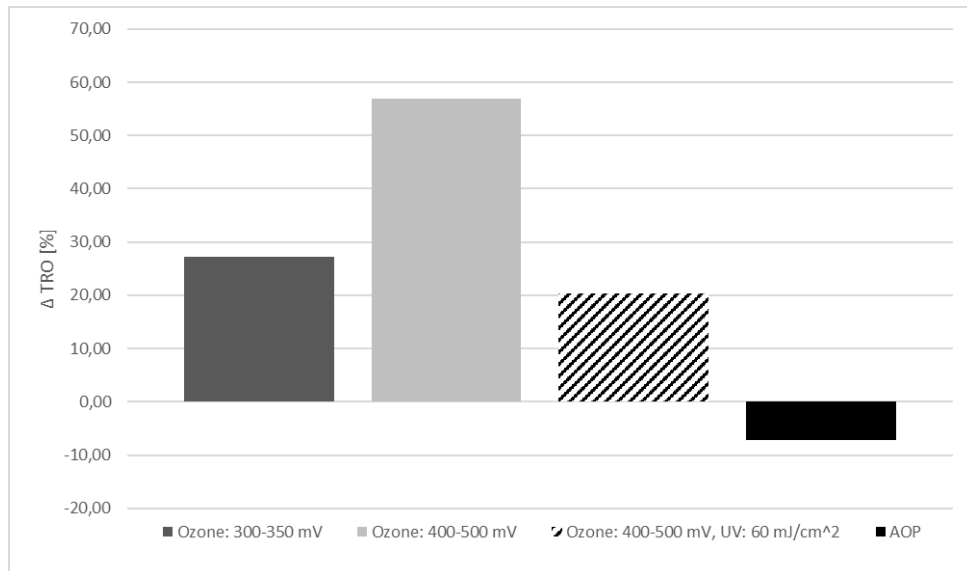
Figure 8: Change in TRO [%] for each configuration (run), with low TSS and filter mesh size of 40 [μm]. Results are average of two samplings, at start (0 min) and end (20 min) at each sampling point.

Presented in table 6 are the results in [%] increase/decrease of TRO in test 1, including run 1

and 2, where only filter and UV was used. A possible aspect to monitor more closely are as mentioned that when using UV after ozone, the UV seems to stagnate the increase of TRO, when using high ozone doses.

Table 6: Results from test 1 with low TSS and 40 [μm] filter.

Run	Treatment	Production/Reduction in TRO [%]
1	Filter then UV	8,82
2	Filter then UV	1,35
3	Ozone then filter	27,27
4	Ozone then filter	56,86
5	Ozone then filter, UV	20,34
6	Filter then AOP	-7,23

Test 4 is similar to test 1, except TSS is set to high, 20 [mg/L], so comparing them can be interesting. As in test 1, we see that using a ozone dose of 400-500 [mV] increases the TRO levels considerably, see figure 9. In figure 10 is the change in TRO [%] of test 4.

Figure 9: Change in TRO [mg/L] throughout test 4, with high TSS and filter mesh size of 40 [μm]. Results are average of the two samplings, at start (0 min) and end (20 min) at each sampling point.

As for low TSS, use of UV after ozone seems to stagnate the formation of TRO.
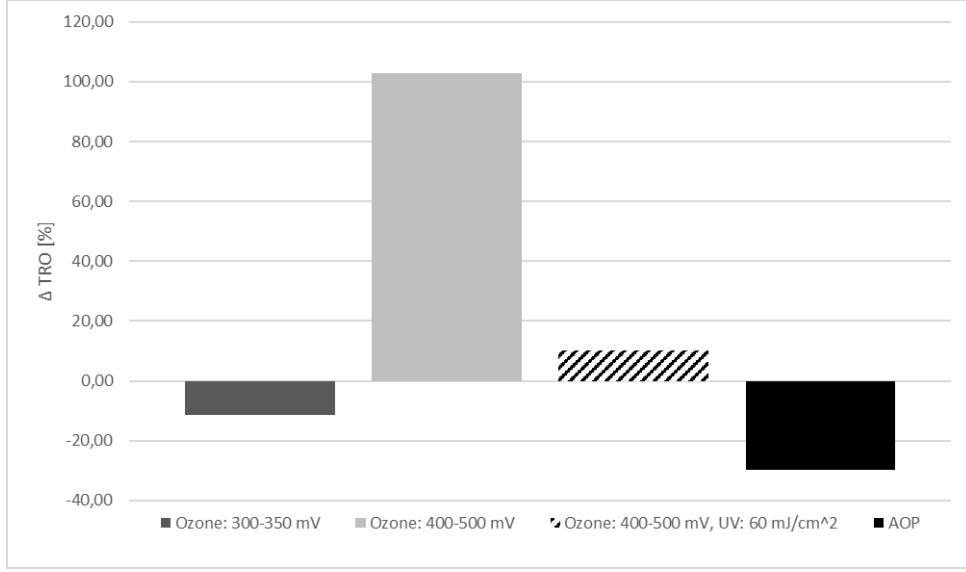
Figure 10: Change in TRO [%] for each configuration (run 3-6), with high TSS and filter mesh size of 40 [μm]. Results are average of the two samplings, at start (0 min) and end (20 min) at each sampling point.

Table 7 show the result of all the runs in test 4, including run 1 and 2, where only filter and UV was used. Results from all the tests can be seen in the appendix 7 A.

Table 7: Results from test 4 with high TSS and 40 [μm] filter.

Run	Treatment	Production/Reduction in TRO [%]
1	Filter, UV	-29.80
2	Filter, UV	-27.97
3	Ozone, filter	-11.28
4	Ozone, filter	102.83
5	Ozone, filter, UV	10.00
6	Filter, AOP	-29.89

Table 8: Results from all tests, sorted by run. The average is of formation/reduction in each run, independent of TSS and filter-mesh size. This represent the main results of the different system configurations with respect to production/reduction of TRO.

	Test						
Run	1	2	3	4	5	6	Average [%]
1	8.8	-	-	-29.8	5.8	2.0	-3.3
2	1.4	-	-	-28.0	1.6	3.8	-5.3
3	27.3	0.0	9.8	-11.3	24.7	44.9	15.9
4	56.9	93.8	51.5	102.8	40.5	71.6	69.5
5	20.3	11.9	18.9	10.0	15.9	25.1	17.0
6	-7.2	-	-7.0	-29.9	4.6	24.0	-3.1

To sum up the results of the different system configurations, table 8 presents all the tests with their runs, including an average value of each run, independent of TSS value and filter mesh size. As expected run 3, 4 and 5, using ozone, increased the level of TRO throughout the system, while only using UV and AOP seemed not to affect TRO levels.

The average with and without UV, with the same ozone dose, are 17.0% and 69.5% increase, independent of TSS and filter mesh size. The difference is interesting for further tasks regarding use of ozone in combination with UV treatment, related to total residual oxidants and possible disinfection by-products. It is difficult to give a clear answer to why this difference appear, but one possible hypothesis is the fact that UV and remaining oxidants might react to create hydroxyl radicals (HO$\bullet$) and by that reduce the level TRO. By applying ozone doses of both low and high will lead to toxic values when looking at $LC_{50} = 0.068$ [mg/L] as BrO^- for trout (Fisher et al., 1999).

5.2 TSS and Ozone

The effect of TSS in the water with respect to TRO is clear in this case, as seen in figure 11. Higher TSS gives a higher level of TRO in the inlet water. As mentioned in chapter 3.1.2 higher amount of organic material will decrease the ORP value of the water, resulting in need of a higher ozone dose to get the desired disinfection level. But this is before any ozone is injected, which means there has to be something related to the components of the wastewater from the fish-tanks at the research station. Because of this increase in TRO in the intake water the starting point of disinfection is quite different, as expected.
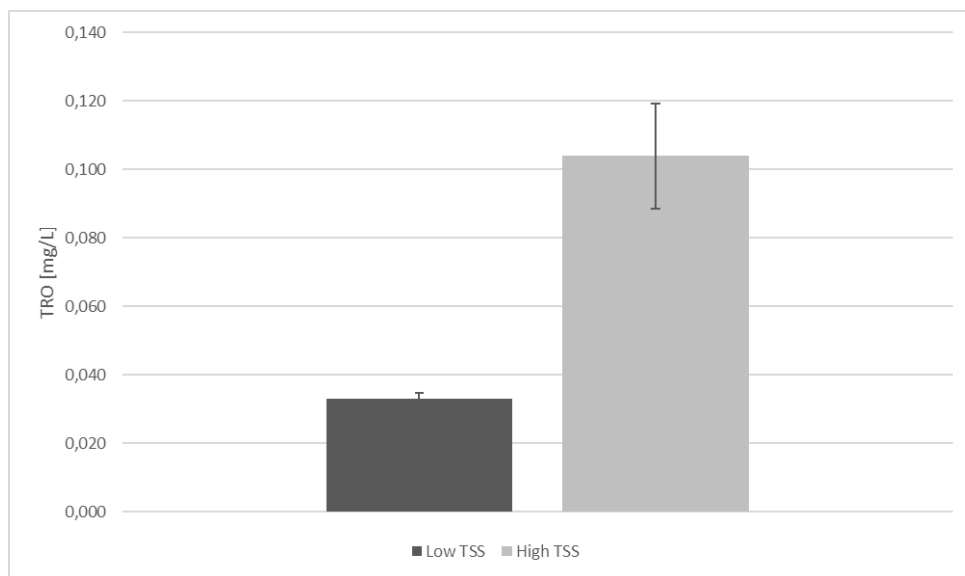

Figure 11: Average TRO measured for low and high TSS at inlet before treatment. Raw seawater.

If we look at the combination of TSS and ozone doses used we can see that higher ozone dose gives an increase of TRO, as expected.
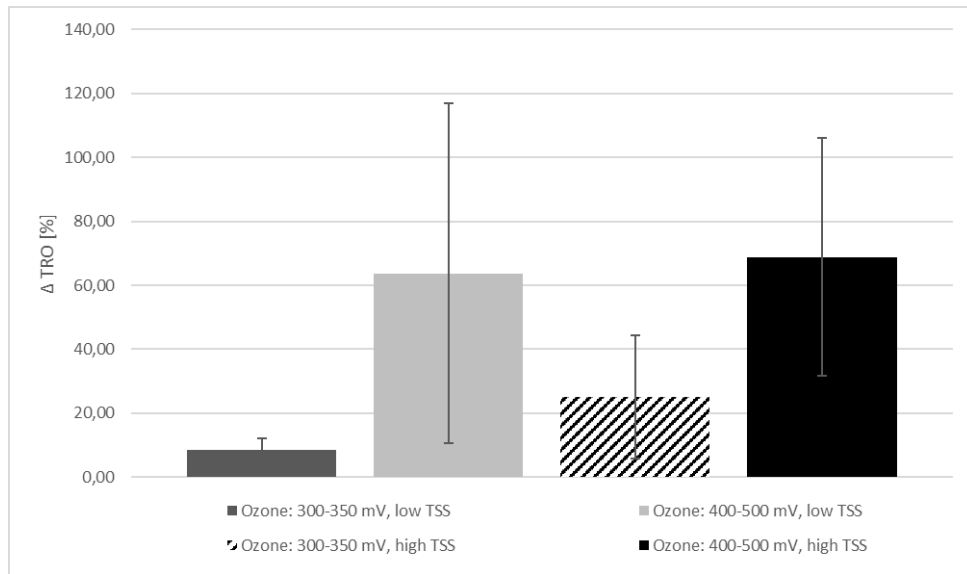
Figure 12: Change in TRO [%] across ozonation tank, at different TSS values with two different ozone doses.

As mentioned in section 4.3 higher ozone doses was also tested to try to find a threshold for increase in level of TRO. The result is presented in figure 13 together with the average from running with ozone doses of 300-350 [mV] and 400-500 [mV]. The increase in TRO [%] when increasing the ozone dose is distinct. Because it was only conducted single samples for the higher ozone doses it is no standard deviation plotted and the results are only indications of a trend. Ozone doses of 630 and 690 [mV] gave high TRO values, 0.217 and 0.349 [mg/L], respectively.
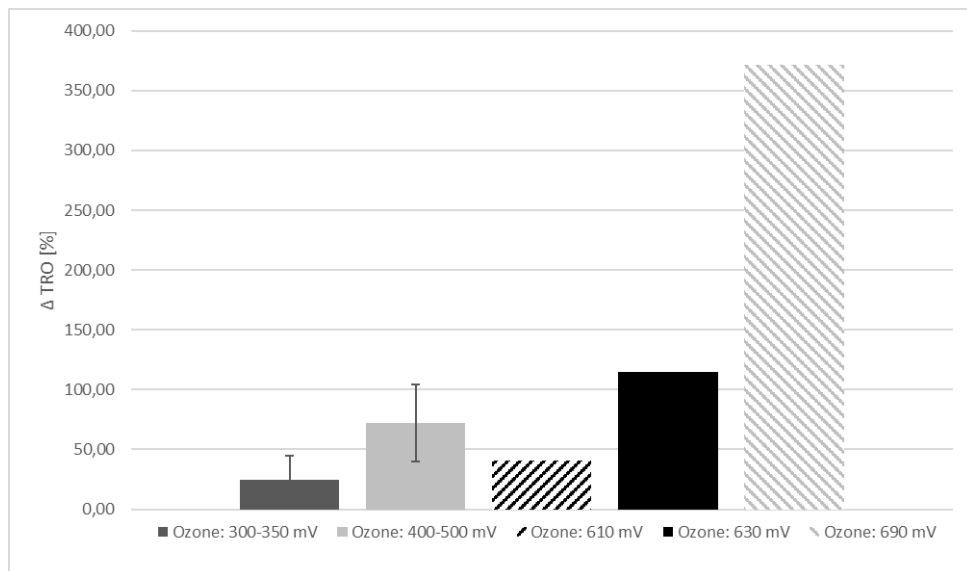
Figure 13: Change in TRO for the different ozone doses tested. Doses of 300-350 and 400-500 [mV] were tested for multiple runs, hence the standard deviation plotted. For the high ozone doses it was only done single sampling. TSS is high, at 20 [mg/L].

Presented in a table, an ozone dose of 690 [mV] ORP gave a maximum TRO value at 0.349

[mg/L]. In table 9 the values are presented together. We observe a possible exponential relation between ozone dose and TRO levels reaching ozone doses above 630 [mV].

Table 9: Change of TRO in [mg/L] and [%] with increasing ozone dose.

	Ozone dose [mV]				
	300-350	400-500	610	630	690
TRO [mg/L]	0,115	0,167	0,144	0,217	0,349
Δ TRO [%]	25,04	72,27	40,49	114,85	371,62

It is important to understand that these results only gives indications, since TRO measurements are highly unstable, combined with the difficulty to obtain a continuous and stable ozone dose.

5.3 Filter mesh size effecting TRO levels

Tests where executed with different mesh sizes on the drum filter. For a filter mesh size of 40 [μm], see figure 14, it was a reduction through all runs when TSS was high. It seems particles removed, when TSS are high, are including residual oxidants, and that way reduces the overall level of TRO over the drum-filter.
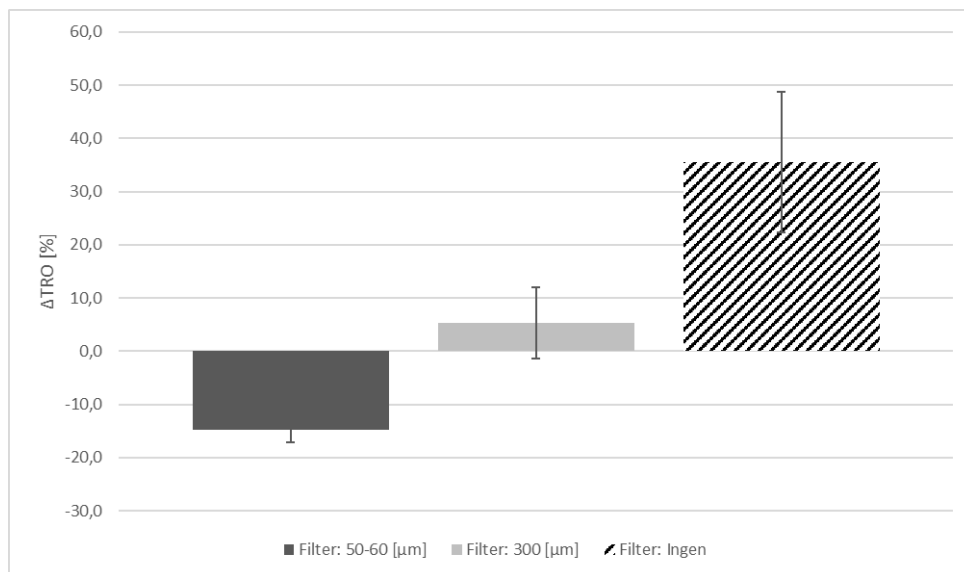

Figure 14: The effect of filter mesh size, when TSS is high and including different ozone doses. Change in TRO [%] across the filter.

Effects of the filter will be more interesting when looking into the different microorganisms removed with different mesh size. This might be of interest for further tasks and should be discussed in section 7.

5.4 Effect of UV treatment

In figure 15 the average of run 4 and 5 are compared. The only difference is the use of UV treatment after ozonation. UV dose is 60 [mJ/cm^2]. This show the percentage change from seawater to after UV.
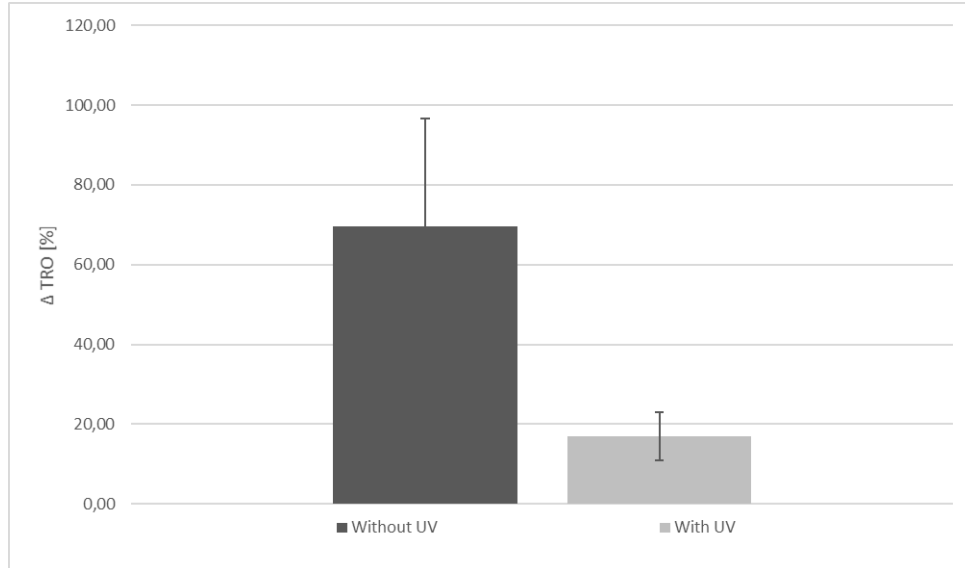

Figure 15: Comparing average of run 4 and 5 of all six tests, both runs with ozone dose of 400-500 [mV] Run 5 included a UV treatment of 60 [mJ/cm^2] after ozonation step, while Run 4 did not include UV treatment.

As shown in table 8, run 5 gave a significant lower formation of TRO than run 4 throughout all tests. In figure 16 we look at the concentration of TRO across the system to show it more clear. Even though it is more difficult to give UV treatment all the credit for the stagnation of TRO we see the difference between using UV and not.
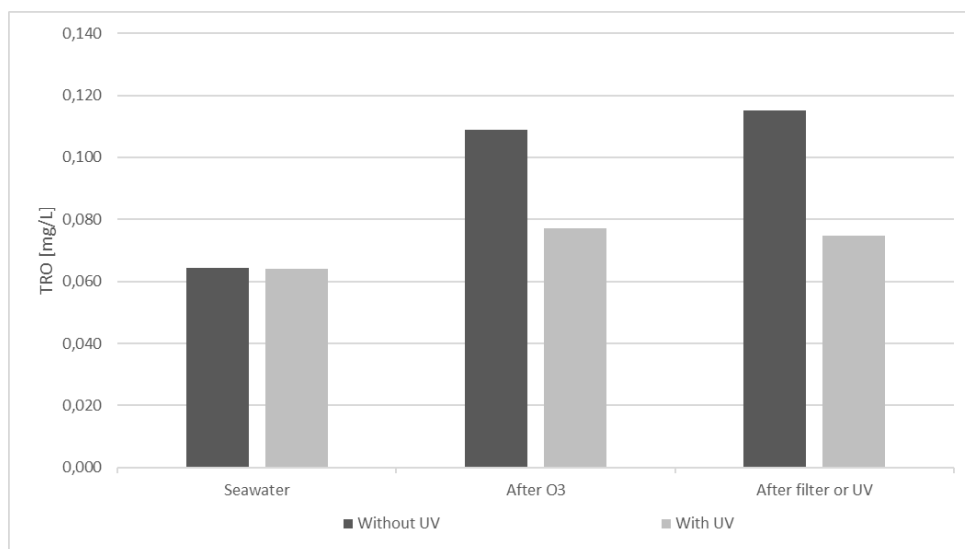
Figure 16: Measured concentration of TRO with high ozone dose, but with and without UV. Ozone dose of 400-500 [mV].

5.5 AOP - combining UV and Titanium Dioxide

According to Brookman and Gagnon (2011) the use of an AOP combining UV and titanium dioxide to treat seawater gave no increase in the disinfection by-products bromate and bromoform. If no disinfection by-products(DBP) was found this could mean that all the active bromine, TRO, had been destroyed and couldn't contribute to formation of the DBP. Our results show that the AOP did not contribute to an increase in TRO. As expected, but now confirmed by results from testing, see figure 17 and 18.
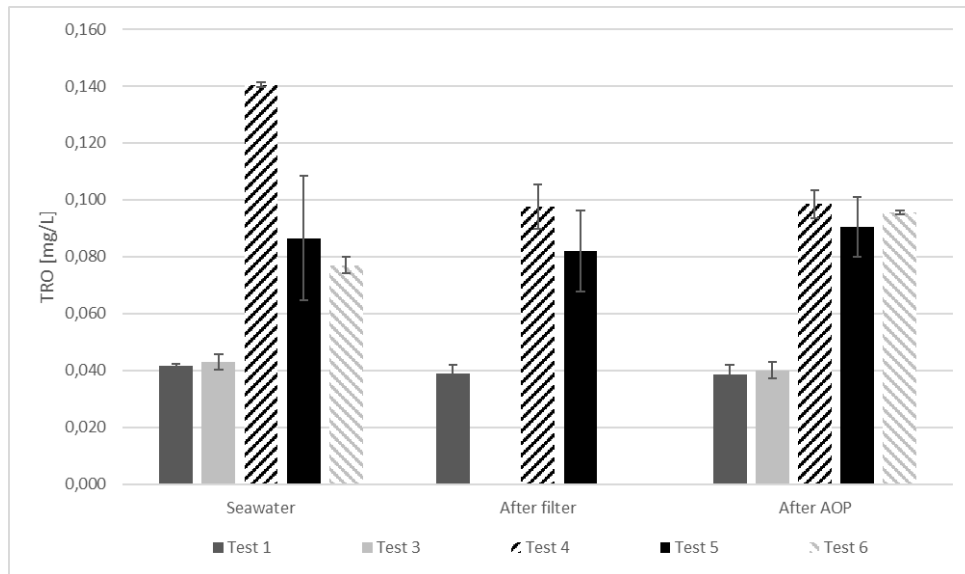
Figure 17: All runs using AOP. Presented as change throughout the whole system. Test 2 is not represented since TRO measurements were not conducted in test 2, run 6 (AOP).
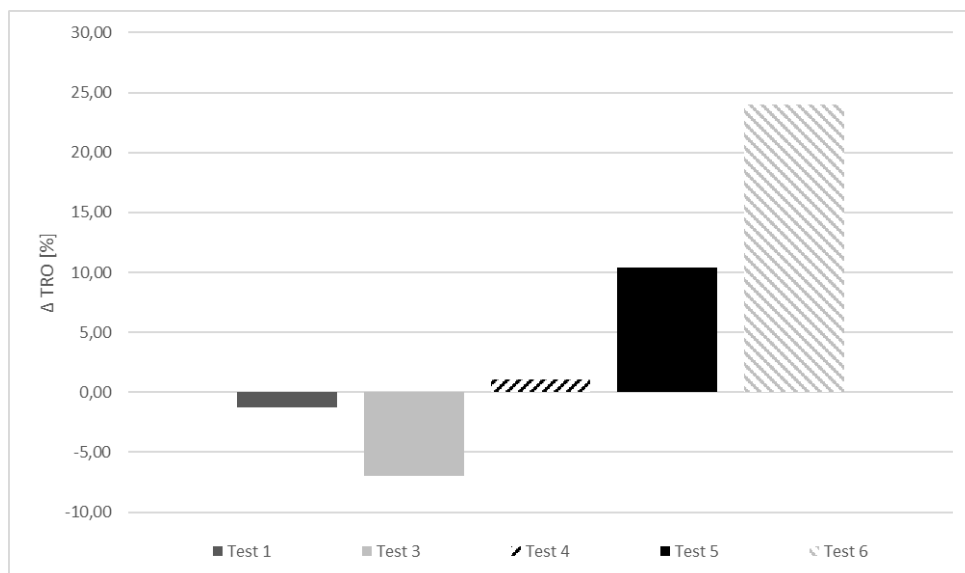

Figure 18: Change in TRO [%] over the AOP treatment unit for different tests.

5.6 Summary regarding TRO level

To sum up all the results considering the TRO levels lets look at the average change in [%] over each treatment. In figure 19 the average change over each treatment unit, except filter, is presented. Use of ozone increases the TRO most, as expected.
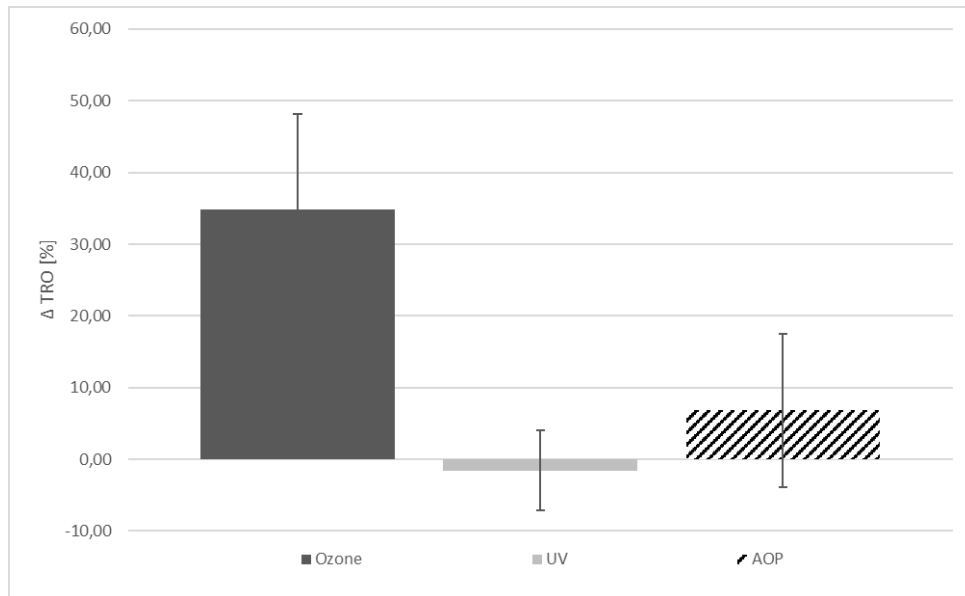

Figure 19: Average change in [%] over each treatment unit, except filter.

It is somewhat difficult to say that the results highlight any trend, but the amount of samples analysed should give an indication. Next it is important to look at the results for the whole system, since the treatment units are coupled in series they affect each other throughout. At the same time it is interesting trying to understand what treatment unit that affect the TRO levels the most. In figure 20 the average values from table 8 is presented in a graph.

With respect to the problem description all three configurations, only UV, ozone and UV, and AOP will be discussed and compared in further analysis. If ozone and UV is used there should be treatment, preferable a GAC, to remove or reduce the TRO beneath possibly toxic levels.
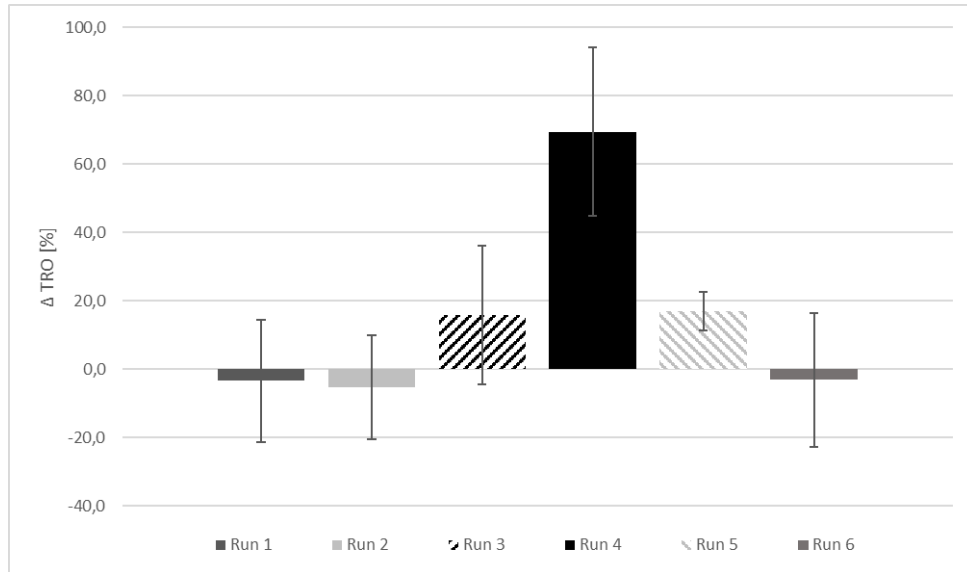
Figure 20: Average of each run over all tests. Related to values in table 8.

In the following section other results will be mentioned, as the amount of active bacteria (CFU) surviving the treatment process.

5.7 Other results from experiment

Choosing a treatment system for production of fish is not based only on results of total residual oxidants. There are a lot of other important aspects with higher level of importance related to production of living organisms. For instance analysis of bacterias removed, the amount of active bacteria surviving the treatment and what kind of bacterias surviving. Since this is not in the scope of this project these topics have not been thoroughly presented, but they are highly relevant when choosing what kind of technology to use in future aquaculture systems.

Colony Forming Unit (CFU) analysis is done to see how many bacteria that are viable and then could be harmful for the fish. Viable meaning the ability to multiply. The method of checking if the bacteria are active is by cultivation and then count how many that are viable.

Results from the CFU show that UV treatment, AOP and ozone followed by UV had the highest removal rate. AOP and ozone followed by UV showed the highest removal rate throughout all the tests. In figure 21 and 22 the change in CFU is presented for AOP and ozone followed by UV for test 6. These two configurations showed the same result for every test, independent of TSS and filter.
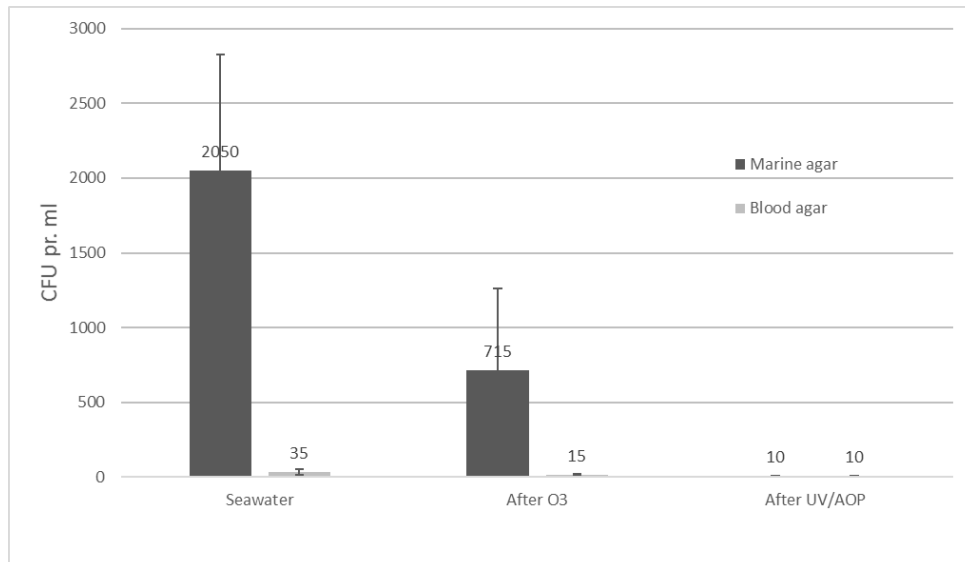
Figure 21: Test 6 run 5, ozone high dose, no filter and 60 mJ/cm^2 UV. TSS is high.
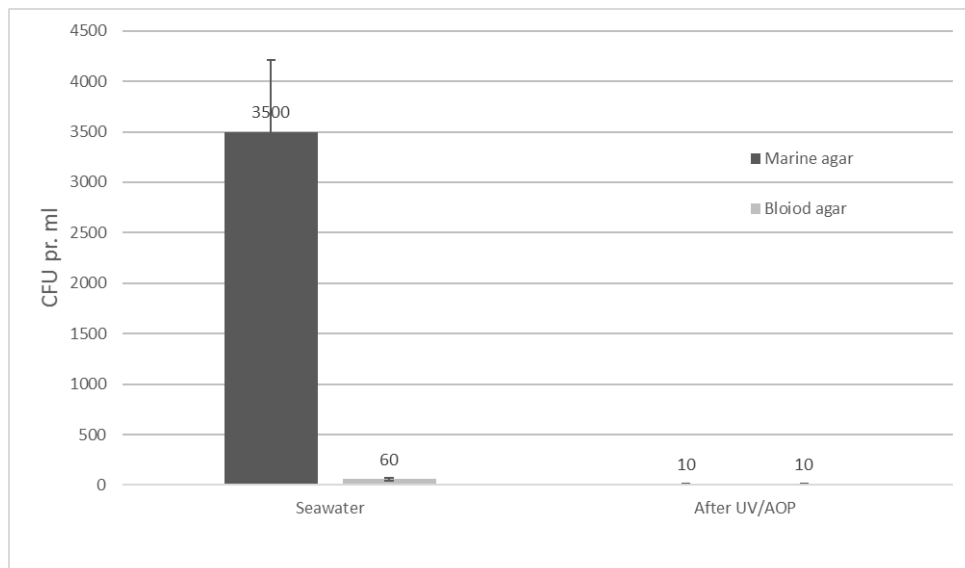

Figure 22: Test 6 run 6, no filter and AOP. TSS is high.

Other important parameters to be analysed, which will give a better understanding before choosing system configuration are; turbidity and UVT, TSS, TOC and types of bacteria and virus. All these results will most certain be ready during spring 2017. It would definitely be interesting to get more results from the experiment to see if it is the same system configurations that do best.

If the AOP inactivate at the same level of ozone with UV it might be interesting to have focus on these two runs when analysing the remaining results. At the same time it would be interesting to compare AOP with UV.

6 Conclusion

The amount of data retrieved from the experiment at Sunndalsøra made it extremely important to sort out important data to present in this report. Focus has all the time been disinfection by-products and the measure of the biocidal component of active bromine as [mg/L] TRO. Especially the use of AOP combining UV and titanium dioxide with seawater is exciting, since there has not been done much like this before towards aquaculture.

Results related to TRO levels indicates that there are systems more preferable than others. Using ozone to oxidise particles before UV disinfection gives decent results regarding TRO levels. Only using UV or AOP gives good results. Results related to TRO levels alone will not give a definite conclusion, but if the other analysis gives results matching with those presented here we might end up comparing only two or three different systems. TRO levels are only one aspect of the total performance of the system. A final conclusion or decision need to address all important water quality parameters as well as the degree of removal regarding microorganisms.

Besides the results from the experiment, the literature presented in section 2 and 3 show that many of the traditional treatment technologies used in drinking water and wastewater treatment is applied in aquaculture. Treatment system with UV and titanium dioxide seems to be the least tested system with little information available.

One important aspect to keep in mind is that the proposed system solutions should be able to be installed and operate in sea. This is mentioned in the introduction, but is not covered thoroughly in this report, but should be taken into account in further work. What works well in landbased fishfarming, might not be possible to get functional in systems in sea, making solutions like AOP very interesting.

7   Further tasks of interest

One possible approach in the master thesis next semester will be to see how a treatment system can affect the production in a semi-closed system in sea. Implementing water treatment would effect the production rate and should give positive effects for fish health and welfare. At the same time introducing a technological system into the existing production system can be costly and acquire adaption of design. Main reason for introducing water treatment will be to ensure water without pathogenic microorganisms, as safety. Comparing different type of treatment configurations from this experiment and evaluate their influence in production and as a whole would be interesting. Results of TOC, turbidity, TSS and micro-bacterial analysis will be of interest if approaching this kind of problem.

Another approach could be to look into design of the water treatment system with the goal of being functional in sea. It could be interesting to look at different design alternatives and see if there is existing solutions to take advantage of.

As shown in the results of the experiment, it could be beneficial to treat the intake water to reduce the risk of microorganisms entering the rearing unit, ref. CFU results, but how could the technologies mentioned be implemented in full scale semi-closed production units? What are the limitations for a full scale system? Focus can either be of the total system, the treatment units or a combination.

If the AOP is chosen as one of the possible options it can be interesting to look deeper into what is the mechanisms and reactions in progress, limitations and the possibility of optimisation. Design of the treatment unit can also be of interest. Is it CFD analysis of similar design available and what are the parameters important when simulating a system like this to reach maximum efficiency.

Introducing a water treatment will increase the energy demand and could in the end be a trade-off between cost and gained safety and increased production rate. What will be the energy required? How well does the different options perform related to energy and cost?

In the scope of this project the specific microorganisms wanted removed are not discussed in depth, and this could be of interest to include in the master thesis when the results from Pharmaq are ready. Especially the removal efficiency of organisms mentioned in section 4.4.1, like *Tenacibaculum sp, Flavobacterium, Yersinia ruckeri, Aeromonas salmonicida, Moritella viscosa*, Poxvirus and IPNV, *Paramoebae perurans* (AGD).

It would definitly be beneficial to continue the cooperation with CtrlAQUA and write the master thesis together with them and their research partners.

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A   Appendix - Tables with change[%]

Table 10: Results from test 1 with low TSS and 50-60 [μm] filter.

Run	Treatment	Production/Reduction in TRO [%]
1	Filter, UV	8.82
2	Filter, UV	1.35
3	Ozone, filter	27.27
4	Ozone, filter	56.86
5	Ozone, filter, UV	20.34
6	Filter, AOP	-7.23

Table 11: Results from test 2 with low TSS and 300 [μm] filter.

Run	Treatment	Production/Reduction in TRO [%]
1	Filter, UV	-
2	Filter, UV	-
3	Ozone, filter	0.00
4	Ozone, filter	93.75
5	Ozone, filter, UV	11.94
6	Filter, AOP	-

Table 12: Results from test 3 with low TSS and no filter.

Run	Treatment	Production/Reduction in TRO [%]
1	Filter, UV	-
2	Filter, UV	-
3	Ozone, filter	9.80
4	Ozone, filter	51.52
5	Ozone, filter, UV	18.92
6	Filter, AOP	-6.98

Table 13: Results from test 4 with high TSS and 50-60 [μm] filter.

Run	Treatment	Production/Reduction in TRO [%]
1	Filter, UV	-29.80
2	Filter, UV	-27.97
3	Ozone, filter	-11.28
4	Ozone, filter	102.83
5	Ozone, filter, UV	10.00
6	Filter, AOP	-29.89

Table 14: Results from test 5 with high TSS and 300 [μm] filter.

Run	Treatment	Production/Reduction in TRO [%]
1	Filter, UV	5.79
2	Filter, UV	1.62
3	Ozone, filter	24.69
4	Ozone, filter	40.49
5	Ozone, filter, UV	15.91
6	Filter, AOP	4.62

Table 15: Results from test 6 with high TSS and no filter.

Run	Treatment	Production/Reduction in TRO [%]
1	Filter, UV	1.96
2	Filter, UV	3.76
3	Ozone, filter	44.90
4	Ozone, filter	71.59
5	Ozone, filter. UV	25.13
6	Filter, AOP	24.03

Table 16: Results from all tests, sorted by run.

	Test						
Run	1	2	3	4	5	6	Average [%]
1	8.8	-	-	-29.8	5.8	2.0	-3.3
2	1.4	-	-	-28.0	1.6	3.8	-5.3
3	27.3	0.0	9.8	-11.3	24.7	44.9	15.9
4	56.9	93.8	51.5	102.8	40.5	71.6	69.5
5	20.3	11.9	18.9	10.0	15.9	25.1	17.0
6	-7.2	-	-7.0	-29.9	4.6	24.0	-3.1

B   Appendix - CFU results ozone with UV
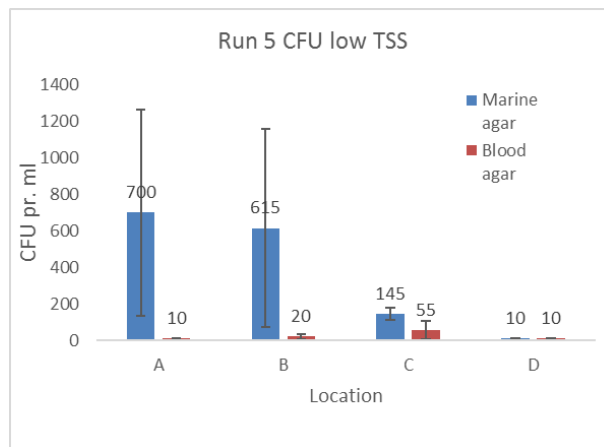
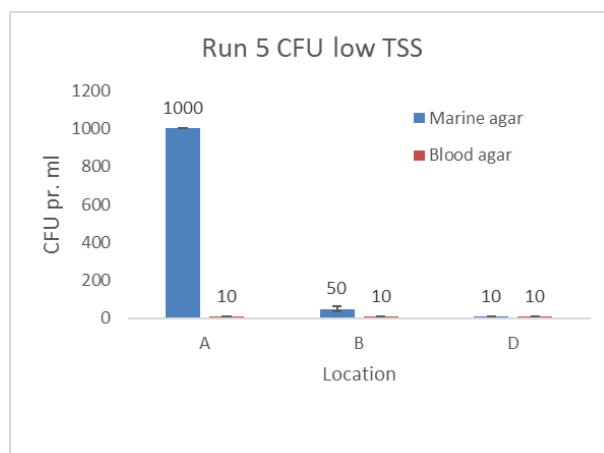Figure 23: CFU Test 1, run 5
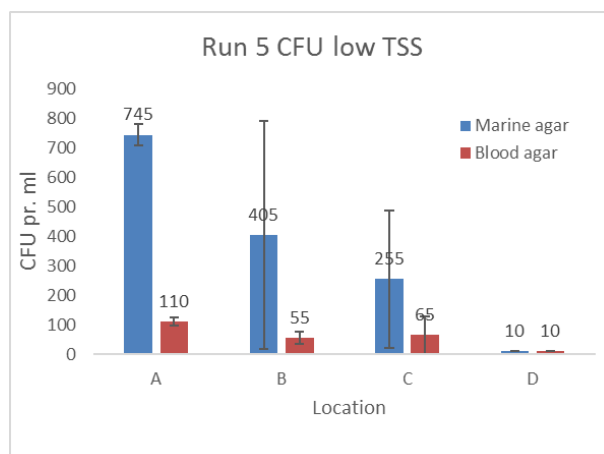
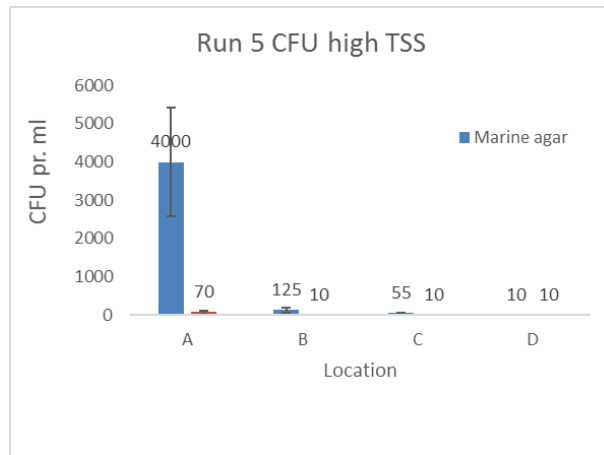Figure 24: CFU Test 2, run 5

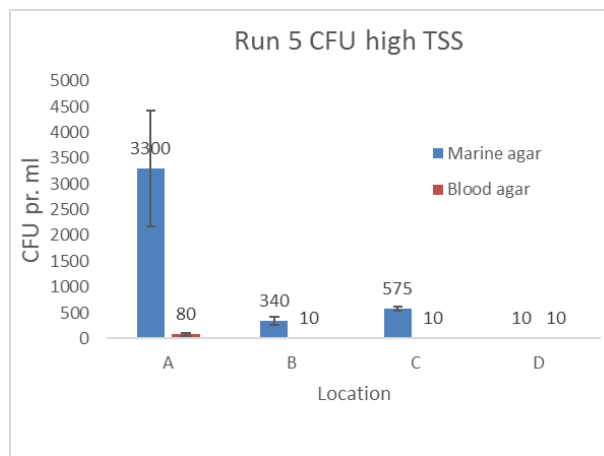Figure 25: CFU Test 3, run 5

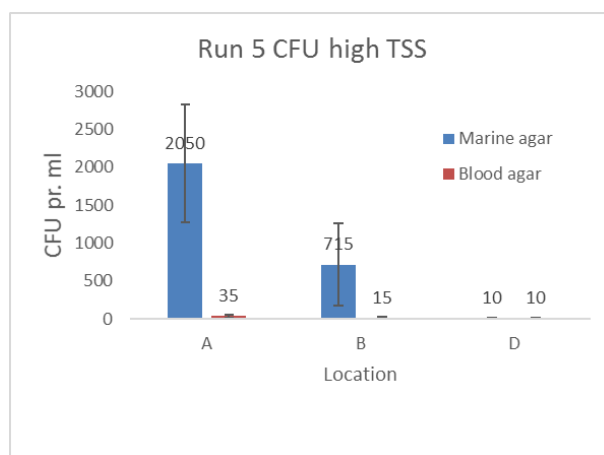Figure 26: CFU Test 4, run 5

Figure 27: CFU Test 5, run 5

Figure 28: CFU Test 6, run 5

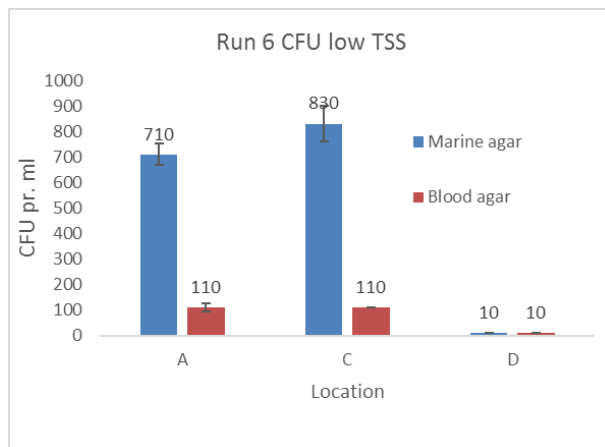# C Appendix - CFU results AOP

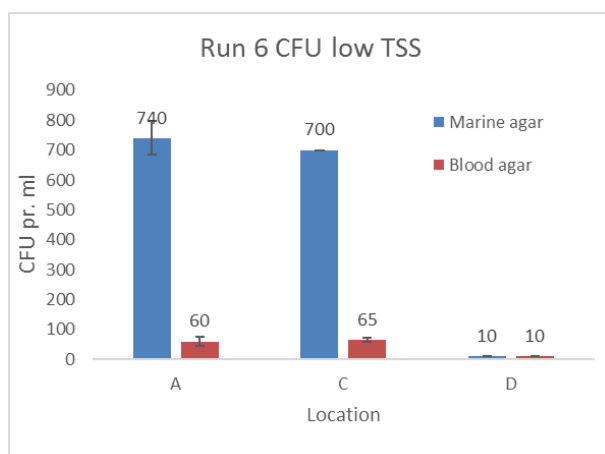
Figure 29: CFU Test 1, run 6
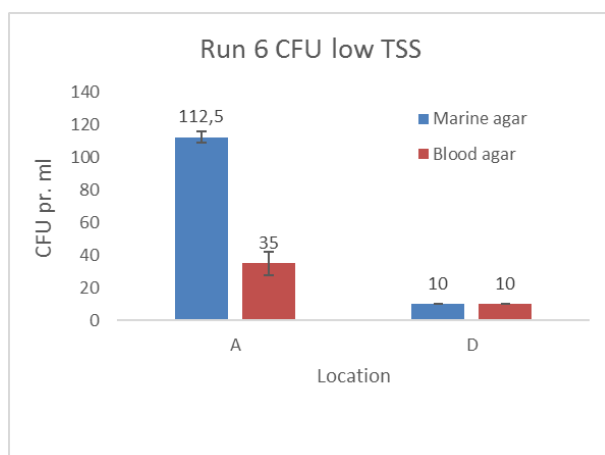

Figure 30: CFU Test 2, run 6
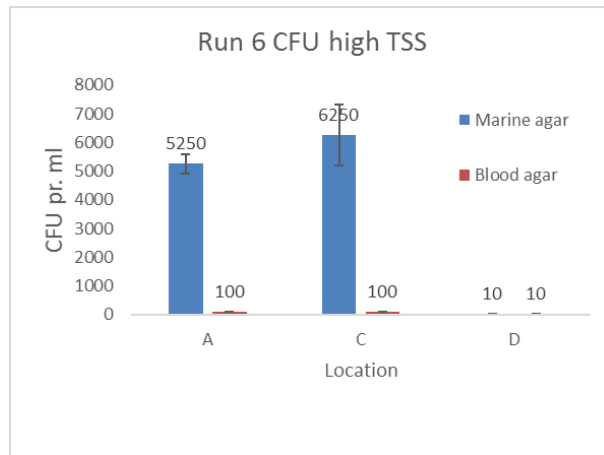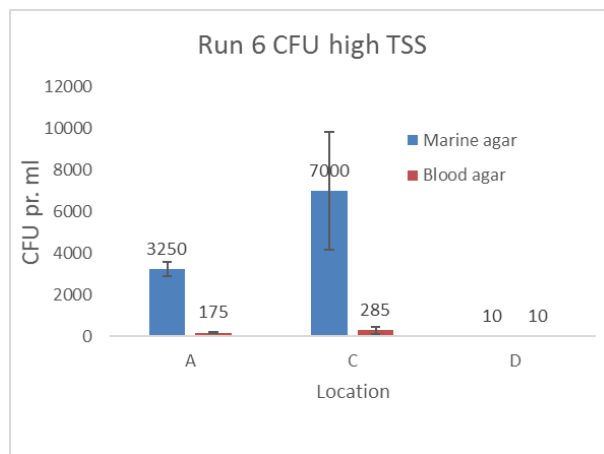

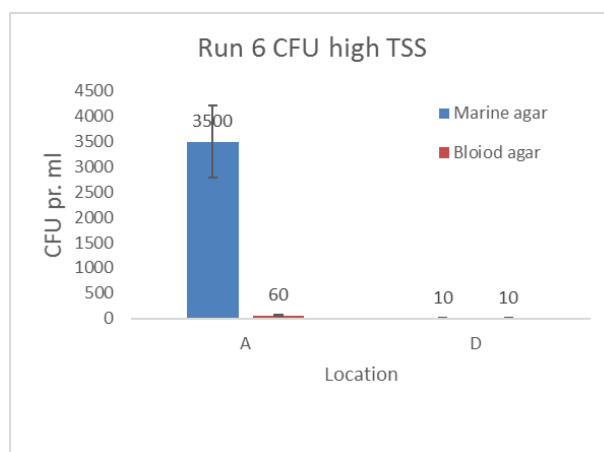Figure 31: CFU Test 3, run 6

Figure 32: CFU Test 4, run 6

Figure 33: CFU Test 5, run 6

Figure 34: CFU Test 6, run 6