



**THE REMOVAL OF TASTE AND ODOR COMPOUNDS FROM AQUACULTURE WATERS  
BY MEANS OF DIFFERENT ADVANCED OXIDATION PROCESSES**

Lappeenranta–Lahden teknillinen yliopisto LUT

Kemiantekniikan kandidaatintyö

2022

Saara Wahlberg

Tarkastaja: Postdoctoral Researcher Kinga Skalska

## TIIVISTELMÄ

Lappeenrannan–Lahden teknillinen yliopisto LUT

LUT Teknis-luonnontieteellinen

Kemiantekniikka

Saara Wahlberg

### **Hajuja ja makuja aiheuttavien yhdisteiden poistaminen kalankasvatusvesistä erilaisten hapetusmenetelmien avulla**

Kemiantekniikan kandidaatintyö

44 sivua, 13 kuvaa ja 6 taulukkoa

Tarkastaja: Postdoctoral Researcher Kinga Skalska

Avainsanat: Recirculating aquaculture systems, geosmin, 2-methylisoborneol, advanced oxidation processes, ozonation, UV-photocatalysis, kierto-vesiviljely, edistyneet hapetusmenetelmät

Geosmiini ja 2-metyyli-isoborneoli (MIB) ovat yhdisteitä, jotka aiheuttavat mutaista ja ummehtunutta sivumakua ja -hajua vesiin. Ihminen kykenee maku- ja hajuaistillaan havaitsemaan nämä yhdisteet, vaikka niiden pitoisuudet olisivat nanogrammoja litrassa. Geosmiini ja MIB ovat erityisen ongelmallisia kierto-vesiviljelmissä, sillä niitä on hyvin vaikea saada poistettua. Tämän seurauksena yhdisteet kertyvät kierto-vesiviljeltyihin kaloihin, joista valmistetut kalatuotteet eivät ole kuluttajille miellyttäviä.

Kahta erilaista edistynyttä hapetusmenetelmää, otsonointia ja titaanidioksidilla katalysoitua UV-fotokatalyysia, tutkittiin mahdollisina ratkaisuin tähän ongelmaan. Tavoitteena oli selvittää, ovatko nämä menetelmät tehokkaita poistamaan geosmiinia ja MIB:a tislatuslaboratoriovedestä, sekä vertailla menetelmiä keskenään. Kokeita suoritettiin kahdella eri otsonipitoisuudella: 20 mg/l ja 40 mg/l. Fotokatalyysikokeissa taas käytettiin 8 ja 20 tunnin altistusajoja UV-valolle. Kokeista kerätyt näytteet esikäsiteltiin neste-nesteuutolla ja analysoitiin kaasukromatografia-massaspektrometrilla.

Kromatografianalyysien tulokset osoittivat, että kaikki kokeet johtivat geosmiinin ja MIB:n pitoisuuksien pienemiseen. 20 tunnin fotokatalyysikoe osoittautui tehokkaimmaksi vaihtoehdoksi, sillä sen avulla saatiin 99.5 % MIB:sta ja 96.6 % geosmiinista poistettua. Vastaavasti 40 mg/l otsonikonsentraation avulla saatiin MIB:n konsentraatio pienemmäksi 98.6 % ja geosmiinin 96.3 %. 8 tunnin fotokatalyysikokeen avulla saavutettiin yli 84 % konsentraation pieneminen kummallekin yhdisteelle ja tehottomin menetelmä, 20 mg/l otsonikonsentraatio, johti alle 60 % poistoasteeseen. Näiden tulosten perusteella voidaan todeta, että jatkotutkimusten toteuttaminen olisi kannattavaa. Käytettyjä menetelmiä voitaisiin tutkia lisää käyttämällä näytteinä oikeita kierto-vesiviljelyvesiä. Nämä vedet

eroavat tislatuslaboratoriovedestä muun muassa siksi, että ne sisältävät typen ioneja ja luonnollisia orgaanisia aineita sekä niiden pH on korkeampi. Kaikilla näillä tekijöillä on vaikutusta sekä otsonoinnin että fotokatalyyysin hapetustehoon. Toinen mahdollinen tutkimuskohde on 8 tunnin fotokatalyyssikoe, sillä se oli ennustettua tehokkaampi. UV-valon ja titaanioksidikatalyytin vaikutuksia poistoasteeseen tulee tutkia erikseen. Voittaisiin myös suorittaa kokeita eri kokoisilla katalyytipinta-aloilla ja tutkia, kuinka se vaikuttaa poistoasteeseen.

## ABSTRACT

Lappeenranta–Lahti University of Technology LUT

School of Engineering Science

Chemical Engineering

Saara Wahlberg

### **Removal of taste and odor compounds from aquaculture waters by means of different advanced oxidation processes**

Bachelor's thesis

2022

44 pages, 13 figures, and 6 tables

Examiners: Postdoctoral Researcher Kinga Skalska

**Keywords:** Recirculating aquaculture systems, geosmin, 2-methylisoborneol, advanced oxidation processes, ozonation, UV-photocatalysis, kiertovesivilyjely, edistyneet hapetusmenetelmät

Geosmin (GSM) and 2-methylisoborneol (MIB) are compounds, that cause earthy muddy, and musty off-flavors and -odors in waters and can be detected by human senses at nanograms per liter concentrations. These compounds are particularly problematic in recirculating aquaculture systems (RAS), as they are hard to remove and they accumulate in fish cultivated in the RAS, thus making the fish product unappealing to consumers.

Two different advanced oxidation processes (AOPs) were studied as potential solutions to this issue: ozonation and titanium dioxide catalyzed UV-photocatalysis. The goal was to determine whether these methods are effective in removing GSM and MIB from deionized (DI) laboratory water and to compare the two methods. Two ozone doses were used for the experiments: 20 mg/l and 40 mg/l. Two different exposure times were used for UV-photocatalysis: 8 hours and 20 hours. The collected samples were pretreated by liquid-liquid extraction and analyzed by gas chromatography-mass spectrometry (GC-MS)

The results from GC-MS showed, that all experiments resulted in reductions in GSM and MIB concentrations. The 20-hour photocatalysis experiment provided the most efficient removal with a removal rate of 99.5 % for MIB and 96.6 % for GSM. Similarly, the 40 mg/l ozone dose resulted in 98.6 % of MIB and 96.3 % of GSM removed. The 8-hour photocatalysis trial resulted in over 84 % removal rates for both compounds and the least effective method, 20 mg/l ozone dose, resulted in under 60 % removal rates. These results indicate that it would be beneficial to further research this issue by applying

the used AOPs to real RAS waters. RAS water differs from DI-water, for example, RAS water contains nitrogen ions and natural organic matter and has a higher pH. All these factors affect the oxidizing abilities of both ozonation and photocatalysis. Another possible research subject would be the 8-hour photocatalysis experiment since it removed the compounds surprisingly well. The effects of the UV light and the catalyst should be examined separately. It could also be experimented on how the area of the catalyst affects the removal rate.

## SYMBOLS AND ABBREVIATIONS

### Symbols

$O_{3abs}$  = ozone concentration absorbed into the sample [mg/l]

$O_{3in}$  = ozone concentration going into the reactor [mg/l]

$O_{3out}$  = ozone concentration coming out of the reactor [mg/l]

$t$  = time [s]

$\dot{V}$  = gas flowrate [l/h]

$V$  = reactor volume [l]

### Abbreviations

AOP      Advanced oxidation process

DCM      Dichloromethane

DI-water      Deionized water

GC-MS      Gas chromatography-mass spectrometry

Geosmin-d3      Deuterated geosmin

GSM      Geosmin

IBMP      2-isobutyl-3-methoxypyrazine

IPMP      2-isopropyl-3- methoxypyrazine

LLE      Liquid-liquid extraction

MIB      2-Methylisoborneol

NOM      Natural organic matter

RAS      Recirculating aquaculture system

TCA      2,4,6-trichloroanisole

## Contents

Tiivistelmä

Abstract

Symbols and abbreviations

1	Introduction.....	8
2	Aquaculture systems.....	10
	2.1 Recirculating aquaculture systems.....	11
	2.1.1 The history of recirculating aquaculture systems.....	11
	2.1.2 The facilities of a recirculating aquaculture system.....	12
3	Taste and odor compounds.....	15
	3.1 Geosmin and 2-Methylisoborneol.....	15
	3.1.1 Microorganisms.....	16
	3.2 The issues caused by taste and odor compounds in RAS.....	17
4	Advanced Oxidation Processes.....	19
	4.1 Ozonation.....	21
	4.2 Photocatalysis.....	22
	4.2.1 Electron-hole pairs.....	22
	4.2.2 Titanium dioxide.....	23
5	Materials and methods.....	25
	5.1 Chemicals.....	25
	5.1.1 Samples.....	25
	5.2 Equipment.....	26
	5.2.1 Ozonation unit.....	26
	5.2.2 UV photocatalysis reactor.....	27
	5.3 Laboratory experiments.....	28
	5.4 Analytics.....	29
	5.4.1 Liquid-liquid extraction.....	30
	5.4.2 Gas chromatography-mass spectrometry.....	31
6	Results and discussion.....	33
7	Conclusions.....	38
	References.....	40

# 1 Introduction

World hunger is a constantly rising issue in multiple developing countries. As the world population grows, there is a high demand for environmentally friendly and efficient food production methods to ensure food security. Blue food, meaning fish, bivalves, seaweed, and other aquatic foods, is an overlooked category of food sourcing. It has excellent potential to be an option for environmentally friendly, sustainable, and healthy eating. (Gephart et al. 2021.) The fish industry has grown to be a significant industry over the past 30 years. In 2018, about 156 million tonnes of blue food were consumed globally. Expanding and advancing onshore aquaculture methods is required rather than expanding marine and freshwater fishing for this growth to continue sustainably. (FAO 2020.) Due to problems caused by commercial fishing, such as overfishing and the disturbance in food chains, recirculating aquaculture systems could come in as a potential option (Marsh 2020). Recirculating aquaculture systems (RAS) are operated by purifying the water used in the cultivation tanks to be used again (Blue Ridge Aquaculture 2022). RAS waters tend to have issues with off-flavors and odors that affect the quality of the fish (Helmer 2020).

Research has shown that the off-flavors and odors are mainly caused by two compounds, geosmin (GSM) and 2-methylisoborneol (MIB). These compounds cause earthy, muddy, and musty tastes and odors in aquaculture waters. The off-flavors and odors are then accumulated in the fatty tissue of fish. (Zimba et al. 2011.) Since GSM and MIB are hard to remove from RAS waters, the fish are placed in a different tank with clean water, where the impurities accumulated in their fatty tissue diffuse into the clean water. This method is called depuration. Depuration requires separate facilities and uncontaminated water reserves, making this method impractical and expensive. (Lindholm-Lehti and Vielma 2018.) However, there have been some promising results with utilizing advanced oxidation processes (AOPs) as removal methods. (Antonopoulou and Konstantinou 2017.) A study on removing GSM and MIB with UV photocatalysis using titanium dioxide found it effective on spiked laboratory water and actual aquaculture water. Up to 90 % reduction in taste and odor compounds was reached in RAS waters. (Pestana et al. 2014.) Ozonation has been another possibility for the purification of RAS waters since it has already been used for municipal water treatment. It was found that low-dose ozone addition of 0.25 – 0.8



milligrams per liter did not reduce the compounds remarkably. It was also stated that a higher dose might work better. (Schrader et al. 2010.) Similarly, Yuan et al. (2013) found that using an ozone dose of 4.19 milligrams per liter reduced the amount of both GSM and MIB by 90 %. These findings are consistent with Schrader et al. (2010). While current research has shown potential, more research is still needed to utilize these methods on a larger scale effectively.

This bachelor's thesis investigated the practicality of GSM and MIB removal from water with titanium dioxide photocatalysis and ozonation. It was also explored whether these methods differed performance-wise. The focus of the literature review is on getting a general understanding of the topic before diving into the experimental part of the thesis. The practical part involves removing GSM and MIB from spiked laboratory waters with UV photocatalysis using titanium dioxide as a catalyst and ozonation. The GSM and MIB levels are determined by pre-treating the samples with liquid-liquid extraction and analyzing them with gas chromatography-mass spectrometry (GC-MS).

## 2 Aquaculture systems

Aquaculture systems can be classified in four different ways. The first category is the structure of the fish farm. The most commonly used alternatives are ponds, cages, raceways, tanks, and pens. Aquaculture systems can also be classified by their water usage, ranging from open to recirculated. The remaining factors to consider are the intensity of culturing, from intensive to extensive, and whether the cultivation method used is monoculture or polyculture. (Soltan 2016.)

Open-net pens consist of floating frames with nets attached under them. These structures are situated offshore in marine waters and freshwater lakes. The issues with open-net pens are that it is impossible to control the growing conditions of the fish. Bacteria or pollution could easily affect the fish. Additionally, predators, such as marine mammals, can be attracted to the fish and get tangled in the nets and drown. (SeaChoice 2022.)

Closed cages, like open-net pens, are used in marine waters and lakes. Unlike open-net pens, cages are fully closed and submerged. The fish are not as susceptible to the unstable conditions of surface waters. There is also a lower risk of escaping fish with closed cages. The challenges with this kind of fish farming are that the fish experience hindered growth rates compared to open-net pens. The environmental conditions become less optimal at the deeper depths. (Sievers 2021.) It might also be more challenging to ensure an adequate feed rate of fish in submerged cages.

Ponds are partly or fully closed bodies of water. They can be formed naturally by land shapes or by levees. The advantages of pond farming lie in the simpleness and low energy requirements. Apart from the actual harvesting of the fish, pond culturing requires a modest labor force. The problems of pond raising fish include the susceptibility to disease, weather conditions, and predators such as birds. It has been reported that birds have ruined whole pond farms. (New World Encyclopedia 2022.) Pond culturing can also have devastating effects on the environment. For example, pond-raising shrimp has been estimated to be responsible for more than a third of the total loss of mangrove forests, due to cutting down the trees. The altered land usage has also led to blockages to the natural water flow of the mangroves, which has led them to be damaged. (Das et al. 2016.)

There are some disadvantages to traditional fish farming. For example, Lazzari and Baldisserotto (2008) state that nitrogen and phosphorus found in fish metabolites are the most significant sources of harmful waste from aquaculture since they cause eutrophication. Large amounts of these accumulated compounds are harmful to the fish and if released into the surrounding environment. Considering the severity of the aforementioned disadvantages, other options are needed. Recirculating aquaculture systems provide an option for monitored fish culturing. As closed systems, they are not dependent on weather conditions, and it is known what the fish are ingesting. This thesis focuses on solving some problems regarding recirculating aquaculture systems.

## 2.1 Recirculating aquaculture systems

Recirculating aquaculture systems (RAS) are a technology for farming fish, aquatic plants, and other seafood. The water used goes through filtrations and purifications and is recycled back into use. This method of food production utilizes minimal water reserves and land area and allows high-density culturing. (Ahmed and Turchini 2021.) Therefore, RAS could be a potential option for food production for heavily populated areas or areas without access to natural waters.

### 2.1.1 The history of recirculating aquaculture systems

The research on recirculating aquaculture systems was first conducted in the 1950s in Japan. Still, the most significant growth and development started in the 1980s, when the Danes built the first commercial production system. After this achievement, many European countries, North America, and China began developing their recirculating aquaculture systems. (Ahmed and Turchini 2021.) Even though the development of RAS started in the 1950s, it was not recognized until the mid-1980s that it is essential to monitor water quality parameters, such as chemical oxygen demand and total ammonia nitrogen. Discoveries were made about methods that reduced the accumulation of sludge and nitrate in the aquaculture waters, causing the water quality to stabilize. These findings were a step forward in commercializing the use of RAS. (Espinal and Matulić 2019, 36–37.)

### 2.1.2 The facilities of a recirculating aquaculture system

Recirculating aquaculture systems require many different components to work. The most obvious of these components is the tank where the fish is grown. These tanks vary in size from 1 to 400 cubic meters. The size of the tanks is based on the density of the farmed fish. The most common farming density of fish for human consumption is about 60 kilograms per cubic meter. The most common aquaculture tanks are circular, rectangular, and raceway tanks. Raceway tanks are rectangular and have an extra wall installed in the middle of the tank.

The circulation of the water is an essential component of RAS. The flow rate of circulation varies from 42 to 84 liters per minute per kilograms of the daily feed ration. There are different options for producing the water flow, such as centrifugal pumps, axial flow pumps, and airlifts. Airlifts can also be used for aeration, which means oxygen addition. Aeration is essential to ensure that fish have sufficient oxygen since the water is constantly recirculated through the system. There are other methods for aeration, such as using a diffuser hose or a surface aerator.

Other critical components for RAS are carbon dioxide degassers, filtration, denitrification units, and disinfecting units. The aquaculture tanks also require temperature control, which means heating systems. These components purify the water and keep the conditions optimal for the cultivation of the fish. (Malone 2013.) The flowchart of a typical RAS is shown in figure 1.

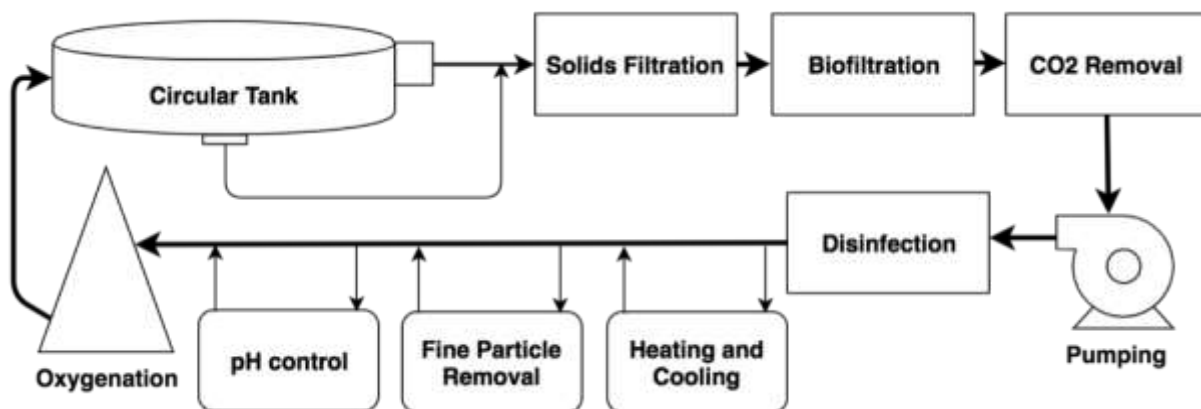


Figure 1 A flowchart of a typical recirculating aquaculture system with the water quality ensuring devices described above (Wikimedia commons 2018).

These steps ensure that the RAS has optimal conditions for raising fish. General water quality parameters of a RAS are presented in Table 1.

Table 1 The parameters related to RAS waters, their normal ranges, and the thresholds for harmful quantities (Bregnballe 2015)

<b>Parameter [unit]</b>	<b>Normal range</b>	<b>Disadvantageous level</b>
Temperature [°C]	10-35	It depends on the species
pH [-]	6.5-7.5	< 6.2 and > 8.0
Oxygen saturation [%]	70-100	< 40 and > 250
Nitrogen saturation [%]	80-100	> 101
Carbon dioxide [mg/L]	10-15	> 15
Ammonia [mg/L]	<0.01	> 0.025
Nitrite [mg/L]	0-0.5	> 0.5
Nitrate [mg/L]	100-200	> 300
Suspended solids [mg/L]	25	> 100

Table 1 shows that the normal range for the water temperature in RAS is quite broad. This is due to the different needs of different species of fish. For example, the optimal temperature for the Atlantic cod (*Gadus morhua*) is 12 °C, whereas the Whiteleg shrimp (*Penaeus vannamei*) thrives at 30 °C. The water temperature is adjusted with the optimal growth rates for the stock in mind. (Bregnballe 2015.)

Two factors must be considered when adjusting the pH levels of a RAS: its effect on the biofilter efficiency and the formation of ammonia. A pH under 7 reduces the rate of bacterial nitrifying, where the biofilter bacteria convert harmful ammonia into nitrite and further into harmless nitrate. However, the higher the pH, the higher the formation of free ammonia. Ammonia is also a metabolite of fish. It has been determined that a pH of around 7 is the best considering both these factors. (Bregnballe 2015.)

The oxygen saturation of the RAS waters must be kept at a sufficient level for the fish to get enough oxygen into their bloodstream. Another reason oxygenation is important is that anaerobic conditions caused by elevated nitrogen saturation and carbon dioxide allow the formation of highly toxic hydrogen sulphide that is lethal to fish even in small concentrations. (Bregnballe 2015.) An oxygen saturation level too high leads to bubble gas disease, which is also lethal to the fish (Máchová et al. 2017).

The elevation in nitrite concentration is harmful to fish since nitrite enters their bloodstream through the gills and replaces oxygen. If nitrite levels are elevated, the fish may seem to be gasping for air even though the oxygen saturation appears to be at a sufficient level. Therefore, the biofilters must convert nitrite into nitrate. Nitrate is mainly harmless to fish, but high levels seem to stunt the growth rates. The levels of suspended solids, which consist of undigested feed and feces, must be monitored since they offer a breeding ground for harmful bacteria. (Bregnballe 2015.) The suspended solids in RAS are challenging since the smaller particles cannot be removed with standard filtration. This causes the small particles to accumulate in the system. The particles are known to harm gill structures and elevate stress levels in fish. (Dahle et al. 2020.)

### 3 Taste and odor compounds

Since water is a solvent, it catches taste and odors from dissolved chemical compounds and the surrounding air. The most frequently reported compounds to cause off-flavors and odors in drinking waters are geosmin (GSM), 2-methylisoborneol (MIB), 2,4,6-trichloroanisole (TCA), 2-isopropyl-3-methoxypyrazine (IPMP), and 2-isobutyl-3-methoxypyrazine (IBMP). TCA is a chlorinated benzene derivative, and it has been reported to cause moldy taste and odor in drinking waters. IPMP and IBMP are pyrazines, benzene derivatives with nitrogen atoms replacing two of the carbons. They are found to cause an odor resembling rotten vegetables. However, GSM and MIB are the most common of these harmful compounds in RAS waters. (Antonopoulou and Konstantinou 2017.)

#### 3.1 Geosmin and 2-Methylisoborneol

GSM and MIB are tertiary semi-volatile alcohols (Antonopoulou and Konstantinou 2017). This tertiary alcohol structure is challenging to break down since it holds great resistance towards oxidation (Kutschera et al. 2009). Antonopoulou and Konstantinou (2017) suggest that MIB is more difficult to break down than GSM due to the stronger hindrance in the chemical structure.

Geosmin (trans-1,10-dimethyl-trans-9-decalol) has been reported to be the number one cause of earthy off-flavor and odor in municipal and RAS waters. GSM has a bicyclic tertiary alcohol structure, as depicted in Figure 2.

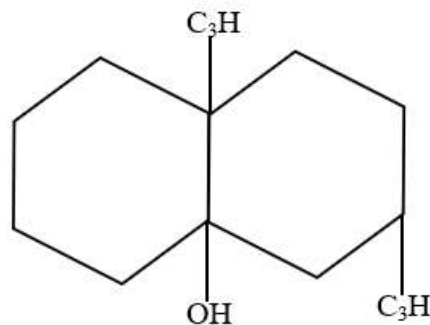


Figure 2 The chemical structure of GSM. The tertiary structure can be seen in the hydroxyl group attached to a carbon that is linked to three other carbon chains.

The earthy-muddy taste of GSM can be detected by human taste and smell at very low levels. The odor threshold is 4 nanograms per liter, and the taste threshold is 7.5 nanograms per liter. (Antonopoulou and Konstantinou 2017.)

While GSM is the leading cause of earthy flavors and odors in RAS waters and fish, 2-methylisoborneol has been reported to cause a musty off-flavor. The flavor and odor thresholds for MIB are also low. It can be detected by smell at 15 ng/L and taste at 2.5 ng/L. (Antonopoulou and Konstantinou 2017.)

MIB belongs to the group of terpenoids, which are bacteria metabolites and are constructed of isoprene units. (Pandey et al. 2021). The cyclic structure is depicted in Figure 3.

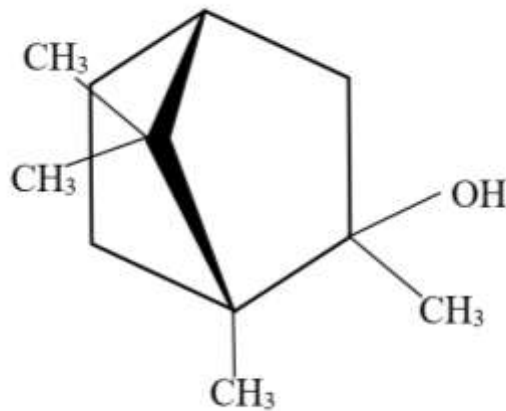


Figure 3 The chemical structure of 2-methylisoborneol. MIB is also tertiary alcohol which can be seen by the placement of the hydroxyl group.

### 3.1.1 Microorganisms

GSM and MIB are the metabolite products of various microorganisms (Antonopoulou and Konstantinou 2017). Microorganisms are microscopically tiny living beings, including bacteria, archaea, protists, and fungi (University of Bergen 2010). It has been discovered that the actinomycetes and cyanobacteria are the main bacteria that produce GSM and MIB (Whangchai et al. 2017).

Actinomycetes are a group of bacteria that spread by forming a network of filaments and spores (Ortenberg and Telsch 2003). GSM and MIB are produced by the most extensive genus of actinomycetes



called *Streptomyces*. These bacteria are earthborn, and in addition to tainting waters with off-flavors and smells (Zaitlin et al. 2003), they are known to have harmful effects on fish gills (Lewis et al. 2009).

Cyanobacteria have also been found to metabolize GSM and MIB (Whangchai et al. 2017). Cyanobacteria, formerly known as blue-green algae, are prototrophic bacteria, which means they can perform photosynthesis. Cyanobacteria are commonly found in waters but have also been found in soil and in symbiosis with other plants and primitive animals. Mass occurrences, also known as water blooms of cyanobacteria, can frequently be found in eutrophic waters, and they are toxic to humans and animals. (Sivonen 2009.)

### 3.2 The issues caused by taste and odor compounds in RAS

The issue with RAS lies in the taste and odor compounds formed in the aquaculture waters that affect the taste of the fish. There have been significant financial losses due to consumers rejecting the tainted fish products. (Pestana et al. 2014.) As mentioned before, the main compounds causing these off-flavors and odors are GSM and MIB. Priyantha Indrajith and Kenneth (2016) found in their experiment on the accumulation of GSM and MIB in RAS waters that their levels ranged from 5 to 40 nanograms per liter and 10 to 17 nanograms per liter, respectively. In the study conducted by Lindholm-Lehto et al. (2020), it was shown that these levels ranged from 6.8 to 49 nanograms per liter and 39 to 58 nanograms per liter. The GSM concentrations appear similar between the two experiments, but the MIB concentrations vary significantly. This might be caused by different species of fish cultivated in the RAS or differences between the conditions of the experiments.

Methods such as coagulation, sedimentation, and chlorination have been deemed ineffective in removing the taste and odor compounds in question. The most used removal method utilizes activated carbon, either powdered or granulated. The problems with this method lie in the natural organic matter (NOM) present in the water. NOMs cause the excessive formation of sludge, which increases operating costs. Activated carbon has also been less effective on MIB removal than GSM removal. The doses of activated carbon needed to get under the human threshold of these compounds are high, which is not practical and increases costs. (Srinivasan and Sorial 2011.)

Another method experimented with is adsorption by zeolite. So far, zeolite has only been used in laboratory-scale testing. Some issues were faced in these experiments, like the formation of biofilm and reduced efficiency, similar to activated carbon. It was also found to be expensive. (Lindholm-Lehto and Vielma 2018.)

The method most widely used to purify the fish from the off-flavors is depuration. The fish are placed in a separate depuration tank filled with water not contaminated by GSM or MIB. The fish are kept in this independent tank while metabolizing the GSM and MIB out of their system. (Davidson et al. 2020.) This is inefficient because separate depuration tanks require more extensive facilities, equipment, and increased water usage. Additionally, they cause a decrease in fish weight and delay in selling the fish. This means increased expenses and decreased income. (Lindholm-Lehto and Vielma 2018.)

## 4 Advanced Oxidation Processes

Advanced oxidation processes (AOPs) were first introduced to treat drinking waters in the 1980s. This ignited broad research on the usage of AOPs in wastewater treatment. AOPs can be defined as oxidation processes that produce reactive oxygen species, most commonly hydroxyl radicals. Hydroxyl radicals have an oxidation potential of 2.8 eV, making them highly effective in degrading harmful organic compounds. (Garrido-Cardenas et al. 2019.) The different kinds of AOPs are shown in Figure 4. Deng and Zhao (2015) describe four pathways for OH-radicals to attack organic compounds: hydrogen abstraction, electron transfer, radical combination, and radical addition.

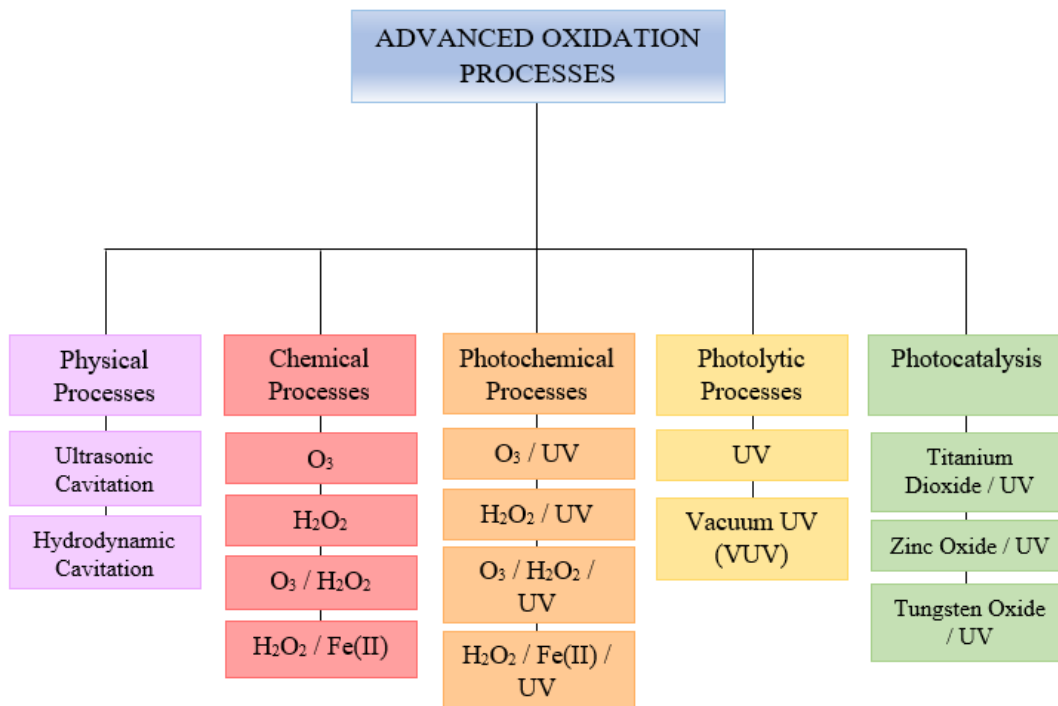


Figure 4 Different methods of advanced oxidation processes categorized by the active oxidizing element (Chirwa and Bamuzza-Pemu 2010).

As shown in Figure 4, the methods of AOPs mainly rely on UV radiation or a strong oxidizing agent to produce the hydroxyl radicals needed for oxidation. The physical processes presented rely on a phenomenon called cavitation, where the static pressure in the liquid drops below its vapor pressure. This

creates tiny vapor-filled bubbles, which form reactive hydrogen atoms and hydroxyl radicals when they collapse in contact with a higher pressure. Ultrasonic cavitation utilizes ultrasound to create these bubbles, and hydrodynamic cavitation uses hydraulic devices such as valves respectively. (Chirwa and Bamuza-Pemu 2010.)

Photolytic processes depend on the absorption of UV radiation. The radiation can be direct or indirect, and it can be generated by an artificial source, or it can be solar energy. The direct radiation absorbs into organic compounds and causes them to either become inert or cause self-degradation. The indirect radiation produces photosensitizers such as oxygen and hydroxyl radicals through photolysis. These photosensitizers then decompose the organic compounds. (Chirwa and Bamuza-Pemu 2010.) The difference between UV and vacuum UV radiation is that UV radiation happens at 254 nanometres, and VUV has a wavelength of below 200 nanometres (Buchanan et al. 2004).

The oldest systems rely on having one strong oxidant in the process. These include hydrogen peroxide and ozone. Even though hydrogen peroxide is a fairly strong oxidant, it has been found to have limited applications in degrading complex organic compounds by itself since there are some kinetic limitations in moderate peroxide concentrations. A search for improvement found that hybrid processes, such as hydrogen peroxide combined with ozone or Fenton's reagent, could improve the oxidizing properties. (Chirwa and Bamuza-Pemu 2010.)

The ozone-hydrogen peroxide system, also known as the peroxone system, generates far more hydroxyl radicals than hydrogen peroxide alone due to the interaction between hydrogen peroxide and ozone. This type of process has shown great potential for the complete removal of organic pollutants and their degradation intermediates from solutions. (Chirwa and Bamuza-Pemu 2010.)

The Fenton process utilizes an oxidative catalytic mixture and an oxidant, usually a ferrous salt and hydrogen peroxide. The Fenton process has been used in increasing amounts to treat organics in waters and soils. The decomposition of hydrogen peroxide by ferrous ions produces highly reactive and non-selective hydroxyl radicals, which means it has strong oxidizing powers. The advantages of the Fenton process include relatively low costs, since iron is abundant, and it is also non-toxic. The downside is that this process has a narrow pH range of 2-4 for application, making it a poor choice for natural waters. (Chirwa and Bamuza-Pemu 2010.)

UV radiation can be applied to all the aforementioned chemical processes making them photochemical processes. UV radiation accelerates the production of hydroxyl radicals, thus improving the oxidizing qualities of the chemical oxidants. (Chirwa and Bamuza-Pemu 2010.) This literature review will focus on two methods of AOPs: photocatalysis and the chemical process of ozonation.

#### 4.1 Ozonation

Ozonation is a chemical water treatment method where ozone is infused into the water. The disinfecting properties of ozone were found in the late 1800s, and it has been used for water treatment ever since. Ozone ( $O_3$ ) is produced from oxygen by exposing it to electric current or UV light. The oxygen molecule is split into two oxygen atoms. These atoms attach to oxygen molecules forming the supercharged ozone. The structure of ozone is highly unstable and creates hydroxyl radicals when it breaks down, which is the cause of its oxidizing potential. The benefit of using ozonation is that there are no harmful residuals since the breaking down of ozone produces oxygen as a by-product. It has also been found to kill bacteria and viruses effectively. (WaterProfessionals 2022.) The disadvantages of ozonation include the high cost of generating sufficient ozone levels to degrade organic compounds completely (Chirwa and Bamuza-Pemu 2010.) The selectivity of ozone is very dependent on the pH of the water. Ozone tends to be more selective in acidic waters due to direct oxidation by ozone. In alkaline waters, the degradation of ozone to hydroxyl radicals is faster. Hydroxyl radicals are less selective and have a higher oxidation potential, which is why ozonation is more effective in alkaline waters. (Yuan et al. 2013.) A major issue with ozonizing natural waters and seawater-operated RAS is the presence of bromide (Chirwa and Bamuza-Pemu 2010). Bromide and ozone react, forming bromate, a highly toxic compound labeled as a probable human carcinogen (Aljundi 2011).

Yuan et al. (2013) conducted a study where ozonation at 4.19 milligrams per liter was applied to laboratory waters spiked with 100, 200, and 500 nanograms per liter of GSM and MIB. The contact time used was 20 minutes. They found that ozonation at this rate managed to degrade over 93 % of both compounds from the 100 nanograms per liter solution. The effectiveness was significantly lower with higher concentrations of GSM and MIB: the ozonation degraded only 66.4 % of the MIB and 72 % of the GSM.

## 4.2 Photocatalysis

Photocatalysis is described as a process where a semiconductor is exposed to light, which accelerates the rate of a chemical reaction. Photocatalysis can be divided into two categories: homogenous and heterogeneous photocatalysis. Homogenous photocatalysis uses a semiconductor and a reactant in the same phase, while heterogeneous photocatalysis utilizes semiconductors and reactants that are in different phases. (Ameta and Ameta 2018.) For the semiconductor catalyst to be sufficient for photocatalysis, it should be inert, photostable, and easily produced and used. The most used semiconductors are zinc oxide, tungsten trioxide, and titanium dioxide, the latter of which appears to be most suitable for photocatalysis. None of these options meet all of the expectations looked for in a semiconductor catalyst. The oxidizing power of semiconductor photocatalysis is based on the generation of electron-hole pairs since the holes have a redox potential of 1.0-3.5 electron volts. (Chirwa and Bamuza-Pemu 2010.) An electron volt (eV) is a unit of energy described as the energy an electron gains when the electrical potential is increased by one volt (Britannica 2014). Photocatalysis as a method attacks the organic pollutants and degrades them into carbon dioxide, water, and mineralized acids. (Chirwa and Bamuza-Pemu 2010.)

### 4.2.1 Electron-hole pairs

When exposed to a sufficient amount of light, an electron-hole pair forms on the surface of a semiconductor. An electron-hole pair consists of a free electron and its matching hole, which is the position of an atom or atom lattice, where an electron could exist, but one does not. (Connor 2019.) The valence band is the outermost electron orbital of an atom, where electrons reside in their ground state. When the electrons absorb energy and are excited, they move from the valence band to the conduction band, the outermost orbital excited electrons can inhabit, leaving behind an electron hole. (Donev et al. 2018.) A bandgap signifies the energy required to excite the electrons enough to move from the valence band to the conduction band (Bowden and Honsberg 2022). The bandgap differences between a typical conductor, semiconductor, and insulator, are shown in Figure 5.

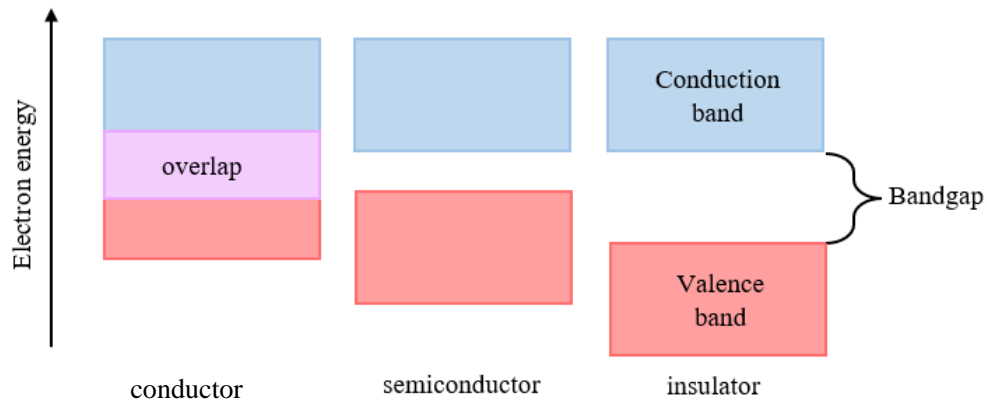


Figure 5 The differently located conduction and valence bands of conductors, semiconductors, and insulators. The further away the bands are from each other, the more energy is required to excite the valence electrons. (Connor 2019.)

Semiconductors work for catalytic purposes due to the suitable size of the bandgap between the conduction and valence bands. The overlap in the conduction and valence band in conductors, such as metals, allows the electrons to move freely between the two bands. The bandgap of insulators is large so the electrons would need a considerably higher amount of energy to be able to move to the conduction band. (Donev et al. 2018.) This thesis will focus on UV photocatalysis with the semiconductor titanium oxide as the catalyst.

#### 4.2.2 Titanium dioxide

Titanium dioxide has been found to be the most suitable catalyst option for photocatalysis. Depending on its crystalline form, it has a bandgap energy of 3.05-3.25 eV. (Chirwa and Bamuza-Pemu 2010.) It is also non-toxic and has great properties, such as high activity and stability to light illumination, and low price. Titanium oxide can be used as a powder or in an immobilized form, for example, as a titanium cylinder with a titanium oxide coating. The benefit of using immobilized titanium oxide is that there is no need for additional post-treatment to recover the particles remaining in the sample. (Thiruvengkatahari et al. 2007.) An acceptable daily intake of titanium dioxide has not been determined, which is why the catalyst has recently been banned from food use due to the possibility of it having genotoxic tendencies.

(Food ingredients 1<sup>st</sup> 2022.) Since the titanium dioxide semiconductor is a solid and the reactant, RAS-water, is a liquid, it can be said that this thesis focuses on a heterogeneous UV-photocatalysis reaction.

In a study conducted by Pestana et al. (2014), two kinds of samples were used: laboratory water spiked with 100 nanograms per liter of GSM and MIB and actual RAS waters. The samples were treated with UV photocatalysis with pelletized titanium dioxide as the catalyst. The reactor used was a continuous flow-packed bed reactor with a flow rate of 2 liters per minute. It was found that the amount of GSM and MIB was reduced by 97 % in the laboratory water and by almost 90 % in the RAS water.



## 5 Materials and methods

In the experimental part, two different AOPs were applied to two different GSM and MIB solutions concentrations. This section will present the materials, equipment, and analytic methods used in the laboratory experiments.

### 5.1 Chemicals

Dichloromethane SupraSolv<sup>®</sup> (DCM) from Sigma-Aldrich with an assay of  $\geq 99.8\%$  was used for the extractions. EMSURE<sup>®</sup> ACS reagent grade sodium sulfate granules were used to bind off any remaining water from the extracted samples.

#### 5.1.1 Samples

The chemical used for the samples was a mixture of geosmin and 2-methylisoborneol, with both having a concentration of 100 mg/l. The chemical and physical properties of GSM and MIB are presented in Table 2.

Table 2 Physical and chemical properties of geosmin (ACS 2020) and 2-methylisoborneol (Chemical Book 2017 and TGSC 2021).

Property [unit]	Geosmin	2-Methylisoborneol
Molar mass [g/mol]	182.3	168.3
Melting point [°C]	47	168
Boiling point [°C]	270.5*	212*
Water solubility [mg/l]	160*	305*

\* Predicted values

## 5.2 Equipment

The equipment used for the experiments were an ozonation unit and a UV-photocatalysis reactor with a titanium dioxide catalyst.

### 5.2.1 Ozonation unit

A simplified flow chart of the ozonation device is presented in Figure 6.

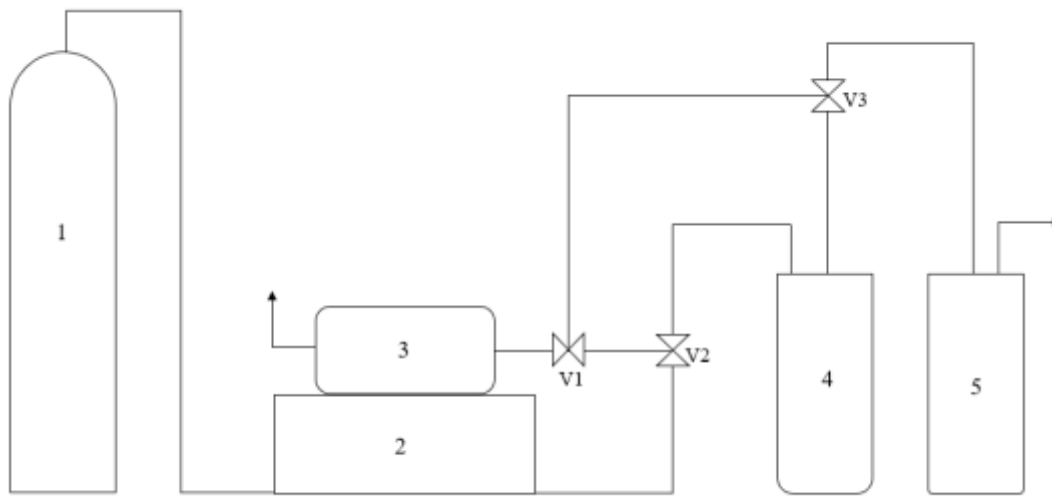


Figure 6 Schematic example of the ozonation equipment used for the experiments.

A gas line goes from the oxygen supply (1) to the Modular 4HC lab ozonation unit by Wedeco AG Water Technology (2) that converts the oxygen into ozone. The ozonated gas flow then travels through a valve (V2) into a reactor (4); meanwhile, some of the gas makes its way through valve V1 to the ozone analyzer BMT 964 (3). Gas flow from the reactor travels through valve V3 into a scrubber (5) that removes the remaining ozone from the flow.

The ozone concentration absorbed into the sample was determined by first viewing the reading of the ozone analyzer, ozone flow into the reactor, while the valve V2 is open. Then, valve V2 was closed, and valve V3 opened concurrently, which resulted in the reading of ozone flow out on the ozone analyzer. This whole process was timed, and the total amount of absorbed ozone was calculated using formula 1.

$$O_{3abs} = \frac{t \cdot \frac{\dot{V}}{3600} \cdot (O_{3in} - O_{3out})}{V}$$

where

$O_{3abs}$  = ozone concentration absorbed into the sample

$t$  = time, s

$\dot{V}$  = gas flowrate, l/h

$O_{3in}$  = ozone concentration going into the reactor, mg/l

$O_{3out}$  = ozone concentration coming out of the reactor, mg/l

$V$  = reactor volume, l

### 5.2.2 UV photocatalysis reactor

A picture of the UV reactor used for the photocatalysis experiments is presented in Figure 7.



Figure 7 The UV-photocatalysis unit used for the experiments.

The two 8 W lamps (1a and b) emit UV radiation at 365 nm. The fan (2) ensures air circulation and cooling inside the unit. The thermometer (3) and the aeration device (4) go into the reactor (5). Inside the reactor hangs the titanium dioxide-coated catalyst (6), and on the bottom is a magnetic mixer (7). The outside surface area of the titanium dioxide coating is 50,17 cm<sup>2</sup>, and it was determined with a digital caliper.

### 5.3 Laboratory experiments

Five liters of the two diluted solutions were made from the original solution: 1000 ng/l and 500 ng/l. Four different experiments were conducted for both solutions. Two 400 ml reactors were used for the ozonation experiments instead of one 800 ml reactor to achieve a larger area for the gas flow. The parameters of the ozonation experiments are presented in Table 3.

Table 3 Parameters for ozonation experiments

Experiment	Pressure [bar]	O <sub>2</sub> flow [l/h]	Gas flow to ozone analyser [l/h]	c <sub>0</sub> (GSM/MIB) [ng/l]	c <sub>CO<sub>2</sub></sub> [mg/l]	pH <sub>0</sub>	pH <sub>final</sub>
1	0.5	30	0.2	1000	20	5.60	5.64
2	0.5	30	0.2	1000	40	5.60	5.56
3	0.5	30	0.2	500	20	6.24	6.01
4	0.5	30	0.2	500	40	6.24	5.81

An ozone detector was used during all the ozonations to ensure a safe work environment.

For the UV-photocatalysis experiments, the volume of the used reactor was 800 ml. The reactive area of the used titanium dioxide catalyst was 50,17 cm<sup>2</sup>. The parameters of the UV-photocatalysis experiments are presented in Table 4.

Table 4 Parameters for the UV-photocatalysis experiments

Experiment	c <sub>0</sub> (GSM/MIB) [ng/l]	t [h]	pH <sub>0</sub>	pH <sub>final</sub>	T <sub>0</sub> [°C]	T <sub>final</sub> [°C]
5	1000	21	5.60	5.19	18.6	26.6
6	1000	8	5.60	5.74	14.7	28.0
7	500	20	6.24	5.76	15.6	28.1
8	500	8	6.24	5.90	16.6	27.4

Two parallel samples of 100 ml were taken from each experiment for the analytics.

#### 5.4 Analytics

The samples collected from the ozonation and UV-photocatalysis experiments were analyzed by gas chromatography-mass spectrometry (GC-MS). GC-MS requires the samples to be easily vapourised by heat, which water is not, so liquid-liquid extraction (LLE) was needed as a pre-treatment.

### 5.4.1 Liquid-liquid extraction

Liquid-liquid extraction (LLE), otherwise known as solvent extraction, can be used for two immiscible solvents, the other of which is aqueous and the other organic (Sandtorv 2021). The samples acquired from the experiments were pre-treated by LLE as illustrated in Figure 8.

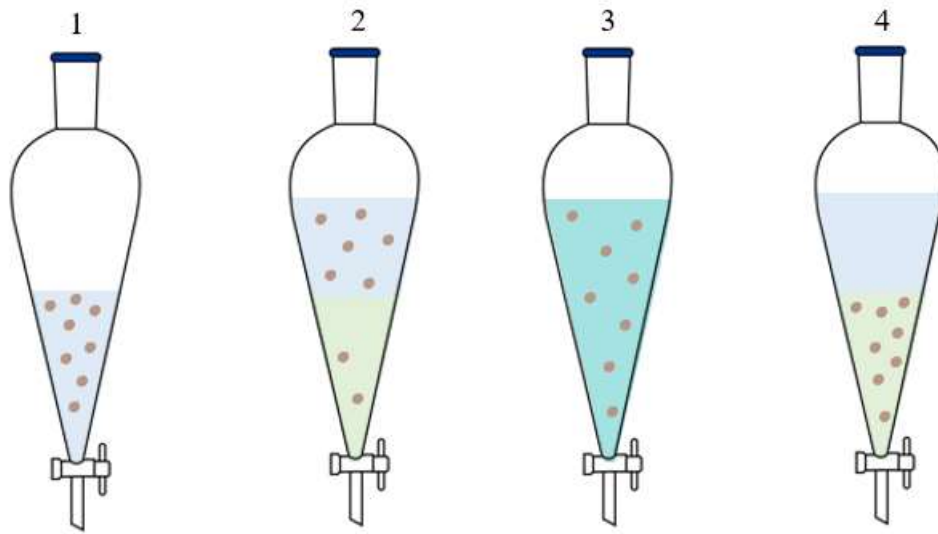


Figure 8 The process of liquid-liquid extraction.

Step 1 shows the sample that has been poured into the separatory funnel. In the second step, the organic solvent DCM is poured into the funnel. As it is denser than water, it sinks to the bottom of the funnel. An internal standard, 100  $\mu\text{l}$  of deuterated geosmin (geosmin- $\text{d}_3$ ) with a concentration of 100 ng/ml was measured in each sample. Its chemical structure is presented in Figure 9.

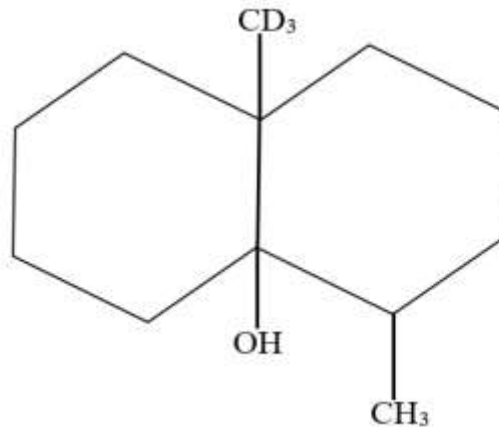


Figure 9 The chemical structure of deuterated geosmin. One of the methyl group's hydrogens have been replaced with the heavier isotope of hydrogen: deuterium (Britannica 2020).

After the addition of the internal standard, the extractions were done by shaking the funnel. Step 3 shows the two layers before separating. Step 4 shows the separated layers and the analytes that have been extracted from the aqueous solvent. The samples were collected from the funnels into Erlenmeyer flasks and sodium sulfate was added to them to bind any remaining water. The samples were then filtered through glass wool to remove the sodium sulfate crystals. Finally, the samples were concentrated by evaporation in a 36.5 °C water bath with a gentle nitrogen gas flow to a 0.5-1 ml volume and pipetted into vials. The samples were then analyzed by gas chromatography-mass spectrometry.

#### 5.4.2 Gas chromatography-mass spectrometry

A gas chromatograph (GC) is used to analyze samples that contain compounds easily vaporized by heat. The samples can be gas, liquid, and even solids, and the solvent used must also be able to vaporize with heat. The sample is injected into the analysis machine and transported to the column by a carrier gas, an inert gas, acting as the mobile phase. The column is surfaced with a stationary phase, a liquid or a solid that does not vaporize easily. The sample components interact with the stationary phase at different rates, which causes them to separate from each other. The different components travel through the column and reach the detector at different times, causing peaks in the chromatogram. The sizes of the peaks correspond with the concentration of the compound. These chromatograms are then compared to known standard peaks to determine what compounds were in the injected sample. (Shimadzu 2020.) Mass

spectrometry is an analytic method, often paired with a GC, that can be used to determine the molecular weight of the components of a sample. The mass spectrometer (MS) ionizes the sample with an electron beam. These charged particles are accelerated in the electric field and then directed into a magnetic field. The different particles hit the detector at different places, and their mass-to-charge ratio ( $m/z$ ) is determined. (Beynon and Brown 2020.) Extracts from LLE were analyzed by an Agilent 6890N gas chromatograph coupled with an Agilent 5975 quadrupole mass spectrometer. DB-5MS column was used ( $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ ). A simplified flow chart of the used GC-MS is shown in Figure 10.

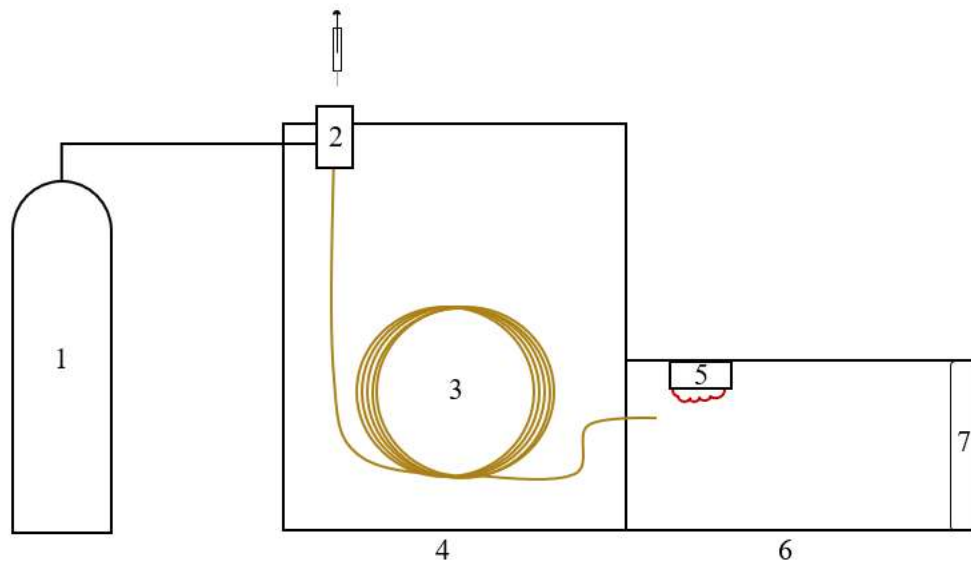


Figure 10 A simplified schematic of a GC-MS. Adapted from Wu et al. (2012).

The carrier gas was high purity helium at a constant flow of 1.3 mL/min (1). Samples (1 $\mu$ L) were injected at 250 °C splitless (2). The sample and carrier gas travel through the column (3) and separate. The oven (4) temperature was kept at 40 °C for 2 min, then ramped to 110 °C by 20 °C/min and held for 10 minutes. After which the temperature was ramped to 250 by 10 °C/min and finally to 300 °C by 40 °C/min and kept for 10 minutes. The transfer line temperature was 280 °C, the ion source temperature was 230 °C and the quadrupole temperatures were 150 °C. The electron ionization source (5) was operated at 70 eV. After traveling through the mass spectrometer (6), the analytes were identified by selected ion monitoring (SIM) (7), 95, 112, and 115  $m/z$  for MIB, GSM, and geosmin-d3 (internal standard), respectively.



## 6 Results and discussion

The concentrations of GSM and MIB in the samples were found by integrating their respective peaks in the chromatogram and comparing them to the peak areas of geosmin-d3. An example of the chromatograms and integrated peaks is presented in Figure 11.

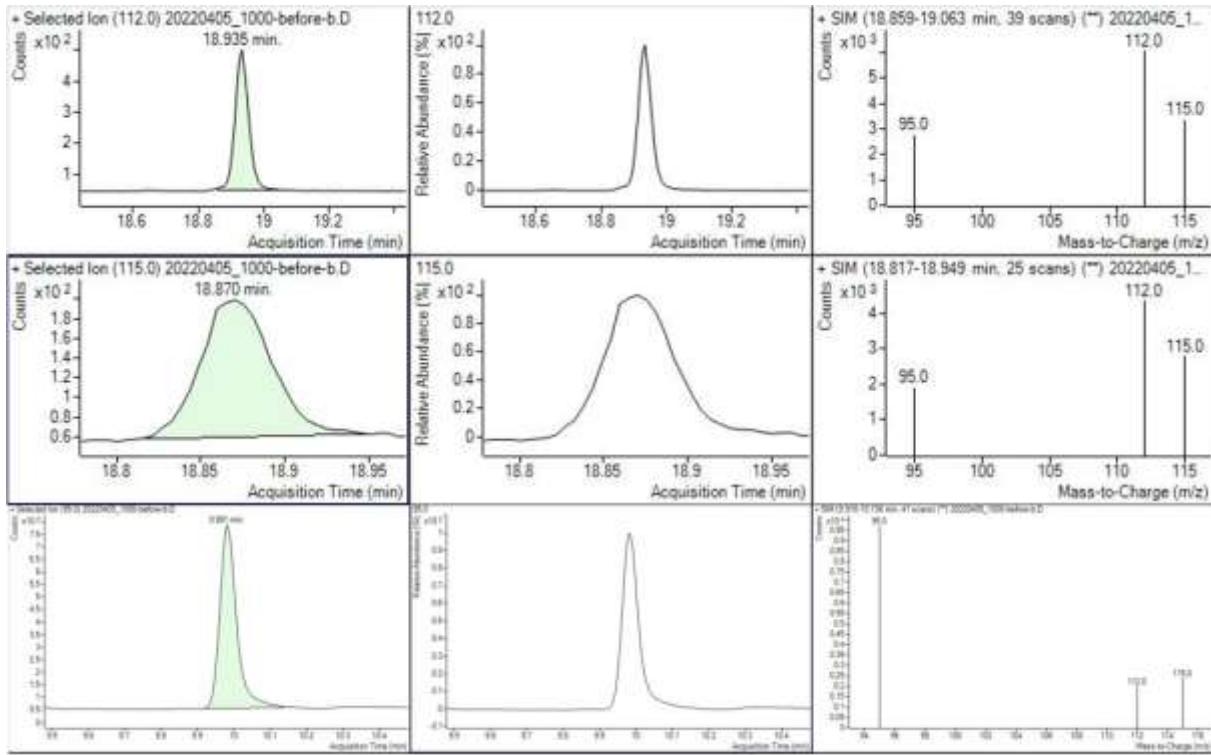


Figure 11 The GSM, geosmin-d3, and MIB peaks in the sample with 1000 ng/l concentration without AOPs applied.

The concentrations of GSM and MIB in the samples were calculated by dividing their peak areas by the peak area of geosmin-d3. This was done under the assumption that the peak of geosmin-d3 would be proportional to GSM. This was not the case, which is why correction factors were needed. The correction factors were calculated with the help of the known concentrations of 1000 and 500 ng/l. The correction factor for GSM was 3.076 and 1.395 for MIB. The concentrations of GSM and MIB in each sample are presented in Table 5.

Table 5 The concentrations of GSM and MIB in the samples. The concentrations are the means of the two parallel samples coupled with their standard deviation.

Sample name	GSM (ng/l)	MIB (ng/l)
Blank	30.69 ± 4.50	3.29 ± 1.84
500 ng/l	496.07 ± 12.06	484.61 ± 10.75
500 ng/l, 8 h UV-light	110.54 ± 3.52	77.98 ± 5.15
500 ng/l, 20 h UV-light	31.29 ± 4.62	2.44 ± 0.50
500 ng/l, 20 mg/l ozone	287.21 ± 11.27	268.05 ± 10.73
500 ng/l, 40 mg/l ozone	42.78 ± 11.70	19.33 ± 4.02
1000 ng/l	1028 ± 28.53	890.77 ± 103.57
1000 ng/l, 8 h UV-light	127.96 ± 2.38	134.59 ± 10.08
1000 ng/l, 20 h UV-light	34.90 ± 6.25	4.27 ± 0.14
1000 ng/l, 20 mg/l ozone	417.78 ± 0.46	458.04 ± 37.57
1000 ng/l, 40 mg/l ozone	38.47 ± 0.98	12.29 ± 0.18

As these concentrations show, the applied AOPs were effective in removing GSM and MIB from the deionized (DI) laboratory water. However, as mentioned earlier, the taste and odor thresholds for GSM and MIB are extremely low. These concentrations were not reached even though the removal by percentage seemed effective. The blank sample appeared to contain over 30 ng/l of GSM, which indicates some error since there should not be any GSM in DI-water. The error might have occurred due to cross-contamination between the other samples, which is why working with clean glassware is of extreme importance when working with such small concentrations. Due to this error, it cannot be explicitly stated whether there were any differences in the removal efficiencies between GSM and MIB.

The effectiveness of the different ozone doses and UV-photocatalysis times were inspected by normalizing the concentrations of GSM and MIB. It was also studied whether the initial concentration of the solution had any impact on the effectiveness of the AOPs. Finally, the two AOPs were compared on how much of the compounds were removed by them. The normalized concentrations in the ozonation experiments are presented in Figure 12.

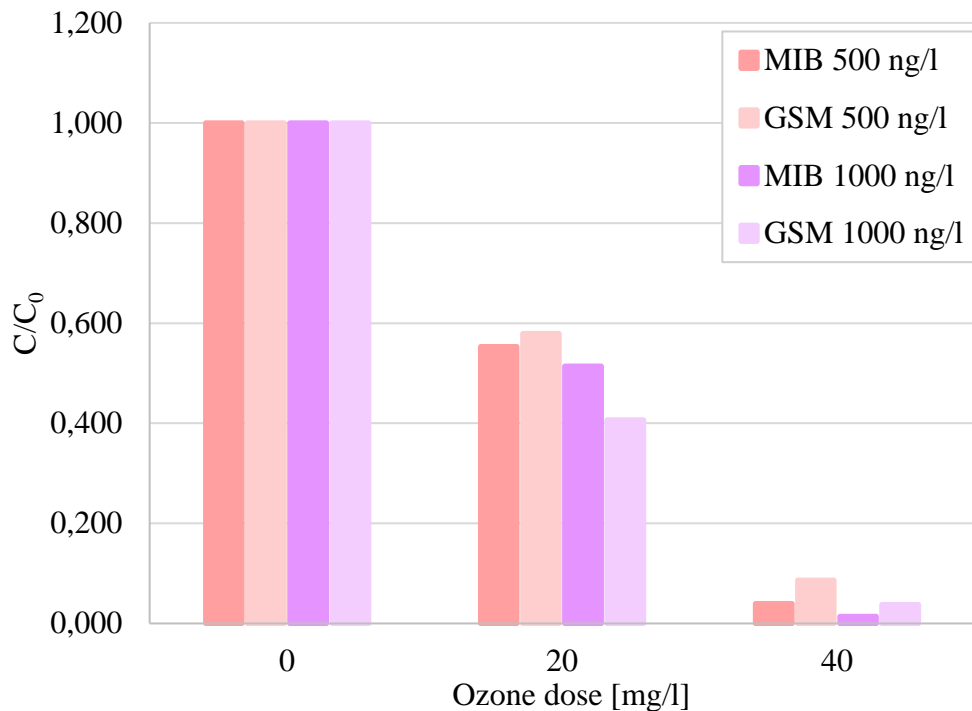


Figure 12 The effect of the used ozone dose on GSM and MIB normalized concentrations.

The graph shows that the 20 mg/l ozone dose did not remove much of the compounds since over 40 % of them remained in all the samples. Better results were achieved with a 40 mg/l ozone dose as under 10 % of the compounds remained in the samples. Even though the higher ozone concentration was significantly more effective, it also took more time to achieve the desired concentration. The removal rate of GSM and MIB appears to decrease as the initial concentration decreases, which indicates that the removal rate in actual RAS waters would be even lower since the initial concentrations of GSM and MIB are significantly lower. Yuan et al. (2013) conducted a study, where GSM and MIB were attempted to be removed from synthetic water. They managed to reach a removal rate of over 93 % with a solution that had been concentrated with 4.19 mg/L of ozone. Since the experimental setting was different than for this thesis, the results cannot be explicitly compared to each other. However, the effect of pH should be considered when comparing these results. The pH used in the experiments of Yuan et al. (2013) was 7.3. As mentioned earlier, a more alkaline pH results in better oxidative properties of the ozone. Yuan et al. also did a trial with a pH of 5.4, which is closer to the pH levels of the samples analyzed for this thesis. The lower pH resulted in only a 47.1 % removal rate of MIB and 88.5 % for GSM.

UV-photocatalysis on the other hand ended up being fairly effective with the shorter experiment. The normalized concentrations of GSM and MIB in the UV-photocatalysis experiments are shown in Figure 13.

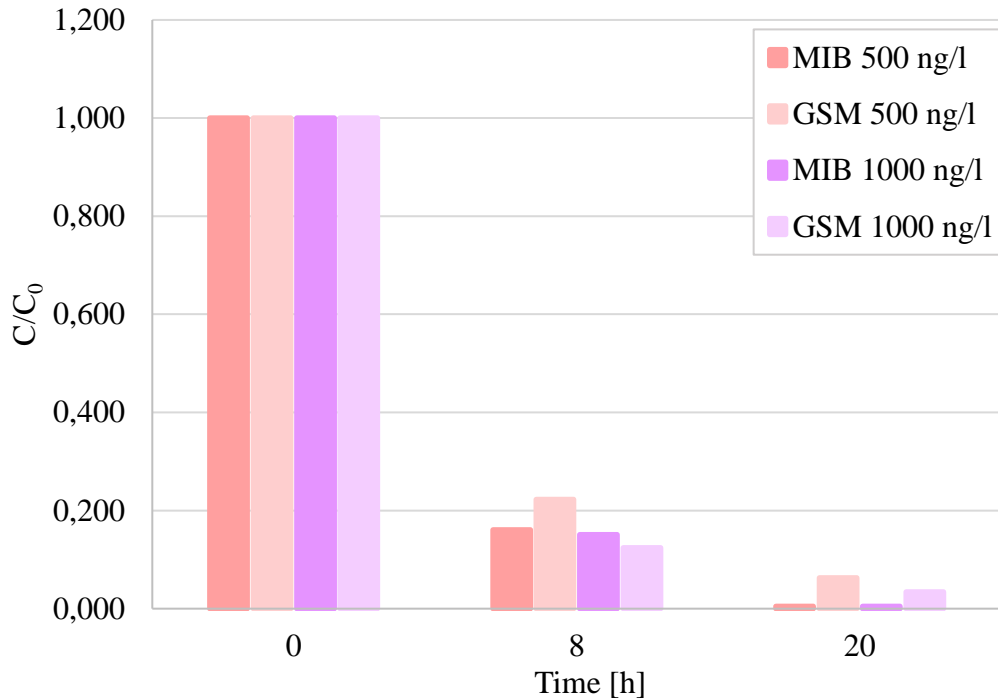


Figure 13 The effect of the UV light exposure time on the normalized concentrations of the compounds.

It can be seen from the graph that under 25 % of the compounds remained in the samples of the 8-hour experiment. For MIB, almost a 100 % removal was achieved with the 20-hour experiment and for GSM a bit less, but as stated earlier, there is no certainty whether this is the case or if there was some error. Yuan et al. (2013) and Antonopoulou and Konstantinou (2017) both suggest that GSM should be easier to remove than MIB, which further confirms the suspicion of some error in the GSM results. The UV-photocatalysis also seemed to work better for the higher initial concentration. Pestana et al. (2013) found similar results in their experiment, which was executed with a fixed bed reactor with pelletized titanium dioxide. The experiment was done on a pilot-scale reactor and a 96 % reduction rate was achieved with a 4-liter working fluid volume and a 2-minute residency time in the reactor. They also found that the smaller initial concentration of GSM and MIB resulted in a smaller reduction rate. This experiment shows

that it could be possible to have good outcomes in larger-scale experiments with an immobilized titanium dioxide catalyst as well.

Ozonation and UV-photocatalysis were also compared with each other. The graphs in Figures 12 and 13 showed that the larger ozone concentration and the longer UV-light exposure were the more effective ones, which is why they were chosen for this comparison. It was also found that the removal was more effective for the solution with an initial concentration of 1000 ng/l. The removal percentages of GSM and MIB from the 1000 ng/l solution by the aforementioned methods are presented in Table 6.

Table 6 The comparison of ozonation and photocatalysis on their abilities to remove MIB and GSM from DI-water.

Experiment	GSM removed	MIB removed
Ozonation, 40 mg/l	96.3 %	98.6 %
Photocatalysis, 20 h	96.6 %	99.5 %

The table shows that photocatalysis was more effective in removing both GSM and MIB. Photocatalysis also requires less attending to and might be safer since no pressured gas tanks of oxygen are needed for it.

## 7 Conclusions

This thesis aimed to explore whether the AOPs ozonation and titanium dioxide catalyzed UV-photocatalysis were effective in removing GSM and MIB from DI-water and whether it would be beneficial to apply these methods to RAS waters. The compounds were attempted to be removed with ozonation by adding 20 mg/l and 40 mg/l of ozone into 500 ng/l and 1000 ng/l solutions. It was found that the lower ozone concentration did not achieve very high removal rates, they remained under 60 % for all experiments. The 40 mg/l ozone concentration reached over 90 % reduction. For the photocatalysis experiments, the samples were exposed to 8 hours and 20 hours of UV light in the presence of a titanium dioxide catalyst with an area of 50.17 cm<sup>2</sup>. The collected samples were analyzed with GC-MS and the results showed especially photocatalysis to have great potential to be used on a larger scale. The removal percentage for the 8-hour experiments was over 75 % and for the 20-hour experiments over 93 % removal was achieved.

These experiments, though very promising, caused some questions and more research ideas. Since the experiments were done using DI-water, experimenting with actual RAS waters is needed to get more realistic results. The water matrix of RAS water differs from DI-water, meaning there are other compounds such as nitrogen ions and NOMs. NOMs are known to compete in the photocatalytic decomposition process thus taking away from the oxidizing capacity that should be directed towards GSM and MIB (Pestana et al. 2013). The pH of RAS waters is usually also more alkaline than the pH of the samples used for this thesis. This could have enhancing effects, especially on the reduction rate of ozonation. Additionally, the GSM and MIB levels are significantly lower than 500 ng/l in RAS waters. Pestana et al. (2013) found that the GSM level in the RAS water they used was 18.5 ng/l and the MIB level was 14.0 ng/l. As stated earlier, the lower initial concentration leads to lowered reduction rates.

As it was not expected for the 8-hour UV-photocatalysis experiment to be as efficient as it was, it needs to be further studied. Experiments should be conducted by exposing a sample to UV light for 8 hours without using a catalyst to find out whether photolytic decomposition of the analytes happens. The role of the adsorption process at the surface of the catalyst could be determined by placing the catalyst into another sample for 8 hours without any UV light exposure. By conducting such experiments, it could be studied whether the UV light of the catalyst is contributing more to the effectiveness. It could also be

studied how the area of the used catalyst affects the removal rate since Pestana et al. achieved removal rates of 96 % with a 2-minute contact time with the use of 22 kilograms of titanium dioxide pellets for 4 liters of sample volume. It could also be explored whether these two methods could be combined and if this would result in better removal rates with less ozone and shorter UV light exposure time since 8 hours is still an unrealistically long time to work for an actual RAS.

## References

- Ahmed N, Turchini G.M. (2021) Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. *Journal of Cleaner Production* 297.
- Aljundi I.H. (2011) Bromate formation during ozonation of drinking water: A response surface methodology study. *Desalination* 277(1): 24-28.
- American Chemical Society (2020) *Molecule of the Week Archive: Geosmin*. Retrieved [20.3. 2022] Available at: <https://www.acs.org/content/acs/en/molecule-of-the-week/archive/g/geosmin.html>.
- Ameta S.C. and Ameta R, (2018) *Advanced Oxidation Processes for Waste Water Treatment*. London: Academic Press.
- AndyParadise (2018) *English: A Flow Chart for the various Water Treatment Processes Needed in a Recirculating Aquaculture System*. Retrieved [6.3.2022] Available at: [https://commons.wikimedia.org/wiki/File:Recirculating\\_aquaculture\\_system\\_flow\\_chart.png](https://commons.wikimedia.org/wiki/File:Recirculating_aquaculture_system_flow_chart.png).
- Beynon J.H. and Brown L, (2020) *Mass Spectrometry | Definition, Applications, Principle, & Facts | Britannica*. Retrieved [19.3.2022] Available at: <https://www.britannica.com/science/mass-spectrometry>.
- Blue Ridge Aquaculture (2022) *What is Recirculating Aquaculture?* Retrieved [17.1.2022] Available at: <http://www.blueridgeaquaculture.com/recirculatingaquaculture.cfm>.
- Bregnballe J (2015) *A Guide to Recirculation Aquaculture*. the Food and Agriculture Organization of the United Nations. Copenhagen, Denmark: the Food and Agriculture Organization of the United Nations (FAO) and EUROFISH International Organisation.
- Britannica (2020) *Deuterium | Definition, Symbol, Production, & Facts | Britannica*. Retrieved [27.3.2022] Available at: <https://www.britannica.com/science/deuterium>.
- Britannica (2014) *Electron Volt | Unit of Measurement | Britannica*. Retrieved [27.3.2022] Available at: <https://www.britannica.com/science/electron-volt>.
- Buchanan W, Roddick F, Porter N, Drikas M (2004) Enhanced biodegradability of UV and VUV pre-treated natural organic matter. *Water Science and Technology: Water Supply* 4.
- Chemical Book (2017) *2-Methylisoborneol*. Retrieved [20.3.2022] Available at: [https://www.chemicalbook.com/ChemicalProductProperty\\_EN\\_CB7766668.htm](https://www.chemicalbook.com/ChemicalProductProperty_EN_CB7766668.htm).
- Chirwa E and Bamuzi-Pemu E, (2010) *Investigation of Photocatalysis as an Alternative to Other Advanced Oxidation Processes for the Treatment of Filter Backwash Water*.



- Connor N (2019) *What is Electron-Hole Pair in Semiconductors - Definition*. Retrieved [11.2. 2022] Available at: <https://www.radiation-dosimetry.org/what-is-electron-hole-pair-in-semiconductors-definition/>.
- Dahle S. W, Bakke I, Birkeland M, Nordøy K, Dalum AS, Attramadal K. J. K (2020) Production of lumpfish (*Cyclopterus lumpus* L.) in RAS with distinct water treatments: Effects on fish survival, growth, gill health and microbial communities in rearing water and biofilm. *Aquaculture* 522: 735097.
- Das P, Das A, Roy S (2016) Shrimp fry (meen) farmers of Sundarban Mangrove Forest (India): a tale of ecological damage and economic hardship. *International Journal of Agricultural and Food Research* 5(2): 32.
- Davidson J, Grimm C, Summerfelt S, Fischer G, Good C (2020) Depuration system flushing rate affects geosmin removal from market-size Atlantic salmon *Salmo salar*. *Aquacultural Engineering* 90: 102104.
- Deng Y, Zhao R (2015) Advanced Oxidation Processes (AOPs) in Wastewater Treatment. *Current Pollution Reports* 1(3): 167-176.
- Donev J, Hanania J, Stenhouse K, (2018) *Valence Band*. Retrieved [11.2.2022] Available at: [https://energyeducation.ca/encyclopedia/Valence\\_band](https://energyeducation.ca/encyclopedia/Valence_band).
- Espinal C. A and Matulić D, (2019) *Recirculating aquaculture technologies*. In: Anonymous Aquaponics Food Production Systems. : Springer, Cham, 35-76.
- Food and Agriculture Organization of the United Nations, FAO (2020) *The State of World Fisheries and Aquaculture 2020. Sustainability in Action*. FAO. Rome, Italy: FAO.
- Food ingredients 1st (2022) *Titanium Dioxide Ban Comes into Force, Companies have Six Months to Adjust*. Retrieved [10.3.2022] Available at: <https://www.foodingredientsfirst.com/news/titanium-dioxide-ban-comes-into-force-companies-have-six-months-to-adjust.html>.
- Garrido-Cardenas J. A, Esteban-García B, Agüera A, Sánchez-Pérez J. A, Manzano-Agugliaro F (2019) Wastewater Treatment by Advanced Oxidation Process and Their Worldwide Research Trends. *International Journal of Environmental Research and Public Health* 17(170).
- Gephart, Jessica A. et al. (2021) Environmental performance of blue foods. *Nature* 597: 360-364.
- Helmer J (2020) *Efforts to Address Off-Flavors in RAS Fish are Off the Charts - Responsible Seafood Advocate*. Retrieved [17.1.2022] Available at: <https://www.globalseafood.org/advocate/efforts-to-address-off-flavors-in-ras-fish-are-off-the-charts/>.
- Honsberg C and Bowden S, (2022) *Bandgap*. Retrieved [11.2.2022] Available at: <https://www.pveducation.org/pvc/drom/pn-junctions/band-gap>.
- Instruments SS (2020) *GC Fundamentals - what is Gas Chromatography*. Retrieved [16.3.2022] Available at: <https://www.ssi.shimadzu.com/>.

- Konstantinou I, Antonopoulou M (2017) AOPs Methods for the Removal of Taste and Odor Compounds. : 180-206.
- Kutschera K, Börnick H, Worch E (2009) Photoinitiated oxidation of geosmin and 2-methylisoborneol by irradiation with 254 nm and 185 nm UV light. *Water Research (Oxford); Water Res* 43(8): 2224-2232.
- Lawton L.A, Robertson P.K.J, Robertson R.F, Bruce F.G. (2003) The destruction of 2-methylisoborneol and geosmin using titanium dioxide photocatalysis. *Applied Catalysis B: Environmental* 44: 9-13.
- Lazzari R, Baldisserotto B (2008) Nitrogen and phosphorus waste in fish farming. *Boletim do Instituto De Pesca* 34: 591-600.
- Lewis J.W, Morley N.J, Drinkall J, Jamieson B.J, Wright R, Parry J.D. (2009) Toxic effects of *Streptomyces griseus* spores and exudate on gill pathology of freshwater fish. *Ecotoxicology and Environmental Safety* 72(1): 173-181.
- Lindholm-Lehto P, Vielma J (2019) Controlling of geosmin and 2-methylisoborneol induced off-flavors in recirculating aquaculture system farmed fish—A review. *Aquaculture Research* 50(1): 9-28.
- Lindholm-Lehto P, Koskela J, Kaseva J, Vielma J (2020) Accumulation of Geosmin and 2-methylisoborneol in European Whitefish *Coregonus Lavaretus* and Rainbow Trout *Oncorhynchus Mykiss* in RAS. *Fishes*.
- Máchová J, Faina R, Randak T, Valentova O, Steinbach C, Kocour Kroupova H, Svobodova Z (2017) Fish death caused by gas bubble disease: A case report. *Veterinární Medicína* 62: 231-237.
- Malone R (2013) *Recirculating Aquaculture Tank Production Systems: A Review of Current Design Practice*. Southern Regional Aquaculture Center. Uniter States of America: Southern Regional Aquaculture Center.
- Marsh J (2020) *Pros & Cons of Commercial Fishing*.
- National Fisheries Development Board (2020) Recent Trends in Aquaculture: Recirculatory Aquaculture System (RAS).
- New World Encyclopedia (2022) *Aquaculture*. Retrieved [13.2.2022] Available at: <https://www.newworldencyclopedia.org/entry/Aquaculture>.
- Ortenberg E and Telsch B, (2003) 42 - *Taste and odour problems in potable water*. In: Mara D and Horan N (eds) *Handbook of Water and Wastewater Microbiology*. London: Academic Press, 777-793.
- Pandey K.D. Patel A.K. Singh M Vandana Kumari A, Jalaluddin, (2021) *Chapter 2 - Secondary metabolites from bacteria and viruses*. In: Sinha Rp and Häder D (eds) *Natural Bioactive Compounds*. : Academic Press, 19-40.

Pestana C.J, Robertson P.K.J, Edwards C, Wilhelm W, McKenzie C, Lawton L.A (2014) A Continuous flow packed bed photocatalytic reactor for the destruction of 2-methylisoborneol and geosmin utilizing pelletised TiO<sub>2</sub>. *Chemical Engineering Journal* 235.

Priyantha Indrajith H, Davey K (2016) Experimental validation of a time-dependent model for chemical taste taint accumulation as geosmin (GSM) and 2-methylisoborneol (MIB) in commercial RAS farmed barramundi (*Lates calcarifer*). *Ecological Modelling* 340: 17-27.

Sandtorv A (2021) 2.3: *Liquid-Liquid Extraction*. Retrieved [21.3.2022] Available at: [https://chem.libretexts.org/Bookshelves/Organic\\_Chemistry/Book%3A\\_How\\_to\\_be\\_a\\_Successful\\_Organic\\_Chemist\\_\(Sandtorv\)/02%3A\\_COMMON\\_ORGANIC\\_CHEMISTRY\\_LABORATORY\\_TECHNIQUES/2.03%3A\\_LIQUID-LIQUID\\_EXTRACTION](https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Book%3A_How_to_be_a_Successful_Organic_Chemist_(Sandtorv)/02%3A_COMMON_ORGANIC_CHEMISTRY_LABORATORY_TECHNIQUES/2.03%3A_LIQUID-LIQUID_EXTRACTION).

Schrader K.K, Davidson J.W, Rimando A.M, Summerfelt S.T (2010) Evaluation of ozonation on levels of the off-flavor compounds geosmin and 2-methylisoborneol in water and rainbow trout *Oncorhynchus mykiss* from recirculating aquaculture systems. *Aquacultural Engineering* 43(2): 46-50.

SeaChoice (2022) *Aquaculture Methods*. Retrieved [13.2.2022] Available at: <https://www.seachoice.org/info-centre/aquaculture/aquaculture-methods/>.

Sievers M, Korsøen Ø, Warren-Myers F, Oppedal F, Macaulay G, Folkedal O, et al., (2021) *A Review of Marine Fish Aquaculture in Submerged Cages*. Retrieved [13.2.2022] Available at: <https://www.globalseafood.org/advocate/a-review-of-marine-fish-aquaculture-in-submerged-cages/>.

Sivonen K (2009) *Cyanobacterial Toxins*. In: Schaechter M (ed) *Encyclopedia of Microbiology* (Third Edition). Oxford: Academic Press, 290-307.

Soltan, M. (2016) *Aquaculture Systems*.

Srinivasan R, Sorial G. A (2011) Treatment of taste and odor causing compounds 2-methyl isoborneol and geosmin in drinking water : A critical review. *Journal of Environmental Sciences (China); Journal of Environmental Sciences* 23(1): 1-13.

TGSC (2021) *2-Methyl Isoborneol*, 2371-42-8. Retrieved [20.3.2022] Available at: <http://www.thegoodscentscompany.com/data/rw1384871.html>.

Thiruvengkatachari R, Vigneswaran S, Moon IS (2008) A review on UV/TiO<sub>2</sub> photocatalytic oxidation process. *Korean Journal of Chemical Engineering* 25(1).

University of Bergen (2010) *What are Microorganisms?* Retrieved [8.3.2022] Available at: <https://www.uib.no/en/geobio/56846/what-are-microorganisms>.

Whangchai N, Pimpimon T, Sompong U, Suwanpakdee S, Gutierrez R, Itayama T (2017) Study of Geosmin and 2-Methylisoborneol (MIB) Producers in Phayao Lake, Thailand. *International Journal of Bioscience, Biochemistry and Bioinformatics* 7.

Wu S, Lyu G, Lou R, (2012) *Applications of Chromatography Hyphenated Techniques in the Field of Lignin Pyrolysis*. In: Anonymous.

Yuan B, Xu D, Li F, Fu M (2013) Removal efficiency and possible pathway of odor compounds (2-methylisoborneol and geosmin) by ozonation. *Separation and Purification Technology* 117: 53-58.

Zaitlin B, Watson SB, Ridal J, Satchwill T, Parkinson D (2003) Actinomycetes in Lake Ontario: Habitats and geosmin and MIB production. *American Water Works Association Journal* 95(2): 113.

Zimba P.V, Schrader K.K, Hyldig G, Strobel B.W, Jorgensen NOG (2011) Evaluation of geosmin and 2-methylisoborneol off-flavour in smoked rainbow trout fillets using instrumental and sensory analyses. *Aquaculture Research* 43: 149-153.