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Reduction of Acrylamide in Reject Water from Sludge Dewatering

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Reduction of Acrylamide in Reject Water from Sludge Dewatering

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Degree Project in Environmental Engineering and Sustainable Infrastructure

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Sammanfattning

Norrvatten producerar och levererar dricksvatten till cirka 700 000 människor i norra delen av Region Stockholm i Sverige. I deras vattenverk Görvälnverket renas vatten från Mälaren. När sjövattnet renas produceras slam. För att slammet ska kunna användas som landfyllnadsmaterial måste det avvattnas. Polyakrylamid används som flockningsmedel i det syftet. I rejektvattnet som lämnar slammet och släpps ut i Mälaren finns ofta akrylamid monomerer kvar som en restprodukt från framställning av polyakrylamid. Problemet med detta är att akrylamid är toxiskt för levande organismer.

Syftet med detta masterexamensarbete var att evaluera föreslagna vattenreningstekniker för att reducera akrylamid i rejektvattnet som lämnar Görvälnverket och inte överstiga en akrylamidkoncentration på 0,10 µg/l som är Norrvattens mål. Vattenreningsteknikerna som evaluerades var ozonering, biofilmreaktor med rörlig bädd och biobädd. Utöver det var oxidativ stress samt genotoxicitet från ozoneringen analyserad. Dessutom var nedbrytningsförmågan av akrylamid i Mälaren testad. Ozonerings försöken utfördes i en pilotanläggning i ett laboratorium hos IVL Svenska Miljöinstitutet, testerna med biofilmreaktor med rörlig bädd och biofilter utfördes i pilotanläggningar på Görvälnverket och nedbrytningstesterna i Mälarvatten utfördes också på Görvälnverket.

Resultaten visade på att ozonering av rejektvattnet kan reducera akrylamid och uppfylla Norrvattens mål om en akrylamidkoncentration på mindre än 0,10 µg/l. När en ozondos på 0,70 mg/l applicerades i rejektvattnet med en akrylamidkoncentration på 2,5 µg/l reducerades mer än 98 % av akrylamiden. Vidare tycktes inte ozoneringen bidra till oxidativ stress eller genotoxicitet. Biofilmreaktorn med rörlig bädd och biofiltret reducerade akrylamid med 80,77 % respektive 94,7 %. Resultaten visade att dessa tekniker skulle kunna användas för att nå Norrvattens mål. Utöver detta visade studien att vatten från Mälaren kan bryta ner akrylamid i 15 °C på 4 dagar och i 8 °C på 13 dagar och nå målet.

Nyckelord

Vattenreningstekniker, Ozonering, Biofilmreaktor med rörlig bädd, Biobädd, Biologisk nedbrytning, Mälaren

Abstract

Norrvatten produces and delivers drinking water to approximately 700 000 people in the northern part of the Stockholm region in Sweden. In their water treatment plant Görvälnverket, water from Lake Mälaren is purified. During the purification, sludge is produced. To be able to use the sludge as landfilling material, it must be dewatered. Polyacrylamide is used as a flocculant for this purpose. However, in the reject water leaving the sludge and discharged into Lake Mälaren, acrylamide monomers are often left as a rest product from the manufacturing of polyacrylamide. The problem is that acrylamide is toxic to living organisms.

The aim of this master thesis was to evaluate proposed water treatment techniques to reduce acrylamide in the reject water leaving Görvälnverket and reach Norrvatten´s goal of an acrylamide concentration below 0.10 µg/l. The water treatment techniques evaluated were ozonation, moving bed biofilm reactor and trickling filter. Along with that, oxidative stress and genotoxicity from the ozone were analysed. Also, the ability of Lake Mälaren to degrade acrylamide was evaluated. The ozone tests were performed in a pilot plant in a laboratory at IVL Swedish Environmental Research Institute (IVL Svenska Miljöinstitutet), the moving bed biofilm reactor- and the trickling filter test were performed in pilot plants at Görvälnverket and the degradation tests in water from Lake Mälaren were performed at Görvälnverket as well.

The results showed that ozonation of the reject water could reduce acrylamide in the reject water and fulfil Norrvatten´s goal of below 0.10 µg/l acrylamide. When an ozone dose of 0.70 mg/l was applied to the reject water with an acrylamide concentration of 2.5 µg/l, more than 98 % of the acrylamide was reduced. Furthermore, no oxidative stress or genotoxicity seemed to be generated from the ozonation. The moving bed biofilm reactor and the trickling filter did reduce the acrylamide by 80.77 % and 94.7 % respectively and the results suggested that they could be used to reach Norrvatten´s goal. Finally, the results indicated that water from Lake Mälaren could degrade acrylamide at a temperature of 15 °C in 4 days and in 8 °C in 13 days and reach the goal.

Keywords

Water treatment techniques, Ozonation, Moving bed biofilm reactor, Trickling filter, Biological degradation, Lake Mälaren

Acknowledgements

My master thesis has been written for Norrvatten as a contribution to their future drinking water plant and their project about Norrvatten´s future drinking water production. With this master thesis I will end my master program Environmental Engineering and Sustainable Infrastructure at The Royal Institute of Technology in Stockholm.

Firstly, I want to thank my supervisor at Norrvatten, Ewelina Basiak-Klingspetz for the engagement in both the practical work at Görvälnverket and in my report. I would also like to thank Daniel Hellström at Norrvatten for the opportunity to write my master thesis for Norrvatten. Further, I am grateful for the support from all people at Norrvatten, especially Philip Warman for helping me with some of my practical work.

The consultant company Ramboll has also been a part of the project about Norrvatten´s future drinking water production and is the leading consultant company dealing with the design of the future drinking water plant and future processes. I want to thank Susanne Forsberg at Ramboll for your support at the beginning of my master thesis project.

Finally, I would like to thank my supervisor at The Royal Institute of Technology, Zahra Kalantari for your support and your good advice on my report.

Table of Contents

Abbreviations

MBBR - Moving Bed Biofilm Reactor

Leca - Light Expanded Clay Aggregate

EBCT - Empty Bed Contact Time

ROS - Radical Oxygen Species

tBHQ - Tertiary Butyl Hydroquinone

1. Introduction

When purifying water to get drinking water in a water treatment plant, sludge is produced. In order to use the sludge from the drinking water production, for example as landfilling, it must be dewatered and thickened (Jutterström, 2015). Polymers are common to use in the water treatment processes for this specific purpose and have been for over 50 years (Eveborn et al, 2008; Nyyssölä & Ahlgren, 2019).

A polymer is a chain-molecule which is either a cation or an anion. At water treatment plants, the polymers function as a flocculant because it drags particles to it and creates flocs and separates the solid sludge from the water which makes the sludge thicker (Norrvatten, 2010). Polymers which are strictly ionic are called polyelectrolytes. Polyacrylamide is such a polyelectrolyte. Polyacrylamide is synthetized from acrylamide monomers when manufactured (Guezennec et al, 2015). The chain in polyacrylamide consists of such monomers (Eveborn et al, 2008).

A side effect of using polyacrylamide as a flocculant for dewatering sludge streams is that there might be acrylamide monomers left in the reject water leaving the sludge facility and being discharged into a nearby recipient. This is an ongoing problem at Norrvatten´s water treatment plant Görvälnverket in Stockholm, which discharge reject water containing acrylamide into Lake Mälaren (Hellström, 2022). Lake Mälaren is the drinking water source for Norrvatten´s water treatment plant (Heldt, 2021). The reason for it to be a problem is that acrylamide monomers have a toxic effect on aquatic life (Spraggs et al, 1982; Krautter et al, 1986), and is likely to cause cancer and could affect the neurological systems in humans (U.S EPA, 2010). Acrylamide has been found in aquatic environments nearby industries that use it (Weideborg et al, 2001) which makes this potential problem real and substantial.

The population in Stockholm is growing which means that the water demand is rising. Today, Norrvatten´s water treatment plant has a maximum capacity of 200 000 m^3/day. To be able to continue delivering healthy drinking water until 2030, the capacity needs to increase to 220 000 m^3/day, and even more in the years after. As a result, Norrvatten will build a new water treatment plant, which is the purpose of the project *Norrvattens Framtida Vattenproduktion* (NFVP) or The Future Water Production by Norrvatten. Another reason to expand the plant is that there is too little protection against chemical contaminants from Lake Mälaren and that the microbiological treatment is not good enough today (Heldt, 2021).

Currently, there is no purification of the reject water containing acrylamide since there is no legal requirement, but Norrvatten considers changing this as they will expand their water production and build a new water treatment plant. This could be done by either treating the reject water or by using polymers not containing acrylamide, like

biopolymers (Basiak-Klingspetz, 2023). This master thesis project will focus on treatments of the reject water. Treatments that are proposed are ozonation and biological treatments including moving bed biofilm reactor (MBBR) and trickling filter (Basiak-Klingspetz, 2023).

Norrvatten has a goal for the acrylamide concentration in their reject water to not exceed the limit of drinking water quality, which is 0.10 µg/l (SLVFS 2001:30). Today, they are not fulfilling this goal (Basiak-Klingspetz, 2023). In order to do that at the new drinking water plant, research about techniques for reducing the acrylamide content is required. Therefore, this master thesis project is relevant for NFVP.

A lot of research has been done on the toxicity of acrylamide for humans and for living organisms. The U.S Environmental protection agency (2010) has evaluated the toxic effects of acrylamide in detail and presents the average intake in Sweden. Peivasteh-Roudsari et al (2022) also give a review of the potential toxic effects on humans from acrylamide in food. Additionally, Shimamura et al (2022) did some research on the effects of acrylamide on DNA.

Furthermore, many reports have investigated and reviewed the environmental hazards of both polymers such as polyacrylamide and acrylamide monomers. However, most of the research seems to focus on the environmental hazards of polyacrylamide. Murgatroyd et al (1996) did a comprehensive review of polyelectrolytes effects on the environment and environmental organisms, as well as the environmental fate of both polyelectrolytes and acrylamide monomers. They describe the biological degradation of these compounds and also their ability to adsorb to particles in the environment and their accumulation capability. Wahlberg and Paxéus (2003) wrote a report for VA-Forsk about the same subjects with a focus on polyelectrolytes. Along with that Eveborn et al (2008) did research about the environmental hazards of polymers and acrylamide for the Institution for Agriculture and Environmental Technique (JTI) in Sweden. Other reports worth mentioning are Brown et al (1982), Biesinger and Stokes (1986), Bolto and Gregory (2007), as well as Guezennec et al, (2015) and Joshi and Abed (2017) who also mentioned photodegradation of polyacrylamide in the environment.

Brown et al (1982) conclude that acrylamide is biologically degradable in natural environments, but also mentions uncertainties when it comes to determining it. However, Krautter (1986) did some studies on the biological degradation of acrylamide in rivers to try to measure the degradation. Guezennec et al (2015) present the process of the degradation by bacteria and which products are left afterwards. Also, they announce the conditions most favorable for a rapid degradation. Furthermore, Joshi and Abed (2017) also mention the importance and dependency of the bacterial culture when it comes to the biological degradation of acrylamide.

Emmanuel et al (2013), Lakshmikandan et al (2014) and Bedade and Singhal (2017) have all done studies and experiments on certain bacterial strains which can degrade acrylamide faster than other bacteria. Along with that, they acknowledge the degradation time for the specific bacterial strains, the most effective conditions and the compounds that remain after the degradation.

Literature about the potential spontaneous polymerization of acrylamide monomers is crucial to look at since polymers are less toxic than acrylamide. Polymerization means that the acrylamide can form polymer molecules. In a water treatment plant that uses polymers for dewatering the sludge, this research is of big importance. If acrylamide gets polymerized spontaneously, it will change the acrylamide concentration at different measuring points in the sludge treatment process and change the concentration in the discharge of reject water into recipients. Kazantsev et al (2003, 2004) did study the spontaneous polymerization of methacrylamide in concentrated aquatic solutions. Moreover, Bol'shakov and Kiryukhin (2007, 2008, 2010) did write three reports where they did experiments on the spontaneous polymerization of acrylamide in glycerol.

The degradation of both polyacrylamide and acrylamide is dependent on the water and the bacterial culture (Emmanuel et al, 2013; Lakshmikandan et al, 2014; Bedade & Singhal 2017; Joshi & Abed, 2017). How well microorganisms in Lake Mälaren in Stockholm degrade acrylamide discharge from a water treatment plant is unknown. Given that, this master thesis will, except to test techniques to reduce acrylamide, also provide a study on the biological degradation in Lake Mälaren of acrylamide from Görvälnverket.

The general principle for discharges of acrylamide in the environment today is "as low as reasonably achievable" (Tritscher, 2004; Capuano & Fogliano, 2011), because there are no known thresholds for acrylamide in the environment (Felsot, 2002). Consequently, there are no regulations on the concentration of acrylamide in the reject water that leaves a water treatment plant in Sweden (Basiak-Klingspetz, 2023). Little research has been done on treatments to reduce acrylamide from reject water. This master thesis will also begin to fill that gap. Even though this master thesis project is written for Norrvatten and focuses on Görvälnverket, this study could contribute to developing other water treatment plants as well as gain knowledge about the fate of acrylamide in Lake Mälaren and similar recipients.

Ramboll which is a part of the NFVP project is a consultant company dealing with the design of the future drinking water plant and future processes for Görvälnverket (Ramboll, 2021). In a complementary PM for the NFVP project, Ramboll (2022) recommends that tests with ozone applied to the reject water leaving the sludge facility at Görvälnverket should be done to investigate possible solutions for the new expanded drinking water plant for Norrvatten. Along with that, biological degradation tests of acrylamide in water from Lake Mälaren at different temperatures are suggested to

measure the degradation time in the actual Lake after the reject water is discharged. This master thesis project follows those recommendations very well.

The expected outcome of this master thesis is that it will contribute to research for the NFVP project about which new techniques to implement at Görvälnverket to reduce acrylamide in the reject water in the future when expanding the drinking water plant. The contribution from this project will be about the actual reduction of acrylamide and potential bi-products, not cost, used volume in the plant, energy consumption, used chemicals or climate impact.

1.1 Aim and Research Questions

The aim of this master thesis project is to better understand how Norrvatten could decrease their discharges of acrylamide into Lake Mälaren by investigating potential technologies capable to degrade acrylamide from the reject water from the sludge dewatering facility at Görvälnverket.

This master thesis project will contribute to answering the following research questions:

- Do the techniques of ozonation, moving bed biofilm reactor and trickling filter have the potential to reduce the acrylamide concentration in the reject water from Görvälnverket, and reach Norrvatten´s goal of below 0.10 µg/l acrylamide in the reject water?
- Is there a risk that ozonation of the reject water could generate oxidative stress or genotoxicity to the reject water?
- What happens with acrylamide when it ends up in Lake Mälaren at a 5 m depth near Görvälnverket at the temperatures of 8 °C and 15 °C with retention times of 4 days and 13 days?

2. Background Theory

2.1 Polyacrylamide

Synthetic polyacrylamide is manufactured by acrylamide monomers and acrylic acid, which is formed from acrylate (Guezennec et al, 2015). The polyacrylamide molecule consists of a chain which consists of acrylamide monomers (Eveborn et al, 2008). Synthetic polyacrylamide could contain residual acrylamide monomers from manufacturing if the acrylamide used is not fully polymerized into polyacrylamide (Eveborn et al, 2008; Guezennec et al, 2015). For this reason, acrylamide can be present in the reject water after the use of polyacrylamide (Bolto & Gregory, 2007). However, most of the cationic polyacrylamides have a content of non-polymerized free

acrylamide below 0.1% (Wahlberg & Paxéus, 2003). Besides, according to the European Parliament (1999), polyacrylamide in EU is not allowed to contain more than 0.1% acrylamide.

Polyacrylamide can be degraded both through hydrolysis and through biological degradation (Wahlberg & Paxéus, 2003). Research have shown that the biological degradation of polyacrylamide seems to increase with higher temperatures and higher pH (Soponkanaporn & Gehr, 1988; Wahlberg & Paxéus, 2003). When polyacrylamide is degraded, the chain of polyacrylamide is broken and the molecule size, weight and rheological properties will change (Guezennec et al, 2015). However, Wahlberg and Paxéus (2003) state that there is a low possibility that acrylamide is formed after degradation of polyacrylamide, since double bindings will need to be recreated. Experiments with polyacrylamide mixed with activated sludge, which should degrade polyacrylamide, did not result in the formation of acrylamide (Murgatroyd et al, 1996). On the contrary, some research suggests that acrylamide could be formed after photodegradation of polyacrylamide by UV light (Smith & Oehme 1993; Smith et al, 1996, 1997; Wen et al, 2010). Also, Spraggs (1982) and Krautter (1986) state that acrylamide could be formed after degradation of polyacrylamide.

In the aquatic environment, polyacrylamide is not very bioavailable since the molecule is too big to pass through cell membranes of living organisms (Wahlberg & Paxéus, 2003; Guezennec et al, 2015). The toxicity of polyacrylamide in the aquatic environment also decreases since the polyacrylamide will be adsorbed to dissolved organic matter, which makes it even less bioavailable (Cary et al, 1987; Murgatroyd et al, 1996). The acrylamide monomers that are a part of the polyacrylamide are even more toxic than the polymer itself (Bolto & Gregory, 2007).

2.2 Norrvatten´s Sludge Dewatering Process

At Görvälnverket, sludge from the sedimentation tank with a total solid content of 1.5% and the sludge from the flotation and pulsator with a total solid content of 0.1% and 0.2% respectively are mixed before entering the sludge dewatering facility (Norrvatten, 2010). At the beginning of the dewatering process, 0.80 kg polymer/ton TS (Hugg, 2023) is added to the sludge stream in a mixing tank. After that, the sludge stream enters two parallel lamella separators where the sludge particles sediment on the lamella discs. Now, the total solid content of the sledge has risen to 2-3 % (Norrvatten, 2010). Following, the sludge stream is pumped to a sludge tank before it reached a centrifuge where 8.0 kg polymer/ton TS (Hugg, 2023) is added again. If the polymer dosage is increased, the total solid content will rise. The centrifuge force makes the solid particles separate from the water and the sludge reaches a total solid content of 15-20 %. The final dewatered sludge is transported to a storage and the water from the centrifuges is led back to the lamella separators. To clarify, the water from the centrifuges and the water from the lamella separators are mixed and discharged into Lake Mälaren. This water is called reject water (Norrvatten, 2010).

Norrvatten are using a polymer called Superfloc C-492PWG which is a polyacrylamide delivered by Kemira Oyj (Basiak-Klingspetz, 2022). Superfloc C-492PWG is a cationic polyacrylamide flocculant and is delivered as a dry granular powder. This flocculant is used because it works at various pH values and is effective at low dosages. Moreover, the molecule has a high molecular weight which is 0.75 kg/l bulk density and a very low relative charge (Kemira Oyj, 2015). At Görvälnverket, the polyacrylamide powder is mixed with water in a preparation container and is added to the sludge stream after a certain time. The polyacrylamide concentration in the containers is automatically controlled. The polyacrylamide dosages to the lamella separators and to the centrifuge are dependent on the total solid content and the flow of the sludge stream. Furthermore, the polyacrylamide dosage is increased if the total solids content or the flow is increased (Norrvatten, 2010). The water from the centrifuge contains polyacrylamide residuals and acrylamide monomers. This means that the reject water discharged into Lake Mälaren also contains acrylamide monomers (Hugg, 2023).

Water samples from the centrifuges and samples from the reject water leaving the lamella separators and later is discharged into Lake Mälaren have been analysed by Eurofins for their acrylamide concentration during 1 year before this master thesis project began (Fig. 1). The results illustrate that the acrylamide concentration in both the water leaving the centrifuges and the lamella separators often is higher than the drinking water limit of 0.10 µg/l (SLVFS 2001:30). The average acrylamide concentration in the reject water leaving the lamella separators during the period 2022-03 to 2023-02 is 1.25 µg/l (Norrvatten, 2023).

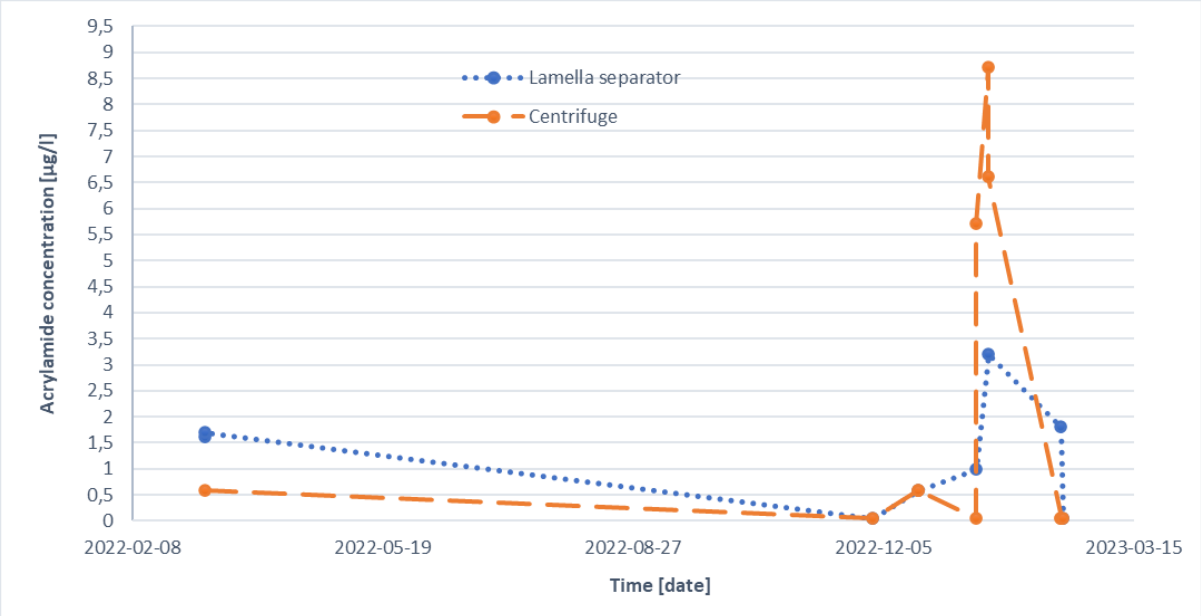

Figure 1: The acrylamide concentration in the reject water leaving one of the lamella separators, and the acrylamide concentration in the water leaving the centrifuge between 2022-03-09 to 2023-02-15.

The mean value of incoming sludge flow to the two lamella separators between February 2022 and February 2023 is 4.58 l/s respectively (Fig. 2). This means that the average sludge flow to the dewatering facility is 9.16 l/s (Norrvatten, 2023).

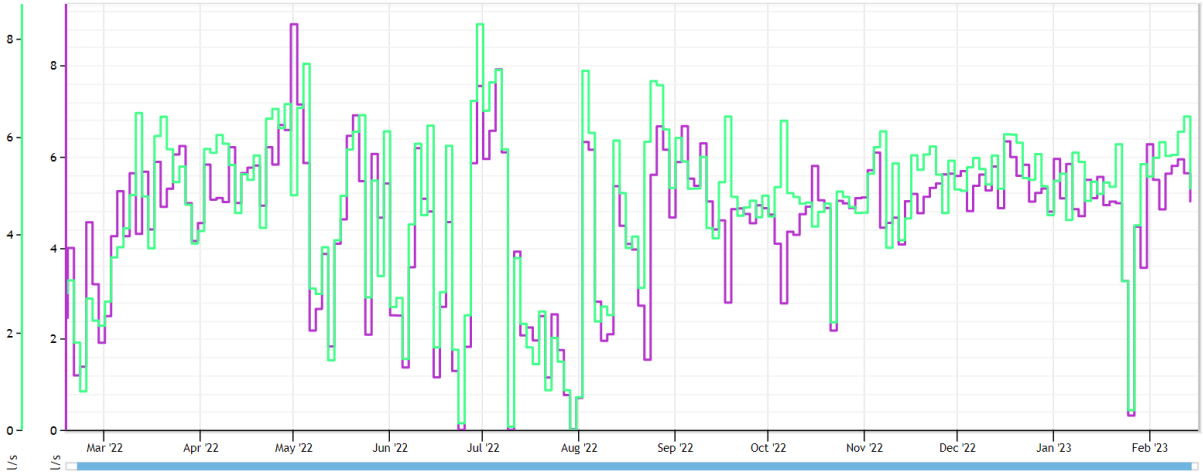

Figure 2: The sludge flow [l/s] into lamella separator 1 (pink graph) and into lamella separator 2 (green graph) between 2022-02 and 2023-02 (Norrvatten, 2023).

2.3 The Flocculation Process

According to Bolto and Gregory (2007), cationic polyacrylamide is present as coils in water. They do adsorb to particle surfaces with a negative charge through electrostatic attraction, which is why they are used as flocculants (Fig. 3). This adsorption will be stronger if the charge density of the polyacrylamide molecule is high. Charge density refers to how many percent of the acrylamide monomers in the polymer chain that contain a charged group (Guezennec et al, 2015). A high charge density will make the loops small since a bigger part of the molecule is attached to the particle surface. The opposite happens if the charge density is low (Bolto & Gregory, 2007). The adsorption process is irreversible and can be fast if the polymer chain is small enough (Bolto & Gregory, 2007; Guezennec et al, 2015). Nevertheless, it can take seconds for a long-chain polymer to achieve an equilibrium after adsorption with the particle surface (Bolto & Gregory, 2007).

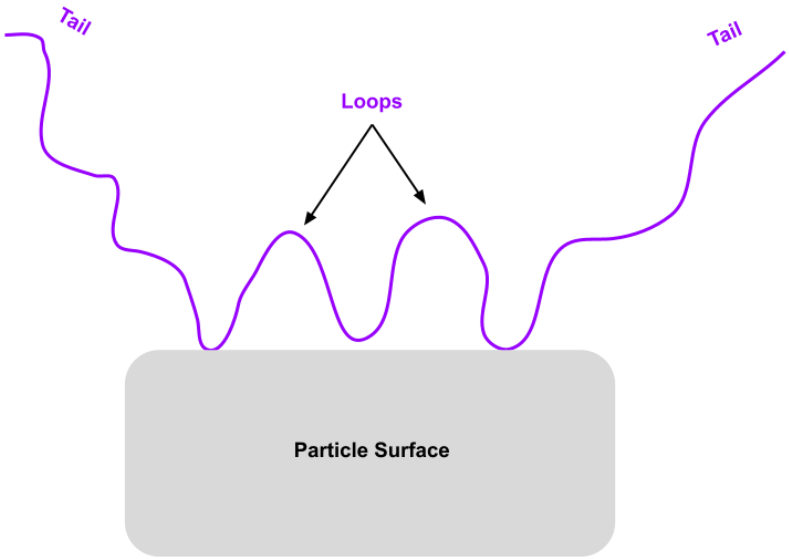

Figure 3: Illustration of polyacrylamide adsorption to a particle surface with the names of the different parts of the polyacrylamide molecule chain highlighted (Aspegren, 2023).

The phenomenon called bridging creates big flocs with many particles and polymers (Bolto & Gregory, 2007; Guezennec et al, 2015). This means that the loops and tails which are reaching out in the solution from the particle surface can be adsorbed to other particles as well (Fig. 4). Bolto and Gregory (2007) explain that the adsorption should not be too strong if bridging will occur, which means that the charge density should be lower. Now, it is favorable that the loops and tails are reaching out in the water. Also, the polymer chain must be long to enhance long tails and big loops. Along with that, linear chains are optimal for bridging as well as polymers with high molecular weights of up to several million. Bridging is a strong force which creates strong flocs. This might be because the molecule chains in this formation are flexible and elastic and not easily destroyed. However, the bridging process could result in particles being restabilised. This means that a too big part of the particle surface is covered with polymer chains (Bolto & Gregory, 2007) and available particle surfaces for bridging are decreased (Sato & Ruch, 1980). For an optimal process, half of the particle surface should be covered (Mer, 1966). The optimal dosage of polymers is usually 1 mg polymer/g of suspended solids (Bolto & Gregory, 2007).

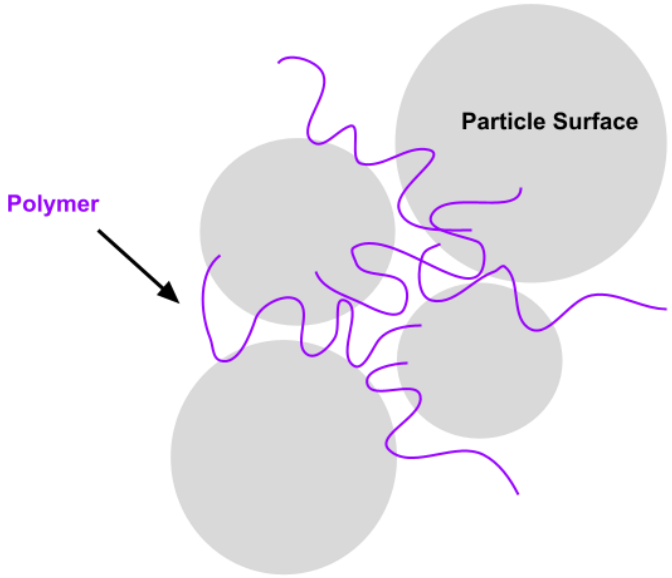

Figure 4: Illustration of the flocculation phenomenon called bridging with polymers (Aspegren, 2023).

On anionic particle surfaces where cationic polymers are adsorbed, patches of positive charge are created on the negatively charged particle surface. This means that these patches attract other particles by electrostatic attraction. A positive patch on a particle surface can bind to a negative patch on another particle (Fig. 5). For this process to occur, high charge density is required by the polymer molecule. Also, the polymer chain needs to lay flat on the particle surface, otherwise bridging will happen instead (Bolto & Gregory, 2007).

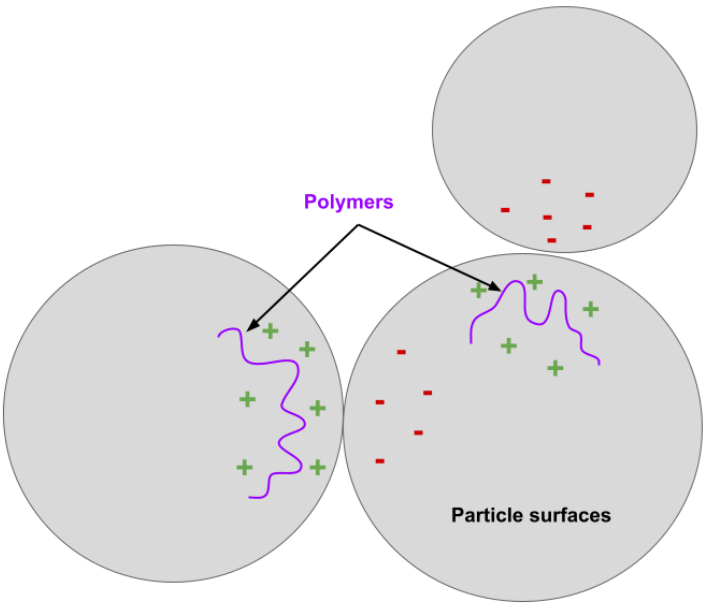

Figure 5: Illustration of electrostatic attraction between particles when cationic polymers have been adsorbed so the particle surfaces (Aspegren, 2023).

In the end, after the flocculation, all the polymers are found on solid particles in the sludge. (Anderson et al, 1993) However, a part of the easy-bound polyelectrolytes can be found in the reject water, these are called extracellular polymers. These will occur to a bigger extent if centrifuges are used for sludge thickening (Sanin & Veslind, 1994). At optimal dosage of polymers to the sludge, 95 % of the polymers are stuck in the sludge, and not in the reject water (Dentel et al, 2000).

2.4 Acrylamide

The molecular formula for acrylamide is C_3H_5NO (Fig. 6) (US EPA, 2010). Residual acrylamide can be left in the reject water after the use of polyacrylamide as a flocculant agent (Bolto & Gregory, 2007). Since acrylamide is not adsorbed by the sludge very well, 90% of the residual acrylamide will be found in the reject water leaving the plant. Just a small part gets stuck in the pore water in the dewatered sludge (Brown et al, 1980a; Brown et al, 1980b; Wahlberg & Paxéus, 2003). Besides, acrylamide has a high water solubility (Cherry et al, 1956; Brown et al, 1980a; Lee & Chang, 1989; Seybold, 1994; Merck Index, 1996; Murgatroyd et al, 1996; US EPA, 2010; Guezennec et al, 2015), which support the statement that most acrylamide will be found in the reject water**.** To be precise, the water solubility is 2.155 g/ml at 30°C (Cherry et al, 1956; Brown et al, 1980a; US EPA, 2010). Moreover, the small size of acrylamide monomers makes it difficult to adsorb to particle surfaces in the sludge (Guezennec et al, 2015), which further support the statement. As a result, acrylamide does transfer easily in water (Cherry et al, 1956; Brown et al, 1980a; US EPA, 2010; Guezennec et al, 2015). However, the fact that acrylamide is efficiently biodegradable could restrict the transfer in natural waters (Guezennec et al, 2015). Also, the volatilization potential for acrylamide in water is low (US EPA, 2010).

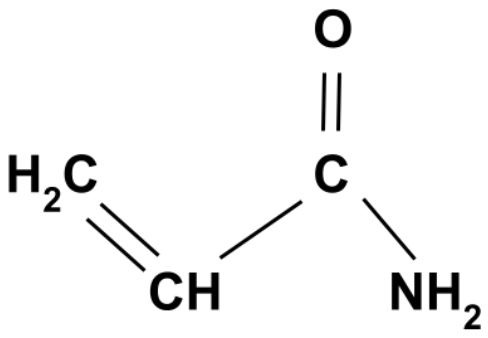

Figure 6: Illustration of the acrylamide molecule (Aspegren, 2023).

2.5 Environmental Aquatic Effects of Acrylamide

Acrylamide monomers itself could have moderate toxicity to aquatic life (Spraggs, 1982; Krautter 1986). A study by Brown et al (1982) confirms this. The study was about the toxicity of acrylamide continuously discharged in a river with a concentration of 6 µg/l during 10 weeks in winter and autumn, resulted in adverse effects on most of the aquatic invertebrates. When the concentration in the river reached 50 µg/l, the diversity of species was reduced in only 5 hours (Brown et al, 1982). Because of the small molecule of acrylamide, it can pass through cell membranes and be present in the bodies of living organisms, which makes it more toxic than for example polyacrylamide (Buranasilp & Charoenpanich, 2011). To only test the toxicity of acrylamide in labs is not enough, since acrylamide could have a sub-lethal effect that is not detected in labs. There is little data on the effects of low concentrations of acrylamide discharged in surface water from waterworks. Consequently, there are uncertainties about the actual effects of acrylamide in surface waters (Murgatroyd et al, 1996).

2.6 Toxicity of Acrylamide

Acrylamide is a chemical compound hazardous to health. When acrylamide is entering the human body, it bounds to the chromosomes and the risk for tumors to form is then increased (Eveborn et al, 2008). Acrylamide can enter the human body via food, especially fried food and food heated up to high temperatures (Tareke et al, 2002; Eveborn et al, 2008; Peivasteh-Roudsari et al, 2022). It is likely that acrylamide is cancerogenic to humans (Hogervorst et al, 2007; U.S EPA, 2010; Pan et al. 2020; Shimamura et al, 2022). However, it is unclear how acrylamide impacts the human body and especially affects cancer, because there are no reliable results of cancer caused by chronic exposure to acrylamide (U.S EPA, 2010; Zhivagui et al, 2019).

Studies on the toxicity of acrylamide show that it can be neurotoxic to humans at high concentrations and cause adverse effects on cognitive functions and memory for example (Erkekoglu & Baydar, 2014; Matoso et al. 2019). Studies also suggest that acrylamide is genotoxic (Fuhr et al, 2006; Hogervorst et al, 2007; Nowsheen & Yang, 2012; Hemgesberg et al, 2016). According to U.S Environmental Protection Agency (2010) just inhalation or dermal contact has been shown in animal studies to have neurological impacts and dermal contact itself cause germ cell impairment. Oral intake of acrylamide could cause changes in peripheral nerves (U.S EPA, 2010) and have negative effects on the reproduction for males (Kumar et al, 2018). Other effects of acrylamide exposure are hallucination, disorientation, and confusion (Zamani et al. 2017), as well as DNA damage and mutations (Fuhr et al, 2006; Nowsheen & Yang, 2012; Hemgesberg et al, 2016). Additionally, if acrylamide already exists in the human body, it could also be present in breast milk (Semla et al. 2017).

The human oral slope factor of acrylamide is 0.5 $(mg/kg - day)^{-1}$ and the daily intake in Sweden in 2002 in the age group 18-74 years was 0.45 µg/kg according to U.S Environmental Protection Agency (2010). Further, Eveborn et al (2008) state that the average intake of acrylamide by Swedish people is 35 µg/day. In general, the human exposure to acrylamide in the environment is very low (EU, 2002).
The limit for acrylamide in drinking water in Sweden is 0.10 µg/l (SLVFS 2001:30).

2.7 Biological Degradation of Acrylamide

Microorganisms in the natural environment can degrade acrylamide (Croll et al, 1974; Brown et al, 1980a; Guezennec et al, 2015) and particularly in natural surface waters (Seybold, 1994). Wahlberg and Paxéus (2003) and Brown et al (1982) state that acrylamide is easily biological degradable. Most studies show that the biological degradation of acrylamide in the natural environment is an aerobic process, although some recent studies show that anaerobic bacteria are involved as well. For instance, nitrate reducers seem good at degrading acrylamide (Guezennec et al, 2015).

Biological degradation of acrylamide forms carbon dioxide, acrylic acid and ammonia (Guezennec et al, 2015; Joshi & Abed, 2017). On the other hand, at complete degradation of acrylamide, which only some bacteria are capable of, carbon dioxide and water are the only end products according to Joshi and Abed (2017).

In addition, the microbial composition is an influencing factor since experiments show that biological degradation varies with different types of water used (Guezennec et al, 2015). Tests present that in non-acclimated river water, the degradation is done after 4 days and in acclimated river water after 24 hours (Krautter, 1986). Guezennec et al (2015) confirms that minimum one day are required for complete degradation of acrylamide in natural waters, but usually some more days. On the other hand, Seybold (1994) states that the interval is much longer, between 4 and 29 days (Seybold, 1994). Alike Seybold (1994), Brown et al (1980a) approve that the degradation time can vary a lot and states that the half-life for acrylamide could range between weeks and months in a river. To summarize, it is hard to know how good the degrading performance will be if the water is continuously affected by discharges of acrylamide (Brown et al, 1982).

The enzyme acrylamidase, which is a part of the group hydrolases is important for the biological degradation of acrylamide. Some of these enzymes are involved in the first steps of the biological degradation of acrylamide by catalyze the hydrolysis of acrylamide which forms ammonia and acrylate (Shanker et al, 1990; Zabaznaya et al, 1998; Shukor et al, 2009; Sharma et al. 2009; Cha & Chambliss, 2011; Guezennec et al, 2015; Joshi & Abed, 2017). The enzyme is a part of some bacteria, which produce it (Shukor et al, 2009; Cha & Chambliss, 2011; Guezennec et al, 2015).

2.8 Bacterial Strains Capable of Degrading Acrylamide

Bacteria are required for biological degradation of acrylamide, and the degradation depends highly on the bacterial culture (Seybold, 1994; Joshi & Abed, 2017). Acrylamide can be used as the only source of nitrogen and carbon for bacteria. Bacteria known to be able to degrade acrylamide through hydrolysis are *Enterobacter aerogenes, Xanthomonas maltophilia, Helicobacter pylori, Ralstonia eutropha* and *Rhodopseudomonas palustris* (Nawaz et al., 1993; Van Vliet et al., 2003; Wampler & Ensign, 2005; Buranasilp and Charoenpanich, 2011; Cha and Chambliss, 2011). Also, *Rhodococcus sps*., (Nawaz et al, 1994), *Bacillus clausii* (Jain, 2012), *Pseudomonas aerugenosa* (Sathech & Thatheyus, 2007), *Aspergillus oryzae* (Wakaizumi et al. 2009), *Pseudomonas sp, Geobacillus thermoglucosidasius, Kluyvera georgiana, Bacillus licheniformis* and *Variovorax boronicumulans* are able to degrade acrylamide (Nawaz et al., 1993; Van Vliet et al, 2003; Wampler et al., 2005; Prabu and Thatheyus, 2007; Cha & Chambliss, 2011; Buranasilp and Charoenpanich, 2011; Richi et al, 2012; Thanyacharoen et al., 2012; Cha & Chambliss, 2011, 2013; Liu et al, 2013; Emmanuel et al., 2013). Additionally, X. *maltophilia, E. aerogenes, R. eutropha, V. boronicumulans*. can degrade acrylamide with the enzyme acrylamidase even at high concentrations of acrylamide (Shanker et al., 1990; Nawaz et al., 1992; Prabu and Thatheyus, 2007; Cha & Chambliss, 2011; Buranasilp and Charoenpanich, 2011; Liu et al, 2013).

However, there are bacteria which can degrade acrylamide much faster than others. Among those are *Moraxella osloensis MSU11* which is a gram negative diplobacillus (Emmanuel et al, 2013). Included are also *Stenotrophomonas acidaminiphila MSU12* which is a gram-negative bacilli strictly aerobic (Lakshmikandan et al, 2014) and is present in both artificial and natural environments (Boonchan et al, 1998; Zheng et al, 2007; Dwivedi et al, 2010). Also, *Arthrobacter sp. DBV1* which is an aerobic bacterial strain which is gram positive is among these fast-degrading bacteria (Robert et al, 1994). Finally, the *Bacillus cereus strain DRY135* (Shukor et al, 2009) which is a gram-positive aerobic bacteria (Granum, 2002; Bottone, 2010), however, able to grow in anaerobic conditions as well (Granum, 2002) and is spread in the environment (Bottone, 2010), as well as *Cupriavidus oxalaticus* (Bedade & Singal, 2018; Bedade et al, 2019) which is a gram-negative strain (Makkar & Casida, 1987). For both *Moraxella osloensis MSU11* and *Stenotrophomonas acidaminiphila MSU12*, total degradation of acrylamide occurs after 54 hours (Emmanuel et al, 2013; Lakshmikandan et al, 2014), but for *Cupriavidus oxalaticus* it takes 24 hours to degrade 60 mM acrylamide (Bedade & Singal, 2018).

All these three bacterial strains produce the important enzyme acrylamidase which catalyze the hydrolysis of acrylamide and its carboxylic amides (Lakshmikandan et al, 2014; Bedade & Singhal, 2017). Moreover, acrylamidase uses acrylamide as its only source of carbon and nitrogen while hydrolysing acrylamide (Nawaz et al., 1993; Van Vliet et al, 2003; Wampler et al., 2005; Prabu and Thatheyus, 2007; Cha & Chambliss,

2011; Buranasilp & Charoenpanich, 2011; Richi et al, 2012; Thanyacharoen et al, 2012; Cha & Chambliss, 2013; Liuet al., 2013; Emmanuel et al., 2013; Guezennec et al, 2015).

After the hydrolysis, the final products are ammonia and the non-toxic acrylic acid (Emmanuel et al, 2013; Lakshmikandan et al, 2014; Bedade & Singhal, 2017). The ammonia is released during the degradation (Lakshmikandan et al, 2014). As a result, the ammonia release, which implies acrylamide degradation, is correlated to bacterial growth (Bedade & Singhal, 2017). Both *Moraxella osloensis MSU11* and *Arthrobacter sp. DBV1* are able to use the acrylic acid after the degradation of acrylamide for their growth (Emmanuel et al, 2013; Bedade & Singhal, 2017). This statement corresponds to Guezennec et al (2015) who confirms that acrylate, which is formed from acrylic acid, possibly are used for bacterial growth and as an energy source, for other bacteria degrading acrylamide as well (Bedade & Singhal, 2017).

The best temperature for fast acrylamide degradation by these bacteria are around 30 °C (Lakshmikandan et al, 2014; Emmanuel et al, 2013; Shukor et al, 2009; Bedade & Singal, 2018). For *Bacillus cereus*, bacterial growth is decreased significantly when the temperature sinks from 25 °C to 20 °C (Shukor et al, 2009). This indicates that the minimum temperature for degradation of acrylamide for this bacterial strain is 20 °C. Similar, Bedade and Singal (2018) showed that *Cupriavidus oxalaticus* could degrade acrylamide at a temperature of 20 °C, and that this temperature was even better than 40 °C.

The optimal concentration of acrylamide was 5.6 mM for *Bacillus cereus* (Shukor et al, 2009), 30 mM for *Stenotrophomonas acidaminiphila MSU12* and *Arthrobacter sp. DBV1* (Lakshmikandan et al, 2014; Bedade & Singhal, 2017), 40 mM for *Moraxella osloensis MSU11* (Emmanuel et al, 2013) and 60 mM for *Cupriavidus oxalaticus* (Bedade & Singal, 2018). For these bacteria, a pH of around 7 is optimal for degrading acrylamide (Shukor et al, 2009; Emmanuel et al, 2013; Lakshmikandan et al, 2014; Bedade & Singal, 2018). According to Nawaz et al (1998) a pH of 7 is the best for most bacteria. The reason is that the acrylamidase enzyme is most active at a pH around 7, independent of the bacterial strain in the water (Bedade & Singhal, 2017). However, the hydrolysis of acrylamide begins at pH 8,5 according to Smith-Palmer et al (1994). The degradation process is very pH dependent, at least when *Stenotrophomonas acidaminiphila MSU12* is present (Lakshmikandan et al, 2014). Furthermore, the presence of iron not higher than 10 mM does seem to favor the degrading process. Nickel and cobalt however, are decreasing the degradation speed (Nawaz et al, 1998). Furthermore, it is hard to know if immobilized cells or non-immobilized cells have a better degrading capacity (Emmanuel et al, 2013; Guezennec et al, 2015; Joshi & Abed, 2017). However, Emmanuel et al (2013) suggest that immobilized cells could be slightly better. Correspondingly, Nawaz et al (1998) and Prabu and Thatheyus (2007) did show that immobilized cells did degrade acrylamide faster than free cells.

2.9 Spontaneous Copolymerization of Acrylamide

It could be possible for acrylamide to form polyacrylamide. According to Kazantsev et al (2004, 2003), N-(3-dimethylaminopropyl)methacrylamide and other methacrylamides with different structures and charges, can spontaneously get polymerized in water. But special conditions are required. The pH needs to be less or equal to 8. The more acidic, the faster the process. An acid like sulfuric acid or formic acid, which are anions, must be added. To clarify, anions need to be present in the solutions to neutralize the monomers (Kazantsev et al, 2004). Additionally, the concentration of monomers in the solution needs to be high (Kazantsev et al, 2004; Kazantsev et al, 2003). Moreover, Bol'shakov and Kiryukhin (2007) conclude that active centers like radicals and ions need to be formed to start the spontaneous polymerization and growth of polymer chains. Finally, the formation of monomeric associates is a requirement as well. In addition, higher temperatures speed up the polymerization, but a temperature of 50-70 °C is enough. However, even if all requirements are fulfilled, the polymerization is dependent on the actual type of monomers (Kazantsev et al, 2004). For example, the structure of the amide groups in the monomers seems to influence the polymerization, as well as how well (meth)acrylamides can bind to hydrogen atoms (Kazantsev et al, 2003)

The polymerization begins with a redox reaction by the anions from the added acid and the C=C bonds in the monomers, which breaks. The polymer formed after the polymerization does not contain any C=C bonds. However, the polymerization is only noticeable when 80 % of the amino groups in the monomers are neutralized by the acid (Kazantsev et al, 2004). The polymers from the polymerization of methacrylamide are not soluble in water (Kazantsev et al, 2003).

2.10 Ozonation

Ozonation is an oxidation treatment and one of the techniques which best reduce hard degradable substances. Ozonation also decreases the content of suspended material in water (Baresel et al, 2017). When applying ozone to water, hydroxyl radicals are formed, which degrade organic compounds, like dissolved organic matter (Baresel et al, 2019; Fu et al, 2019). Polyacrylamide impacted by hydroxyl radicals could potentially lead to a rise of acrylamide monomers in the water, when the chain of polyacrylamide is broken under certain conditions (Xiong et al, 2018).

Toxic bi-products could be created after ozonation of water (Baresel et al, 2017; Baresel et al, 2019; Fielding et al., 1999). Often they decrease with time, but if the ozonation dosage is high, more stable bi-products could be produced. Ozonation tests on wastewater by IVL Swedish Environmental Research Institute in a pilot-plant with an ozonation dose of 7.5 mg/l did not however show any toxic effects on crustacean *Nitocra spinipes* or algae *Selenastrum capricornutum*. A small effect was seen for the bacteria *Vibrio fisheri Microtox*. The toxic effects seem to be dependent on the dose of ozone (Baresel et al, 2017).

Hydroxyl radicals generated while ozonation could cause oxidative stress for living organisms. Hydroxyl radicals is a reactive oxygen species (ROS). When ROS in cells of living organisms rises, the cell could be affected by oxidative stress, if it does not have enough defense in terms of antioxidants (Pizzino et al, 2017; BioCell Analytica, 2023a). If a cell is affected by oxidative stress, it can lead to damage to the cell and in the long run end up in various diseases like cancer, inflammation and diabetes (Pizzino et al, 2017; BioCell Analytica, 2023a).

2.11 Trickling Filter

A trickling filter consists of a containment with some sort of biofilm carriers or media, where the biofilm grows and a distribution system with sprinklers and ventilation. The water is distributed on the top of the biofilm media and flows downwards through the media (Séguret et al, 2000; Daigger & Boltz, 2011). The water is either pumped to the trickling filter or flows there by gravity. The media have a big specific surface where microorganisms can grow and accumulate (Daigger & Boltz, 2011; Zhu et al, 2016), and it has a porosity which prevents clogging and favor aeration. It is the biofilm that grows on the media that is responsible for the biological degradation of contaminants (Daigger & Boltz, 2011).

2.12 Moving Bed Biofilm Reactor

A MBBR is often used to treat wastewater for reduction of phosphorus, nitrogen, COD and BOD (Tsitouras et al, 2021), but is also used to treat hospital wastewater (Casas et al, 2015; Bakar et al, 2018; Barathi et al, 2022) and industrial wastewater. It is a biological water treatment method with bacteria like in a trickling filter. Unlike a trickling filter, these bacteria grow and create a biofilm on plastic carriers with a big specific surface, created for MBBR specifically (Maurya et al, 2023). These carriers move in the water with the help of bubbles or a stirrer (Abdelfattah et al, 2020). The shape, porosity and size of the carriers are influencing the treatment efficiency (Kostrytsia et al, 2022).

3. Methodology

For the background part of the report, literature research was done. To answer the research questions, experimental tests were carried out in pilot plants and on a bench-scale and water samples were taken and sent for analysis. The water treatment techniques tested were ozonation and biofiltration including trickling filter and MBBR. Moreover, a biological bench-scale test was performed to evaluate the effectiveness of the trickling filter. Finally, tests in water from Lake Mälaren were performed to investigate the impact of the current situation. The following text describes the study area, the data collection and the method used for the experimental tests.

3.1 Study Area

This master thesis will be written for the municipal federation Norrvatten. They are in charge of producing and distributing drinking water to approximately 700 000 people in the northern part of the Stockholm region. There are 14 municipalities which together own Norrvatten. Furthermore, Norrvatten has a drinking water plant named Görvälnverket located on the island Skäftingeholmen in Lake Mälaren in Järfälla municipality in Stockholm, Sweden. Norrvatten is distributing drinking water through water mains to water supply networks owned by each of the municipalities (Norrvatten, 2022).

3.2 Data

The data needed to answer the research questions were the acrylamide concentration in the reject water from the lamella separators and in the treated water from the treatments that were tested, namely ozonation and biofiltration. The same data was needed for the tests in water from Lake Mälaren. Eurofins analyzed the concentration of acrylamide in the water samples and the results from the samples took two weeks to get. Eurofins were analysing 100 ml samples by In house method (210) with LOQ of 0.05 µg/l and a measurement error of 20 %. The result of the analysis was the acrylamide concentration in ug/l.

Furthermore, genotoxicity and oxidative stress were measured in a few samples from the water treated with ozone. Moreover, measurements of background parameters such as pH, dissolved oxygen, conductivity, temperature and dissolved organic matter were done with a digital device from Hach called HQ Series Multi and YSI multiparameter sonde EXO2 during the test periods.

3.3 Ozonation

The ozonation tests aimed to evaluate if ozonation of the reject water would reduce the acrylamide concentration to levels below the drinking water limit of 0.10 ug/l, and if so, which ozone concentrations reduce the acrylamide concentration the most. Another reason for the ozone tests was to compare the acrylamide reduction with biological treatments.

Three ozonation tests were performed in a pilot plant in a laboratory at IVL Swedish Environmental Research Institute in Stockholm. The materials used were 100 mg acrylamide powder, an E-flask, a digital device from Hach called Hach multimeter HQ2100, plastic hoses, a 1 l plastic colon, glass beakers of different sizes, an ozone generator (OGK-3G), a flow measurer, an ozone content generator (BMT 964 C) and 30 l reject water from one lamella separator. The ozone flows through a glass filter (porosity 4) to the water colon (Fig. 7). Photographs of the ozone pilot plant are shown in Appendix I, Fig. 28.

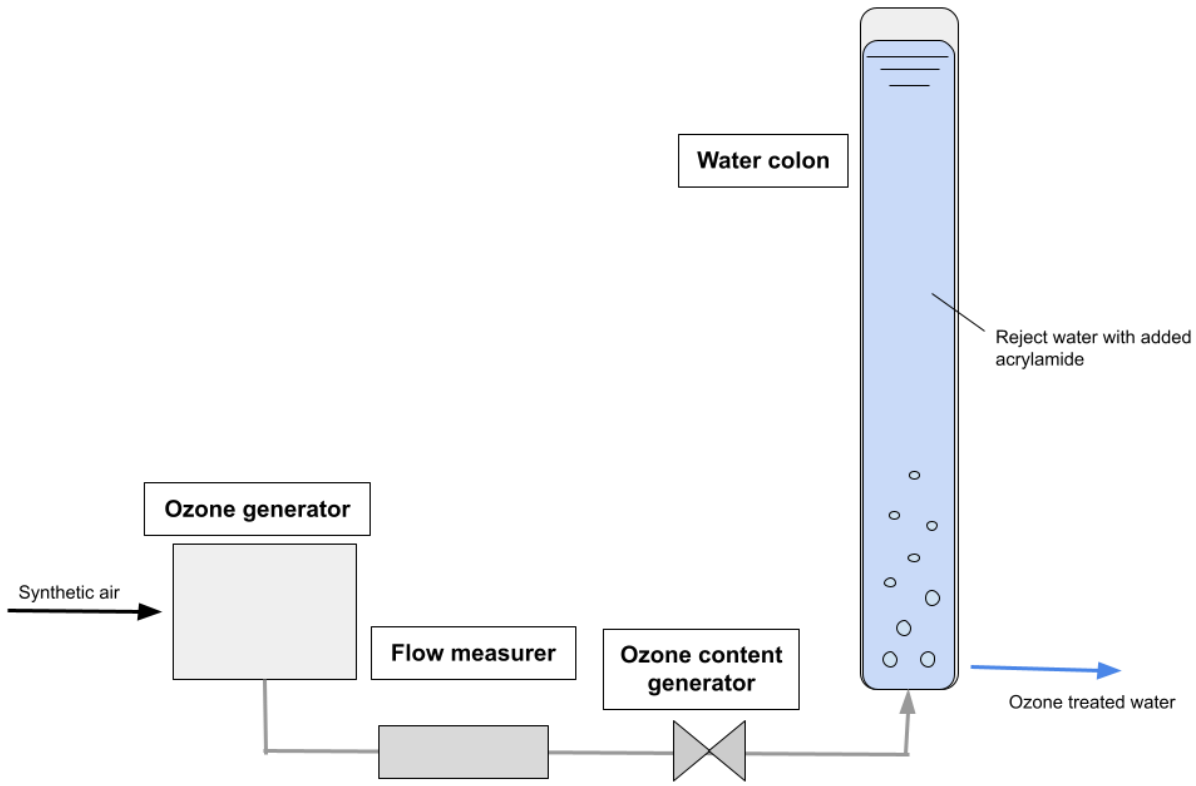

Figure 7: Illustration of the ozone pilot plant in the laboratory at IVL Swedish Environmental Research Institute (Aspegren, 2023).

In ozone test 1, the 100 mg acrylamide powder was mixed with 1 l of water in a ventilation chamber. The solution did then contain 0.01% acrylamide or 100 mg/l acrylamide. The solution was stored cold (≤4°C) during the whole experimental period. 2.5 ml of the acrylamide solution (100 mg/l)was then mixed with 25 l reject water collected the day before to get a 10 µg/l concentration of acrylamide. The reject water was stored cold until the test was initiated. The ozone was applied to this solution. Before the ozonation was applied, the pH, conductivity, dissolved oxygen concentration and temperature were measured three times in the mixed acrylamide solution with Hach multimeter HQ2100. On average this solution had a pH of 6.45, a conductivity of 270 µS/cm, a dissolved oxygen concentration of 10.88 mg/l and a temperature of 11.7 °C.

After that, six ozone dosages were applied to the solution (Table. 1). For every dosage, 1 l of the mixed acrylamide solution was used in the plastic colon and ozone from the ozone generator was applied to the bottom of the colon. To clarify, for every new ozone dose, a new batch of the initial mixed acrylamide solution was used. The ozone generator generated ozone from synthetic air and did deliver a constant flow of 0.34 l O^3/min with a content of 14 g/Nm^3 with a transmission coefficient of 0.439 throughout all the tests. The ozone dosage depended on the time in seconds while ozone was applied, which started when small ozone bubbles were seen at the bottom of the water colon. Before collecting the sample, some treated water from the bottom of the colon was used to rinse the hoses in order not to contaminate the current sample with leftovers from the previous test. 100 ml of the treated water was taken as a sample via a plastic hose from the bottom of the colon which later was sent for analysis of acrylamide concentration. Also, some of the treated water was put in a bigger glass bottle via a plastic hose from the bottom of the colon so that the pH, conductivity and temperature could be measured after every of the 6 ozone applications.

Table 1. Demonstration of how the ozone concentration was correlated to the application time in ozone test 1.

Sample	Ozone concentration [mg/l]	Time for application [s]
1	0.7	22
2	1.4	45
3	2.1	67
4	2.8	89
5	3.5	111
6	7	222

In ozone test 2 performed four weeks later, 1.24 ml of same acrylamide solution (100 mg/l) was mixed with 25 l of reject water collected two days before. The expectation was to get a solution with 5 µg/l acrylamide. However after analysis by Eurofins, the real concentration was 2.5 µg/l. The pH, conductivity and temperature were measured three times in this solution before the ozonation tests began. On average this solution had a pH of 6,54, a conductivity of 272 µS/cm and a temperature of 11,07 °C. The same procedure of ozonation as for the previous test was done (Table. 2), except that the measurement of time while ozone was applied started when bigger ozone bubbles than during the first test were observed at the bottom of the colon, which was 1-2 seconds later than during the first test. Another exception was that the treated water from the test colon was collected in a glass beaker after ozonation, which then was used for sampling and measurements.

Table 2. Demonstration of how the ozone concentration was correlated to the application time in ozone test 2.

Sample	Ozone concentration [mg/l]	Time for application [s]
1	0.7	22
2	0.9	30
3	1.2	38
4	1,4	45
5	6,9	222
6	18,7	600

During ozone test 2, three 1-l samples were sent for analysis for genotoxicity and oxidative stress. The first sample was from the reject water with added acrylamide, the second from the third ozone sample and the third from the fifth ozone sample. Because a sample volume of 1 L is required for genotoxicity and oxidative stress analysis and the test colon holds only 1 L of water, the ozonation had to be done twice to have enough sample volume for genotoxicity samples, acrylamide concentration samples and measurements. The genotoxicity and oxidative stress were analysed by BioCell Analytica. To analyse the oxidative stress, a reference substance called tertiary butyl hydroquinone was used to compare with the oxidative stress the actual samples generated. The unit for the oxidative stress was then given in biological equivalent concentration (tBHQ) in ug/l. Nuclear transcription factor erythroid 2-related factor 2 (Nrf2) which regulates the cell response to oxidative stress, was used as a factor to measure oxidative stress (BioCell Analytica, 2023a). The genotoxicity was measured by in vitro micronucleus test. The water samples were concentrated 25- and 50 times for the analysis (BioCell Analytica, 2023b).

Finally, for ozone test 3, the same procedure as for ozone test 2 was done on the same day (Table. 3), except that this time 2.5 ml of the acrylamide solution (100 mg/l) was mixed with 25 l Milli-Q deionized water. The expected acrylamide concentration to get was 10 ug/l. However after analysis by Eurofins, the real concentration was 6.6 ug/l. The main reason for using Milli-Q water was that it does not contain any polyacrylamide or dissolved organic matter that could react with the ozone. The expectation was that a high acrylamide reduction could be generated with low ozone dosages.

Table 3. Demonstration of how the ozone concentration was correlated to the application time in ozone test 3.

Sample	Ozone concentration [mg/l]	Time for application [s]
1	0.7	22
2	0,9	30
3	1,2	38
4	1,4	45
5	2.1	67
6	2.8	89

3.4 Biofiltration Tests

3.4.1 Trickling Filter

The test with the trickling filter aimed to evaluate if the trickling filter would degrade acrylamide to levels below the drinking water limit of 0.10 µg/l.

The materials used for the trickling filter were a 1000 *l* plastic container with a lid, spreaders for the plastic container, a flow meter, a valve, 600 *l* Light Expanded Clay Aggregate (leca) granules, hoses and one small portable water pump. For measurements of electrandemical parameters directly in the water, Hach multimeter HQ2100 and YSI multiparameter sonde EXO2 were used.

The leca granules were put in the plastic container. The pump was connected to the reject water from one of the lamella separators. At the inlet to the trickling filter, the valve and the flow meter were installed, to enable adjustment and control of the flow and retention time of the reject water in the filter. After that, spreaders were connected to this pipe in order to spread the reject water evenly over the leca granules. The treated water did flow out at the bottom of the trickling filter on the opposite side of the intake (Fig. 8). Photographs of the trickling filter pilot plant are shown in Appendix, Fig. 26. The flow from the spreaders was set to around 5 l/min and the empty bed contact time (EBCT) was then 2 hours.

Samples from the trickling filter inlet- and outlet water were taken 4 times during the 13-week operation period and sent to Eurofins for analysis of the concentration of acrylamide. The reason was that the biofilm developed more and more since the reject water from the lamella separator did flow continuously through the trickling filter. Together with that, the temperature, pH, dissolved oxygen and conductivity of the intake reject water as well as the treated outlet water was measured two times per week from the sixth week of the trickling filter treatment with Hach multimeter HQ2100 or YSI multiparameter sonde EXO2.

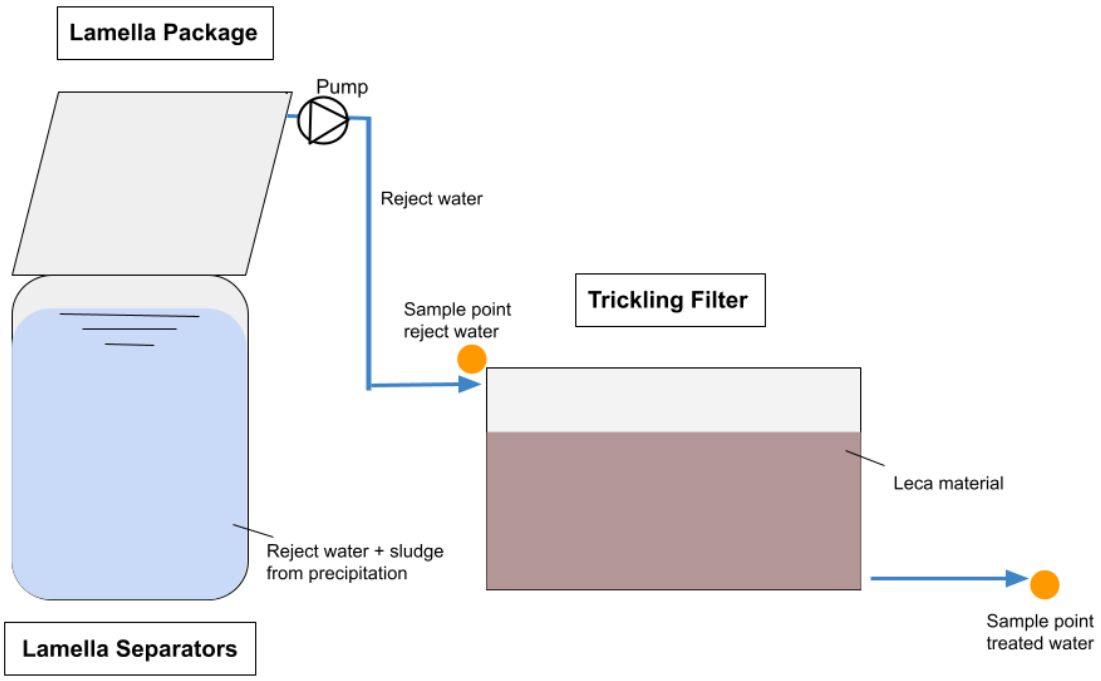

Figure 8: Illustration of the trickling filter pilot plant at Görvälnverket (Aspegren, 2023).

3.4.2 Biological Bench-Scale Tests

Biological bench-scale tests were done to evaluate the effectiveness of the leca granules in the trickling filter to degrade acrylamide in at two different temperatures (8 °C and 15 °C) and three different retention times (40-, 80- and 120 minutes) to degrade acrylamide. Therefore, the biological bench scale tests were done 12 weeks after the trickling filter was put in operation, for the bacteria to be mature enough.

The material used was two 2 l beakers, 2 l leca granules with mature biological film from the trickling filter, one water bath with a temperature of 15 °C and one with a temperature of approximately 8 °C, a solution with an acrylamide concentration of 100 mg/l, a 1 ml pipette, a 100 ml pipette and a Hach multimeter HQ2100 for measurement of electrandemical parameters.

5 l of reject water from one lamella separator was collected. Then the 1 ml pipette was used to mix 0.15 ml of the acrylamide solution with the 5 l reject water. The acrylamide concentration in the 5 l mixture aimed to be 3.0 µg/l but the real concentration ended up in 8.3 µg/l because of acrylamide in the reject water. By adding acrylamide, a detectable reduction of acrylamide would be possible to measure. A sample of the 5 l mixed acrylamide solution was taken, to later confirm the acrylamide concentration. After that 2 l leca granules with biofilm from the trickling filter were put in the two plastic containers. Coupled with that, 1,3 l of the 5 l mixed acrylamide solution was put in each beaker, but with a 30 min time difference (Fig. 9). The first beaker was put in a water bath with a temperature of about 8 °C, and the other breaker was put in a water bath with a temperature of 15 °C. Samples of 100 ml were taken from each beaker after 40 minutes, 80 minutes and 120 minutes with the 100 ml pipette. The beakers were stirred every five minutes and the pH, conductivity, oxygen and temperature were measured at the beginning and at the end of the tests with the Hach multimeter HQ2100.

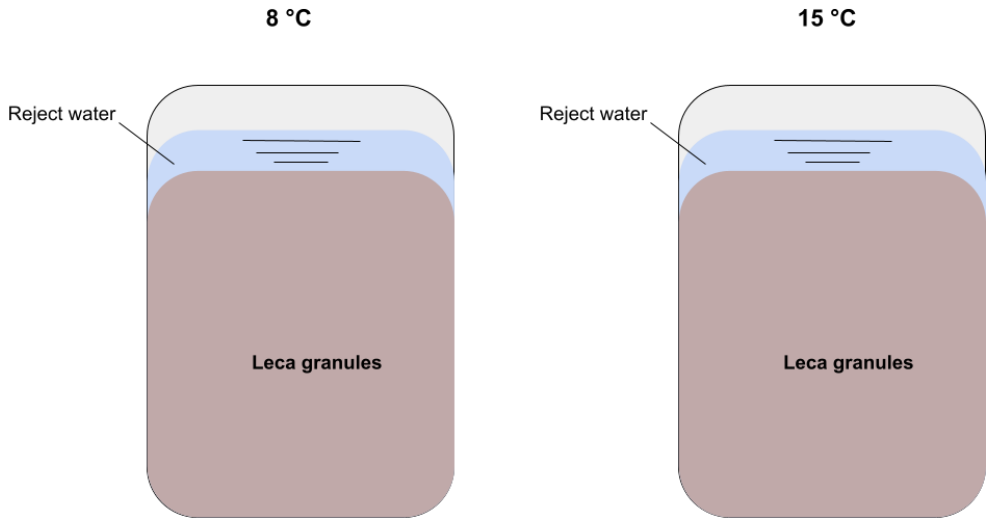

Figure 9: Illustration of the bench-scale tests (Aspegren, 2023).

3.4.3 Moving Bed Biofilm Reactor

The purpose of the MBBR test was to evaluate if the biofilm present in the MBBR pilot plant could biologically degrade acrylamide to levels under the drinking water limit of 0.10 µg/l.

Tests in a MBBR pilot plant took place at Görvälnverket as a biological treatment for the reject water leaving one of the lamella separators. The materials used for the MBBR were two 60 l plastic tanks, hoses, one U-bended pipe, valves, one flow meter, two aquarium pumps for air supply and stirring and 30 l Kaldnes biofilm carriers K5. For

measurements of electrandemical parameters directly in the water, Hach multimeter HQ2100 and YSI multiparameter sonde EXO2 were used.

The MBBR pilot plant was supplied with the same reject water from the lamella separator as the trickling filter. A valve and a flow meter were installed at the inlet of the first plastic tank, to regulate the water flow. A U-bended pipe was installed between the two tanks to connect them and to regulate the water level in the first tank. The reject water did flow through the first tank to the second tank and ended up in the drain (Fig. 10). Photographs of the MBBR pilot plant are shown in Appendix 1, Fig. 27. After the installation, 5,5 l of plastic biofilm carriers were placed in each of the two tanks. After 16 days 9.5 l new biofilm carriers were placed in each tank in a net to avoid them floating to the water surface. After 11 more days, the new biofilm carriers were let out of the net. The inlet- and outlet flow were regulated so that the water level in the two tanks was about the same and constant. The water flow was about 2.75 l/min in and out.

Capacity monitoring was done for the MBBR to measure a difference in the reduction of acrylamide after certain time periods, and if the reduction increased with time since the biofilm did mature. Samples of the inlet- and outlet water were taken 2 times during the 5 weeks of operation and sent to Eurofins for analysis of acrylamide concentration.

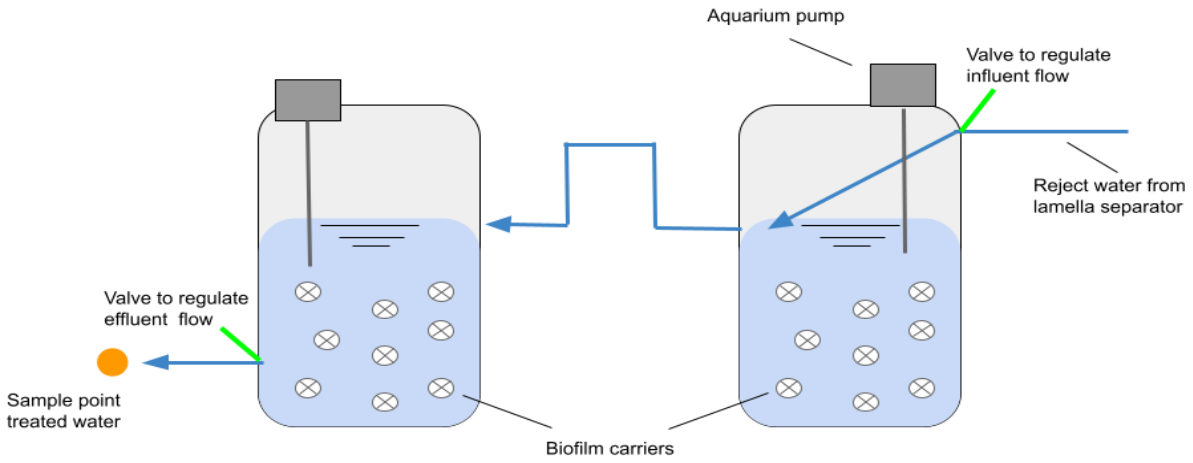

Figure 10: Illustration of the MBBR pilot plant at Görvälnverket (Aspegren, 2023).

3.5 Biological Tests in Water From Lake Mälaren

Biological tests with water from Lake Mälaren mixed with reject water from one of the lamella separators with added acrylamide solution were performed at Görvälnverket. The reason for the teats was to investigate the ability of the bacteria in the Lake to degrade acrylamide at 2 different temperatures (8 °C and 15 °C), since the reject water is discharged into the Lake The materials used were two 30 *l* plastic containers, a 1 ml pipette, a solution with an acrylamide concentration of 100 mg/l, Hach multimeter HQ2100 and YSI multiparameter sonde EXO2.

The tests began by mixing 0.2 ml of the acrylamide solution (100 mg/l) with 2.5 l reject with the help of a 1 ml pipette. After that, the plastic containers were both filled with 7.5 l of water from a 5 m depth in Lake Mälaren. The water came from a 5 m depth since the reject water containing acrylamide is discharged at that depth right outside the drinking water plant. The degradation ability of acrylamide in that specific part of Lake Mälaren was therefore of interest. The acrylamide and reject water mixture (2.5 l) was then put in each of the containers. The aim was to obtain an acrylamide concentration of 2 µg/l. The containers had a hole in them so that the bacteria always had oxygen access. One of the containers was put in a water bath with a temperature of 15 °C (Appendix I, Fig. 29), and the other was put in a location with a temperature of approximately 8 °C. The intention was to keep the water in this container at a temperature between 5 and 10 °C (Fig. 11).

The pH, temperature, dissolved oxygen content and conductivity were measured continuously every 15 minutes with YSI multiparameter sonde EXO2 throughout the test period in the container with 8 °C, and on occasions in the container with 15 °C. Samples of 100 ml were sent to Eurofins for analysis of the acrylamide concentration after 0, 4 and 13 days from each container.

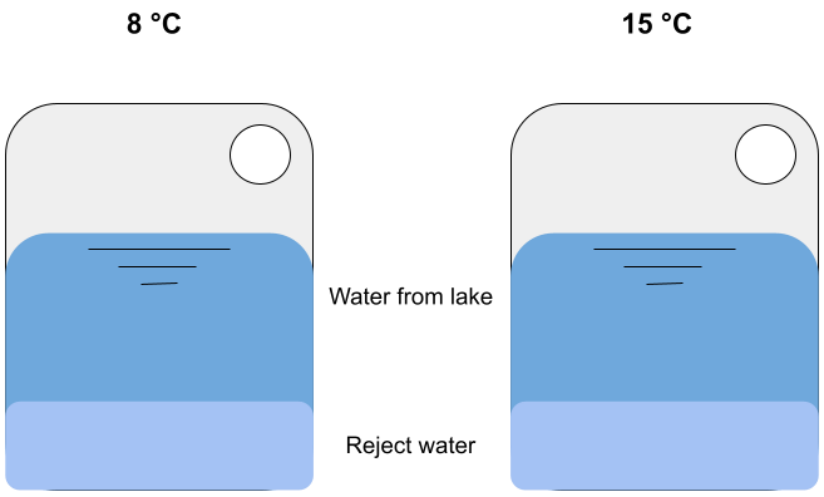

Figure 11: Illustration of the biological degradation tests in water from Lake Mälaren (Aspegren, 2023).

4. Results

The result part will present the results from the tests: ozonation, biofiltration, as well as the degradation tests in water from Lake Mälaren. The biofiltration tests include trickling filter, MBBR and biological bench-scale tests with leca granules from the trickling filter.

4.1 Ozonation

4.1.1 Ozone Test 1

In this section, the results from ozone test 1 are illustrated in two figures (Fig. 12a, Fig. 12b). In ozone test 1, reject water with an initial acrylamide concentration of 12 µg/l was exposed to 6 ozone doses. The results show a reduction of acrylamide concentration in the reject water when ozone is applied, which indicates that ozonation can be used to reduce acrylamide in water (Fig. 12a). What stands out is the significant decline of acrylamide concentration in the reject water between the ozone dose of 0.7 and 1.4 mg/l. This suggests a decrease in acrylamide concentration from 12 to 3.6 µg/l, a reduction of 8.4 µg/l. This corresponds to an acrylamide reduction of 70 %. The acrylamide concentration in the reject water is stagnating between the ozone dose of 2.1 and 7.0 mg/l and reaches a bottom low of 0.41 ug/l at the ozone dose of 3.5 mg/l (Fig. 12b) with an acrylamide reduction of 11.59 µg/l compared to the initial concentration. This is a reduction of 96.6 %. Interestingly, when 7 mg/l ozone is applied, the acrylamide concentration rises from 0.41 to 0.49 µg/l resulting in a decrease in reduction with 0.08 µg/l. For exact ozone doses, acrylamide concentrations and reduction, see Appendix II Table. 5.

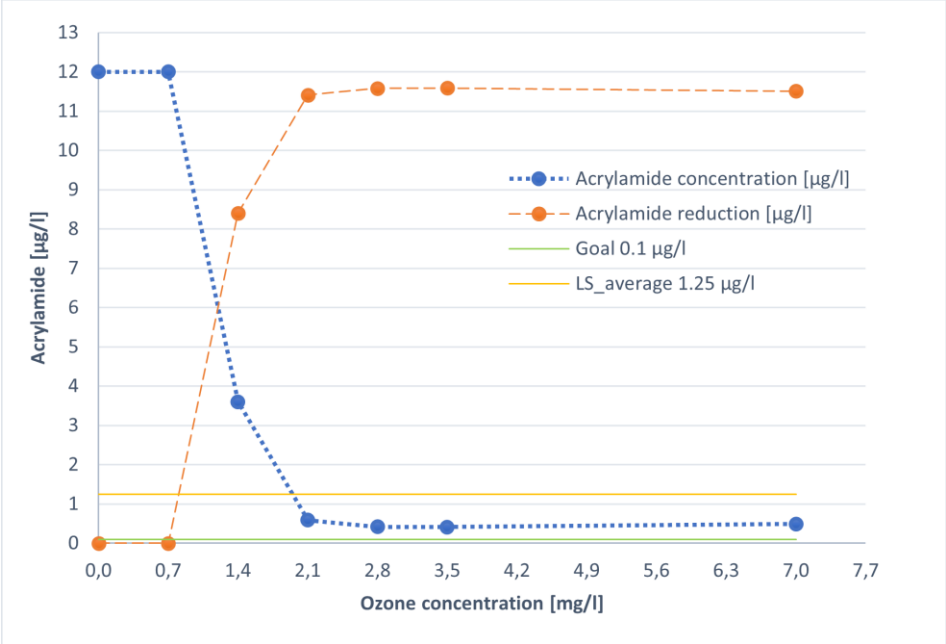

Figure 12a

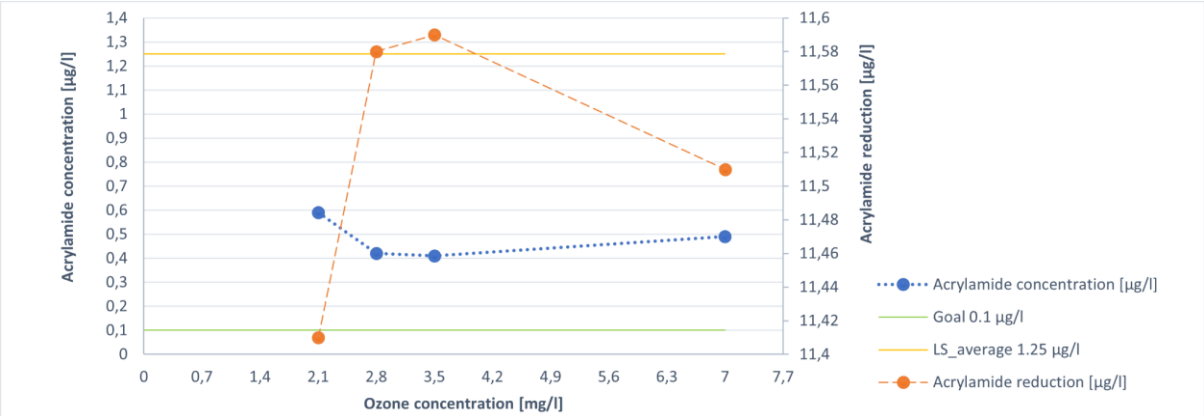

Figure 12b

Figure. 12: Ozone test 1 (initial level 12 µg/l). a) Levels of acrylamide concentration for ozone concentrations between 0.0 and 7.0 mg/l. Acrylamide reduction is the difference in acrylamide concentration between the reject water and the ozonated reject water and LS_average 1.25 µg/l is the average acrylamide concentration in the reject water the last year. b) A zoom in at the acrylamide concentrations between ozone doses 2.1 and 7.0 mg/l.

The temperature in the reject water between the ozone doses of 0.7 and 7.0 µg/l is on average 15.7 °C and varies between 14.47 °C and 16.67 °C (Fig. 13). The pH between the same ozone doses is stable with an average of 7.72. The conductivity in the reject water decreases between the ozone doses of 0.7 and 1.4 mg/l. This is a similar pattern to the acrylamide concentration (Fig. 12a), thus the conductivity and the acrylamide reduction could be related. The average conductivity is 254.67 µS/cm.

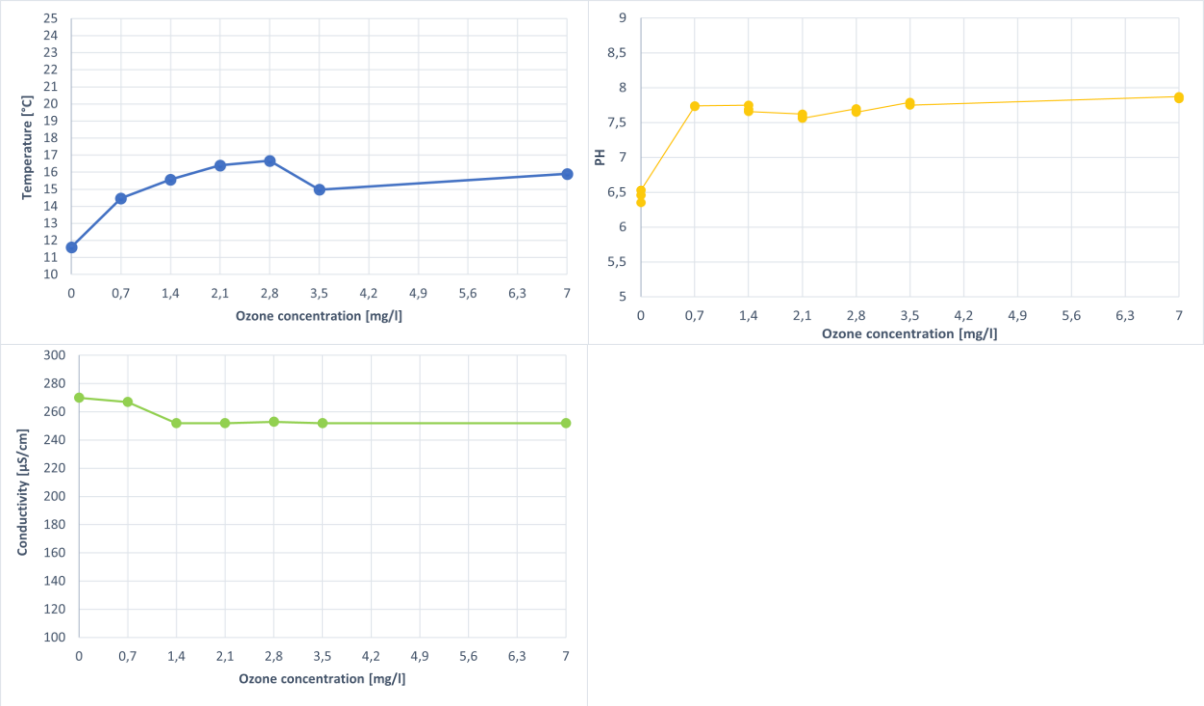

Figure. 13: Ozone test 1 (initial level 12 µg/l). Levels of temperature, pH and conductivity for ozone doses between 0.0 and 7.0 mg/l.

4.1.2 Ozone Test 2

In ozone test 2, reject water with an initial acrylamide concentration of 2.5 µg/l was exposed to 6 ozone doses. The most interesting part of this test is that the acrylamide concentration decreases to <0.05 µg/l when 0.7 mg/l ozone is applied, an acrylamide reduction of 2.45 µg/l (Fig. 14). This corresponds to a reduction of 98 %. This is the highest reduction during ozone test 2. However, the acrylamide concentration rises to 0.68 µg/l when the ozone dose of 1.2 mg/l is applied. At the ozone dose of 1.2 mg/l, the acrylamide reduction is 1.82 µg/l (72.8 %) which is the lowest reduction during ozonation test 2. After this, the acrylamide concentration is gradually decreasing with higher ozone doses. But between the ozone dose of 6.9 and 18.7 mg/l, the acrylamide concentration only decreases with 0.02 ug/l, from 0.13 ug/l to 0.11 ug/l. For exact ozone doses, acrylamide concentrations and reduction, see Appendix II, Table. 6.

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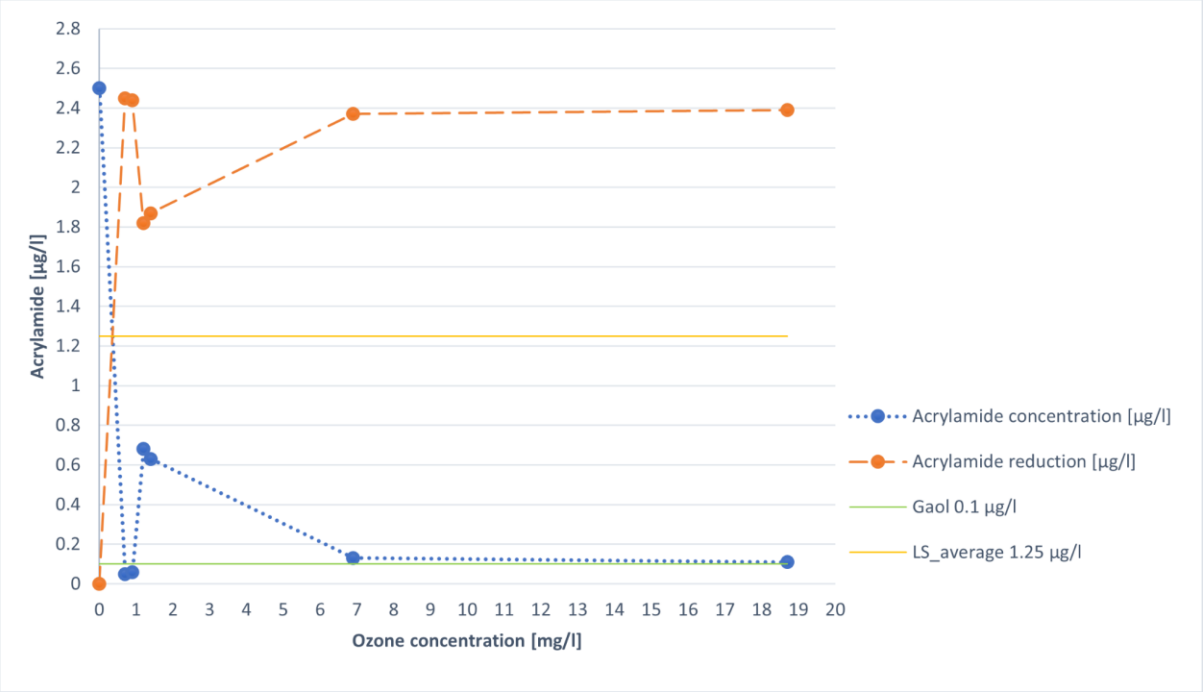

Figure 14: Ozonation test 2 (initial level 2.5 µg/l) . Levels of acrylamide concentration for ozone concentrations between 0.0 and 18.7 mg/l. Acrylamide reduction is the difference in acrylamide concentration between the reject water and the ozonated reject water and LS_average 1.25 µg/l is the average acrylamide concentration in the reject water the last year.

The temperature is relatively stable and is on average 15.3 °C between the ozone doses of 0.7 mg/l and 18.7 mg/l and varies between 14.2 °C and 16.4 °C. Further, the pH is stable and is on average 6.88. Finally, the conductivity is stable as well and on average 268.25 µS/cm (Fig. 15).

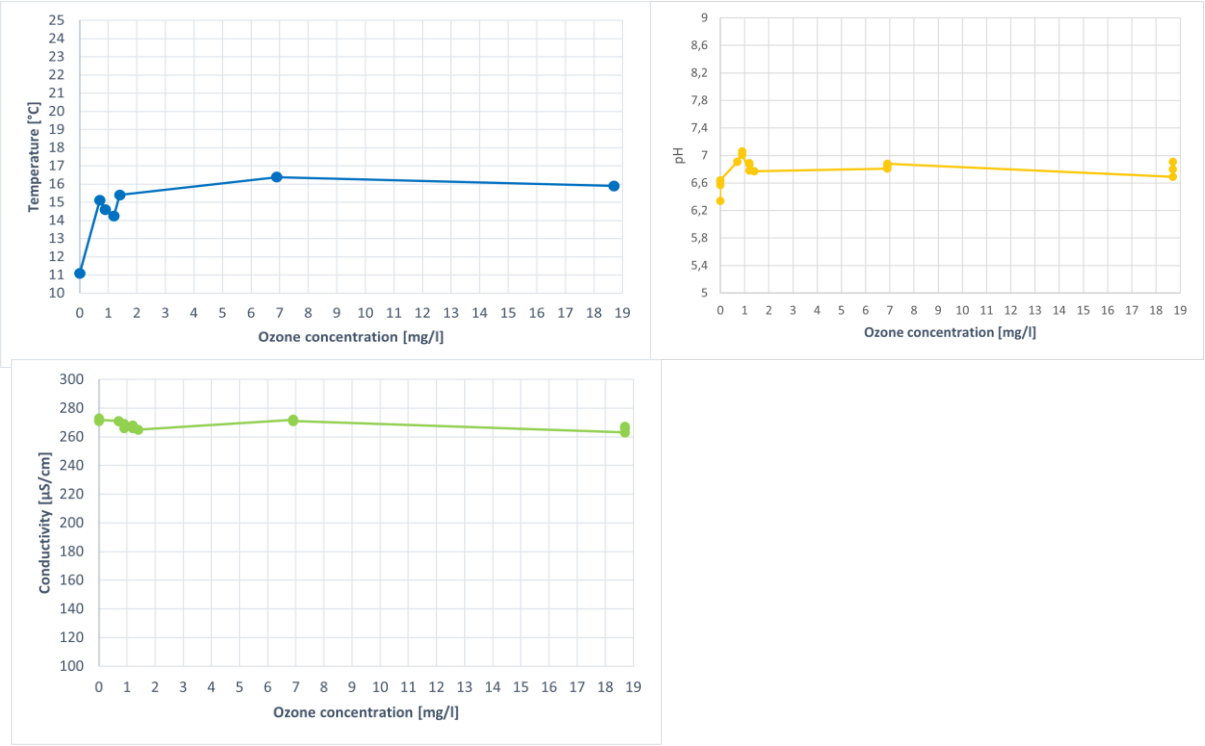

Figure 15: Ozone test 2 (initial level 2.5 µg/l. Temperature, pH and conductivity at ozone doses between 0.0 mg/l and 18.7 mg/l.

The results from the oxidative stress analysis from ozone test 2 reveal that the reject water with 2.5 µg/l acrylamide generates the most oxidative stress, precisely 16.4 µg/L tBHQ-equivalents (Table. 4). The samples treated with an ozone dose of 1.2 mg/l and 6.9 mg/l from ozone sample 3 and 5 respectively, do generate oxidative stress lower than 9.15 µg/L tBHQ-equivalents. It is not possible to know the exact result for these 2 samples because the limit for detection is 9.15 µg/l tBHQ-equivalents. The results for genotoxicity are shown in Table 4 as well.

Table 4: Ozone test 2 (initial level 2.5 µg/l. Oxidative stress presented in biological equivalent concentration with the reference substance tertiary butyl hydroquinone (tBHQ) and genotoxicity for three samples.

Sample	Oxidative stress tBHQ-equivalents [µg/L]	Genotoxicity
Ozone test 2 (reject water + added acrylamide)	16.4	Yes
Ozone test 2, sample 3 (1.2 mgO_3/l)	<9.15	Yes
Ozone test 2, sample 5 (6.9 mgO_3/l)	<9.15	No

4.1.3 Ozone Test 3

In ozone test 3, deionized water with an initial acrylamide concentration of 6.6 ug/l was exposed to 6 ozone doses. What stands out in the results is that the acrylamide concentration reaches a level below 0.05 µg/l when 0.7 mg/l ozone is applied (Fig. 16). This is similar to ozone test 2. Furthermore, the results reveal that ozone doses up to 2.8 mg/l do end up in the same acrylamide concentration, namely <0.05 µg/l. For exact ozone doses, acrylamide concentrations and reduction, see Appendix II, Table. 7.

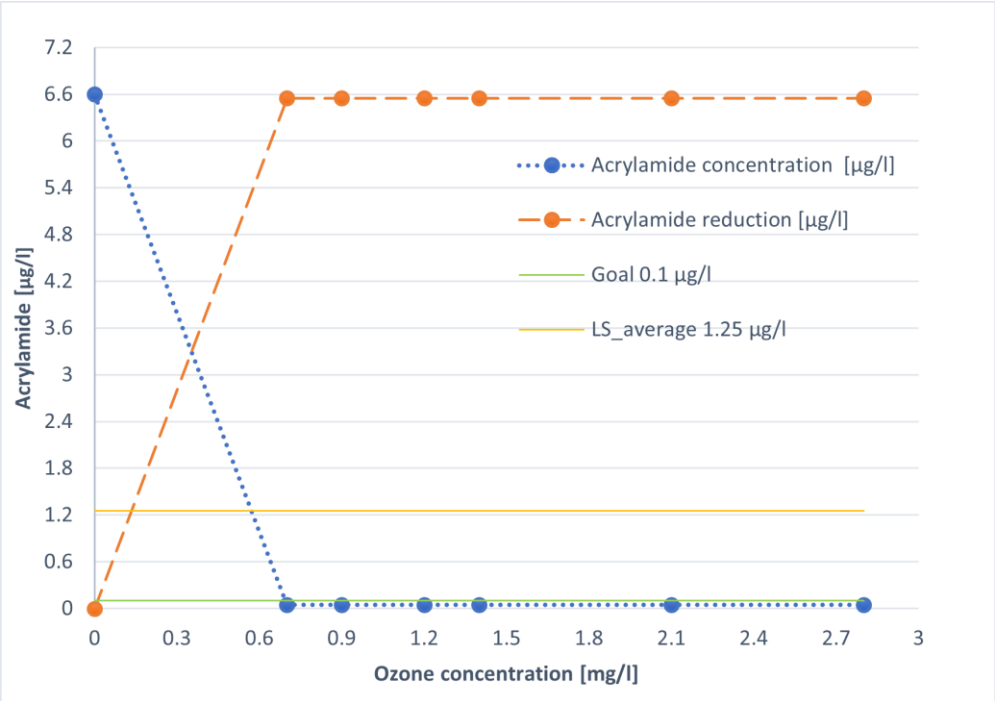

Figure 16: Ozonation test 3 (initial level 6.6 µg/l). Levels of acrylamide concentration for ozone concentrations between 0.0 mg/l and 2.8 mg/l. Acrylamide reduction is the difference in acrylamide concentration between the reject water and the ozonated reject water and LS_average 1.25 µg/l is the average acrylamide concentration in the reject water the last year.

The temperature in ozone test 3 is on average 19.9 °C between the ozone doses of 0.7 mg/l and 2.8 mg/l and varies between 19.0 °C and 20.6 °C (Fig. 17). Moreover, the pH is in average 6.35 and varies between 5.87 to 7.30. The conductivity is low compared to the other ozone tests. The reason is the deionized water used.

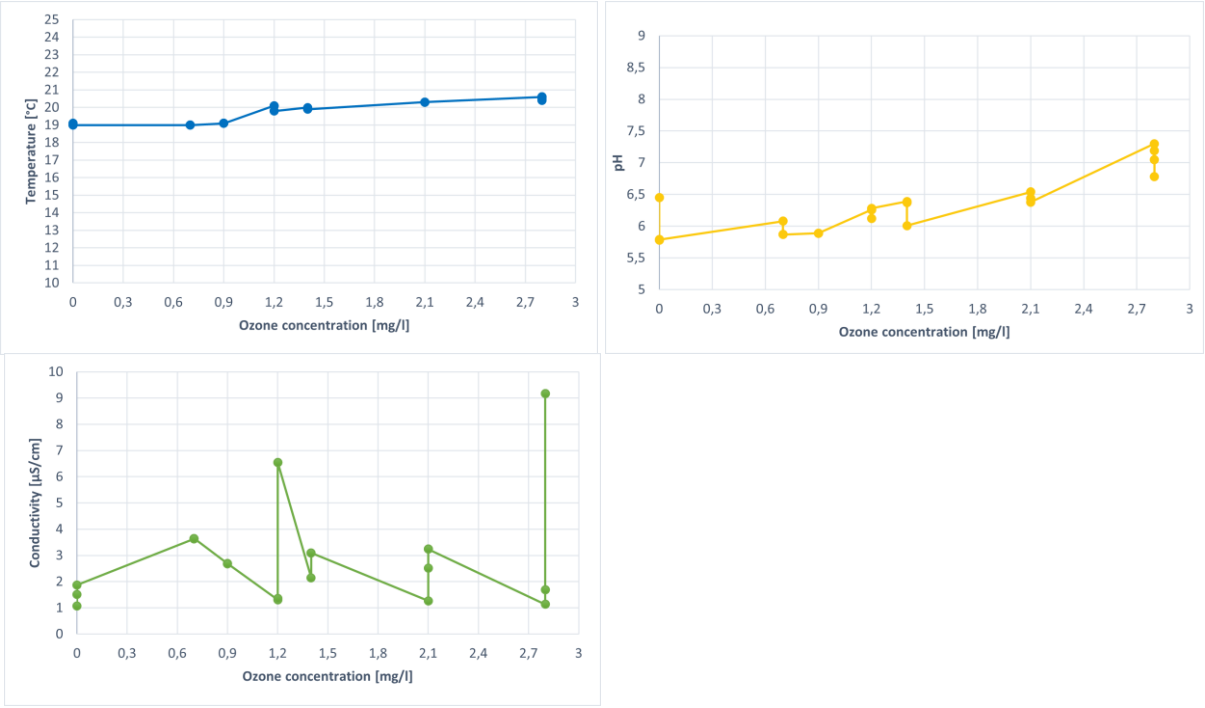

Figure 17: Ozone test 3 (initial level 6.6 µg/l). Temperature, pH and conductivity at ozone doses between 0.0 mg/l and 2.8 mg/l.

4.2 Biofiltration tests

4.2.1 Trickling Filter

The result from the biological degradation test in the trickling filter show a reduction of acrylamide in the reject water after 29 days, to clarify, a reduction of 0.12 µg/l or 71 %, and the reduction gradually increases until day 52 (Fig. 18). After 52 days the reduction is at its peak of 5.59 µg/l or 94.7 %. This suggests that the trickling filter successfully reduces acrylamide. Interestingly, the reduction is then decreases and reaches a level of 1.6 µg/l or 37.21 % after 85 days.

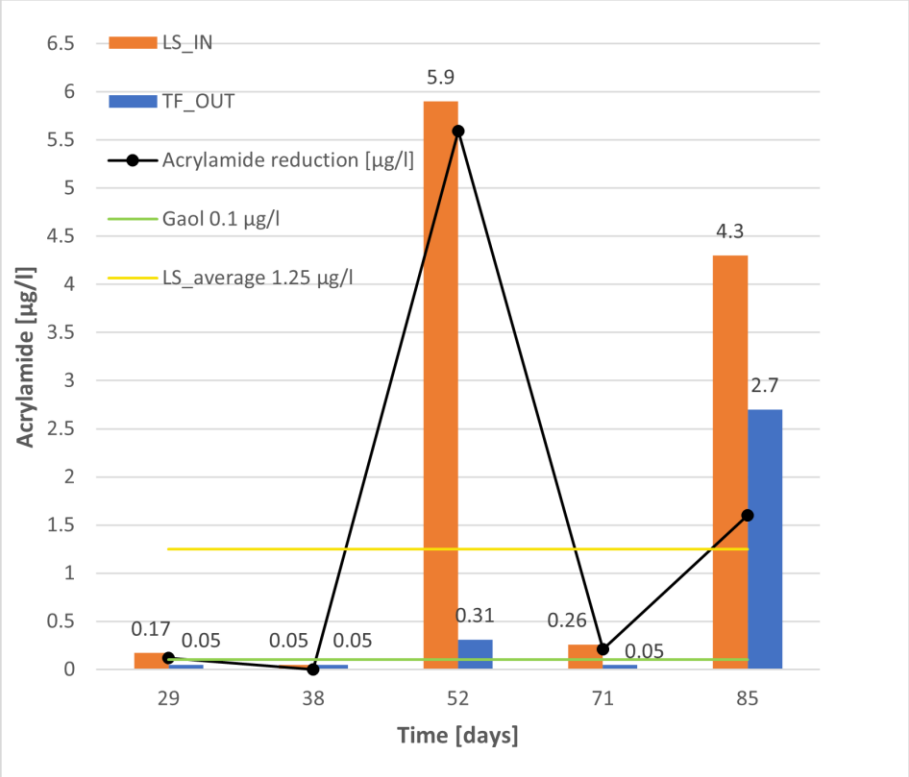

Figure 18: Acrylamide reduction in the trickling filter between 0 and 85 days. LS_IN is the acrylamide concentration in the inlet water from the lamella separator, TF_OUT is the acrylamide concentration in the outlet water leaving the trickling filter, Acrylamide reduction is the difference in acrylamide concentration between the inlet and outlet water and LS_average 1.25 µg/l is the average acrylamide concentration in the reject water the last year.

The temperature in the inlet and outlet water of the trickling filter does not differ very much and is relatively stable (Fig. 19). The average temperature in the inlet water is 4.6 °C and in the outlet water 5.5 °C. Further, the pH in the outlet water is on average 2.49 % higher than the pH in the inlet water after 2 months of operation and forward. The average pH in the inlet water is 6.62. The conductivity in the inlet and outlet water is stable and is on average 156.12 µS/cm in the outlet water and 152.17 in the inlet water. Finally, the dissolved oxygen in the outlet water is following the same pattern as the dissolved oxygen in the inlet water as expected. Interestingly, the dissolved oxygen in the outlet water leaving the trickling filter is on average 5,6% lower than in the inlet water.

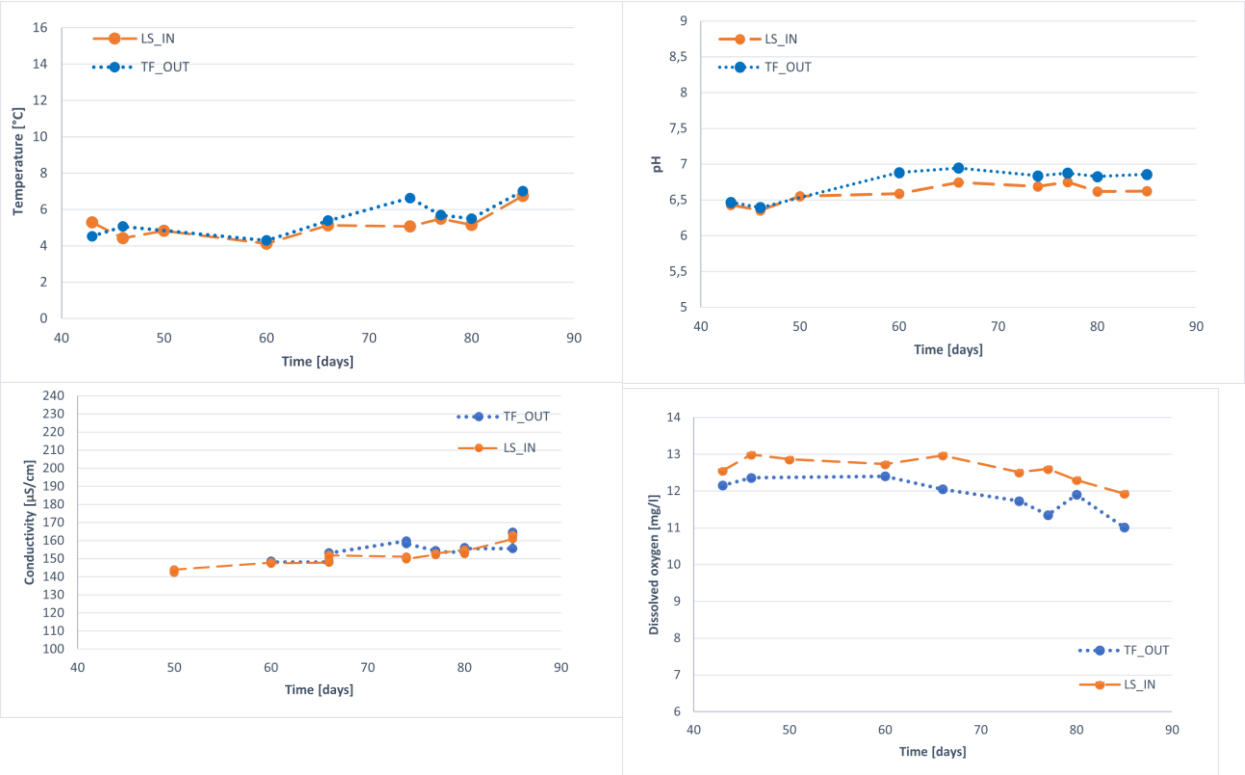

Figure 19: Levels of temperature, pH, conductivity and dissolved oxygen in the inlet and outlet water of the trickling filter between 2023-03-14 to 2023-04-25. LS_IN represents the inlet water from the lamella separator. TF_OUT represents the outlet water leaving the trickling filter.

4.2.2 Biological Bench-Scale Tests

The purpose of the biological bench-scale tests were to evaluate the biological degradation of acrylamide in 2 different temperatures (8 °C and 15 °C) and 3 different retention times (40-, 80- and 120 minutes). The leca granules from the biofilter were used for the degradation. The results from the biological bench scale tests show that in both temperatures, the acrylamide with an initial concentration of 8.3 µg/l was reduced to below 0.05 µg/l after 40 minutes (Fig. 20). This is a reduction of 99.4 %. The acrylamide concentration is stable at 0.05 µg/l after 80 minutes and 120 minutes as well. Because of the detection limit for acrylamide, it is not possible to get a more accurate result than this.

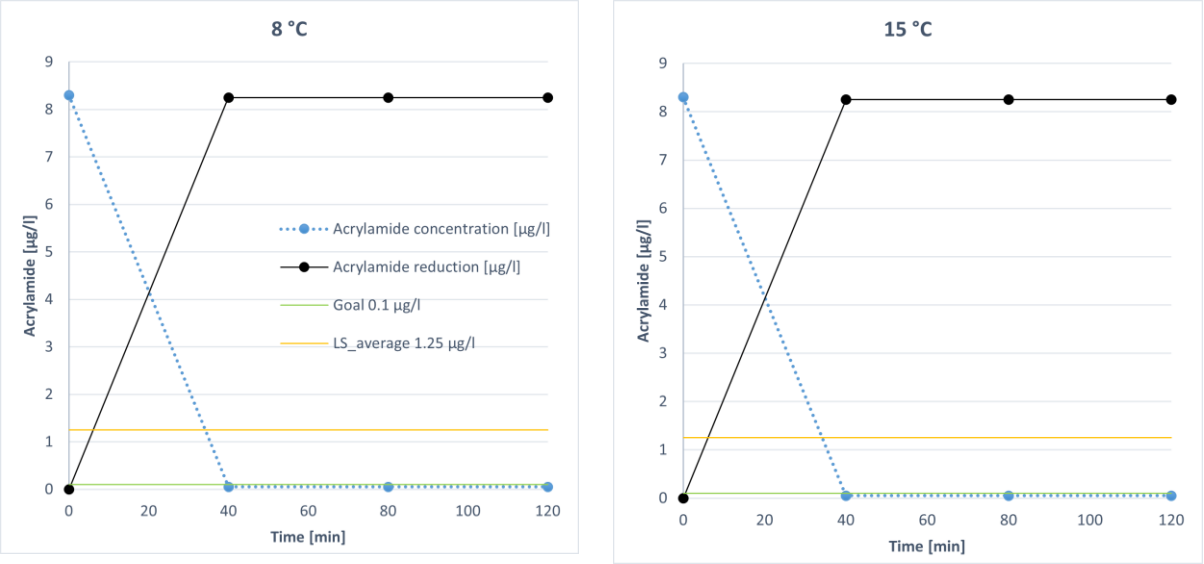

Figure 20: Acrylamide concentration in the biological bench-scale tests with a temperature of 8 °C and 15 °C between 0 and 120 minutes. Acrylamide reduction is the difference in acrylamide concentration between the inlet and outlet water and LS_average 1.25 µg/l is the average acrylamide concentration in the reject water the last year.

The temperature, dissolved oxygen, pH and conductivity are relatively stale throughout the test period for both tests. For the test with a temperature of 8 °C, the average temperature is 8.0 °C, the average dissolved oxygen concentration is 10.58 mg/l, the average pH is 6.51 and the average conductivity is 383.14 µS/cm (Fig. 21a). For the test with a temperature of 15 °C, the average temperature is 12.3 °C, the average dissolved oxygen concentration is 10.16 mg/l, the average pH is 6.75 and the average conductivity is 274.57 µS/cm (Fig. 21b).

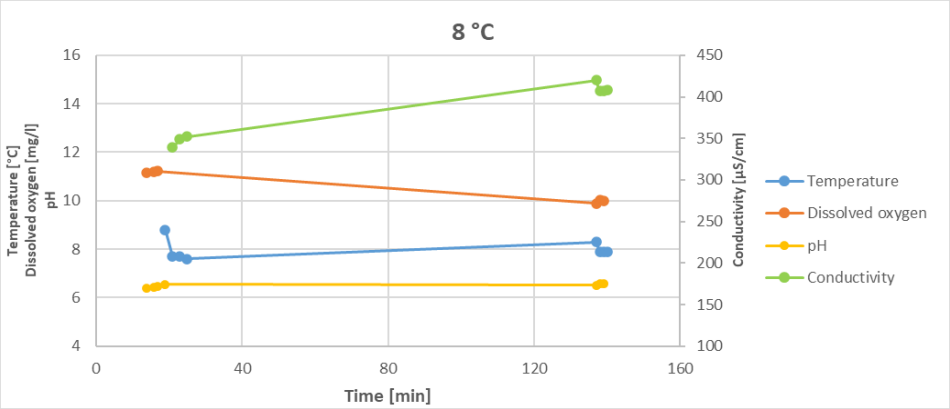

Figure 21a

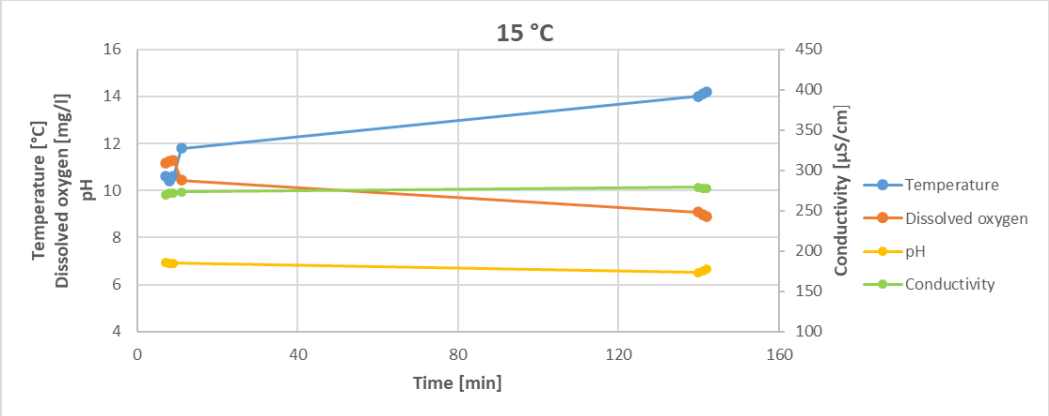

Figure 21b

Figure 21: a) Levels of temperature, dissolved oxygen, pH and conductivity for the bench-scale test with a temperature of 8 °C. b) Levels of temperature, dissolved oxygen, pH and conductivity for the bench-scale test with a temperature of 15 °C.

4.2.3 Moving Bed Biofilm Reactor

The MBBR test results imply that there is a reduction of the acrylamide concentration in the reject water going through the MBBR pilot plant (Fig. 22). After 21 days of operation, the acrylamide reduction is 0.21 µg/l or 80.77 %. After 35 days, the acrylamide reduction is 1.4 µg/l or 32.56 %.

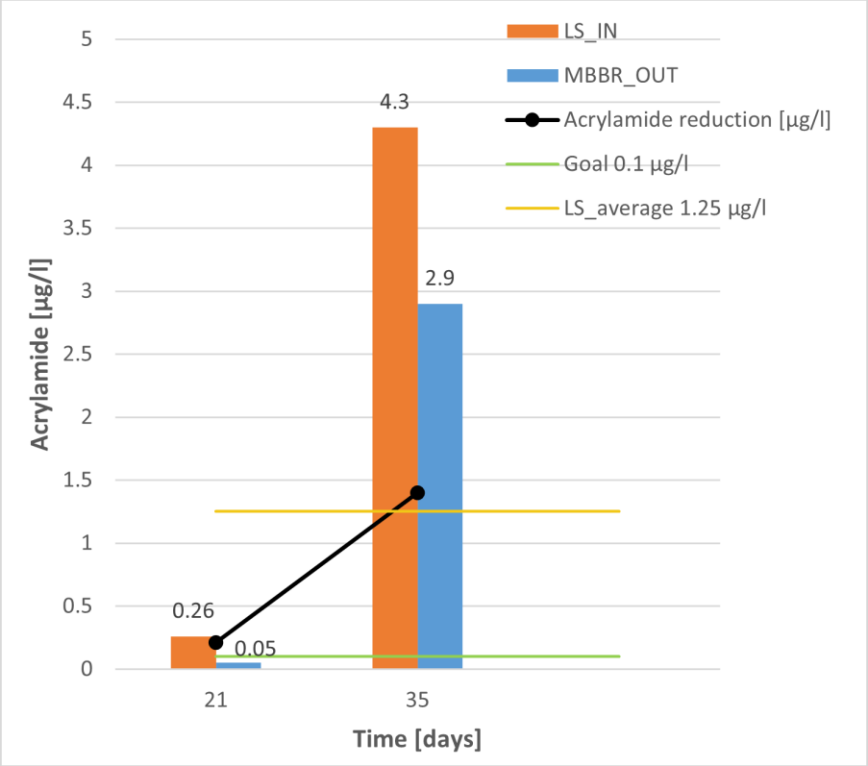

Figure 22: Acrylamide reduction in the MBBR pilot plant between 21 and 35 days. LS_IN is the acrylamide concentration in the inlet water from the lamella separator, MBBR_OUT is the acrylamide concentration in the outlet water leaving the MBBR, Acrylamide reduction is the difference in acrylamide concentration between the inlet and outlet water and LS_average 1.25 µg/l is the average acrylamide concentration in the reject water the last year.

The temperature in the inlet water from the lamella separator has an average of 5.1 °C and varies between 4.1 °C and 6.7 °C (Fig. 23). The outlet water has an average temperature of 5.8 °C and varies between 4.3 °C and 7.5 °C. Furthermore, the pH in the inlet and the outlet water is stable, but is on average 2.84 % higher in the outlet water than in the inlet water from 1 month of operation and forward. The conductivity in the outlet water is on average 2,39 % higher than in the inlet water during the test period. Finally, the dissolved oxygen in the outlet water from the MBBR starts to decrease 2023-04-06, which is 16 days after the start of the test. Between 2023-04-14 and 2023-04-25 there is a decrease (9.68%) in dissolved oxygen in the outlet water from 12.7 to 11.48 mg/l.

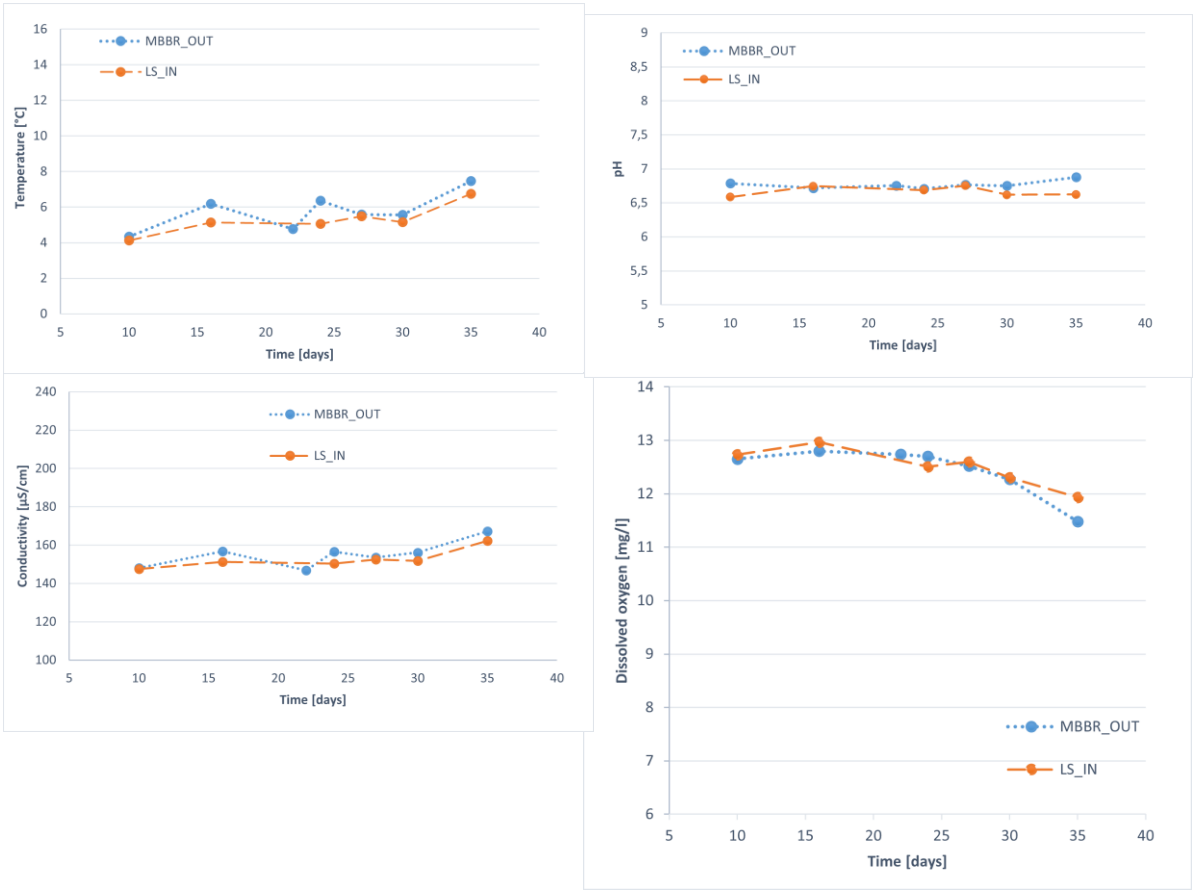

Figure 23: Levels of temperature, pH, conductivity and dissolved oxygen in the inlet and outlet water of the MBBR between 2023-03-14 to 2023-04-25. LS_IN represent the inlet water from the lamella separator. MBBR_OUT represent the outlet water leaving the MBBR.

4.3 Biological Tests in Water From Lake Mälaren

Even though the initial acrylamide concentration is lower than planned, it is possible to observe a degradation of acrylamide in both temperatures (8 °C and 15 °C). In the test with 15 °C, the acrylamide is reduced from 0,09 µg/l to less than 0.05 µg/l after 4 days as well as after 13 days (Fig. 24). In the test with 8 °C, a rise of acrylamide concentration is observed after 4 days from 0,1 µg/l to 1,2 µg/l followed by a drop to less than 0,05 µg/l after 15 days.

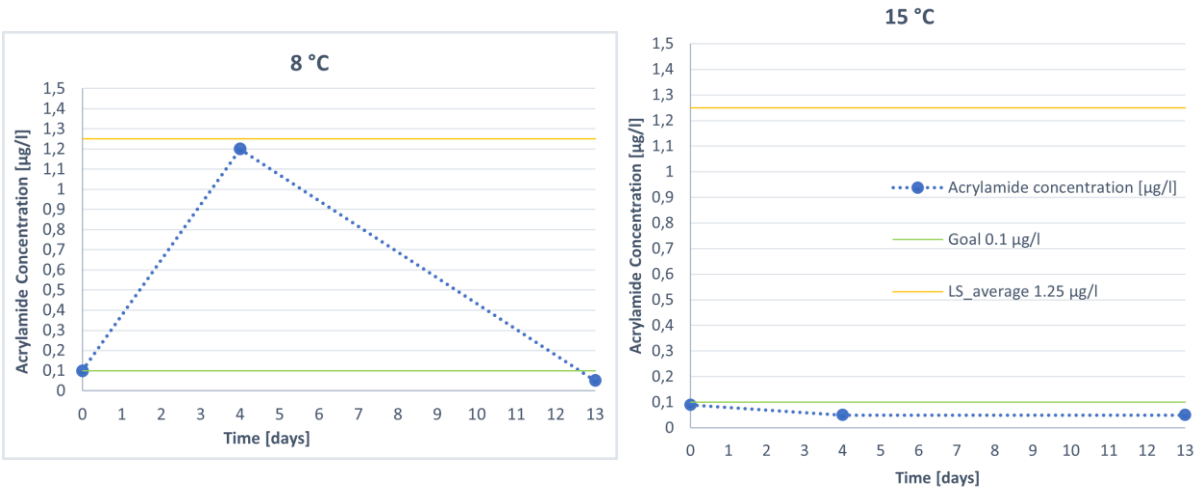

Figure 24: Levels of acrylamide concentration in water from Lake Mälaren with a temperature of 8 °C and15 °C between 0 and 13 days. LS_average 1.25 µg/l is the average acrylamide concentration in the reject water the last year.

The background parameters for both tests are stable during the test period. For the test with a planned temperature of 8 °C, the average temperature is 9.0 °C, the average dissolved oxygen concentration is 11.33 mg/l, the average pH is 7.65 and the average conductivity is 254.52 µS/cm (Fig. 25a). For the test with a planned temperature of 15 °C, the average temperature is 15.1 °C, the average dissolved oxygen concentration is 10.27 mg/l, the average pH is 7.95 and the average conductivity is 201.09 µS/cm (Fig. 25b).

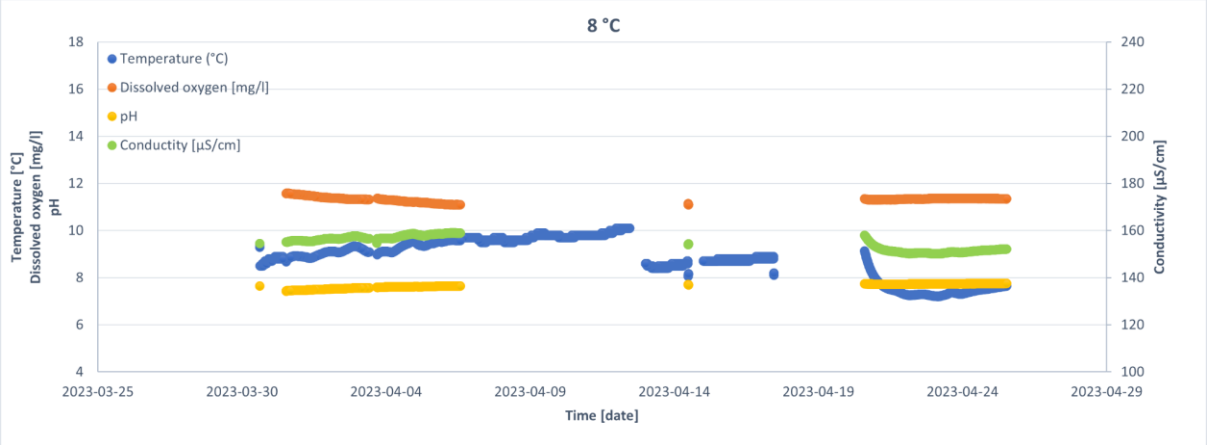

Figure 25a

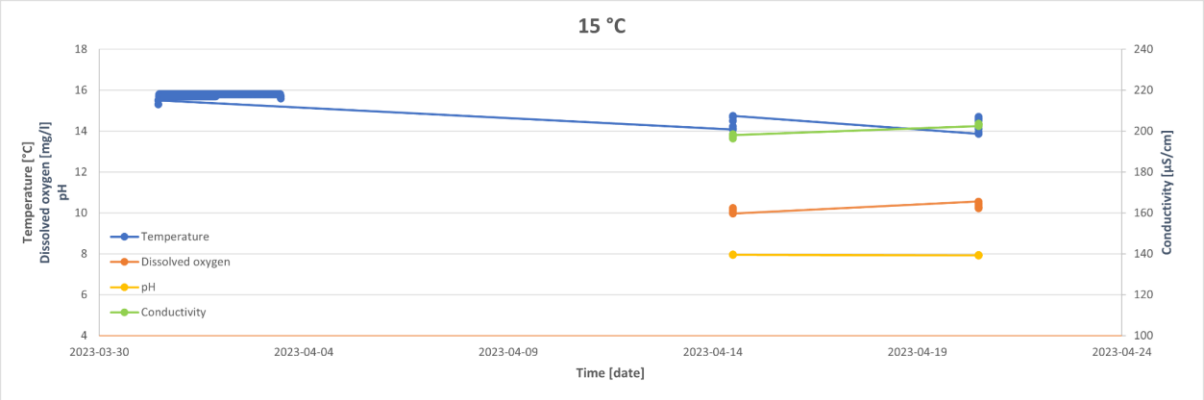

Figure 25b

Figure 25: a) Levels of temperature, dissolved oxygen, pH and conductivity for the test in water from Lake Mälaren with a temperature of 8 °C, between 2023-03-30 and 2023-04-25. b) Levels of temperature, dissolved oxygen, pH and conductivity for the test in water from Lake Mälaren with a temperature of 15 °C, between 2023-03-30 and 2023-04-25.

5. Discussion

5.1 Ozonation

All ozone tests showed a reduction of acrylamide present if reject water when ozone was used. However different applied doses gave different reduction rates. The fact that the acrylamide concentration increases in ozone test 2 when the ozone doses of 1.2 mg/l and 1.4 mg/l are applied to the reject water could be a result of polyacrylamide present in the reject water. The hydroxyl radicals from the ozone could break the polymer chain and a release of acrylamide could occur as Xiong et al. (2018) explain. The same phenomenon could explain the increase in acrylamide concentration in ozone test 1 when 7.0 mg/l ozone is applied. This explanation is strengthened in ozone test 3. The Milli-Q water does not contain any polyacrylamide and the acrylamide reduction in that water is stable throughout all ozone doses. Studies also states that acrylamide can be formed after degradation of polyacrylamide in general (Spraggs, 1982; Krautter, 1986). However, there are contradicting research suggesting that there is a low risk that acrylamide is formed after polyacrylamide is degrading (Murgatroyd et al, 1996; Wahlberg & Paxéus, 2003).

In ozone tests 2 and 3, the highest ozone doses do not degrade acrylamide better than lower ozone doses. In ozone tests 2 and 3, the acrylamide reduction is 2.45 µg/l and 6.55 µg/l respectively when 0.7 mg/l ozone is applied, but in ozone test 1, 0.7 mg/l ozone does not degrade acrylamide at all. The content of dissolved organic matter in the reject water 2 days before the collection of reject water for ozone test 1 was on average 83.31 QSU, and on average 42.87 QSU 1 day after the collection for ozone tests 2 and 3 (Appendix III. Fig. 30). According to Baresel et al (2019) and Fu et al (2019), ozone does break down dissolved organic matter. Consequently, the ozone might only have degraded the dissolved organic matter instead of the acrylamide in the reject water used in ozone test 1 because of the high content of dissolved organic matter in the reject water. In ozonation test 3, the acrylamide is degraded to a concentration below 0.05 µg/l when 0.7 mg/l up to 2.8 mg/l ozone is applied. This is expected since the water used was Milli-Q water which does not contain any organic matter for the ozone to degrade instead of the acrylamide.

The potential degradation of dissolved organic matter in the reject water might also lead to an increase in acrylamide. As Cary et al (1987) and Murgatroyd et al (1996) explain, polyacrylamide does adsorb to dissolved organic matter easily. If the ozone break down the dissolved organic matter, the adsorbed polyacrylamide could potentially get free in the water, and then degraded by the ozone to acrylamide monomers. However, further experiments have to be done to evaluate this.

In ozone test 1, the high increase in reduction between the ozone doses of 0.7 mg/l and 1.4 mg/l is corresponding to the rapid drop in conductivity between those ozone doses. In other words, the number of dissolved ions is decreasing, like dissolved organic

matter that degrades by the ozone according to Baresel et al (2019) and Fu et al (2019). A change in conductivity indicates that something is happening in the water, which could also be the degradation of acrylamide. However, the correlation between conductivity and acrylamide degradation needs to be further studied.

In ozone test 1, the acrylamide concentration in the reject water did not reach Norrvatten´s goal of 0.10 µg/l, which is the limit for drinking water quality in Sweden (SLVFS 2001:30). On the other hand, the initial acrylamide concentration of 12 µg/l is very high compared to the average concentration at Görvälnverket the last year, which is 1.25 µg/l (Norrvatten, 2023). When an ozone dose of 1.4 mg/l is applied to the reject water, a reduction in acrylamide concentration of 8.4 µg/l is obtained. To reduce the average acrylamide concentration at Görvälnverket to 0.10 µg/l, the reduction needs to be at least 1.15 µg/l. The reduction obtained from 1.4 mg/l ozone is more than enough. In ozone test 2 with reject water with an initial concentration of 2.5 µg/l of acrylamide, the goal of 0.10 µg/l acrylamide is reached when 0.7 mg/l and 0.9 mg/l ozone is applied.

When ozone is applied to the reject water used for ozone test 2 with 2.5 µg/l acrylamide, the oxidative stress decreases compared to the untreated reject water. This suggests that the untreated reject water could generate more oxidative stress than water treated with ozone. To clarify, the ozone might not rise the oxidative stress of the reject water. When the reject water is exposed to an ozone dose of 6.9 mg/l, there is no genotoxicity in the water. However, the other 2 samples (reject water with added acrylamide and the same water treated with 1.2 mg/l ozone) were genotoxic. This suggests that higher ozone doses do not generate new genotoxicity and also degrade the initial genotoxicity present in the reject water caused by acrylamide. This is similar to other ozone tests done by IVL Swedish Environmental Research Institute where an ozone dose of 7.5 mg/l did not show any toxic effects on crustacean Nitocra spinipes or algae Selenastrum capricornutum. In the same report by IVL Swedish Environmental Research Institute, it is stated that stable bi-product could be generated from high ozone doses (Baresel et al, 2017). However, the current results indicate that high ozone doses does not generate bi-products that cause oxidative stress or genotoxicity at least.

The results should be interpreted with caution since the exact ozone dosages are uncertain. The time of application is the only source of measurement for the ozone doses applied. However, the results provide a comparison between low and higher ozone doses and can be used to identify patterns, as well as pave the way for further in-depth experiments.

The expected initial acrylamide concentrations in ozone tests 2 and 3 were lower than expected. In ozone test 2 the expected initial acrylamide concentration was 5 µg/l, but only half of it (2.5 µg/l) was detected by Eurofins. In ozone test 3, the expected initial acrylamide concentration was 10 µg/l but only 6.6 µg/l was detected, It is possible

that the acrylamide added to the reject water in the beginning of the tests did degrade before the analysis of the acrylamide concentration by Eurofins began. Moreover, another possible explanation is that the acrylamide solution of 100 mg/l was not stable. The acrylamide might have broken down until ozone tests 2 and 3 began. This explanation is strengthened since the initial acrylamide concentration in ozone test 1 was expected. Further research should be done to study the stability of acrylamide in water and how fast it can be degraded.

5.2 Biofiltration Tests

5.2.1 Trickling Filter

The reduction of acrylamide in the trickling filter is declining after it reaches the peak of 5.59 µg/l reduction after 52 days. One explanation could be that the inlet flow into the trickling filter was not totally stable, due to the flow meter. This means that the inlet flow could have been higher sometimes during the test period. As a result, more acrylamide would flow into the trickling filter, and the bacteria might not have been able to degrade everything.

Another explanation could be that the acrylamide concentration in the inlet water is not enough for all bacteria to consume at the end of the test period. The number of bacteria is probably higher and the bacteria itself more mature at the end of the test period compared to the start. Besides, if the acrylamide-degrading enzyme acrylamidase is a part of these bacteria, which it is for other acrylamide-degrading bacteria, the acrylic acid which is left after the acrylamide degradation could speed up the bacterial growth (Shanker et al, 1990; Zabaznaya et al, 1998; Shukor et al, 2009; Sharma et al. 2009; Cha & Chambliss, 2011; Emmanuel et al, 2013; Guezennec et al, 2015; Bedade & Singhal, 2017; Joshi & Abed, 2017). More bacteria means that they need more acrylamide to consume. If the acrylamide concentration in the inlet water is not enough for all the bacteria, not enough acrylic acid is produced and the bacteria might die because of the lack of that.

The fact that the reduction of acrylamide rises in the first 52 days correlates to the trend in dissolved oxygen in the water leaving the trickling filter. The result shows that the dissolved oxygen is higher in the inlet water than in the outlet water, which indicates that there are aerobe bacteria in the trickling filter which consumes oxygen while degrading acrylamide. Since the bacteria in the trickling filter are maturing and the number is growing with time, more oxygen is also consumed by the bacteria. This matches the statement by Guezennec et al (2015) that most degradation of acrylamide is aerobic processes, at least in the natural environment. Besides, some of the fastest acrylamide-degrading bacterial strains are aerobic (Robert et al, 1994; Granum, 2002; Bottone, 2010; Lakshmikandan et al, 2014). In addition, the dissolved oxygen consumption suggests that the bacteria in the trickling filter are active, which is crucial when it comes to degrading acrylamide.

The temperature of the inlet water as well as the outlet water did rise during the test period. But a warmer climate should not affect the bacteria negatively, rather the other way around since 20 °C to 30 °C is optimal for many acrylamide-degrading bacteria according to other research (Lakshmikandan et al, 2014; Emmanuel et al, 2013; Shukor et al, 2009; Bedade & Singal, 2018). Since the average temperature in the inlet water is as low as 4.6 °C, the high reduction of 94.7 % or 5.59 µg/l is a positive result, and means that the bacteria in the trickling filter are able to degrade acrylamide even at low temperatures.

The results of the acrylamide reduction indicate that the trickling filter is able to reach Norrvatten´s goal of an acrylamide concentration of 0.10 µg/l in the reject water. When the acrylamide concentration in the inlet water is 5.9 µg/l and 4.3 µg/l, the acrylamide reduction is 5.59 µg/l and 1.6 µg/l respectively. This is more than enough since the average acrylamide concentration in the reject water the last year was 1.25 µg/l (Norrvatten, 2023). This means that the trickling filter could be enough to reach the goal.

The tests done to measure the real retention time indicate that the retention time in the trickling filter is shorter than expected, approximately 30 minutes, whereas the empty bed contact time is calculated to be 2 hours. Still, the results show a high reduction of acrylamide after 52 days, which is surprisingly successful. This is a promising finding for the biological degradation of acrylamide. To clarify, biological degradation of acrylamide in a trickling filter can be done relatively easily, probably with retention times much shorter than 2 hours.

On the other hand, a limitation is that the retention time is not exact. Further studies of acrylamide degradation in a trickling filter when the retention time is better understood are therefore recommended. It is conceivably hypothesised that the reduction of acrylamide is correlated to the retention time and a longer retention time would results in a higher reduction after fewer days than these results show. This is an important issue for further research about the biological degradation of acrylamide in a trickling filter.

5.2.2 Biological Bench-Scale Tests

An unexpected result is that the acrylamide concentration reached 0.05 µg/l in the reject water after only 40 minutes of degradation. This provides further support that the bacteria growing on the leca granules in the trickling filter are able to degrade acrylamide well in a short period of time.

When it comes to the temperatures, one can argue that a higher temperature is preferable according to research (Lakshmikandan et al, 2014; Emmanuel et al, 2013; Shukor et al, 2009; Bedade & Singal, 2018). However, it seems that the temperature did not have a significant impact on the acrylamide degradation, since both tests did degrade acrylamide to 0.05 µg/l after only 40 minutes. But it is most probable that the degradation is still quicker in the water with 15 °C and would have been observed if samples were taken more frequently within the first 40 minutes.

Still, further research should be done to evaluate the minimum temperature for acrylamide-degrading bacteria and how the temperature is affecting the degradation, since most studies show that it does. This is particularly important if a biological filter will be used in a water treatment plant. Yet, the results suggest that the minimum temperature for acrylamide-degrading bacteria is lower than 8 °C, unlike the bacterial strain Bacillus cereus whose minimum temperature for degrading acrylamide is 20 °C according to Shukor et al, 2009.

Both tests reduce the acrylamide with 8.25 µg/l and reach Norrvatten´s goal of 0.10 µg/l acrylamide in the reject water. This provides support for the bacteria on the leca granules in the trickling filter to degrade acrylamide in a short period of time and at a temperature of 8 °C.

5.2.3 Moving Bed Biofilm Reactor

The fact that the dissolved oxygen in the outlet water from the MBBR pilot plant is lower than in the inlet water at the end of the test period could mean that the bacteria in the MBBR are aerobic and consumes oxygen while degrading acrylamide. 2023-04-17 there is a notable reduction of dissolved oxygen in the outlet water. A reason could be that the new biofilm carriers which were put in each of the barrels were released from the net to be free in the barrels at that time, which made them more available for biofilm growth.

The acrylamide concentration in the outlet water after 21 days reaches the goal of 0.10 ug/l acrylamide. Yet, the acrylamide concentration in the inlet water coming from the lamella separator at that time was 0.26 µg/ and lower than the average acrylamide concentration in the lamella separator the last year which is 1.25 µg/l. However, after 35 days, the acrylamide reduction is 1.4 µg/l, which should be enough to reduce normal acrylamide concentrations to reach the goal.

One factor that could have limited the reduction at the beginning of the test period is that only 5.5 l of biofilm carriers were placed in each barrel in the first 16 days before 9.5 l more biofilm carriers were put in each barrel. Hypothetically, an even higher reduction would have happened if all of the 15 l carriers had been used from the start.

Another uncertainty is the water level in the barrels. The water level was most of the time higher in the first barrel, which could have been a result of the too small perforated pipe that connected the first barrel with the second one. There is a chance that the biofilm carriers did block the water from flowing into the pipe sometimes. In addition, the outlet valve did not have any flow measurer, which made it difficult to adjust the outlet flow to the inlet flow, and in that way regulate the water levels in the barrels.

5.2.4 Biological Degradation

The results suggest that acrylamide is easily biological degradable by aerobic bacteria. This is strengthened by studies (Brown et al., 1982; Wahlberg & Paxéus, 2003; Guezennec et al., 2015).

The pH in both the inlet water for the trickling filter and the MBBR is on average 6.62. Moreover, pH 7 is preferable for most acrylamide degrading bacteria (Nawaz et al, 1998; Lakshmikandan et al, 2014; Emmanuel et al, 2013; Shukor et al, 2009; Bedade & Singal, 2018) since the acrylamidase enzyme is most active then (Bedade & Singal, 2017). In both the trickling filter and the MBBR, the pH is often higher in the outlet water than in the inlet water. This means that something is happening with the pH when the water passes through the biofilter. This could indicate for example acrylamide degradation, but more studies on the relation between pH and acrylamide degradation have to be done to conclude that. Moreover, the higher conductivity in the outlet water in the trickling filter and MBBR could possibly have to do with acrylamide degradation, but more research has to be done regarding that.

5.3 Biological Tests in Water From Lake Mälaren

It is hard to compare the results for water with a temperature of 8 °C and 15 °C since the acrylamide concentration rises in the water with less than 8 °C after 4 days. It could be argued that this surprising result is due to an error in the analysing by Eurofins. To resolve this ambiguity, a similar test with water from Lake Mälaren should be repeated in the future. Another explanation is that some polyacrylamide was left in the water and later did degrade into acrylamide by the bacteria which increased the acrylamide concentration. The reason for this not being observed in the test with 15 °C could be that a higher temperature is preferable for acrylamide-degrading bacteria (Soponkanaporn & Gehr, 1988; Wahlberg & Paxéus, 2003). This would make the degradation process speed up, the degradation of polyacrylamide and the rise in acrylamide would happen sooner and is not observed after 4 days.

Still, the fact that the acrylamide concentration is less than 0.05 µg/l after 13 days in the colder water provides support for the hypothesis that bacteria in water from Lake Mälaren at a temperature of 8 °C can degrade acrylamide. Furthermore, this suggests that Lake Mälaren can degrade acrylamide during a big part of the year. This result is similar to other research, for instance, that bacteria in natural surface water are able to degrade acrylamide (Guezennec et al, 2015; Seybold, 1994; Brown et al, 1980a; Croll et al, 1974). In addition, the results also confirm the statement that complete acrylamide degradation in natural waters will take a minimum of one day (Guezennec et al, 2015), but often between 4 and 29 days (Seybold, 1994).

In the test with a temperature of 8 °C, the acrylamide concentration after 4 days is 1.2 µg/l, which is similar to the average acrylamide concentration in the reject water the last year (1.25 µg/l). After 9 days, the acrylamide concentration is reduced to less than 0.05 µg/l. This supports the ability of the bacteria on the leca granules in the trickling filter to reach Norrvatten´s goal of 0.10 µg/l acrylamide in the reject water.

6. Conclusions

One of the purposes of this study was to investigate if the techniques ozonation, MBBR and trickling filter could reduce acrylamide in the reject water at Görvälnverket and reach Norrvatten´s goal of 0.10 µg/l acrylamide in the reject water.

This study suggests that ozonation of the reject water with an ozone dose of 0.7 mg/l can reduce the acrylamide concentration by 2.45 µg/l when the initial acrylamide concentration is 2.5 µg/l in the reject water. With this ozone dose, Norrvatten´s goal of below 0.10 µg/l acrylamide in the reject water is reached. Since the average acrylamide concentration in the reject water the last year is 1.25 µg/l, this result indicates that ozone could be used to reach this goal in reality.

The results of this study also identified that a trickling filter could reduce the acrylamide concentration with 5.59 µg/l. Furthermore, the goal of below 0.10 µg/l acrylamide can be reached when the initial acrylamide concentration in the reject water is 5.9 µg/l and 4.3 µg/l, which is higher than the average acrylamide concentration the last year. A similar conclusion can be used for the MBBR, which should be able to reach the goal with an initial acrylamide concentration of 4.3 µg/l in the reject water. The study indicates that a trickling filter and a MBBR could be used separately to reach the goal.

The second purpose of this study was to determine if ozone would generate oxidative stress or genotoxicity. The analysis of oxidative stress from ozonation did reveal that ozonation of the reject water does not seem to generate oxidative stress, nor genotoxicity.

The final aim of the study was to investigate what happens with acrylamide when it ends up in Lake Mälaren. The results propose that the bacteria in Lake Mälaren at a depth of 5 m right outside Görvälnverket are able to degrade acrylamide in 4 days. The best reduction seems to happen in water with a temperature of 15 °C.

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Appendices

Appendix I: Photographs of the pilot plants and the biological test in water from Lake Mälaren

Figure 26a

Figure 26b

Figure 26: Photographs of the trickling filter in the sludge dewatering facility at Görvälnverket. a) The green hose is where the inlet reject water from the lamella separator is flowing. The red hose on the floor is where the outlet treated water flows out. b) The leca granules are seen and the water spreaders have a blue color. At the top left corner of the container, the flow meter is seen in a red color (Aspegren, 2023).

Figure 27a

Figure 27b

Figure 27: Photographs of the MBBR pilot plant in the sludge dewatering facility at Görvälnverket. a) The yellow hose is connected to the green hose and gets the same inlet reject water from the lamella separator as the trickling filter b) The biofilm carriers which are covered in a microbiological film are seen in the water. The aquarium pump for air supply and stirring is placed on the outside of the blue tank and the air is flowing into the water through the white hose (Aspegren, 2023).

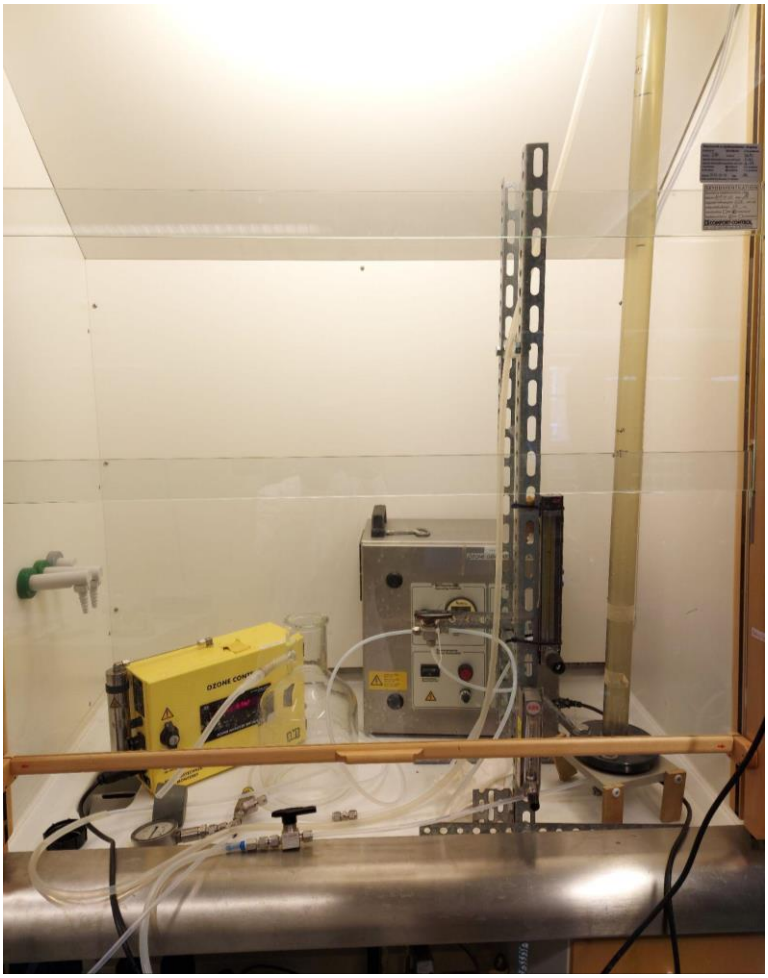

Figure 28a

Figure 28b

Figure 28c

Figure 28d

Figure 28: Photographs of the ozone pilot plant in the laboratory at IVL Swedish Environmental Research Institute. a) A photograph of the ozone pilot plant b) A photograph of the ozone content generator c) A photograph of the ozone generator. d) A photograph of the water colon. The bubbles in the water are ozone, applied to the water in the colon (Aspegren, 2023).

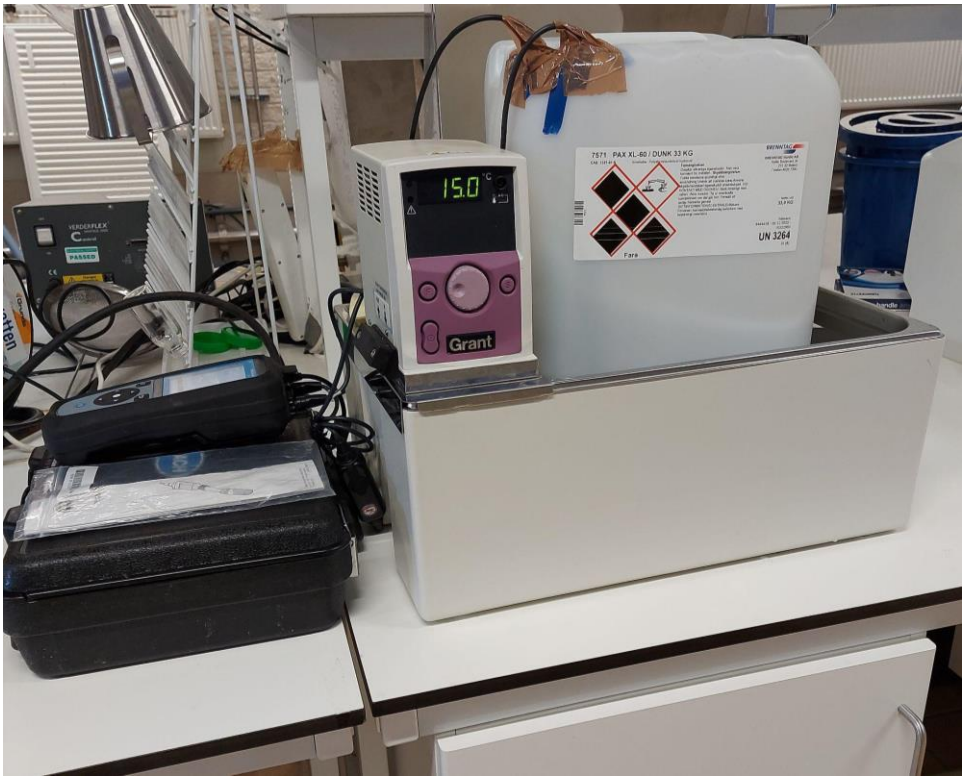

Figure 29: A photograph of one of the plastic containers for the biological degradation tests in water from Lake Mälaren. The container is placed in a water bath with a temperature of 15°C at Görvälnverket. Hach multimeter HQ2100 is seen to the left of the water bath and is measuring electrostatic parameters in the water (Aspegren, 2023).

Appendix II: Tables for ozone concentrations, acrylamide concentrations and acrylamide reduction for ozone tests 1,2 and 3

Table 5: Ozone test 1 (initial level 12 µg/l). Exact levels of acrylamide concentration and the reduction of acrylamide for ozone concentrations between 0.0 and 7.0 mg/l.

Ozone Concentration [mg/l]	Acrylamide Concentration [µg/l]	Reduction of acrylamide [µg/l]
0	12	0
0.7	12	0
1.4	3.6	8.4
2.1	0.59	11.41
2.8	0.42	11.58
3.5	0.41	11.59
7.0	0.49	11.51

Table 6: Ozonation test 2 (initial level 2.5 µg/l). Exact levels of acrylamide concentration and the reduction of acrylamide for ozone concentrations between 0.0 and 18.7 mg/l.

Ozone Concentration [mg/l]	Acrylamide Concentration [µg/l]	Reduction of acrylamide [µg/l]
0	2.5	0
0.7	<0.05	2.45
0.9	0.06	2.44
1.2	0.68	1.82
1.4	0.63	1.87
6.9	0.13	2.37
18.7	0.11	2.39

Table 7: Ozonation test 3 (initial level 6.6 µg/l). Exact levels of acrylamide concentration and the reduction of acrylamide for ozone concentrations between 0.0 and 2.8 mg/l.

Ozone Concentration [mg/l]	Acrylamide Concentration [µg/l]	Reduction of acrylamide [µg/l]
0	6.6	0
0.7	<0.05	6.55
0.9	<0.05	6.55
1.2	<0.05	6.55
1.4	<0.05	6.55
2.1	<0.05	6.55
2.8	<0.05	6.55

Appendix III: Dissolved organic matter in the reject water from the lamella separator

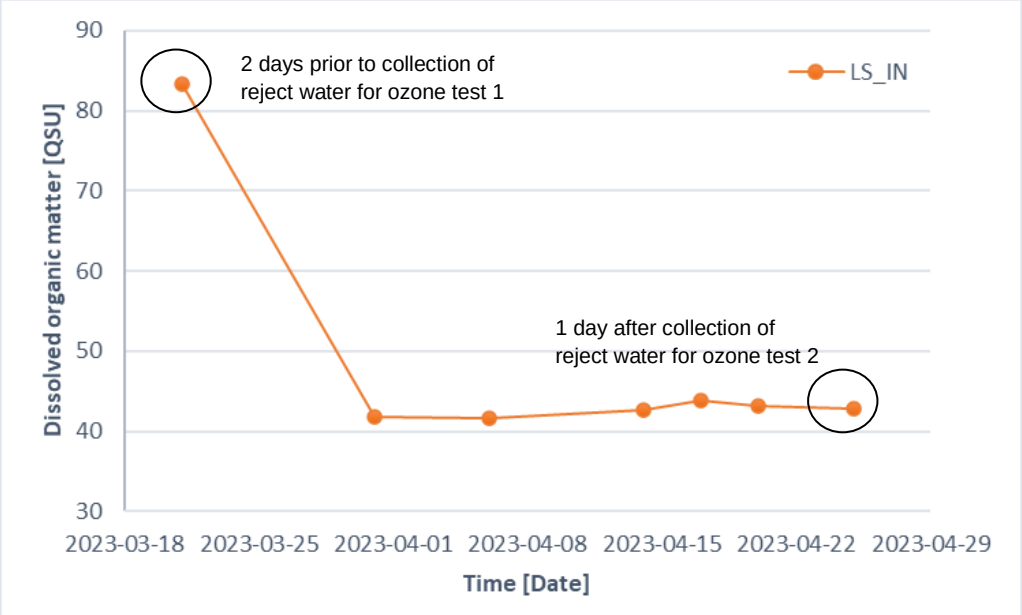

Figure 30: Levels of dissolved organic matter in the reject water in the lamella separator between 2023-03-21 and 2023-04-25.

TRITA-ABE-MBT-23456