Wageningen University & Research

Master thesis

The reactivity of dissolved organic carbon, present in the effluent of a wastewater treatment plant, with ozone.

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0. Abstract

Current wastewater treatment techniques are not sufficient in the removal of micro pollutants. New techniques, such as advanced oxidation with ozone, are being studied to improve the removal of micro pollutants. The problem with these systems is that ozone does not only react with the targeted micro pollutants, but also with the dissolved organic carbon present in the wastewater. This increases the amount of ozone needed to remove the micro pollutants and thus the costs of the process.

Removing the most interfering dissolved organic carbons as a pre-treatment would lower the costs. This thesis investigates the reaction of dissolved organic carbon with ozone, to find the most interfering organic matter in the ozonation of micro pollutants.

In this thesis the dissolved organic carbon in the effluent of the wastewater treatment plant in Bennekom is fractioned into four different fractions using the XAD-8 resin. Afterwards the four fractions; hydrophobic bases, hydrophilics, hydrophobic acids and hydrophobic neutrals, were each ozonated with four different concentrations of ozone: 0 mg/L, 1.2 mg/L, 2.4 mg/L and 4.8 mg/L.

All the samples were analysed using a Liquid Chromatography-Organic Carbon Detection, fluorescence and ultraviolet absorbance, to determine the composition of the dissolved organic carbon in the effluent and analyse the reaction of the organic matter with ozone.

The biggest fraction present in the effluent is the hydrophilic group followed by the hydrophobic neutrals, hydrophobic acids and hydrophobic bases. From the fractions the hydrophilics and hydrophobic acids are the most reactive with ozone and will probably be the most interfering in the removal of micro pollutants using ozonation.

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1. Abbreviation list

COD	-	Chemical oxygen demand
DOC	-	Dissolved organic carbon
EfOM	-	Effluent organic matter
FEEM	-	Fluorescence excitation emission matrix
HI	-	Hydrophilics
HOA	-	Hydrophobic acids
HOB	-	Hydrophobic bases
HOC	-	Hydrophobic components
HON	-	Hydrophobic neutrals
LC-MS	-	Liquid chromatography mass spectroscopy
LC-OCD	-	Liquid chromatography organic carbon detection
LMW	-	Low molecular weight
MP	-	Micropollutant
MW	-	Molecular weight
OCD	-	Organic carbon detection
OND	-	Organic nitrogen detection
SUVA	-	Specific ultraviolet absorbance
UV	-	Ultraviolet
WWTP	-	Wastewater treatment plant

2. Introduction

2.1 Micropollutants

Since a few decades micro pollutants (MPs), also termed emerging contaminants, have been an increasing concern for the environment. This group consist of chemicals such as: pharmaceuticals, personal care products, steroid hormones, industrial chemicals and pesticides. These chemicals are commonly found in the aquatic environment in concentrations ranging from ng/L to µg/L (Luo et al., 2014). It has already been shown that this low concentration of MPs has an effect on the re-productivity of fish (Alan et al., 2008, Tetreault et al., 2011). However, it is unknown what the effect of long-term exposure has on human health. Due to the risk of negative effects on human health this exposure should be limited (Margot et al., 2013). Therefore, there are technologies needed which can remove MPs from the environment.

A lot of the MPs end up in the wastewater before entering the environment. Wastewater treatment plants (WWTPs) are therefore a good target point to remove MPs. Unfortunately conventional treatment is not capable of removing MPs completely and MPs remain in the effluent of WWTPs (Eggen et al., 2014). New techniques are needed to remove the MPs remaining in the effluent of the WWTPs. Advanced oxidation is a new technique showing a lot of potential in the removal of MPs. In this new technique the MPs are broken down using chemical oxidation (Andreozzi et al., 1999). One of the chemical oxidants studied in this new technique is ozone.

2.2 Ozone treatment of effluent

The treatment of wastewater with ozone has been studied before, but the reaction mechanics behind ozone treatment are still not completely understood (Snyder et al., 2007; Gardoni et al., 2012). However, it is known that ozone can oxidise organic matter, including MPs, via two possible reactions: direct reactions and indirect reactions (Figure 1). Direct reactions are reactions which involve the ozone molecule. Ozone contains two delocalised electrons making it a very reactive compound which can act both as a electrophile or nucleophile. Ozone reacts mostly with electron rich groups such as double bonds and amines (Khadhraoui et al. 2009). Indirect reactions involve the reaction of a hydroxyl radical (OH·) with the organic matter. The hydroxyl radicals are formed by the decomposition of ozone and can be stimulated by photochemical pathways or other catalysts like hydrogen peroxide and titanium dioxide (Shon et al., 2007). In contrast with ozone, the hydroxyl radical reacts rather non selectively and immediately with any compound (Von Gunten, 2003).

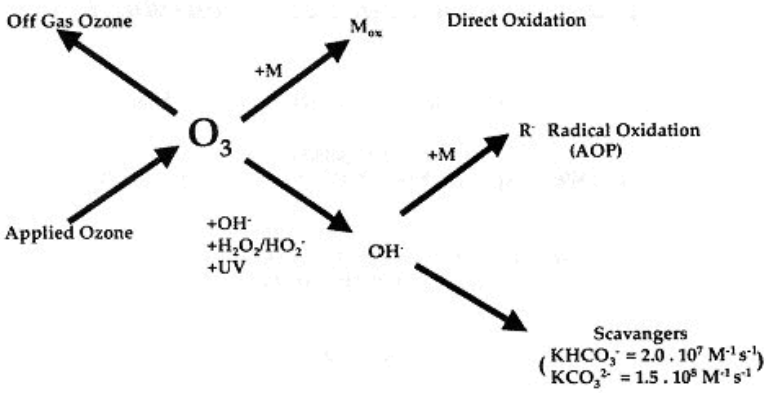

Figure 1. Applying ozone gas to remove compound M has two possible reactions, either directly with ozone or indirectly with a formed radical, OH·. Part of the ozone is lost in the off gas and some of the radicals are lost due to scavengers (from Von Gunten, 2003).

Although the high reactivity of hydroxyl radicals is an advantage for breaking down a large range of different kinds of MPs, the disadvantage is that an extra inducer is needed to form the radicals. Moreover, the radicals will also react more easily with other compounds then MPs which are also present in the effluent of WWTPs. Therefore, the reaction of ozone itself is preferred, this reaction targets mostly the more electron rich groups making it a more specific reaction towards organic matter, such as MPs. However, there is also other electron rich organic matter present in the effluent.

For instance, it is known that humic acids contain a lot of aromatic structures, which are electron rich, and are therefore very reactive with ozone (Schulten and Plage, 1991). This will result in a competition between the organic matter and the MPs for the ozone. The organic matter will lower the efficiency of the removal of the MPs with ozone. Thereby increasing the total amount of ozone needed to degrade the MPs, and thus the costs. More insight is needed into the reaction of organic matter with ozone to improve the process.

2.3 Organic matter in effluent

The total amount of organic matter present in the effluent of WWTPs is called effluent organic matter (EfOM). The EfOM contains three kinds of organic matter, based on their origin: natural organic matter, synthetic organic compounds and soluble microbial products (Shon et al., 2007). Natural organic matter is the organic matter which is frequently found in surface water bodies and consist mainly of humic substances (Chen et al., 2002). Synthetic organic compounds are man-made products derived from naturally organic carbons, such as plastics made from oil (Postigo and Barcelo, 2015). Soluble microbial products is organic matter formed during the treatment of wastewater due to the growth and decomposition of microorganisms (Dewes and Fox 1999).

Within these groups there are soluble and non-soluble particles. Therefore, EfOM can be classified in two other different groups based on size: Particulate organic matter and dissolved organic carbons (DOC). All the organic matter that is retained in an 0.45 µm filter is regarded as insoluble and falls into the first category. The organic matter that can pass an 0.45 µm filter is soluble and falls in the latter category, DOC.

Multiple studies have researched the composition of DOC in the effluent of WWTPs (Aiken et al., 1992; Imai et al., 2002; Jin et al., 2016; Qi et al., 2019). However, based on the origin of the wastewater and the treatment processes used in the WWTP, the composition of the DOC can differ. To get a better insight in the composition of the DOC, it is separated into subgroups. One of the methods used to look at the composition of DOC is the fractioning using XAD-8 resin (Imai et al., 2002). Using the XAD-8 resin the DOC is fractioned based on the adsorption of the organic matter to the resin. It is possible to form four different fractions using the XAD-8 resin: hydrophobic acids (HOA), hydrophobic bases (HOB), hydrophobic neutrals (HON) and hydrophilics (HI) (Jin et al., 2016; Zhang et al., 2008). It has been shown that it is possible to separate the HI fraction even further by using cation exchange resins. This fractions the HI into 3 different groups: hydrophilic acids, hydrophilic bases and hydrophilic neutrals (Qi et al., 2018; Imai et al., 2002; Swietlik et al., 2004).

DOC is a broad group of organic compounds with different sizes, chemical properties and reactivity with ozone (Westerhof et al., 1999). Due to the broad range of compounds in DOC, its reaction chemistry with ozone proves difficult (Zhang et al., 2008). The application of advanced oxidation processes in WWTP is not yet used widely. This is due to two reasons: the formation of toxic by-products and the costs of the ozone generation (Wert et al., 2007 and Wilt et al., 2018). The reaction of organic matter with ozone can form toxic by-products, which have to be removed afterwards. While the reaction of DOC with ozone increases the amount of ozone needed to remove MPs, increasing the costs.

2.4 Bio-ozone-bio system

A new process combining the use of bioreactors with the technique of advanced oxidation using ozone tries to overcome the disadvantages of ozone treatment. This system is the bio-ozone-bio system, which consists of a biological reactor followed by an oxidation process with ozone and finishes with a second biological reactor (Figure 2). The first reactor of the bio-ozone-bio system removes the readily biodegradable DOC and MPs in the effluent of WWTPs by degradation with microorganisms. The remaining MPs and DOC are oxidized with ozone, forming smaller and more oxidized products. These smaller and more oxidized products are more susceptible to biodegradation (Snyder et al., 2007). The system is designed to not only remove the MPs but also the toxic by-products formed during the ozonation of the effluent. In the second bioreactor there is a second biodegradation, which should remove any toxic by-products formed during the ozonation.

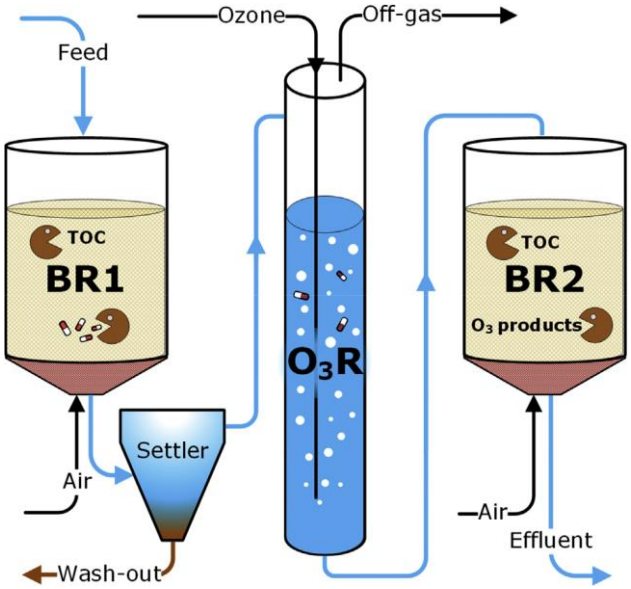

Figure 2. schematic of the experimental set-up. BR1 represents the first biological reactor, O_3R the ozone reactor and BR2 the second biological reactor (from de Wilt et al., 2018).

While this system seems promising there are still some challenges to overcome. During the ozonation there is a competition between the DOC and the MPs to react with the ozone, increasing the total amount of ozone needed to degrade all the MPs. Secondly, the second bioreactor should be able to remove the toxic by-products formed in the ozonation step. This research will focus on the first challenge, the competition between the DOC and MPs during ozonation.

2.5 Research aim

This research will investigate the reactivity of DOC in the effluent of WWTPs with ozone. This will give more insight in which specific group of DOC should be removed in the first bioreactor of the bio-ozone-bio system. If the first bioreactor is more efficient in the removal of the interfering DOC, the ozone use in the ozonation is lowered and possibly less toxic by-products are formed. The overall goal of the thesis is to determine how the DOC in the effluent of WWTPs influences the degradation of MPs during ozonation. The following research questions were formulated. The main research question is:

- How does the DOC present in the effluent of WWTPs influence the ozonation of micropollutants?

With the following sub questions:

1. What is the composition of the DOC in the effluent from the WWTP in Bennekom?
2. How do the resin based DOC fractions react with ozone?
3. How do resin based fractions of DOC influence the removal efficiency of micropollutants during ozonation?

3. Materials and method

To investigate the three sub-questions experiments were conducted. The DOC in the effluent from a WWTP was fractioned in four groups using XAD-8 resin. The obtained fractions were spiked with MPs and ozonated with different concentrations of ozone. Afterwards the reaction between the ozone and the DOC in the fractions was studied. Using LC-MS the removal efficiency of the MPs would be determined. However, due to the intensive use of the LC-MS machine the results were not in at the time of writing of this report.

The following section will explain the methods and materials used to fraction the DOC in effluent into four different fractions, how the DOC in these fractions was ozonated and how the samples were analysed.

3.1 Materials

3.1.1 Wastewater treatment plant effluent

The effluent studied in this research was taken from Bennekom's WWTP (The Netherlands). The WWTP was designed to treat around 20.000 residential houses and no industrial wastewater. The wastewater was treated with a settler followed by biological degradation in an aerobic and anaerobic tank. After the biological degradation, part of the sludge was recovered in a secondary settler. Sampling was performed after this settler. The DOC concentration present in the effluent was around 10 mg/L, but this was influenced by the seasons and weather conditions. To avoid dilution of the sample the effluent was sampled on a dry day. Before the fractioning procedure the effluent was filtered through an 0.45 µm PES filter to remove any insoluble organic matter. The filtered sample was concentrated two times by placing the effluent in a beaker inside a flow chamber and letting the water evaporate over a period of a week.

3.1.2 Micropollutants

Different MPs were chosen based on consumption patterns, occurrence in the environment and wastewater, variety in physico-chemical properties and analytical limitations (De Voogt et al., 2009). The MP stock solution was prepared in HPLC grade methanol and consisted of desphenyl-Chloridazon, opamidol, rimethoprim, dimetridazol, metoprolol tartrate, tetracycline, caffeine, benzotriazole, propranolol hydrochloride, BAM, chloridazon, sulfamethoxazole, irbesartan, carbamazepine, furosemide, bentazon, naproxen, 2,4-D, mecoprop, ibuprofen, diclofenac sodium salt, gemfibrozil, clarithromycin, hydrochlorothiazide, sotalol hydrochloride, 4- methylbenzotriazol and 5-methylbenzotriazol. More information about the MPs, such as the chemical formula and application, can be found in appendix A.

3.1.3 Resin

For the fractioning of the DOC, Amberlite XAD-8 resin (40-60 mesh) was used, which was provided by the Soil Chemistry and Chemical Soil Quality chair group at Wageningen University & Research.

3.2 Methods

3.2.1 Ozone concentration determination

To determine the concentration of ozone dissolved in water, two different methods were compared: a continuous absorbance measuring system called Oceanview and the indigo method from Bader and Hoigné., (1980). The set-up for the Oceanview system consisted of a DH-2000-BAL Lightsource using a halogen lamp, a OceanOptics USB 2000+ connecter and a Tp 300-UV-VIS spectrophotometer. The Oceanview system was set to measure in real time at an absorbance of 254nm which is the absorbance peak for ozone (Parisse et al., 1996).

The indigo method was used to determine the stability of ozone dissolved in water inside of a beaker over time. Samples of indigo reference were made after 1, 2, 4, 8, 12 and 25 min to determine the decrease in ozone concentration over time.

3.2.2 DOC fractioning

The DOC present in the effluent was fractioned into four different fractions using a modified procedure from Imai et al., (2002). Three grams of XAD-8 resin was used to isolate the following fractions: hydrophobic acids (HOA), hydrophobic neutrals (HON), hydrophobic bases (HOB) and hydrophilic compounds (HI) (Figure 3). The XAD-8 resin was washed before the procedure to lower the background DOC. The washing was performed with three times a litre of demi water, 0.1M NaOH and 0.1M HCl, ending with a litre of demi water. A 50 ml syringe and an 0.45 µm PES filter were washed before the fractioning with demi water. A total of 3 grams of XAD-8 resin was added to the 50 ml syringe which was used for the fractioning. A background sample of demi water was taken from the syringe containing the XAD-8 resin with a 0.45 µm filter attached. This background sample was named: background filter+resin. This sample was taken to analyse the DOC concentration coming of the material used in the procedure.

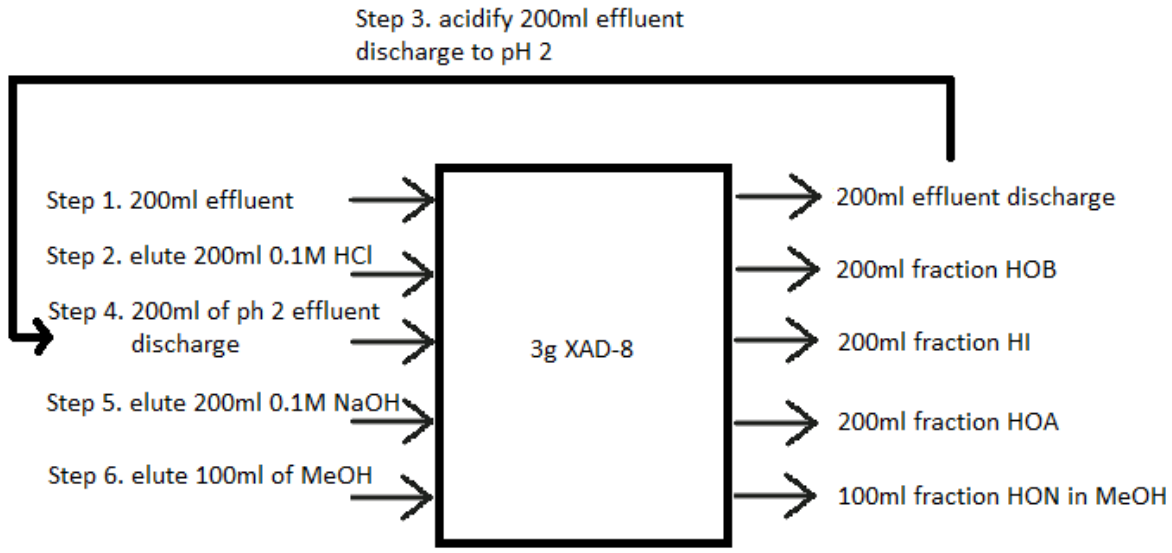

Figure 3. Schematic overview of the fractioning of DOC in effluent from WWTP Bennekom using XAD-8 resin.

- Step 1: With the syringe 50 ml of 0.45 µm filtered effluent was taken up and then shaken to homogenise the liquid with the XAD-8 resin. To prevent discharging the XAD-8 resin with the liquid a 0.45 µm filter was placed on the syringe while discharging, letting through only the liquid phase. Afterwards the liquid phase was discharged in a beaker glass for pH adjustment. This was repeated 3 times for a total of 200ml of effluent.
- Step2: Afterwards the XAD-8 resin was washed with 200ml 0.1M HCl in demi water (fraction HOB) in steps of 50 ml.
- Step 3: The pH of the discharged effluent was adjusted to pH 2 with HCl.
- Step 4: This was reapplied on the XAD-8 resin, the washout was labelled fraction HI. After 100ml of the HI fraction, a new 0.45 µm filter was placed on the syringe due to clogging of the first filter. A separate background sample was taken for this filter without the XAD-8 resin, this background sample was named: background 2nd filter. The procedure was carried out afterwards in the same method as described above to wash out the rest of the 200m.
- Step 5: The XAD-8 resin was washed with 200ml of 0.1M NaOH, the discharge was labelled fraction HOA.

- Step 6: The XAD-8 resin was washed with 100ml of methanol and the discharged methanol was labelled fraction HON.

To remove the methanol from the HON fraction, the methanol was evaporated by leaving the sample in a flow chamber overnight. Afterwards the HON fraction was re-dissolved in ultrapure water. The pH of each fraction was adjusted to pH 3 ± 0.2.

3.2.3 Micropollutant spiking

To the four fractions HON, HOB, HOA and HI a fifth sample was added. This fifth sample consisted of the concentrated effluent before the fractioning. This sample was diluted 2 times to reach a more similar DOC concentration.

Before spiking with MPs from each fraction 1 ml was taken and kept separate for MP analysis to compare to spiked fractions. The rest of the fractions was spiked with MPs from a stock solution using pure methanol and beakers. First 0.5 ml of pure methanol was pipetted into a clean beaker and into this methanol 0.2 µl micro pollutant stock mix 03-4 (appendix A) was added. After the methanol was evaporated one of the fractions was added to the beaker and stirred, resulting in a concentration of around 12 ng/L of each micro pollutant in the fraction. This was done in a clean beaker for all the fractions.

3.2.4 Fraction ozonation

The total volume of each fraction was divided in four parts of 40 ml in tubes of 50 ml to apply four different ozone dosages. The four different ozone dosages were aimed at 1 g O_3/g DOC, 0.5 g O_3/g DOC, 0.25 g O_3/g DOC and 0 g O_3/g DOC respectively. A new background sample was made from ultrapure water in the same 50 ml tubes as the fractions. This was done to determine the DOC concentration coming of the tubes, this background sample was named: background tubes.

To produce ozone stock solution, 1L of ultrapure water was bubbled with ozone in a Schott bottle placed in a fridge (Figure 4). Ultrapure water was spiked with ozone inside a fridge to keep the temperature low, thus increasing the solubility of ozone in water. Based on earlier measurements, the concentration of ozone in the ultrapure water was stable around 45 mg/L. This meant that 4 ml, 2 ml and 1 ml of ozonated water was added to the 40 ml fractions aiming for the dosages of 1 g O_3/g DOC, 0.5 g O_3/g DOC and 0.25 g O_3/g DOC respectively.

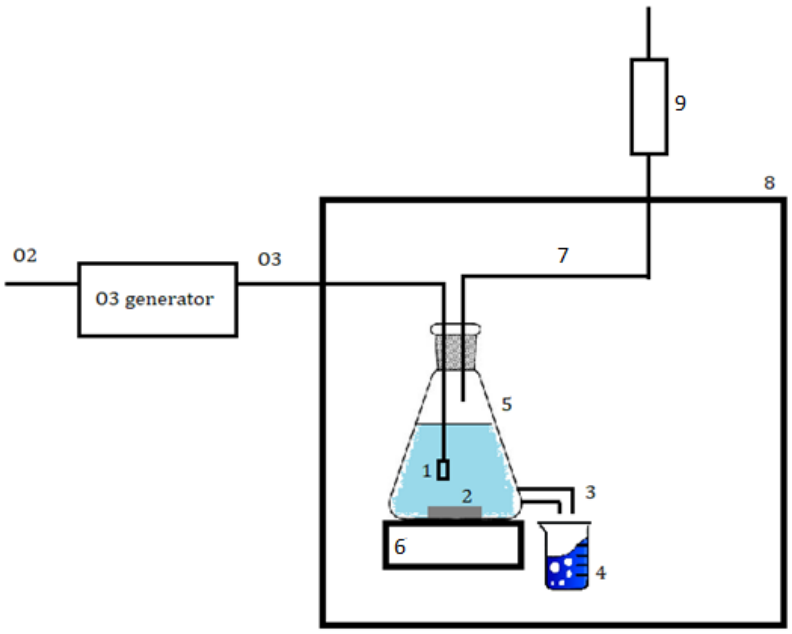

Figure 4 Schematic set-up of the ozonation of ultrapure water with: (1) aeration diffuser, (2) magnetic stir bar, (3) glass tap, (4) beaker glass, (5) Schott flask containing ozone stock, (6) magnetic stirrer, (7) off gas tube containing left over ozone, (8) fridge and (9) ozone catalyst which reacts ozone to O_2. The ozone generator was supplied with pure oxygen.

Pipette tips were pre-ozonated with some of the ozone stock to have minimal reaction between the pipette and the ozone. Before the application of ozone dosage, a triplicate sample of indigo reference was made to determine the ozone content of the ultrapure water in the beaker glass. From each fraction a sample was dosed with 4, 2, 1, or 0 ml of ozone stock. The ozone dose was applied by tapping the water from the ozone stock in a beaker and subsequently pipetting from the beaker into the samples. After each dosing of a sample a new indigo reference was made, to check the stability of the ozone concentration and recalculate the exact applied ozone concentration afterwards. After all the samples aiming for a 1 g O_3/g DOC were dosed with 4 ml of ozone stock, the ozone stock in the beaker was refreshed with new ozone stock. The ozone stock in the beaker was also refreshed after all the 0.5 g O_3/g DOC samples were dosed with 2 ml of ozone stock. After applying all the ozone doses all the samples were filled with ultrapure water to a total volume of 44 ml to keep the dilution the same.

The samples were stored for 24h before sampling was performed for the LC-OCD. The measurement of the LC-OCD was performed 5 week after the ozonation. UV and COD measurements were performed 48h after the ozonation. Fluorescence measurements using the Infinite m200 plate reader were performed 3 weeks after the ozonation and the fluorescence measurements using the Perkin Elmer LS50B were performed 7 weeks after the ozonation.

3.2.5 Analyses

3.2.5.1 Ozone concentration

Indigotrisulfonate reference was used for the determination of the ozone concentration in the ozone stock. This method is based on the method from Bader and Hoigné., (1980). However the extinction coefficient of indigotrisulfonate was calculated rather than taken from Bader and Hoigné's., (1980) method because of degradation of indigo sulfonate over time, indicated by Gordon et al., (2000). The indigo reference for determination of the extinction coefficient was prepared by pipetting 1 ml of 1 mM indigo stock solution and 0.5 ml of 1.5 M H_3PO_4 into a 25 ml volumetric flask and filled with demi water. Afterwards the absorbance at 600 nm was measured and with the law of Beer-Lambert the extinction coefficient was calculated.

To determine the ozone concentration another 25ml volumetric flask was prepared with indigo stock solution and H_3PO_4. To this flask 1 ml of ozone stock was added, after which the flask was filled with demi water. The absorbance at 600 nm was measured and the concentration ozone in the ozone stock in mg/L was calculated using $O3 = \frac{V_f \Delta A}{f b V_t}$ [mg. L^{-1}]. Equation 1.

$O3 = \frac{V_f \Delta A}{f b V_t}$ [mg. L^{-1}]. Equation 1

Where f is a ratio of ε/M_{O3} in L $cm^{-1}mg^{-1}$, b is the path length of the cuvette in cm, Vf is the volume of the volumetric flask, Vt is the volume of the individual sample (ozone stock) added to Vf, and ΔA is difference in absorbance between the reference indigo solution and the indigo reference with ozone stock added.

3.2.5.2 Dissolved organic carbon concentration

The DOC content of the samples was measured using the Liquid Chromatography-Organic Carbon Detection (LC-OCD) at Wetsus (Leeuwarden, Netherlands) (Figure 5) (Huber et al., 2010). Samples were applied to a size exclusion column, Toyopearl HW-50S, 30 µm, 250 mm, to separate the organic compounds present in the sample based on size. Part of the sample bypasses the columns and is used for total organic carbon detection. After the column first a fixed wavelength UV detection at 254 nm was performed, followed by both a continuous Organic Carbon Detection (OCD) and Organic Nitrogen Detection (OND). Based on the time it takes for compounds to go through the column the organic carbon detection can be separated in the following components with different sizes: biopolymers MW > 20000, humic substances MW = ±1000, neutral components MW < 350 (LMW neutrals) and acidic compounds with MW <350 (LMW acids). The organic matter which stays on the column (total minus the measured groups) are the hydrophobic organic compounds (HOC). The UV and OND detection is performed using a Agilent 1260 Infinity. Reagents used for the column are a mobile phase of 28 mMol phosphate buffer and for the acidification Phosphoric acid, pH 1,5.

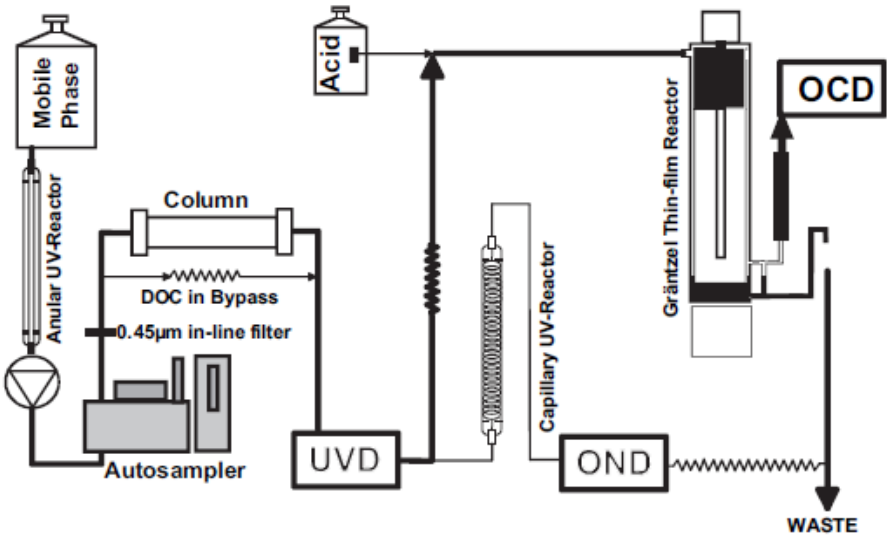

Figure 5 Schematic overview of the LC-OCD with an UV detector (UVD), organic nitrogen detector (OND) and organic carbon detector (OCD).

All samples of each fraction and the samples of an non-fractioned effluent sample were analysed using the LC-OCD. The results from the LC-OCD were corrected for dilutions of the samples during the fractioning and ozonation process and for blank measurements of DOC coming of the material used. The corrected results for the LC-OCD can be found in appendix B. It should be noted that there was a pressure drop during the measurement of the LC-OCD, this results in a overestimate of the total DOC and HOC values and underestimate in the values obtained for the size components (Biopolymers, Humic acids and LMW compounds). Moreover the humic acids were not measured for the non-fractioned effluent sample with 0 mg/L ozone dose due to technical failure.

3.2.5.3 Fluorescence of humic-, tryptophan- and tyrosine structures

Using fluorescence it is possible to determine the presence of different chemical groups in the samples. Based on literature from Jin et al., (2016) and Li et al., (2019) three different chemical groups were chosen for analysing: humic structures, tryptophan structures and tyrosine structures. The literature used the technique Fluorescence excitation and emission matrix (FEEM) to generate 3d graphs of the fluorescence peaks of the groups with excitation ranging from 230 to 500nm. However the FEEM fluorescence machine available for this research was not able to reach beneath 250nm and thus could not measure the peak which correlates to the presence of tyrosine structures. Instead a cross section of the peaks was measured by making one emission and one excitation scan (Table 1). These measurements were performed on two fluorescence machines. The two different fluorescence machines used were the Infinite m200 pro plate reader, and the Perkin Elmer LS50B.

Table 1. settings for the fluorescence measurement to make a cross section of the fluorescence peak of humic-, tyrosine- and tryptophan-structures.

	Emission scan	Excitation scan
Humic structures	Ex: 340nm Em: 380-480 nm	Em: 425nm Ex: 280-420nm
Tryptophan structure	Ex: 280nm Em: 300-400 nm	Em: 345nm Ex: 230-360nm
Tyrosine structure	Ex: 230nm Em: 300-400 nm	

3.2.5.4 COD and NO_2 concentrations

The COD and NO_2 concentrations of all the samples after ozonation were measured using Hach Lange kits: LCK 341 for the NO_2 concentration and LCK 1414 and LCK 314 for the COD concentrations.

3.2.5.5 Specific ultraviolet absorbance

Using the Infinite m200 pro the UV 254nm absorbance was measured of the samples and together with the obtained DOC values from the LC-OCD, the specific ultraviolet absorbance (SUVA) value was calculated by dividing the UV absorbance by the DOC measured by the LC-OCD.

3.2.5.6 MPs concentration

Using the LC-MS at Rikilt (Wageningen University & Research) the concentration of the micropollutants would be determined before and after spiking with MPs. The removal efficiency of MPs by ozonation would be calculated by dividing the ozonated sample of a fraction by the non-ozonated sample of the same fraction. However due to the busy schedule for the machine the results of these analyses are not included in this report.

4. Results and discussion

The HOB, HI, HOA, HON and a non-fractioned effluent samples were analysed using a LC-OCD, fluorescence techniques, UV absorbance and COD analyses. This section will state the observations observed in the data after ozonation of the fractions using different ozone dosages. First the results of the ozone concentrations measurement will be explained after which the observations per analyses technique will be discussed. Finally the observations of each technique will be combined for a more elaborate discussion on the reaction of DOC with ozone.

4.1 Ozone concentration in ozone stock

To determine the ozone concentration in the ozone stock two different methods were compared: the Oceanview system and the indigo method. The Oceanview system was unstable during the measurements and therefore the indigo method was used for the determination of ozone concentration during ozonation of the samples (Appendix C).

To determine if the ozone concentration would decrease over time when no ozone was being bubbled through the ozone stock, multiple indigo references were made over time from a beaker containing water from the ozone stock (Appendix C). It was decided that the decrease in ozone concentration should be taking into account when dosing samples with ozone stock. Furthermore fresh ozone stock had to be used after a few minutes to ensure that similar concentrations of ozone were used.

After the ozonation of the fractions the exact ozone dose per sample was determined using the indigo method. The concentration in the ozone stock was steady during the pipetting of the ozone stock. The amount of ozone applied to each sample was, from highest dose to lowest, 4.8 mg/L ozone, 2.4 mg/L, 1.2 mg/L, and 0 mg/L (appendix D).

4.2 LC-OCD

4.2.1 DOC composition of the effluent

Based on the total DOC measurements, the HI fraction has the highest DOC concentration of the four fractions in the effluent. This fraction contains over a third of the DOC present in the effluent (Table 2). The total DOC measurement for the HI fraction was over the range of the detection limit. Therefore, the DOC is likely higher than the measured 36% of DOC present in the HI fraction. To determine the composition total DOC of the HI fraction, this was calculated based on the sum of the size components plus the HOC. This showed that almost 41% of the DOC present in the effluent is present in the HI fraction (Table 2).

Table 2. Comparison of the composition of the effluent from the WWTP in Bennekom in percentage. The first column is the composition based on the DOC measurement performed by the LC-OCD. The second column is the composition based on the calculations of the sum of the size components to calculate the DOC total per fraction.

	Measured DOC	Calculated DOC
HOB	6%	5%
HI	36%	41%
HOA	26%	24%
HON	32%	30%
Total	100.00%	100%

The second biggest fraction is the HON fraction, which contains almost a third of the total DOC. This is followed by the HOA fraction which contains around a fourth of the total DOC. The smallest fraction is the HOB fraction, this fraction hardly contains DOC from the effluent of the WWTP in Bennekom.

Effluents used in different studies shows similar composition of the DOC using the same XAD-8 resin for fractioning (Table 3). Various studies found that the HI fraction was the biggest fraction (Zhang et al., (2008), Qi et al., (2018) and Imai et al., (2002)). Swietlik et al., (2004) found a significant lower HI fraction, which is due to the separation of the humic acids from the fractions. Humic acids are mainly found in the HI fraction and removing these separately lowers the percentage. Only Jin et al., (2016) found HOA as the biggest fraction rather than the HI fraction. They also showed that the DOC composition of effluent is highly site specific, as the use of a sand filtration in the WWTP increased the HOA and HON fractions. Thus changing the composition of the DOC found in the fractions. Like in this thesis, the HOB fraction is in all the literature the smallest of the four fractions. The second biggest fraction in this thesis is the HON fraction, while the HOA fraction is the second biggest in literature (Imai et al., 2002, Zhang et al., 2008, Qi et al., 2018).

*Table 3. Comparison of the DOC composition of effluents from WWTP's. Values are obtained from literature using similar fractioning procedures. Swietlik et al., 2004 separated the humic acids in the sample before the fractioning process, thus the humic acids are mentioned as an extra fraction. *Some of the literature fractioned the HI fraction into smaller sub-fractions, for easier comparison these were summed up to a single HI fraction.*

	HOB	HI*	HOA	HON	Humic acid
Effluent of WWTP of Bennekom	5%	41%	24%	30%	
Zhang et al., 2008	4%	46%	30%	20%	-
Qi et al., 2018	5%	49%	26%	20%	-
Imai et al., 2002	1%	77%	18%	4%	-
Jin et al., 2016	9%	21%	42%	28%	-
Swietlik et al., 2004	0%	15%	54%	12%	19%

4.2.2 DOC composition of the fractions

Comparing the DOC concentration of the four fractions combined to the non-fractioned effluent, shows that the four fractions combined have almost a three times higher concentration as the non-fractioned effluent (Table 4). This would indicate that there is an increase in the DOC concentration during the fractioning process. This is contradicting to other work who reported a loss of DOC after fractioning (Maurice et al., (2002) and Jin et al., (2016)).

This contradiction seems to originate from the LMW acids and neutrals. The effluent contains around 1.1 mg/L of LMW components (acids and neutrals combined) while the total of the four fractions reaches a total of 15.5 mg/L. The concentration LMW components in the total of the four fractions is more than the total amount of DOC in the non-fractioned effluent. This indicates that either a lot more LMW components were coming of the XAD-8 resin during the fractioning, or the values are incorrect due to the pressure drop during the measurements.

An analysis on effluents from five different locations in The Netherlands showed similar composition for the size components: Biopolymers are present at less than 10%, humic acids are present at 25-50%, LMW components for around 20-50% and HOC for around 5-10% (Hofman-Caris et al., 2017). The LC-OCD used in this thesis was not capable of determining the size components 'building blocks' as found in Hofman et al., (2017), this group would fall under the LMW acids and neutrals. The biopolymers and humic acids are present in similar amounts, though HOC is more present in the samples used in this thesis. In this thesis the LMW components are present for more than 50% in the total of the four fractions combined. Confirming there is most likely a measurement error in the LMW components.

*Table 4 LC-OCD results for the non-fractioned effluent, the sum of the fractions (total) and each fraction. The sum of the fractions was calculated by adding up the measurement of the four different fractions. Humic acids – C for the non-fractioned effluent is the measurement of the effluent sample with the 1.2 mg/L ozone dose (red), due to failure to measure the non-fractioned effluent 0 mg/L dose. The measurements highlighted with * were either over or under the range of detection limit.*

	Effluent 0 mg/L O3	Total of the 4 fractions	HOB 0 mg/L O_3	HI 0 mg/L O_3	HOA 0 mg/L O_3	HON 0 mg/L O_3
Biopolymers – C	1.01	0.62	0.01*	0.54	0.07	0.01*
Biopolymers - N	0.20	0.58	0.01*	0.54	0.02	0.01*
Humic acids – C	4.47	4.42	0.03	3.00	1.22	0.18
Humic acids – N	0.34	0.36	0.01*	0.27	0.07	0.01*
LMW acids	0.14	2.17	0.16	0.23	1.11	0.68
LMW neutrals	0.98	13.37	0.19	5.32	2.67	5.21
HOC	3.83	9.73	1.21	3.35	2.28	2.89
DOC	10.98	28.81	1.76	10.42*	7.50	9.13

The highest concentration of the biopolymers is found in the HI fraction. While almost no biopolymers are found in the other three fractions. There is a loss of 40% of the biopolymers from the non-fractioned effluent compared to the total of the four fractions. This could be due to a loss during the fractioning process. Higher MW organic matter is more hydrophobic and could remain on the hydrophobic XAD-8 resin during the washing steps in the fractioning. This correlates with the observation that almost no biopolymers are found in in the three hydrophobic fractions HOA, HON and HOB. If the hydrophobic biopolymers can not be washed of the XAD-8 resin, there will be no biopolymers in fractions that are obtained after a washing step in the fractioning process, which are the hydrophobic fractions.

The HOA fraction consists mainly of the humic acids and LMW acids. This is expected in a group which should consist out of acids. The HON group contains mostly LMW neutrals, although the measured value is rather high. The HI fraction contains mostly the humic acids. The humic acids are expected to be hydrophilic, because of the presence of carboxylic acid groups. The HOB fraction contains less DOC than the other fractions and consist mainly out of HOC.

4.2.3 Ozonation effect on DOC

To understand the effect of ozonation on the DOC in the fractions, 4 different doses of ozone were applied. Based on literature it is expected that the reaction of ozone with organic matter reduces the size of the organic matter. The change in size is shown by the decrease of bigger size components and the increase in the smaller size components form the LC-OCD measurements. An overview of the LC-OCD results with observations can be found in appendix B. The most significant trends in the increase or decrease of size components are presented in the figures below.

The biopolymers decreases in concentration in the non-fractioned effluent with increasing ozone dose, with a higher decrease at a dose of 4.8mg/L ozone (Figure 6). This decrease correlates with an increase in the humic acids and HOC with increasing ozone dosages in the non-fractioned effluent samples (Figure 7, Figure 8). It can be concluded that the Biopolymers are broken down into smaller organic compounds by the reaction with ozone. This also makes part of the organic matter more hydrophobic which results in the organic matter remaining in the column of the LC-OCD.

The biggest part of the biopolymers are found in the HI fraction, but there is no decrease in the biopolymers concentration in the HI fraction with increasing ozone dosage. This indicates that only the

hydrophobic biopolymers are broken down into smaller components in the non-fractioned effluent sample (Figure 6, Figure 7).

The HI fraction shows no increase or decrease in any of the size components in the LC-OCD measurements. This could be due to two reasons: either the hydrophilic DOC is not reacting with the ozone, or the reactions do not results in significant size changes and are therefore not visible with a size depending DOC measurement.

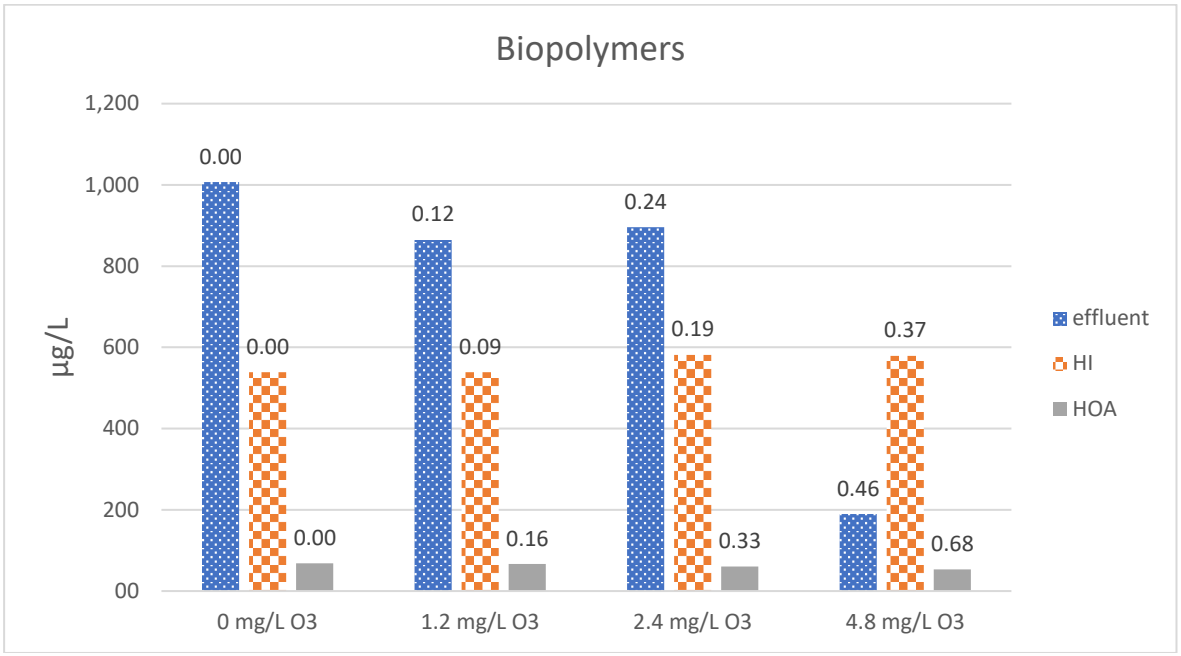

Figure 6. Biopolymers measured in non-fractioned effluent, HI and HOA fractions with increasing ozone dose. The ozone dose in mg O_3/mg TOC is displayed above the bars.

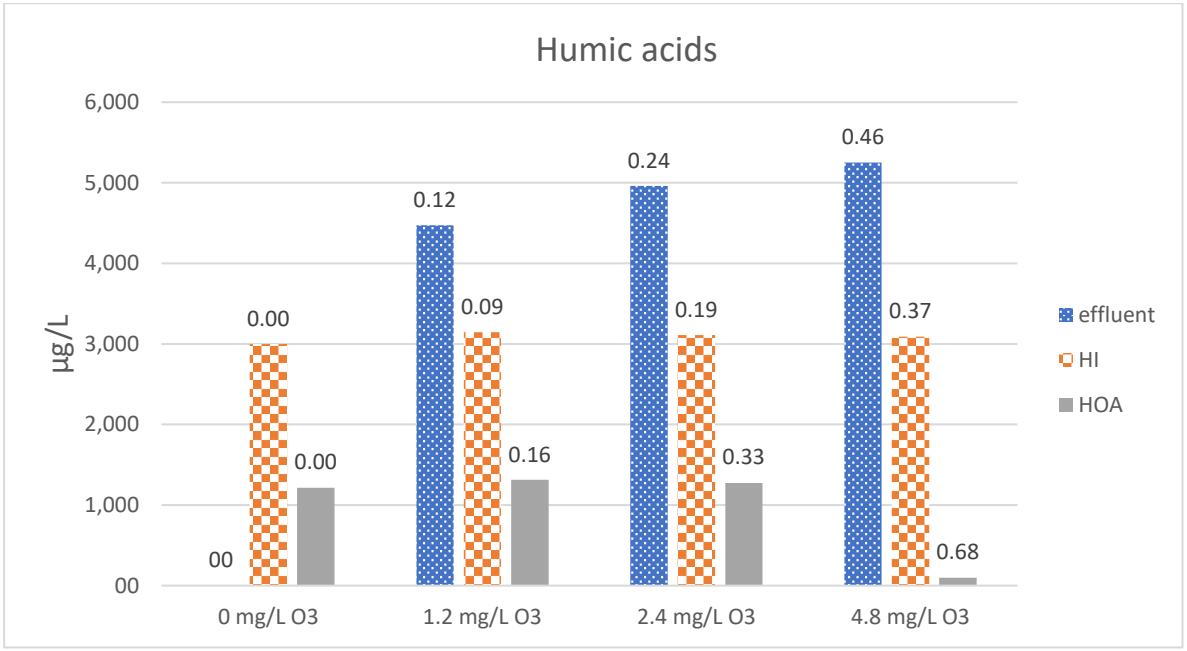

Figure 7. Humic acids measured in non-fractioned effluent, HI and HOA fractions with increasing ozone dose. The effluent measurement at 0 mg/L O_3 is missing due to failure of the machine to measure the sample. *The ozone dose in mg O_3/mg TOC is displayed above the bars.*

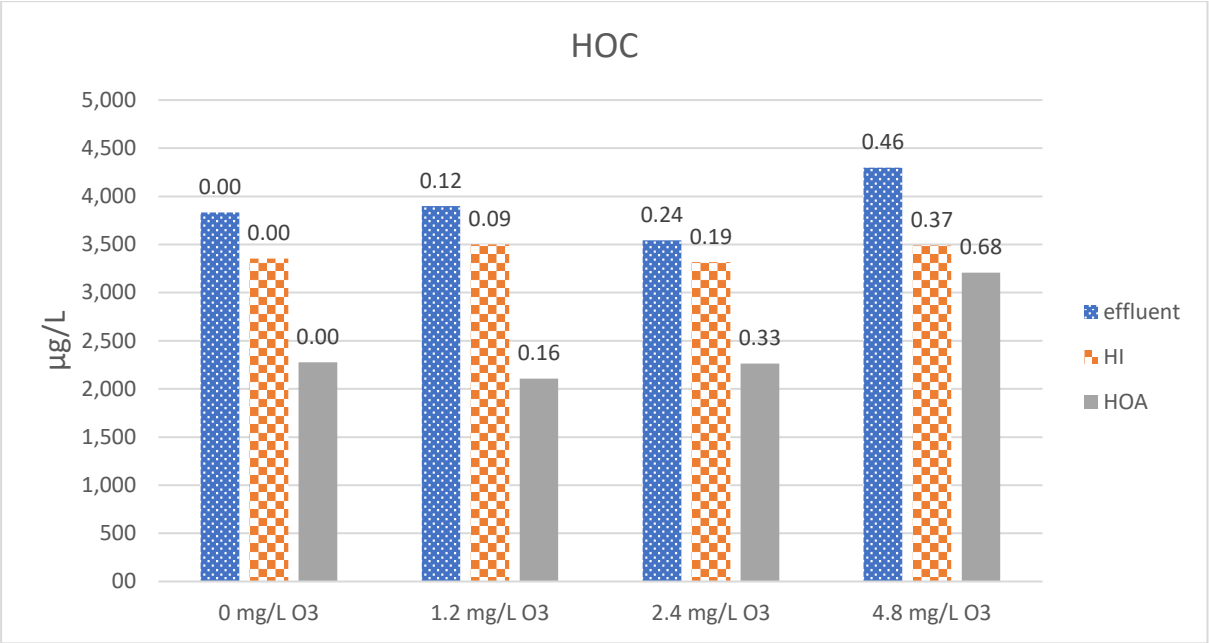

Figure 8. HOC in non-fractioned effluent, HI and HOA fractions with increasing ozone dose. The ozone dose in mg O_3/mg TOC is displayed above the bars.

In contrast to the non-fractioned effluent, the HOA fraction has an decrease in concentration of humic acids rather than an increase (Figure 7). This decrease correlates with the increase in the HOC (Figure 8). Swietlik et al., (2004) also reported a decrease in the HOA fraction after ozonation of the organic matter. That decrease corresponded with the loss of organic matter during the fractioning process. They reported this is due to the change in the psychical and chemical properties during the ozonation, increasing the interactions of the organic matter with the XAD-8 resins, which results in a loss of organic matter during the fractioning. This same trend is observed in this thesis in the humic acids of the HOA fraction. An increase in HOC indicates an physical or chemical change, which increases the interaction between the organic matter and the column.

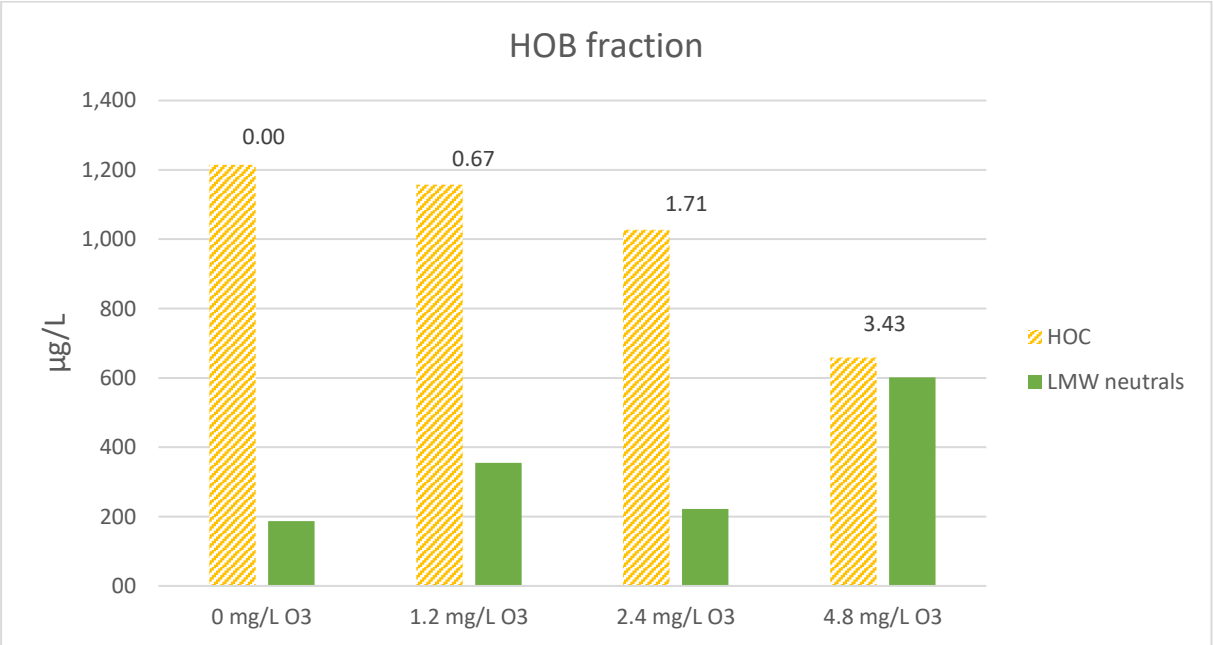

Figure 9. The decrease in HOC and increase of LMW neutrals for the HOB fraction with increasing ozone dosage. The ozone dose in mg O_3/mg TOC is displayed above the bars.

There is also a decrease in HOC in the HOB fraction (Figure 9). This decrease with increasing ozone dosages seems to correlate with an increase in the LMW neutrals. The decrease in HOC confirms that hydrophobic organic matter is reactive with ozone.

4.3 Fluorescence

4.3.1 Spectrophotometer selection

Using fluorescence it is possible to characterise different chemical groups present in the effluent. To determine the different groups five different excitation and emission scans were measured (see M&M Table 1). The scans were performed on two different machines, the Infinite m200 pro plate reader and the Perkin Elmer LS50B. The Infinite m200 pro plater reader showed instability during the measurements and therefore only the results of the Perkin Elmer LS50B are used in the following result section. An example of the instability of the Infinite m200 pro plate reader is shown in appendix E.

There was no fluorescence observed for any of the samples on the excitation scan at an emission of 345nm on either the Perkin Elmer LS50B or the Infinite m200 pro. This indicates that there is no tyrosine or tryptophan structures in the samples. All the other scans were corrected for a blank using ultrapure water and dilution factors during the fractioning process.

4.3.2 Characterization of aromatic groups

In the HI and HOA fraction and the non-fractioned effluent sample, there is fluorescence on the emission scan at 425nm fluorescence. This shows a peak at an excitation of 335nm (Figure 10A). This peak corresponds with the fluorescence area for humic substances. The emission observed in the emission scan in Figure 10C shows this same peak at around 425nm. The fraction HI shows the highest fluorescence in both Figure 10A and C, followed by the HOA fraction. The HOB and HON fractions do not show fluorescence at these wavelengths. This indicates that there are no humic acids present in the HON and HOB fractions.

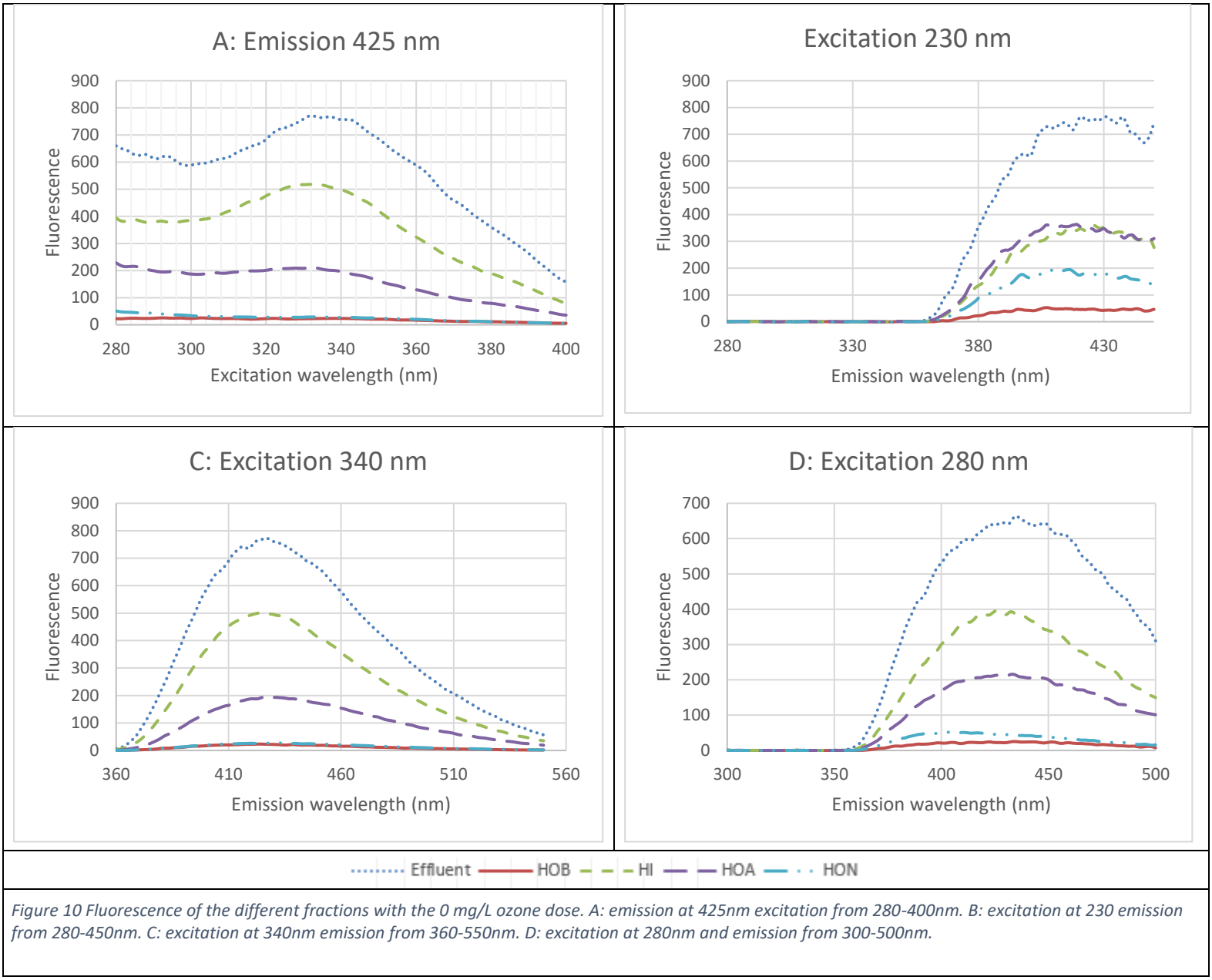

Figure 10 Fluorescence of the different fractions with the 0 mg/L ozone dose. A: emission at 425nm excitation from 280-400nm. B: excitation at 230 emission from 280-450nm. C: excitation at 340nm emission from 360-550nm. D: excitation at 280nm and emission from 300-500nm.

There is no peak observed in the emission scan at 345nm with an excitation of 230nm (Figure 10B). This peak corresponds with the presence of tyrosine like structures in the samples. The absence of this peak indicates that there are no tyrosine like structures in the non-fractioned effluent or the fractions.

Figure 10D shows fluorescence starting at and emission of around 360nm with the peak at 438nm. This fluorescence originates from the same group as in Figure 10A and C, the humic substances. The lack of any fluorescence at an emission of 345nm indicates that there are no tryptophan structures present in the samples.

There is fluorescence present starting at an emission of around 360nm with the peak at 425nm in Figure 10B. This fluorescence should originates from the humic substances in the samples. However, the HON fraction also shows fluorescence, while no fluorescence was observed in Figure 10A and Figure 10C. Yu et al., (2011) report that fulvic acids show an emission peak between 420-440nm at excitations between 240-260nm. Although an excitation of 230nm is lower than the reported excitation range the fluorescence could still be visible though not at maximum intensity. Li et al., (2019)

also reports that fluorescence observed with an excitation of 230nm corresponds either to the presence of proteins, when beneath an emission of 400nm, or fulvic acids, with emissions above 400nm. This indicates that the observed fluorescence in Figure 10B could originate from either fulvic acids or humic acids.

Due to the overlapping emission peaks of humic acids and fulvic acids it is hard to determine if the fluorescence observed is originating from either the humic acids, the fulvic acids or a combination. However, the HON fraction only has fluorescence at the lowest excitation of 230nm indicating that the HON fraction does contain fulvic acids but no humic acids which should also be excited by higher wavelengths.

Jin et al., (2016) reported the presence of both tyrosine and tryptophan like structures which were mainly present in the HOB and HI fraction. The fluorescence peak of the tyrosine structures had a higher intensity than the humic substances for both the fractions. Jin et al., (2016) concluded based on these data that the HI fraction consisted mainly out of protein-like organic matter rather than humic acids. This is contradicting the observation found in this thesis for the HI and HOA fractions. This could indicate that the presence of proteins could have an effect on the fractioning distribution of humic acids.

4.3.3 Ozonation effect on humic substances

All the fractions show a decrease in fluorescence with increasing ozone dosage (Figure 11). For the HI and HOA fractions and the non-fractioned effluent the fluorescence keeps decreasing with increasing ozone dose. Even after the highest dose of ozone the fluorescence is not completely lowered to zero for the non-fractioned and HI and HOA fractions. This indicates that the humic substances are present in such high concentrations that there is no complete conversion of all the fluorescent structures reacting with ozone.

The maximum intensity of the HOB and HON fractions is at least 2 times lower when compared to the HOA and HI fractions. This lower fluorescence shows there is little fluorescent organic matter present which could react with ozone. This is confirmed with the almost complete loss of fluorescence after the first ozone dosage. This indicates that there are hardly humic substances present in the HOB and HON fractions.

However, the HON fraction does show some fluorescence even after the highest ozone dose at an excitation of 230 nm. The fluorescence of the HON fraction does not decrease below 90 at an excitation of 230nm. Even after the highest ozone dose, this fluorescence is present in the sample. It was hypothesized that this fluorescence could be originating from fulvic acids instead of humic acids.

The fulvic acids appear less reactive with ozone than humic acids. Since the fluorescence of the fulvic acids does not decrease. This is due to difference in size and presence of aromatic structures, fulvic acids are smaller and contain less aromatic structures (Harvey et al., 1983). Anderson et al., (1985) also reported that the reaction of ozone with fulvic acids results in lower concentrations of products compared to other oxidants, showing the lower reactivity of ozone with fulvic acids. Humic acids contain more aromatic structures and therefore should have a higher reactivity with ozone. The HON fraction only shows a sharp decrease in fluorescence after the first ozone dose. After the first ozone dose the fluorescence does not decrease indicating that fulvic acids are less reactive with ozone than humic acids.

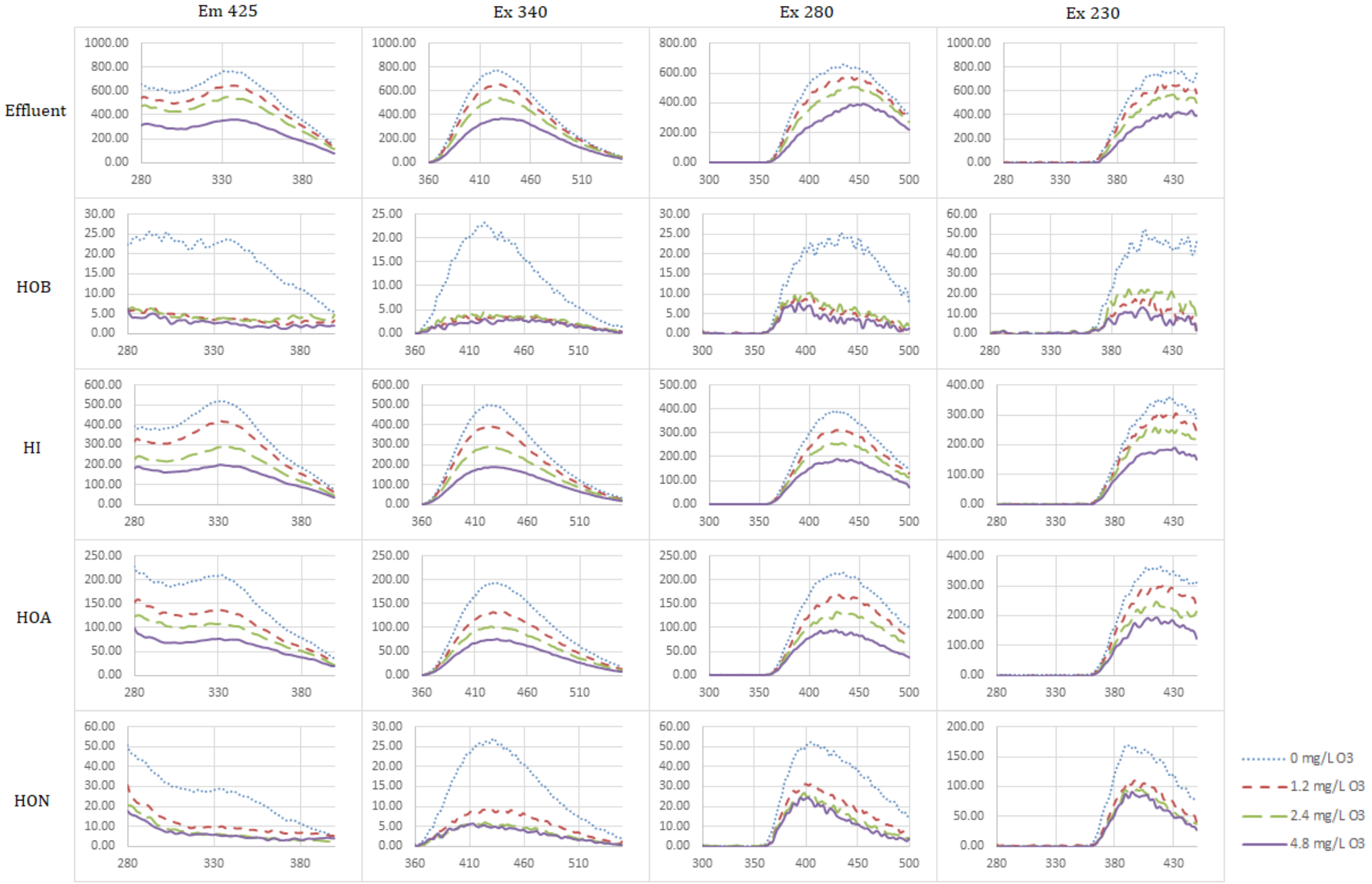

Figure 11. Fluorescence spectra for all the samples ordered per fraction with the four different ozone dosages per graph. On the Y-axis the fluorescence is depicted and the X-axis the wavelength in nm.

4.4 COD, UV absorbance and NO_2 concentration

The values for the COD, UV absorbance and NO_2 measurements were all corrected for dilutions during the fractioning and ozonation process (Table 5). The DOC values in the table were not adjusted for the possibility that the LMW components and or HOC measurements in the LC-OCD could be incorrect due to the pressure drop.

The COD values of the non-fractioned effluent samples are lower than the COD values combined for the four fractions. This could be because the COD values were not adjusted for organic matter coming of the XAD-8 resin, syringe and filter in the fractioning process. However, even accounting for possible COD originating from the XAD-8 resin, syringe and filter, an increase of a factor of four seems highly unlikely. No possible explanation for this increase has been found yet.

All the fractions show a decrease in the COD with increasing ozone dosage. However, after the 2.4 mg/L ozone dose, the COD values do not decrease any further and even increase. This observation is the opposite of what is expected, since the COD is not depleted after the 2.4 mg/L of ozone a further decrease is expected. There could be a possible limit on the reaction of ozone with organic matter before the COD value is not lowered anymore, with increasing ozone dose. This would require experiments with higher ozone doses to confirm.

Table 5 The applied O_3 concentration, the COD concentrations, the DOC concentrations (LC-OCD) and O_3 dosage per DOC concentration.

	Dosage O_3 (mg/L)	**COD (mg/L)**	**DOC (mg/L)**	**Dosage (mg O_3/mg DOC)**
effluent	0	29.2	10.4	0.00
	1.2	27.5	10.0	0.12
	2.4	27.0	10.2	0.24
	4.8	26.2	10.5	0.46
HOB	0	10.9	1.6	0.00
	1.2	10.1	1.8	0.67
	2.4	9.3	1.4	1.75
	4.8	9.4	1.4	3.50
HI	0	46.2	12.4	0.00
	1.2	45.3	12.9	0.09
	2.4	44.3	12.9	0.19
	4.8	44.9	13.0	0.37
HOA	0	27.8	7.3	0.00
	1.2	26.3	7.3	0.16
	2.4	25.1	7.3	0.33
	4.8	25.4	7.1	0.68
HON	0	40.1	9.0	0.00
	1.2	38.6	8.7	0.14
	2.4	37.9	8.6	0.28
	4.8	38.2	9.4	0.51

The UV absorbance of the fractions decreases with increasing ozone dose (Table 5, Figure 12), indicating there is loss in the complexity of the organic matter structure. This decrease is most notable in the non-fractioned effluent, HI and HOA fractions. The non-fractioned effluent and HOA and HI fractions show a linear decrease in the UV with increasing ozone dosage (Figure 12). This linearity is not observed in the HON and HOB fractions. This indicates that the HOB and HON fractions contain

either less complex organic matter or almost no organic matter at all. Zhang, T et al., (2008) showed a similar reduction in UV absorbance, the HOA and HON fraction showed a decrease of 45.5% and 41.7% of UV absorbance at an dose of 1 mg O_3/mg DOC. While the decrease in the HOA fraction and HON fraction in this thesis were 47% and 19% respectively. The reduction in this thesis of the HON fraction is 2 times lower, however the mg O_3/mg DOC was also 2 times lower (Table 5). The UV absorbance in the HOA fraction already shows a higher reduction in the UV absorbance at a lower ozone dose of 0.68 mg O_3/mg DOC. This could indicate that there is a difference in reactivity of the HOA fraction, based on the organic matter present in the HOA fraction.

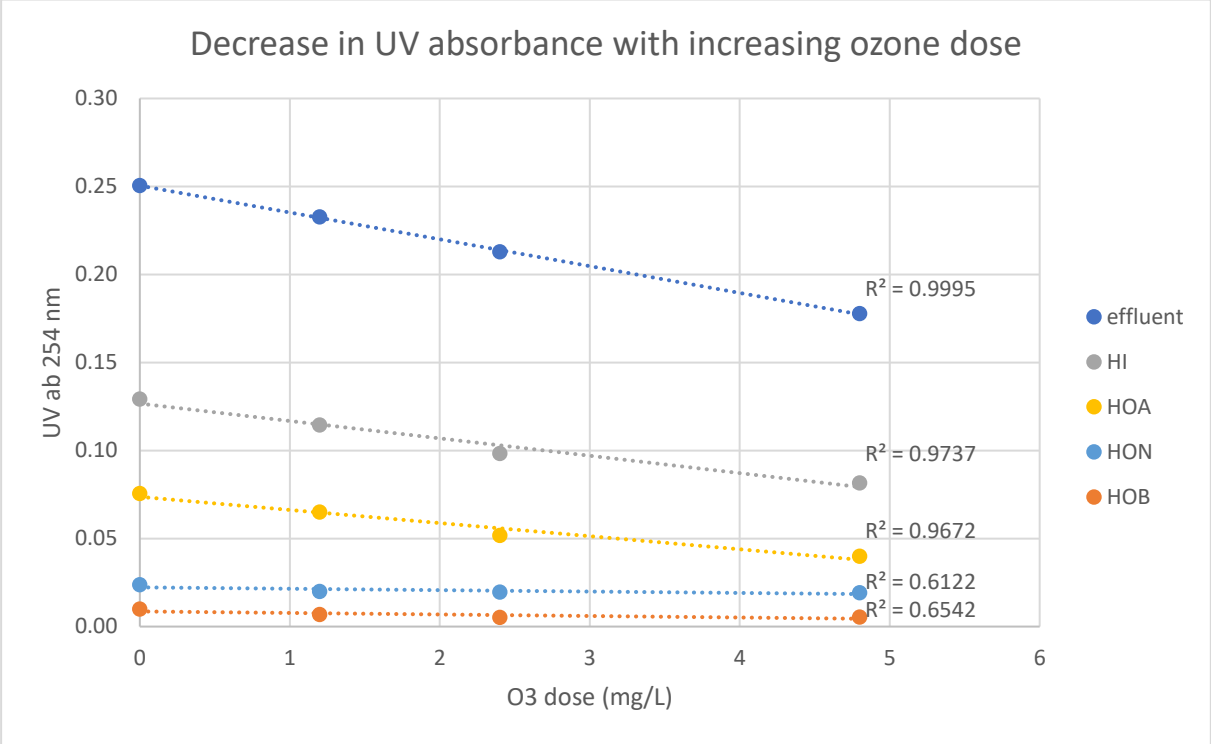

Figure 12. UV absorbance with increasing ozone dose.

Only in the non-fractioned effluent samples the NO_2 concentration was above the minimum detection limit. This likely indicates that NO_2 is converted or lost during the fractioning process.

4.5 Correlations between different parameters

4.5.1 Reactivity of hydrophilics

Starting with the biggest fraction, the LC-OCD results showed no changes between ozone dosages in the size components composition of the HI fraction (Figure 6, Figure 7 and Figure 8). This indicated that the hydrophilic organic matter is not reactive with ozone, however the fluorescence and UV measurements show that this is not the case (Figure 10 and Figure 12). The fluorescence in the HI fraction decreased with increasing ozone dose and a similar decreasing trend was observed for the UV absorbance. This indicates that there is a reaction between hydrophilic organic matter and ozone. Combing these results show that this reaction does either: form smaller organic matter but the new sizes still fall under the same size category in the LC-OCD, or the reaction does not change the size but rather the unsaturated structure of the organic matter lowering the fluorescence and absorbance values.

4.5.2 Reactivity of hydrophobic acids

The LC-OCD showed that the HOA fraction lost around 90% of the humic acids after the highest ozone dose (Figure 7). Although the fluorescence of the HOA fraction also decreases, the fluorescence of the HOA fraction remains around 40-50% of the initial value (Figure 11). The UV absorbance also shows a decrease with increasing ozone dosage, however also this measurement retains over 50% of the initial value even after the highest ozone dosage. The fluorescence and UV absorbance indicate that are unsaturated structures present in the organic matter in the HOA fraction, which could react with ozone.

4.5.3 Reactivity of hydrophobic neutrals

Both the fluorescence and the UV absorbance show a decrease with the first ozone dose, but do not see a decrease after this initial dose (Figure 11 and Figure 12). The LC-OCD did not see changes for the HON fraction with increasing ozone dosages. The HON fraction has a higher DOC concentration than the HOA fraction, nevertheless there was almost no UV absorbance in the HON fraction (Table 5). This indicates that the DOC in the HON fraction contains less unsaturated bonds and aromatic structures compared to DOC in the HOA and HI fraction. The fluorescence does show the presence of fulvic acids in the fraction. The fluorescence does not decrease even at the highest ozone dosage indicating these compounds are not very reactive with ozone.

The low values for the UV absorbance and fluorescence indicate that the DOC in the HON fraction consist mainly out of saturated bonds. There are some fulvic acids present according to the fluorescence observed, but the fulvic acids seem to react only to a limited extend with ozone. This fraction is hypothesised to interfere less with the removal of MPs in the ozonation due to the low amount of unsaturated bonds that are present.

4.5.4 Reactivity of hydrophobic bases

The HOB fraction has low UV absorbance, fluorescence and DOC concentration (Figure 12, Figure 11 and Table 5). The low UV absorbance and fluorescence indicate that, the HOB fraction contains less unsaturated bonds and aromatic structures than the HI and HOA fractions just as the HON fraction. The HOB fraction is the only fraction that shows an increase in the LMW neutrals (Figure 9). This indicates that the ozone can react with saturated organic matter to break the molecules down into smaller molecules. However, the ozone dose per mg of DOC in this fraction was 7 times higher than the other three fractions. This could indicate that higher dosages of ozone are required to break down organic matter into LMW components.

4.5.5 Reactivity of organic matter with ozone

The HI fraction is the most reactive fraction of the four fractions followed by the HOA fraction. The HON fraction has a higher DOC concentration than the HOA fraction but does not show more reactivity with the ozone. Although the HON fraction contains more DOC then the HOA fraction, the HON fraction contains mainly saturated bonds and is therefore less likely to interfere in the removal of MPs during ozonation. The smallest fraction HOB is present in a very low concentration and does also not show reactivity with the ozone. This results indicate that more hydrophilic organic matter, the HI fraction and the acids of hydrophobic fractions (HOA), have a higher reactivity with ozone. This can be explained with the preference of ozone to react with electron rich bonds. Electron rich regions in a molecule are present when there are more polar covalent bonds. Polar covalent bonds also increase the van der Waals interactions between the molecule and the water increasing the solubility in water. The ozone therefore reacts more with the fractions which are more hydrophilic.

It is highly likely that the results from the LC-OCD are incorrect due to the pressure drop during the measurements. The total DOC found in the four fractions combined was almost three times higher

than the non-fractioned effluent sample. The main mistakes in the measurements are probably in the LMW neutrals and acids and in the HOC measurement. These measurement are bigger in the four fractions combined than in the non-fractioned effluent sample. The biopolymers and humic acids measurements do not show a higher concentration for the fractions combined and are therefore more likely to be correct. Thus the results from the humic acids and biopolymers from the LC-OCD have a higher chance to represent the actual reaction with ozone.

The LC-OCD measurement showed a loss in the biopolymers after the fractioning (Table 4). The four fractions combined contained less biopolymers then the non-fractioned effluent sample. The highest concentration of biopolymers was found in the HI fraction. The biopolymers present in the HI fraction showed no decrease in concentration after ozonation (Figure 8). The non-fractioned effluent did show a decrease in concentration of the biopolymers after ozonation. From this comparison was concluded that the more hydrophobic biopolymers were broken down into smaller molecules while the HI fraction seemed unreactive. The fluorescence and the UV absorbance show actually that the more hydrophilic organic matter is more reactive then the hydrophobic organic matter. The decrease of the biopolymers in the non-fractioned effluent is thus not due to the hydrophobic biopolymers being broken down. Applying a higher ozone dose to the HI fraction could give insight into, if these biopolymers are indeed broken down into smaller molecules after sufficient ozone dose is applied.

The HI and HOA fractions contain the most unsaturated organic matter as shown by the fluorescence and the UV absorbances. This unsaturated organic matter shows a reactivity with the ozone indicated by the decrease in both the fluorescence and the UV absorbance with increasing ozone dosage. The LC-OCD did not show a difference in size components for the HOA and HI fractions with any ozone dose. The only difference was the increase in HOC in the HOA fraction. Therefore the reaction with ozone results most likely first into a structural change rather than a size change of the organic molecules.

Between the fractions the mg O_3/mg DOC was different due to the difference in concentration of DOC in the fractions (Table 5). However the only fraction with a large difference between the doses is the HOB fraction due to the lower DOC concentration in this fraction. Moreover the trends in decrease in fluorescence and UV are linear with the increase in ozone dose for the HI and HOA fractions. The results are therefore showing that, although the mg O_3/mg DOC dose was not the same for the fractions, the linear decrease in UV absorbance and fluorescence indicate the reaction of DOC with ozone.

5. Conclusion

This research shows that the DOC present in effluent in Bennekom could be divided into four different fractions using the XAD-8 resin. The biggest fraction of DOC is present in the HI fraction followed by the HOA and HON fraction and the HOB fractions is the smallest fraction. From the four fractions the HI and HOA fractions are the most reactive with the ozone and will probably be the most interfering fractions during the ozonation of MPs. Although that the HON fraction is bigger than the HOA fraction, the LC-OCD, UV absorbance and fluorescence show little indication this fraction has interaction with ozone. The LC-OCD measurements are likely to contain some errors which seem to originate from the LMW acids and neutrals measurements. The most reactive organic matter with ozone contains unsaturated bonds and is more hydrophilic. This organic matter is more present in the HI and HOA fractions, which are therefore the more reactive fractions with ozone.

6. Recommendations

Testing the MP removal efficiencies, as was proposed in the research aim, can give more insight in the reactivity of the ozone with organic matter. If the HI and HOA fraction have indeed lower removal efficiency MPs, the most interfering DOC is the unsaturated organic matter. According to the LC-OCD results this kind of organic matter consist mainly out of humic acids biopolymers. The pre-treatment of the first biological reactor of the bio-ozone-bio system should then mainly focus on the removal of humic acids and biopolymers. Comparing the removal efficiencies of MPs in the fractions to the removal efficiency of MPs in a water sample, could also give more insight in whether there is indeed interference for the HOB and HON fractions. If the removal efficiencies of MPs in the water sample are equal to the removal efficiencies in the HOB and HON fraction there is no interference of saturated organic matter.

Confirming whether humic acids are the most interfering group of organic matter could give insight into if the HI and HOA fractions show the most reaction with ozone, due to containing more humic acids than the HOA and HOB fractions. According to Swietlik et al., (2004) the humic acids can be precipitate at very low pH over a period of 24h. Removing the humic acids from the HI and HOA fractions would significantly lower their total DOC. Comparing the removal efficiencies of MPs of a HI fraction containing humic acids with a HI fraction without humic acids, would give more insight if the humic acids are the most interfering organic matter for ozonation of MPs.

In the effluent of the WWTP of Bennekom there was no indication for the presence of proteins. However Li et al., (2019) and Jin et al., (2016) showed that there is the possibility that there are protein like structures present in the effluent of WWTPs. Addition of proteins to an effluent sample of the WWTP of Bennekom could give insight in the reactivity of proteins with ozone. Comparing the results of such an experiment to the results in this thesis, could show whether or not ozone has a preference reaction with proteins over MPs. If this is indeed the case the removal efficiencies of MPs in a sample with and without proteins can be compared to investigate the interference of proteins in the removal of MPs during ozonation.

During the thesis many fluorescence measurements were performed, which can be time consuming to perform for multiple samples. The UV absorbance and the fluorescence of humic acids showed similar decrease in percentage with increasing ozone dose. This indicates that the UV absorbance gives the same information, the decrease of complexity of organic matter, as the fluorescence measurements. Since both measurements give the same kind of information, the UV absorbance is the easier and more reliable measurement. Since it requires less data analysis and is quicker overall to measure.

The fractionation performed in this thesis can be improved with the following adjustment. The syringe used in the fractioning process should be a smaller volume then 50 ml. This is because the syringe showed clogging issues, due to the XAD-8 resin leaking through the adapter part where the filter is placed. Using a smaller syringe should decrease the amount of XAD-8 resin leaking. Moreover it will simplify the uptake of sample, this required a lot of force for the 50 ml syringe.
It is also recommended to dilute the samples to the same DOC concentration before ozonation. This makes comparison between the fractions more reliable and gives more insight into the specific chemistry of ozone with the fractions rather than the total amount of reaction between organic matter and ozone.

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8. Appendix

Appendix A – MPs stock mix 03-4.

This appendix contain a list of the MPs used to spike the DOC fractions with information on the chemical structure, water solubility, original application and the concentration in the stock (Table 6).

Table 6. Synonym, chemical formula, solubility in water, application and concentration of the micropollutants in stock 03-4

Synonym	**Chemical formula**	**Water solubility (mg/L at 20-25 degrees C)**	**Application**	**concentration (mg / L)**
Dimetridazole	C5H7N3O2	-	Antiparasitic	12.54151294
Trimethoprim	C14H18N4O3	400	antibiotic	12.7520846
Gemfibrozil	C15H22O3	11	Fibrate (lower fat)	12.96867711
Ibuprofen	C13H18O2	21	Painkiller	12.97903548
Naproxen	CH3OC10H6CH(CH3)CO2H	15.9	Anti inframatory (non steroid)	12.66536154
Propranolol	C16H21NO2	61.7 / 5000	beta blocker	12.76512333
Chloridazon	C10H8ClN3O	400	Herbicide	11.61851936
Desphenyl-chloridazon	C4H4ClN3O	-	degradation product of chloridazon	10.86499544
Iopamidol	C17H22I3N3O8	120000	contrast liquid	11.98796267
BAM	C7H5Cl2NO	2730	Herbicide	11.99031216
2,4-D	Cl2C6H3OCH2CO2H	0.677	Herbicide	12.92791245
Bentazone	C10H12N2O3S	570	Herbicide	12.36968192
Tetracycline	C22H24N2O8	231	antibiotic	13.76123707
Metoprolol	C15H25NO3	16900	beta blocker	12.39224327
Caffeine	C8H10N4O2	21600	Stimulant	13.26400187
Benzotriazole	C6H5N3	19800	Corrosion inhibitor	12.27527226
Sulfamethoxazole	C10H11N3O3S	610	Antibiotic	14.30989626
Irbesartan	C25H28N6O	0.059	Lower blood pressure	12.05393751
Carbamazepine	C15H12N2O	17.7	Antiepileptic	12.8839414
Furosemide	C12H11ClN2O5S	73	Loop diuretic (increases urine excretion)	11.57444598
Mecoprop	C10H11ClO3	880	Herbicide	12.336

Diclofenac	C14H11Cl2NO2	2.37	non-steroidal anti inflammatory	13.33575442
Clarithromycin	C38H69NO13	0.33	antibiotic	12.264
Hydrochlorothiazide	C7H8ClN3O4S2	-	Thiazide	12.68522747
sotalol hydrochloride	C12H20N2O3S	0.782	Beta blokker	11.48336252
4-methylbenzotriazol	C7H7N3	5500	Corrision inhibitor derivative	12.24708015
5-methylbenzotriazol	C7H7N3	6000	xenobiotic	12.07528517

Appendix B – LC-OCD results

This appendix contains all the LC-OCD measurements summarised in five tables, one for each fraction and for the non-fractioned effluent. The most important observations are mentioned per table.

In the effluent samples is a decrease of the biopolymers with increasing ozone dosage, while there is an increase in humic acids with increasing ozone dosage. The other groups do not show a trend with increasing ozone dosage.

Table 7. The LC-OCD measurement for the effluent samples with either 0, 1.2, 2.4 and 4.8 mg/L of ozone applied.

	effluent 0 mg/L O3	effluent 1.2 mg/L O3	effluent 2.4 mg/L O3	effluent 4.8 mg/L O3
Biopolymers - C	1007.0	864.4	895.6	189.4
Biopolymers - N	199.6	172.4	22.3	160.4
Humic acids - C	-	4471.1	4961.2	5250.8
Humic acids - N	340.9	303.0	340.9	347.5
LMW acids	144.1	77.3	72.8	133.0
LMW neutrals	983.4	669.3	687.1	615.8
HOC	3831.90	3898.73	3542.28	4299.75

Table 8 shows the data for the HOB fraction. This fraction shows low values for all the groups and shows an increase in LMW neutrals with increasing ozone dosage. Furthermore there is a significant decrease in HOC with increasing ozone dose.

Table 8 The LC-OCD measurement for the HOB fraction samples with either 0, 1.2, 2.4 and 4.8 mg/L of ozone applied.

	HOB 0 mg/L O3	HOB 1.2 mg/L O3	HOB 2.4 mg/L O3	HOB 4.8 mg/L O3
Biopolymers - C	6.2	6.2	6.2	6.2
Biopolymers - N	12.3	12.3	12.3	12.3
Humic acids - C	33.3	70.3	34.5	23.4
Humic acids - N	12.3	29.7	12.3	14.2
LMW acids	159.3	197.5	81.5	81.5
LMW neutrals	187.1	355.0	221.7	601.8
HOC	1214.48	1157.71	1026.88	659.08

The HI fraction is shown in table 8, most of the groups seem unaffected by treatment with ozone dosage. There is a decrease in Biopolymers-N after ozone treatment however this seems to be a duplicate of the Biopolymers-C measurement.

Table 9 The LC-OCD measurement for the HI samples with either 0, 1.2, 2.4 and 4.8 mg/L of ozone applied.

	HI 0 mg/L O3	HI 1.2 mg/L O3	HI 2.4 mg/L O3	HI 4.8 mg/L O3
Biopolymers - C	538.9	538.9	581.2	578.9
Biopolymers - N	538.9	84.6	84.1	84.1
Humic acids - C	2996.7	3145.4	3111.1	3087.9
Humic acids - N	267.7	278.0	273.5	264.3
LMW acids	227.2	277.5	310.7	423.9
LMW neutrals	5315.0	5418.0	5555.3	5417.4
HOC	3352.46	3501.21	3318.14	3489.42

The HOA fraction shows an increase in HOC with the highest ozone dosage (table 9). The humic acids - C show the same stable trend for the first 2 ozone dosages but at the highest ozone dosage the humic acids - C concertation decreases.

Table 10 The LC-OCD measurement for the HOA samples with either 0, 1.2, 2.4 and 4.8 mg/L of ozone applied.

	HOA 0 mg/L O3	HOA 1.2 mg/L O3	HOA 2.4 mg/L O3	HOA 4.8 mg/L O3
Biopolymers - C	68.3	66.5	60.2	53.4
Biopolymers - N	18.4	15.5	14.4	14.2
Humic acids - C	1215.1	1312.0	1275.6	98.7
Humic acids - N	65.9	66.1	68.2	62.8
LMW acids	1105.7	1092.4	1076.7	1093.6
LMW neutrals	2666.6	2751.4	2606.1	2642.4
HOC	2276.37	2106.85	2264.26	3208.71

In table 10 the LC-OCD results for the HON fraction are shown. There is a small increase in humic acids – C with increasing ozone dosage. And the HOC group is decreasing with each ozone dosage.

Table 11 The LC-OCD measurement for the HON samples with either 0, 1.2, 2.4 and 4.8 mg/L of ozone applied.

	HON 0 mg/L O3	HON 1.2 mg/L O3	HON 2.4 mg/L O3	HON 4.8 mg/L O3
Biopolymers - C	5.6	5.6	5.6	11.2
Biopolymers - N	11.2	11.2	11.2	22.3
Humic acids - C	177.5	204.3	236.7	293.6
Humic acids - N	11.2	11.2	11.2	22.3
LMW acids	682.0	641.8	638.4	675.3
LMW neutrals	5205.5	5105.0	5071.5	5875.2
HOC	2890.83	2779.22	2667.60	2555.99

Appendix C – Ozone measurements

This appendix contains the measurement performed by the Oceanview system and the decay in ozone concentration overtime.

The Oceanview system showed an increase in absorbance each time a pipette sample was taken from the ozone stock (Figure 13). After pipetting the system sometimes measured higher values then before the pipetting, which would imply an increase in ozone concentration. However, this was not possible since no new ozone was being bubbled through the sample. Therefore it was decided to not use the Oceanview system to measure the ozone concentration during the ozonation.

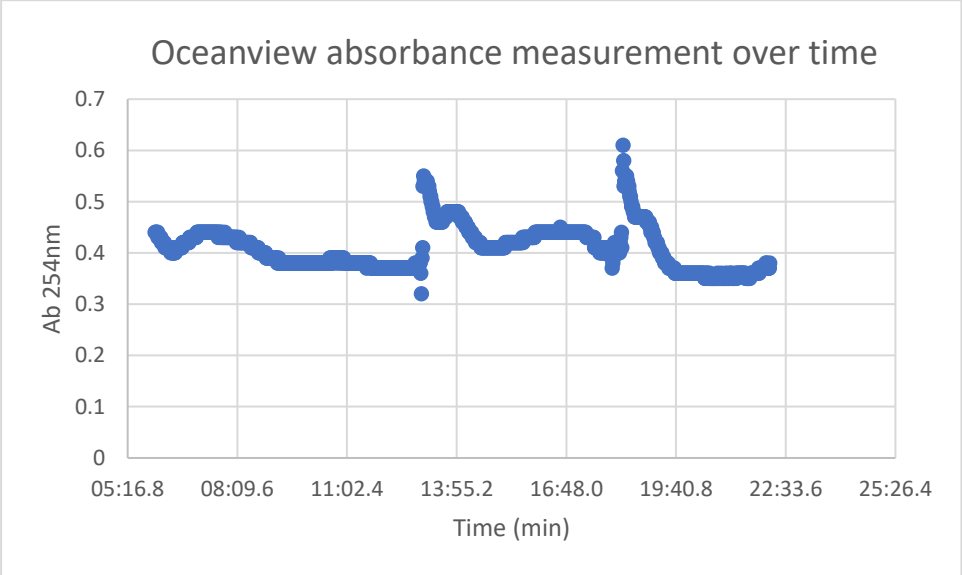

Figure 13. Realtime measurement of the absorbance at 254 nm using the Oceanview system.

The concentration of ozone in the ozone stock decreased over time. This was measured using the indigo method taking samples after 2, 4, 8, 12, 20 and 25 min (Figure 14).

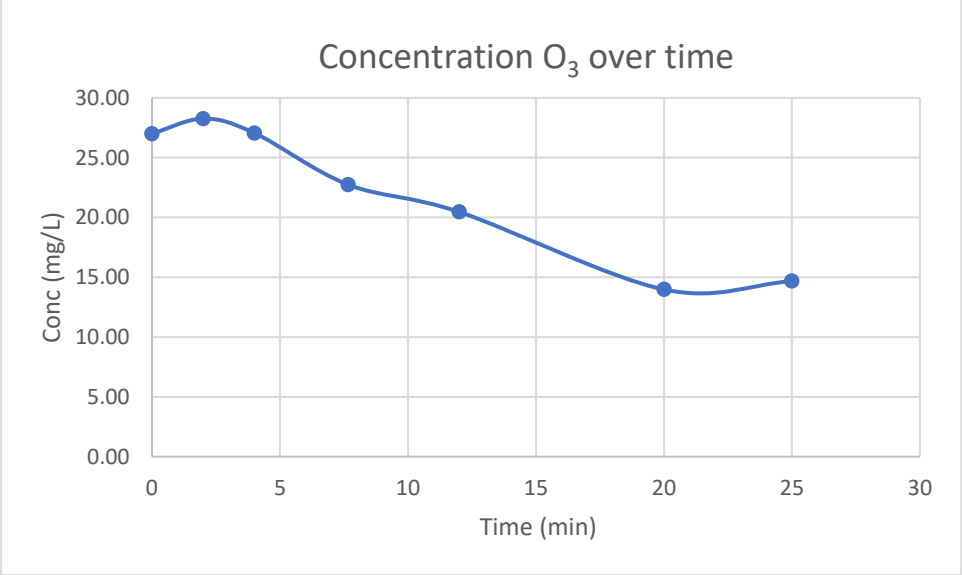

Figure 14. concentration of ozone in water inside of a beaker over time determined using indigo reference.

Appendix D – Ozone dose per sample

Based on the measurement of the indigo samples the concentration of ozone added to the samples was calculated (Table 12).

Table 12. The name of the indigo sample, corresponding absorbance at 600nm, concentration of ozone in the ozone stock and the eventual ozone dose applied to the samples.

Indigo sample	Absorbance 600nm	Conc O_3 (mg/l)	Ozone dose (mg/L)
REF	0.606	0.000	
1T1	0	48.000	
1T2	0	48.000	
1T3	0	48.000	
1A	0.003	47.762	1.2
1B	0	48.000	1.2
1C	0.013	46.970	1.2
1D	0	48.000	1.2
1E	0.004	47.683	1.2
2T1	0.002	47.842	
2T2	0	48.000	
2T3	0	48.000	
2A	0.002	47.842	2.4
2B	0	48.000	2.4
2C	0	48.000	2.4
2D	0	48.000	2.4
2E	0	48.000	2.4
4T1	0.003	47.762	
4T2	0	48.000	
4T3	0	48.000	
4A	0	48.000	4.8
4B	0	48.000	4.8
4C	0.001	47.921	4.8
4D	0.001	47.921	4.8
4E	0.016	46.733	4.8

Appendix E – Infinite m200 pro plate reader

The infinite m200 pro measurements were performed several times due to instability of the system. The duplo measurement of the same sample would sometimes result in two different values which could be five times higher, an example is shown in Figure 15. Moreover sometimes the blank would measure more fluorescence then the samples making correction for the blank impossible. The results of the Infinite m200 pro were therefore not used in this thesis.

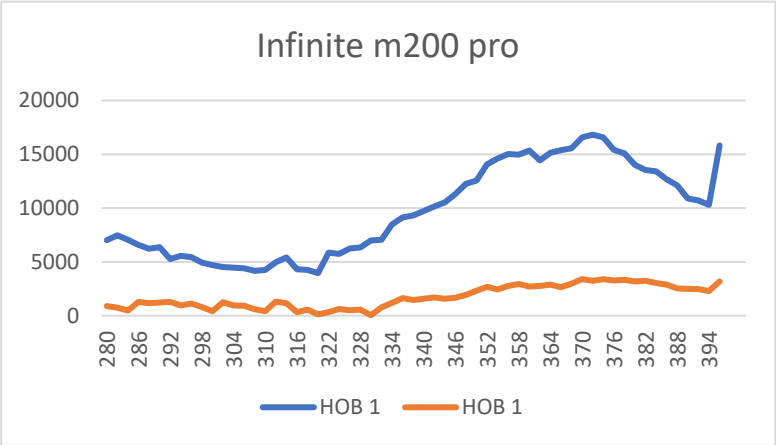

Figure 15. Duplo fluorescent measurement of the same sample in the Infinite m200 pro. The sample was the HOB fraction dosed with the 1.2 mg/L ozone dose.