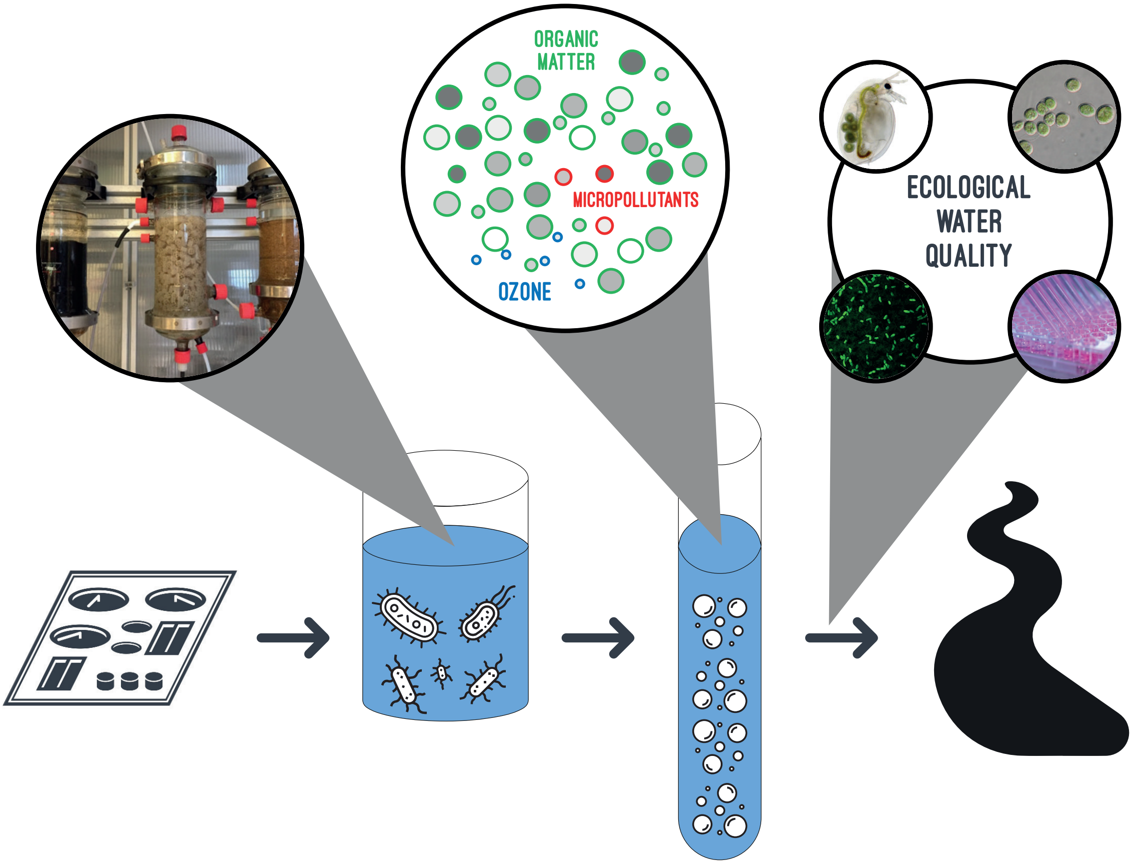
The BO_3 process for removal of micropollutants from wastewater treatment plant effluent

Exploring the synergies between biological treatment and ozonation



Koen van Gijn

Propositions

1. Hybrid processes are more effective and efficient than single processes for micropollutant removal
(this thesis)

2. Characterization of background organic matter is crucial for the optimization of micropollutant removal technologies
(this thesis)

3. Being amazed by what we do not understand is the basis of science

4. A socially fair and sustainable world is incompatible with economic models based on infinite growth

5. Allowing the mind to be distracted is a prerequisite for true creativity and innovation

6. Genuine positive feedback is required to stimulate development and growth in people

Propositions belonging to the thesis, entitled

The BO_3 process for removal of micropollutants from wastewater treatment plant effluent

Koen van Gijn

Wageningen, 13 January 2023

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Exploring the synergies between biological treatment and ozonation

Koen van Gijn

Thesis

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Together we can find a balance between ourselves and our environment

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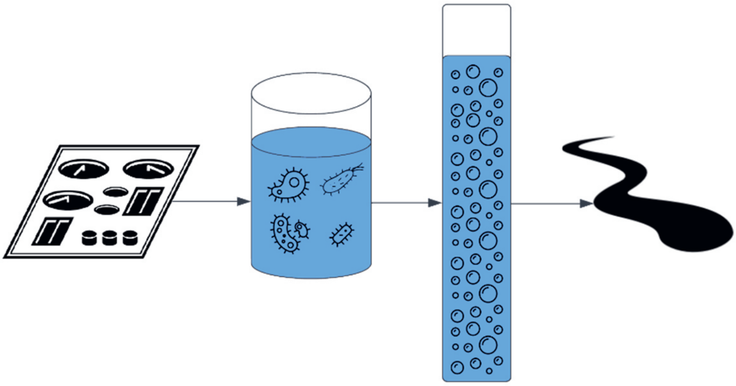
Chapter 1

General introduction



1.1 Micropollutants

Due to the improvement of analytical techniques, it became possible to detect micropollutants (MPs), or chemicals of emerging concern, at their low concentrations (nanogram – microgram per liter range) around the year 2000. Since then MPs have been detected in wastewater, surface water, ground water, sea water and even drinking water all over the world [1–6]. The growing scientific and political concern about these anthropogenic compounds and their widespread presence is illustrated by the increase of scientific attention that MPs have received in the last decades [7, 8] and by the fact that several MPs have recently been included in the European watchlist [9]. Most recently, even the media are giving more attention and increase in social awareness regarding the presence and the consequences of MPs in our environment [10–12].

MPs are a large group of different types of compounds characterized mainly by their low prevalent concentrations in aquatic systems (nanogram – microgram per liter range). The list of possible MPs is currently already extensive and continuously growing. MPs belong to a wide range of chemical classes such as pharmaceuticals, pesticides, industrial by-products, polybrominated flame retardants, surfactants, by-products originating from water treatment processes, food additives, ionic liquids, life-style and personal care products [13]. The number of chemicals used and produced by humans has been and is increasing exponentially, now almost reaching 200,000,000 compounds [14]. On top of that, the number of MPs increases with numerous biological and chemical transformation products (TPs) that can be formed from the parent compounds in various ways. All these different MPs have different properties and part of them are likely to cause risks for us or for our environment.

1.1.1 Risks

When only taking into account their prevalent concentrations, MPs might seem harmless. Nevertheless, several years after we became aware of the presence of MPs, a number of examples where MPs caused clear adverse environmental effects became evident. Predation of vultures on cows that were fed an anti-inflammatory (diclofenac) resulted in a decrease of the vulture population in Pakistan [15] and the presence of synthetic estrogens caused the collapse of a fish population in Canada [16]. Additional to the environmental effects that were observed, there are significant uncertainties regarding the toxic risk of MPs in the aquatic environment. Because it is challenging to reliably asses the toxic risk of MPs, larger safety factors should be included in risk assessment, and action to reduce MP concentrations should be taken out of precaution. The toxicological uncertainties around MPs are illustrated by a study that compared the theoretical toxicity (based on the chemically measured MPs and the available toxicity data) with the toxicity directly measured in a wastewater with a bioassay. Escher et al. [18] found that the theoretical toxicity (based on nearly 100 MPs) could explain only 1% of the total measured toxicity with the microtox assay, this number dropped even further to 0.0025% after advanced treatment of the WWTP effluent. There are four reasons that make it difficult to accurately determine the toxic risk of MPs: i. The large number of MPs, including parent compounds and TPs results in lack of hazard assessments for many compounds [14,19–21], ii. the continuous presence of the MPs in the environment requires the use of chronic tests, which are more expensive and more complex to perform and to interpret [22–24], iii. uncertainties regarding the combined effects of MPs. MPs are present in complex mixtures, which makes interpretation of available toxicity data is more difficult, because most available data are single compound based tests [25–27], and iv. bioaccumulation of non-polar MP that can increase the exposure concentrations within organisms by orders of magnitude [28,29].

Toxic effects of MPs for human health are also a point of concern, mainly through uptake via drinking water or via food. Because MPs end up in ground waters and surface waters, which are used for the production of drinking water, there is a risk for the MPs to end up in drinking water. For the case of drinking water production, MPs are already monitored for a longer time than for wastewater [30]. Especially for drinking water production sites that use surface water, extensive treatments trains are already implemented to remove the MPs that are present [31,32]. For the case of ground water, currently MP concentrations are low enough to not pose a risk, though strategies to reduce MP concentrations in ground waters are already being studied [32–34]. By the application of wastewater, wastewater sludge or animal manure, MPs can reach agricultural fields, where they can be taken up by plants and end up in human food [35–37]. Even though current concentrations of MPs in drinking water and crops are low, the uncertainties mentioned in the previous paragraph are also valid for human toxicity of MPs [38].

There are significant environmental and human concerns regarding the prevalence of MPs in the environment and yet their concentrations are expected to increase further in the future. The global consumption of chemicals increases with an increasing world population and increasing welfare [39] and all of these chemicals can end up in the environment as MPs via waste or wastewater. Moreover, climate change results in more extreme draughts that result in less dilution of WWTP effluents into surface waters [40]. This will result the emissions of even more different MPs and also in higher final concentration in the environment. The combination of the uncertainties around toxicological risks of MPs and the expected increase of MPs and their concentrations in the future explains the attention that the topic has received in the last decades and underlines that the precautionary principle should be applied and the emissions of MPs into the environment should be reduced even in places where serious environmental and human impacts are not (yet) apparent [41].

1.1.2 Removal

Reducing the emissions of MPs towards natural waters can be done at the “source” (decreasing production and consumption) or “end-of-pipe” (the place where the MPs enter the environment). Source solutions have several advantages but are not always feasible for the case of MPs. For example, preventing the use of pharmaceuticals or pesticides can have serious impact on health and food security which results in ethical dilemmas. This means that in addition to source solutions, end-of-pipe solutions for MPs are and will be necessary. Many MPs end up in wastewater after production and consumption and, when not properly removed by wastewater treatment plants (WWTPs), consequentially enter the environment: their end-of-pipe is the WWTP. Conventional WWTPs use activated sludge to remove bulk organic matter (OM) and nutrients like ammonium and phosphate. This activated sludge consists of a large community of microorganisms that remove some MPs from the wastewater by sorption to the sludge or by biological conversions. Unfortunately this removal is very limited or nonexistent for a large number of MPs, which makes WWTPs major emission points of MPs into the environment [42,43]. Because of this, improving the MP removal of conventional WWTPs is an effective option to reduce MP emissions into the environment.

1.2 Advanced treatment

There are a number of technologies that aim to improve the MP removal in WWTPs and while many of these show the potential to remove a large number of MPs, most technologies still have their limitations. The two most commonly implemented technologies are (powder and granular) activated carbon treatment and ozonation. These technologies have already proven their potential and are implemented in full-scale in several countries [44–48]. Additionally, many new technologies based on other sorption processes, advanced oxidation, advanced biological treatment and membrane filtration are being researched [49–52]. Unfortunately, all of these treatment processes (including the established ones) have limitations that hinder implementation at full-scale. These limitations are mainly related to high energy requirements, costs, greenhouse-gas emissions, the formation of TPs, saturation of sorption materials, or the generation of concentrated streams that remain after treatment and have their own environmental concerns. Lastly, even though most advanced technologies achieve higher MP removal than conventional WWTPs, these methods still cannot remove all MPs due to the large variety and diversity of MPs that are present in wastewaters. Hybrid treatments combine multiple advanced treatment processes and can therefore remove an even broader range of MPs than single advanced treatment processes [53].

This thesis focusses on a hybrid treatment technology combining biological treatment with ozonation in the BO_3 process. The synergies between these two treatment processes have the potential to minimize all of the limitations of individual biological and ozone treatment, reducing both energy demand and release of formed ozone transformation products (OTPs) and increasing the range of MPs that can be removed. Advanced biological treatment differs from conventional wastewater treatment in the way that the biomass is retained in the reactor using different types of carriers and by the adaptation of the biomass to the available substrates, namely organic compounds and MPs that were not removed in the activated sludge process and MPs. The carriers are used because wastewater effluents contain limited amounts of substrate and nutrients (compared to wastewater influent) to support sludge growth. Suspended biomass would quickly wash out of the reactor, but due to the carriers, biofilm can grow that remains in the reactor almost indefinitely. During ozonation, ozone gas is bubbled though the water which enables the reactive ozone molecules to dissolve into the water and to react with the dissolved pollutants.

1.2.1 Biological treatment

Biological treatments use microorganisms to convert pollutants (such as OM), MPs and nutrients) and are well established in wastewater treatment due to their energy efficiency. The microorganisms make many conversions possible at relatively low temperatures (where heating up the wastewater is not necessary) and without much external inputs, by using enzymes to lower activation energies of chemical reactions. However, microorganisms only have selective pressure to produce the right enzymes when it is beneficial for the them to convert the pollutants. The microorganisms need energy profit from each enzymatic conversion and the amount of substrate (i.e. pollutant) available at any time should be large enough to provide for sufficient growth to compete for this and other substrates with the other microorganisms present in the water. Also sorption to biomass can play a role in biological treatments, but in advanced biological treatment with high sludge retention times, this is limited because sorption equilibria are quickly reached. Biological treatments have the advantage of limited of energy and chemical requirements, and are applicable for a large number of organic compounds present in wastewater. For MPs, biodegradation is applicable but the potential is limited by the chemical diversity of compounds which results in a wide variance in biodegradability. To remove an MP in a bioreactor, the MP needs to react with the biologically produced enzymes at a rate that fits the retention time of the MP in the reactor This retention time is controlled by the size of the reactor and by the flow rate of the water through the reactor.

Microorganisms have shown to be able to gain energy from different types of conversions, depending on the availability of specific conditions such as electron donors and carbon sources (Figure 1.1). The most simple division in redox conditions is to distinguish aerobic and anaerobic and conditions, whereas the latter can be subdivided in e.g. nitrate reducing, sulfate reducing and methanogenic conditions. Moreover, the chemical structure is a factor that makes MPs susceptible or recalcitrant to biodegradation (Figure 1.1). For degradation of MPs, especially the concentration of substrate (or MP) is a limiting factor because the MPs are by definition present at low concentration. In other words, the concentrations of MPs are too low for microorganisms to set up MP degradation oriented enzyme systems. Research suggests that the main mechanism behind biodegradation of MPs is co-metabolic degradation [54–56]. Co-metabolic degradation of MP's is mediated by enzymes generated for other processes than MP degradation. This means that the occurrence and speed of degradation depend on the presence of other compounds than the MPs themselves (e.g. ammonium for nitrifiers), which makes it challenging to adapt microbial communities to degrade bio-recalcitrant MPs. Combining different types of redox and substrate conditions can increase the range of MPs that can be removed with biological treatment, though a relatively large group of MPs remain that resist biodegradation [42,57,58].

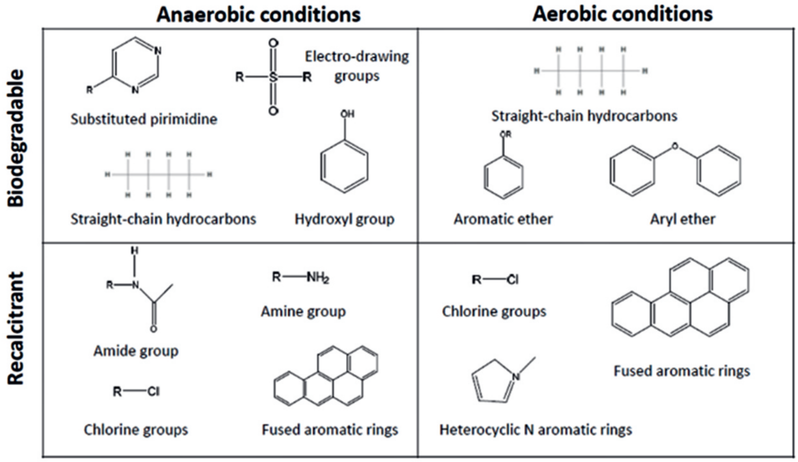


Figure 1.1 Overview of types of molecular groups that can be biodegraded in different conditions (adapted from van Loosdrecht & Kleerebezem [59])

1.2.2 Ozonation

Ozone is a strong oxidant that can be mixed with water to oxidize pollutants (such as OM and MPs) and inactivate pathogens. Degradation of pollutants during ozonation can occur via direct reaction with ozone, or via indirect processes through the formation of radicals (e.g. ·OH) that can in turn react with pollutants. For the direct reaction, ozone reacts fastest with compounds that contain nucleophilic moieties (e.g. phenols, anilines, olefins, reduced sulphur, and deprotonated amine groups) (Figure 1.2) [60]. Radicals react fast with almost all compounds, but are normally formed to a low extent (10^8 times lower than free ozone concentrations [61,62]). Radical formation can be increased by adding chemicals (e.g. hydrogen peroxide, peroxymonosulfate, sodium thiosulfate, or hydroxylamine) or UV light, but this directly increases the operational costs of the treatment, which makes it less attractive [63]. Ozonation has shown its potential to remove a broad range of MPs from WWTP effluents at full-scale, but in many countries further implementation is limited by the high required energy and cost inputs and the formation of potentially toxic OTPs [45,64].

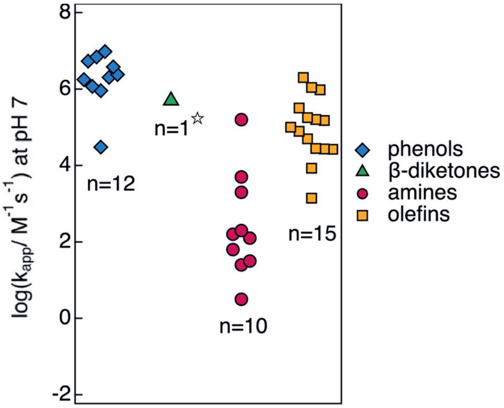


Figure 1.2 Reactivity (first order reaction constant, K_{app} in mole/s) of different types of moieties with ozone at neutral pH (adapted from Houska et al. [65])

The main reason for the high energy and costs for ozonation of WWTP effluents is the presence of effluent organic matter (EfOM) in the water. EfOM is a complex mix of organic compounds that remain after the regular WWTP process and that can all react with ozone at a faster or slower rate. Total organic carbon (TOC) can be used to quantify EfOM and is generally found in WWTP effluents at concentrations ranging from 4–40 mg/L [45,66,67]. The MPs are present at 10^3-10^6 times lower concentrations, which means that most ozone reacts with EfOM and not with MPs. Therefore, the required amount of ozone to remove MPs in a WWTP effluent is several orders of magnitude higher than the required amount of ozone to remove the same MPs in an OM free matrix. In practice, the required ozone dose for a water is usually based on the amount of OM present in the water [66,68].

During ozonation, OTPs are formed. Pollutants are oxidized, but generally not mineralized to carbon dioxide and water (Figure 1.3). Only at higher ozone doses and with the stimulation of radical formation, mineralization can occur to a significant extent (roughly more than 10%) [69]. This means that even though a reaction with ozone results in the removal of a parent compound, it also results in production of one or more OTPs. The number of potential OTPs formed per parent compound can be large [70], resulting in an even larger number of compounds adding to the already large number of MPs present in WWTP effluents. Because of this, chemical monitoring of MPs and OTPs is a challenge [71]. In some cases, ozone OTPs can pose are larger toxicological risk than their parent compounds, which underlines the importance of monitoring all of the MPs and formed OTPs in WWTP effluents treated with ozone [72,73]. Toxicity tests or bioassays have the potential to complement chemical analysis to identify the presence of toxic compounds in a complex water matrix. Such bioassays also screen for unknown chemicals in the samples, and are therefore a valuable tool to monitor ozonated samples in order to monitor the formation of unknown toxic OTPs.

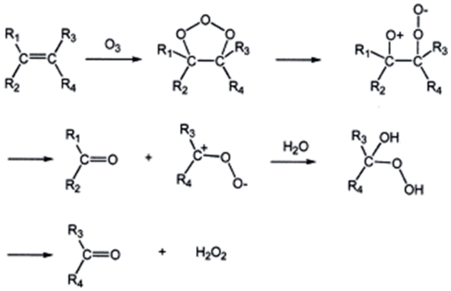


Figure 1.3 Example reaction of an olefin with ozone and the formation of OTPs during this reaction (adapted from von Gunten et al. [74])

1.3 Synergies between biological treatment and ozonation

In a combined treatment, the advantages of biological treatment and ozonation can help to overcome the disadvantages of the single treatment technologies. The limited removal of bio recalcitrant compounds in biological treatment can be compensated by the power of ozonation and the high energy demand and formation of unknown OTPs during ozonation can be negated by energy efficiency of biological treatment (Figure 1.4) [75]. The high energy demand of ozone treatment is caused by the large amount of ozone that reacts with EfOM instead of reacting with MPs (section 1.2.2). Removing part of the EfOM in a biological treatment directly reduces the amount of ozone that has to be generated to remove the MPs and therefore limits the required energy input. OTPs formed during ozonation are generally more biodegradable than their parent compounds and can therefore be removed in a biological post treatment [76]. There are two research areas related to the synergies between biological treatment and ozonation: i) the presence of EfOM, how it can be removed during biological treatment and how it interferes with ozonation, and ii) the formation of OTPs, how toxic these are and whether it is necessary to use a consecutive (biological) treatment to remove the OTPs.

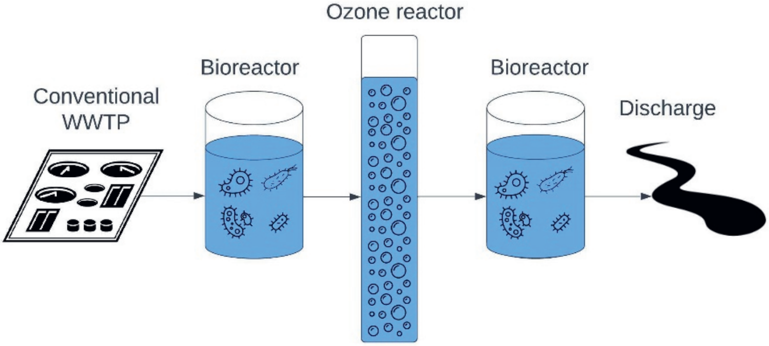


Figure 1.4 Schematic overview of the bio ozone bio (BO_3B) process (adapted from de wilt et al. [75])

1.3.1 Effluent organic matter

The effectivity of EfOM removal in the first bioreactor directly affects the amount of energy saved in the consecutive ozone treatment. Therefore, optimizing this first biological step directly results in a more efficient overall treatment. Both the type of biological reactor and the operational conditions can be optimized to ensure that the biomass can grow and convert as much organic matter (OM) as possible. For the type of reactor, biomass retention and mass transfer conditions are the most important factors [77]. Biomass growth in a reactor fed with WWTP effluent will be slow, because all the easily degradable substrates are already consumed in the conventional WWTP. The biomass will wash out from the reactor if it is not retained, which makes high biomass retention especially important in reactors treating WWTP effluent. For mass transfer, the flow pattern of the reactor will determine the concentration gradients and the diffusion, and therefore the availability of substrate and electron acceptors for the biomass. Mixed reactors result in a homogeneous distribution of substrate and electron acceptors, ensuring utilization of the entire reactor. Plug flow reactors can reach lower effluent concentrations, if the conditions (substrate, electron donor and carbon source) are not limiting. Next to the reactor type, also operational conditions have to ensure that substrate and electron donors are sufficiently supplied to support the growth of the biomass. This can be done by changing the flow rate through the system and by adding electron acceptors such as oxygen.

While EfOM is the main cause of the high energy requirement for MP ozonation, the composition of OM can be of influence as well. However, the effects of OM composition are not broadly studied in WWTP effluents. Up to recently, only chemical oxygen demand (COD) was measured to quantify EfOM, mainly because regulations are focused on this parameter.

COD is an ideal parameter to determine the required amount of aeration in conventional WWTPs, but it does not give information about the ozone reactivity of the OM. TOC is often used to determine the required dosing of ozone [45,66]. However, also TOC is a very general parameter and does not take into account the preference of ozone to react with OM that contains electron rich moieties. Getting more specific insight in EfOM is challenging and therefore characterization methods often focus on (semi) quantifying parts, or fractions, of the OM instead of trying to identify all unique chemicals that are present in the complex matrix.

Several types of OM characterization methods have been developed and used to get insight in the composition of EfOM. Fractionation methods use a separation step, for example with a hydrophobic resin or with membranes, followed by quantification of the TOC and UV absorbance in the fractions [78–81]. UV and fluorescence methods use optical properties of the organic matter and quantify different parts of the OM based on the way the molecules absorb and emit light [82,83]. Size exclusion chromatography uses a specific type of column to give insight in the distribution of molecular weights in the OM matrix [84]. More detailed characterization is possible by coupling liquid chromatography and mass spectrometry to ‘fingerprint’ the OM, after which the large amount of data can be interpreted using online databases [76,85]. Insights in the composition of EfOM have been gained using these characterization methods, nevertheless, experimental information about the effect of the different types OM in WWTP effluents on MP ozonation is not available. The reason for this is the relative novelty of the MP topic in water technology research (10-15 years) and that, developing and applying OM characterization methods and interpreting the results is complex due to the complexity of the EfOM matrix.

Because of the strong relevance of OM for MP ozonation, investigating the effect of the different EfOM types on MP ozonation is of crucial importance to make the implementation of MP removal treatment more efficient and more feasible.

1.3.2 Transformation products

Ozone reacts with both organic and inorganic compounds in a WWTP effluent matrix. Reactive products formed from OM are often referred to as (organic) OTPs, while products from reaction with inorganics are in most cases unintentional and are therefore referred to as by-products. While some of these OTPs can have adverse effects, for the majority of the OTP toxicological information and even chemical identity is unknown.

During ozonation of WWTP effluents, the most important inorganic by-products that can be formed are bromate (from bromide) and nitrosamines (e.g. N-nitrosodimethylamine (NDMA)) [62]. NDMA can be biodegraded relatively easily in a biological filter after the ozonation [44], while bromate can only be removed with an anaerobic biofilter, or with advanced reduction processes [86,87]. Both of these options are difficult to achieve in the oxygen rich conditions that remain after ozonation. Additionally, bromate is highly toxic at low concentrations (e.g. low µg/L range from bromate), which makes this compound a serious environmental concern. One advantage is that bromate is generally only formed at higher ozone doses due to the low reaction rate of bromide with ozone (160 M^{-1} s^{-1}) [86,88]. Because of its recalcitrance to removal and its toxic threat, the formation of bromate should be monitored and prevented in treatment systems that apply ozonation.

Ozonation without additions to stimulate ·OH formation does not result in full mineralization of the organic compounds. Ozone reactive moieties (e.g. phenols, anilines, olefins, reduced Sulphur, and deprotonated amines) consume ozone and are generally converted into more hydrophilic and more biodegradable OTPs [76]. Formaldehydes are an example of OTPs from OM after ozonation. Formaldehydes are toxic, though also biodegradable and can be easily removed after ozone treatment using a biological filter [72,89,90]. Because the WWTP effluent matrix is so complex, it is practically impossible to chemically identify all compounds that are present, let alone to identify all the OTPs formed during ozonation.

To know whether hazardous parent compounds or OTPs are present in a WWTP effluent, bioassays can be performed additional to the chemical analyses. Bioassays use a test organism, expose this test organism to a sample, and monitor an endpoint (for example growth or mortality) in the organism. These assays come in many different types, using different organisms, different endpoints, and different durations. Because many uncertainties regarding the compounds present in WWTP effluents exist, it is challenging to select which organisms and endpoints should be monitored. For the durations, chronic (longer lasting) assays are generally more realistic (because WWTPs discharge effluents the whole year around), but these longer assays are also more complex to perform and interpret. Studies into the toxicity of WWTP effluents after ozonation have shown contradicting results, since both decreases [91] and increases [72,73] in toxicity are observed after ozonation [92]. The results of these studies are challenging to compare because generally limited amounts of samples are taken and limited number of endpoints and bioassays are used. To judge whether OTPs formed during ozonation are toxic or not, a broad range of bioassays should be tested to ensure that any possible harmful effect can be detected, and measures can be taken to mitigate the effect if necessary.

While both organic OTPs and inorganic by-products can be toxicologically relevant, there are more knowledge gaps regarding organic OTPs due to the complexity of the initial OM matrix.

1.4 Thesis outline

Combining biological treatment and ozonation in in a new MP removal treatment technology (the BO_3 process) offers the potential to utilize the advantages and mitigate the shortcomings of the two individual processes. The hybrid treatment technology can remove a broad range of MPs, at reduced energy input while also dealing with possible toxic organic OTPs, as has been suggested by previous researcher [75]. To prove and improve the feasibility of the BO_3 process, several knowledge gaps related to the effect and behavior of OM and OTPs in the system have to be investigated. The aim of this thesis is to gain more insight in the following three questions: i) which type and operation strategy for the bioreactor results in the highest OM removal, ii) how do different types of OM in WWTP effluents affect the ozonation of MPs, and iii) what is the effect of biological pre-treatment and ozone dose on the formation of toxic OTPs. Answering these questions will contribute to optimizing the synergies between biological treatment and ozonation in an effective hybrid treatment that achieves high MP removal with limited energy requirements.

In Chapter 2, OM removal in three different types of bioreactors, a biological activated carbon filter, a sand filter and a moving bed bioreactor, are investigated at different flowrates resulting in hydraulic retention times from 0.25-4 hours. Additionally, the contribution of biodegradation and sorption processes to the removal in the BAC filter are discussed.

Chapter 3 and 4 discuss the effect of EfOM type on MP ozonation. Several WWTP effluents are fractionated using membranes and a hydrophobic resin and the produced fractions are ozonated to compare the interference of the OM fractions with MP removal during ozonation. Next to the MPs, also absorbance and fluorescence based characteristics of the OM fractions are measured to elucidate their composition and to find out which characteristics are related to differences in interference with MP ozonation.

Chapter 5 demonstrates the combination of the BAC and ozone for treatment of the effluent of a WWTP in Bennekom, the Netherlands. The removal of OM and MPs, and the formation of toxic OTPs is monitored at different ozone doses. A broad range of bioassays is used to give a reliable image of the toxicity of the treated samples. Additionally, the formation of bromate is monitored in a separate experiment where the feed was spiked with bromide.

In Chapter 6, the results of the separate chapters are combined and put into context. Scientific limitations and ideas for future research are discussed and the potential of the BO_3 process for implementation in practice is underlined.

Chapter 2

Optimizing biological effluent organic matter removal for subsequent micropollutant removal

This chapter is published as:

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Abstract

Wastewater treatment plant effluent is an important point source for micropollutants emissions into the environment. These micropollutants can pose risks for ecosystems and humans; therefore, wastewater treatment plants should be upgraded to improve their micropollutant removal. Micropollutant removal can be improved by implementing tertiary treatment such as ozonation or activated carbon filtration. However, organic matter in the wastewater treatment plant effluent interferes with the micropollutant removal in these tertiary treatments, resulting in high energy demand and costs. To decrease the energy demand of tertiary treatment, biological pre-treatment can be introduced to remove effluent organic matter in an energy efficient manner. This biological pre-treatment was optimized by comparing three types of bioreactors; biological activated carbon, sand filter, and moving bed bioreactor. The reactors were operated at five flow rates (0.25, 0.5, 1, 2 and 4 L/h) in a continuous setup. The biological activated carbon filter achieved higher effluent organic matter removal than the sand filter and moving bed bioreactor (up to 72, 41, and 21% respectively). Additionally, effluent organic matter removal was negatively correlated to the flow rate in the biological activated carbon filter and the sand filter. The biological activated carbon filter also achieved average removal of 85% for the 18 analyzed micropollutants, although how long this high micropollutant removal can be sustained is unclear. To conclude, the biological activated carbon filter as pre-treatment can achieve high effluent organic matter removal, which would decreases the energy demand and cost of subsequent treatment for micropollutant removal.

2.1 Introduction

The occurrence of micropollutants (MPs), such as pharmaceuticals, personal care products, pesticides and industrial chemicals, is a growing global concern [9,117,118]. MPs occur in fresh and saline water systems at concentrations ranging from ng to µg /L. Despite their low concentrations, toxicological risks of MPs are of concern for three reasons: the vast amount of compounds, synergistic mixture effects, and chronic or long term exposure. The number of chemical compounds used in our society is increasing. For instance, the CAS registry has grown from 100 million in 2015 to over 160 million chemical compounds registered in 2020 [14]. Many of the chemicals that people use end up in sewage, or directly drain into natural waters. Moreover, every MP can be broken down by biological, chemical or physical processes, forming transformation products (TPs) that are sometimes difficult to mineralize into carbon dioxide and water [119–122]. Especially the toxicity of TPs formed during strong oxidative treatment such as ozonation have giving rise to concern [123,124]. Assessing the long term toxicological risk of a complex environmental mixture with a large number MPs and TPs is difficult, because most available toxicological information is related to individual compounds and short term effects [4,125–127]. Due to the widespread occurrence of MPs and the uncertainties related to their toxicological risks the precautionary principle should be applied to reduce emissions of MPs into the environment [128].

A large portion of MP discharge into the environment occurs at wastewater treatment plants (WWTPs) [96]. Conventional WWTPs remove MPs to a limited extent because these WWTPs were only designed for bulk organic matter (OM) and nutrient removal [42]. MP removal in WWTPs can be improved by implementing oxidation, sorption or filtration based tertiary treatments, for example ozonation, catalyzed UV treatment, activated carbon filtration and membrane filtration. Although these treatment types have proven their effectiveness to remove MPs, they are associated with high energy and costs; the production of ozone and UV radiation has a high energy demand, activated carbon treatment requires renewal or regeneration of the carbon and membrane filtration is hindered by membrane fouling and the need to treat the concentrate stream [45,64,129,130].

The high energy demand and costs of these tertiary treatments results from interference of effluent organic matter (EfOM) with the MP removal. The concentrations of EfOM are three to six orders of magnitude higher than the concentrations of MPs, which means that the largest part of the treatment capacity is not used for the MPs but for the EfOM [131]. This complex group of OM can be quantified as dissolved organic carbon (DOC), chemical oxygen demand (COD) or absorbance at 254 nm (UV254). The OM quantity is frequently used to determine the required ozone or UV dose [66,132], the time an AC filter can run until regeneration [133] and the fouling rate during membrane filtration [134]. Therefore, reducing EfOM concentrations directly improves the efficiency of a tertiary treatment.

Even though EfOM has already passed through a conventional biological treatment, advanced biological treatment using biofilm reactors can further degrade EfOM, possibly because of the higher sludge retention times in biofilm reactors compared to conventional activated sludge systems. Therefore, biofilm reactors can be used as pre-treatment to reduce EfOM concentrations and therefore reduce the energy and cost requirement of ozonation for MP removal. Biofilm reactors can be designed as a bio-filter or as a mixed tank reactor, for example sand filters (SF) and biological activated carbon filters (BAC) or moving bed biofilm reactors (MBBRs) respectively. Filters have an added value that they also remove suspended particles and generally have a higher biofilm area per reactor volume than MBBRs. However, filters need to be backwashed regularly to avoid clogging. On the one hand, MBBRs provide better mixing compared to filters. On the other hand, MBBRs have more turbulent conditions in the reactor, due to air bubbles and collisions between carrier particles, that can affect the biofilm growth. A BAC is a bio-filter with a high specific surface area, that utilizes saturated granular activated carbon (GAC) as base for biofilm growth. It is hypothesized that high local substrate concentrations as a result of sorption and desorption can stimulate biological activity in a BAC filter [135,136]. The main difference between BAC and GAC treatment is that in GAC reactors the activated carbon has to be regenerated or replaced periodically due to saturation of the filter bed and BAC does not have to be regenerated. Regeneration or replacement of the filter bed accounts for the majority of the energy demand associated with GAC treatment [130]. The three types of biofilm reactors have been studied separately in literature, but they have not been studied together in the same conditions.

The high energy demand of tertiary MP removal can be reduced by first removing OM from WWTP effluent in a pre-treatment step. Biofilm reactors have the potential to act as a pre-treatment and remove this EfOM but little is known about their effectiveness as pre-treatment. In our work, the EfOM removal of three different biofilm reactors is studied at lab scale in continuous mode. Additionally, the effect of flow rate on the EfOM removal efficiency was investigated. The results of this study can be used to design and optimize a biological-pre-treatment to reduce the energy requirement of the successive MP removal step and facilitate implementation.

2.2 Materials and methods

2.2.1 Inoculum and feed

All three bioreactors were inoculated with a mix of biological active sludges collected at four WWTPs in the Netherlands one year prior to the experiments. These four WWTPs were selected because they treat wastewaters containing complex organic compounds, for example hospital wastewater, industrial wastewater or domestic WWTP effluent (see section 2.5.1).

Effluent of the secondary clarifier from the WWTP in Bennekom (the Netherlands) was used as feed for the three reactors. The EfOM characteristics in the feed were on average: UV254 levels of 0.6±0.02, COD levels of 24±3.4 mg/L and TOC levels of 6.9±1.0 mg/L. Nutrient concentrations in the feed were on average: ammonium levels of <0.02 mg/L (below detection limit), nitrite levels of 0.10±0.15 mg/L, nitrate levels of 9.02±3.9 mg/L and phosphate levels of 0.57±0.18 mg/L. The feed was stored in a 3 m^3 tank kept at 4 °C and continuously stirred with a metal rod. The tank was refilled with fresh dry weather effluent once a month, to avoid fluctuations in EfOM levels due to rain.

The used MPs (a selection of pharmaceuticals, pesticides and an industrial chemical) were chosen due for their occurrence in WWTP effluent. Pharmaceuticals are frequently found in wastewater influents and effluent at concentrations of several hundreds of ng/L to several µg/L [44,64,137]. Pesticides are found less frequently and at lower concentrations than pharmaceuticals (tens to hundreds of ng/L). Despite the lower concentrations, this class of MPs is relevant to include in the current study due to their recalcitrance to advanced treatment (e.g. oxidative technologies) and their ecotoxicological risk [29,64,138]. Therefore, these pesticides can also act as model compounds for more recalcitrant MPs. The industrial chemical benzotriazole is detected in a broad range of concentrations, up to tens or hundreds of µg/L, depending on the presence and type of industry at the wastewater source [44,139]. Furthermore, the selected MPs have a broad range of susceptibilities to conventional and advanced treatments, ranging from 0 to 100% removal. The reactors were fed with feed spiked with a mix of 16 MPs with approximately 2 µg/L: benzotriazole, caffeine, carbamazepine, clarithromycin, chloridazon, desphenyl chloridazon, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,6-dichlorobenzamide (BAM), diclofenac, mecoprop, 4 & 5 methyl benzotriazole, metoprolol, naproxen, propranolol, sulfamethoxazole and trimethoprim (octanol partitioning coefficients and ozone reactivity coefficients of these MPs are presented in (Table S2.2). Spiking was done to ensure the comparability between different measurement days. In order to avoid the presence of solvent of the MP mix (acetonitrile) in the feed, the MP solution was added to a smaller tank of 20 L, the solvent was evaporated under a stream of nitrogen gas, and finally the WWTP effluent was added to dissolve the MPs.

2.2.2 Reactor setup

The feed was pumped from the feed tank via the ‘sampling point 1’ into the pre-aerator where pure oxygen gas was added (Figure 2.1). From the pre-aerator, the flow was split in three equal parts and pumped into the bottom of the up flow reactors. All three reactors had a total volume of 1.7 L. The bottom of the BAC filter and the SF consisted of a stabilization layer with gravel and sand (from bottom to top, particles with diameters of: 8-12 mm, 1.0-2.0 mm, and 0.7-1.25 mm). The BAC filter was filled with 1.2 L FILTRASORB® TL830 granular activated carbon (GAC) used for 38,000 bed volumes in a drinking water treatment facility of Evides (Kralingen, the Netherlands). The SF was filled with 1.2 L sand (0.4-0.8 mm diameter) from a drinking water treatment facility of Vitens (De Meern, the Netherlands). The MBBR was filled with 0.85 L Kaldness K1 carriers (section 2.5.1) and was continuously aerated with pressurized air to keep the bed moving. The outflow of the three reactors was pumped via the ‘sampling point 2’ into the sewer (Figure 2.1).

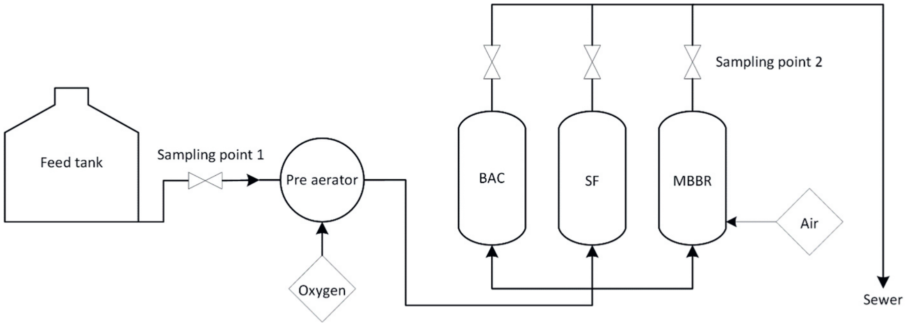

Figure 2.1 Schematic representation the three reactors: biological activated carbon (BAC) filter, sand filter (SF) and moving bed bioreactor (MBBR)

2.2.3 Reactor operation

The three reactors were tested at five different flow rates in a random order (approximately 1, 0.5, 0.25, 2, and 4 L/h) (Table S2.3 and Table S2.4). Each flow rate was run for two weeks and after this, three of the flow rates were repeated for one week each (0.25, 1, 4 L/h) to test the reproducibility of EfOM removal in the reactors (Table S2.1). Samples were taken three times per week for UV254 absorbance, COD and TOC analysis. The top of the SF was washed before the experiments, to remove accumulated brown fluffy material. This was not needed for the BAC (Figure S2.5). Additional samples were taken for MP analysis at the end of week 1 and week 12 (during operation at 1 L/h). Prior to sampling for MP analysis, the reactors were fed with fresh MP spiked effluent for three hours to homogenization of MP concentrations in the system. MP spiking done in order to ensure the presence of the same MPs at the different sampling times.

2.2.4 Analytical methods

Three properties of the EfOM were quantified: UV254 absorbance, COD and TOC. UV254 absorbance was analyzed using an Infinite M200 Pro multimode plate reader (Tecan). COD was analyzed with Hach kits (LCK 1414). TOC was analyzed with a non-purgeable organic carbon detection method on a TNM-L TOC analyzer (Shimadzu).

Ammonium was measured using Hach kit LCK 304 and negatively charged nutrients were measured using ion chromatography. The used ion chromatograph was a Dionex ICS-2100 with a Dionex ionpac AS10 column. The mobile phase (KOH in demi water) was ramped from 5-20 mM over 10 minutes at a constant flow speed of 1mL/min.

Oxygen was measured in the pre-aeration tank and in the three reactors using non-invasive oxygen sensors (Spot SP-Pst3, Presence Precision Sensing) and an oxygen meter (Fibox 4).

The flow rate of each reactor was determined once a week for each reactor by measuring the time needed to fill a 100 mL measurement cylinder.

MPs were analyzed using Ultra High Performance Liquid Chromatography coupled with tandem quadrupole mass spectrometry (3Q UPLC-MS). Chromatographic separation of conjugates was performed on a Waters Acquity UPLC with a phenyl-hexyl column, 1.7 µm, 2.1x100 mm. Flow rate 0.3 mL/min with a column temperature of 35ºC. The LC mobile phase consisted of solution A (UPLC-MS quality water with 1% formic acid (v/v)) and solution B (UPLC-MS quality acetonitrile with 1% formic acid (v/v)). Compounds were eluted according to the following gradient: 0-0.5 min. 0%B; 0.5-13 min. linear increased to 80% B; 13-16 min. 80%B; 16-17 min. linear decreased to 0%B; 17-23 min. 0%B. From the conjugate sample, 50 µl was injected (partial loop with needle overfill and load ahead). The MS system switched between positive and negative ion ionization during analysis. The capillary voltage was set to 0.8 kV, the cone voltage was adjusted to 25 V, cone gas 150 L per hour. The source temperature was 150˚C and the desolvation temperature was 500˚C. The flow of the desolvation gas was 800 L per hour. The LM 1 Resolution was 2.8 and the HM Resolution was 15.0. The Ion Energy 1 was set to 0.0 and the Ion Energy 2 to 0.6. Data processing was done with MassLynx V4.1 software. The limit of quantification (LoQ) for all 16 MPs was 200 ng/L. R^2 values of the standard curves can be found in Table S2.6.

2.3 Results and discussion

2.3.1 Effluent organic matter removal in different bioreactors

Three bioreactors were assessed for their OM removal from a real WWTP effluent. The BAC filter achieved reduction in UV254 absorbance and COD of over 60% and reduction in TOC of approximately 40% at a flow rate of 1.2 L/h (Figure 2.2). TOC is commonly used as guiding parameter in tertiary treatment, while UV254 absorbance and COD can give more insight into the complexity of the EfOM [68,140]. Specifically for ozone treatment, UV254 absorbance and COD have also been suggested as parameters to determine the required dose [60,66,68]. The high reduction of these three parameters in the BAC filter would directly improve the efficiency of subsequent MP removal treatment and results in significant reduction of energy demand and costs.

Reduction in UV254 absorbance, COD and TOC in the BAC filter was more than two times higher than in the SF and the MBBR (Figure 2.2). A possible explanation for the higher EfOM removal in the BAC filter is a synergy between sorption and biodegradation, where sorption can increase the local concentration and influx of substrate (EfOM in this case) into the cells and thereby stimulate the biodegradation [135]. This hypothesis is supported by the long use times BAC, a sum of 42,000 bed volumes in drinking water treatment and WWTP effluent treatment combined. The MBBR showed little EfOM removal and did not achieve reduction in UV254 absorbance, COD or TOC removal above 10%. The reason for this low removal most likely is the lower surface area of the MBBR and the turbulent conditions in this continuously mixed reactor. To illustrate, the MBBR had two and nine times lower external surface area than the BAC filter and the SF respectively (see section 2.5.1). The turbulent conditions, for instance shear stress caused by air bubbles and collisions of the carriers in the MBBR, likely resulted in higher stress on the biofilm and higher decay of the biomass [141,142].

The BAC filter and SF reactors removed more COD than TOC (Figure 2.2). The reduction in COD/TOC ratio indicates that incomplete mineralization occurred. This means that saturated and less complex compounds with a high COD are transformed into more oxidized compounds with a lower COD, without lowering the TOC. The BAC filter achieved higher reduction of UV254 absorbance than reduction of TOC, which indicates a selective removal of aromatic organic matter compared to non-aromatic constituents. Whether this is an effect of non-biological removal by sorption or of biological removal processes by the biofilm is yet unclear. Compounds that absorb at 254 nm contain aromatic groups are often considered as bio-recalcitrant [81], in this experiment these seem to be more reactive than the more saturated organic structures that are removed to a lower extent. This indicates removal by sorption or by a specialized biological community that can target compounds that are often considered as bio-recalcitrant. The high reduction in UV254 absorbance and COD is especially beneficial for a pre-treatment before ozonation, since these two parameters are related to ozone demand [68,111] .

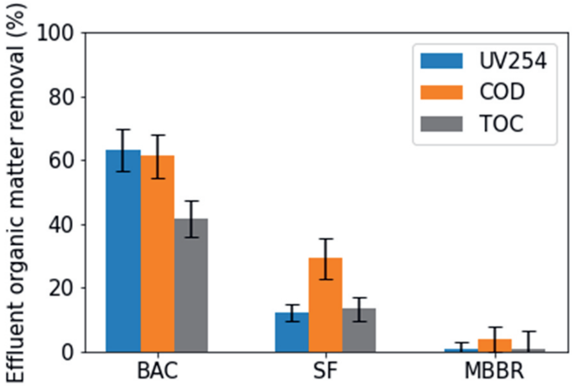

Figure 2.2 Reduction of UV254 absorbance, COD and TOC in the biological activated carbon filter (BAC), sand filter (SF) and moving bed bioreactor (MBBR) at a flow rate of 1.2 L/h. Averages and standard deviations represent 9 datapoints

2.3.2 Effluent organic matter removal at different flow rates

EfOM removal increased with increasing empty bed contact time (EBCT) for the BAC filter and the SF (Figure 2.3 A and B). In the BAC filter, reduction of UV254 absorbance and COD increased from approximately 40% at an EBCT of 0.3h up to 70% at an EBCT of 4.9h. This increase in EfOM removal indicates that contact time of the water is a limiting factor in the pre-treatment. A logarithmic regression fitted the reduction of all three parameters better than a linear regression (Table S2.5), which indicates that at higher EBCTs, other parameters than the contact time start to become limiting. Logarithmic correlation coefficients between EfOM removal and flow rate ranged from 0.52 to 0.82 for all three EfOM characteristics in the BAC filter and the SF (Table S2.5). These high correlation coefficients indicate that flow rate is an important design parameter to control EfOM removal in the BAC filter and the SF.

Observed removal in the BAC filter and SF in this research are in line with results from similar studies. Reungoat et al. [143] compared a BAC and a SF for MP and DOC removal from WWTP effluent and found DOC removals of 34-47% in their BAC and DOC removals from 11 to 22% in their SF at EBCTs from 0.5 to 2 hours. Reungoat et al. [143] did not find a correlation between DOC removal and EBCT, such as observed in our experiments, however they hypothesized that this lack of correlation was caused by oxygen limitation in the BAC filter. Additionally, Pipe-Martin (2018) [144] did find a clear correlation between DOC removal and EBCT in a BAC and a SF for EBCTs ranging from 0.5 – 2.67 hours. At higher EBCT, micro-organisms have more time to degrade the EfOM, resulting in higher removal, which explains the observed trend in our results [145,146].

Average EfOM removal in the MBBR was below 10% at all tested flow rates (Figure 2.3). This is in line with the results of [147], who measured DOC removal in a MBBR treating conventional WWTP effluent.

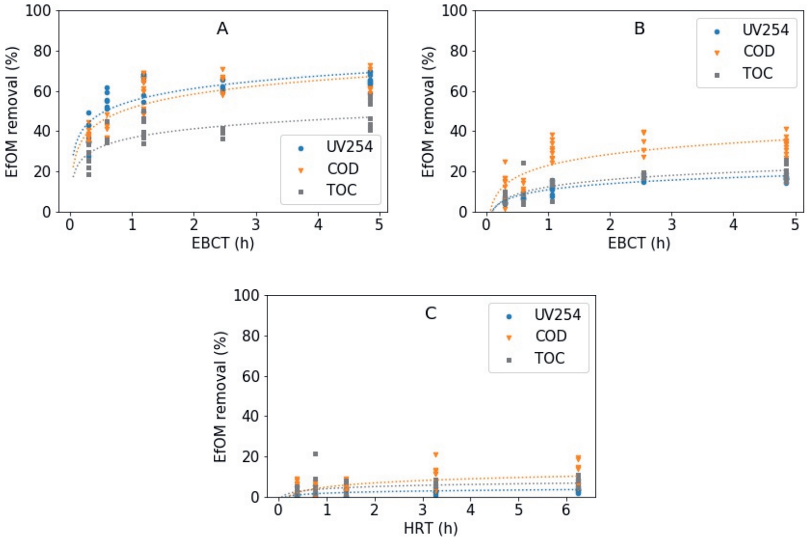

Figure 2.3 Removal of effluent organic matter in the biological activated carbon filter (A), sand filter (B) and moving bed bioreactor (C) at all tested flow rates. Three characteristics of effluent organic matter were quantified: UV absorbance at 254 nm (UV254), chemical oxygen demand (COD) and total organic carbon (TOC). Dotted lines represent a logarithmic regression model

Results of our study indicate that BAC is a promising technology to remove OM from WWTP effluent. Lower OM concentrations result in a reduction of energy demand and costs for subsequent treatment such as ozonation, AC filtration and membrane filtration [66,109,148,149]. Additionally, flow rate is a key parameter to control the OM removal in biological pre-treatment, lower flow rates result in higher EBCT and higher EfOM removal. However, lower flow rates require larger reactor volumes resulting in higher capital and operational costs. For implementation in practice, a trade-off between the capital and operational costs has to be made. The regressions made in Figure 2.3 can be used for this purpose.

2.3.3 Micropollutant removal in different bioreactors

The BAC filter removed on average 92% of all MPs, which is three times higher than the MP removal in the SF and the MBBR (Figure 2.4). The sorption capacity of the BAC was expected to be saturated since it had already treated 38,000 bed volumes of surface water in a drinking water treatment facility and 6,000 bed volumes of WWTP effluent in the current setup. Still, the high removal of bio-recalcitrant MPs (for example, Carbamazepine, 4 & 5 methyl benzotriazole and diclofenac [43,58,150]) indicates that sorption is still taken place in the BAC. The reason for this could be bio regeneration of the sorption capacity of the AC, which would free sorption sites for the bio-recalcitrant MPs [151]. In this case it would be expected that more hydrophilic MPs (with low k_{ow} (Table S2.2)) show the lowest removals, though that is not the case. Alternatively, the high removal of bio-recalcitrant MPs could also be explained by optimized biological conditions in the BAC or by catalyzed chemical oxidation on the BAC surface [135,152]. This raises the question whether the observed MP removal in the BAC filter can be sustained for prolonged operation times or whether the AC would saturate for MPs at some point.

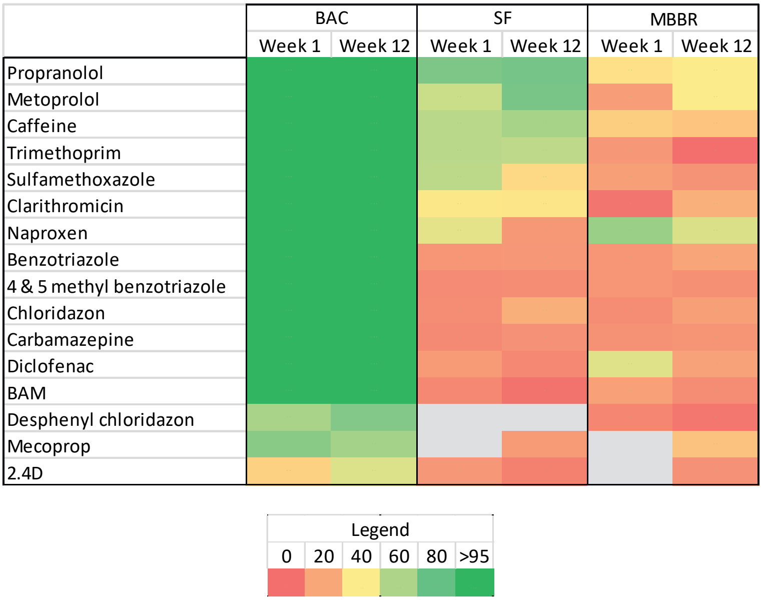

Figure 2.4 Heatmap of micropollutant removal in the biological activated carbon (BAC) filter, sand filter (SF) and moving bed bioreactor (MBBR)

2.3.4 Implications for application

Three biological reactors were compared for their suitability as pre-treatment before tertiary MP removal treatment. The BAC reactor showed the best performance and reduced UV254 absorbance, COD and TOC by up to 70%. Whether the high observed removal of the BAC filter continues without the need to replace the GAC is crucial for the cost effectiveness of the reactor. Continuation of the observed removal is determined by the type of removal processes taking place in the reactor. Sorption dominated removal would mean saturation is reached over time and therefore removal capacity decreases, while biological dominated removal would not reduce over time.

The BAC filter shows indications that both sorption and biological processes play a role in the observed removal. The used GAC was previously used in a drinking water treatment plant, fed with surface water for 38,000 bed volumes (DOC 2-3 mg/L). Thereafter, the GAC was used in this study with WWTP effluent for approximately 6,000 bed volumes. A breakthrough of DOC was not observed with this GAC, while Ma et al. [153] found a breakthrough of DOC after 25,000 bed volumes of surface water treatment (with an DOC concentrations between 3 and 6 mg/L) and Fundneider et al. [133] found a breakthrough of DOC after approximately 3,000 bed volumes of WWTP effluent treatment. In addition, the BAC filter consumed on average 42% times more oxygen than the SF (Figure S2.6), which also indicates a high biological activity in the BAC. Alternatively, this high oxygen consumption can be related to chemical oxidation occurring on the BAC surface. The surface of AC can catalyze the formation of oxidative radicals that chemically react with EfOM [152,154,155]. The BAC achieved over 90% removal for 14 out of 18 measured MPs, some of which are considered bio-recalcitrant in other studies. This indicates the influence of sorption and/or chemical based processes for the observed MP removal. It is possible that biological processes are responsible for the observed OM removal while the MP removals caused by sorption. Benstoem et al. [131] and Sundaram et al. [156] found that sorption of MPs onto GAC can continue after saturation of DOC has already been reached, which could mean that the observed OM removals in this study are based on biological. Overall, extended use times show the potential of the BAC filter without regeneration, while the processes responsible for the observed OM and MP removal remain under debate.

A biological OM removal treatment is foreseen to enable a significant reduction in costs (investment and operation), energy consumption and CO_2-footprint for subsequent MP-removal technologies such as oxidation, sorption and filtration. These technologies are strongly affected by the OM content, higher OM (UV254, COD or TOC) resulting in higher oxidant dosage, higher consumption of AC and fouling and reduced fluxes of membranes. Oxidant dosage and AC consumption are the main the operational costs and a significant part of the total costs of oxidation and adsorption technologies and the largest part of the energy use and CO_2 footprint, approximately 40%, 80% and 80% for the example of ozonation [157,158]. Therefore, a biological pre-treatment removing up to 70% of the EfOM could reduce the operational costs of a subsequent treatment with 30% and reduce the energy use and CO_2 footprint with 50%.

A treatment train that combines different types of removal processes would be considered most promising for the removal of a broad range of MPs [159]. We suggest a combination of a BAC (biological and sorption processes) and ozone (advanced oxidation processes). Due to the sustained high removal in the BAC after extended use times, a combination of a BAC and a low dose ozone treatment provides a robust post-treatment for MP removal with the following advantages. The BAC filter removes EfOM in the WWTP effluent, which reduces the energy and costs of the ozonation. The double barrier removes both compounds that be removed with ozone, but not with BAC and vice versa (e.g. BAM, 2,4-D and benzotriazole (Table S2.2), even after saturation of MPs would occur in the BAC. Therefore, operational costs in a BAC – ozone combination are lower compared to single GAC or ozone treatment where the GAC has to be replaced more frequently or where higher ozone doses are required. Ozonation with a BAC pre-treatment can remove a broad range of MPs at relatively low energy input and costs.

2.4 Conclusions

In both the BAC filter and the SF, an increasing empty bed contact time resulted in an increasing effluent organic matter removal. Flow rate controls the removal, therefore flow rate is an important parameter for the design of the biological pre-treatment reactor.

Implementing a BAC filter before a tertiary micropollutant removal treatment results in approximately 30 % reduction in the total implementation costs of the tertiary micropollutant removal treatment. The BAC filter achieved high reduction in UV254 absorbance, COD and TOC (up to 70%) at the lowest flow rate of 0.25 L/h. The BAC showed higher effluent organic matter removal than the SF and the MBBR at all tested flow rates (0.25 L/h – 4 L/h). Micropollutant removal in the BAC filter was approximately four times higher than in the other two reactors and showed an average removal of 85% for 18 analyzed micropollutants.

Indications for both biological processes and other processes, such as sorption or advanced oxidation, were found in the BAC filter. Operation time in the current study was longer than activated carbon breakthrough times in other studies, which shows the potential for long use time without the need to replace the filter bed. Combined with an additional advanced treatment, the BAC filter can improve the effectivity and efficiency of micropollutant removal from wastewater treatment plant effluent.

Acknowledgement

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2.5 Supplementary information

2.5.1 Supplementary materials and methods

Inoculum

All three reactors were started using a mix of sludges from the following four locations:

- Membrane bioreactor sludge collected from the hospital wastewater treatment facility at Reinier de Graafziekenhuis (Delft, the Netherlands)
- Biological granular activated carbon sludge collected from the post treatment system of wastewater treatment plant Horstermeer (Nederhorst den Berg, the Netherlands)
- Activated sludge from the wastewater treatment plant treating industrial chemicals in Moerdijk (Moerdijk, the Netherlands)
- Activated sludge from the wastewater treatment plant treating a mix of industrial and domestic wastewater in Bath (Rilland, the Netherlands)

Reactor filling

The biological activated carbon (BAC) reactor, sand filter (SF), and moving bed bioreactor (MBBR) were filled with the following materials:

- BAC: coal-based FILTRASORB® TL830 granular activated carbon was obtained from a drinking water plant of Evides (Kralingen, the Netherlands). The porosity of the granular activated carbon was 60 %, mean diameter 1.4 mm [160]. Based on this data, an external surface area of 1700 m2/m3 was calculated for the BAC bed. Before the granular activated carbon was added to the reactor, it had been in use for 2 years (approximately 38,000 bed volumes)
- SF: Sand with a diameter of 0.4-0.8 mm was obtained from a drinking water treatment plant of Vitens (Meern, the Netherlands) and acted as carrier media. The porosity of the sand was 40 %. Based on this data, an external surface area of 6000 m2/m3 was calculated for the SF bed.
- MBBR: polyethylene Kaldness K1 carriers were obtained from Evolution Aqua (Wigan, the United Kingdom). These carriers have a protected surface area of 950 m2/m3 with a porosity of 81 %. The density of the carrier material is similar to that of water

Figure S2.5 Pictures of brown fluffy material in the reactors. The material only visible for the BAC in the settler after the reactor (right most picture)

Table S2.1 Outline of the different flow rates applied in the reactors

Week number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Flow rate (L/h)	1		0.5		0.25		2		4		0.25	1	4

Table S2.2 Log(K_{ow}) and Log(KO_3) values of the used MPs found in literature. *To the authors best knowledge these values could not be found in literature

Common name	log(K_{ow})	log(K_{ow}) source	log(KO_3)	Log(kO_3) source
2,4-D	2.66	[161]	1.34	[113]
4&5-methylbenzotriazole	1.71	[162]	2.66	[45]
BAM	0.77	[163]	*	
Benzotriazole	1.03	[164]	1.34	[114]
Caffeine	− 0.07	[165]	2.81	[138]
Carbamazepine	2.5	[165]	5.48	[60]
Chloridazon	2.2	[166]	*	
Clarithromycine	3.16	[167]	4.60	[168]
Desphenyl-chloridazon	*		*	
Diclofenac	4.4	[165]	6.00	[60]
Mecoprop	0.1	[161]	2.05	[45]
Metoprolol	1.9	[165]	2.52	[45]
Naproxen	3.3	[165]	5.30	[169]
Propranolol	2.47	[170]	5.00	[114]
Sulfamethoxazole	0.89	[165]	6.30	[60]
Trimethoprim	0.9	[165]	5.61	[116]

2.5.2 Supplementary results

Table S2.3 Actual flow rates compared to intended flow rates in the BAC, SF and MBBR in L/h

	1	0.5	0.25	2	4
BAC (L/h)	1.01 ± 0.06	0.49 ± 0.01	0.25 ± 0.00	2.02 ± 0.01	3.97 ± 0.03
SF (L/h)	1.13 ± 0.01	0.47 ± 0.01	0.25 ± 0.00	2.03 ± 0.02	3.99 ± 0.04
MBBR (L/h)	1.10 ± 0.06	0.47 ± 0.00	0.25 ± 0.00	2.01 ± 0.00	4.01 ± 0.01

Table S2.4 Actual EBCT (for BAC and SF) and hydraulic retention time (for MBBR) in hours

	1	0.5	0.25	2	4
BAC (EBCT in h)	1.19	2.46	4.85	0.59	0.30
SF (EBCT in h)	1.07	2.54	4.86	0.59	0.30
MBBR (hydraulic retention time in h)	1.40	3.27	6.25	0.77	0.38

Table S2.5 Correlation coefficients of the regression analysis between effluent organic matter removal and flow rates

	Logarithmic			Linear		
	UV254	COD	TOC	UV254	COD	TOC
BAC	0.62	0.64	0.59	0.36	0.39	0.46
SF	0.82	0.59	0.67	0.63	0.43	0.57
MBBR	0.22	0.22	0.09	0.30	0.20	0.15

Table S2.6 R^2 values of 5 point low (200 – 1000 ng/L) and high (1000 – 5000 ng/L) concentration standard curves for micropollutant analysis at the start and end of the LC-MS run

	start		end	
	R2 low	R2 high	R2 low	R2 high
Propranolol	0.9992	0.9864	0.9634	0.9729
Metoprolol	0.9574	0.9904	0.8561	0.9781
Caffeine	0.9211	0.9851	0.9561	0.9998
Trimethoprim	0.9551	0.9967	0.9719	0.9925
Sulfamethoxazole	0.9973	0.9997	0.9896	0.9824
Clarithromycin	0.9769	0.9788	0.9783	0.9578
Naproxen	0.9171	0.9333	0.9427	0.9901
Benzotriazole	0.9915	0.9997	0.9992	0.9992
4 & 5 methyl benzotriazole	0.9998	1.000	0.9998	0.9982
Chloridazon	0.9992	0.9997	0.9987	0.996
Carbamazepine	0.9961	0.9993	0.998	0.9978
Diclofenac	0.999	0.9994	0.9996	0.9999
BAM	0.9996	0.9969	0.9994	0.9987
Desphenyl chloridazon	0.9936	0.9892	0.9775	0.9996
Mecoprop	0.6606	0.9929	0.9364	0.9840
2,4-D	0.7029	0.9756	0.9057	0.9563

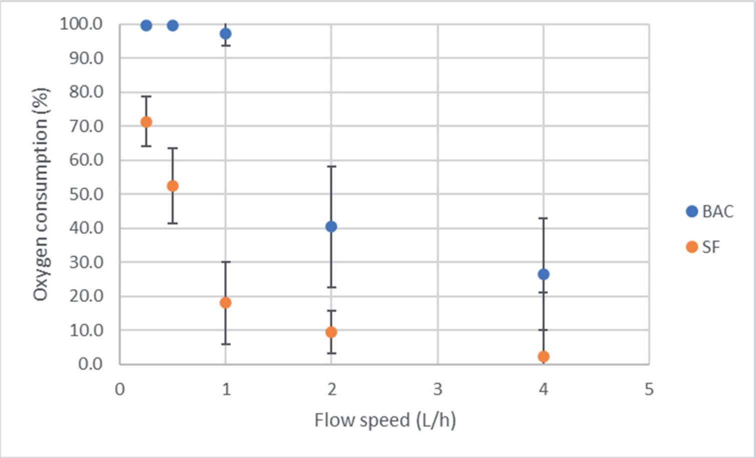
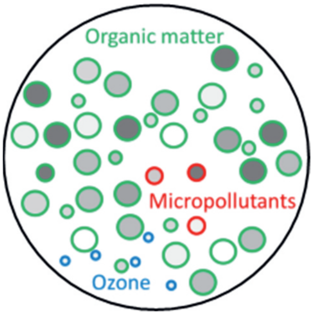


Figure S2.6 Oxygen consumption in BAC and SF at different flow rates. Averages and standard deviations represent 6 datapoints

Chapter 3

Optimizing micropollutant removal by ozonation; interference of effluent organic matter fractions



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Abstract

Ozonation for micropollutant removal from wastewater treatment plant effluent is energy and cost intensive because of competition between background organic matter and micropollutants. This study aims to elucidate the interference of different organic matter fractions during the ozonation of micropollutants. Wastewater treatment plant effluent was fractionated using membranes and XAD-8 resin. All membrane and resin fractions were spiked with 18 micropollutants (2 µg/L) and ozonated with 0.25, 0.5 and 1 g O_3/g TOC. Results show that these fractions differ in their interference with the ozonation of micropollutants. Interference was lower in the smallest size fraction (< 1 kDa) than in all other fractions for micropollutants with low and medium ozone reactivity. The hydrophobic neutrals and hydrophilics resin factions showed a high interference for ozonation of micropollutants with medium and high ozone reactivity respectively. The four parameters that were analysed (specific UV absorbance at 254 nm, fluorescence, chemical oxygen demand and nitrite) could not elucidate the differences in micropollutant removal. Still, we conclude that understanding the type of organic matter present in the matrix, is essential to optimize micropollutant ozonation and other tertiary micropollutant removal treatments.

3.1 Introduction

All over the world, micropollutants (MPs) are detected in various types of water systems, such as surface water, groundwater and wastewater [93,94]. The presence of MPs in these water systems raises concerns regarding ecosystems and human health [15,16,36,37]. Both human population and the consumption of chemicals like pharmaceuticals are increasing, hence the discharge of MPs into the environment will also increase. Therefore, the precautionary principle should be applied to reduce emissions of MPs into the environment [41].

Wastewater treatment plants (WWTPs) are suitable places to intercept and reduce emissions into the environment because they are important point sources of MPs. Conventional WWTPs remove part of the MPs present in wastewater via adsorption to sludge and biological degradation, but cannot remove all MPs [95,96]. Hydrophilic and bio-recalcitrant MPs can be removed in a tertiary treatment step for instance, ozonation or activated carbon filtration [45,64,97]. However, these tertiary treatments are often associated with high energy use and high costs.

The high energy requirement for ozonation of WWTP effluent results from competition of other organic matter (OM) with MPs for reaction with ozone. All of the OM in WWTP effluent can react with ozone and MPs only form a small fraction of the total. In general, the total effluent organic matter (EfOM) concentrations (in the range of mg/L) are 10^3 to 10^6 times higher than target MP concentrations (in the range of ng-µg/L). Hence, required ozone doses are based on EfOM concentrations, using specific ozone dosages such as g O_3/g TOC or g O_3/g COD, and not on MP concentrations [66,68,98,99]. Both TOC and COD are bulk parameters that mainly give information about the quantity of EfOM. However, also the type of OM in WWTP effluent strongly affects the reactivity of OM with ozone [78,80,100]. Still, EfOM composition and its interactions with the ozonation of MPs is poorly understood. Insight in these processes is key to improve the energy and cost efficiency for ozone treatment to remove MPs from WWTP effluent.

In order to gain insight in the composition of EfOM, it can be fractionated by for example, particle size using membranes, or by functional groups using resins. Additionally, fingerprinting methods such as LC-OCD or novel MS based fingerprinting can be used to elucidate EfOM structure [101,102]. Although, for fingerprinting techniques it is not possible to collect different parts of the OM separately and conduct experiments regarding their specific behavior. Previous studies found that EfOM fractions have different reactivity towards ozone. Gonzales et al. [81] showed that size fractions have different ozone reactivity and OH· formation potential. Resin fractionation experiments showed that hydrophobic-neutral and -acid compounds are removed during ozonation, while hydrophilic compounds are produced [78,100]. Still, it is not clear if and if so how differences in ozone reactivity of EfOM fractions affect the ozonation of MPs.

We hypothesize that the type of EfOM affects efficiency of MP ozonation, whereas currently, only the quantity of EfOM is taken into account for the ozonation of MPs in WWTP effluent. To investigate this hypothesis, WWTP effluent was fractionated using both membrane and resin based fractionation methods. Produced fractions were diluted to a standardized TOC level to ensure that effects of the type was compared and not the quantity of the EfOM. Standardized fractions were spiked with a mix of MPs and ozonated with four different ozone doses. In addition to the MP concentrations, several parameters were analyzed to understand the differences in OM composition between the fractions: fluorescence, specific UV absorbance at 254 nm (SUVA), chemical oxygen demand (COD) and nitrite. Additionally, the size fractions were analyzed using liquid chromatography coupled to organic carbon detection (LC-OCD) to give more insight in their composition, since these fractions provided the most interesting results. The results are discussed in the context of improving MP removal by ozonation of WWTP effluent.

3.2 Materials and methods

3.2.1 Wastewater treatment plant effluent

A volume of 5 L of WWTP effluent was collected from the WWTP in Bennekom, the Netherlands. This WWTP is designed for organic carbon and biological nutrient removal and treats approximately 1,000 m^3/h municipal wastewater per hour (35.000 P.E.). The effluent was collected on a dry day to minimize dilution by rainfall and stored in a glass Schott bottle, wrapped in aluminium foil at 4 °C until further use. The WWTP effluent was used for the membrane experiment one week after sampling and for the resin experiment three months after sampling.

3.2.2 Micropollutants

The MP mix used for this study contained 18 MPs dissolved in methanol: 2,4-D, 4 & 5 methylbenzotriazole, BAM, bentazone, benzotriazole, carbamazepine, chloridazon, desphenyl-chloridazon, dimetridazole, furosemide, iopamidol, irbesartan, mecoprop, metoprolol, propranolol, sotalol, sulfamethoxazole and trimethoprim (

Table S3.1). The MPs are organized in three classes based on their ozone reactivity: class I - high ozone reactivity ($kO_3 > 10^4$ M^{-1} s^{-1}), class II - medium ozone reactivity ($10^2 > kO_3 > 10^4$ M^{-1} s^{-1}) and class III - low ozone reactivity ($kO_3 < 10^2$ M^{-1} s^{-1}) (Table S3.2). Note that, to the authors best knowledge, kO_3 values for four of the used MPs (bentazone, chloridazon, desphenyl-chloridazon and 2,4-D) are not available in literature. Therefore, these four MPs were placed in the three classes solely based on the removal observed in our study and interpretation of their classification should be done with caution.

3.2.3 Fractionation

Two fractionation methods were used: fractionation with membranes to separate fractions based on particle size and fractionation with XAD-8 resin to separate fractions based on functional groups. Cross-flow filtration using three hollow fibre membranes with a molecular weight cut-off of 1.08, 11.4 and 99.8 kDa was used to separate the effluent sample into four size fractions. In addition to the size fractions, a sample of unaltered effluent (later referred to as 'Total') and effluent that was passed through a 0.45 µm membrane (later referred to as 'Filter') were included in the experiment. Two litre of 0.45 µm filtered effluent was circulated through the cross-flow setup (Figure S3.8). For each of the following steps, the effluent was circulated until at least 200 mL of permeate was collected. First, the permeate was collected of the 1.08 kDa membrane (F4). Second, the 1.08 kDa membrane was replaced with the 11.4 kDa membrane, and the permeate was collected (F3). Finally, the permeate from the 99.8 kDa membrane was collected (F2) and the remaining liquid in the circulating setup was collected (F1) (Figure S3.9).

The resin fractionation procedure was adjusted from Imai et al. [103] and Qi et al. [78] (Figure S3.10). Fractionation using XAD-8 resin combined with acidity adjustments was used to fractionate the effluent sample into four resin fractions: hydrophilics (HI), hydrophobic acids (HOA), hydrophobic neutrals (HON) and hydrophobic bases (HOB). In addition to the resin fractions, a sample of non-fractionated effluent ('Total') was included in the experiment. First, 3.1 g of XAD-8 resin (wet weight) was washed subsequently with demi water, 0.1 M KOH and 0.1 M HCl to reduce background OM release from the resin. The washed resin was placed in a 50 mL syringe and flushed with 20 mL of demi water, that was collected as a blank for the experiments. Next, 400 mL of 0.45 µm filtered effluent sample was eluted through the resin and collected as first eluate for later use. The resin was washed with 200 mL 0.1M HCl in demi water and the second eluate was collected (HOB). The first eluate was adjusted to pH 2, flushed through the resin again and collected as HI. The resin was washed with 200 mL of 0.1M KOH and collected (HOA). Finally, the resin was washed with 100 mL of methanol. The methanol was evaporated in a flow cabinet overnight after which the non-evaporated organics were dissolved in 200 mL MQ water (HON). After fractionation, all resin fractions were adjusted to pH 7. All pH adjustments mentioned above were done with 1 M HCl or 1 M KOH in demi water. The detailed fractionation process is described in the supplementary information.

3.2.4 Ozonation experiment

Prior to the ozonation, all fractions were diluted to a TOC concentration of 3.7 mg/L. For each fraction, 27 µL of MP mix was added to a glass beaker the methanol was evaporated under a gentle flow of nitrogen gas in a flow cabinet for half an hour. After all methanol was evaporated, the diluted fractions were added to the glass beakers with MPs to reach final MP concentrations of approximately 2 µg/L per MP and the beakers were shaken to dissolve the MPs.

Each diluted and spiked fraction was split into four equal parts of 40 mL and each part was transferred into a 50 mL Greiner tube. The spiked fractions were ozonated at different doses: 0, 0.25, 0.5 and 1 g O_3/g TOC. The ozone was added using an ozone stock (as described in [104]) with a concentration of approximately 45 mg O_3/L. Ozone stock was prepared by guiding pure oxygen gas through an Anseros COM-AD ozone generator and into a cooled modified Schott bottle (Figure S3.10). After saturating the ozone stock, it was transferred from the Schott bottle into a clean glass beaker and from there pipetted into the samples. A beaker with ozone stock was used to pipet no more than 6 times to limit the effect of evaporation of ozone from the ozone stock. The first and sixth pipetting were used to measure ozone concentration with the indigo method, in order to calculate the exact amount of ozone dosed (Table S3.3).

3.2.5 Analyses

Ozone concentration in the ozone stock was analysed using the indigo method based on Bader and Hoigné (1985) [88]. For each measurement, 1 mL of indigo trisulfonate stock (0.97 mM) and 0.5 mL of H_3PO_4 stock (1.5 mM) were added to a 25 mL volumetric flask. 1 mL of ozone stock was added and the flask was shaken briefly to let the ozone react with the indigo. Afterwards, the volumetric flask was filled up to 25 mL with MQ water and absorbance was measured at 600 nm. Similarly, a reference was made without ozone stock. Using the reference, ozone concentration was calculated using the following equation:

$$[O_3\ ] = \frac{\mathrm{V_f}\ \Delta\mathrm{A}}{\mathrm{f\ b\ V_t}}\ [\mathrm{mg/L}]$$

, where $[O_3]$ is the ozone concentration in solution (mg/L), V_f is the volume of the volumetric flask, ΔA is difference in absorbance between the reference indigo solution and the indigo reference with ozone stock added, f is the ratio between absorbance change and ozone concentration (ε/O_3 in L/cm/mg), b is the path length of the cuvette in cm and V_t is the volume of the individual sample (ozone stock) added to V_f.

MPs were analysed using liquid chromatography coupled to high-resolution accurate-mass mass spectrometry (LC-HRAM-MS). The LC consisted of an Ultimate 3000 coupled through a

Hesi II electrospray source to a QExactive Orbitrap MS (Thermo Scientific, San Jose, CA, USA). Sample volumes of 50 µL were injected onto a phenyl-hexyl column, oven temperature at 35 °C. Elution flow was set to 0.3 mL/min with a gradient of the following two eluents (A): UPLC-MS quality water with 1% formic acid (v/v) and (B): UPLC-MS quality acetonitrile with 1% formic acid (v/v), both UHPLC grade, purchased from Actu-All (the Netherlands). The gradient applied was: 0-0.5 min stable at 0% B, 0.5-13 min linear increase of eluent to 80% B, 13-16 min stable at 80% B, 16-17 min linear decrease to 0% B, 17-23 min stable at 0% B. Micropollutants were detected in positive ionisation and negative mode using electrospray. Full-scan windows were applied for each MP. The following MS conditions were applied: spray voltage 3.5 kV, sheath and sweep gas flow rates 48 and 2 respectively, capillary temperature 256 °C, aux gas heater temperature 413 °C and resolution 70000. MS was calibrated for each series according to manufacturer protocol using a Pierce™ LTQ Velos ESI Positive Ion Calibration Solution (Thermo Scientific). Peak identification and quantification was done with Thermo Xcalibur (version 2.2) software.

Fluorescence intensity analyses was based on Jin et al. [79]. A Perkin Elmer Luminescence Spectrometer LS50B was used to scan the following ranges: emission from λ_{em} 280 – 550 nm with 0.5 nm intervals and excitation from λ_{ex} 220 – 480 nm with 10 nm intervals. The slit width for both emission and excitation was 5 nm and the scanning speed was 1300 nm/min. Region integration was done based on Chen et al. [105], to calculate the fluorescence in five regions: Region I indicating tyrosine like proteins (ex 220-250 nm, em 280-325 nm), Region II indicating tryptophan like proteins (ex 220-250 nm, em 325-380 nm), Region III indicating fulvic-like compounds (ex 220-250 nm, em 380-550 nm), Region IV indicating microbial by-products (ex 250-400 nm, em 280-3380 nm) and Region V indicating humic-like compounds (ex 250-400 nm, em 380-550 nm) (Figure S3.11). The StaRdom package in R was used to remove and interpolate the Rayleigh scattering bands for the emission excitation matrices.

Total organic carbon (TOC) was analysed with the non-purgeable organic carbon method on a TNM-L TOC analyser (Shimadzu). Ultra violet absorbance at 254 nm (UV_{254}) was analysed using an Infinite M200 Pro multimode plate reader (Tecan). Specific ultraviolet absorbance (SUVA) was calculated by dividing the UV_{254} values by the TOC values. Chemical oxygen demand (COD) and nitrite concentrations were analysed with dr. Hach-Lange kits, LCK 1414 and LCK 341 respectively. Size fractions were analysed using LC-OCD to quantify biopolymers, chromatographic DOC, hydrophobic organic carbon, humic acids, low molecular weight acids and low molecular weight neutrals. The LC-OCD used a Toyopearl HW-50S column and was coupled with both a UV and an organic nitrogen detector (Figure S3.12).

3.3 Results and discussion

3.3.1 Fractionation

To investigate the effect of differences in organic matter type in WWTP effluent on the ozonation of MPs, the effluent was fractionated with two methods: membrane based fractionation to produce four size fractions and XAD-8 resin based fractionation to produce four functional group based fractions.

Fractionation with membranes produced four size fractions with larger (F1) to smaller (F4) EfOM. Due to the used fractionation process, smaller molecules smaller molecules can still be present in the larger fractions. The TOC of F1 was highest, followed by F2, F4 and F3 (Figure 1A). This is different compared to Gonzales et al. [81] who found that for each of the four effluents they fractionated, 75% of the DOC was present in the fraction smaller than 10 kDa. In our case the sum of F3 and F4 is only 46% of the 'Total' TOC. This difference could be due to the use of different effluents, or due to the different type of membrane separation that was applied. We used cross-flow filtration whereas Gonzales et al. [81] used dead end filtration. Cross-flow filtration results in less fouling of the membrane resulting in the high TOC recovery rate of 97%. The disadvantage of the technique is that part of the small particles or molecules, can end up in the larger fractions, resulting in a slight overestimation of the large fractions and underestimation of the small fractions.

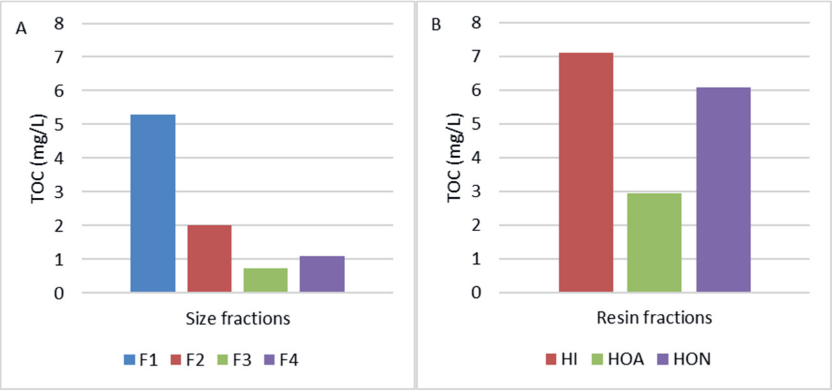


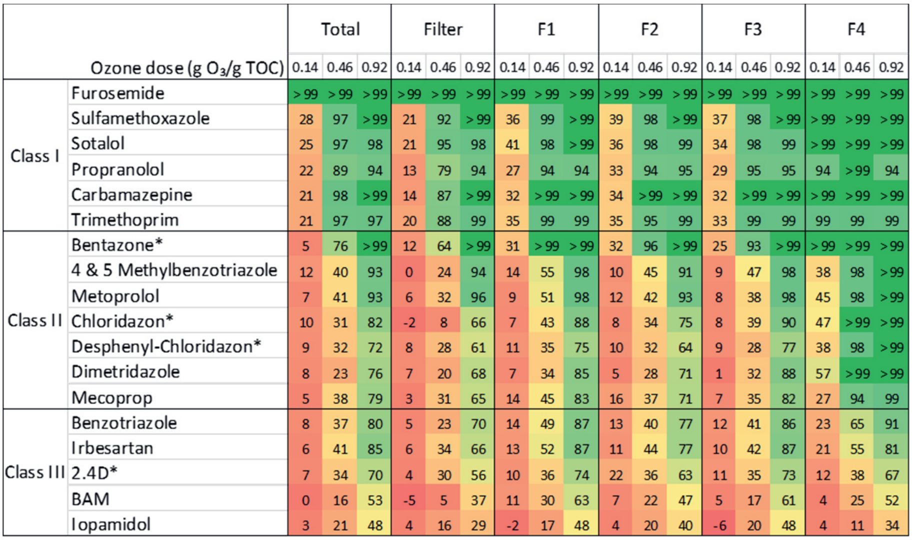
Figure 3.1 Composition of complete wastewater treatment plant effluent: size fractions (A) and resin fractions (B). The TOC of the resin fraction HOB was lower than the TOC of the blank, therefore this fraction is not included in the figure

Fractionation with resin also produced four fractions: hydrophilics (HI), hydrophobic acids (HOA), hydrophobic neutrals (HON) and hydrophobic bases (HOB). The TOC of the HOB fraction was lower than the TOC of the blank, therefore this fraction is not included in further analysis. The HI fractions was the largest based on TOC, followed by the HON fraction and the

HOA fraction. (Figure 1B). Other studies using resin fractionation found similar effluent compositions [78,100,103,106]. One difference we found, is that the HON fraction had a higher TOC than the HOA fraction, whereas the other studies found the opposite. This difference could be explained by variation between effluents from different WWTPs was added. The TOC recovery of the resin fractionation was close to 100% (102%), similar to that of Jin et al. [100], which indicates that OM was not lost or released from the resins. This means that the used washing, sorption and desorption steps onto and from the resin were appropriate to prevent loss of OM during the fractionation process.

3.3.2 Micropollutant removal

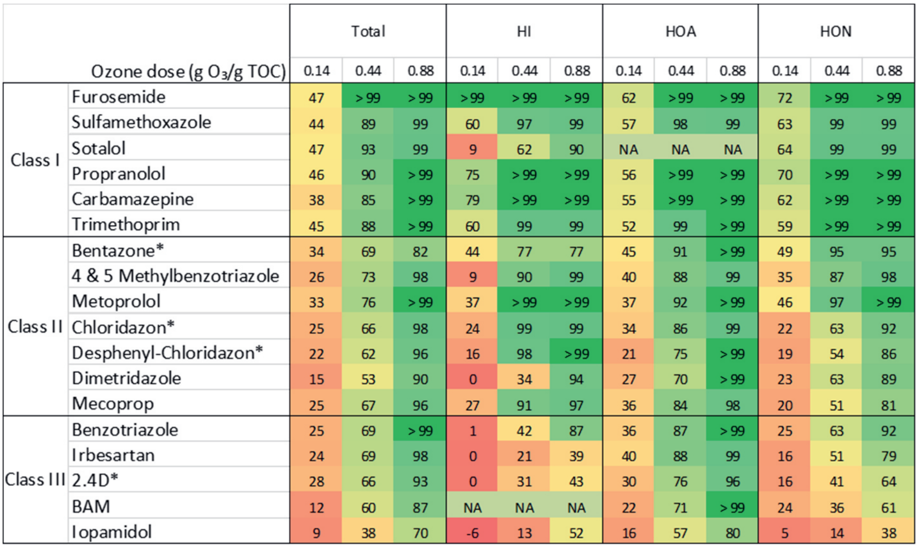
The smallest size fraction (F4) showed the highest MP removal compared to the other size fractions (Figure 2). This higher removal was only observed for class I and class II MPs, but not for the MPs with low ozone reactivity in class III. The five MPs in class III (benzotriazole, irbesartan, 2,4-D, BAM and iopamidol) showed similar removal in all size fractions. This shows that the OM in F4 is less reactive with ozone than most MPs, resulting in high removal of class I and class II MPs in this fraction. Only class III MPs are less reactive with ozone than the OM in F4, resulting in similar removal behavior of these compounds in all size fractions.



		Total			Filter			F1			F2			F3			F4		
	Ozone dose (g O_3/g TOC)	0.14	0.46	0.92	0.14	0.46	0.92	0.14	0.46	0.92	0.14	0.46	0.92	0.14	0.46	0.92	0.14	0.46	0.92
Class I	Furosemide	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99	> 99
	Sulfamethoxazole	28	97	> 99	21	92	> 99	36	99	> 99	39	98	> 99	37	98	> 99	> 99	> 99	> 99
	Sotalol	25	97	98	21	95	98	41	98	> 99	36	98	99	34	98	99	> 99	> 99	> 99
	Propranolol	22	89	94	13	79	94	27	94	94	33	94	95	29	95	95	94	> 99	94
	Carbamazepine	21	98	> 99	14	87	> 99	32	> 99	> 99	34	> 99	> 99	32	> 99	> 99	> 99	> 99	> 99
	Trimethoprim	21	97	97	20	88	99	35	99	99	35	95	99	33	99	99	99	99	99
Class II	Bentazone*	5	76	> 99	12	64	> 99	31	> 99	> 99	32	96	> 99	25	93	> 99	> 99	> 99	> 99
	4 & 5 Methylbenzotriazole	12	40	93	0	24	94	14	55	98	10	45	91	9	47	98	38	98	> 99
	Metoprolol	7	41	93	6	32	96	9	51	98	12	42	93	8	38	98	45	98	> 99
	Chloridazon*	10	31	82	-2	8	66	7	43	88	8	34	75	8	39	90	47	> 99	> 99
	Desphenyl-Chloridazon*	9	32	72	8	28	61	11	35	75	10	32	64	9	28	77	38	98	> 99
	Dimetridazole	8	23	76	7	20	68	7	34	85	5	28	71	1	32	88	57	> 99	> 99
	Mecoprop	5	38	79	3	31	65	14	45	83	16	37	71	7	35	82	27	94	99
Class III	Benzotriazole	8	37	80	5	23	70	14	49	87	13	40	77	12	41	86	23	65	91
	Irbesartan	6	41	85	6	34	66	13	52	87	11	44	77	10	42	87	21	55	81
	2.4D*	7	34	70	4	30	56	10	36	74	22	36	63	11	35	73	12	38	67
	BAM	0	16	53	-5	5	37	11	30	63	7	22	47	5	17	61	4	25	52
	Iopamidol	3	21	48	4	16	29	-2	17	48	4	20	40	-6	20	48	4	11	34

Figure 3.2 Heatmap with micropollutant removal (%) at three different ozone doses (0.14, 0.45 and 0.93 g O_3/g TOC) in the size fractions. Micropollutants were divided in three classes: class I - high ozone reactivity ($kO_3 > 10^4$ M^{-1} s^{-1}), class II - medium ozone reactivity ($10^2 > kO_3 > 10^4$ M^{-1} s^{-1}) and class III - low ozone reactivity ($kO_3 < 10^2$ M^{-1} s^{-1}). The colour scale is from red (0% removal) to green (100% removal). For micropollutants with an *, kO_3 values were not found in literature so the division of these compounds into the classes is solely based on the results of our study

The resin fraction HI showed lower removal of class III MPs compared to the other resin fractions and similar removal of class II and class III compounds (Figure 3). This indicates that OM from the HI fraction interferes more with the removal of MPs with low kO_3 ($< 10^2$ M^{-1} s^{-1}). The resin fraction HON showed lower removal of class II MPs than all other resin fractions and lower removal of class III MPs than the resin fractions 'Total' and HOA. The HON fraction interferes more with the ozonation of class II MPs than other resin fractions.



		Total			HI			HOA			HON		
	Ozone dose (g O_3/g TOC)	0.14	0.44	0.88	0.14	0.44	0.88	0.14	0.44	0.88	0.14	0.44	0.88
Class I	Furosemide	47	> 99	> 99	> 99	> 99	> 99	62	> 99	> 99	72	> 99	> 99
	Sulfamethoxazole	44	89	99	60	97	99	57	98	99	63	99	99
	Sotalol	47	93	99	9	62	90	NA	NA	NA	64	99	99
	Propranolol	46	90	> 99	75	> 99	> 99	56	> 99	> 99	70	> 99	> 99
	Carbamazepine	38	85	> 99	79	> 99	> 99	55	> 99	> 99	62	> 99	> 99
	Trimethoprim	45	88	> 99	60	99	99	52	99	> 99	59	> 99	> 99
Class II	Bentazone*	34	69	82	44	77	77	45	91	> 99	49	95	95
	4 & 5 Methylbenzotriazole	26	73	98	9	90	99	40	88	99	35	87	98
	Metoprolol	33	76	> 99	37	> 99	> 99	37	92	> 99	46	97	> 99
	Chloridazon*	25	66	98	24	99	99	34	86	99	22	63	92
	Desphenyl-Chloridazon*	22	62	96	16	98	> 99	21	75	> 99	19	54	86
	Dimetridazole	15	53	90	0	34	94	27	70	> 99	23	63	89
	Mecoprop	25	67	96	27	91	97	36	84	98	20	51	81
Class III	Benzotriazole	25	69	> 99	1	42	87	36	87	> 99	25	63	92
	Irbesartan	24	69	98	0	21	39	40	88	99	16	51	79
	2.4D*	28	66	93	0	31	43	30	76	96	16	41	64
	BAM	12	60	87	NA	NA	NA	22	71	> 99	24	36	61
	Iopamidol	9	38	70	-6	13	52	16	57	80	5	14	38

Figure 3.3 Heatmap with micropollutant removal (%) at three different ozone doses (0.14, 0.45 and 0.93 g O_3/g TOC) in the resin fractions. Micropollutants were divided in three classes: class I - high ozone reactivity ($kO_3 > 10^4$ M^{-1} s^{-1}), class II - medium ozone reactivity ($10^2 > kO_3 > 10^4$ M^{-1} s^{-1}) and class III - low ozone reactivity ($kO_3 < 10^2$ M^{-1} s^{-1}). The colour scale is from red (0% removal) to green (100% removal). For micropollutants with an *, kO_3 values were not found in literature so the division of these compounds into the classes is solely based on the results of our study

MP removal in the 'Total' sample was consistently higher in the resin experiment than in the membrane experiment. This was not expected because the 'Total' sample was taken from the same batch for both experiments. This can be explained by the difference in storage time of the water prior to the experiments, which was longer for the resin fractionation than for the membrane fractionation experiments, as discussed further in paragraph 3.3.

Observed differences in interference of MP ozonation were not consistent for all analysed MPs. Grouping the MPs in classes based on their kO_3 values shows that EfOM fractions interfere only with MPs in specific classes. To illustrate, differences in interference of the smallest size fraction (F4) were only observed for class I and class II MPs, while interference in F4 was similar to interference in the fraction 'Total' for class III MPs (Figure 3.2).

Additionally, differences in interference observed in the resin fractions HON and HI were only observed for class II and class III MPs respectively (Figure 3.3). This means that the interference of OM during ozonation of MPs does not only depend on the type of OM, but also on the ozone reactivity of the MPs themselves.

Uncertainties introduced in the different experimental steps were less than 5% for the individual steps. TOC recoveries of the fractionation steps were 100% (102 and 97%), which means that OM loss during the fractionation procedures was negligible. For the ozonation step, the standard deviation in the measured ozone dose was always less than 5% of the applied dose (Table S3.3). Finally, the average R2 of the standard curves for MP measurements were close to 1 (0.996 for membrane and 0.994 for the resin fractions), illustrating that the MP quantification is reliable. Finally, the different ozone doses served as replicates for each other in the ozonation and analysis steps and confirm the observed differences between the fractions.

Generally, only the quantity of EfOM is used as indication for the interference of EfOM with MP ozonation. To illustrate, EfOM quantity (DOC) is often used to determine the required ozone dose [66,99,107]. Our results show that also the type of EfOM is important to consider for the ozonation of MPs. For example, class I and class II MP removal was over 3 times higher in the smallest membrane fraction (F4) compared to the membrane fraction ‘Total’ (Figure 3.2). Furthermore, in the resin fractions, class III MP removal was 1.3 lower for HON and 2 times lower for HI, when compared to the ‘Total’. This shows that, at the same DOC concentration, different types of OM have different interference with MP ozonation. Therefore, additional research is needed into the variations of EfOM type in WWTP effluents, for example spatial and temporal variation, and their effect on MP ozonation.

Understanding of the complex EfOM in effluents can confirm which OM fractions and characteristics interfere with the ozonation of which MPs. For example, WWTP effluent can be screened for the type and characteristics of EfOM to decide whether the effluent is suitable for ozone treatment, or other treatment should be considered. Moreover, insight in the characteristics of highly interfering EfOM can be used to develop online sensors to control ozone dosing and prevent over and under dosing. Finally, this insight can aid the optimisation of treatments preceding ozonation treatment, e.g. conventional WWTPs or small specialized treatment modules. A pre-treatment that reduces the most interfering OM fractions and characteristics, will result in a lower energy and cost demand for the ozone treatment.

3.3.3 Organic matter characteristics

Three parameters were used to characterise the EfOM in the analysed fractions: specific UV absorbance at 254 nm (SUVA) as indication for double bonds and aromatic compounds, fluorescence as indication for humic- and fulvic-like organics and protein like compounds

[108], and COD as indication for total oxidizable potential in the samples. In addition to the three EfOM parameters, we analysed nitrite because it can scavenge ozone and affect MP removal [66]. We aim to use these analyses to get insight in the compositional differences of the fractions.

SUVA

SUVA correlated well to the applied ozone dose, showing a decrease with increase in ozone dose in all fractions (Figure 4). This indicates that SUVA values are a strong tool to monitor and screen ozone reactions, which is in line with other studies [80,109,110]. The size fraction 'Total' showed 1.8 times higher SUVA values than the resin fraction 'Total'. The most likely reason for this were instabilities in the TOC measurement, used for the dilutions of the fractions, as explained further in paragraph 3.4.

Similar SUVA values of 2.4 ± 0.14 L/mg TOC/m were found in most size fractions at the zero ozone doses. The smallest fraction, F4 with compounds smaller than 1 kDa, was the only exception to this and showed a lower SUVA of 0.19 L/mg TOC/m. Another study that used membranes to fractionated four different WWTP effluent found similar SUVA values, but did not find lower SUVA values in their smaller than 1 kDa fractions [81]. This indicates that the nature of the smaller than 1 kDa fraction in our study is very different from the respective fractions of [81]. An explanation for this could be different source waters for the WWTPs from which the effluents were taken.

The resin fractions 'Total', HI and HOA showed similar SUVA values around 1.3 L/mg TOC/m. The HON fraction showed a lower SUVA of 0.39 L/mg TOC/m and also hardly any decrease in SUVA with increasing ozone dose. Qi et al. [78] also found that SUVA of the HON fraction does not change after ozonation. This indicates that UV_{254} absorbing compounds, for example aromatic compounds, in the HON fraction of WWTP effluents generally are not reactive with ozone, unlike most other UV_{254} absorbing EfOM [111].

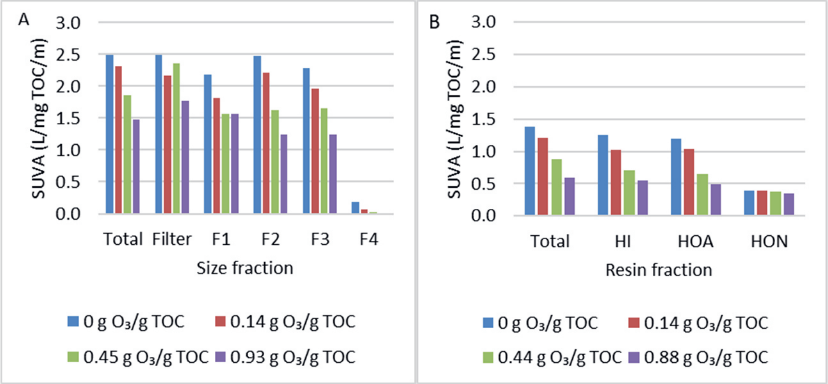


Figure 3.4 SUVA measurements in different effluent organic matter fractions at four ozone doses for the size fractions (A) and resin fractions (B)

Fluorescence

The highest fluorescence was found in region III and region V in most samples (Figure 3.5). These regions indicate the presence of humic- and fulvic-like compounds respectively [105]. None of the analyzed samples showed fluorescence around in region I and little fluorescence was observed in regions II and IV, this indicates absence of protein-like compounds [108]. A clear decrease in fluorescence with increasing ozone dose was found in both the size fractions and the resin fractions (Figure S3.13-1.8).

Approximately 1.2 times higher fluorescence values were found in the resin fraction ‘Total’ compared to the size fraction ‘Total’. This difference could be caused by lower SUVA values in the size fractions (Figure 3.5). Lower SUVA causes a lower inner filter effect and therefore higher fluorescence values in the resin fractions. The inner filter effect is absorption of part of the emitted fluorescence during fluorescence measurement by particles or molecules in the sample. This causes the measured fluorescence to be lower than the actual fluorescence of a sample. The inner filter effect is stronger in samples with high absorption values [112].

Most non-ozonised size fractions showed similar fluorescence intensities (Figure 3.5). The smallest size fraction (F4) is the only exception and shows almost no fluorescence. For the resin fractions at zero ozone doses, ‘Total’ and HI showed similar fluorescence intensities. Organic matter in the HOA fraction showed lower fluorescence, mainly at the humic-like compounds peak, and the HON fraction showed lower fulvic- and humic-like content than the ‘Total’ and the HI. The low fluorescence intensities in size fraction F4 and resin fractions HON and HOA indicate that these three fractions contain less fulvic- and humic-like compounds than the other studied fractions.

We observed a decrease in SUVA with increasing ozone dose in almost all fractions (Figure 3.4). This should also have resulted in a decrease in inner filter effect and therefore an increase in fluorescence. However, the opposite was found: a decrease in fluorescence with increasing ozone dose (Figure S3.14). This indicates that fluorescent groups are faster depleted than UV_{254} absorbing groups and therefore either less abundant or more reactive with ozone than UV_{254} absorbing groups.

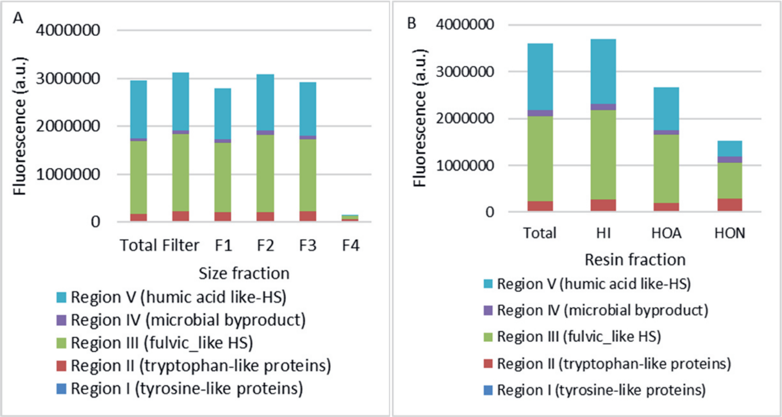


Figure 3.5 Fluorescence measurements in the size fractions (A) and resin fractions (B) without ozone addition

COD

COD decreased with ozone dose for all size and resin fractions except for the size fraction 'Filter' (Figure 3.6). The observed decrease in COD was similar to the absolute ozone concentration dosed (0.5, 1.6 and 3.3 mg O_3/L relating to the low medium and high doses respectively). Approximately 2.3 times lower COD values in the resin fraction 'Total' than in the size fraction 'Total'. This is probably due to instabilities in the NPOC measurement used for the dilutions as explained in paragraph 3.3 – SUVA.

A more than three times higher COD was measured in the size fraction 'Filter' at the highest ozone dose compared to the zero ozone dose. This COD value of 35 mg O_2/L was confirmed by duplicate measurements at different days by different people, and cannot be explained. Therefore, this value is not presented in Figure 3.6.

COD measured in the resin fraction HON was approximately two times higher than in the 'Total', HI and HOA fractions, indicating that the HON fraction contains many unsaturated groups that have higher COD. This is because more saturated organic molecules, with less double bonds, have higher COD values than unsaturated groups. The low saturation in the

HON fraction is confirmed by the low SUVA values of the this fraction (Figure 3.4), as SUVA mainly detects saturated bonds, for example in aromatic groups.

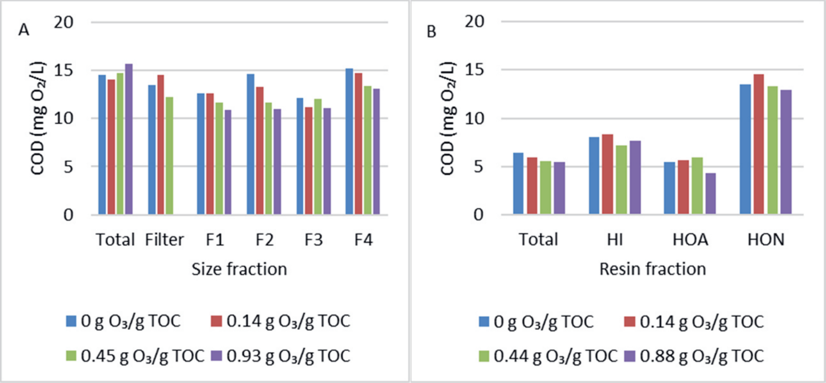


Figure 3.6 COD measurements in different effluent organic matter fractions at four ozone doses for the size fractions (A) and resin fractions (B). For the size fractions, several samples were measured twice to confirm the stability of the analysis, averages are presented

Nitrite

A rapid decrease of nitrite concentrations was observed with increasing ozone dose in the size fractions (Figure 3.7). In all size fractions except ‘Filter’, nitrite was removed by ozonation to below the detection limit of 0.051 mg NO_2/L at the highest ozone doses. Surprisingly, nitrite concentrations in the smallest size fraction (F4) were below the detection limit at the zero ozone dose. We did not expect that the naturally present nitrite would be rejected by the membrane with cutoff 1.08 kDa since nitrite molecules are more than 20 times smaller (0.046 kDa). Possibly, nitrite was rejected due to interactions of nitrite with positively charged compounds.

There are compounds that have a stronger affinity for ozone than nitrite. At the lowest ozone dose of 0.14 g O_3/g TOC or 0.5 mg O_3/L, not all nitrite was removed from the fractions, even though 0.05 mg O_3/L would theoretically be sufficient for the complete oxidation of the nitrite that was present. This indicates that ozone reacts first with other compounds in the effluent, before reacting with nitrite. These other compounds are most likely organic because a decrease in SUVA was also observed at the lowest ozone dose (Figure 3.4). Moreover, reduction in MP concentrations was also observed at the lowest ozone dose (Figure 3.2 and Figure 3.3). After reaction with these other compounds, nitrite is completely removed, as was observed at the two highest ozone doses.

Nitrite levels in all of the resin fractions were below the detection limit of 0.05 mg NO_2/L. We assume that this lack of nitrite in the resin fractions is caused by longer storage time of the effluent before use. Samples for the resin and membrane fractionation experiments were taken from the same batch that was stored at 4 °C. The resin experiment was done three months after the membrane experiment, during this time, biological nitrification depleted all nitrite in the resin fractionation samples.

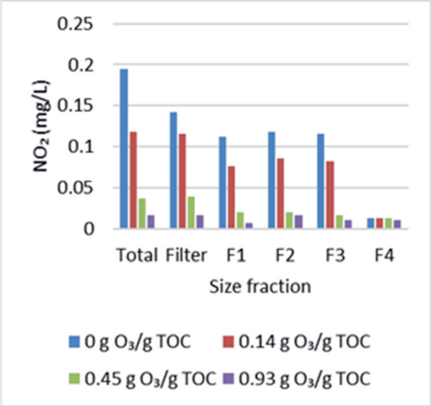


Figure 3.7 Nitrite measurements in different size fractions at four ozone doses. Results for the resin fractions are not shown because they were all below the detection limit of 0.05 mg NO_2/L

LC-OCD

The size fractions were further analysed using LC-OCD to quantify biopolymers, chromatographic DOC, hydrophobic organic carbon, humic acids, low molecular weight acids and low molecular weight neutrals (Figure S3.16). No trends in any of these components with increasing ozone dose were observed. The smallest size fraction F4 showed the most differences with the other analysed fractions at the zero ozone doses. This fraction contained very few biopolymers: 28 µg C/L in F4 compared to 310 ± 85 µg C/L for the other size fractions, very few humic acids: 41 µg C/L in F4 compared to 1890 ± 208 µg C/L for the other size fractions and many low molecular weight neutrals: 2560 µg C/L in F4 compared to 650 ± 98 µg C/L for the other size fractions. Biopolymers and humic acids are large molecules that were retained by the 1 kDa membrane, while the small low molecular weight neutrals could easier pass through [84]. The hydrophobic organic carbon was approximately 12% of the total DOC of the unfractionated effluent, which is typical for EfOM [84].

3.3.4 Relations between MP removal and EfOM characteristics

The size fraction F4 and the resin fraction HON showed the largest differences in MP removal and concentrations of the other parameters at zero ozone doses compared to the other fractions. The size fraction 'Total' and the resin fraction 'Total' showed consistent differences in the measured parameters, which was not expected because they were taken from the same effluent. These three fractions are further analysed below combining all measured parameters to give more insight in their constituents. Finally, we analyse which of the measured parameters can be used to explain the observed differences in MP removal.

Total

We expected both 'Total' samples to behave similarly because both samples were taken from the same batch of effluent, stored in a 4 °C room. The size fraction 'Total' showed more than three times higher fluorescence and SUVA values and more than two times lower COD values than the resin fraction 'Total'. We did not expect these differences because both 'Total' samples were taken from the same batch. We expect that these differences are due to the difference in storage time before the experiments as also discussed in paragraph 3.3.

Size fraction F4

Higher removal of class I and II MP was observed in the smallest size fraction (F4) compared to the other fractions. This indicates that the EfOM in this fraction interferes less with ozonation of ozone reactive MPs than the EfOM in other fractions. Organic matter in F4 also showed very low fluorescence, SUVA and nitrite, while the OM in this fraction had similar COD levels as other size fractions. LC-OCD analysis showed that this fraction contains mainly low molecular weight neutrals and no humic acids or biopolymers. The lack of humic compounds is confirmed by the SUVA and fluorescence measurements. Low SUVA values indicate the absence of aromatic groups, which are unsaturated [80]. Because F4 has similar overall saturation as other size fractions, but a lower aromaticity content, this fraction must contain other types of unsaturated groups, such as alkenes.

Another study that used membrane fractionation on four different effluents found that the EfOM fraction < 1kDa has a higher ozone reaction coefficient than the other fractions of < 10 kDa, < 5 kDa and < 3 kDa [81]. Our study shows a high MP removal in the < 1 kDa fraction (F4). This indicates that the highly reactive EfOM in this fraction stimulates MP ozonation. However, the SUVA values of the < 1 kDa in our study were lower than those of Gonzales et al. [81], 0.2 and 1.8 ± 0.22 L/mg C/m respectively. This indicates that the OM in the < 1kDa fraction in our study was different from that in the respective fractions of Gonzales et al. [81].

Resin fraction HON

The OM in the resin fraction HON has low fluorescence and SUVA, and high COD values. SUVA in the HON fraction also did not decrease with increasing ozone dose as was seen in the other fractions. Despite this, we did not observe high MP removal in the HON fraction, which was expected because of the low fluorescence and SUVA values. The high COD values indicate that OM in this fraction contains OM with a lower level of saturation, which matches with the low SUVA values. Another study that ozonated and fractionated WWTP effluent also found low and stable SUVA values in the HON fraction [78]. Jin et al. [100] found a more than twofold decrease in UV_{254} absorbance at their lowest ozone dose of 0.42 g O_3/g TOC and stable SUVA with higher ozone doses. This shows that the HON fraction is made up of a very different type of OM than the other fractions, while it only shows slight differences in MP removal.

Explaining parameters

Because the smallest size fraction (F4) showed low interference with the ozonation of MPs and low SUVA, fluorescence and nitrite values, all three parameters can be used to explain the low interference in this fraction. This indicates that effluents with low fluorescence, SUVA and nitrite values need less ozone to reach the same MP removal. Moreover, other studies find that UV_{254} absorbing compounds and nitrite are highly reactive with ozone and are therefore likely to interfere with MP ozonation [66,111]. On the other hand, all resin fractions showed nitrite levels below detection limit and MP removal in these fractions was not as high as in F4 of the size fractions. Therefore, our results indicate that the OM characteristics have a larger effect on MP ozonation than nitrite concentrations. Furthermore, fluorescence values in the resin fraction 'Total' were approximately two times higher than fluorescence values in the size fraction 'Total', whereas MP removal was higher in the resin fraction 'Total'. This indicates that fluorescence is not a suitable parameter to explain the observed differences in MP removal. Finally, low SUVA correlates with high MP removal in most samples. The only exception to this rule is the resin fraction HON, that had low SUVA values and did not have lower MP removal other resin fractions. Therefore, we conclude that none of the measured parameters are sufficient to predict all variation in MP removal by ozonation.

3.4 Conclusions

In general, the quantity of organic matter is important for ozonation of micropollutants. Our study shows that micropollutant removal is also affected by the type of organic matter (different organic matter fractions). Comparing all size fractions, the smallest size fraction (< 1 kDa) had the lowest interference with the ozonation of micropollutants. For the resin fractions, hydrophobic neutrals and hydrophilics had higher interference than other resin fractions. This shows that size and resin fractionation give insight in the type of organic matter, which provides understanding in the interference of organic matter during micropollutant ozonation. Further research on effluents of various matrix compositions is needed to study the consistency of this interference in effluents of different origin.

The degree of interference by organic matter fractions depends on the ozone rate constants of individual micropollutants. For micropollutants with high and medium ozone rate constants, the smallest size fraction (< 1 kDa) had low interference on their removal. This and other size fractions had the same interference for micropollutants with low ozone rate constants. The higher interference in the resin fractions hydrophobic neutrals and hydrophilics only occurred for micropollutants with medium and high ozone rate constants, respectively. Therefore, when studying the interference of the type of organic matter on the ozonation of micropollutants, micropollutants must be grouped based on their ozone rate constants.

The four parameters: specific UV absorbance at 254 nm, fluorescence, chemical oxygen demand or nitrite were used to elucidate the composition of organic matter in the fractions. Still, these four parameters were insufficient to predict the differences in micropollutant removal in the various fractions. Therefore, other characterisation methods are needed to understand and predict the interference of organic matter with micropollutant ozonation.

To conclude, understanding of the type of organic matter in wastewater is essential to optimize micropollutant ozonation from for example, wastewater treatment plant effluent. Insight in the type organic matter can be used to decide which effluents are suitable to implement ozonation and to determine the required ozone dose for specific effluents.

Acknowledgement

This work was funded by the Dutch “Topsector Water” and Royal HaskoningDHV. We would like to thank Wiebe de Vos and Bob Siemerink from the Membrane Science and Technology group at the Technical University of Twente (the Netherlands) for their help with the membrane fractionation and Gerlinde Vink from the Soil Chemistry and Chemical Soil Quality group at Wageningen University & Research (the Netherlands) for their help with the resin fractionation. We would also like to thank Uwe Hübner and Sona Fajnorova from the Urban Water Systems Engineering group at the Technical University of Münich (Germany) with their help on the ozone stock setup. Finally we would like to thank Victor Ajao and Mieke Kersaan-Haan from Wetsus in Leeuwarden (the Netherlands), for their help with the LC-OCD analyses.

3.5 Supplementary information

3.5.1 Membrane fractionation

The cross-flow setup used for the membrane fractionation (Figure S3.8) consisted of a Verder internal rotary gear pump (1), with tubing attached to two sides. One side of the pump was connected with a tube (2) to a 2 L Schott bottle (3), wrapped in aluminium foil, that contained 2 L of previously filtrated WWTP effluent. The other side of the pump was connected to a pressure gauge (5), followed by a hollow fibre membrane (5). The membrane was encased in a rubber tube with a hole in one side to allow the permeate to flow out into a 500 mL Schott bottle wrapped in aluminium foil (6). The top of the 2 L Schott bottle, as well as the top of the 500 mL Schott bottle, were covered with aluminium foil to reduce the loss of water due to evaporation. After the permeate was flown through the membrane, the retentate was led back into the 2 L Schott bottle with a different tube (7) to allow recirculation of the WWTP effluent. Before each filtration step, the membrane to be used was first flushed with MQ water. Three hollow fibre membranes with different MWCO's were used to fractionate the WWTP effluent.

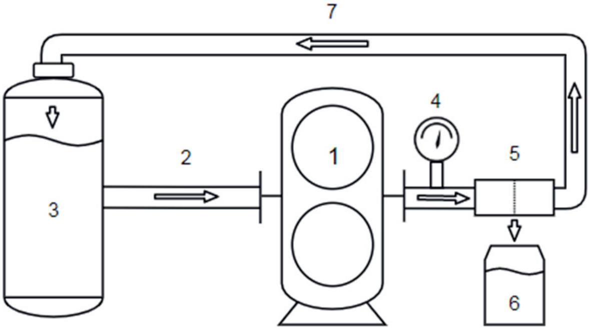


Figure S3.8 Schematic representation of the hollow fibre membrane filtration set-up. The arrows indicate the flow of the WWTP effluent. 1 = 2 L Schott bottle containing WWTP effluent; 2 = tube connecting Schott bottle to pump; 3 = internal rotary gear pump; 4 = pressure gauge; 5 = hollow fibre membrane; 6 = 500 mL Schott bottle for collection; 7 = tube connecting hollow fibre membrane to 2 L Schott bottle

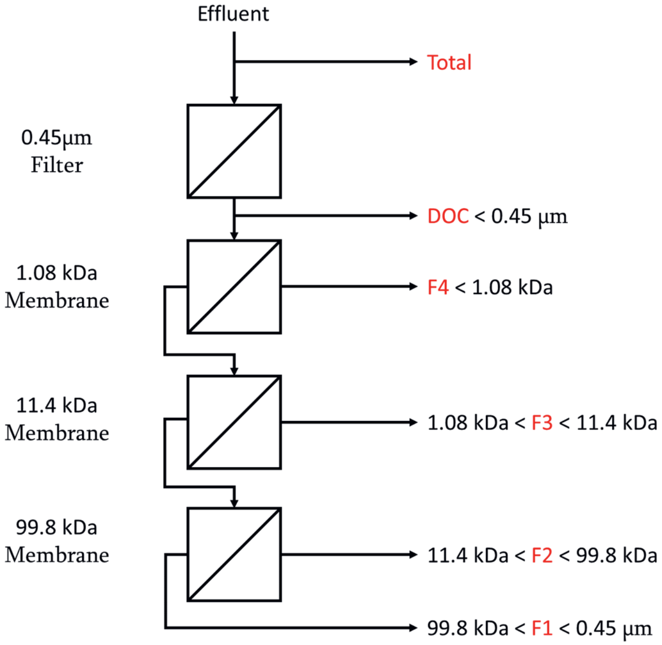


Figure S3.9 Schematic representation of the membrane fractionation procedure. Permeate of the membranes was collected as the different membrane fractions indicated in red

3.5.2 Resin fractionation

Materials

- g XAD-8 resin per sample (SOC)
- 50 mL syringe, one per sample
- Millipore SA 5.0 μm filter with filter holder that fits on the syringe
- Schott bottles / greiner tubes to collect the fractions and blanks, four fractions + total
  + blank per sample
- 0.1M HCl solution for acidification, 1.5 L for wash + 200 mL per sample
- 0.1M KOH solution for de-acidification, 1.5 L for wash + 200 mL per sample
- pH meter

Methods

- Preparation

  - Label all bottles / tubes clearly to avoid confusion later on
  - Filter sample with 0.45 µm PES filter (protocl for 400 mL non-up-concentrated sample)
    - Wash the XAD-8 resin with demi water, 0.1M KOH and 0.1M HCl;
    - Add liquid to resin container, stir for several seconds
    - Use vacuum to speed up flushing
    - Repeat for a total of: 1 L demi, 1 L KOH, 1 L demi, 1 L HCl, 1 L demi, 0.5 L KOH, 0.5 L demi, 0.5 L HCl, 1 L demi
  - Weigh empty scott bottles (to determine the volume of the fractions in the end)
- Step 1:
  - Place the XAD-8 resin in the syringe and note exact weight added
  - Add the SA filter with filter holder to prevent washout of resin
  - Wash with 20 mL of demi water (resin blank)
  - Take in sample and homogenise the liquid with the XAD-8 resin (suck in sample + a bit of air and shake for several seconds)
  - Discharge the first eluent in a beaker glass for pH adjustment
  - Repeat if not all sample fits in one syringe
- Step2:
  - Wash the XAD-8 with 200ml 0.1M HCl in demi water
  - Collect the second eluent (fraction HOB)
  - Repeat if not all sample fits in one syringe
- Step 3:
  - Adjust the pH of the first eluent from step 1 to pH 2 with 1 M HCl and 1 M KOH
  - Note volume of acid and base added (dilution)
  - Reapply the pH adjusted discharge on the XAD-8 resin > liquid loses some colour
  - Collect the eluent (fraction HI).
- Step 4:
  - Wash the XAD-8 resin with 200ml of 0.1M KOH,
  - Collect the eluent (fraction HOA) > first wash has dark colour
- Step 5:
  - Wash the XAD-8 resin with 100ml of methanol
  - Collect the eluent
  - Evaporate the methanol by leaving the schott bottle in a flow cabinet overnight
  - Dissolve leftovers in 200 mL MQ water (fraction HON)

- Adjust pH of all samples to 7
- Weigh all fraction bottles to determine final volume of fractions (after evaporation and dissolving of HON!)

-

3.5.3 Micropollutants background information

Table S3.1 micropollutants supplier information

Common name	Chemical name	CAS number	Supplier
2,4-D	2,4-Dichlorophenoxyacetic acid	94-75-7	Sigma Aldrich Chemie B.V.
4 methylbenzotriazole	4-methyl-2H-benzotriazole	29878-31-7	VWR international B.V.
5 methylbenzotriazole	5-methyl-2H-benzotriazole	136-85-6	VWR international B.V.
BAM	2,6-Dichlorobenzamide	2008-58-4	Sigma Aldrich Chemie B.V.
bentazone	1H-2,1,3-Benzothiadiazin-4(3H)-one, 3-(1-methylethyl)-,2,2-dioxide	25057-89-0	Sigma Aldrich Chemie B.V.
benzotriazole	1H-1,2,3-Benzotriazole	95-14-7	Sigma Aldrich Chemie B.V.
carbamazepine	5H-Dibenz[b,f]azepine-5-carboxamide	298-46-4	Sigma Aldrich Chemie B.V.
chloridazon	5-amino-4-chloor-2-fenylpyridazine-3(2H)-on	1698-60-8	Sigma Aldrich Chemie B.V.
desphenyl-chloridazon	5-amino-4-chlor-3(2H)-pyridazinon	6339-19-1	AKOS consulting & solutions GMBH
dimetridazole	1,2-Dimethyl-5-nitroimidazole	551-92-8	VWR international B.V.
furosemide	4-Chloro-2-[(2-furylmethyl)amino]-5-sulfamoylbenzoic acid	54-31-9	Sigma Aldrich Chemie B.V.
iopamidol	(S)-N,N'-bis(2-Hydroxy-1-(hydroxymethyl)ethyl)-2,4,6-triiodo-5-lactamidoisophthalamide	60166-93-0	Sigma Aldrich Chemie B.V.
irbesartan	2-butyl-3-[p-(o-1H-tetrazol-5-ylfenyl)benzyl]-1,3-diazaspiro[4.4]non-1-een-4-on	138402-11-6	TCI Europe N.V.
mecoprop	2-(4-Chloro-2-methylphenoxy)propanoic acid	93-65-2	Sigma Aldrich Chemie B.V.
metoprolol	1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]-2-propanol	56392-17-7	Sigma Aldrich Chemie B.V.
propranolol	1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride	318-98-9	Sigma Aldrich Chemie B.V.
sotalol	4'-(1-Hydroxy-2-(isopropylamino)ethyl)methane sulfonanilide	959-24-0	Thermo Scientific
sulfamethoxazole	4-Amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide	723-46-6	Sigma Aldrich Chemie B.V.
trimethoprim	2,4-Pyrimidinediamine, 5-[(3,4,5-trimethoxyphenyl)methyl]	738-70-5	Sigma Aldrich Chemie B.V.

Table S3.2 Ozone rate constants of the analyzed micropollutants

Micropollutant	kO_3 value	Reference
2,4-D	2.19E+01	[113]
4 & 5 methylbenzotriazole	4.60E+02	[45]
BAM	*	
bentazone	*	
benzotriazole	2.20E+01	[114]
carbamazepine	3.00E+05	[60]
chloridazon	*	
desphenyl-chloridazon	*	
dimetridazole	3.30E+02	[115]
furosemide	6.80E+04	[116]
iopamidol	1.00E+00	[116]
irbesartan	2.40E+01	[45]
mecoprop	1.11E+02	[45]
metoprolol	3.30E+02	[45]
propranolol	1.00E+05	[114]
sotalol	1.90E+04	[116]
sulfamethoxazole	2.00E+06	[100]
trimethoprim	4.10E+05	[116]

* no rate constants are available to the authors best knowledge

3.5.4 Ozone stock setup

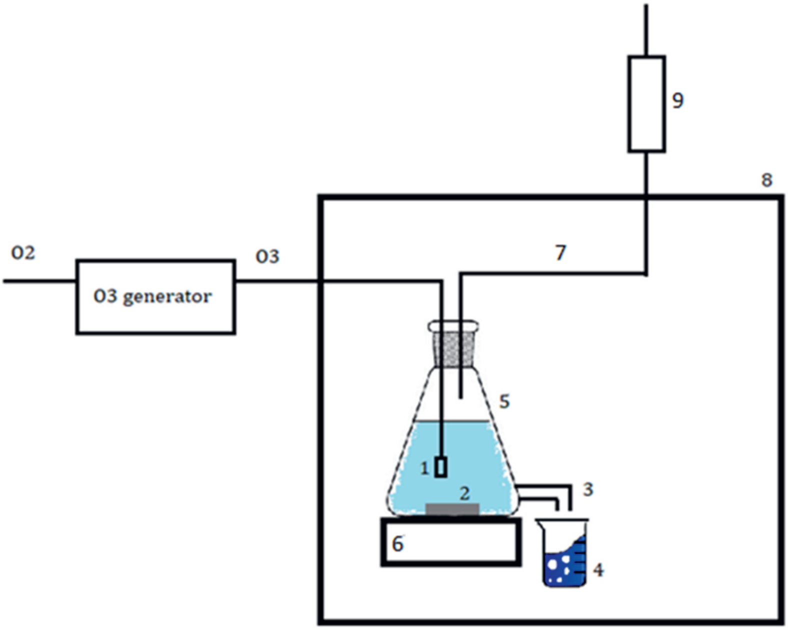


Figure S3.10 Schematic set-up of the ozonation of ultrapure water with: (1) aeration diffuser, (2) magnetic stir bar, (3) glass tap, (4) beaker glass, (5) Schott flask containing ozone stock, (6) magnetic stirrer, (7) off gas tube containing left over ozone, (8) fridge and (9) ozone catalyst to destroy excess ozone. The ozone generator was supplied with pure oxygen

3.5.5 Fluorescence intensity analysis

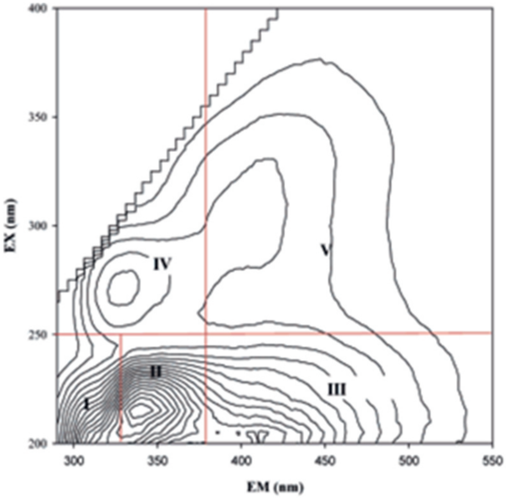


Figure S3.11 Regions used for region integration adapted from Chen et al. [105]

3.5.6 LC-OCD analysis

For the LC-OCD analysis, a volume of 28 mmol phosphate buffer was used as mobile phase and Phosphoric acid at pH 1,5 as acidic solution with the following settings:

- Flow: 1.1 mL/min
- Nitrogen pressure: 1.5 bar
- NDIR detector: 0 – 20 vpm
- UV-detectors: 220 nm and 254 nm

Immediately after injecting 0.9 mL sample, 5% was sent to the detector for DOC measurement. The remaining 95% of the sample was pumped through the size exclusion chromatography column and then led to both an organic carbon detector and an organic nitrogen detector. The setup (Figure S3.12) contained the following equipment:

- LC-OCD, Model 8
- NDIR-detector, Siemens Ultramat 6E, built into the LC-OCD
- Column, Toyopearl HW-50S, 30 µm, 250 mm
- UV-detector, Agilent 1260 Infinity
- OND-detector, Agilent 1260 Infinity
- Auto sampler, Agilent 1260 Infinity
- Isocratic pump, Agilent 1260 Infinity
- Degasser, Agilent 1260 Infinity

- Interface, Agilent 35900E
- Software Open Lab

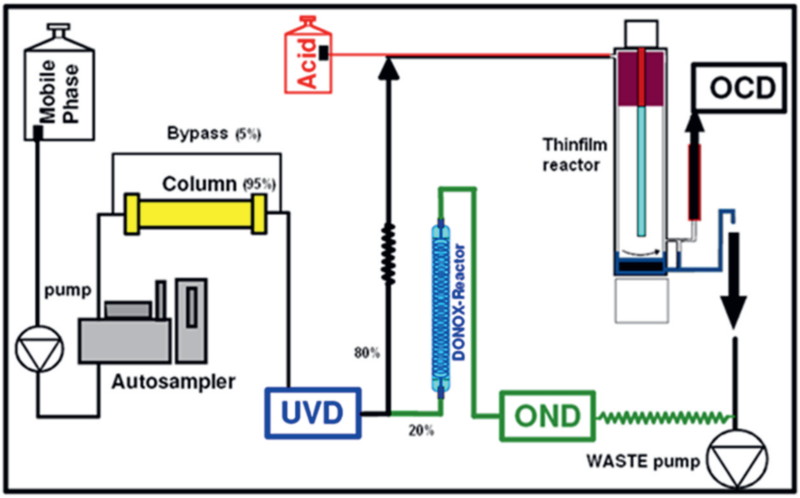


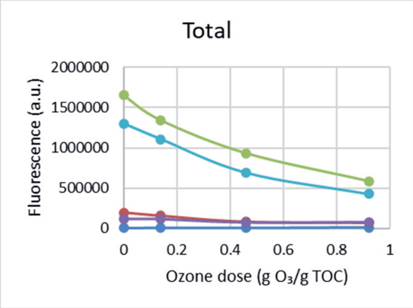
Figure S3.12 Schematic representation of the LC-OCD setup

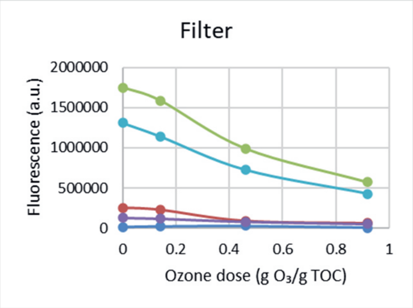
3.5.7 Measured ozone doses

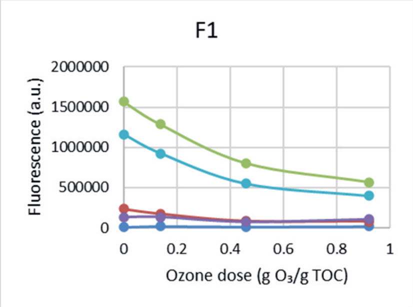
Table S3.3 Measured ozone doses in size and resin fractions

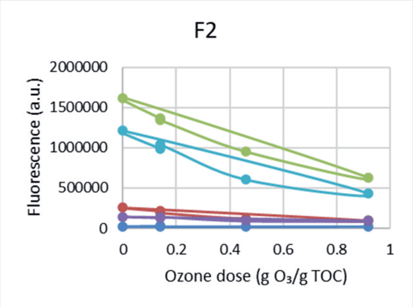
	Ozone dose size fractions (mg O_3 / mg TOC)	Ozone dose resin fractions (mg O_3 / mg TOC)
Low dose	0.14 ± 0.006	0.14 ± 0.006
Medium dose	0.46 ± 0.009	0.44 ± 0.001
High dose	0.92 ± 0.017	0.88 ± 0.012

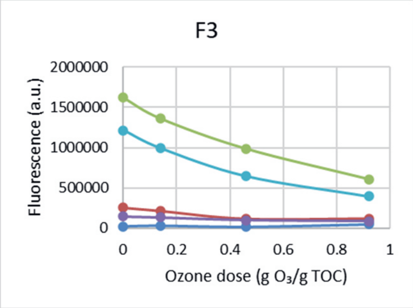
3.5.8 Fluorescence results

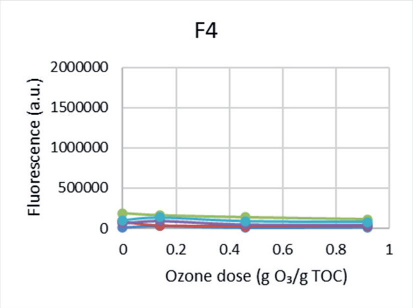


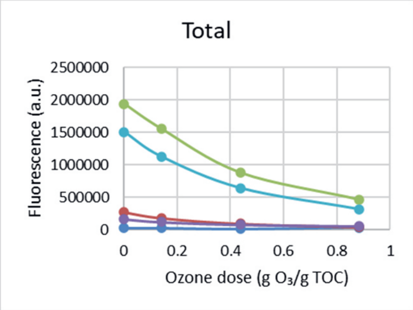


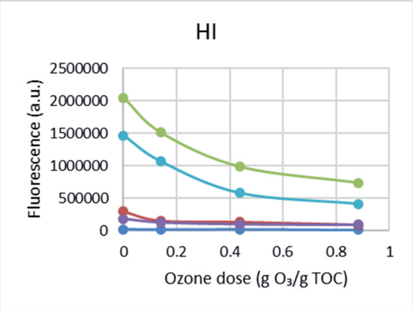












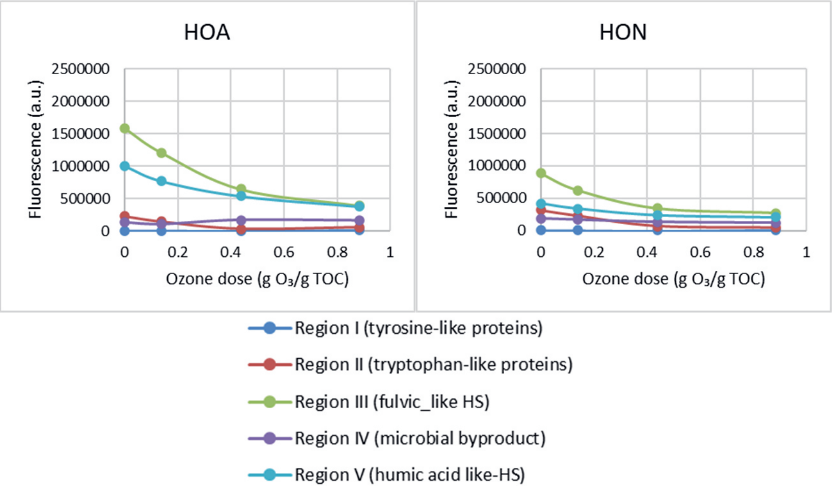


Figure S3.13 Fluorescence of the five integrated regions in the size (Total, Filter, F1, F2, F3 and F4) and resin (Total, HI, HOA and HON) fractions with increasing ozone dose

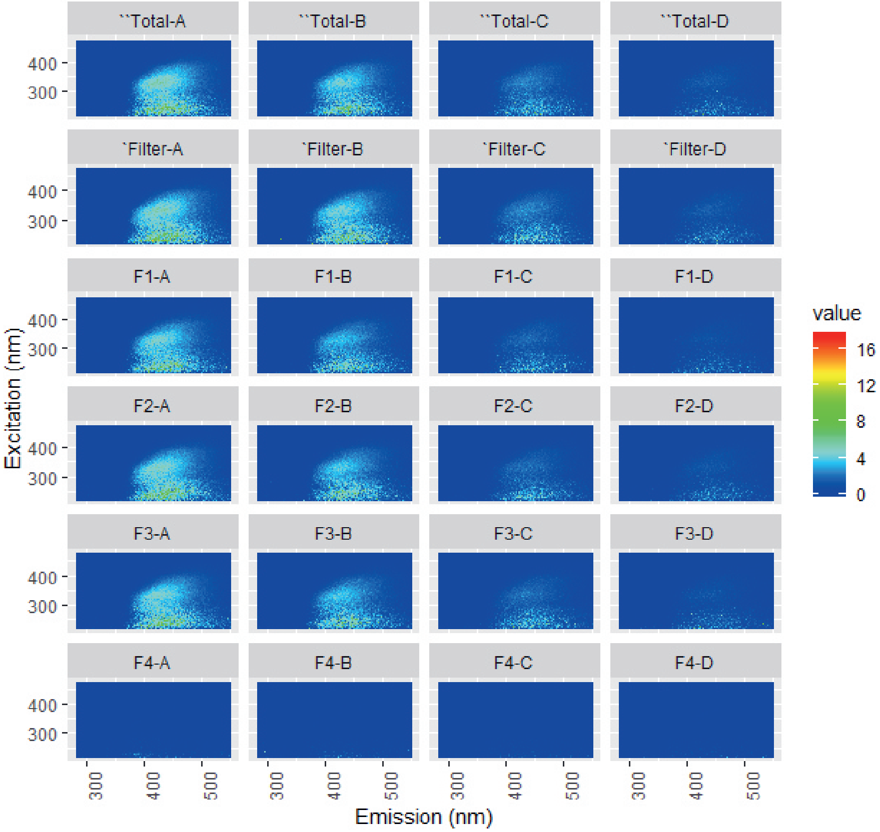


Figure S3.14 Fluorescence measurements of different size based effluent organic matter fractions at four ozone doses. Size fractions are: 100 kDa - 0.45 µm (F1), 11 - 100 kDa (F2), 1.1 - 11 kDa (F3) and < 1.1 kDa (F4). Applied ozone doses are 0 g O_3/g TOC (A), 0.14 g O_3/g TOC (B), 0.45 g O_3/g C (C), 0.93 g O_3/g C (D)

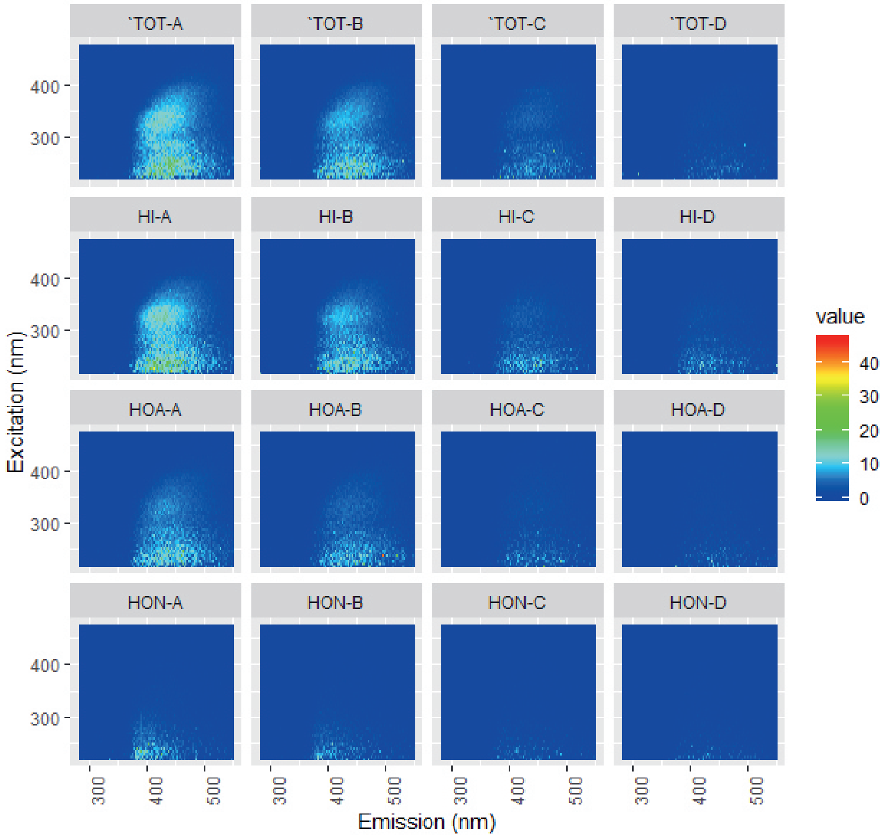


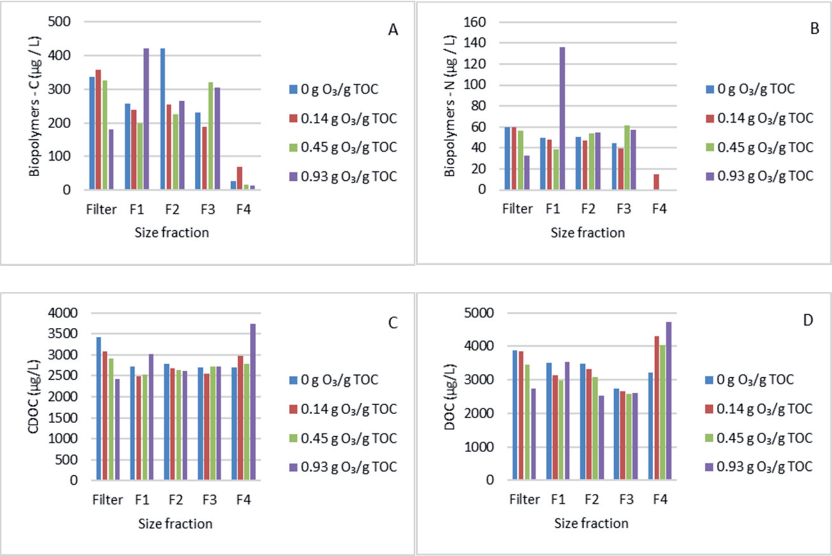
Figure S3.15 Fluorescence measurements of different resin based effluent organic matter fractions at four ozone doses. Resin fractions are hydrophilics (HI), hydrophobic acids (HOA) and hydrophobic neutrals (HON). Applied ozone doses are A: 0 g O_3/g TOC, B: 0.14 g O_3/g TOC, C: 0.45 g O_3/g C, D: 0.93 g O_3/g C

3.5.9 LC-OCD results

The different components separated by the LC-OCD column that are presented in Figure S3.16 are explained below:

- Biopolymers (polysaccharides amino sugars, polypeptides, proteins; “extracellular polymeric substances”): This fraction is very high in molecular weight (100.000 – 2.000.000 g/mol), hydrophilic, not UV-absorbing. Polysaccharides exist only in surface waters.
- CDOC (Chromatographic DOC): This is the OC value obtained by area integration of the total chromatogram. Subfractions of CDOC are either natural organic matter or soil organic matter (see below).
- DOC: dissolved organic carbon

- HOC (Hydrophobic OC): Calculated as difference DOC minus CDOC (CDOC= Chromatographic DOC). Therefore, all OC retained on the column is defined as „hydrophobic“. This could be either dissolved hydrocarbons etc. or microparticulate (“humins” in ground waters).
- Humic substances (HS): In LC-OCD measurements there is a tight definition for HS based on retention time, peak shape and SAC. Calibration on the basis of „Suwannee River“ Standard IHSS-FA and IHSS-HA. In addition, statistical data are given, like number-averaged molecular mass (Mn) and aromaticity (SAC/OC).
- LMW (low molucular weight) Organic-Acids: In this fraction all aliphatic low-molecular-mass organic acids co-elute due to an ion chromatographic effect. A small amount of HS may fall into this fraction and has to be subtracted on the basis of SAC/OC ratios.
- LMW Neutrals: According to theory, only low-molecular weight weakly charged hydrophilic or slightly hydrophobic (“amphiphilic”) compounds appear in this fraction, like alcohols, aldehydes, ketones, amino acids. The hydrophobic character increases with retention time, e. g. pentanol at 120 min, octanol at 240 min. However, compounds eluting after 200 min are rated “hydrophobic” (HOC).



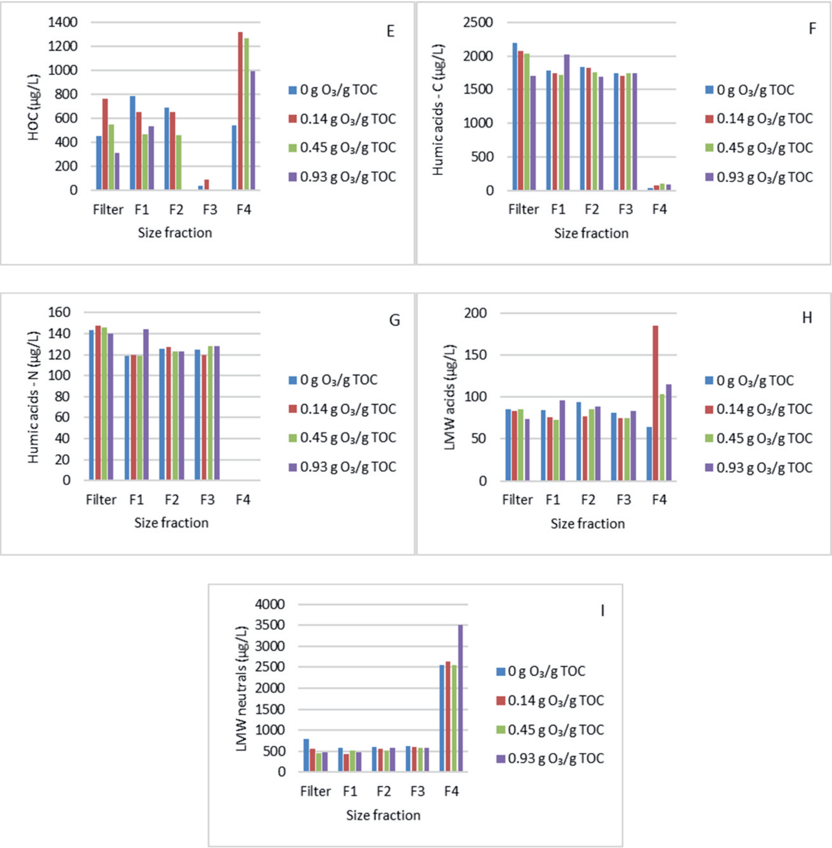
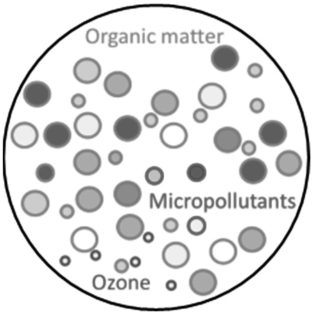


Figure S3.16 LC-OCD measurements in different effluent organic matter fractions at 4 ozone doses for the size fractions. The different components that are separated in the LC-OCD column are: biopolymers (A and B), chromatographic DOC (C), DOC (D), hydrophobic OC (E), humic substances (F and G), LMW acids (H), LMW neutrals (I)

Chapter 4

The effect of organic matter fractions on micropollutant ozonation in wastewater effluents



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Abstract

Organic matter (OM) is the most important factor influencing the effectivity and efficiency of micropollutant (MP) ozonation in wastewater effluents. The importance of the quantity of OM is known, because of this, total organic carbon (TOC) is generally used to determine the required ozone dose for any water sample. Still, the effect of OM type on MP ozonation is not well understood. In this study, effluents from five wastewater treatment plants were collected and the organic matter in these effluents was fractionated using membranes (F1-4) and resin (HI, HOA, HON and HOB). Fractions were diluted to the same TOC concentration, spiked with MPs and ozonated at three ozone doses. Our results show that all five effluents had comparable OM compositions and similar MP removal, confirming the suitability of OM quantity (TOC) to compare the ozone requirements for wastewater effluents. From the 19 analyzed MPs, three groups were identified that showed similar removal behavior. The strongest differences between the groups were observed around MP ozone reactivities of 10^2, 10^4 and 10^6 M^{-1} s^{-1}. This indicates the presence of three OM groups in the samples that interfere with the removal of different MPs. MP removal in the resin fraction HON were higher for MPs with high and medium ozone reactivity, indicating a low interference of OM in this fraction with MP ozonation. OM in the resin fractions HOA and HI showed higher interference with MP ozonation. Therefore, removing the HOA and HI fractions prior to ozonation would result in a lower required ozone dose and a more efficient removal of the MPs. MP removal correlated with the OM characteristics A300, SR and fluorescence component comp 2. These characteristics can be used as inline tools to predict the required ozone dose in water treatment plants.

4.1 Introduction

Micropollutants (MPs) are found in waters across the world and pose a threat to ecosystems and possibly human health [1,37,171,172]. These pollutants enter the environment via the effluent of wastewater treatment plants (WWTPs), because WWTPs are not designed to remove MPs and only remove them to a limited extent [42,58,120]. To improve the MP removal in conventional WWTPs, an advanced post-treatment such as ozonation can be added.

Ozonation has shown its potential to remove MPs from wastewater effluent on large scale [45]. Ozone itself readily reacts with electron rich moieties such as aromatic and amine groups and it can also form small amounts of ·OH radicals that react with any compound [60,173]. The downside of the high reactivity of ozone and the formed radicals is that they also react with other compounds than MPs in the water matrix. Other organic compounds in a WWTP effluent (effluent organic matter, or EfOM) are generally present at 10^3 – 10^6 times higher concentrations than the MPs (mg/L compared to µg and ng/L) (Table S4.) [66,174,175]. As a result, the majority of the dosed ozone reacts with EfOM instead of the target MPs. Because of this ozone consumption by EfOM, the required amount of ozone is generally based on the quantity of EfOM in the water (total organic carbon TOC, or chemical oxygen demand COD) and not only on the volume of water [44,45,68,176].

Not only the quantity of organic matter (OM), but also the composition of this complex matrix affects the ozonation process. Studies have used size (with membranes) and resin (with a hydrophobic resin) based fractionation methods to elucidate the composition of EfOM and its interactions with ozone. The effects of size and resin fractions on ·OH formation have been confirmed [80,81]. In addition, the effect of resin fractions on by-product formation [78], and the effect of ozone on the fraction compositions has been studied [100]. Van Gijn et al. [177] showed that size and resin based fractions show differences in interference during MP ozonation. Still, the variations of these fractions in different effluents and the reasons why these fractions interfere differently are unclear.

In this study, the interference of size- and resin based fractions from different WWTP effluents on MP ozonation was investigated and related to the characteristics of the fractions. Aiming to elucidate whether different WWTP effluents contain different types of OM and how these different OM types interfere with MP ozonation.

4.2 Materials and methods

4.2.1 Chemicals and Reagents

A mix of 19 MPs was selected based on their occurrence in WWTP effluents and their reactivity with ozone (Table S4.2). Secondary clarified effluent was obtained from five Dutch

WWTP, selected for their variety in treated wastewaters: Bath, Bennekom, Ede, Epe, and Nieuwveen (Table 4.1). Effluent samples were collected over 24 hours on a day without precipitation to ensure dry weather conditions. All effluent samples were filtered through a 0.45 µm membrane filter and were stored at 4 °C until use for the experiments.

Table 4.1 Wastewater types and treatment processes of the five effluents

Sample name	Source wastewater	Treatment process	Hydraulic capacity (m^3/h)
Bath	Domestic, petrochemical industry, tanneries	Conventional activated sludge	20,000
Bennekom	Domestic	Conventional activated sludge	1,000
Ede	Domestic, cheese industry	Conventional activated sludge	6,750
Epe	Domestic, slaughter industry	Aerobic granular sludge	1,000
Nieuwveen	Domestic (sewer in peat area)	Conventional activated sludge	1,000

4.2.2 Fractionation

Membrane fractionation was performed based on van Gijn et al. [177] with an updated method (section 0). A Mexplorer test unit (NX Filtration BV, the Netherlands), and three hollow fiber membranes (Pentair, USA) with molecular weight cut-off of 150 kDa, 10 kDa, and 1 kDa were used to separate EfOM into four size ranges: 0.45 µm -150 kDa (F1), 100-10 kDa (F2), between 10 and 1 kDa (F3) and smaller than 1 kDa (F4).

Resin fractionation was based on van Gijn et al. [177] and Imai et al. [103] with minor modifications, i.e. doubling the starting volumes to increase OM concentrations in the final fractions (section 0). EfOM was fractionated with XAD-8 resin into four resin fractions: hydrophilic compounds (HI), hydrophobic acids (HOA), hydrophobic neutrals (HON) and hydrophobic bases (HOB).

The precision of both fractionation methods was confirmed with triplicates using Bennekom effluent (Figure S4.7).

4.2.3 Dilution, spiking and ozonation

All fractions were diluted to a TOC concentration of 3.2 mg/L (to standardize the TOC concentrations) and spiked with the 17 MPs to final concentrations of approximately 1 µg/L per MP. A dilution experiment was performed to confirm that the made dilutions did not affect the MP ozonation (Figure S4.9). The MP mix in acetonitrile was spiked in a glass beaker and acetonitrile was evaporated under a gentle flow of nitrogen gas in a flow cabinet for ten minutes. 200 mL of the diluted fraction was added to the glass beaker and shaken to dissolve the MPs. The spiked fractions were split into four equal parts of 40 mL and transferred to 50 mL tubes for ozonation at four doses: 0, 0.2, 0.4, and 0.8 g O3/g TOC. Ozonation was done by spiking the samples with a concentrated ozone stock solution, as described in Hoigné et al. [88] (section 4.5.4). Actual applied ozone doses were confirmed to be close to the intended doses using the indigo method (section 4.5.4 and Table S4.3).

4.2.4 Analyses

Organic matter

Four EfOM characteristics were measured to gain insight in the types of functional groups present in the OM: absorbance (230 – 800 nm), TOC, size distribution, and fluorescence. Absorbance was measured by a microplate reader (Infinite® 200 PRO, Tecan) with 10 mm quartz cuvettes. Total organic carbon (TOC) was measured by a TNM-L TOC analyzer (Shimadzu) using a non-purgeable organic carbon method.

OM size distribution was analyzed by HPSEC on an Ultimate 3000 HPLC system (Dionex, Sunnyvale, CA, USA). Samples were centrifuged for 5 minutes at 15000 rpm prior to analysis. 10µl of the sample was injected into three TSK-Gel columns connected in series (4000-3000-2500 SuperAW; 150 × 6 mm), preceded by a TSK Super AW-L guard column (35 × 4.6 mm) (Tosoh Bioscience, Tokyo, Japan). The columns covered a molecular mass range from 0–250 kDa. Samples were eluted with 0.2 M NaNO3 at 55 °C with a flow rate of 0.6 mL/min. Eluate of the column was monitored using a Shodex RI-101 refractive index detector (Showa Denko, Tokyo, Japan) and a UV detector (254 nm). Pullulan standards (Polymer Laboratories, Palo Alto, CA, USA) were used for calibration.

Fluorescence emission excitation matrixes (FEEM) were measured in a quartz cuvette using Perkin Elmer Luminescence Spectrometer LS50B at the following scan ranges: emission 280 – 550 nm with 0.5 nm intervals and excitation 220 – 480 nm with 5 nm intervals. The slit width for both emission and excitation was 5 nm and the scanning speed was 1300 nm/min. The StaRdom package in R was used to analyses FEEM results [178]. The raw data were corrected for blanks, for the inner filter effect using the absorbance scan and Raleigh scattering bands were removed and interpolated. Parallel factor analysis (PARAFAC) was used to determine and quantify four fluorescence components (comp 1-4) in the samples (Figure S4.10).

Fluorescence fingerprints of the components were compared with the online database openfluor to gain insight in their identity (Table S4.4).

Micropollutants

MP analysis was done with an ultra-high performance liquid chromatograph (ExionLC AD-30 System) equipped with a tandem mass spectrometer (Triple QuadTM 5500+ System), both from SCIEX. MPs were separated on the LC with a phenyl-hexyl based column using water and acetonitrile-based eluents as mobile phase, both with 0.1% (v/v) formic acid. Quantification was done with calibration standards from 50 to 1000 ng/L. Matrix effect correction was done for the MPs with the strongest sensitivity to the matrix, using internal standards (Table S4.6). Obtained data was processed using SCIEX OS 1.7 software. For the calibration curves R^2 values of 0.989 ± 0.01 were obtained (Table S4.7). Detailed LC and MS settings and the R^2 values of the standard curves can be found in section 4.5.6.

Other parameters

Electrical conductivity and pH were measured using a Hach Lange HQ440d—Multi-parameter pH meter.

Statistics

Statistical significance was tested using a Mann-Whitney test in R. Correlations between MP removal and OM characteristics were investigated using single correlation coefficients calculated in excel. Two other methods were tested for this purpose (unconstrained-suppl-vars analysis in Canoco and multi component regression with the sklearn package in python), but were found unsuitable due to the large number of measured variables and limited number of samples.

4.3 Results and discussion

4.3.1 Organic matter characteristics

Effluents from five WWTPs were fractionated, the produced fractions were diluted to the same TOC concentration and used in ozonation experiments. The five WWTPs had varying TOC, COD, UV254, nitrite and bromide levels, while their pH was relatively similar (Table S4.8). This confirms the dissimilarity of the selected effluents. In spite of their different characteristics, the five effluents had similar TOC and UV254 based fraction compositions for both the size fractions, and the resin fractions (Figure 4.1 and Figure S4.11) and also similar fluorescence based compositions (Figure S4.13). For the size fractionation, the smaller fractions (F3, 1-10 and F4 <1 kDa) were dominant, while the fractions with molecules larger than 10 kDa were not significantly present in the tested WWTP effluents. HP-SEC measurements and other literature findings confirm the absence of large molecules (>10 kDa) in wastewater effluents (Figure S4.14) [179]. For the resin fractionation, HI and HOA were dominant in all effluents, HON was present to a lower extent, and HOB was not present (Figure 4.1 and Figure S4.11). Other studies found similar resin fraction composition for WWTP effluents (**Figure S4.15**).

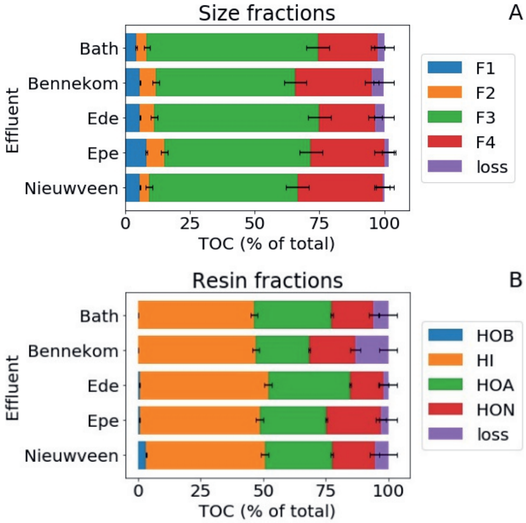

Figure 4.1 TOC based size (a) and resin (b) fraction compositions of the five WWTP effluents. Error bars represent standard deviations are based on the stability test shown in Figure S4.7

4.3.2 Micropollutant removals

The removal of the MPs was affected by the applied ozone dose and the ozone reactivity of the individual MPs (Figure 4.2). Based on the observed removals in this study and kO_3 values found in literature (Table S4.2), the MPs were divided into three reactivity groups: group 1 with highly reactive MPs ($kO_3 > 10^4$ M^{-1} s^{-1}), group 2 with moderately reactive MPs ($10^4 > kO_3 > 10^2$ M^{-1} s^{-1}) and group 3 with low reactive MPs ($10^2 > kO_3$ M^{-1} s^{-1}). Interestingly, despite the large range of kO_3 values within each group (100-fold), the differences in MP removal between the three groups are larger than the removal differences within each group. This is the same for all fractions and all WWTPs (section 4.5.8). Because there is a gap in removal between sotalol and atenolol, and mecoprop and irbesartan, while there is no gap in kO_3 values between these compounds, we conclude that there must be other compounds present in the effluent matrix that cause interference around these values. These other compounds are most likely organic compounds, and the combination of their kO_3 and concentration causes interference with removals of MPs that have kO_3 values around 10^2 and 10^4 M^{-1} s^{-1}. Additionally, the group 1 MPs were not fully removed at the ozone dose of 0.2 g O_3/g C, which indicates that the wastewater effluents also contain another OM group that causes interference with the MPs that have kO_3 values around 10^6 M^{-1} s^{-1}. This insight can be used to select relevant indicator MPs to monitor ozonation processes and to help decision makers to set removal criteria for specific (groups of) MPs.

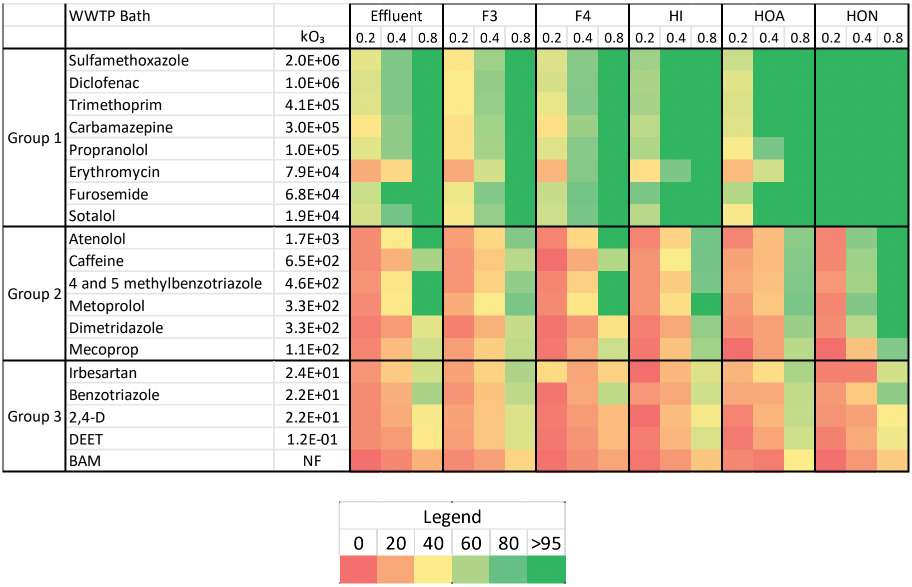

Figure 4.2 MP removal in the size and resin fractions of the effluent from Bath WWTP at three ozone doses. Removal in percentage is shown from 0 (red) to 100 (green). kO_3 values are presented in M^{-1} s^{-1}, see Table S4.2 for references. NF means not found

MP removal was similar in the five WWTP effluents for all three MP groups and at all applied ozone doses (Figure 4.3). This means that the similar fraction composition of the OM in the five effluents (see section 4.3.1) results in a similar interference with the ozonation of MPs. Because the effluents of different WWTPs have a similar OM composition, the OM quantity (TOC) is the most important factor to discriminate between the effluents. Comparing different water matrixes based on their TOC values is a reliable option for MP removal with ozonation, which is also confirmed by literature [66,176].

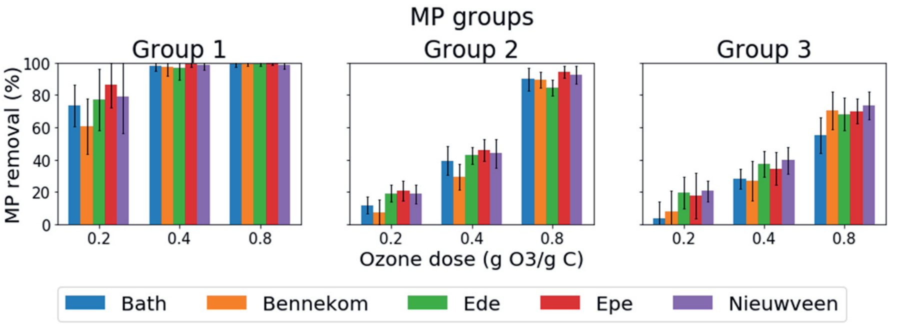

Figure 4.3 MP removal of the five unfractionated WWTP effluents. Average removals and standard deviations (error bars) are shown per MP group defined in paragraph 4.3.2

The fractions showed different interference behavior with the ozonation of MPs. Removals of group 1 and group 2 MPs were higher in the resin fraction HON than in the other fractions for all five WWTP effluents (Figure 4.4). The reason for this is that the OM in the HON has a lower interference with the ozonation of these MPs. Or more specifically, the OM groups that interfere with the removal of group 1 and group 2 MPs have a lower abundance in the HON fraction than in the other fractions. Low values for most fluorescence and absorbance characteristics and high COD were observed in the HON (Table S4.10 and Table S4.11), which indicates that this OM fraction contains saturated hydrocarbons with low abundance of (ozone reactive) aromatic moieties and low reactivity to ozone [80,180]. The resin fraction HI showed slightly lower interference than the other fractions. Statistical significance of the differences in MP removal in the resin fractions were confirmed (Table S4.12). Only for the highly reactive MPs at high ozone doses and for the low reactive MPs at low ozone doses the differences between the resin fractions were not significant. The lower interference of HON and HI could be due to differences in the ozone reactivities (kO_3 values) of the OM that is present in the fractions. A lower abundance of highly ($kO_3 > 10^6$) and moderately ($kO_3 \approx 10^4$) reactive OM would result in higher MP removal for groups 1 and 2.

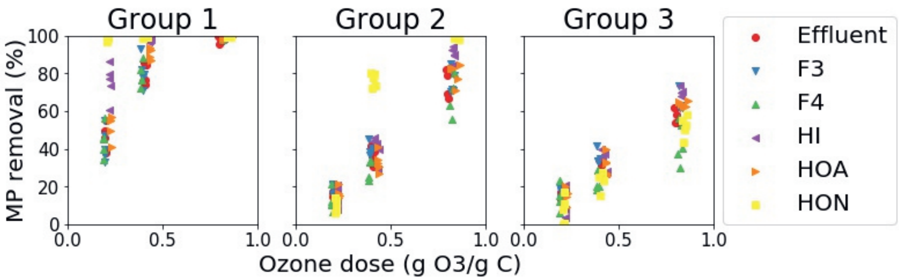

Figure 4.4 MP removal of size and resin fractions of the five WWTP effluents. Average removals are shown per MP group defined in paragraph 4.3.2

The size fractions did not show a significant difference in their interference with MP ozonation (Figure 4.4). This result is different from our previous study where the smallest size fraction (F4) showed a lower interference than the other fractions [177]. The reason for this difference could be a change in the used membranes, resulting in different pressure and cross-flow velocity conditions (section 0). This means that the results of membrane fractionation experiments are difficult to reproduce. The challenges of consistently creating size OM fractions are also underlined in other studies [181].

4.3.3 Optimizing micropollutant ozonation

Comparing effluents

The five WWTPs selected in this study showed little variation in OM compositions and in interference with MP ozonation (after TOC standardization). Note that in this study only Dutch WWTPs were tested that all make use of a biological treatment process for the removal of OM, nitrogen and phosphorus (Table 4.1). Because all studied effluents behaved similarly, we conclude that using a standardized ozone dose (g O_3/g TOC), is a reliable way to compare MP ozonation results from different WWTPs. This conclusion is confirmed by another study that compared MP ozonation at 10 Swedish WWTP [66]. Reported OM concentrations in WWTP effluents around the world vary from 3 – 23 mg /L dissolved organic carbon (DOC) (Table S4.1). Note that DOC and TOC are comparable in WWTP effluent. Therefore, applying ozonation for MP removal at different WWTPs would result in a factor 7 difference in the required ozone input depending on the OM levels in the WWTP. This underlines the importance of monitoring OM in WWTP effluents during MP ozonation.

Removing fractions in pre-treatment

The produced resin fractions showed consistent differences in their interference with MP ozonation. The HON fraction showed lower interference with the ozonation of MPs with high and moderate ozone reactivity, while the HOA and HI fractions showed the highest interference. This means that specifically reducing the HOA and HI fractions before ozonation would result in a reduction of the required ozone for the removal of MPs. Several studies suggested OM removing pre-treatment before ozonation [75,175,177]. Biological treatment would be a preferred pre-treatment because of its low energy use. On the other hand, using physical treatment such as ion exchange be more effective at removing OM with specific charge properties (e.g. HOA) but also have a higher energy demand [175,182]. Further studies are needed to find the optimal balance between a pre-treatment that can specifically remove the HOA and HI fractions, with lower energy requirements than the alternative direct ozone treatment. This will reduce the required ozone input for MP ozonation and make implementation of MP abatement technology more feasible. Additionally, further studies using non-target MS screening could be used to shed further light on the molecular compositions of the most interesting fractions [76,85]

OM characteristics for inline monitoring

A single correlation analyses between the OM characteristics of the samples and the observed MP removals was performed to investigate which OM characteristics have the most potential to predict MP removals (Table S4.13). The three characteristics with the strongest relations with MP removal (R^2 up to 0.7) were: absorbance at 300 nm (A300), fluorescence component 2 (comp 2) and the ratio of the absorbance slopes at 275-295 and 350-400 nm (SR) **Table 4.2**. A300 showed the strongest correlation for MPs with high ozone reactivity (group 1) at the low and medium ozone dose. This relation was not evident for group 1 MPs at the highest ozone dose because these MPs were removed for 100% at this dose. Note that the commonly used parameter absorbance at 254 nm (A254) showed slightly lower R^2 values (approximately 0.1 point lower for the relevant MPs) (Table S4.13). Comp 2 showed slightly lower R^2 values than A300 for most group 1 MPs, but a slightly stronger potential to predict group 2 MP removal (Table 4.2). SR also showed high R^2 values for group 2 MPs at an ozone dose of 0.4 g O_3/g C ($R^2 > 0.7$), but not for any of the other MP groups and ozone doses (Table 4.2). Contrary to the other mentioned correlations, SR showed a positive correlation, higher MP removal relating to higher SR measurements. None of the measured OM characteristics showed correlations (R^2 all below 0.4) with the removal of group 3 MPs (Table S4.13). This indicates that OM type does not affect the removal of group 3 MPs. Group 3 MPs are more likely to be removed via reaction with ·OH radicals than via direct reaction with ozone [180,183–186]. Based on literature, specifically for size fractions, it is expected that the type of OM does affect formation of ·OH [81,187]. This could mean that in this study, changes in ·OH formation were compensated by scavenging of ·OH by the OM. Further studies that combine measurements of ·OH exposure and MP removal in OM fractions are needed to confirm this hypothesis.

In contrast to the other fluorescence components, fluorescence component comp 3 showed no correlation with the MP removal which indicates that comp 3 OM does not interfere with MP ozonation (). Comp 3 most likely consists of terrestrial or microbial humic like OM (Table S4.4). The low interference of comp 3 is confirmed by the observation that only in the resin fraction with the lowest interference (HON), comp 3 is dominant (Figure S4.13). The reason for the low interference most likely is that the OM with this characteristic has a low reactivity with ozone.

Table 4.2 R^2 values of single correlations between OM and effluent characteristics and MP removal in the different samples. Three OM characteristic that showed the strongest correlations were selected (absorbance at 300 nm, fluorescence component 2 and SR), shows all values . Cells are highlighted from 0 (red) to 1 (green). Explanations of the used characteristics can be found in Table S4.10

	Ozone dose (g O_3/g C) >	0.2			0.4			0.8		
	OM characteristic >	A300	Comp 2	SR	A300	Comp 2	SR	A300	Comp 2	SR
Group 1	Sulfamethoxazole	0.67	0.61	0.41	0.42	0.43	0.18	0.07	0.17	0.03
	Diclofenac	0.72	0.71	0.50	0.34	0.38	0.13	0.01	0.01	0.05
	Trimethoprim	0.71	0.70	0.54	0.43	0.38	0.17	0.02	0.05	0.08
	Carbamazepine	0.65	0.62	0.48	0.38	0.43	0.17	0.13	0.18	0.10
	Propranolol	0.70	0.70	0.56	0.50	0.45	0.22	0.17	0.04	0.03
	Erythromycin	0.67	0.62	0.60	0.76	0.44	0.33	0.16	0.04	0.08
	Furosemide	0.63	0.56	0.31	0.29	0.40	0.11	0.22	0.28	0.35
	Sotalol	0.74	0.60	0.61	0.51	0.32	0.16	0.00	0.03	0.09
Group 2	Atenolol	0.21	0.11	0.07	0.53	0.64	0.77	0.54	0.28	0.29
	Caffeine	0.16	0.12	0.16	0.54	0.70	0.77	0.60	0.67	0.35
	4 and 5 methylbenzotriazole	0.12	0.08	0.12	0.42	0.53	0.70	0.60	0.42	0.36
	Metoprolol	0.07	0.06	0.09	0.51	0.63	0.77	0.55	0.28	0.27
	Dimetridazole	0.06	0.02	0.02	0.44	0.65	0.77	0.53	0.68	0.39
	Mecoprop	0.28	0.19	0.15	0.06	0.24	0.32	0.59	0.71	0.45
Group 3	Irbesartan	0.03	0.07	0.12	0.08	0.04	0.05	0.03	0.00	0.03
	Benzotriazole	0.05	0.02	0.08	0.10	0.03	0.02	0.26	0.35	0.18
	2,4-D	0.05	0.03	0.00	0.19	0.15	0.25	0.05	0.02	0.17
	DEET	0.19	0.18	0.21	0.13	0.12	0.31	0.05	0.01	0.14
	BAM	0.31	0.26	0.17	0.18	0.12	0.14	0.06	0.02	0.16

The OM characteristics A300 and SR showed the strongest correlations for group 1 and group 2 MPs respectively, while fluorescence component comp 2 showed strong correlations for both MP groups. Therefore, these measurements can be used to control the applied ozone dose in an ozone reactor for optimal MP removal. For application in practice, absorbance and fluorescence measurements have the advantage over TOC that it is easier to measure them inline in a WWTP.

4.4 Conclusions

The five tested unfractionated wastewater effluents all showed similar organic matter compositions and similar interferences with MP ozonation. This indicates that the quantity of organic matter (TOC) is the most relevant difference between wastewater effluents for ozonation of MPs. Additionally, three groups of MP that showed similar removal behavior within the groups were identified. The groups existed around ozone reactivities of the MPs of 10^2, 10^4 and 10^6 M^{-1} s^{-1} in all samples. This indicates the presence of three OM groups with distinct interference behavior.

The resin fractions HOA and HI showed the highest interference with MP ozonation and were abundant in all tested effluents. Removing these fractions before ozonation will likely increase the efficiency of the ozonation process. Further studies are needed to determine which pre-treatments are most suitable to remove the HOA and HI fractions.

Several organic matter characteristics correlated strong ($R^2 \approx 0.7$) with the MP removal in the different fractions, depending on the kO_3 values of the MPs. Removal of MPs with higher ozone reactivities correlated best to absorbance at 300 nm, while removal of MPs with medium ozone reactivities correlated best to SR. Fluorescence component 2 correlated relatively well to both MP groups ($R^2 \approx 0.6$). These three organic matter characteristics are useful as inline monitoring tools to continuously determine the required dose and improve the effectivity and efficiency of MP ozonation.

Acknowledgement

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4.5 Supplementary information

4.5.1 Organic matter in wastewater effluents

Table S4.1 Dissolved organic matter (DOC) concentrations in effluents of wastewater treatment plants found in other studies. Note that DOC and TOC (used in this manuscript) are comparable in wastewater effluents. CAS means conventional activated sludge, SF means sand filter, MBBR means moving bed bioreactor, NF means not found.

DOC (mg/L)	Type of process	Location	Country	Reference
5.2	CAS	South France	France	[188]
9.9-11.3	CAS	Berlin	Germany	[189]
8.8-11.2	CAS	Schoenerlinde	Germany	[190]
19 (1.5-38)	CAS	Thessaloniki	Greece	[67]
20	CAS	Panheel	Netherlands	[175]
20	NF	Daspoort	South Africa	[191]
6.6-8.6	CAS	Madrid	Spain	[192]
19-23.4	CAS	El ejido	Spain	[193]
9.2-11.8	CAS	Landskorna	Sweden	[194]
7	NF	Kallby	Sweden	[194]
5.2	NF	Björnstorp	Sweden	[194]
8.1	NF	Öresundsverket	Sweden	[194]
13.7	NF	Sjölunda	Sweden	[194]
8.4	NF	Nykvarnsverket	Sweden	[194]
13.9	CAS + SF	Sterno	Sweden	[66]
10.6	CAS	Sjohog	Sweden	[66]
11.4	CAS + SF	Nyvangsverket	Sweden	[66]
9.5	CAS	Torekov	Sweden	[66]
11	CAS + MBBR	Sjolunda	Sweden	[66]
9.8	CAS	Kallby	Sweden	[66]
13.1	CAS	Ellinge	Sweden	[66]
8.4	CAS + SF	Kavlinge	Sweden	[66]
13.3	CAS	Svedala	Sweden	[66]
11.4	CAS	Vastra Stranden	Sweden	[66]
3.5-6	CAS + SF	Neugut	Switzerland	[45]
7.2-8.2	CAS	Kloten- Opfikon	Switzerland	[195]

4.5.2 Used micropollutants

Table S4.2 Used micropollutants with their molecular formulas, kO_3 values and k·OH values from literature. NF means not found

Micropollutant	Mol. formula	kO_3 (pH=7)	Reference	k·OH	Reference
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	5.50E+05	[116]	8.50E+09	[99]
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	6.80E+05	[62]	7.50E+09	[99]
Trimethoprim	$C_{14}H_{18}N_4O_3$	2.70E+05	[62]	6.90E+09	[99]
Carbamazepine	$C_{15}H_{12}N_2O$	3.00E+05	[196]	8.80E+09	[99]
Propranolol	$C_{16}H_{21}NO_2$	1.00E+05	[197]	1.00E+10	[198]
Erythromycin	$C_{37}H_{67}NO_{13}$	7.9E+04	[116]	5.00E+09	[116]
Furosemide	$C_{12}H_{11}ClN_2O_5S$	6.80E+04	[116]	3.40E+09	[199]
Sotalol	$C_{12}H_{20}N_2O_3S$	1.90E+04	[197]	1.00E+10	[116]
Atenolol	$C_{14}H_{22}N_2O_3$	1.70E+03	[99]	8.00E+09	[99]
Caffeine	$C_8H_{10}N_4O_2$	6.50E+02	[138]	5.90-8.50E+09	[200]
4 and 5-methyl benzotriazole	$C_7H_7N_3$	7.80E+02	[116]	8.60E+09	[116]
Metoprolol	$C_{15}H_{25}NO_3$	2.49E+03	[201]	7.3E+09	[198]
Dimetridazole	$C_5H_7N_3O_2$	3.30E+02	[202]	7.30E+09	[203]
Mecoprop	$C_{10}H_{11}ClO_3$	1.11E+02	[45]	NF	
Benzotriazole	$C_6H_5N_3$	2.00E+01	[185]	9.00E+12	[203]
Irbesartan	$C_{25}H_{28}N_6O$	2.20E+01	[45]	7.60E+09	[116]
2,4-D	$C_8H_6Cl_2O_3$	2.19E+01	[113]	NF	
DEET	$C_{12}H_{17}NO$	1.23E-01	[204]	8.40E+12	[203]
BAM	$C_7H_5Cl_2NO$	NF		NF	

4.5.3 Fractionation procedures

Membrane fractionation

A circulating setup containing a Mexplorer test unit (NX Filtration BV, the Netherlands) and three hollow fibre membranes (Pentair, USA) were used to separate fractions by size (Figure S4.5). The Mexplorer test unit's flow meter was removed to decrease volume loss. Before using the setup for a new effluent, the filtration system was rinsed with demi water three times. In the circulation process, the membrane was replaced in the following order:

1. The 150 kDa membrane's permeated liquid was collected until there was no permeate flow. Approximately 200 ml of water was used to backwash the system, mixed with the retentate liquid and collected as F1.
2. The 150 kDa membrane was replaced with the 10 kDa membrane, and the above process was repeated with the permeated liquid from the 150 kDa membrane. The retentate liquid and backwash water in the second step was collected as F2.
3. The 10 kDa membrane was replaced with the 1 kDa membrane, the permeate liquid of the 1 kDa membrane was collected as F4. The retentate liquid and backwash water were collected as F3.

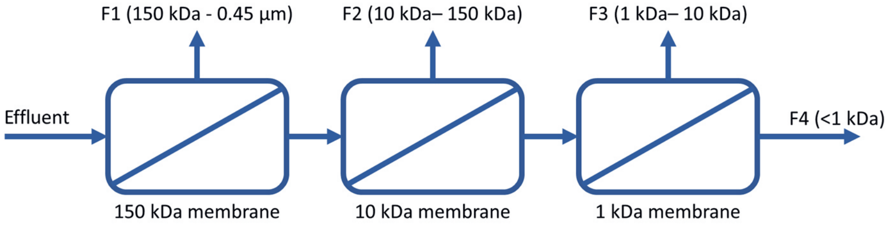


Figure S4.5 Schematic overview of the membrane fractionation process

Resin fractionation

Effluents were separated by XAD-8 resin into four resin fractions: hydrophilic compounds (HI), hydrophobic acids (HOA), hydrophobic neutrals (HON) and hydrophobic bases (HOB). This method was based on van Gijn et al. [177] and Imai et al. [103], and was adjusted to obtain higher organic matter concentrations in the fractions (by increasing the starting volume in step 1 below). Prior to fractionation, 6 g of XAD-8 resin (wet weight) was washed subsequently with 1.5 L of demi water, 0.1 M KOH and 0.1 M HCl. The washed resin was placed in a plastic 60 mL syringe with a membrane holder and flushed with 50 mL of demi water which was collected as a blank.

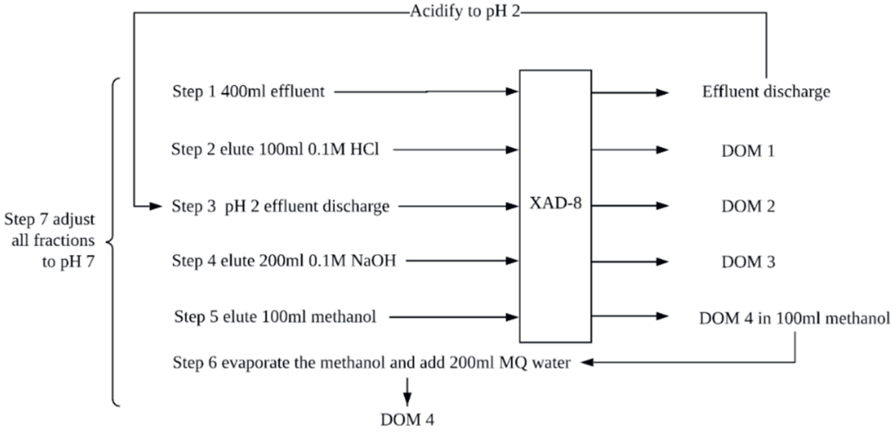


Figure S4.6 Schematic overview of the resin fractionation

The resin fractionation procedure contains 7 steps (Figure S4.6):

1. 400 mL of 0.45 µm filtered effluent was eluted through the resin and collected as first elute for later use.
2. The resin was eluted with 100 mL 0.1M HCl and the effluent was collected as second elute (HOB).
3. The effluent discharge was adjusted to pH 2, eluted through the resin again and collected (HI).
4. The resin was eluted with 200 mL of 0.1M KOH and collected (HOA).
5. The resin was eluted with 100 mL of methanol.
6. The methanol was evaporated in a flow cabinet, and the residual matter was dissolved in 200 mL MQ water (HON).
7. All resin fractions were adjusted to pH 7 using 1 M HCl or 1 M KOH.

After fractionation, TOC and UV 254 were measured for DOM fractions 1–4 and the blank samples. TOC and UV 254 of each resin fraction were calculated as follows:

$$\text{HOB} = (\text{DOM 1} - \text{BL}) * (\text{collected volume}/\text{sample volume}) \quad (1)$$

$$\text{HI} = (\text{DOM 2} - 2*\text{BL}) * (\text{collected volume}/\text{sample volume}) \quad (2)$$

$$\text{HOA} = (\text{DOM 3} - \text{BL}) * (\text{collected volume}/\text{sample volume}) \quad (3)$$

$$\text{HON} = (\text{DOM 4} - \text{BL}) * (\text{collected volume}/\text{sample volume}) \quad (4)$$

, where the collected volume refers to the final collected fractions volume in ml (after dilutions), the sample volume refers to the initial effluent volume used for resin fractionation in ml and DOM 1-4 refers to the absorbance (cm^{-1}) or TOC (mg/L) of the collected DOMs (Figure S4.6).

Stability test

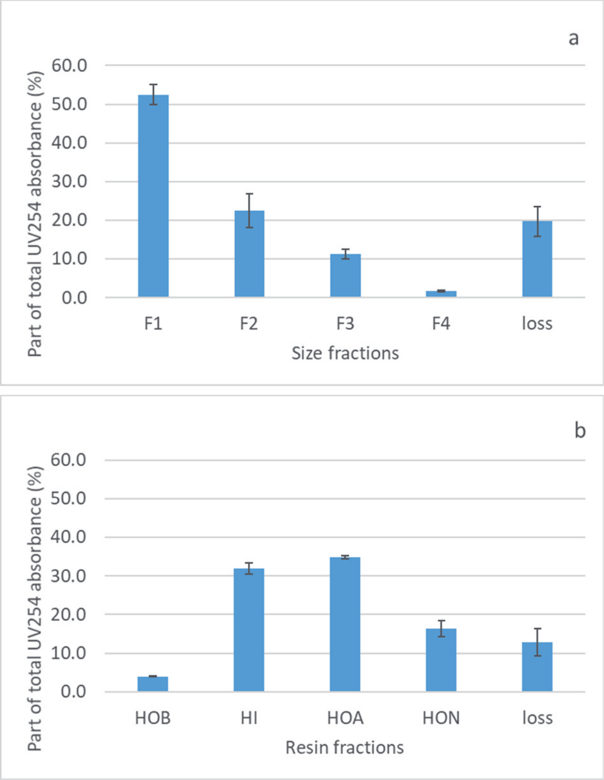


Figure S4.7 UV254 based composition of the size (a) and resin (b) fractions from the triplicate with Bennekom WWTP effluent. Error bars represent standard deviations

4.5.4 Ozonation procedure

Ozone stock

The ozone stock was produced in a 1L glass Schott bottle with MQ water (Figure S4.8). In this setup, an ozone generator (1) was used to transform pure oxygen into ozone. The ozone sparged through the MQ water (kept at 4 °C in a fridge) (5) with an aeration diffuser and magnetic stirrers. Off gas passed through a catalyst (7), which converted ozone back to oxygen before releasing it into the ventilation system. The ozone stock solution was taken from the tap (6).

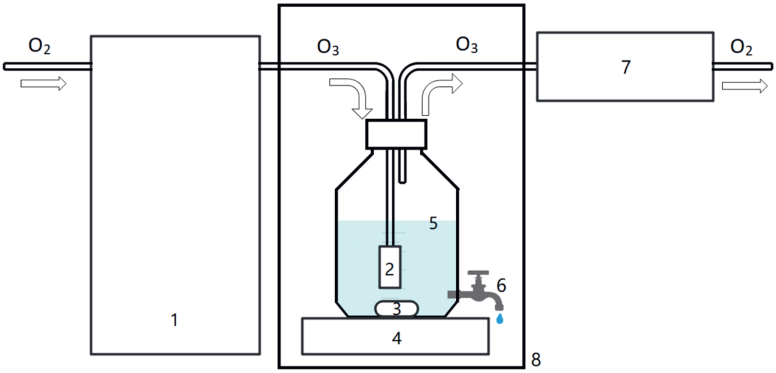


Figure S4.8 Setup of the ozone stock. The arrows indicate the flow of the gas. 1 = ozone generator; 2 = aeration diffuser; 3 = magnetic stir bar; 4 = magnetic stirrer; 5 = Schott flask containing ozone stock, 6 = glass tap; 7 = ozone catalyst which reacts O3 to O2; 8 = refrigerator

Ozone concentration measurement (indigo)

Ozone concentration in the ozone stock was measured using the indigo method, based on Hoigné et al. [88]. Potassium indigo trisulfonate reacts quickly with ozone and decolorizes. The absorbance changes of potassium indigo trisulfonate at 600 nm were measured by spectrophotometer (DR3900, Hach) to calculate the ozone concentration.

First, 1 mL indigo stock (1mM) and 0.5 mL H_3PO_4 stock (1.5 mM) was added in a 25 mL volumetric flask. Then, 1 mL of ozone stock was added in the flask and shaken briefly to let the ozone fully react with the indigo. Finally, the volumetric flask was filled up to 25 mL with Milli-Q water and the absorbance at 600 nm was measured. Quantification was done using a one point calibration. The ozone concentration was calculated using the following equation:

$$[O_3] = \frac{V_f \Delta A}{f b V_t}$$

, where Vf refers to the volume of the volumetric flask, ΔA refers to the difference in absorbance of the reference and the sample, f refers to sensitivity coefficient (ε/O3 in L/cm/mg), b refers to path length of the cuvette in cm and Vt refers to the volume of the ozone stock added to the volumetric flask

The sensitivity coefficient (f) was calculated using the following equation:

$$f = \frac{Abs}{b * [indigo\ stock] * M\ O_3}$$

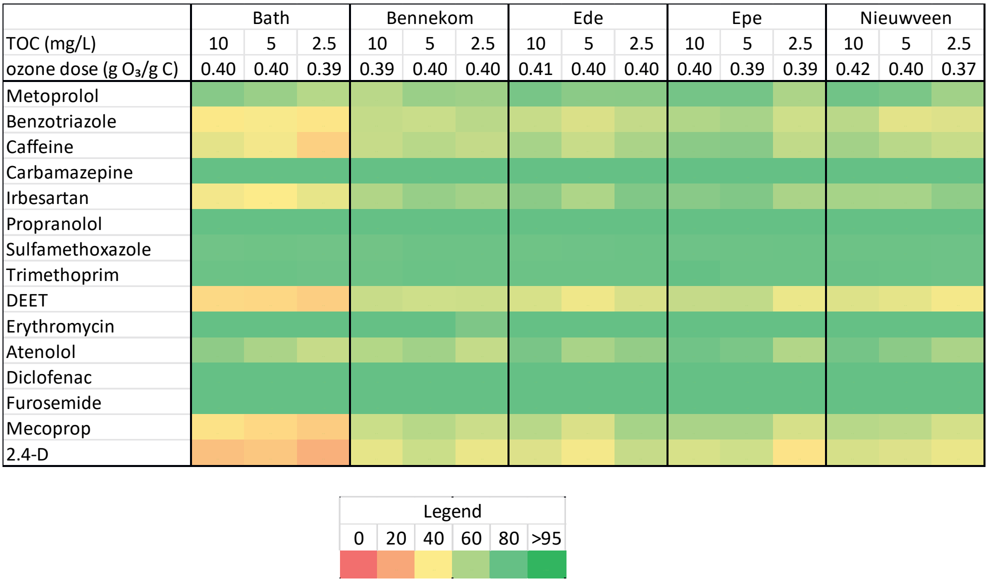
, where Abs refers to the absorbance of the calibration standard, b refers to path length of the cuvette in cm, [indigo stock] refers to concentration of indigo stock solution in mg/L and MO_3 refers to molar mass of ozone, 48g/mol.

Measured ozone doses

Table S4.3 Measured ozone doses (g O_3/g C) in al fractions and effluents

	Intended dose	Bath	Bennekom	Ede	Epe	Nieuwveen
Effluent	0.8	0.81	0.81	0.80	0.80	0.79
Effluent	0.4	0.41	0.41	0.40	0.40	0.40
Effluent	0.2	0.20	0.20	0.20	0.20	0.19
F3	0.8	0.83	0.83	0.82	0.82	0.81
F3	0.4	0.40	0.40	0.39	0.39	0.39
F3	0.2	0.20	0.19	0.19	0.19	0.19
F4	0.8	0.83	0.83	0.82	0.82	0.81
F4	0.4	0.39	0.39	0.39	0.39	0.38
F4	0.2	0.19	0.19	0.19	0.19	0.19
HI	0.8	0.84	0.83	0.83	0.83	0.82
HI	0.4	0.44	0.43	0.42	0.42	0.41
HI	0.2	0.22	0.22	0.22	0.22	0.22
HOA	0.8	0.87	0.85	0.84	0.83	0.81
HOA	0.4	0.44	0.44	0.43	0.43	0.43
HOA	0.2	0.23	0.23	0.23	0.22	0.22
HON	0.8	0.86	0.85	0.85	0.84	0.84
HON	0.4	0.42	0.42	0.41	0.40	0.39
HON	0.2	0.22	0.21	0.21	0.21	0.21

Dilution experiment



	Bath			Bennekom			Ede			Epe			Nieuwveen		
TOC (mg/L)	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5	10	5	2.5
ozone dose (g O_3/g C)	0.40	0.40	0.39	0.39	0.40	0.40	0.41	0.40	0.40	0.40	0.39	0.39	0.42	0.40	0.37
Metoprolol															
Benzotriazole															
Caffeine															
Carbamazepine															
Irbesartan															
Propranolol															
Sulfamethoxazole															
Trimethoprim															
DEET															
Erythromycin															
Atenolol															
Diclofenac															
Furosemide															
Mecoprop															
2.4-D															

Legend					
0	20	40	60	80	>95
🟥	🟧	🟨	🟩	🟩	🟩

Figure S4.9 MP removal in the dilution experiment. The five effluents were diluted to three different TOC concentrations (10, 5 and 2.5 mg/L) and ozonation at the same ozone dose (0.4 g O_3/g C). Removal in percentage is shown from 0 (red) to 100 (green)

4.5.5 PARAFAC method

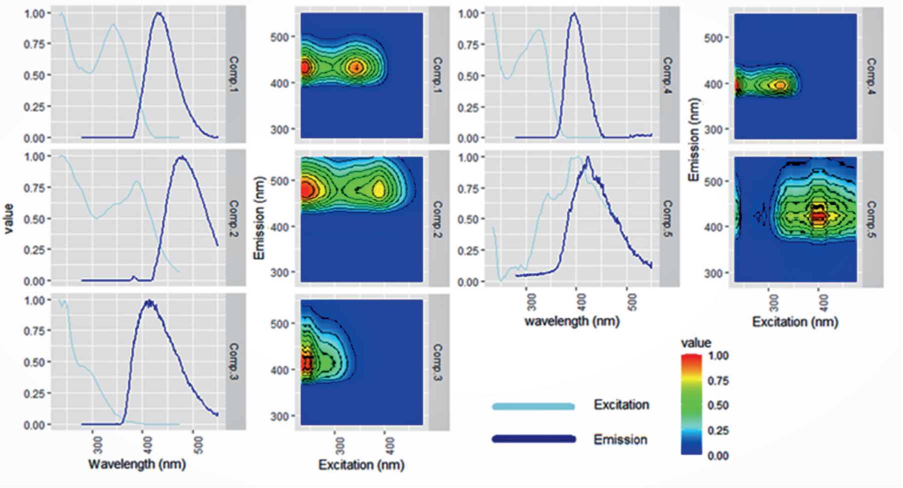


Figure S4.10 Fluorescence fingerprints of the PARAFAC components comp 1-5, note that comp 5 was not significantly present in any of the samples and is therefore not included in the discussion

Fit Information:

- SSE = 56695
- $R^2 = 0.983$
- GCV = 0.0280
- EDF = 2605

Table S4.4 Sources for identity of PARAFAC components based on openfluor database (https://openfluor.lablicate.com/)

Component	Ex/Em wavelengths*	Score	Component identity	References
C1	240/435 nm & 344/435 nm	0.99	Terrestrial humic-like	[205] (C2)
		0.97	Terrestrial humic-like (similar to syringaldehyde)	[206] (C1)
		0.97	Terrestrial fulvic acids	[207] (C2)
		0.96	Terrestrial humic-like compounds,	[208] (C1)
C2	240/475 nm & 390/475 nm	0.99	High-molecular terrestrial compounds	[209] (C3)
		0.98	Terrestrial humic-like	[210] (C2)
		0.98	Terrestrial humic-like	[206] (C3)
		0.98	Humic-like	[211] (C4)
C3	245/415 nm	0.99	Microbial humic-like	[210] (C3)
		0.98	Terrestrial delivered Reprocessed OM	[82] (C2)
		0.97	terrestrial humic acid, found in agricultural-influenced streams and estuaries	[212] (C3)
C4	325/400 nm	0.97	Humic-like; low molecular weight	[213] (C2)

4.5.6 LC-MS method

Components were quantified against a calibration curve in the range of 50 to 1000 ng/l and processed with SCIEX OS 1.7 software. During sampling, influent and effluent samples were pre-mixed with acetonitrile to a concentration of 2.5% (v/v) to keep the less polar compounds in solution during storage in a freezer. Prior to analysis, the samples were thawed and centrifuged at 15000 rpm for 10 minutes, to remove any particles before injection. 975 µL supernatant was mixed with 25 µL internal standard (ISTD) mix in acetonitrile (Table S4.6) to achieve final ISTD concentrations of 500 ng/L.

LC method

The ExionLC AD-30 was configurated as a low pressure gradient system equipped with autosampler (injection volume 25 µL) and column oven (set to 35°C). Eluent A (UPLC-MS quality water with 0.1% formic acid in volume) and eluent B (UPLC-MS quality acetonitrile with 0.1% formic acid in volume) were used to apply the following gradient, where T0 is the time of injection: 0-0.5 min constant 5% B; 0.5-3.5 min. linear increase to 80% B; 3.5-7.5 min. constant 80% B; 7.5-8.5 min. linear decrease to 5% B; 8.5-12.4 min. constant 5% B. The flow through the column was kept constant at 0.4 ml/min. The used analytical column was a Waters Acquity UPLC CSH Phenyl-Hexyl 1.7 µm 2.1*150 mm; the guard column Security guard ULTRA UHPLC phenyl 2.1 mm ID was used to delay the deterioration of the stationary phase. The autosampler temperature was kept at 5°C to prevent sample degradation; injection volume was 25 µl.

MS method

After chromatographic separation, components were detected through triple quad mass spectrometry. Ionisation took place, both in positive and negative mode, by Electro Spray Ionisation (ESI): The IonSpray voltage was set at 1500 V for positive ionisation and -2000 V for negative ionisation and a nebulizer gas esd set at 50 psi. For solvent evaporation, a heater gas at fixed temperature of 725°C was set at 60 psi. In order to prevent neutrals from entering the orifice and contaminating the ion optics, a curtain gas was set at 20 psi. To minimize solvent cluster entering the vacuum chamber, de-clustering potential (DP) was applied at the orifice; this and other component dependent parameters can be found in Table S4.5.

The Triple QuadTM 5500+ System was operated in Scheduled Mass Reaction Monitoring mode (S-MRM): in this mode, specific transitions are monitored only at the retention times in which the related target components are expected, improving sensibility. In Table S4.5 all the component specific parameters are listed; here follows a short description of each parameter:

- Target ion (m/z): mass over charge ratio of the ion formed after ionisation of the target molecule
- Fragment ion (m/z): mass over charge ratio of the ion formed after fragmentation in the collision cell
- De-clustering potential (DP): voltage applied at the orifice to minimize solvent cluster
- Entrance Potential (EP): voltage difference between the so called Q0 region and the ground, to focus ions in the first quadrupole

- Collision Energy (CE): voltage difference between Q0 region and the collision cell, to accelerate the ions against the collision gas for fragmentation
- Collision gas exit potential (CXP): voltage applied to guide the fragmented ions in the last quadrupole (Q3)
- Collision activated Gas (CAD): pressure of the gas in the collision cell for ion fragmentation
- Retention Time (RT): centre of a 30 second time window in which the target component was expected to elute from the LC and the specific transition was monitored.

Table S4.5 Component dependent MS parameters

Component name	Cas number	Fragment ion (m/z)	Target ion (m/z)	RT (min)	DP	EP	CE	C1XP
Positively ionized components								
4-methylbenzotriazole and 5-methylbenzotriazole (not separated)	29878-31-7	134.1	106	4.45	100	10	25	12
	136-85-6	134.1	79	4.45	100	10	12	10
Atenolol	29122-68-7	267.1	145.1	2.3	96	10	37	18
	29122-68-7	267.1	190.1	2.3	96	10	27	10
Benzotriazole	95-14-7	120.0	65	4.15	90	10	31	8
	95-14-7	120.0	92	4.15	90	10	25	12
Caffeine	58-08-2	195.0	138	4.08	66	10	27	12
	58-08-2	195.0	110.1	4.08	66	10	33	14
Carbamazepine	298-46-4	237.0	194.1	4.9	95	10	29	10
	298-46-4	237.0	193.1	4.9	95	10	47	14
Dimetridazole	551-92-8	142.0	96	3.7	70	12	23	11
	551-92-8	142.0	95	3.7	70	12	34	10
	551-92-8	142.0	81	3.7	70	12	36	12
Erythromycin	114-07-8	734.3	158.2	4.76	130	10	39	10
	114-07-8	734.3	576.3	4.76	130	10	29	26
Irbesartan	138402-11-6	429.1	207.1	5.22	95	10	35	10
	138402-11-6	429.1	195.1	5.23	95	10	31	10
Metoprolol (tartrate)	56392-17-7	268.1	116.1	4.36	95	10	27	14
	56392-17-7	268.1	191.1	4.36	95	10	27	10
Propranolol (hydrochloride)	318-98-9	260.1	116.1	4.87	100	10	25	12
	318-98-9	260.1	183	4.87	100	10	27	16
Sotalol	959-24-0	273.1	255.2	2.47	94	10	18	18
	959-24-0	273.1	213.3	2.47	94	10	26	12
	959-24-0	273.1	133.3	2.47	94	10	36	10
Sulfamethoxazole	723-46-6	254.0	156	4.61	76	10	23	16
	723-46-6	254.0	92	4.61	76	10	39	10
	723-46-6	254.0	108.1	4.68	76	10	35	10

Trimethoprim	738-70-5	291.0	230.1	4.18	121	10	33	12
	738-70-5	291.0	261.1	4.18	121	10	35	18
Positively ionized Internal standard								
Benzotriazole D-4		124.0	69.2	4.11	151	10	33	10
		124.0	96.2	4.11	151	10	27	10
Caffeine D-9		204.1	144.1	4.46	66	10	53	8
		204.1	89.3	4.46	66	10	39	8
Carbamazepine D-10		247.1	204.1	4.93	161	10	29	6
		247.1	202.1	4.93	161	10	51	16
Irbesartan D-6		435.2	213.2	5.22	166	10	35	16
		435.2	195.2	5.22	166	10	33	14
Propranolol (hydrochloride) D-7		267.2	189.2	4.37	16	10	27	18
		267.2	116.2	4.37	16	10	29	16
Sulfamethoxazole D-4		258.0	160.2	4.68	86	10	23	16
		258.0	96.1	4.68	86	10	35	10
		258.0	112.1	4.68	86	10	35	12
Negatively ionized components								
2,4-D (dichlorophenoxyacetic acid)	94-75-7	218.9	161	5.2	-55	-10	-20	-13
	94-75-7	218.9	125	5.2	-55	-10	-38	-11
Diclofenac	15307-86-5	293.9	249.9	5.68	-65	-10	-16	-21
	15307-86-5	293.9	34.9	5.68	-65	-10	-58	-17
Furosemide	54-31-9	328.9	285	4.92	-110	-10	-22	-22
	54-31-9	328.9	204.9	4.92	-110	-10	-34	-34
Mecoprop (MCPP)	93-65-2	212.8	141	5.35	-65	-10	-20	-14
	93-65-2	212.8	143	5.35	-65	-10	-20	-14
Negatively ionized internal standard								
Diclofenac D-4		297.9	254	5.68	-60	-10	-16	-16
		297.9	218	5.68	-60	-10	-28	-28
Furosemide D-5		333.9	290.2	4.91	-110	-10	-22	-22
		333.9	209.9	4.91	-110	-10	-34	-34
Mecoprop (MCPP) D-6	93-65-2	218.9	147.1	5.32	-55	-10	-22	-22
	93-65-2	218.9	146.5	5.32	-55	-10	-22	-22

The concentrations of the components for which in Table S4.5 a deuterated internal standard is available, were corrected for internal standard response. Each internal standard was present both in calibration standards and in samples at a concentration of 500 ng/l and as response factors in the calibration line. The ratio between the response of the target component and the one of the respective deuterated internal standard was used to quantify the measured peak areas. This allows to minimize the effect of matrix on quantification (so called ion suppression or ion enhancement).

Table S4.6 Used internal standards (ISTDs) for the quantification of the MPs

Micropollutant	ISTD
Benzotriazole	Benzotriazole D4
Carbamazepine	Carbamazepine D10
Irbesartan	Ibersartan D6
Propranolol	Propranolol D7
Sulfamethoxazole	Sulfamethoxazole D4
Trimethoprim	Carbamazepine D10
Diclofenac	Diclofenac D4
Caffeine	Caffeine D9
Furosemide	Furosemide D5
Mecoprop	Mecoprop D6

Table S4.7 R^2 values of the MP calibration curves of the four measurement days

Micropollutant	1	2	3	4
Metoprolol	0.997	0.995	0.988	0.986
Benzotriazole	0.998	0.996	0.975	0.992
Caffeine	0.999	0.999	0.990	0.988
Carbamazepine	0.991	0.976	0.976	0.995
Irbesartan	0.968	0.986	0.992	0.955
Propranolol	0.996	0.998	0.986	0.989
Sulfamethoxazole	0.999	0.995	0.993	0.984
Trimethoprim	0.999	0.999	0.986	0.985
DEET	0.995	0.998	0.983	0.986
Erythromycin	0.978	0.975	0.979	0.970
Atenolol	0.998	0.999	0.990	0.988
4 and 5 methylbenzotriazole	0.998	0.999	0.986	0.985
BAM	0.999	0.998	0.985	0.983
Dimetridazole	1.000	0.999	0.985	0.988
Sotalol	0.995	0.999	0.987	0.986
Diclofenac	0.979	0.995	0.947	0.972
Furosemide	0.988	0.991	0.989	0.987
Mecoprop	0.994	0.997	0.998	0.988
2,4-D	0.999	0.998	0.986	0.976

4.5.7 Organic matter characteristics

Effluent characteristics

Table S4.8 Characteristics of the five WWTP effluents. NA means not detected.

	TOC ($mg \cdot L^{-1}$)	COD ($mg \cdot L^{-1}$)	UV 254 (cm^{-1})	SUVA ($L \cdot mg^{-1} \cdot m^{-1}$)	pH	Nitrite (mg/L)	Bromide (mg/L)
Bath	12.1	43.5	0.28	2.31	8.14	NA	1.40
Bennekom	9.9	30.4	0.23	2.32	7.87	0.330	NA
Ede	19.1	60.3	0.54	2.83	7.58	0.968	0.36
Epe	10.1	34.1	0.20	1.98	7.48	0.490	0.08
Nieuwveen	12.9	42.7	0.40	3.10	7.78	0.341	0.20

Fraction compositions

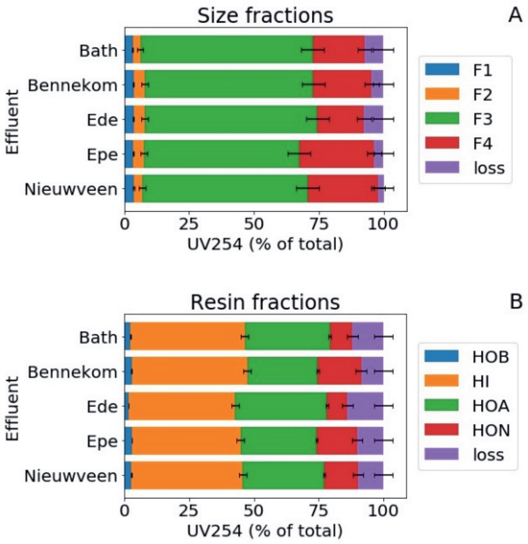


Figure S4.11 UV254 based size (A) and resin (B) fraction compositions of the five WWTP effluents

Fluorescence compositions

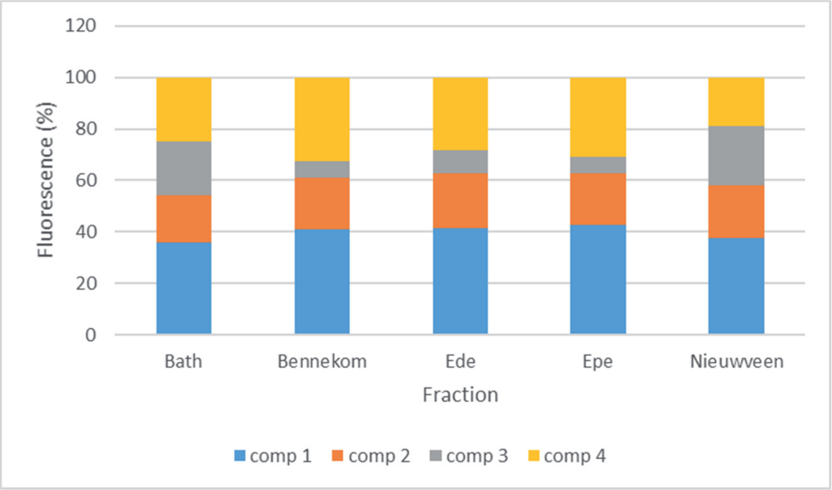


Figure S4.12 PARAFAC component composition of the five effluents

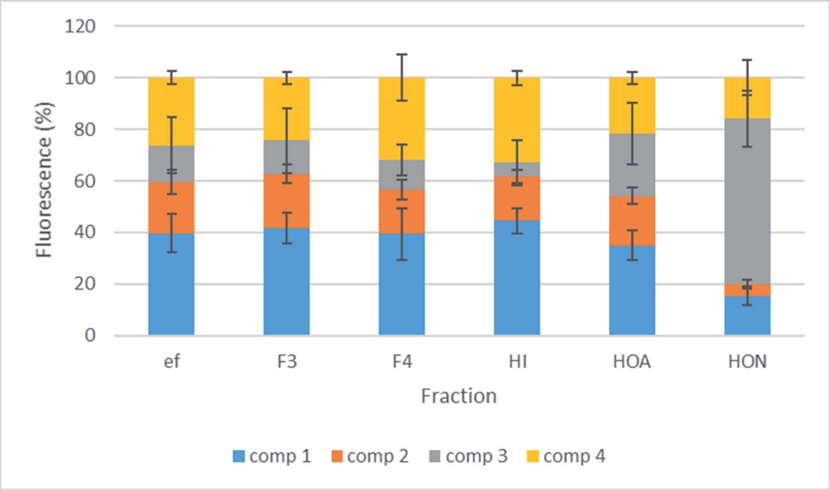


Figure S4.13 Fluorescence component compositions of the size and resin fractions. Averages are taking from the five used WWTPs, error bars represent standard deviations

HPSEC measurements

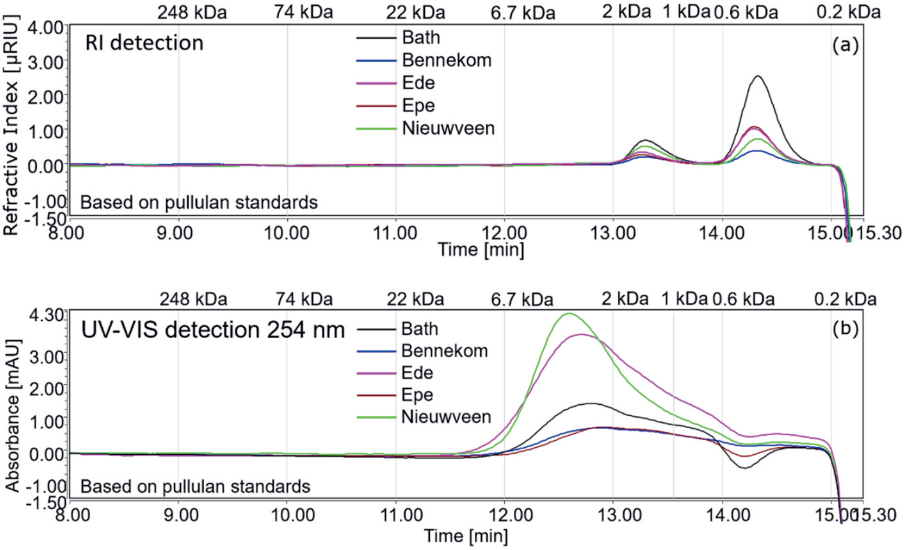


Figure S4.14 High-pressure size exclusion chromatogram of undiluted EfOM from 5 WWTPs, a: refractive index detector's results; b: UV absorbance detector's results at 254nm

Literature based resin fraction compositions

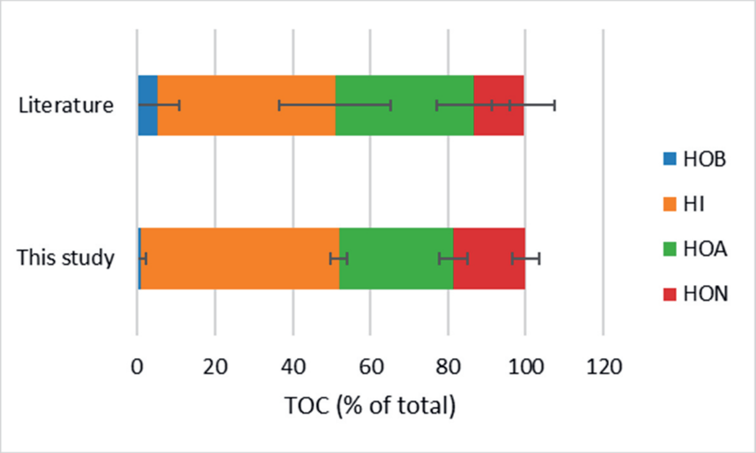


Figure S4.15 TOC composition of resin fractions in this study and in literature. Literature used to make the averages is presented in Table S4.9 below. Note that loss was not included in this figure because the loss was not included in the found literature

Table S4.9 Overview of resin fraction proportions found in literature

Proportion (%)				
HI	**HOA**	**HON**	**HOB**	**Reference**
57.0	31.0	-	21.0	[214]
36.0	50.0	10.0	4.0	[215]
27.2	47.7	19.8	-	[216]
47.0	39.0	9.0	5.0	[217]
31.0	34.0	11.0	-	[218]
61.0	28.0	8.0	3.0	[103]
57.0	28.0	13.0	2.0	[103]
41.6	26.0	24.4	1.7	[219]
30.0	23.0	14.0	-	[218]
-	38.0	8.0	1.0	[220]
64.0	32.0	4.0	2.0	[217]
45.0	43.0	8.0	4.0	[221]
68.0	21.0	6.0	5.0	[221]
53.0	43.0	-	-	[222]
45.0	50.0	-	-	[222]
22.0	37.0	32.0	9.0	[223]

Overall characteristics

Table S4.10 Explanations of all the OM characteristics

Characteristic	Meaning	Calculation
Comp1	First component from the PARAFAC analysis, related to terrestrial humic-like compounds	PARAFAC (see Figure S4.10 for fingerprint and sources)
Comp2	Second component from the PARAFAC analysis, related to high molecular weight terrestrial humic-like compounds	
Comp3	Third component from the PARAFAC analysis, related to microbial humic-like compounds	
Comp4	Fourth component from the PARAFAC analysis, related to low molecular weight humic-like compounds	
Fmax	Sum of all fluorescence components	
bix	Ratio of albuminoid and biological components	em380 divided by em430 at ex = 310
b	Protein-like (tyrosine-like)	ex/em ranges 225–237/309–321 and 275/310
t	Protein-like (tryptophan-like)	ex/em ranges 225–237/340–381 and 275/340
a	Humic-like	ex/em ranges 237–260/400–500
m	Marine humic-like	ex/em ranges 312/380–420
c	Humic-like	ex/em ranges 300–370/400–500
fi	Distinguish DOM sources from terrestrial or microbial sources	em450 divided by em500 at ex370
hix	Degree of humification of dissolved organic matter	em 435-480 divided by em 300–345 at ex254
A254	Absorbance at 254 nm	
A300	Absorbance at 300 nm	
E2_E3	Characterization of the humification. When E2/E3 < 3.5, humic acid > fulvic acid. When E2/E3 > 3.5, fulvic acid > humic acid	abs250 divided by abs365
E4_E6	Degree of polymerization of the carbon skeleton of benzene rings	abs465 divided by abs665
S275_295	The ratio of fulvic acid to humic acid	Exponential spectral slope from 275-295 nm
S350_400	Spectral slope from 350-400 nm	Exponential spectral slope from 350-400 nm
S300_700	Spectral slope from 300-700 nm	Exponential spectral slope from 300-700 nm

SR	Related to molecular weight and photochemical degradation of OM. SR < 1 indicates terrestrial OM. SR > 1.5 indicates oceanic and photodegraded terrestrial OM	Spectral slope 275-295 divided by spectral slope 350-400
COD	Chemical oxygen demand (mg/L)	
pH	pH	
Conductivity	Conductivity (µs/cm)	
Ozone dose	Measured ozone dose (g O_3/g C)	

Table S4.11 Overview of all absorbance and fluorescence characteristics in the unfractionated effluents, size fractions and resin fractions. Averages and standard errors are calculated from the five effluents. Colors for the average indicate the highest value (green) and lowest value (red) for that specific characteristic

		Comp1	Comp2	Comp3	Comp4	Fmax	bix	fi	hix	b	t	a	m	c	A254	A300	E2_E3	E4_E6	S275_295	S350_400	S300_700	SR	COD	pH	Conductivity
Average	Effluent	0.53	0.27	0.19	0.35	1.33	0.44	1.97	0.9988	0.0004	0.0011	0.63	0.53	0.57	18.09	10.47	5.12	4.40	0.014	0.018	0.016	0.80	10.6	7.77	313
	F3	0.49	0.25	0.15	0.29	1.18	0.38	1.99	0.9987	0.0006	0.0006	0.57	0.47	0.53	19.48	11.31	5.01	15.28	0.014	0.017	0.016	0.79	10.1	7.32	81
	F4	0.69	0.30	0.20	0.56	1.74	0.53	2.13	0.9989	0.0007	0.0008	0.77	0.72	0.73	15.74	9.10	6.26	-0.31	0.015	0.020	0.021	0.76	10.5	7.82	893
	HI	0.46	0.18	0.06	0.34	1.04	0.47	2.25	0.9988	0.0004	0.0008	0.46	0.45	0.49	12.71	6.88	6.37	2.90	0.016	0.021	0.018	0.77	10.5	7.00	1335
	HOA	0.40	0.22	0.28	0.25	1.16	0.40	1.77	0.9987	0.0004	0.0006	0.58	0.46	0.43	16.68	9.23	5.40	-6.15	0.015	0.018	0.016	0.82	13.1	7.15	7612
	HON	0.05	0.02	0.23	0.06	0.35	0.57	2.14	0.9980	0.0002	0.0003	0.18	0.13	0.11	7.70	4.09	4.32	3.06	0.015	0.011	0.011	1.41	17.2	7.19	336
Std error (%)	Effluent	19	23	77	10	21	14	8	0.018	73	45	30	20	16	16	14	21	158	15	16	17	11	6	3.3	45
	F3	14	17	96	10	19	13	7	0.029	108	86	27	18	12	9	9	23	160	17	13	15	10	13	3.3	37
	F4	25	23	52	28	21	8	10	0.012	121	71	19	23	26	19	20	15	1316	7	11	21	13	9	2.0	36
	HI	11	14	160	9	14	16	10	0.035	16	61	22	13	9	17	12	22	103	13	13	16	9	13	3.8	35
	HOA	16	17	50	11	18	14	8	0.043	99	59	26	17	14	15	16	20	381	13	12	11	8	12	2.8	30
	HON	24	41	17	43	22	13	17	0.064	139	90	22	23	15	16	18	18	47	13	17	28	8	6	4.1	11

4.5.8 Micropollutant removals

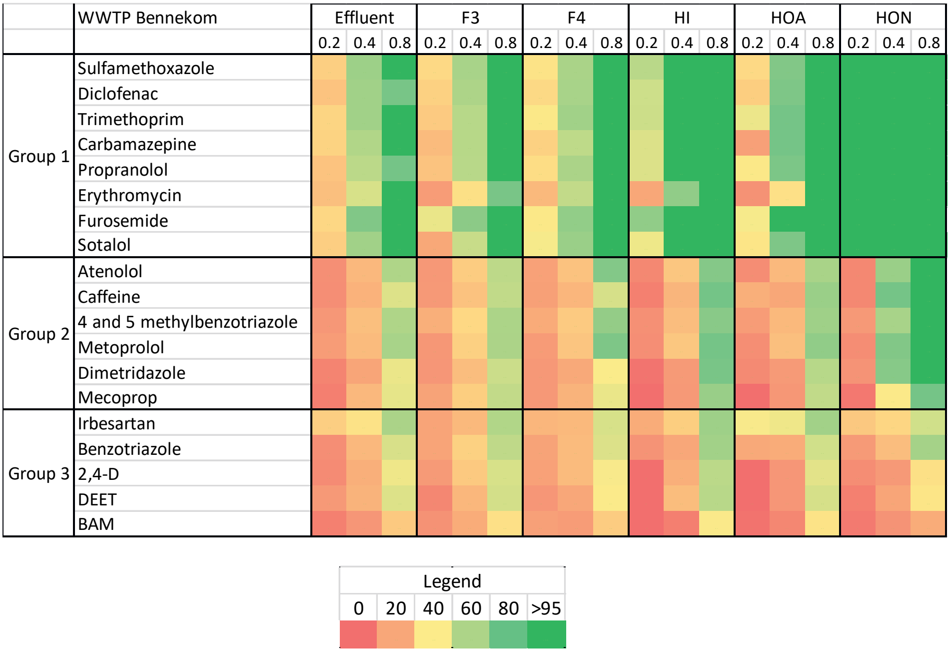


Figure S4.16 MP removal in the size and resin fractions of the effluent from Bennekom WWTP at three ozone doses. Removal in percentage is shown from 0 (red) to 100 (green)

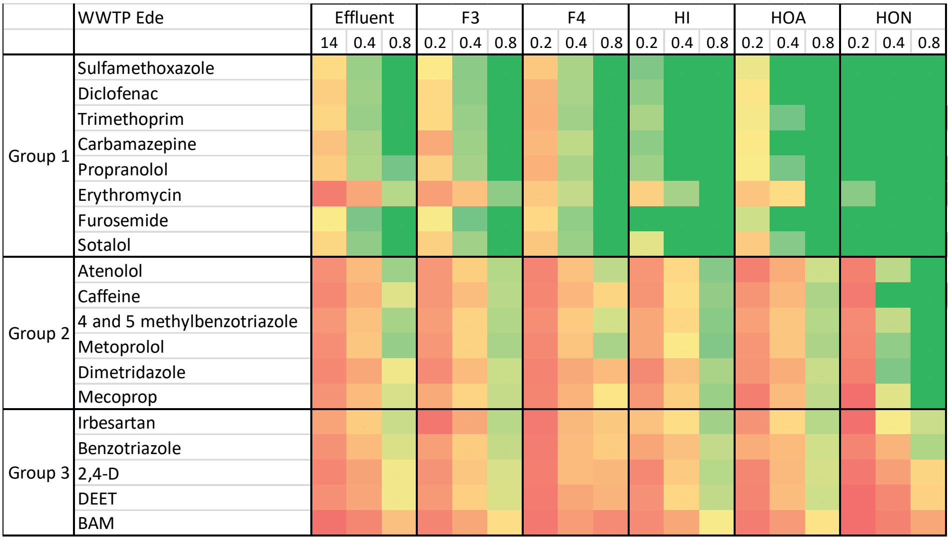


Figure S4.17 MP removal in the size and resin fractions of the effluent from Ede WWTP at three ozone doses. Removal in percentage is shown from 0 (red) to 100 (green)

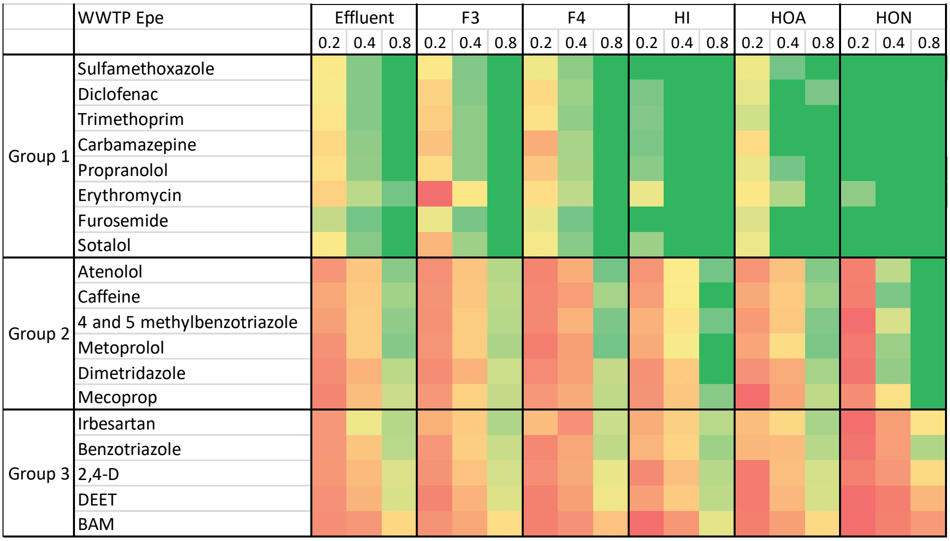


Figure S4.18 MP removal in the size and resin fractions of the effluent from Epe WWTP at three ozone doses. Removal in percentage is shown from 0 (red) to 100 (green)

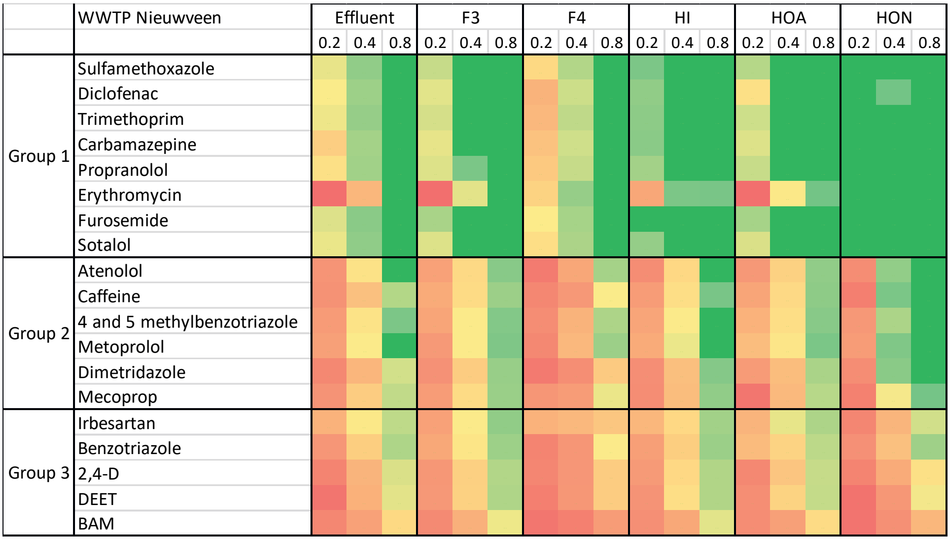


Figure S4.19 MP removal in the size and resin fractions of the effluent from Nieuwveen WWTP at three ozone doses. Removal in percentage is shown from 0 (red) to 100 (green)

4.5.9 Statistics

Table S4.12 P values of the Mann-Whitney test comparing the statistical significance of the size and resin fractions for the three MP groups and at the three tested ozone doses. P values smaller than 0.05 indicate significance and are highlighted in red

	Ozone dose (g O_3/g C)	0.2						0.4						0.8					
MP group	Fraction	Effluent	F3	F4	HI	HOA	HON	Effluent	F3	F4	HI	HOA	HON	Effluent	F3	F4	HI	HOA	HON
Group 1	Effluent	1E+00	6E-01	3E-01	9E-10	1E-02	6E-15	1E+00	9E-01	1E-01	6E-13	5E-08	3E-15	1E+00	1E+00	6E-01	1E-01	8E-01	1E-01
	F3	6E-01	1E+00	8E-01	6E-10	5E-03	6E-15	9E-01	1E+00	1E-01	1E-10	5E-05	4E-13	1E+00	1E+00	7E-01	1E-01	8E-01	2E-01
	F4	3E-01	8E-01	1E+00	4E-10	1E-03	6E-15	1E-01	1E-01	1E+00	1E-13	2E-08	3E-15	6E-01	7E-01	1E+00	3E-01	8E-01	4E-01
	HI	9E-10	6E-10	4E-10	1E+00	6E-08	3E-12	6E-13	1E-10	1E-13	1E+00	5E-06	2E-02	1E-01	1E-01	3E-01	1E+00	2E-01	8E-01
	HOA	1E-02	5E-03	1E-03	6E-08	1E+00	6E-15	5E-08	5E-05	2E-08	5E-06	1E+00	7E-10	8E-01	8E-01	8E-01	2E-01	1E+00	2E-01
	HON	6E-15	6E-15	6E-15	3E-12	6E-15	1E+00	3E-15	4E-13	3E-15	2E-02	7E-10	1E+00	1E-01	2E-01	4E-01	8E-01	2E-01	1E+00
Group 2	Effluent	1E+00	4E-04	3E-02	5E-01	1E-01	6E-04	1E+00	4E-03	5E-03	2E-02	2E-01	6E-14	1E+00	1E+00	4E-01	7E-05	2E-01	3E-10
	F3	4E-04	1E+00	1E-05	2E-02	3E-01	2E-08	4E-03	1E+00	8E-07	7E-01	1E-04	5E-13	1E+00	1E+00	5E-01	2E-09	4E-02	2E-11
	F4	3E-02	1E-05	1E+00	3E-02	2E-02	3E-01	5E-03	8E-07	1E+00	3E-05	7E-02	7E-11	4E-01	5E-01	1E+00	4E-05	9E-02	7E-10
	HI	5E-01	2E-02	3E-02	1E+00	5E-01	1E-03	2E-02	7E-01	3E-05	1E+00	8E-04	2E-12	7E-05	2E-09	4E-05	1E+00	8E-07	1E-09
	HOA	1E-01	3E-01	2E-02	5E-01	1E+00	7E-04	2E-01	1E-04	7E-02	8E-04	1E+00	3E-15	2E-01	4E-02	9E-02	8E-07	1E+00	2E-11
	HON	6E-04	2E-08	3E-01	1E-03	7E-04	1E+00	6E-14	5E-13	7E-11	2E-12	3E-15	1E+00	3E-10	2E-11	7E-10	1E-09	2E-11	1E+00
Group 3	Effluent	1E+00	5E-02	2E-01	1E+00	8E-01	1E-03	1E+00	3E-02	3E-02	2E-01	6E-01	2E-02	1E+00	7E-02	1E-03	1E-02	6E-02	2E-01
	F3	5E-02	1E+00	2E-02	3E-01	4E-01	4E-06	3E-02	1E+00	4E-06	6E-01	2E-01	3E-05	7E-02	1E+00	2E-06	4E-01	9E-01	2E-02
	F4	2E-01	2E-02	1E+00	7E-01	4E-01	5E-02	3E-02	4E-06	1E+00	2E-04	8E-03	8E-01	1E-03	2E-06	1E+00	2E-07	3E-06	1E-01
	HI	1E+00	3E-01	7E-01	1E+00	9E-01	4E-02	2E-01	6E-01	2E-04	1E+00	4E-01	8E-04	1E-02	4E-01	2E-07	1E+00	1E-01	2E-03
	HOA	8E-01	4E-01	4E-01	9E-01	1E+00	2E-02	6E-01	2E-01	8E-03	4E-01	1E+00	1E-02	6E-02	9E-01	3E-06	1E-01	1E+00	2E-02
	HON	1E-03	4E-06	5E-02	4E-02	2E-02	1E+00	2E-02	3E-05	8E-01	8E-04	1E-02	1E+00	2E-01	2E-02	1E-01	2E-03	2E-02	1E+00

Table S4.13 Correlation coefficients between organic matter characteristics of the initial fractions and micropollutant removals at the three ozone doses. R² values are colored from low (white) to high (green), the highest R² per MP is colored red. Explanations of the used characteristics (no. 1-25) can be found in Table S4.11

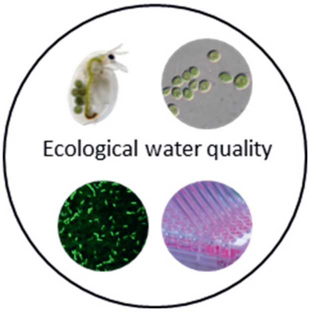
Ozone dose	MP	Comp1	Comp2	Comp3	Comp4	Fmax	bix	b	t	a	m	c	fi	hix	A254	A300	E2_E3	E4_E6	S275_295	S350_400	S300_700	SR	COD	pH	Conductivity	Ozone dose
0.2	Sulfamethoxazole	0.5	0.6	0.0	0.4	0.5	0.1	0.1	0.0	0.4	0.4	0.5	0.1	0.2	0.5	0.7	0.0	0.0	0.1	0.1	0.1	0.4	0.4	0.2	0.0	0.2
	Diclofenac	0.6	0.7	0.0	0.5	0.6	0.2	0.0	0.1	0.5	0.5	0.6	0.1	0.3	0.6	0.7	0.0	0.0	0.1	0.2	0.1	0.5	0.4	0.2	0.0	0.2
	Trimethoprim	0.6	0.7	0.0	0.5	0.6	0.1	0.1	0.1	0.5	0.5	0.6	0.0	0.3	0.6	0.7	0.0	0.0	0.1	0.2	0.1	0.5	0.5	0.2	0.0	0.3
	Carbamazepine	0.5	0.6	0.0	0.4	0.5	0.1	0.0	0.0	0.4	0.4	0.5	0.1	0.3	0.5	0.7	0.0	0.0	0.1	0.1	0.1	0.5	0.4	0.2	0.0	0.2
	Propranolol	0.6	0.7	0.0	0.5	0.6	0.1	0.1	0.1	0.5	0.5	0.6	0.0	0.3	0.6	0.7	0.0	0.0	0.1	0.2	0.2	0.6	0.5	0.2	0.0	0.2
	Erythromycin	0.5	0.6	0.0	0.2	0.4	0.4	0.0	0.0	0.5	0.4	0.5	0.1	0.3	0.7	0.7	0.1	0.0	0.0	0.4	0.2	0.6	0.5	0.0	0.0	0.1
	Furosemide	0.4	0.6	0.0	0.4	0.4	0.1	0.0	0.0	0.4	0.4	0.5	0.1	0.2	0.5	0.6	0.0	0.0	0.1	0.1	0.1	0.3	0.3	0.3	0.0	0.3
	Sotalol	0.5	0.6	0.1	0.4	0.4	0.2	0.1	0.0	0.3	0.4	0.5	0.0	0.3	0.6	0.7	0.0	0.0	0.2	0.2	0.1	0.6	0.6	0.1	0.0	0.1
	Atenolol	0.1	0.1	0.0	0.0	0.1	0.2	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0
	Caffeine	0.1	0.1	0.0	0.0	0.1	0.3	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.2	0.2	0.0	0.0	0.0	0.1	0.0	0.2	0.1	0.0	0.3	0.1
	4 and 5 methylbenzotriazole	0.1	0.1	0.0	0.0	0.0	0.3	0.0	0.2	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1
	Metoprolol	0.0	0.1	0.0	0.0	0.0	0.3	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1
	Dimetridazole	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
	Mecoprop	0.2	0.2	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.2	0.0	0.0	0.2	0.3	0.0	0.1	0.1	0.0	0.0	0.2	0.3	0.0	0.3	0.4
	Irbesartan	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0
	Benzotriazole	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2	0.3	0.3
	2,4-D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.3	0.2
	DEET	0.2	0.2	0.0	0.1	0.1	0.1	0.0	0.2	0.1	0.1	0.2	0.0	0.0	0.2	0.2	0.1	0.1	0.0	0.1	0.1	0.2	0.4	0.0	0.1	0.1
	BAM	0.2	0.3	0.0	0.1	0.2	0.2	0.0	0.1	0.2	0.2	0.2	0.0	0.1	0.3	0.3	0.0	0.0	0.0	0.1	0.0	0.2	0.2	0.0	0.0	0.1

Ozone dose	MP	Comp1	Comp2	Comp3	Comp4	Fmax	bix	b	t	a	m	c	fi	hix	A254	A300	E2_E3	E4_E6	S275_295	S350_400	S300_700	SR	COD	pH	Conductivity	Ozone dose
0.4	Sulfamethoxazole	0.4	0.4	0.0	0.5	0.4	0.0	0.0	0.1	0.3	0.4	0.5	0.0	0.1	0.3	0.4	0.0	0.1	0.1	0.0	0.0	0.2	0.3	0.4	0.0	0.3
	Diclofenac	0.4	0.4	0.0	0.4	0.4	0.0	0.0	0.1	0.3	0.4	0.4	0.0	0.0	0.3	0.3	0.0	0.1	0.1	0.0	0.1	0.1	0.3	0.4	0.1	0.4
	Trimethoprim	0.4	0.4	0.0	0.4	0.3	0.0	0.0	0.1	0.2	0.3	0.4	0.0	0.1	0.3	0.4	0.0	0.1	0.1	0.0	0.0	0.2	0.3	0.3	0.1	0.4
	Carbamazepine	0.4	0.4	0.0	0.5	0.4	0.0	0.0	0.1	0.3	0.4	0.5	0.0	0.1	0.3	0.4	0.0	0.1	0.1	0.0	0.1	0.2	0.3	0.4	0.1	0.4
	Propranolol	0.4	0.4	0.0	0.4	0.4	0.0	0.0	0.1	0.3	0.4	0.4	0.0	0.1	0.4	0.5	0.0	0.1	0.1	0.0	0.1	0.2	0.3	0.4	0.1	0.4
	Erythromycin	0.3	0.4	0.0	0.1	0.2	0.4	0.0	0.1	0.3	0.2	0.3	0.2	0.1	0.7	0.8	0.0	0.0	0.1	0.1	0.0	0.3	0.3	0.0	0.0	0.0
	Furosemide	0.4	0.4	0.0	0.4	0.4	0.0	0.0	0.1	0.4	0.4	0.5	0.0	0.1	0.2	0.3	0.0	0.1	0.0	0.0	0.1	0.1	0.2	0.4	0.1	0.4
	Sotalol	0.3	0.3	0.0	0.3	0.2	0.0	0.0	0.0	0.2	0.2	0.3	0.0	0.1	0.4	0.5	0.0	0.1	0.2	0.0	0.0	0.2	0.3	0.2	0.0	0.3
	Atenolol	0.6	0.6	0.0	0.5	0.5	0.2	0.0	0.1	0.4	0.5	0.6	0.0	0.4	0.5	0.5	0.1	0.0	0.0	0.4	0.2	0.8	0.6	0.0	0.1	0.0
	Caffeine	0.6	0.7	0.0	0.5	0.6	0.1	0.0	0.1	0.5	0.6	0.6	0.1	0.4	0.5	0.5	0.1	0.0	0.0	0.5	0.3	0.8	0.6	0.2	0.0	0.0
	4 and 5 methylbenzotriazole	0.5	0.5	0.1	0.5	0.4	0.1	0.0	0.0	0.3	0.5	0.5	0.0	0.4	0.4	0.4	0.1	0.0	0.0	0.4	0.2	0.7	0.5	0.0	0.1	0.0
	Metoprolol	0.6	0.6	0.1	0.6	0.5	0.1	0.0	0.1	0.4	0.5	0.6	0.0	0.4	0.4	0.5	0.1	0.0	0.0	0.4	0.3	0.8	0.6	0.1	0.1	0.0
	Dimetridazole	0.6	0.6	0.0	0.5	0.6	0.1	0.0	0.1	0.5	0.6	0.6	0.1	0.4	0.4	0.4	0.2	0.0	0.0	0.5	0.4	0.8	0.6	0.1	0.1	0.0
	Mecoprop	0.2	0.2	0.0	0.3	0.3	0.0	0.0	0.0	0.2	0.3	0.2	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.3	0.2	0.3	0.1	0.1	0.3	0.1
	Irbesartan	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
	Benzotriazole	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	2,4-D	0.2	0.1	0.0	0.0	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.2	0.2	0.1	0.0	0.0	0.2	0.1	0.2	0.2	0.1	0.0	0.0
	DEET	0.1	0.1	0.0	0.0	0.1	0.3	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.2	0.1	0.1	0.0	0.0	0.3	0.1	0.3	0.3	0.1	0.0	0.0
	BAM	0.1	0.1	0.0	0.0	0.1	0.2	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.2	0.2	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.0	0.0	0.0

Ozone dose	MP	Comp1	Comp2	Comp3	Comp4	Fmax	bix	b	t	a	m	c	fi	hix	A254	A300	E2_E3	E4_E6	S275_295	S350_400	S300_700	SR	COD	pH	Conductivity	Ozone dose
0.8	Sulfamethoxazole	0.2	0.2	0.0	0.3	0.2	0.1	0.0	0.0	0.1	0.2	0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.0	0.1
	Diclofenac	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
	Trimethoprim	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0
	Carbamazepine	0.1	0.2	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.2
	Propranolol	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.1	0.0	0.2
	Erythromycin	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1
	Furosemide	0.3	0.3	0.0	0.2	0.2	0.3	0.0	0.1	0.2	0.2	0.3	0.0	0.1	0.2	0.2	0.0	0.0	0.0	0.2	0.1	0.4	0.3	0.0	0.0	0.1
	Sotalol	0.1	0.0	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0
	Atenolol	0.2	0.3	0.1	0.2	0.2	0.2	0.1	0.0	0.1	0.2	0.2	0.0	0.1	0.4	0.5	0.1	0.0	0.3	0.0	0.0	0.3	0.3	0.0	0.0	0.0
	Caffeine	0.6	0.7	0.0	0.6	0.6	0.1	0.1	0.1	0.5	0.6	0.7	0.0	0.2	0.5	0.6	0.0	0.0	0.1	0.1	0.1	0.3	0.4	0.3	0.0	0.3
	4 and 5 methylbenzotriazole	0.4	0.4	0.1	0.3	0.3	0.1	0.1	0.0	0.2	0.3	0.4	0.0	0.2	0.4	0.6	0.0	0.0	0.3	0.0	0.0	0.4	0.4	0.0	0.0	0.1
	Metoprolol	0.2	0.3	0.1	0.2	0.2	0.1	0.0	0.0	0.1	0.2	0.2	0.0	0.1	0.4	0.6	0.1	0.0	0.4	0.0	0.0	0.3	0.3	0.0	0.0	0.1
	Dimetridazole	0.6	0.7	0.0	0.6	0.7	0.0	0.1	0.1	0.6	0.7	0.7	0.0	0.2	0.5	0.5	0.0	0.0	0.0	0.2	0.2	0.4	0.4	0.4	0.0	0.3
	Mecoprop	0.7	0.7	0.0	0.6	0.7	0.1	0.1	0.1	0.5	0.7	0.7	0.1	0.3	0.5	0.6	0.0	0.0	0.0	0.2	0.2	0.4	0.4	0.4	0.0	0.3
	Irbesartan	0.0	0.0	0.0	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0
	Benzotriazole	0.4	0.3	0.0	0.5	0.4	0.0	0.1	0.0	0.3	0.4	0.4	0.0	0.1	0.2	0.3	0.0	0.0	0.1	0.1	0.0	0.2	0.1	0.1	0.0	0.0
	2,4-D	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.2	0.1	0.2	0.1	0.0
	DEET	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.2	0.1	0.0
	BAM	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.2	0.1	0.2	0.1	0.0

Chapter 5

Removal of micropollutants and ecotoxicity during combined biological activated carbon and ozone (BO_3) treatment



A modified version of this chapter is submitted as:

van Gijn, K., van Dam ,M., de Wilt, H.A., de Wilde, V. Rijnaarts, H.H.M., Langenhoff, A.A.M.. Removal of micropollutants and ecotoxicity during combined biological activated carbon and ozone (BO_3) treatment

Abstract

Ozonation is a viable option to improve the removal of micropollutants (MPs) from wastewater treatment plant (WWTP) effluents. Nevertheless, the application of ozonation is hindered by its high energy requirements and by the uncertainties regarding the formation of toxic transformation products in the process. This study investigated a combination of biological activated carbon (BAC) filtration followed by ozonation (the BO_3 process) to remove MPs at low ozone doses and low energy input, and focused on the formation of toxic organic and inorganic products during ozonation. Effluent from a WWTP was collected, spiked with MPs (approximately 1 µg/L) and treated with the BO_3 process. Different flowrates (0.25-4 L/h) and specific ozone doses (0.2-0.6 g O_3/g C) were tested and MPs, ecotoxicity and bromate were analyzed. For ecotoxicity assessment, three in vivo (daphnia, algae and bacteria) and six in vitro CALUX assays (Era, GR, PAH, P53, PR, andNrf2 CALUX) were used.

Results show that the combination of BAC filtration and ozonation has higher MP removal and higher ecotoxicity removal than only BAC filtration and only ozonation. The in vivo assays show a low ecotoxicity in the initial WWTP effluent samples and no clear trend with increasing ozone doses, while most of the in vitro assays show a decrease in ecotoxicity with increasing ozone dose. This means that for the tested bioassays, feed water and ozone doses, the overall ecotoxicity of the formed transformation products during ozonation was lower than the overall ecotoxicity of the parent compounds. In the experiments with bromide spiking, relevant formation of bromate was observed above specific ozone doses of approximately 0.4 O_3/g C and more bromate was formed for the samples with BAC pre-treatment. This shows the effectivity of the pre-treatment in removing organic matter and making ozone more available to react with other compounds (such as MPs, but also bromide), but also underlines the importance of controlling the ozone dose to be below the threshold to avoid formation of bromate. It was concluded that treatment of the tested WWTP effluent in the BO_3 process at a specific ozone dose of 0.2 g O_3/g C, results in high MP removal at limited energy input and no increase in ecotoxicity, nor formation of bromate was observed. This indicates that the hybrid BO_3 process can be implemented to remove MPs and improve the ecological quality of this WWTP effluent with a lower energy demand than conventional MP removal processes such as standalone ozonation.

5.1 Introduction

In the last decades, micropollutants (MPs) such as pharmaceuticals, pesticides and industrial chemicals, have been detected in water matrixes all over the world [1–5]. The ecotoxicological risks of MPs were indicated clearly in several specific cases [15,224,225], but are hard to measure and predict in most cases due to their prevalence at trace concentrations (ng-µg/L) and in complex mixtures [226,227]. Wastewater treatment plants (WWTPs) are sources from where MPs enter the environment [137]. Therefore, the limited removal of MPs in conventional WWTPs has been addressed as a growing concern [5], which underlines the importance of upgrading WWTPs to increase their MP removal.

A treatment technology that has shown its potential to improve the MP removal capacity of WWTPs is ozonation [45,129]. During ozonation, the MPs are oxidized directly by ozone molecules or indirectly by radicals (e.g. OH·) that are formed during the degradation of ozone [74]. Ozone treatment is widely used for drinking water treatment, but the use of ozonation in wastewater treatment is still limited to a few countries [62,189,228]. The reason for this is that applying ozonation for wastewater treatment has two major challenges: i) producing ozone is energy and cost intensive and the presence of background organic matter (OM) in the WWTP effluent matrix greatly increases (by a factor 100 or more) the required amount of ozone to achieve significant MP removal [177,229] and ii) during ozonation most organic and inorganic compounds are transformed and not mineralized, leaving ozone transformation products (OTPs) and by-products in the water after the ozone treatment. The large number of MPs results in an even larger number of OTPs, which could be as toxic or even more toxic than the parent compound [73,230,231], but are difficult to analyze [232]. Moreover, ecotoxicological information of OTPs is hardly available.

The main reason for the high energy demand and costs of ozone treatment is that OM interferes with the ozonation of MPs. OM is typically present at approximately 5-20 mg/L [233], which is a 10^3 – 10^6 times higher concentration than the concentrations of MPs. Therefore, the bulk of ozone added to the water reacts with OM and not with the target MPs, so the required ozone dose of a water sample is normally based on the quantity of organic matter in the water matrix [66,229]. Reducing the amount of OM in the water before ozonation results in a lower ozone demand and in lower energy and cost input. De Wilt et al. [75] showed the potential of using biological pre-treatment before ozonation (but after the conventional wastewater treatment process) to achieve this. Van Gijn [182] found that a biological activated carbon (BAC) filter is the most suitable bioreactor to remove OM from WWTP effluent and that the flowrate in the bioreactor controls the OM removal. The performance of a combination of BAC filtration with ozone treatment (the BO_3 process) to remove MPs from WWTP effluent with realistic MP concentrations has not yet been tested.

The ecotoxicity of OTPs is difficult to assess and different studies can have contradicting results because of several reasons: the type of MPs (and other background compounds), their concentrations, used sample pre-treatments, used ozone doses and used ecotoxicity assays all vary between studies. Because of this, some studies find an increased ecotoxicity after ozonation [73,234], whereas others find a decrease [92,235,236]. It remains difficult to pinpoint which of the mentioned reasons is the cause of the observed differences. In studies that found an increase in ecotoxicity after ozonation, the increase in toxicity is often nullified with a (biological) post treatment (e.g. sand filtration or activated carbon filtration) after ozonation [45,73,124]. Even though the most toxic OTPs can be removed in this way, a number of OTPs still remain in the water after post-treatment [237]. Post treatment after ozonation is an extra treatment step which adds energy requirement, costs and complexity to the total treatment process. Because of the number of variables that affect the formation of potentially toxic OTPs during ozonation, it is challenging to decide whether post-treatment is required. This can only be confirmed for a specific case when using a broad range of bioassays to make the evaluation.

Bioassays or toxicity tests can be used to get information about the total toxicity of a complex mixture with unknown compounds. By exposing an organism (in vivo) or a cell (in vitro) to a sample, the response can give information about adverse effects of the samples on the organisms or cells. A broad range of bioassays is available ranging from mammals to cells designed with specific receptors. Ideally all relevant species should be tested to know whether a sample is toxic, but normally a selection of the most relevant assays is made due to cost constrains. It can be challenging to make the link between results of a bioassay and environmentally relevant toxicity, therefore the response can be expressed in equivalent of a toxic compounds and the relevance can be shown by comparing the toxic equivalents to effect based trigger values (EBTs) [91,238]. In this way, the environmentally relevant ecotoxicity of the WWTP effluent can be evaluated before and after ozonation.

Next to organic OTPs, also inorganic OTPs (also referred to as by-products) can be relevant during ozonation. The most challenging inorganic ozone OTP is bromate, which formed from bromide. Bromate poses carcinogenic risks for humans at low concentrations (low µg/L range) and is difficult to remove after it has been formed [45,86,239]. The presence of bromide in wastewater originates from various industrial emissions, seaborne aerosol depositions, seawater intrusion into the sewer and background concentrations in tap water. Relevant concentrations are up to about one mg/L [240,241]. When no bromide is present, there is no risk of bromate formation . If bromide is present, the used ozone dose determines the amount of bromate formation [242].

The aim of this study was to evaluate the BO_3 process for the removal of MPs from WWTP effluent. The main focus was on the formation and ecotoxicity of organic and inorganic (bromate) OTPs, and how this affects the potential for full-scale implementation of the BO_3 process. To test this, samples from a WWTP effluent were spiked with MPs, treated with the BAC filter and ozonated at different doses, after which MP removal, ecotoxicity and bromate formation was assessed. The results can be used to assess the MP removal of the BO_3 process, to determine if post-treatment is necessary, and to gain insight in the conditions for which bromate formation occurs.

5.2 Materials and methods

5.2.1 Reactor setup

During the experiments, the feed water (WWTP effluent) was fed through a water bath (set at 15 °C) and through a pre-aerator where the water was aerated with pure oxygen before entering the bioreactors (Figure 5.1). The BAC filter was the same as previously described [182]. Ozone was generated from pure oxygen using an ozone generator (COM-AD-02, Anseros) and the ozone input quantity was controlled and monitored using a mass flow controller (Brooks 5850 series) and an ozone meter (BMT 964). For ozonation without biological pre-treatment, the feed tank was connected directly to the ozone reactor.

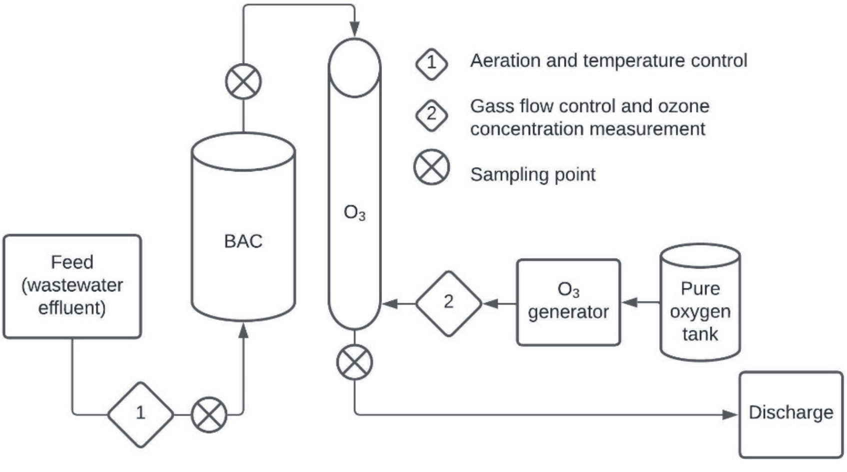


Figure 5.1 Schematic overview of the reactor setup of the BO_3 process. At '1', the feed was aerated with pure oxygen. At '2' a mass flow controller was used to control the gas flow and an ozone meter was used to monitor the ozone concentration in the gas

5.2.2 Experimental design

The BAC filter and the ozone reactor were operated at three flowrates of 0.37, 0.91 and 3.62 L/h (equal to empty bed contact times of 3.21, 1.32 and 0.33 h). After setting each flowrate, a two week stabilization period was applied where only OM removal was monitored in the biofilters, followed by a one week period where the effluent of the biofilters was ozonated and also MP concentrations were measured.

After the experiments at different flowrates, one optimal flowrate was selected and applied in the following tests with the BAC filter and the ozone reactor. In one test, the feed was spiked with bromide (approximately 0.4 mg/L) and formation of bromate was monitored at different specific ozone doses from 0.1 – 0.7 g O_3/g C. In the other test, formation of organic OTPs was monitored using 9 bioassays at specific ozone doses of approximately 0.2, 0.4 and 0.6 g O_3/g C. Additionally, only ozonated samples (without biological pre-treatment) collected using the same ozone reactor and the same feed were included in all experiments as a reference.

5.2.3 Feed water

Effluent from the WWTP in Bennekom, the Netherlands was collected in dry weather conditions and used as feed for the BO_3 process. For the stabilization periods, the WWTP effluent was collected in a $3m^3$ tank that was cooled to 7 °C and from there fed to the reactors. For the measurement periods, WWTP effluent was collected in a 300L container and spiked with a mix of 19 MPs to final concentrations of approximately 1µg/L for each MP. Before spiking, the solvent of the mix (acetonitrile) was evaporated to avoid the presence of solvent in the feed. The MP mix contained 2,4-D, 4 and 5 methyl benzotriazole, BAM, benzotriazole, caffeine, carbamazepine, clarithromycin, DEET, desphenyl chloridazon, diclofenac, dimetridazole, erythromycin, furosemide, irbesartan, mecoprop, metoprolol, propranolol, sulfamethoxazole and trimethoprim. The spiked feed was then fed to the reactors from the 300L container (Figure 5.1).

5.2.4 Analytical methods

Organic matter and micropollutants

UV absorbance at 254 nm, TOC, COD and fluorescence were measured to monitor OM removal in the bioreactors. MPs were measured in bioreactors and in the ozone reactor using liquid chromatography coupled to mass spectrometry. Methods for OM and MP measurements were the same as in our previous study [233]. The tier 1 risk quotient (RQ) of the MPs in the samples [243] were calculated by dividing the measured MP concentration by the lowest predicted no effect concentrations (PNEC) for freshwater obtained from the NORMAN database (Table S5.1).

Ions and nutrients

Bromide, nitrite, nitrate and phosphate were measured using a dionex ICS-6000 dual pump ion chromatography system from thermo scientific with a dionex IonPac AS17-C 2mm IC analytical column, a dionex ADRS 600 2 mm dynamically degenerated suppressor and a conductivity detector. The eluent used was KOH in deionized water with a continuous flow of

0.25 mL/min. KOH gradients were 0-10 min; stable at 5 mM, 10-25 min; linear increase from 5-30 mM, 28-28 min; stable at 30 mM, 28-30 min; linear decrease from 30 to 5 mM. Samples were centrifuged at 15000 rpm for 10 minutes before analyses and an injection volume of 10 µL was used. Chromeleon 7.3 software was used to integrate and quantify the peaks. Ammonium concentrations were quantified using Hach kits LCK304 and LCK303.

Bromate concentrations were quantified with ion chromatography at 'Het Waterlaboratorium', Haarlem, the Netherlands with a method according to NEN-EN-ISO 11206. Limit of detection of the method was 0.2 µg/L, reproducibility was 96.41% and accuracy was 9.69%.

Bioassays

The daphnia inhibition assay, algae growth inhibition assay and microtox test were executed in our laboratory and six CALUX assays (Era, GR, PAH, P53, PR, andNrf2 CALUX) were performed at BDS biodetection systems (Amsterdam, the Netherlands).

The daphnia immobilization assay was performed according to OECD Test No. 202: Daphnia sp. Acute Immobilization Test. Samples were mixed with growth medium in a 1:1 ratio in 6 well plates. Each plate contained four replicates of one sample, one positive control (5 µM $CuSO_4$) and one negative control (growth medium). Five juvenile *Daphnia magna* were added to each well and their activity was monitored after 48 hours. Immobilization was calculated by dividing the number of immobilized daphnia at t=48 hours by the total number of daphnia. See section 5.5.2 for the detailed protocol.

The algae growth inhibition assays was performed according to OECD Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Samples were added to white 96-well plates in four different dilutions (1x, 2x, 4x, and 8x), each dilution in triplicate. Each plate contained six positive controls (200 µM $CuSO_4$) and six blanks (algae growth medium). Algae in their exponential growing phase (*Scenedesmus obliquus*) were added to the samples in a 1:1 ratio and chlorophyl (fluorescence) was measured after 24, 48 and 72 hours. Growth inhibition was calculated by dividing the area under the growth curve for the samples by the area for the controls. See section 5.5.2 for the detailed protocol.

The microtox assay was performed according to ISO 11348-3: Water quality – Determination of the inhibitory effect of water samples on the light emission of *Aliivibrio fischeri* (formally *Vibrio fischeri*) (luminescent bacteria test). Samples were added to white 96-well plates in four different dilutions (1x, 2x, 4x, and 8x), each dilution in triplicate. Samples were mixed with a salt solution (NaCl) to ensure final concentrations of 2.2% NaCl in all wells. Each plate contained six positive controls (200 µM $CuSO_4$) and six blanks (2.2% NaCl). Frozen A*liivibrio fischeri* were reconstituted and dissolved, the solution was added to the samples in a 1:1 ratio using a Tecan plate reader and dispenser. Luminescence was measured after 0 and 15 minutes. Luminescence inhibition of the samples was calculated by comparing the decrease in luminescence over 15 minutes for the samples to the luminescence decrease for the blank. See section 5.5.2 for the detailed protocol.

The CALUX assays performed by BDS were Era (for estrogens), GR (for glucocorticoids), PAH (for polycyclic aromatic hydrocarbons), P53 (for genotoxicity), PR (for progesterone), and Nrf2 (for oxidative stress) CALUX. These assays were selected because of their relevance for surface water quality, illustrated by the fact they are included in the Dutch suggestions for biological effect monitoring [244,245]. Additionally, the Nrf2 assay was included to detect oxidative stress because of its relevance for ozonated samples. Sample were extracted before analysis according to [91].

5.3 Results and discussion

OM and MP removal in the BO_3 process was tested at three flowrates. The tests at three different flowrates showed that MP removal increases with decreasing flowrate in the BAC filter (**Figure 5.2**). The removal stabilized at flowrates higher than 0.91 L/h (empty bed contact time of 1.32 h). This relationship between MP removal and flowrate matches with the results for OM removal in this study (**Table S5.6**) and in our previous study into OM removal in the same reactors [182]. Based on these results, the BAC reactor and a flowrate of approximately 1 L/h (equal to an empty bed contact time of approximately 1h) were selected as the optimal conditions to use in the further experiments.

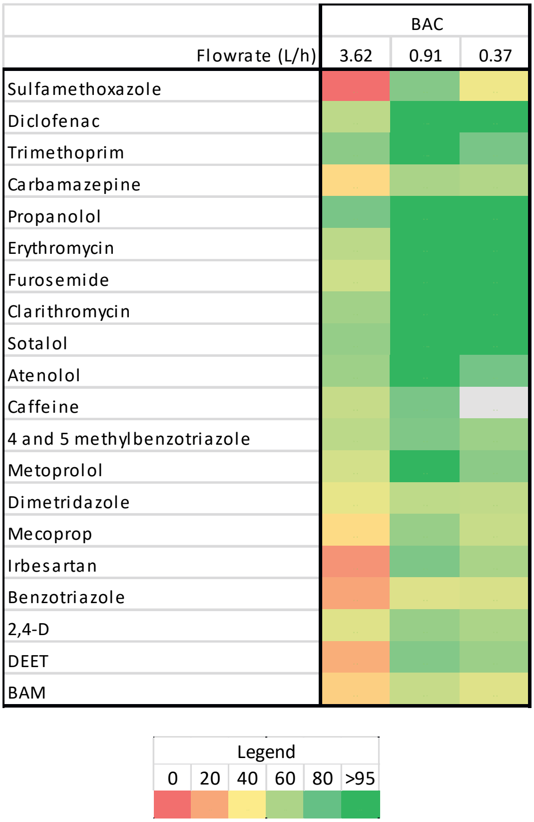


Figure 5.2 Heatmap for MP removal in the BAC filter at three flowrates. Removal in % is shown with a color scale from 0 (red) to >95 (green). NA means not analyzed. Averages are based on triplicates, average standard deviation was 2.7% see Table S5.7 for individual standard deviations)

5.3.1 Ecotoxicity of parent compounds

Risk quotients (RQs) where calculated for the measured MPs to gain insight in the potential toxic risk of the parent compounds in the samples. RQs are used in low tier risk assessment, which means that they are a conservative indicator of whether a toxic effect can be expected. If the RQ is lower than 1, no risk is expected, while if the RQ is higher than 1, a risk could occur so further ecotoxicological assessment is required. Several RQs were higher than 1 in the feed, and some remained higher after only BAC or ozone treatment (Table 5.1). This means that the parent compounds in the feed, BAC and ozone samples could pose an ecotoxicological concern. After treatment with the BO_3 process, RQs where <1 for all MPs at all ozone doses which means that for the measured parent compounds, no toxic effects are expected in these samples. This leaves only the ecotoxicity of the formed OTPs as a potential toxic concern.

Table 5.1 Risk quotients (RQs) of the measured MPs in the feed, BO_3 and O_3 samples at a flowrate of 0.91 L/h at different ozone doses. RQs >1 are highlighted in red

	Feed	BO_3				O_3		
Specific ozone dose (g O_3/g C) >	0	0	0.18	0.36	0.55	0.20	0.39	0.59
Sulfamethoxazole	1.4	0.1	0.0	0.0	0.0	0.1	0.0	0.0
Diclofenac	16.5	0.0	0.0	0.0	0.0	1.4	0.0	0.0
Trimethoprim	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbamazepine	22.4	5.0	0.2	0.2	0.2	3.1	0.5	0.2
Propranolol	2.3	0.0	0.0	0.0	0.0	0.3	0.0	0.0
Erythromycin	5.3	0.1	0.0	0.0	0.0	0.5	0.1	0.0
Furosemide	1.5	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Clarithromycin	6.0	0.2	0.1	0.1	0.1	1.0	0.5	0.4
Atenolol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caffeine	0.9	0.1	0.0	0.0	0.0	0.4	0.2	0.1
4 and 5 methyl benzotriazole	8.4	0.8	0.2	0.1	0.1	3.1	1.7	1.0
Metoprolol	0.3	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Dimetridazole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mecoprop	1.2	0.2	0.1	0.0	0.0	0.5	0.3	0.2
Irbesartan	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzotriazole	0.3	0.1	0.0	0.0	0.0	0.1	0.1	0.0
2,4-D	1.5	0.3	0.1	0.0	0.0	0.7	0.4	0.3
DEET	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BAM	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Desphenyl chloridazon	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

5.3.2 Ecotoxicity of organic transformation products

The ecotoxicity of ozonated WWTP effluent was tested with (BO_3) and without (O_3) the BAC pre-treatment using 3 in vivo bioassays and 6 in vitro CALUX assays. Results from the daphnia immobilization assay show no significant ecotoxicity in any of the samples (Figure 5.3). This means that the formed OTPs at the tested ozone doses were not toxic to daphnia. Another study found formation of formaldehydes during ozonation of WWTP effluent at a concentration that is toxic to daphnia [246] while two other studies found a stable or decreasing ecotoxicity for daphnia with increasing ozone dose [247,248]. These contradicting results from literature underline the complexity and case to case dependency of ecotoxicity of OTPs in WWTP effluent [249].

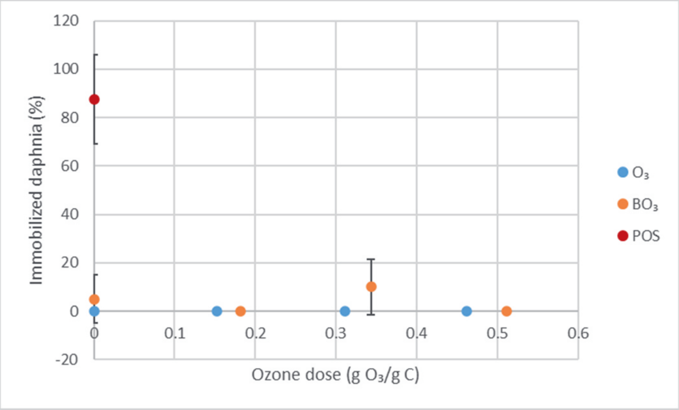


Figure 5.3 Immobilized daphnia after 48h exposure to ozonated samples with (BO_3) and without (O_3) BAC pre-treatment. Error bars represent standard deviations based on 4 replicate wells each

Growth inhibition of algae did not show a clear relation with applied specific ozone dose (Figure 5.4). Slightly higher ecotoxicity (31%) was observed at the highest ozone dose without BAC pre-treatment. This could mean that OTPs toxic to algae are only formed at higher specific ozone doses (higher than 0.5 g O_3/g C). On the other hand, this ecotoxicity at the highest ozone dose was not observed in the 2x, 4x and 8x diluted samples (Figure S5.8). It is clear that ozone treatment preceded by BAC filtration does not result in the formation of OTPs that are toxic to algae (Figure 5.4). Other studies also found stable or decreasing ecotoxicity to algae after ozone treatment [64,250,251].

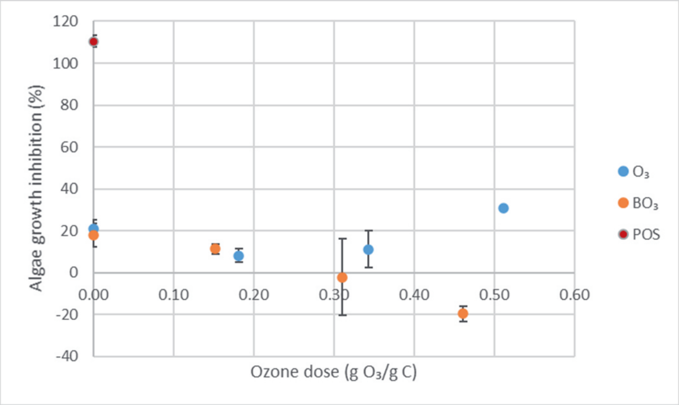


Figure 5.4 Growth inhibition of algae after 72h exposure to ozonated samples with (BO_3) and without (O_3) BAC pre-treatment. Error bars represent standard deviations based on 3 replicate wells each

Inhibition of luminescent bacteria was stable with an increasing ozone dose both with and without BAC pre-treatment (Figure 5.5). This means that ozonation did not reduce the limited ecotoxicity that was present in the WWTP effluent, but the OTPs formed during treatment also were not toxic to the bacteria. Samples with BAC pre-treatment had slightly lower ecotoxicity, although this trend was less clear for the dilutions (Figure S2). Other studies find both increasing [184] and decreasing [143,250] ecotoxicity to luminescent bacteria after ozonation. The reason for these contradiction results could be that in the experiment of Li et al. [184] were performed using a high starting concentration of DEET (200 mg/L) and a high ozone dose (because experiments were performed in clean water without background OM). This means that based on the results of this study and of literature, a stable or decreased ecotoxicity for the microtox assay can be expected after ozonation.

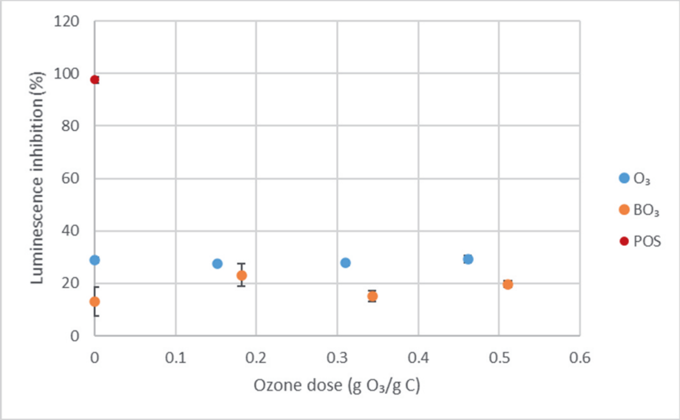


Figure 5.5 Luminescence inhibition of algae after 15 min exposure to ozonated samples with (BO_3) and without (O_3) BAC pre-treatment. Error bars represent standard deviations based on 3 replicate wells each

The in vitro CALUX assays showed a clear decrease in ecotoxicity (Figure 5.6A-D) or stable ecotoxicity (Figure 5.6E and F) with increasing ozone doses. This suggests that the formed OTPs had a lower or equal toxic potential compared to the parent compounds. Nevertheless, the pre-treatment method used for the CALUX assays (SPE) was not tested for the recovery of OTPs which makes it difficult to make strong conclusions. For all assays where ecotoxicity was observed, the ecotoxicity in the samples with BAC pre-treatment (BO_3) showed lower ecotoxicity than the samples without (O_3) (Figure 5.6). This means that the BAC treatment contributed to the removal of compounds that cause adverse effects for the tested endpoints. Concentration equivalent concentrations were below the EBTs for most assays where the EBTs were available (Table S5.9). Only for the glucocorticoids assay (Figure 5.6C) the measured hormonal ecotoxicity in the WWTP effluent was above the EBT of 100 ng Dexamethasone eq./L and was reduced to below the EBT after treatment. Overall, this means that observed in vitro ecotoxicity for the tested assays was decreased to environmentally safe levels after treatment with the BO_3 process.

Results observed in the CALUX assays match with other findings in literature. Phan et al. [91] also found a decrease in Estrogenic and PAH ecotoxicity (Figure 5.6A and C) and fluctuating oxidative stress (Figure 5.6F) with ozonation. Occurrence of genotoxicity using different genotoxicity assays varies strongly in different studies [249]. Therefore the complete absence of genotoxic response in this study is surprising. On the other hand another study into the ecotoxicity of Dutch surface waters also did not find any locations that showed a risk of genotoxicity with the CALUX P53 assay [252].

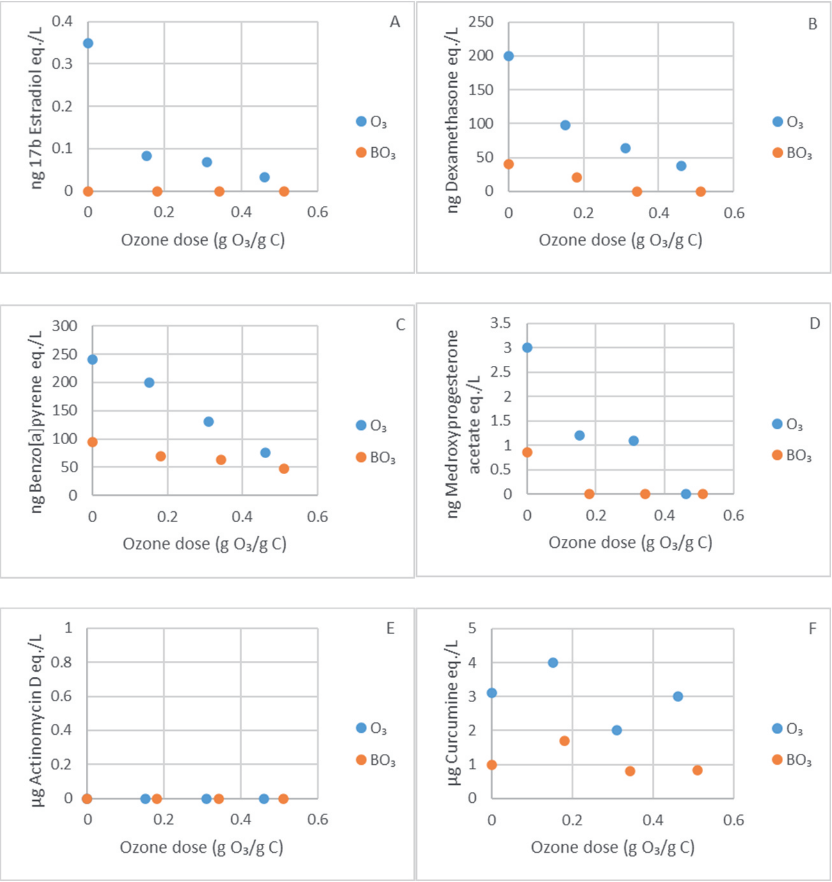


Figure 5.6 Ecotoxicity measured in six in vitro CALUX assays of ozonated samples with (BO_3) and without (O_3) BAC pre-treatment. Toxicity is represented in equivalent concentrations. 17b estradiol is related to estrogens (Era), dexamethasone is related to glucocorticoids (GR), benzo[a]pyrene is related to polycyclic aromatic hydrocarbons (PAH), medroxyprogesterone acetate is related to progesteron ()PR), actinomycin D is related to P53 transcriptional activators (P53, genotoxicity) and curcumine is related to oxidative stress (Nrf2)

5.3.3 Formation of bromate

Bromate formation was tested in a separate experiment where bromide was spiked to the feed tank. Bromide concentrations after spiking were 0.32±0.039 mg/L for the samples with BAC pre-treatment (BO_3) and 0.47±0.011 mg/L for the samples without BAC pre-treatment (O_3).

Bromate concentrations in the BO_3 samples exceeded the lowest drinking water regulatory limit of 1 µg/L at a specific ozone dose of approximately 0.4 g O_3/g C (Figure 5.7). This is a concentration in a WWTP effluent, so before being relevant for drinking water there would be dilution of the concentration in surface and ground waters. Nevertheless, this does illustrate the risk of bromate formation in bromide containing effluents. Figure 5.7 shows that at the lowest specific ozone doses (< 0.4 g O_3/g C) bromate formation remained below the lowest regulatory limits (1 µg/L) and mostly even below the detection limit (0.2 µg/L). If the dose is very low (lower than the instantaneous ozone demand, or initial ozonation demand, defined as the decrease of ozone concentration in the first 30 seconds after dosing), all ozone is consumed by other reactions and bromate formation does not occur [253,254]. This means that as long as the ozone dose can be kept below the initial ozone demand, the risk for bromate formation can be limited.

Bromate formation is also influenced by the initial bromide concentrations. At higher ozone doses, higher bromide concentration (starting from 50 µg/L) result in higher formation of bromate [240]. On the other hand, especially at lower ozone doses, a higher initial bromide concentration can result in lower bromide formation because more ozone is consumed in the initial reaction of Br^- to $HOBr/OBr^-$ (increasing the initial ozone demand) after which insufficient ozone remains to continue the reaction from $HOBr/OBr^-$ to bromate [242,255]. This does mean that $HOBr/OBr^-$ will be remain in the water. $HOBr/OBr^-$ is a disinfectant used for swimming pools that can be converted back to bromide by adding hydrogen peroxide [240].

Figure 5.7 shows that bromate formation in the BO_3 samples is higher than in the O_3 in this study and in other studies that applied ozone treatment [45,256]. This underlines the importance of monitoring and limiting bromate formation by controlling the applied ozone dose. Additionally, it indicates that the availability of ozone (or ozone exposure) is higher for the BO_3 samples, even when the specific ozone dose is used and the ozone dose is corrected for the TOC removal. In other words, in the BAC filter, OM with higher ozone reactivity is removed to a higher extent than OM with average ozone reactivity. Therefore, TOC should not be used to decide the required amount of ozone for the BO_3 process because this can result in overdosing of ozone and in formation of bromate. Ozone doses standardized to absorbance at 254 nm and to fluorescence regions I and II (relating to protein like OM [105]) showed a better potential to predict bromate formation when comparing the samples with and without BAC pre-treatment (Figure S5.10). The remaining difference can be explained by the presence of nitrite (0.78 mg/L) and ammonium (2.0 mg/L) in the O_3 samples, which both were removed to below the detection limit during BAC filtration. Other studies also found that fluorescence indicators (e.g. humic- and vulvic acids) are valuable tools to predict bromate formation [257].

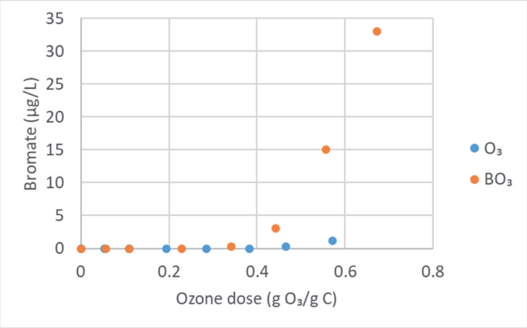


Figure 5.7 Bromate concentrations in ozonated samples with BAC pre-treatment (BO_3) and without BAC pre-treatment (O_3). Limit of detection was 0.2 µg/L and the measurement uncertainty of the method was 4%

5.3.4 Considerations for implementation

Pre-treatment before ozonation

The BO_3 process achieves higher MP removal and ecotoxicity reduction in WWTP effluent compared to conventional standalone ozonation (see also Figures S4-6). Moreover, the OM removal in the BAC filter results in a lower energy requirement for ozonation with BAC pre-treatment, as was discussed thoroughly in our previous paper [182]. Based on results from the bromate formation experiment, it is advised to apply low specific ozone doses (approximately 0.2 g O_3/g C) for treatment of bromide containing waters to avoid the formation of bromate. At this low ozone dose, the high removal potential and low energy requirements make implementation of the hybrid BO_3 treatment for MP removal from WWTP effluents more feasible and more effective than the conventional standalone ozone treatment.

Post treatment after ozonation

Based on the results from this study, ecotoxicity decreased to environmentally safe levels after treatment with the BO_3 process. This suggests that the formed OTPs during treatment had similar or lower toxic potential than the parent compounds present before treatment. Nevertheless, a number of studies recommend to implement a post treatment (for example biological sand filtration) after ozonation because of the worry for formation of OTPs that are more toxic than the parent compounds [45,190]. There are studies that find adverse effects on fish development of ozonated WWTP effluents [234] and even a decrease or increase in adverse effects on a single endpoint (genotoxicity) depending on the specific assay (UmuC or Ames) that was used [73]. It should be noted that the latter two studies used relatively high ozone doses and different feed waters (0.4-1.0 and 0.7 g O_3/g C respectively), which shows the difficulties of comparing ecotoxicological results from different studies. Other broader studies show that in the large majority of the cases, ozonation of WWTP effluents reduces ecotoxicity [46,92,249]. Because of the broad range of MPs spiked and the broad range of bioassays tested in this study, we argue that formation of toxic OTPs are not a risk for a BO_3 treatment applied at most municipal WWTPs. Still, factors such as bromide concentrations in the feed water and applied ozone doses play a big role. Finally, energy consumption and carbon footprint are also becoming more relevant factors to consider for the implementation of tertiary MP removal treatment. Adding an additional post-treatment after ozonation increases the energy demand and should only be used in cases where it is beneficial. For application at full-scale, especially in cases where more industrial water is treated in the WWTP, it is recommended to test the formation of toxic OTPs with a pilot scale plant first. Based on the results from such pilot tests it can be decided whether BO_3 treatment is sufficient, or an additional biological post-treatment (BO_3B) is required [75].

5.4 Conclusions

High micropollutant removal and reduction in ecotoxicity were observed after treatment of WWTP effluent with biological activated carbon filtration and ozonation (the BO_3 process). An average micropollutant removal of 89% was observed at an empty bed contact time in the BAC of approximately 1h and a specific ozone dose of approximately 0.2 g O_3/g C . Ecotoxicity was stable or reduced after ozonation which means that the ecotoxicity of the formed transformation products during ozonation was overall lower than the ecotoxicity of the parent compounds. We conclude that for the tested WWTP effluent and in the tested conditions a biological post-treatment the BO_3 process to remove the formed ozone transformation products is not required.

In the experiments where bromide was spiked to the feed, bromate formation was below all the Dutch regulatory limit (1 µg/L) at specific ozone doses lower than 0.4 g O_3/g C. Additionally, bromate formation in the BO_3 process was higher than in the reference samples with only ozonation and without BAC pre-treatment. This underlines the importance of monitoring bromide and organic matter concentrations in the feed of the ozone reactor to avoid overdosing of ozone which could result in the formation of bromate. Indications were found that absorbance at 254 nm and fluorescence peaks related to protein like compounds are a more reliable inline measurement used to standardize ozone doses compared to the conventional total organic carbon measurement.

Acknowledgement

This work was funded by the Dutch “Topsector Water” and Royal HaskoningDHV. We would like to thank BDS biodetection systems for their help with the CALUX assays and Het Waterlaboratorium for their help with the bromate analysis.

5.5 Supplementary information

5.5.1 Used micropollutants

Table S5.1 Used micropollutants (MPs) with their CAS numbers and predicted no effect concentrations (PNECs) found in the NORMAN database [258]

MP	CAS number	PNEC (ng/L)
Sulfamethoxazole	723-46-6	600
Diclofenac	15307-86-5	50
Trimethoprim	738-70-5	100000
Carbamazepine	298-46-4	50
Propranolol	525-66-6	410
Erythromycin	114-07-8	200
Furosemide	54-31-9	710
Sotalol	3930-20-9	6520
Atenolol	29122-68-7	150000
Caffeine	58-08-2	1200
4 and 5 methyl benzotriazole	136-85-6	150
Metoprolol	37350-58-6	8600
Dimetridazole	551-92-8	29500
Mecoprop	7085-19-0	900
Irbesartan	138402-11-6	704000
Benzotriazole	95-14-7	7770
2,4-D	94-75-7	600
DEET	134-62-3	88000
BAM	2008-58-4	78000
Desphenyl chloridazon	6339-19-1	250000

5.5.2 Bioassay protocols

Daphnia immobilization assay protocol

Materials

- 6-well plates
- Daphnia culture
- Daphnia culture medium (Table S5.2 and Table S5.3)
- Temperature control chamber (20 ˚C)
- Aluminum foil

- Light microscope
- 10 µM $CuSO_4$ in culture medium water as positive control (5 µM final concentration)

Exposure

- Select the juvenile daphnia using a 300 µm sieve
- Add 5 daphnia and approximately 2 mL old culture medium to each well
- Add 5 mL exposure medium to each well. Each plate contains: 1 blank (fresh culture medium), one positive control (5 µM $CUSO_4$ final concentration, diluted in fresh culture medium) and 4 replicates of one sample
- Fill each well with fresh culture medium up to 10 mL
- Cover all wells with aluminum foil and incubate for 48 h in the temperature control chamber (20 ˚C). Note that daphnia are not fed during exposure

Counting

- After the 48 hours of exposure, examine all plates/petri dishes under a light microscope, note the number of
- Moving daphnia
- Immobilized daphnia (animals that are not swimming in 15 seconds observation time after gentle agitation of the plate/petri dish, antennae movement is not counted as movement)
- Stressed daphnia (characterized by black eggs on their back, discoloration or trapping at the water surface)

Table S5.2 Elements in daphnia culture medium, diluted in MQ water

Compound	**Concentration in final medium (mg/L)**
TES buffer	85
$CaCl_2.2H_2O$	39
$NaNO_3$	50
$MgSO_4.7H_2O$	20
$Na_2SiO_3.5H_2O$	10
KCl	10
$CaCO_3$	13
$Ca(OH)_2$	30

Table S5.3 Trace elements in daphnia culture medium, diluted in MQ water

Compound	Concentration in final medium (µg/L)
Na_2-EDTA	500
H_3BO_3	572
$FeCl_3$	193.5
$MnCl_2$.4H2O	72
LiCl	87
KBr	7.5
$Na_2MoO_4.2H_2O$	12.5
$CuCl_2.H_2O$	6.5
$CoCl_2.6H_2O$	20
KI	0.6
$Na_2SeO_3.5H_2O$	0.2

Algae growth inhibition protocol

Materials

- White 96-well plates
- Green algae culture, use algae 3 days after culturing to ensure that they are in exponential growth phase
- Algae growth medium (Table S5.4 and Table S5.5)
- 400 µM CuSO4 in MQ water as positive control (200 µM final concentration)
- Plate reader to measure fluorescence at λemission = 670 nm and λexcitation = 440 nm
- Shaking incubator (90 rpm, 20 ˚C with light source)

Exposure medium

- Add 100 µL blank (algae medium) to the dilution and the blank wells
- Add 200 µL positive control (400 µM CuSO4) to six wells in each plate
- Add 200 µL sample to the undiluted sample wells in the plate
- Transfer 100 µL of the undiluted well (for samples and for pos) to the 2x diluted well, mix by pipetting and continue the (twofold) dilution series

Exposure and measurements

- Dilute the algae to 2·104 cells / mL (in algae growth medium)
- Add 100 µL algae solution (2·104 cells / mL) to each well (with a multi pipet) to a total of 200 µL / well

- Immediately measure fluorescence at the plate reader at λemission = 670 nm and λexcitation = 440 nm
- Incubate the algae in the shaker and measure again after 24, 48 and 72 hours

Data processing

Calculate the area below the growth curves with the following equation:

$$A = \frac{N1 - N0}{2} * t1 + \frac{N1 + N2 - 2N0}{2} * (t2 - t1) + \frac{N2 + N3 - 2N0}{2} * (t3 - t2)$$

, where A is the area below the growth curve, N is the number of cells and t is the time (0-3 would be 0-72 hours)

The growth inhibition is calculated as the difference in area between the control and the samples with the following equation:

$$Is = \frac{Ac - As}{Ac} * 100$$

, where I is the growth inhibition of sample s, Ac is the average area of the controls in the plate and As is the area of the sample.

Table S5.4 Elements in algae growth medium

Compound	Concentration in final medium (mg/L)
K_2HPO_4	8.7
$NaNO_3$	85.0
$MgSO_4.7H_2O$	37.0
$CaCl_2.2H_2O$	36.8
$NaHCO_3$	12.6
$Na_2SiO_3.9H_2O$	28.4
H_3BO_3	24.0

Table S5.5 Trace elements in algae growth medium

Compound	Concentration in ATE stock (mg/L)
Na_2-EDTA.2H_2O	4.36
$FeCl_3.6H_2O$	1.00
$MnCl_2.4H_2O$	0.18
$CuSO_4.5H_2O$	0.001
$ZnSO_4.7H_2O$	0.022
CoCl2.6H_2O	0.012
$NaMoO_4.2H_2O$	0.22
H_2SeO_3	0.0016
Na_3VO_4	0.0018
Biotin	$0.51 \cdot 10^{-3}$
B_{12}	$0.55 \cdot 10^{-3}$
Thiamine HCl	0.10

Microtox assay protocol

Materials

- White 96 well plates
- 220 g/L NaCl solution in MQ water (22%)
- 22 g/L NaCl solution in MQ water (2.2%)
- 400 µM CuSO4 in MQ water with 2.2% NaCl as positive control (200 µM final concentration)
- Vibrio Fischeri bacteria stored at - 20˚C

- Reconstitution liquid
- Dispenser
- Plate reader for luminescence measurement

Exposure medium

- In each plate, add 100 µL blank (2.2% NaCl in MQ water) and 100 µL positive control. 6 wells for each, blank and POS
- Add 180 µL sample and 20 µL 22% NaCl to each undiluted sample well and 100 µL 2.2% NaCl to all dilution wells
- Dilute the wells with sample into the wells with 100 µL 2.2% NaCl

Exposure and measurements

- Pipet 1 mL reconstitution solution into one bacteria container, shake a few times
- Add the bacteria solution to the dispenser and dilute 100 times with 2.2% NaCl
- Immediately add reconstitution fluid at 4˚C (6.2 µL reconstitution fluid per mg bacteria) and mix
- Dispense the bacteria and measure luminescence
- Insert the 96 well plate with the samples, blanks and positive controls
- Start the measurement
- Let the machine dispense 100 µL in each well of the plate
- Shake for 10 s and measure luminescence, measure again 15 minutes after dispensing has finished

Data processing

Calculate the correction factor of the plate (luminescence of blank at time 15 / luminescence of blank at time 0) for all blanks on the plate and take the average (KF)

Calculate the luminescence inhibition with the following equation:

$$I = 100 - 100 * (\frac{L_{15}}{KF * L_0})$$

, where I is the inhibition of the sample in %, L_{15} is the luminescence of the sample at t=15, L_0 is the luminescence of the sample at t=0 and KF is the correction factor (as calculated above).

5.5.3 Removal of organic matter and micropollutants in the BAC

Table S5.6 Average OM removal in the BAC filter. Averages are based on triplicates. See Table S5.7 for standard deviations

Flowrate (L/h)	3.62	0.91	0.37
COD	19	27	29
UV254	20	42	31
TOC	19	30	21

Table S5.7 Standard deviations of OM removal in the BAC filter. NA means not analyzed

Flowrate (L/h)	3.62	0.91	0.37
COD	1	NA	4
UV254	0	2	3
TOC	1	9	7

Table S5.8 Standard deviations of MP removal in the BAC filter at three flowrates. Standard deviation is shown in % and based on triplicate measurements. NA means not analyzed

Bioreactor	**BAC**		
Flowrate (L/h)	3.62	0.91	0.37
Sulfamethoxazole	12.3	3.0	1.0
Diclofenac	6.2	0.0	0.6
Trimethoprim	1.3	0.3	0.5
Carbamazepine	5.7	4.4	2.0
Propranolol	0.7	0.3	0.1
Erythromycin	5.5	0.7	0.4
Furosemide	6.2	0.4	0.2
Clarithromycin	2.4	0.4	1.7
Sotalol	2.1	0.4	0.1
Atenolol	1.4	0.6	0.1
Caffeine	1.3	1.3	NA
4 and 5 methylbenzotriazole	3.1	1.7	0.5
Metoprolol	4.1	0.6	1.3
Dimetridazole	3.7	5.5	0.6
Mecoprop	4.8	4.5	0.4
Irbesartan	12.3	2.6	2.2
Benzotriazole	9.5	5.2	0.0
2,4-D	3.9	4.1	1.3
DEET	6.7	4.0	1.0
BAM	5.9	5.9	1.3

5.5.4 Toxicity of organic transformation products

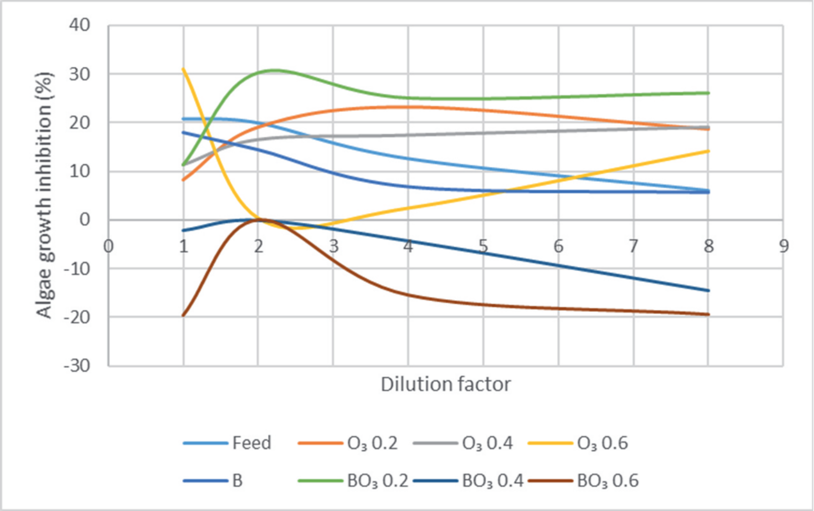


Figure S5.8 Algae growth inhibition of the ozonated samples with and without BAC pre-treatment at the different tested dilution factors. Datapoints are averages of three triplicates. Average standard deviation over all samples was 6.1%

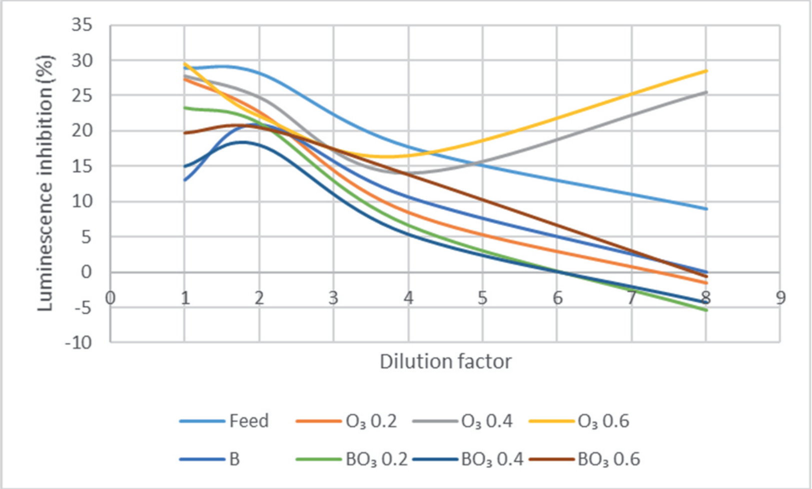


Figure S5.9 Luminescence inhibition of the ozonated samples with and without BAC pre-treatment at the different tested dilution factors. Datapoints are averages of three triplicates. Average standard deviation over all samples was 5.2%

Table S5.9 Effect based trigger values (EBTs) and limits of quantification (LoQs) of the used CALUX assays. Data taken from Escher et al. [238], van der Oost et al. [245] and de Baat et al. [252]. NF means not found

Assay name	EBT	LoQ	Unit
ERa CALUX	14	0.02	ng 17b Estradiol eq./l
GR CALUX	100	8.5	ng Dexamethasone eq./l
PAH CALUX	62.1	1	ng Benzo[a]pyrene eq./l
P53 CALUX	NF	0.0069	ug Actinomycin D eq./l
PR CALUX	NF	1.1	ng Medroxyprogesterone acetate eq./l
Nrf2 CALUX	6.2	10	ug Curcumine eq./l

5.5.5 Formation of bromate

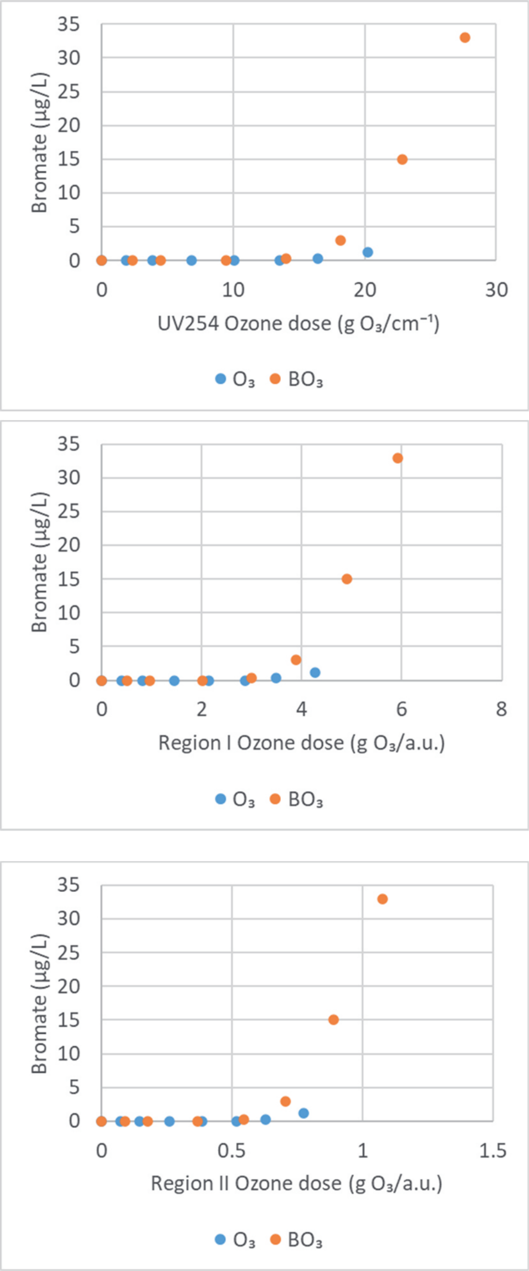


Figure S5.10 Bromate formation related to ozone dose standardized to different absorbance and fluorescence measurements. a.u. means arbitrary fluorescence units. Fluorescence regions I and II are calculated according to Chen et al. [105]

5.5.6 Micropollutant removal in toxicity and bromate experiments

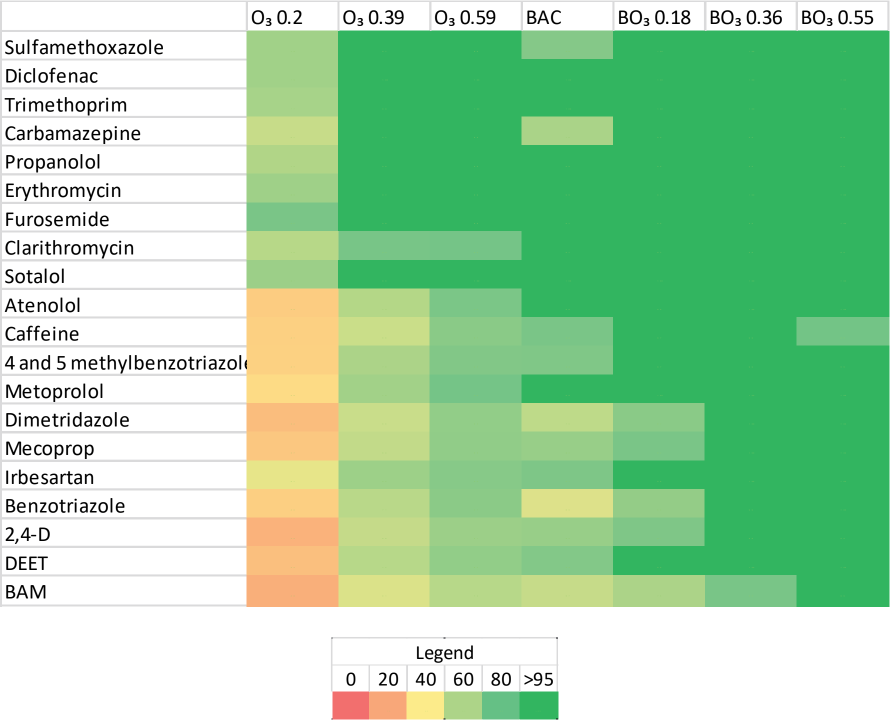


Figure S5.11 Heatmap for MP removal in the BAC filter Heatmap for MP removal in the ozonated samples with (BO_3) and without (O_3) BAC pre-treatment for the flowrate experiments at a flowrate of 0.91L/h. Removal in % is shown with a color scale from 0 (red) to >95 (green).NA means not analyzed. For the samples with BAC pre-treatment averages are based on triplicates, average standard deviation was 1.0 % for all samples combined. Samples without BAC pre-treatment are single measurements

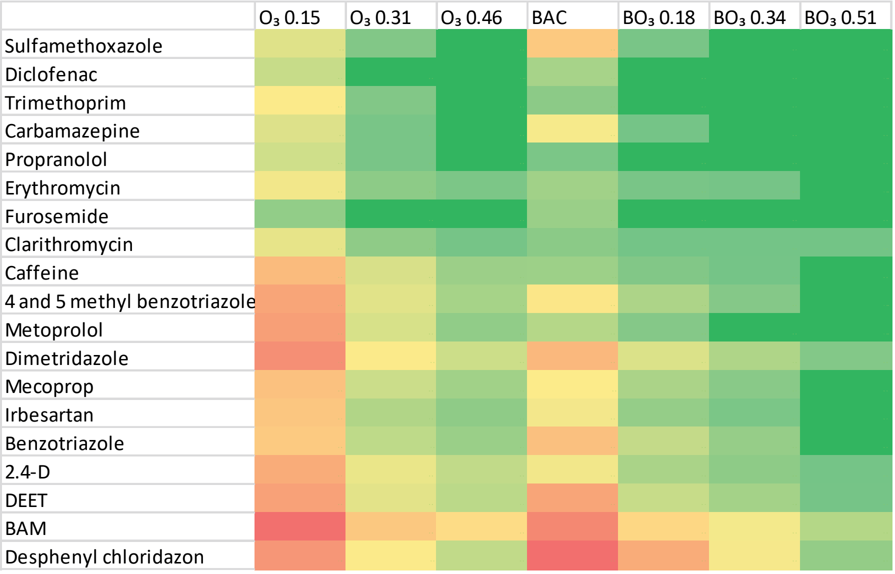


Figure S5.12 Heatmap for MP removal in the ozonated samples with (BO_3) and without (O_3) BAC pre-treatment for the organic transformation products experiment. Flowrate was 1.01 L/h. Removal in % is shown with a color scale from 0 (red) to >95 (green). Measured ozone doses in g O_3/g C are shown in the column names

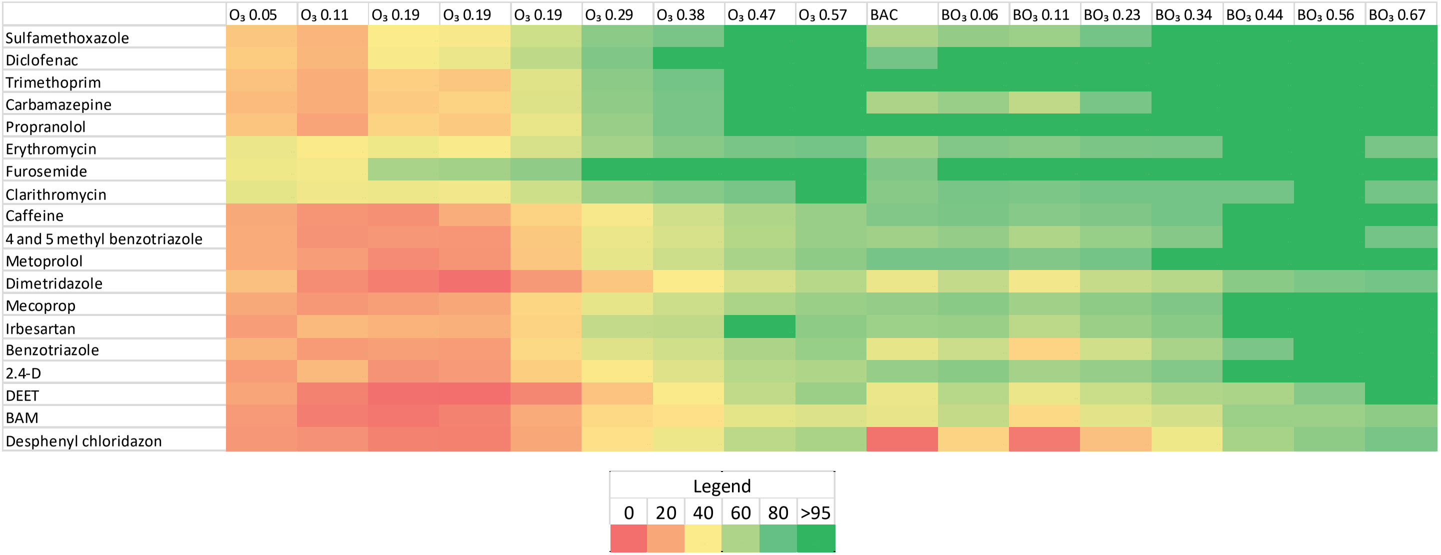
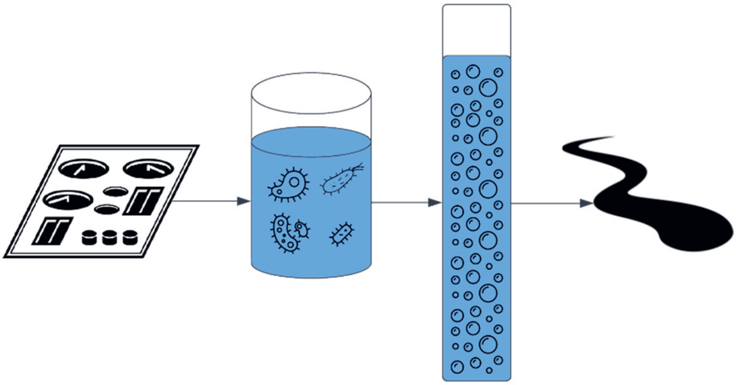


Figure S5.13 Heatmap for MP removal in the ozonated samples with (BO_3) and without (O_3) BAC pre-treatment for the bromate formation experiment. Flowrate was 0.99 L/h. Removal in % is shown with a color scale from 0 (red) to >95 (green). Measured ozone doses in g O_3/g C are shown in the column names

Chapter 6

General discussion



6.1 Main findings

In this thesis, the synergies between biological treatment and ozonation (BO_3) for the removal of micropollutants (MPs) from WWTP effluents are described. It was found that a biological activated carbon (BAC) reactor operated with pre-aeration using pure oxygen has the potential to remove organic matter (OM) from WWTP effluent and therefore reduce the required dose during subsequent ozonation. The retention time of the WWTP effluent in the BAC reactor controlled the extent of removal of both OM and MPs (Chapter 2 and Chapter 5). It was also found that the type of OM impacts its interference with the ozonation of MPs. Specific absorbance and fluorescence characteristics correlated well with the MP removal in effluents from different wastewater treatment plants (WWTPs) and in different OM fractions (Chapter 3 and 4). Indications were found that the BAC is specifically removing these more interfering fractions of the OM. This means that low ozone doses (around 0.2 g O_3/g C) are sufficient to achieve high MP removal when ozonation is combined with a BAC pretreatment. At ozone doses from 0.2-0.6 g O_3/g C in the used municipal WWTP effluent, toxicity decreased or was stable during BAC and ozone treatment for the tested bioassays, which suggests that the formed ozone transformation products (OTPs) during the BO_3 process were less toxic than the parent compounds. In the experiment with bromide spiking, no formation of bromate (< 0.2 µg/L) was observed at the low ozone doses (< 0.3 g O_3/g C), but at higher doses bromate could exceed European regulatory limits if bromide is present in the WWTP effluent (Chapter 5). To summarize, the BO_3 process has shown its potential at laboratory scale to achieve high MP removal, with low required energy input, while reducing the toxicity of the produced effluent.

The studies described in this thesis have increased the understanding on the synergies between biological treatment and ozonation. Insights from the chapters in this thesis can be used to optimize the operation of a BO_3 or BO_3B setup (Figure 6.1). Chapter 2 gives insight in the first B reactor, Chapter 3 and Chapter 4 contribute to understanding of how ozone dosing can be controlled in the O_3 reactor and Chapter 5 discusses whether it is beneficial to apply a second B reactor. Additionally, the results of the studies described in this thesis can be utilized in a broader context. Results from Chapter 2 are beneficial for other OM removal applications (e.g. for water reuse or as biological pre-treatment for other advanced treatments). The concepts and experiments applied in Chapter 3 and Chapter 4 can be utilized for further experiments with other MP removal technologies such as AC filtration and membrane filtration. Finally, insights from Chapter 5 can lead to further discussions on the need for post-treatment after ozone and other advanced oxidation treatments.

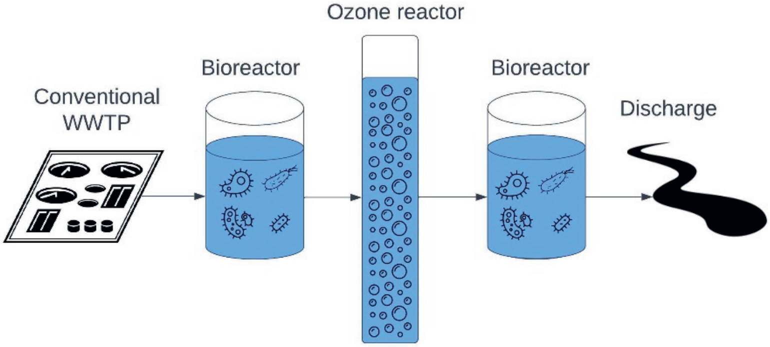


Figure 6.1 Schematic overview of the bio ozone bio (BO$_3$B) process (adapted from de wilt et al. [75])

6.2 Experimental considerations

For all experiments, selections had to be made regarding the conditions that are applied during the experiments, for the number and type of replicates that were included, and which parameters are used to make useful comparisons. Before going into the limitations, future research and the potential for application, the considerations that were made for the experiments that are described in this thesis are discussed.

Especially for applied research, a balance has to be made between how realistic the experiments are and to what extent the variables in the experiments are controlled. In all experiments described in this thesis, real WWTP effluent was used, which was collected during dry weather conditions and spiked with MP at realistic concentrations of approximately 1 µg/L. Real WWTP effluent was selected because the complexity of OM in WWTP effluent is practically impossible to simulate with a synthetic water. The disadvantage of using real WWTP effluent is that the composition changes over time which makes it challenging to compare results from different dates. Therefore, dry weather conditions were selected to improve the stability of the background OM concentrations and MPs were spiked to ensure the presence of a broad list of MPs at detectable concentrations. This setup made sure that it was possible to make comparisons between experiments at different timepoints while working with a realistic and relevant matrix.

The number and type of replicates that are be included in experiments depends on the type of the experiments that are performed. For the work described in this thesis, triplicates were used to determine the stability of the fractionation procedures in Chapter 3 and Chapter 4, time trends were used to check the stabilities of the removals in the bioreactors in Chapter 2 and Chapter 5, and ozone trends (of increasing ozone doses) were used throughout the experiments. For the fractionation experiments, triplicates were used to focus only on the stability of the fractionation procedure and exclude all other variations. Using triplicates is an ideal way to test the stability of experimental methods, but it has less added value when working with continuous reactors. In the case of continuous reactors, time trends give more insight in the stability of the system. In all experiments, ozone trends were preferred over replicates at a single ozone dose because such trends give additional insight in the removal performance at different ozone doses while also showing the stability of the experiments. To sum up, trends are generally preferable over normal replicates because they can provide additional insights in the effects of the trend variable. Nevertheless, trends should only be used when a large number (>3) of points on the trend can be used or if the stability of the process is already well understood.

For the interpretation of the results discussed in this thesis, comparisons had to be made between different types of bioreactors and between ozonation of different wastewaters. In Chapter 2 a BAC filter, a sand filter and a MBBR were compared for their OM removal potential. For a useful comparison, similar total reactor volumes (so not just liquid volume) were selected because reactor size is related to the investment costs for full-scale reactors. It was also considered to make a comparison based on (potential) biofilm area, but it was not possible to quantify this reliably, especially when used to compare different reactor types. To compare different ozonation efficiencies, ozone doses used were based on total organic matter (TOC) concentrations because this is the conventionally used unit (g O_3/g C). Nevertheless, absolute ozone doses (mg O_3/L) can be more useful in situations where total ozone production has to be compared (e.g. comparing stand-alone ozonation to the BO_3 process). An ozone dosed standardized to absorbance at 254 nm (UV254 in g O_3/nm^{-1}) would be useful to quantify ozone doses. The reason is that UV254 is more specific in quantifying ozone reactive OM and is also easier to measure and in my experience more stable than TOC. To be able to use such an UV254 based ozone dose to compare different studies, it is necessary to find a reference compound to calibrate the unit of the UV254 measurement and standardize for differences between spectrophotometers. For example using phenol at a concentration of 1mM as a calibration standard for the UV254 measurement.

6.3 Limitations and future research

In the following paragraphs, the limitations of the work described in this thesis are discussed. There is a focus on subjects that are important to consider in similar studies and I give several suggestions of experiments that are, following up on the research described in this thesis, interesting to continue with.

6.3.1 Removal mechanisms in the BAC filter

Chapter 2 shows that the BAC filter achieved more than two times higher OM and MP removal than the tested sand filter and moving bed bioreactor. As also discussed in Chapter 2 and Chapter 5, the high number of treated BVs shows the long-term potential of a BAC filter. This is an indication that biological degradation plays a significant role in the total removal in this filter. After more than 25,000 BVs of treated WWTP effluent, the BAC filter still shows similar OM removal compared to when the experiments just started, when the AC had only been used for 38,000 BVs in drinking water treatment (Figure 6.2). Nevertheless, also sorption and/or advanced oxidation processes (by reaction of oxygen with the AC surface into radicals [152,154]) could contribute to the total observed removal. The exact contributions of all these processes could not be ascertained in this study.

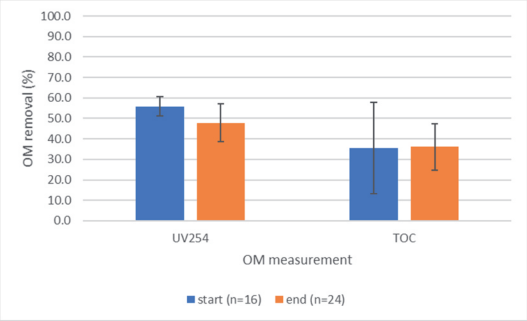


Figure 6.2 Comparison of OM removal in the BAC at the start of the experiments (first year) and at the end of the experiments (3.5 years and 26000 BVs later), experimental conditions were the same as in Chapter 2. Absorbance at 254 nm (UV254) and TOC measurements are used to quantity the OM removal because of their relevance for ozonation. n indicates the number of measurements that the averages and standard deviations are based on. EBCTs were approximately 1 h during both periods

More information on the removal mechanisms contributing to the total removal could be gained with other types of experiments and analytical methods. Using labeled carbon (14C) MPs, transformation and mineralization of the MPs can be traced [259]. By measuring how much of the labeled carbon remains in the parent compounds that are present in the treated effluent, how much is converted into other compounds in the treated effluent, and how much is converted to carbon dioxide gas, a mass balance can be made in which the leftover fraction has to be still present (and probably absorbed) in the BAC. This is assuming that all the stable OTPs that are formed can also be detected. Such experiments with radioactive labeled MPs are costly, but have the advantage that they can be performed under realistic conditions (i.e. with realistic MP concentrations). A limitation of this type of experiment is that it cannot distinguish between biodegradation and chemical oxidation by radicals that could be formed on the AC surface.

The contribution of chemical oxidation in the BAC filter could be studied by adding a scavenger to the feed water to block the radical pathway and compare the removal in the presence of a scavenger to a control where the radical pathways is available. Nielsen et al. [152] showed that oxidation of MPs on the AC surface mainly occurs through the formation of superoxide radicals (O_2^-). Therefore, a superoxide radical scavenger such as tempol (4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), could be added to the feed to ensure that chemical oxidation through superoxide does not occur [260]. For such an experiment, the effect of the superoxide scavenger on the sorption and biodegradation should be tested first. By combining the experiments with radiolabeled isotopes and radical scavengers, it would be possible to quantify the contribution of all three removal mechanisms in the BAC filter.

6.3.2 Removal mechanisms in the ozone reactor

Reactions of ozone with OM and MPs can be direct, with the ozone molecules, or indirect, with radicals (such as ·OH radicals) that are formed during the decomposition of ozone. The relevance of the radical pathway mainly depends on the reactivities of the pollutants with ozone and with radicals and on the ozone exposure time which is related to the applied ozone dose [61,261–263]. The studies described in this thesis did not include experiments with radical scavengers, that can be used to quantify the contributions of direct and indirect ozonation. The reason for this was that at the low ozone doses that are used in the BO_3 process (around 0.2 g O_3/g C), ozone exposure times are low and direct ozonation is expected to be the dominant pathway [180,261]. Nevertheless, putting the results of Chapter 3 and Chapter 4 in a broader context (e.g. of also standalone ozonation, where higher ozone doses are applied), makes it interesting to find out what the effects of different OM fractions are on the contributions of the direct and indirect ozonation to the observed MP removal. Although especially for the MPs with lower ozone reactivity (kO_3), removal via radical pathways can be expected, these MPs did not show differences in their removal in the different fractions in Chapter 3 and Chapter 4. This indicates that the overall contribution of radicals was similar in the different fractions. It seems counterintuitive that other studies do find effects of for example size fractions on the formation of ·OH radicals. How it is possible that different OM fractions have different effects on formation of radicals, but not on the removal of low ozone reactive MPs (that are mainly removed via radicals) could be tested in an additional experiment. This experiment could focus on a single WWTP effluent, because the OM fractions and MP removal were similar in the different effluents in Chapter 4, and use radical scavengers and ozone exposure time measurements to distinguish the contributions of direct ozonation and radical pathways to the total removal.

6.3.3 Effluent organic matter composition

Results from Chapter 3 and Chapter 4 have given new insights in the presence of different OM types in WWTP effluents and how these different OM types affect the ozonation of MPs.

Nevertheless, these studies only gave an initial look in a relatively unexplored area of science. It was shown that the resin fractions hydrophobic acids and hydrophilics have a higher interference with the ozonation of MPs, while the hydrophobic neutrals showed a lower interference (for the MPs with medium and high ozone reactivity). This observation was consistent for all five investigated effluents and also the fraction composition of the different effluents was similar. Fractionating EfOM (or other dissolved organic matter) and using the produced fractions for further experiments is a powerful tool to gain insight into the complex OM matrix in (waste)water samples. The sensitivity of the size fractionation process is indicated by differences in results between Chapter 3 and Chapter 4, where the fraction < 1 kDa showed low interference in Chapter 3 and medium interference in Chapter 4, and other studies [181]. Therefore, for fractionation experiments replicates and quality controls should always be included (i.e. to show the reproducibility of the fractionation process specifically and not only to show the reproducibility of the analysis) and produced fractions should be characterized with additional methods (such as size exclusion chromatography and fluorescence measurements) to ensure the reliability and comparability of different methods.

A novel method to gain more detailed insight in the characteristics of OM fractions or samples is by using MS fingerprinting. By using Fourier-transform ion cyclotron resonance mass spectrometry (FT ICR MS) or by combining liquid chromatography to quadrupole mass spectrometry, thousands or more features can be detected that can be traced back to specific molecules using advanced statistics and large online databases [101,264]. FT ICR MS has been used to elucidate the transformations that occur during ozonation [265]. Additionally, it would be interesting to use such fingerprinting methods to get more detailed insight in the composition of different WWTP effluents and different resin fractions, to understand better why they affect the ozonation of MPs in different ways and to better define the OM interference groups that were found in Chapter 4. Additionally, the hydrophilics resin fraction can be fractionated further (into hydrophilic acids, bases and neutrals) using different types of resins [78,103]. Similar experiments as were described in Chapter 4 could be done with these more specific hydrophilics fractions to find out whether hydrophilic neutrals also have lower interference with MP ozonation (like the hydrophobic neutrals show lower interference) or whether the other hydrophilic fractions show interesting behavior.

There are two other novel topics related to advanced OM characterization that are interesting for future studies: i. how the OM composition changes during BAC filtration (building further on the insights from Chapter 5) and ii. how the OM composition of WWTP effluents varies over time (building further on the insights from Chapter 4). Chapter 5 shows that bromate formation is different in the ozonated water with BAC pre-treatment than in the ozonation water without BAC pre-treatment. This difference could not be explained by the change in TOC concentration alone, nor could it be completely explained by any of the other measured absorbance and fluorescence characteristics. This means that more research into the change of OM composition during BAC filtration, using for example resin fractions or MS fingerprinting techniques, could help to give a more complete understanding of which parts of the OM consume ozone fastest and therefore determine the presence or absence of bromate formation during ozonation of bromide containing waters. Additionally, such a study would also give insight in which parts of EfOM are biodegradable and absorbable and which parts are not. While Chapter 4 compared effluents of five WWTPs, all samples were taken in similar conditions (24h mix samples, dry weather conditions, same season). Other studies show that temporal changes such as rain events, have a large impact on the wastewater influent and the processes in conventional WWTPs, and therefore also on the treated effluent [266,267]. This raises the question how these changes affect the OM composition in WWTP effluents and how these changes in composition affect the processes in the BAC filtration and ozonation treatments. Insights into the effect of temporal variation on OM characteristics in WWTP effluent, could also aid in the effective and efficient implementation of MP removal treatments.

6.3.4 Toxicity of micropollutants and transformation products

To be able to know whether any MP removal treatment actually improves the environmental quality of the treated water, toxicological monitoring using bioassays for MP studies is important. Because of the large number of MPs present in wastewaters it is certain that not all of them can be detected chemically. Therefore, using bioassays, the toxicological relevance of samples can be monitored and if a sample poses a toxicological threat, more detailed chemical studies (non-target screening [232]) can be done to try to identify which compounds cause the toxicological hazard. Using bioassays to determine the usefulness of intensive treatment will become more important in the future when considerations have to be made whether applying advanced treatment is necessary to improve water quality or limiting energy use and CO_2 footprint is more important.

One of the main challenges that hinders the application of bioassays is that interpreting the results of the large list of available bioassays and endpoints can be complex. Especially standardization would be an important tool to overcome this challenge. By using bioanalytical equivalent concentrations (BEQs) and effect-based trigger values (EBTs), the interpretation of bioassays can be simplified [238]. In a BEQ, the toxicological response of a sample is related to a reference compound (by dividing the EC50 of the sample by the EC50 of the reference compound). Then, the BEQ can be divided by the EBT (which indicates the concentration at which the reference compound becomes toxicologically relevant, these values are made for environmental toxicity and humane toxicity separately) that directly shows whether there is a toxicological risk (ratio >1) or not (ratio <1) [238,245,252]. This approach can aid a broad group of users to interpret bioassay results. Nevertheless, the approach also has a disadvantage. Because an EC50 of the sample is needed, in the cases where the direct toxicity of the samples is not very high (which is the case for most WWTP effluents), extraction and concentration is needed to make a full dose response curve and calculate the EC50. In the process of extraction and concentration, unknown compounds can be lost (because the recovery of an unknown compound cannot be tested), while these unknown compounds are the main reason why bioassays are needed. Especially in the case of ozone transformation products (OTP)s, where the OTPs are expected to be more polar and harder to recover in extraction, this can be a serious issue because the non-recovered compounds are not included in the toxicity tests [85]. Therefore, also direct effluent testing (without extraction and concentration) should be applied. Tools to help with the interpretation of direct effluent tests are as far as I know, still lacking.

The broad range of bioassays that exists can also be a limitation. Because different studies use different bioassays (and often only one or a few), it is challenging to compare results of different studies. I suggest that standard lists of bioassays should be made for different applications (for example one for the ozonation and OTPs in WWTP effluent). This way, scientists or engineers who want to test the toxicity of an ozone treated WWTP effluent can easily select which bioassays to use. That means that initially, several extensive toxicological studies are needed to determine which assays are the most relevant to include. This strategy ensures that results of different studies are easier to compare, which improves the reliability of the results. This more structured approach to test for the toxicity of ozonation waters will aid engineers to decide whether post-treatment after ozonation is required (making a BO_3B treatment [75]) or post-treatment is not required where a BO_3 process (Chapter 5) is the preferred option.

6.4 Potential for application

The following paragraphs put the BO_3 process in context by comparing it to other technologies that aim to remove MPs from WWTP effluents, and discuss the most relevant aspects that should be considered when such a hybrid technology is implemented in practice.

6.4.1 Comparison of BO_3 with other technologies

The BO_3 process has several advantages over other single and hybrid technologies to remove MPs from WWTP effluents. The main advantage is related to the limitation of most other effective MP removal treatments: energy use and operational costs. Chapter 5 shows that using BO_3 process, high removal can be reached (average around 90%) for a broad range of MPs at a much lower ozone dose (0.2 g O_3/g C) than is used for conventional ozonation. For a standalone ozone treatment, ozone doses of around 0.7 g O_3/g C and sometimes over 1 g O_3/g C are typically applied [45,62]. Moreover, the OM that is removed in the BAC filter reduces the required amount of ozone even further. Chapter 3 shows that OM can be removed over 50%, which again cuts the required amount of ozone in half. Considering that the generation of ozone (and of pure oxygen, used to generate ozone) comprises 40% of the total cost (operational and investment) and 80% of the CO_2 footprint of conventional ozonation, the large reduction in required ozone demand reduces the costs and CO_2 footprint of the BO_3 process by 33-36% and 64-72% respectively, compared to stand alone ozone treatment [157,158,268].

Another advantage of the low applied ozone dose is that the ozone reactor can be smaller, which also reduces the investment costs of the treatment. A low applied ozone doses means that both the mass transfer of the ozone from the gas to the water and that reactions of ozone with OM and MPs are completed faster. To give a rough estimate for the reaction times, Chapter 5 showed that the ozone dose can stay below the initial (or instantaneous) ozone demand of the treated water, which means that the ozone reacts completely in less than 30 seconds. This reduces the required contact time in the ozone reactor (which is generally 10-40 minutes [45]) resulting in a smaller required reactor volume. A smaller reactor lowers the investment cost for the treatment, reducing the total costs of the BO_3 process even further.

The main advantage of the BAC filter is that it is operated as a biological filter, so the activated carbon (AC) in the reactor is not regenerated or replaced, which is the main cost and energy consumer for standalone AC treatment [158]. Chapter 3 and Chapter 5 show that the BAC filter can achieve high OM and MP removal even when non-biological AC filters show breakthrough. Removal performance of AC filters depends on several factors, such as the empty bed contact time (EBCT), the amount of water AC that it has treated (expressed in the number of bed volumes (BVs) that it has treated) and the influent concentrations of OM and MPs. Altmann et al. [269] observed breakthrough of OM and poorly adsorbable MPs after 5,000 bed BVs and breakthrough of easily adsorbable MPs after 10,000 BVs. Benstoem et al. [131] reviewed 44 studies into granular AC treatment of WWTP effluents and observed both OM and MP breakthroughs (of <20% removal) in the large majority of the studies after 10,000 BVs. The BAC filter in this thesis was operated for 25,000 BVs in WWTP effluent treatment after the 38,000 BVs in drinking water production (this is the situation in Chapter 5), without any regeneration or replacement, and still shows OM removal of up to 50% and removal of many MPs around 90%. This means that, even though it was not possible to quantify the contributions of different removal pathways in Chapter 2, the long-term use potential of the BAC has been confirmed. The most likely reasons for this difference of the BAC with conventional AC filtration are the high retention time (approx. 1h) in the BAC filter and possibly the pre-aeration with pure oxygen that ensures very high (approx. 30 mg/L) oxygen concentrations in the BAC filter influent. When BAC filtration is combined with ozonation the off gas from the ozonation can be used to aerate the feed water of the BAC which limits the additional need for pure oxygen.

Another advantage of the BAC filter is that nitrite is removed to a high extent (Figure 6.3). The removal of nitrite is beneficial for the ozonation process because nitrite is (next to OM) a scavenger of ozone [44]. Ammonium is also removed to a high extent in the BAC (data not shown), which can be expected from aerobic biofilters [270]. It should be noted that very high ammonium concentrations in the BAC feed (> 4 mg NH_4^+/L) can result in an increase in nitrite in the BAC effluent because of incomplete nitrification (data not shown). Ammonium does not affect the ozonation of MPs, but it is a general water quality parameter and has to be almost completely removed in WWTPs to avoid algae blooms in receiving waters. On the other hand, the presence of ammonium could be an advantage in ozone treatment because it reduces the formation of bromate [271]. Nevertheless, as is shown in Chapter 5, the formation of bromate can also be prevented without the presence of ammonium by applying low ozone doses (roughly < 0.4 gO_3/g C), which is the preferred option because of the effects of ammonium on water quality.

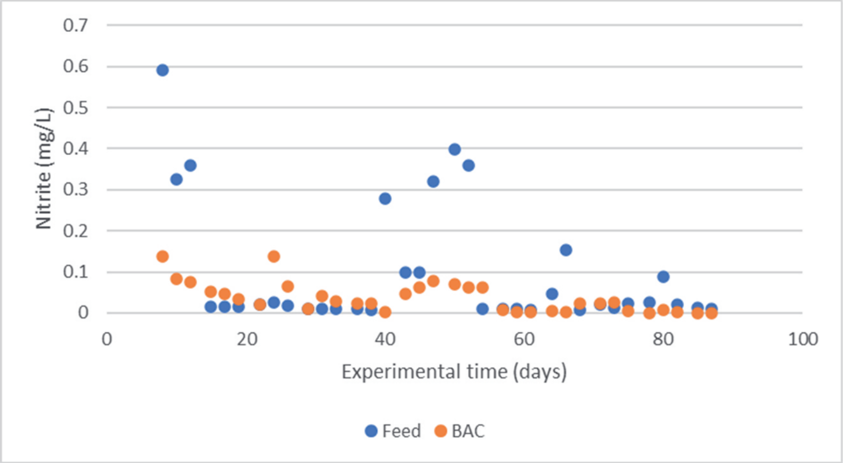


Figure 6.3 Nitrite in the feed and BAC effluent during the experiments in Chapter 3. Limit of quantification (LoQ) was 0.1 mg/L

The high achieved MP removal at low energy inputs of the BO_3 process compared to other MP removal treatments is evident in the feasibility study done by STOWA, the Netherlands in the last years [272]. In this study, data from literature and laboratory scale experiments were used to estimate removal of a specific list of MPs and energy use (expressed and CO_2 footprint) in full-scale. The results are presented in Figure 6.4, where the most promising treatment technologies for MP removal from WWTP effluents were compared based on their MP removal potential and their CO_2 footprints to select which of them should be tested in pilot scale. The BO_3 process (shown as Bio+O3 in Figure 6.4) has been selected to receive funding for pilot tests from STOWA and these pilots are currently ongoing because it showed better potential performance than the three reference technologies (powder activated carbon, ozonation and granular activated carbon).

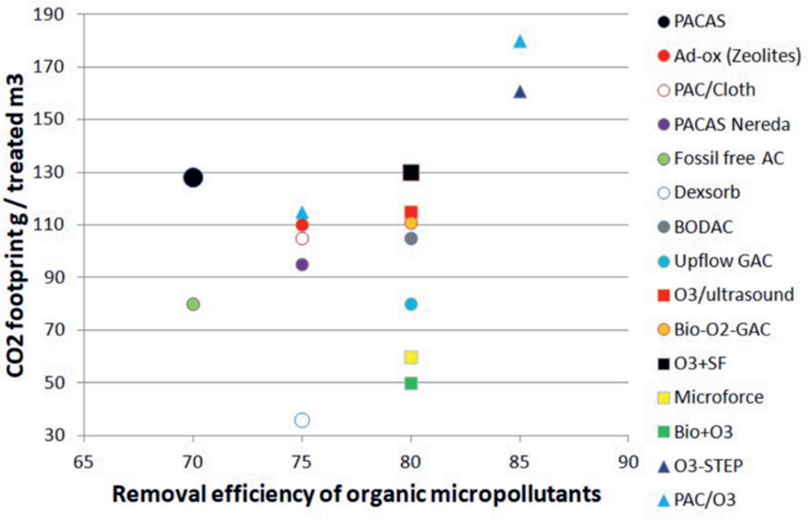


Figure 6.4 Results from the feasibility study for removal of MPs from WWTP effluents from STOWA (the research institute of the Dutch waterboards). 15 technologies were compared based on their MP removal potential and their CO_2 footprint. The BO_3 process is shown in the green square as Bio+O3 [272]

6.4.2 Monitoring organic matter characteristics

All chapters in this thesis underline the importance of the characteristics of OM (e.g. absorbance and fluorescence parameters, resin fractions, size exclusion chromatography) in WWTP effluents for the implementation of ozonation to remove MPs. Other studies have shown the importance of OM characteristics for other advanced treatment processes such as AC filtration, membrane treatment and advanced oxidation processes (AOPs) that aim to remove MPs from different water matrixes. For AC filtration, both in drinking water and wastewater conditions, humic-like compounds interfere less with MP sorption than other types of OM [273,274]. For membrane filtration, the size and properties of the OM, and the interaction between different OM fractions affect the membrane fouling [275] and mainly hydrophilic and hydrophobic acid fractions accumulate on the membranes [276]. Also for other AOPs than ozone (e.g. photocatalysis treatment), OM can act both as inhibitor and as promotor of MP removal, depending of the characteristics of the OM. As MP removal treatments are applied more and more, structured information about OM characteristics of the waters that have to be treated becomes more valuable. Such information can be used to decide which locations are most suitable for which MP removal treatments and can teach us more about process control and temporal/seasonal effects on the MP removal processes.

Unfortunately, currently mainly chemical oxygen demand (COD) and sporadically TOC are measured in conventional WWTPs, which underlines the importance of expanding the generally applied OM characterization methods in WWTPs.

Which OM characterization methods should be applied and how frequently they should be done, depends on the relevance of MP removal at the specific location. For locations with medium relevance (where the presence of MPs in the water poses a medium risks for humans or the environment), inline absorbance and fluorescence measurements can be implemented with probes. These measurements are easy to implement, need little maintenance and give insight in temporal variation of OM characteristics. For the locations with high relevance, or where MP treatment is already implemented, more specific measurements should be applied so the MP treatments can be further optimized in these locations. Examples of such specific OM characterization methods are size exclusion chromatography [84], but also fractionation methods or LC-MS fingerprinting could be included. Such measurements could be implemented on a weekly or biweekly basis in an intensive monitoring year at different WWTPs wo. Such measurement plan would expand insights in the variation of OM characteristics in different water matrixes, different locations and changes over time. With that information, the implementation of MP removal treatment could be improved throughout the world. Next to the relevance of monitoring OM characteristics from a scientific perspective, inline monitoring can also contribute directly to the reliability of MP removal treatment in practice by improving the ozone dosing (i.e. reducing overdosing and underdosing of ozone).

6.4.3 Inline measurements to control ozone dosing

When the BO_3 process is applied in practice there are several factors that affect the composition of WWTP effluents and in turn affect the processes in the BAC filter and ozonation steps. The concentrations of MPs, OM and inorganic compounds vary over time and per WWTP. These aspects have to be monitored continuously because they affect the MP removal and also the formation of OTPs in the reactors. If the right parameters are selected for monitoring, the ozone dose can be adjusted continuously to ensure that the ozone dose is high enough to remove MPs, but also low enough to avoid formation of the toxic compound bromate.

The BO_3 process achieves high MP removal at low ozone doses, because less ozone is scavenged by OM and therefore more ozone is available to react with MPs. This also means that more ozone is available to react with bromide to form the hazardous compound bromate (Chapter 5). Because bromate is harmful at low concentrations, but only formed at higher ozone doses, it is crucial to use low ozone doses and avoid overdosing of ozone when the water contains bromide [86]. Chapter 5 shows that using the conventional ozone dose standardization (based on TOC) does not give sufficient insight in the formation of bromate to compare between normal WWTP effluent and BAC treated WWTP effluent. This means that an alternative parameter (additional to the water flow rate) is needed to continuously assess how much ozone is required to treat the incoming water. Especially in a real wastewater treatment plant, where OM quantity and type varies over time, continuous monitoring of the incoming OM and adjustment of the ozone dose is necessary to ensure stable removal of MPs and to prevent formation of bromate (in the cases where bromide is present in the water).

Results from Chapter 4 show that OM in WWTP effluent can be classified in different groups based on their ozone reactivity and their interference with the ozonation of MPs. These insights can be related to current knowledge about the formation of bromate, i.e. that bromate formation only occurs if the ozone dose is higher than the initial ozone demand (IOD) of the water [253]. I hypothesize that the initial ozone demand is mainly related to the two OM groups with the highest ozone reactivity (and to nitrite if it is present at relevant concentrations). Therefore, better understanding of these OM groups can aid both in optimizing MP removal with ozone, and also in avoiding the formation of bromate. Chapter 4 shows that the resin fraction hydrophobic neutrals has a relatively low abundance of these OM groups with high ozone reactivity, while the resin fraction hydrophobic neutrals contains mainly saturated organic compounds with low absorbance and fluorescence properties. This suggests that the OM group with high ozone reactivity mainly consists of compounds that absorb light and have fluorescent properties. In Chapter 5 similar indications are found, because this chapter shows that absorbance and fluorescence properties give a more reliable prediction of bromate formation than the TOC. Nevertheless, which absorbance and fluorescence properties should be monitored is not yet clear. Chapter 5 shows that especially fluorescence related to protein like compounds is relevant to predict bromate formation, while the results of Chapter 4 indicate that fluorescence related to humic-like compounds are more reliable to predict MP removal with ozonation. The use of these parameters to control ozone dosing should be applied in larger scale tests to find out which are most useful.

The reactions of bromide with ozone and hydroxyl radicals that result in the formation of bromate are complex, and have been extensively studied [240,242,253,277] (Figure 6.5). Studies show that the presence of ammonium and hydrogen peroxide can reduce or prevent the formation of bromate during ozonation. Ammonium inhibits bromate formation without interfering with ozone reactions with MPs, because it does not react with ozone itself, but it reacts with the intermediate product in bromate formation ($HOBr/OBr^-$) and forms bromide [271] (Figure 6.6). Nevertheless, a more recent study shows that ammonium addition increases the formation of organic bromated compounds and through that also the toxicity of the treated water [278]. Hydrogen peroxide addition can also limit bromate formation but can also increase the formation of organic bromide products [271]. Adding either ammonium or hydrogen peroxide is a chemical input which results in additional costs and also in (some degree of) pollution of the water. The results from Chapter 4 and Chapter 5 suggest that, if the right parameters are selected, monitoring OM characteristics and adjusting the ozone dosing accordingly can completely prevent the formation of bromate. Moreover, a more reliable control of the ozone dosing has the advantage that it results in a more stable removal of MPs over time and a lower overall energy use. Therefore, optimizing monitoring and control of ozone dosing is preferred over adding ammonium or hydrogen peroxide to avoid formation of bromate.

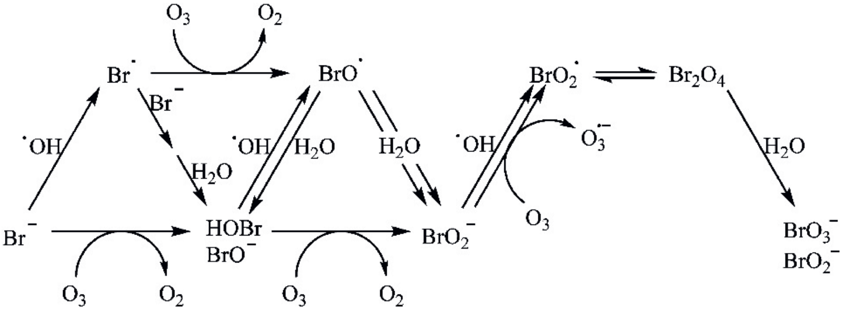


Figure 6.5 Formation pathways of bromate in ozone reactors via reactions with ozone and ·OH radicals. Adapted from Fischbacher et al. [277]

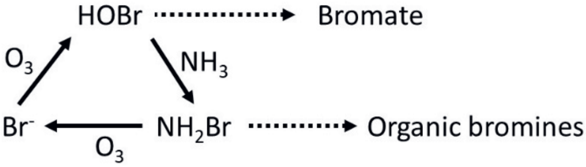


Figure 6.6 The effect of ammonium on the formation of bromate. Dotted lines indicate that multiple reactions are involved. Adapted from von Gunten [240] and Wu et al. [278]

6.5 Final considerations

This PhD project was a collaboration between a university (Wageningen University) and a consultancy and engineering company (Royal HaskoningDHV). A governmental fund from TKI Topsector Water and Royal HaskoningDHV both contributed to the total funding and, supervision was shared between Wageningen University and Royal HaskoningDHV. Such a collaboration of a university and the private sector has the advantages that more points of view are included in the research process (e.g. for setting research questions and choosing methodologies) and that chances are higher that the results of the research will be used after the PhD project has been completed. On the other hand, the challenge is that the focus of the research has to be divided over both the more curiosity based questions from science and the more practical questions that come from the private sector. For this to work successfully, it is especially important that expectations of all involved stakeholders are shared in the beginning of the PhD project (during the writing of the proposal). Moreover, during the research regular meetings are needed, in of this PhD that was weekly meetings with two daily supervisors (one from WUR and one from Royal HaskoningDHV) and meetings twice per year with all stakeholders together, where the progress and adjustments of the focus are discussed. As is shown by the example of this PhD thesis, a collaboration between a university and the private sector can be beneficial for all involved parties by resulting in new scientific insights that can be further applied in practice.

This thesis has shown the potential of the BO_3 process for removal of MPs from WWTP effluent and has given indications that will aid the optimization of the process, i.e. to increase MP removal and to minimize energy consumption. New insights in biological OM removal from WWTP effluent were presented (Chapter 2), further understanding of the effects of different OM types on MP ozonation were gained (Chapter 3 and Chapter 4) and the toxicity of OTPs formed in the BO_3 process was assessed (Chapter 5). Paragraph 6.4.1 also shows the advantages of the BO_3 process with regard to removal potential and energy efficiency compared to other MP removal technologies. Based on this thesis, a patent application has been filed for the BO_3 process (reference number P6106531) and the next steps are the larger scale studies where the BO_3 process is being tested further. These steps show that the potential of the BO_3 process for application in practice is recognized and that the development of the technology will continue beyond the scope of this thesis.

List of abbreviations

Abbreviation	Definition
AC	Activated carbon
BAC	Biological activated carbon
BO_3	Bio ozone
BO_3B	Bio ozone bio
BV	Bed volumes
CAS	Conventional activated sludge
COD	Chemical oxygen demand
DOC	Dissolved organic carbon
EBCT	Empty bed contact time
EBT	Effect based trigger value
EfOM	Effluent organic matter
FEEM	Fluorescence emission excitation matrixes
HI	Hydrophilics
HOA	Hydrophobic acids
HOB	Hydrophobic bases
HON	Hydrophobic neutrals
ISTD	Internal standard
LC	Liquid chromatography
LC-OCD	liquid chromatography coupled to organic carbon detection
LoQ	Limit of quantification
MBBR	Moving bed bioreactor
MP	Micropollutant
MS	Mass spectrometry
NF	Not found
OM	Organic matter
OTP	Ozone transformation product
PARAFAC	Parallel factor analysis
PNEC	Predicted no effect concentration
RQ	Risk quotient
SF	Sand filter
SUVA	specific UV absorbance at 254 nm
TOC	Total organic carbon
TP	Transformation product
UV254	Absorbance of ultraviolet light at 254 nm
WWTP	Wastewater treatment plant

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Summary / samenvatting

Summary

Micropollutants, characterized by their prevalent concentrations ranging from ng/L to µg/L, are found in water bodies all over the world and cause harm to the environment. Therefore, anthropogenic emissions of micropollutants have to be reduced. A major source of micropollutants into the environment is via wastewater treatment plants. Most of the chemicals used by households and industries end up in wastewater and are only removed to a limited extent in conventional wastewater treatment processes. Therefore, conventional wastewater treatment plants have to be upgraded to improve their removal of micropollutants.

Several advanced treatment technologies have been developed that can remove a broad range of micropollutants from the effluent of conventional wastewater treatment plants. The most applied examples are ozonation, activated carbon, tertiary biological treatment and membrane filtration. Nevertheless, these processes have their limitations, often related to high energy use and costs, which hinder their application in practice. Combining multiple treatments in a hybrid treatment further broadens the range of micropollutants that can be removed.

The focus of this thesis is on the bio-ozone (BO_3) process that utilizes a combination of biological treatment and ozonation to remove a broad range of micropollutants from wastewater treatment plant effluent at limited energy input. During conventional ozonation, only a small fraction of the ozone reacts with micropollutants. The majority of the applied ozone reacts with background organic matter (all organic matter except micropollutants), which is present in wastewater treatment plant effluent at 3-6 orders of magnitudes higher concentrations than the micropollutants. Ozonation is an energy and cost intensive treatment because producing ozone is an energy intensive process and most of the ozone reacts with background organic matter. Part of this background organic matter can be removed in an energy efficient biological treatment prior to ozonation to reduce the required amount of ozone. Another limitation of the application of ozonation is the formation of ozone transformation products during the ozone treatment. These transformation products can pose a hazard to the environment and are in some cases even more toxic than the parent compounds. Most ozone transformation products are more biodegradable than their parent compounds and can therefore be removed in an additional biological treatment after ozonation. Nevertheless, it is not yet well understood under which conditions ozone transformation products do and do not pose environmental risks. The aim of the studies described in this thesis is to optimize and improve our understanding of the synergies between biological treatment and ozonation in the BO_3 process.

In the study described in Chapter 2, three different bioreactors where compared for their potential to remove organic matter from wastewater treatment plant effluent. A biological activated carbon filter, a sand filter and a moving bed bioreactor were tested at different flowrates in a lab scale setup where they were continuously fed with real wastewater treatment plant effluent. The biological activated carbon filter showed the best performance and reached organic matter removal up to 72% at the highest tested empty bed contact time of 4.85 h. This means that the biological activated carbon filter is the best option to use as pre-treatment prior to ozonation. Indications were found that both biological processes and sorption contributed to the observed removal in the biological activated carbon filter.

The studies described in Chapter 3 and Chapter 4 explored the interference of different types of organic matter on the ozonation of micropollutants. Organic matter from different wastewater treatment plant effluents was fractionated based on size using membranes and based on functional groups using a resin, diluted to the same total organic carbon concentration, and ozonated to compare the micropollutant removal. Micropollutants were classified in three groups based on their ozone reactivities (high, medium and low ozone reactivities). The organic matter fractions showed different interference with micropollutant removal, especially for the micropollutants with high and medium ozone reactivity. The micropollutants with low zone reactivity showed similar removal in all fractions. These insights can be used to further optimize the pre-treatment before ozonation by ensuring that the most interfering organic matter fractions are removed in the pre-treatment. Several absorbance and fluorescence based organic matter characteristics in the fractions showed high correlations with the micropollutant removal ($R^2 \approx 0.7$), depending on the ozone reactivities of the micropollutants and on the applied ozone doses. These characteristics can be used as inline measurements to determine the required ozone dose in a full-scale ozone reactor.

The BO_3 process as biological activated carbon filtration followed by ozonation was tested in the study described in Chapter 5 to monitor the performance for micropollutant removal and to assess whether a final biological treatment step to remove ozone transformation products is necessary to improve the biological quality of the effluent. The BO_3 process showed high micropollutant removal at a low ozone dose of 0.2 g O_3/g C. The tested in vivo assays that used daphnia, algae and luminescent bacteria showed no changes in toxicity with increasing ozone doses. The used in vitro assays (CALUX assays for estrogens, glucocorticoids, polycyclic aromatic hydrocarbons, genotoxicity, progesterone, and oxidative stress) showed a decreasing or stable toxicity with biological activated carbon filtration and with increasing ozone doses. These results show that for the used bioassays, wastewater treatment plant effluent and ozone doses, the toxicity of the ozone transformation products was overall lower than the toxicity of the parent compounds. These results suggest that an additional biological treatment step after ozonation is not required in the tested conditions. In the experiment where bromide was spiked to the feed, no relevant bromate formation was observed at ozone doses below 0.4 g O_3/g C. This means that bromate formation in the BO_3 process can be prevented by avoiding the use of higher ozone doses.

Finally, in Chapter 6, the limitations of the used experiments and ideas for future research are discussed. Additionally, the potential of the BO_3 process for application in practice is underlined.

Samenvatting

Microverontreinigingen, zo genoemd vanwege de concentraties waarin ze voorkomen (van ng/L tot µg/L), worden wereldwijd gemeten in waterlichamen en kunnen schadelijk zijn voor het milieu. Dit is een van de redenen dat antropogene emissies van deze microverontreinigingen verminderd moeten worden. Een van de belangrijkste bronnen van microverontreinigingen die in het milieu terecht komen zijn afvalwaterzuiveringsinstallaties. Een groot deel van de chemicialien die worden gebruikt door huishoudens en industrieën komen in het afvalwater terecht worden onvoldoende verwijderd in de afvalwaterzuiveringsinstallaties en zijn daarom microverontreinigingen. Om de verwijderingen van microverontreiniging te verhogen, moeten afvalwaterzuiveringsinstallaties aangepast en verbeterd worden.

Er zijn al een aantal geavanceerde zuiveringstechnologieën ontwikkeld die een breed scala aan microverontreinigingen uit afvalwaterzuiveringseffluent kunnen verwijderen. De meest toegepaste technologieën zijn ozonisatie, actief kool filtratie, tertiaire biologische behandeling en membraan filtratie. Desalniettemin hebben deze technologieën hun beperkingen, die in de meeste gevallen gerelateerd zijn aan een hoog energie verbruik en hoge kosten, en daarmee de toepassing in de praktijk in de weg staan. Het samenvoegen van verschillende technologieën tot een hybride zuiveringstechnologie kan het aantal microverontreinigingen dat verwijderd kan worden nog verder verhogen.

De focus van dit proefschrift is het bio ozon (BO_3) proces. Het BO_3 proces gebruikt een combinatie van biologische behandeling en ozonisatie om een breed scala aan microverontreinigingen te kunnen verwijderen uit afvalwaterzuiveringseffluent, terwijl het energieverbruik beperkt is. Tijdens ozonisatie van afvalwaterzuiveringseffluent reageert maar een klein deel van de toegepaste ozon met de microverontreinigingen. De meerderheid van de ozon reageert met achtergrond organisch materiaal (al het organisch materiaal behalve microverontreinigingen), omdat de concentraties van dit achtergrond organisch materiaal is 3-6 ordes van grote hoger zijn dan de concentraties van de microverontreinigingen. Ozonisatie kost veel energie en geld omdat het produceren van ozon veel energie kost en omdat het grootste deel van de ozon wordt verbruikt door reacties met achtergrond organisch materiaal. Een deel van dit achtergrond organisch materiaal kan worden verwijderd in een energie efficiënte biologische behandeling vóór de ozonisatie om de benodigde hoeveelheid ozon te verminderen. Een andere limitatie van ozonisatie is de vorming van ozontransformatieproducten tijdens de ozonisatie. Deze transformatieproducten kunnen een gevaar vormen voor het milieu en zijn in sommige gevallen nog schadelijker dan de originele stoffen. De meeste ozontransformatieproducten zijn beter afbreekbaar dan de originele stoffen en kunnen daarom verwijderd worden in een biologische nabehandeling na de ozonisatie. Het is niet duidelijk onder welke condities de gevormde ozontransformatieproducten wel en niet schadelijk zijn in het milieu. Het doel van het onderzoek dat wordt beschreven in deze proefschrift is om de synergiën tussen biologische behandeling en ozonisatie beter te begrijpen en optimaal te benutten.

In de het onderzoek dat wordt beschreven in Hoofdstuk 2 zijn drie verschillende bioreactoren vergeleken om organisch materiaal uit afvalwaterzuiveringseffluent te verwijderen. Een biologische actief kool filter, een zandfilter en een 'moving bed bioreactor' (MBBR) zijn vergeleken bij verschillende stroomsnelheden in een lab opstelling, waarbij de reactoren continue gevoed werden met afvalwaterzuiveringseffluent. De biologische actief kool filter behaalde de hoogste organisch materiaal verwijdering (tot 72%) bij de hoogste geteste empty bed contact time van 4.85 uur. Dit betekent dat de biologische actief kool filter de beste optie is als voorbehandeling voor de ozonisatie. Verder bleek dat biologische processen en sorptie allebei een rol spelen bij de gemeten verwijderingen in de biologische actief kool filter.

De onderzoeken in Hoofdstuk 3 en Hoofdstuk 4 beschrijven de interferentie van verschillende soorten organisch materiaal op de ozonisatie van microverontreinigingen. Organisch materiaal van het effluent van verschillende afvalwaterzuiveringsinstallaties is gefractioneerd met membranen of hars, vervolgens verdund tot dezelfde totaal organisch koolstof concentraties, en geozoniseerd om de verwijdering van microverontreinigingen te vergelijken. Gebaseerd op de resultaten konden de microverontreinigingen ingedeeld worden in drie ozon reactiviteit groepen (hoge, middel, en lage ozon reactiviteit). De organisch materiaal fracties toonden verschillende interferenties met de verwijdering van microverontreinigingen aan. De verschillen in interferentie waren vooral zichtbaar voor de microverontreinigingen met een hoge en middelmatige ozon reactiviteit. De microverontreinigingen met lage ozon reactiviteit gaven weinig verschil in hun verwijderingen. Deze resultaten kunnen worden gebruikt om voorbehandeling voor ozonisatie te optimaliseren door ervoor te zorgen dat de organisch materiaal fracties met de hoogste interferentie worden verwijderd. Een aantal eigenschappen van het organisch materiaal in de fracties hadden sterke correlaties met de verwijdering van microverontreinigingen ($R^2 \approx 0.7$), afhankelijk van de groep microverontreinigingen en de toegepaste ozon dosering. Deze eigenschappen kunnen gebruikt worden om de benodigde hoeveelheid ozon in full-scale reactoren continue te bepalen en de dosering te optimaliseren.

Een studie over het BO_3 proces bestaande uit biologische actief koolfiltratie gevolgd door ozonisatie is beschreven in Hoofdstuk 5. Het doel van dit hoofdstuk is om microverontreiniging verwijdering te monitoren en de afweging te maken of een biologische nabehandeling om ozon transformatieproducten te verwijderen noodzakelijk is of niet. Het BO_3 proces behaalde hoge verwijdering van microverontreinigingen bij een lage ozon dosering van 0.2 g O_3/g C. De gebruikte in vivo bioassays met watervlooien, algen, en lichtgevende bacteriën lieten geen veranderingen in toxiciteit zien bij toenemende ozon doseringen. De gebruikte in vitro bioassays (CALUX assays voor estrogenen, glucocorticoïden, polycyclische aromatische koolwaterstoffen, genotixiciteit, progesteron, en oxidatieve stres) lieten over het algemeen een afname van toxiciteit zien bij toenemende ozon doseringen. Deze resultaten tonen aan dat voor de gebruikte bioassays, afvalwaterzuiveringseffluent en ozon doseringen, de toxiciteit van de gevormde ozon transformatieproducten lager is dan de toxiciteit van het uitgangsmateriaal. Dit suggereert dat een biologische nabehandeling na ozonisatie niet nodig is in de onderzochte omstandigheden. In het experiment waar bromide was toegevoegd aan het influent werd geen bromaatvorming gevonden bij ozon doseringen lager dan 0.4 g O_3/g C. Dit betekent dat bromaatvorming in het BO_3 proces voorkomen kan worden door hoge ozon doseringen te vermijden.

In Hoofdstuk 6 staan de conclusies en beperkingen van de uitgevoerde experimenten beschreven en worden de ideeën voor verder onderzoek bediscussieerd. Daarnaast is de bruikbaarheid van het BO_3 proces in de praktijk benadrukt.

List of publications

Papers

van Gijn, K., et al. "The effect of organic matter fractions on micropollutant ozonation in wastewater effluents." Water Research (2022): 118933.

Moerland, M. J., et al. "Micropollutants removal during high rate thermophilic and hyper-thermophilic anaerobic digestion of concentrated black water." Journal of Environmental Chemical Engineering 10.2 (2022): 107340.

van Gijn, K., et al. "Optimizing biological effluent organic matter removal for subsequent micropollutant removal." Journal of Environmental Chemical Engineering 9.5 (2021): 106247.

van Gijn, K. et al. “Literatuuronderzoek naar bronnen en gedrag van PFAS in afvalwater.” No. 2021-47. Stichting Toegepast Onderzoek Waterbeheer (STOWA), 2021.

van Gijn, K., et al. "Optimizing Micropollutant Removal by Ozonation; Interference of Effluent Organic Matter Fractions." Ozone: Science & Engineering 43.6 (2021): 579-591.

de Wilt, A. et al. "Enhanced pharmaceutical removal from water in a three step bio-ozone-bio process." Water research 138 (2018): 97-105.

Oral presentations

Micropol and Eco hazard, Santiago (may 2022)

Environmental technology for impact, Wageningen (may 2020)

Micropol and Eco hazard, Seoul (October 2019)

Water science and impact, Wageningen (November 2018)

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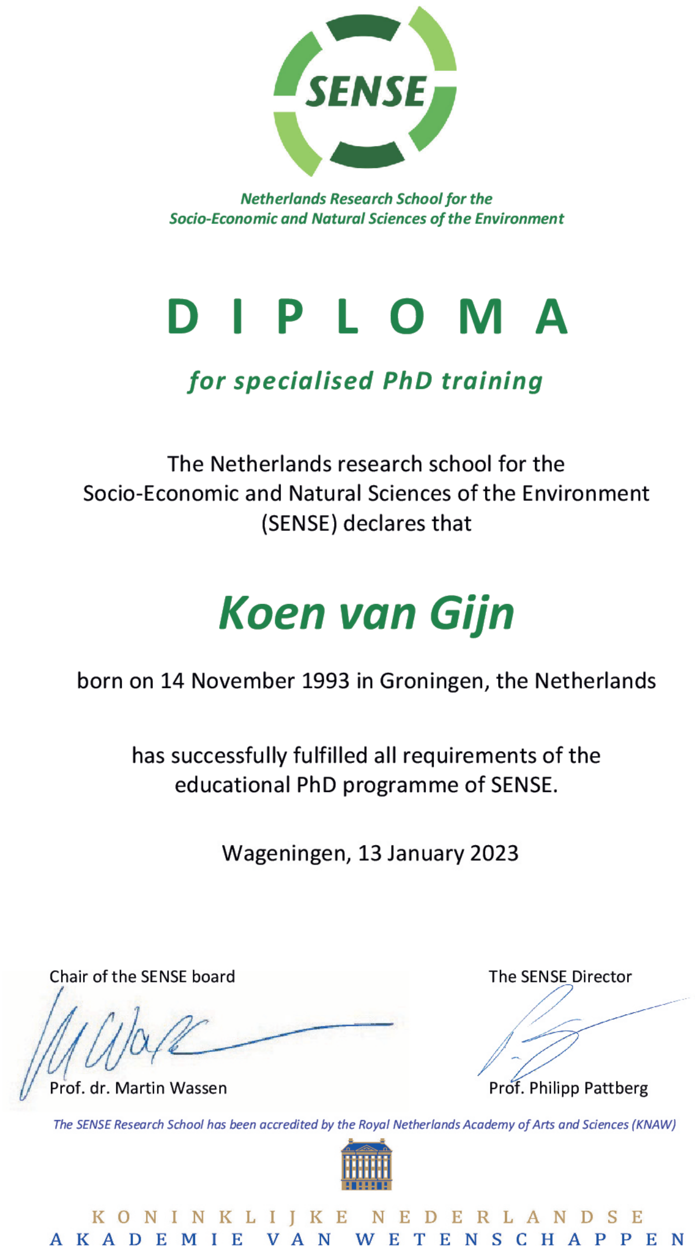
a ustedes aquí estamos e incluso logré terminar mi PhD dentro de lo programado. Muchas gracias, saben que pueden contar conmigo para lo que necesiten y espero algún día pueda retornar la ayuda brindada.

About the author

Koen van Gijn was born in the hospital in Groningen, the Netherlands, on a stormy night on the 14th of November 1993. He moved to Hoogeveen when he was three years old and attended to primary school and secondary school there. At the age of 17 Koen decided to move to Wageningen to study environmental sciences where he could combine all his interests in chemistry, biology, and math. Soon he discovered the field of water sciences and chose get a view on the topic from aquatic ecology, toxicology and environmental technology perspectives. During his studies Koen also contributed to the study association of environmental sciences as a board and committee member for which he got awarded with the environmentalist of the year trophy. After his second Msc thesis at environmental technology about the removal of micropollutants from wastewater treatment plan effluent, and an internship experience in Taiwan where he also learned a bit of Chinese (汙水處理廠), Koen welcomed the opportunity to continue on the thesis topic with a PhD research. During the PhD he studied the bio-ozone(-bio) (BO_3(B)) process and how the synergies between biological treatment and ozonation result in an effective and efficient technology for the removal of micropollutants from wastewater treatment plant. The study focused on different aspects of the synergies, how the organic matter that is removed in the first biological reactor interferes with the ozonation, and whether the formed transformation products during ozonation are very toxic and have to be removed in a final biological reactor. During the PhD Koen also joined the nationaal water traineeship to improve his self-leadership and communication skills and get to know the Dutch water sector more. After his PhD he will start a new function as technologist at the water authority of Rijn and Ijssel where he will support the implementation of new wastewater treatment technologies in practice.



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Other PhD and Advanced MSc Courses

- Toxic Compounds in the Environment, Research Centre for Toxic Compounds in the Environment, Czech Republic (2018)
- Micropollutants: Interdisciplinary look at the problem, INRA & irstea (2018)
- Environmental Biotechnology, Delft University (2019)
- Scientific reviewing, Wageningen Graduate Schools (2018)
- Scientific Writing, Wageningen Graduate Schools (2020)
- Start to supervise BSc & MSc thesis students, Wageningen Graduate Schools (2018)
- PhD competence assessment , Wageningen Graduate Schools (2018)
- Brain friendly working and writing, Wageningen Graduate Schools (2018)
- Supervising BSc and MSc thesis students, Wageningen Graduate Schools (2019)
- Project and Time Management Wageningen Graduate Schools (2019)
- Communication Styles, Wageningen Graduate Schools (2019)

Selection of Management and Didactic Skills Training

- Member of chair groups’ research committee (2018-2022)
- Supervising BSc and MSc students with thesis (2018-2022)
- Teaching in the BSc course ‘Introduction to environmental technology’ (2018-2019) and MSc course ‘Environmental project studies’ (2019-2022)
- Assisting practicals of the MSc course ‘“wastewater treatment’ (2019-2021)

Oral Presentations

- *Removal of micropollutants from wastewater treatment plant effluent, combining the efficiency of biology to the effectivity of ozone*. Water science for impact. 18 October 2018, Wageningen, The Netherlands
- *Combining the efficiency of microorganisms with the effectivity of ozonation*. 11th Micropol & Ecohazard Conference, 20-23 October 2019, Seoul South Korea
- *Interactions between ozone and organic matter and the effects on micropollutant removal*. Environmental technology for impact, 3 May 2020, Wageningen, The Netherlands
- *Optimizing micropollutant ozonation in WWTP effluent*. 12th Micropol & Ecohazard Conference, 6 September 2022S, Santiago, Spain

SENSE coordinator PhD education

Dr. ir. Peter Vermeulen

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