Doctoral Thesis

ADVANCED WASTEWATER TREATMENT WITH OZONATION – ASPECTS FOR PRACTICAL IMPLEMENTATION

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Finally, I would like to express my deepest gratitude to my family and friends for their encouragement, support, and understanding over the years.

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Publications

Significant parts of this thesis are findings of the following two papers that have already been published.

Lam T. Phan, Heidemarie Schaar, Daniela Reif, Sascha Weilguni, Ernis Saracevic, Jörg Krampe, Peter A. Behnisch, Norbert Kreuzinger. Long-Term Toxicological Monitoring of a Multibarrier Advanced Wastewater Treatment Plant Comprising Ozonation and Granular Activated Carbon with In Vitro Bioassays. Water, 13(22). doi:10.3390/w13223245.

Lam Thanh Phan, Heidemarie Schaar, Ernis Saracevic, Jörg Krampe, Norbert Kreuzinger. Effect of ozonation on the biodegradability of urban wastewater treatment plant effluent. Science of The Total Environment, 812, 152466. doi: 10.1016/j.scitotenv.2021.152466.

Further publications are in preparation.

Abstract

Water is an essential natural resource for developing life and human activities. Over the past few decades, water scarcity and water quality have become a significant concern. Large amounts of water are continuously polluted. Restoring water quality is essential to avoid higher pollution levels, dealing with the idea of "zero - pollution" and allowing water to be reused.

Studies show that not all contaminants are removed through conventional biological wastewater treatment plants. One group of these refractory compounds that has gained increasing attention over the last two decades are micropollutants, an emerging class of pollutants composed of highly diverse chemicals that are present at low concentrations (µg/L to ng/L). Micropollutants comprise compounds such as pharmaceuticals, personal care products, steroid hormones, surfactants, industrial chemicals, and pesticides

Implementing advanced treatment steps beyond the conventional biological treatment is one of the measures to reduce micropollutant discharge to receiving water bodies, thus fostering the zero-pollution strategy. Several technologies have been established and implemented in full-scale during the last few years. The two most relevant technologies are ozonation and activated carbon treatment. The ozonation of wastewater treatment plant effluent has shown promising potential as an application for advanced wastewater treatment over the past years. Several studies have demonstrated that many organic micropollutants are removed to a great deal through ozonation.

This thesis aims to extend the existing knowledge to support and extend the practical application of ozonation in the field of advanced wastewater treatment. The work focused on urban wastewater treatment plants in Austria, which are characterized by a high level of treatment performance comprising biological nutrient removal with full nitrification and denitrification.

The experiments presented in this thesis were carried out in three main phases. The first two phases were carried out in laboratory scale, while the third was carried out in pilot scale.

In phase 1, the treated effluent of a wastewater treatment plant was ozonated with different specific ozone doses (0; 0.2; 0.4; 0.6; 0.8; and 1.0 g O_3/g DOC), studying the abatement of micropollutants and bromate formation. Micropollutants were classified into the following three groups based on their response to ozone treatment: highly active compounds (diclofenac, carbamazepine, and sulfamethoxazole), moderately reactive compounds (metoprolol, bezafibrate, benzotriazole, and acesulfame K) and low reactive compounds (ibuprofen and diatrizoic acid dihydrate).

For ozonation, the removal of micropollutants was >80% for three groups at a specific ozone dose of 0.6 - 1.0 g O_3/g DOC. The micropollutant removal was predicted from the second-order kinetics and the oxidant exposure (ozone and $OH^\bullet$). Predicted removal did not coincide with the measured removal for all groups of substances due to mechanistic reasons. Regarding, bromate formation differences were observed, depending on the specific ozone dose and varying between the investigated effluent samples. Bromate formation ranged between 0.65 ± 0.28 and 11.22 ± 9.85 µg/L. The guideline value for drinking water (10 µg/L) was only exceeded at > 0.88 ± 0.05 g O_3/g DOC, which is higher than the usually applied doses for micropollutant removal (0.6 - 0.7 g O_3/g DOC).

In phase 2, the effect of ozonation was studied on organic sum parameters, which are usually measured during conventional wastewater analysis, including biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), dissolved organic carbon (DOC), spectral absorption coefficient at 254 nm (SAC_{254}). The parameters were measured before ozonation and after applying different specific ozone doses (0.4, 0.6, and 0.8 g O_3/g DOC) as well as after exposition to BOD_5 measurement in order to investigate the change in biodegradability after ozonation. The results showed a dose-dependent increase in biological activity after ozonation. This increase is related to the enhanced biodegradability of substances in conventional activated sludge treatment. The highest relative increase was determined for BOD_5, which occurred from 0 to 0.4 g O_3/g DOC for all investigated effluent samples, ranging between 21.33 and 88.75% increase. Increasing the specific ozone doses to 0.6 and 0.8 g O_3/g DOC resulted in less pronounced further increases. DOC did not decrease significantly after ozonation, which is consistent with the low mineralization reported, whereas partial oxidation resulted in a quantifiable reduction of COD (7 to 17%). Delta UV_{254} and specific UV absorption attenuation after ozonation are clearly correlated with the specific ozone doses. In contrast, for COD and biodegradable DOC (BDOC), a clear dose-response pattern was determined after exposure to BOD_5 measurement. Signs of improved biodegradability were further supported by an increase in the BOD_5/COD ratio.

In the final phase, a pilot study on a multibarrier advanced wastewater treatment plant comprising ozonation and granular activated carbon treatment was conducted assessing effects on the effluent toxicity. Eight CALUX *in vitro* bioassays were performed to monitor different modes of action along the toxicity pathway. The toxicity monitoring supported the evaluation of the suitability and robustness of the multibarrier system. Two approaches were followed. First, the signal reduction during the applied advanced treatment steps were monitored. Secondly, the results were compared with currently discussed effect-based trigger values (EBT) as environmental standards. A corresponding decrease in bioequivalence concentrations was observed after the multibarrier system for all investigated modes of action. Although already during ozonation, estrogenic activities decreased significantly below the associated EBT, the potency of toxic PAH - like compounds and oxidative stress still exceeded currently discussed EBT even after advanced treatment. Overall, long-term monitoring confirmed the positive effects of multibarrier systems, which are usually determined only by microcontaminant removal based on chemical analysis. It has been shown that advanced WWTPs designed to eliminate CEC are suitable for significantly reducing toxicological responses.

The results indicate that combining ozonation and biological post-treatment, e.g., granular activated carbon, represents another step towards sustainable water resource management.

Zusammenfassung

Wasser ist eine wesentliche natürliche Ressource für die Entwicklung von Leben und menschlichen Aktivitäten. In den letzten Jahrzehnten sind Wasserknappheit und Wasserqualität zu einem bedeutenden Problem geworden. Große Mengen an Wasser werden ständig verschmutzt. Die Sicherung der Wasserqualität ist unerlässlich, um eine weitere Verschmutzung zu vermeiden, der „Zero-Pollution-Strategie“ Rechnung zu tragen und die Wiederverwendung von Wasser zu ermöglichen.

Studien zeigen, dass nicht alle Abwasserinhaltsstoffe durch konventionelle biologische Kläranlagen entfernt werden. Eine Gruppe dieser refraktären Verbindungen, die in den letzten zwei Jahrzehnten zunehmend an Aufmerksamkeit gewonnen hat, sind Mikroschadstoffe, eine Klasse von Verbindungen, die sich aus sehr unterschiedlichen Chemikalien zusammensetzt und in niedrigen Konzentrationen (µg/L bis ng/L) vorhanden ist. Mikroschadstoffe umfassen Verbindungen wie z. B. Arzneimittel, Körperpflegeprodukte, Steroidhormone, Tenside, Industriechemikalien und Pestizide.

Die Umsetzung weitergehender Behandlungsschritte über die konventionelle biologische Abwasserreinigung hinaus ist eine der Maßnahmen zur Reduzierung der Mikroschadstoffemissionen in die Vorfluter und fördert damit die "Zero-Pollution-Strategie". In den letzten Jahren wurden mehrere Technologien etabliert und großtechnisch eingesetzt. Die beiden wichtigsten Technologien sind Ozonung und Aktivkohlebehandlung. Die Ozonung von Kläranlagenablauf für die weitergehende Abwasserbehandlung hat in den letzten Jahren zunehmend an Bedeutung gewonnen. Mehrere Studien zeigten, dass viele organische Mikroschadstoffe durch Ozonung weitgehend entfernt werden.

Ziel dieser Arbeit ist es, das vorhandene Wissen für die praktische Anwendung der Ozonung im Bereich der kommunalen Abwasserreinigung zu erweitern und damit den praktischen Einsatz weiter voranzutreiben. Basis für diese Studie bildeten kommunale Kläranlagen in Österreich, die sich durch eine hohe Reinigungsleistung, bestehend aus biologischer Nährstoffentfernung mit vollständiger Nitrifikation und Denitrifikation, auszeichnen.

Die in dieser Arbeit vorgestellten Experimente wurden entsprechend der Fragestellungen in drei Phasen unterteilt. Die ersten beiden Phasen wurden im Labormaßstab durchgeführt, während die Dritte im Pilotmaßstab durchgeführt wurde.

In Phase 1 wurde das gereinigte Abwasser mit unterschiedlichen spezifischen Ozondosen (0; 0,2; 0,4; 0,6; 0,8 und 1,0 g O_3/g DOC) behandelt und dabei die Entfernung ausgewählter organischer Spurenstoffe sowie die dabei auftretende Bromatbildung untersucht. Die Spurenstoffe wurden aufgrund ihres Verhaltens in der Ozonbehandlung in die folgenden drei Gruppen eingeteilt: hochreaktive Verbindungen (Diclofenac, Carbamazepin und Sulfamethoxazol), mäßig reaktive Verbindungen (Metoprolol, Bezafibrat, Benzotriazol und Acesulfam K) und ozonresistente Verbindungen (Ibuprofen und Diatrizoesäure Dihydrat).

Bei einer spezifischen Ozondosis von 0,6 - 1,0 g O_3/g DOC betrug die Entfernung von Mikroverunreinigungen für alle drei Gruppen >80%. Die Entfernung wurde mittels Kinetik zweiter Ordnung und der Oxidationsmittelexposition (Ozon und OH•) beschrieben. Der prognostizierte Abbau stimmte aus mechanistischen Gründen nicht für alle Stoffgruppen mit dem gemessenen Abbau überein. Hinsichtlich der Bromatbildung wurden Unterschiede in Abhängigkeit von der spezifischen Ozondosis beobachtet, die zwischen den untersuchten Abwasserproben variierten. Die Bromatbildung lag im Bereich zwischen 0,65 ± 0,28 und 11,22 ± 9,85 µg/l. Der Grenzwert für Trinkwasser (10 µg/L) wurde erst bei > 0,88 ± 0,05 g O_3/g DOC überschritten, was höher ist als die üblicherweise zur Spurenstoffentfernung angewendete Ozondosis (0,6 - 0,7 g O_3/g DOC).

In Phase 2 wurde die Wirkung der Ozonung auf organische Summenparameter untersucht, die normalerweise bei der konventionellen Abwasseranalyse gemessen werden, wie biochemischer Sauerstoffbedarf (BSB_5), chemischer Sauerstoffbedarf (CSB), gelöster organischer Kohlenstoff (DOC), UV-Absorption bei 254 nm (UV_{254}). Die Parameter sowie die untersuchten organischen Spurenstoffe wurden vor der Ozonung und nach Anwendung unterschiedlicher spezifischer Ozondosen (0,4; 0,6 und 0,8 g O_3/g DOC) sowie nach einem biologischen Abbau im Zuge der BSB_5-Messung ermittelt, um die Veränderung der biologischen Abbaubarkeit durch die Ozonung zu untersuchen. Die Ergebnisse zeigten eine dosisabhängige Zunahme des biologischen Abbaus nach der Ozonung. Die höchste relative BSB_5-Veränderung trat für alle untersuchten Abwasserproben zwischen 0 und 0,4 g O_3/g DOC auf und lag bei einer

Zunahme von 21,33 bis 88,75%. Eine Erhöhung der spezifischen Ozondosis auf 0,6 und 0,8 g O_3/g DOC führte zu einem weniger ausgeprägten Anstieg. Der DOC nahm nach der Ozonung nicht signifikant ab, was mit der berichteten geringen Mineralisierung übereinstimmt, während die partielle Oxidation zu einer quantifizierbaren Verringerung des CSB (7 bis 17%) führte. Delta UV_{254} und die Abnahme der spezifischen UV-Absorption nach der Ozonung korrelierten gut mit den spezifischen Ozondosen. Im Gegensatz dazu wurde für den CSB und den biologisch abbaubaren DOC (BDOC) erst nach der BSB_5-Messung eine klare Dosis-Wirkungs-Beziehung festgestellt. Anzeichen einer verbesserten biologischen Abbaubarkeit wurden durch einen Anstieg des BSB_5/CSB-Verhältnisses festgestellt.

In der letzten Phase wurde eine Pilotstudie zu einer modernen Multibarrieren-Abwasserbehandlungsanlage mit Ozonung und anschließender granulierter Aktivkohlebehandlung durchgeführt, um die Auswirkungen auf die Abwassertoxizität zu bewerten. Acht CALUX in vitro Biotests wurden durchgeführt, um verschiedene Wirkungsweisen entlang des Toxizitätspfads zu überwachen. Das Toxizitätsmonitoring unterstützte die Bewertung der Eignung und Robustheit des Multibarrierensystems. Es wurden zwei Ansätze verfolgt. Zunächst wurde die Signalreduktion während der angewendeten weitergehenden Behandlungsschritte überwacht. Zum anderen wurden die Ergebnisse mit aktuell diskutierten effektbasierten Triggerwerten (EBT) als potentielle Umweltqualitätsstandards verglichen. Für alle untersuchten Wirkmechanismen wurde eine entsprechende Abnahme der Bioäquivalenzkonzentrationen nach dem Multibarrierensystem beobachtet. Obwohl die östrogene Aktivität bereits während der Ozonung deutlich unter den damit verbundenen EBT abnahm, lagen die Parameter PAK-ähnliche Verbindungen und oxidativer Stress auch nach der Aktivkohlebehandlung über den aktuell diskutierten EBT. Insgesamt bestätigte das Langzeitmonitoring die positiven Effekte des Multibarrierensystems, die in der Regel nur durch die Entfernung von Mikroschadstoffen auf Basis chemischer Analysen bestimmt werden. Es konnte gezeigt werden, dass eine weitergehende Abwasserbehandlung, die zur Spurenstoffentfernung geeignet ist, auch toxikologische Reaktionen deutlich reduziert.

Die Ergebnisse zeigen, dass die Kombination von Ozonung und biologischer Nachbehandlung, z. B. Aktivkohlegranulat, einen weiteren Schritt in Richtung einer nachhaltigen Wasserressourcenbewirtschaftung darstellt.

Erratum to “Bromate yield”

The author regrets that an error occurred in the calculation of the bromate yield. Too high numbers are given in Table 5.2 (page 57) and Figure 5.6 (page 58).

The text at the end of page 56 has to be changed as follows:
The bromate yield can be defined as the molar ratio of the bromate concentration normalized by the initial bromide concentration.

The following table and figure show the corrected bromate yield:

Table 5.2. (corrected). Bromide and bromate concentration, bromate yield

D_{spec} (g O_3/g DOC)	Bromide (µg/L)	Bromate (µg/L)	Bromate yield* (%)
0.23 ± 0.05	220.00 ± 84.71	0.00 ± 0.00	**0.00**
0.44 ± 0.07	211.75 ± 73.23	0.65 ± 0.28	**0.19**
0.66 ± 0.09	210.00 ± 18.74	2.52 ± 2.35	**0.75**
0.88 ± 0.05	169.25 ± 62.32	5.24 ± 5.38	**1.94**
1.09 ± 0.09	150.00 ± 86.97	11.22 ± 9.85	**4.68**

*Bromate yield = ([bromate]/$[\text{bromide}]_0$)

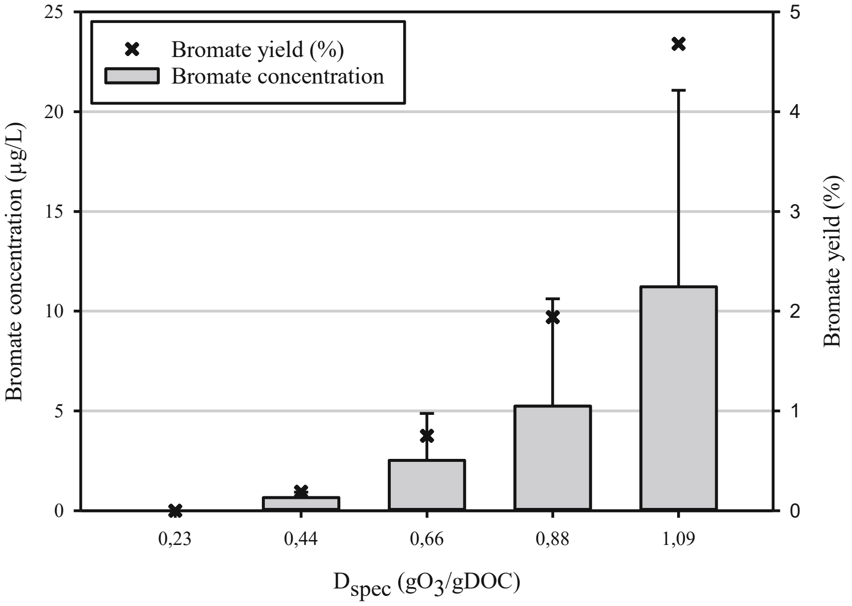

Figure 5.6 (corrected). Relationship between bromate and bromate yield and D_{spec}

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List of Abbreviations

ACE K	Acesulfame K
AhR	Aryl hydrocarbon receptor
AR	Androgen receptor
BAC	Biologically activated carbon
BDOC	Biodegradable DOC
BDS	BioDetection Systems BV
BEQs	Biological equivalent concentrations
BOD	Biological oxygen demand
BrO_3^-	Bromate
BZF	Bezafibrate
BZT	Benzotriazole
CALUX®	Chemical Activated LUciferase eXpression
CAS	Effluent of conventional WWTP
CAR	Constitutive androgen receptor
CBZ	Carbamazepine
CEC	Contaminants of emerging concern
COD	Chemical oxygen demand
DCF	Diclofenac
DHT-EQ	Dihydrotestosterone Equivalents
DOC	Dissolved organic carbon
D_{spec}	Specific ozone dose
DTA	Diatrizoic acid dihydrate
EBT	Effect-based trigger value
ECHA	European Union, the European Chemicals Agency
EDCs	Endocrine disrupting compounds
EEQ	Estrogenic Equivalents
EfOM	effluent organic matter
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
ER	Estrogen receptor
EU	European Union
GAC	Granular activated carbon
GR	Glucocorticoid receptor
HPLC	High Performance Liquid Chromatography

IBP	Ibuprofen
LOD	Limit of detection
LOQ	Limit of quantification
MOA	Mode of action
MS	Mass spectrometry
MTP	Metoprolol
NDMA	N-nitrosodimethylamines
NF	Nanofiltration
NH_4-N	Ammonium nitrogen
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
O_3	Ozone
OH•	Hydroxyl radical
PAC	Powdered activated carbon
PAHs	Polyaromatic hydrocarbons
PCPs	Personal care products
PPCPs	Pharmaceuticals and personal care products
REACH	Registration, evaluation, authorization and restriction of chemicals
RO	Reverse osmosis
RXR	Retinoid X receptor
SAC_{254}	Spectral absorption coefficient at 254 nm
S/N	Signal to noise ratio
SMX	Sulfamethoxazole
SPE	Solid-phase-extraction
SUVA	Specific UV absorbance at 254 nm
TOC	Total Organic Carbon
TR	Thyroid hormone receptor
TrOCs	Trace organic compounds
UV_{254}	Ultraviolet absorbance at 254 nm
WHO	World Health Organization
WWTP	Wastewater treatment plant

1. Introduction

Currently, most people in Europe just need to turn on the faucet to consume clean, clear and safe water but do not know where it comes from and how it has been treated. Such a water supply requires good and a high raw water quality, which is one of the great challenges worldwide in the near future. In fact, emerging organic compounds (pharmaceuticals, industrial chemicals, personal care products, and others) pose a threat to our water resources (Chaturvedi et al., 2021). Conventional municipal wastewater treatment plants (WWTPs) cannot normally treat these compounds and that is why they are released into the aquatic environment (Couto et al., 2019; Zhou et al., 2019b). When these compounds are released to the aquatic environment, they can adversely affect water quality (surface and groundwater) and that raises important questions regarding human health, ecology and economic impacts (Benner et al., 2013). Therefore, with an increasing number of micropollutants being identified in surface water and groundwater, new treatment and management strategies are needed to provide sustainable and cost-effective solutions across Europe.

In recent years, the occurrence of organic micropollutants as e.g., endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCPs) in the aquatic environment was intensively investigated. Since most of these micropollutants are of anthropogenic origin and released into the environment by wastewater, even tertiary treated wastewater effluents are considered to be one of the major point sources for their occurrence in the aquatic environment. Even low residual concentrations (µg/l to ng/l level) of organic micropollutants can show adverse effects to aquatic organisms and may restrict further use as a raw water resource for human demand (Anumol et al., 2016; Salimi et al., 2017; Valitalo et al., 2016). The increasing pressure on water resources due to increased demand for human use on the one hand and decrease of availability due to climate change on the other hand fostered research on technologies to further remove organic trace pollutants from wastewater (Ashauer, 2016; Phattarapattamawong et al., 2018; Rizzo et al., 2020).

Biological processes, such as the conventional activated sludge process, currently represent the majority of applied processes in wastewater treatment plants (WWTPs) worldwide. However, while conventional organic sum parameters such as COD and

BOD are removed to a high degree, others comprising micropollutants are released into the environment unchanged or metabolized (Krzeminski et al., 2019; Quintana et al., 2005). To mitigate this release, particular attention has been directed towards advanced treatment technologies.

Advanced wastewater treatment technologies based on ozone (O_3) and granular activated carbon (GAC), have proven to decrease a broad variety of EDCs in the effluent of WWTPs. (Stalter et al., 2011). A multibarrier system for advanced treatment comprising both O_3 and GAC, may offer an interesting further potential for implementation, since ozonation may destroy adsorbed molecules and regenerate the adsorption capacity of activated carbon. GAC presents a large surface area where ozone and organic pollutants could be adsorbed and react. Although O_3-GAC may be a promising method for reducing or mineralizing organic pollutants in wastewater, complete mineralization of refractory organic matter in effluents will also consume a lot of ozone. To increase the economic efficiency of ozonation, it frequently is combined with a biological process for water and wastewater treatment (Li et al., 2006).

The application of ozone is considered a suitable technology to further remove organic micropollutants from urban wastewater and is already implemented in full scale in several countries (Switzerland, Germany, and Sweden) (Baresel et al., 2016; Bourgin et al., 2018; Itzel et al., 2017). The removal efficiencies for various organic micropollutants are influenced by their reactivity with ozone and spontaneously formed hydroxyl radicals (Zimmermann et al., 2011), the ozone dose (Lee et al., 2013) and the composition of the wastewater (Schindler et al., 2015). To reduce ozone scavenging by the organic fraction in wastewater, ozonation is usually applied after biological treatment (Schaar et al., 2010). In biologically treated wastewater, ozone targets electron-rich moieties, such as olefins, aromatic rings, and amines (von Sonntag et al., 2012) and thus reacts with micropollutants (Lee et al., 2016; Rizzo et al., 2019).

Oxidation byproducts formed from the oxidative transformation of matrix components involve inorganic (e.g., bromate) as well as organic compounds (e.g., nitrosamines, aldehydes) and in some cases are suspected to show a higher toxicological potential as compared to their parent substances. Consequently, the formation of transformation products and/or byproducts is intended to be minimized during the technical operation of ozonation. Beside the chemical matrix and the content of precursor substances in the raw water, the ozone dose is of central importance for the undesired formation of oxidation byproducts. At specific ozone doses below 0.5g O_3/g DOC, only little bromate is formed, as, due to the quick decomposition of ozone, the ozone exposure is low (Lee et al., 2013).

2. Scope and Structure of the Work

This Ph.D. work is carried out as interdisciplinary scientific research covering many areas such as advanced wastewater treatment, toxicology, water quality, etc. The experiments were mainly performed in the laboratories of the Research Unit Water Quality Management, at the Institute for Water Quality and Resource Management, at the TU Wien.

In accordance with the facts and needs presented in Chapter 1 for the use of ozonation in municipal wastewater treatment plants, the objectives of this Ph.D. thesis can be listed as:

- Evaluate the correlation between ozone dose to the effective removal of trace organic compounds (TrOCs) and the formation of oxidation byproducts.
- Evaluate the impact of ozonation on the biodegradability change of recalcitrant COD in treated urban wastewater.
- The toxicological evaluation of the treatment efficiency, general cytotoxicity, and decrease of endocrine activity after ozonation.

Hence this Ph.D. thesis can be divided into three main aspects

Stage 1 is designed to target and test the elimination of TrOCs and the formation of oxidation byproducts (bromate) during ozonation. The effluent of an Austrian WWTP was used. Nine TrOCs usually present in municipal wastewater in wastewater were selected for analysis based on existing and proposed EU legislation, metabolism, and excretion from the human body, known environmental occurrence, persistence during wastewater treatment, and toxicity to aquatic organisms. This includes pharmaceuticals, corrosion inhibitors, and artificial sweeteners. The following research questions needed to be answered during the experiments:

- How is the decomposition performance of ozonation for TrOCs?
- How is the bromate formation in the investigated wastewater related to the ozone dose?

In order to answer the research questions, batch tests were conducted with different nitrite compensated specific ozone doses (0.2, 0.4, 0.6, 0.8, and 1.0 g O_3/g DOC).

Stage 2 was to evaluate the impact of ozonation on the change in biodegradability of recalcitrant COD in urban wastewater after conventional biological treatment. The main parameters of interest were the organic sum parameters BOD_5, COD, DOC and UV absorption at 254 nm (UV_{254}). Additionally, two micropollutants were analyzed to validate the experimental setup for ozonation batch tests. Specifically, the study aimed to answer the following research questions:

- Will an increase of specific ozone doses typically applied for micropollutant abatement from urban wastewater affect organic sum parameters commonly assessed in wastewater treatment and used as quality criteria and threshold for treatment targets in conventional treatment?
- Does ozonation result in an increase in biodegradability of substances previously recalcitrant to biological degradation, and is there a correlation with the specific ozone dose?

Stage 3 focused on the effluent of a WWTP that was treated in a multibarrier system (ozone and GAC) at a pilot-scale plant at a full-scale WWTP. The overall objective was long-term toxicological monitoring of multibarrier advanced wastewater treatment under actual conditions, applying a mode of action (MOA)-based in vitro bioassay battery to target relevant toxicological endpoints. After installation, setup of a proper and robust operation, and training, the WWTP operators were committed to integrating the plant operation into their daily routine. Monthly routine monitoring samplings over one year formed the basis to assess the performance and suitability of the applied technologies for broader implementation. The study aimed to answer the following research questions:

- How is the suitability of the multibarrier system with O_3 and GAC for advanced wastewater treatment with regard to toxicity?
- How is the toxicity abatement of the two treatment technologies in real-life conditions?

In order to answer the research questions, two approaches were employed:

- The biological equivalent concentrations (BEQs) decrease was determined for the various steps of the multibarrier system.
- The BEQs were compared to currently discussed MOA-specific effect-based trigger values (EBTs).

Base on the research questions, this PhD thesis consists of six chapters. The outline of these chapters can be given as

Chapter 1 presents the necessary background information and motivations to perform this thesis.

Chapter 2 describes the scope and the structure of this thesis.

Chapter 3 provides extensive background on micropollutants in wastewater, advanced wastewater treatment technology, and ozonation.

Chapter 4 starts with materials and methods. It describes lab-scale and pilot scale experimental setup used in this thesis.
Chapter 5 reports and discusses the results obtained in experimental investigation.

Chapter 6 provides a summary and conclusion of this Ph.D. thesis.

3. Background

3.1. Micropollutants in wastewater

3.1.1. Micropollutant overview

Water is a precious resource necessary to sustain the life of all living things, and it is closely related to the main activities of human beings. However, several contaminants of emerging concern (CECs), also known as micropollutants, occur in drinking water, surface waters, and groundwater in concentrations ranging from a few ng/L to several µg/L (Barbosa et al., 2016). Micropollutants are also known as trace organic compounds (TrOCs). They can negatively impact human health, the environment, and aquatic life, which is still less explored and, in some cases, completely unknown. These micropollutants include everyday household products, pharmaceuticals, industrial chemicals, personal care products, polycyclic aromatic hydrocarbons (PAHs), endocrine disrupting compounds (EDCs), pesticides, flame retardants, surfactants, as well as metal TrOCs. Pollution caused by these trace substances in the aquatic environment can adversely affect marine organisms and impair human health as part of an ecosystem (Kanaujiya et al., 2019).

The Chemical Abstract Service Registry grew from 20 million to 156 million chemicals between 2002 and 2019 (Escher et al., 2020). In the European Union, the European Chemicals Agency (ECHA) was established to register chemicals under the new EU-wide act (EG 1907/2006) on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). In REACH there are about 22,614 compounds listed so far (June 2021). Currently, there are no urban wastewater discharge regulations and discharge standards for most of these trace substances in the European Union (EU). To protect water resources, the European Union Water Framework Directive 2000/06/CE lists 45 compounds or priority groups of compounds, including pesticides, heavy metals, PAHs, phthalates, EDCs, etc. Furthermore, a trace list of trace substances for EU monitoring was reported in Decision 2015/495/EU of 20 March 2015, covering a wide range of synthetic and natural chemicals (Barbosa et al., 2016).

The presence of micropollutants in aquatic environments has become a global problem. The main sources in the environment are industrial wastewater, agricultural wastewater, wastewater for the medical facilities, wastewater from concentrated livestock operations, etc. Runoff from farmland and livestock areas is also one of the main sources of TrOCs, especially in the case of pesticides used to increase yield and hormonal and antimicrobial steroids are used to maintain livestock (Song et al., 2007). Furthermore, with the reuse of wastewater in crop irrigation, many TrOCs and their transformation products cause pollution to the receiving water in the fields (Barbosa et al., 2016). Other trace sources include wastewater treatment facilities and leaks from landfills, industrial waste streams, and septic tanks (Matthiessen et al., 2006). Domestic wastewater is another major source of many trace substances such as pharmaceutical products (lipid modifiers, anticonvulsants, antibiotics, β-blockers, and stimulants, etc.), care products personalization (perfumes, disinfectants, UV filters, and insect repellents), and steroid hormones (estrogen) (Luo et al., 2014). In addition, small amounts of these compounds are contributed by their domestic uses and applications in various useful products (Kanaujiya et al., 2019). Table 3.1 summarizes the sources of the major categories of micropollutants in the aquatic environment. The categories are shortly described in the following subchapters.

Table 3.1. Micropollutants categories and their major sources (according to Luo et al. (2014), modified)

Category	Important subclasses	Major sources	Examples
Pharmaceuticals	Antibiotics, antidiabetics, analgesics, anticonvulsants, lipid regulators, anticonvulsants, antibiotics, β-blockers, and stimulants	Urban wastewater (excretion) Hospital effluents Farmland waste	Acetaminophen, diclofenac, ibuprofen, ketoprofen, mefenamic acid, naproxen, carbamazepine, bezafibrate, sulfamethoxazole, metoprolol, caffeine, atenolol, etc.
Personal care products	Fragrances, disinfectants, UV filters, and insect repellents	Urban wastewater (bathing, shaving, spraying, swimming and etc.) Industrial waste	Benzophenone, diltiazem, chloroprene, triclosan, methyl benzylidene, chloroprene, tonalite, etc.
Steroid hormones	Estrogens	Urban wastewater (excretion) Hospital effluents Farmlands	Estradiol, estrone, progesterone, testosterone, etc.
Surfactants	Non-ionic surfactants	Urban wastewater (bathing, laundry, dishwashing and etc.) Industrial wastewater (industrial cleaning discharges)	Alkylphenol ethoxylates, alkylphenols (nonylphenol and octyl-phenol), perfluorooctanesulfonates acid, perfluorooctanoic acid
Industrial chemicals	Plasticizers, fire retardants	Urban wastewater (by leaching out of the material)	Benzotriazole, phthalates, polybrominated compounds, dioxin and furans, polycyclic hydrocarbons, trichloroethylene, benzene, toluene, etc.
Pesticides	Insecticides, herbicides and fungicides	Urban wastewater (improper cleaning, run-off from gardens, lawns and roadways and etc.) Agricultural runoff	Diuron, mecoprop, terbuthylazine, etc.

3.1.1.1. Pharmaceuticals

The use of pharmaceutical products is growing exponentially worldwide. The occurrence of more than 200 different medicinal compounds in river water has been reported worldwide (Hughes et al., 2013). The most commonly studied and used pharmaceuticals are anti-depressants, β-blockers, non-steroidal anti-inflammatory drugs and antiepileptic carbamazepine (Petrie et al., 2015). In addition, antibiotics, anti-inflammatory drugs are the most frequently used pharmaceuticals. The presence of pharmaceutical residues in water makes it chronically toxic to humans and animals (Waleng et al., 2022). The prevalence of antibiotics in the environment is very important. Antibiotics are being used to treat bacterial infections in humans and animals, also for meat production in the livestock industry. More than 250 antibiotics and 63,151 tons of antibiotics are being used in human and animal medicine (Ashfaq et al., 2016). It is estimated that ~70% of antibiotics are neither metabolized nor absorbed in the human or animal body and are excreted into the environment through feces (Ahmad et al., 2019b). The widespread use of antibiotics in humans and animals leads to high concentrations in various aquatic environments. Antibiotics can be released into the environment through manufacturing plants, people, patients, sewer lines, and improper handling of these antibiotics. Antibiotics can also be introduced into groundwater through fertilization and leaching (Ahmad et al., 2019a; Kümmerer, 2009).

Antibiotics affect prokaryotic cells by synthesizing the cell envelope, protein, and nucleic acid (DNA/RNA). Exposure of antibiotics to microorganisms (bacteria) can develop resistance to these drugs. The range of antibiotics in soil and water is from a few nanograms to hundreds of nanograms per kilogram and per liter of soil or water, respectively. This concentration may rise in soil or water adjacent to the hospital or animal production farms (Patrolecco et al., 2015; Verlicchi et al., 2015). However, some antibiotics (i.e., penicillin) can be degraded to some extent, while others, such as tetracycline, remain in the environment for more extended periods and cause more environmental effects (Blackwell et al., 2005). The prevalence of pharmaceutical products in the environment increases day by day as they are released continuously and persist for a long time in the environment (Ahmad et al., 2019a).

3.1.1.2. Personal care products (PCPs)

Personal care products include perfumes, cosmetics, shampoos, liquid bath additives, skincare products, oral care products, soaps, sunscreen products, hair styling products, etc., which are used in considerable quantities around the world. Fragrances such as nitro and polycyclic musk and sunscreens, disinfectants and antiseptics, repellents, preservatives are a subset of PCP ingredients. Many personal care products are used as additives. Cosmetic ingredients are lipids or oils (e.g., sunscreens). Therefore, high diversity is typical for many PCP ingredients (Liu et al., 2013).

The wastewater treatment plants have been found as the main sources for the infusion of PCPs into water bodies because some PCPs cannot be entirely degraded in wastewater treatment (Blair et al., 2015; Liu et al., 2013; Meador et al., 2016).

The contamination of water by PCPs is a matter of concern because of their potential toxicity to aquatic ecosystems, humans, and animals. Many reports show that PCPs are persistent, bioactive, bioaccumulative, and endocrine compounds (Niemuth et al., 2015; Yu et al., 2013). In addition, other features related to their release such as the waste stream flow or the PCPs usage patterns, that vary by region and season, also determine the fate and concentration of these compounds in the environment (Montes-Grajales et al., 2017).

3.1.1.3. Surfactants

The widespread use of surfactants in industry and households has accumulated in the environment. According to reports, the annual production of synthetic surfactants has exceeded 12.5 million tons per year (Ahmad et al., 2019a; Edser, 2006). Depending on the charge on the head groups, surfactants are classified as cationic, anionic, nonionic, and amphoteric. Classification of surfactants describes their physicochemical properties and applications. Surfactants and their residues can enter surface water or groundwater via wastewater systems leading to adverse environmental effects (Ivanković et al., 2010). The presence of surfactants can cause physiological, pathological, and biochemical effects on humans, animals, and aquatic life (Ahmad et al., 2019a).

3.1.1.4. Pesticides

Pesticides can be defined as any substance used to protect crops from attack by pests. Pesticides include insecticides, herbicides, fungicides, mollusks, rodenticides, and nematodes. Pesticides can also be used as plant regulators or plant growth promoters (USEPA, 2014). According to the World Health Organization (WHO), there are about 3,000,000 cases of pesticide poisoning and 220,000 deaths every year. As a result, the widespread use of pesticides has resulted in the accumulation of higher levels of pesticide residues in water bodies worldwide. Organochlorine and organophosphorus are among the most critical groups of pesticides (Ahmad et al., 2019a).

Pesticides also affect non-target species, which then cause many harms to humans, animals, and other terrestrial organisms. It has been reported that about 80–90% of pesticides used are converted to vapors and are harmful to plants and other non-target organisms (Sonal et al., 2019). Pesticides can enter the environment through agricultural practices, industrial waste, tank leaks, landfill washout, sewer and septic tank leaks, and many other sources. Pesticides are highly toxic to humans and animals and can disrupt the function of sex hormones and the reproductive system. Pesticides are often called xenohormones because they interfere with endocrine processes. Excessive use of herbicides, fungicides, and pesticides reduces the density of trees and shrubs and causes deforestation (Ahmad et al., 2019a; Sonal et al., 2019).

3.1.1.5. Industrial chemicals

Industrial chemicals used in a wide range of commercial and industrial applications such as corrosion inhibitors, dishwasher detergents, and antifreeze are also among high-concentration micropollutants of 22.1 μg/L and 24.3 μg/L content, respectively (Deeb et al., 2017; Rogowska et al., 2020).

Polyaromatic hydrocarbons (PAHs) represent a group of semi-volatile, cyclic and toxic micropollutants widely distributed in different environments. The majority of PAHs are carcinogenic and mutagenic (Fouillet et al., 1991). PAHs with 2–3 rings are mutagenic, and 4–7 rings are mutagenic and highly carcinogenic (Fernandes et al., 1997). For these reasons, it is essential to understand PAHs' behavior, transport, fate, environmental risks, and ecological impacts. It has been reported that PAHs can alter water-soluble organic carbon and humid content, thereby affecting the transformation and formation of other coexisting contaminants (Ahmad et al., 2019a; Ye et al., 2019).

PAHs enter the environment through human activities and oil and pyrolysis sources. PAHs can be created through waste incineration, petroleum smelting, coke production, coal gasification and liquefaction, vehicle exhaust emissions, non-smart radiation, asphalt pavement, heating equipment, pyrolysis, smoking, incomplete combustion of organic coal and biomass, and fossil fuels and entering the water through industrial and municipal wastewater, atmospheric precipitation, drainage wastewater, marine traffic, running water and rainwater (Imam et al., 2022). It has been reported that anthropogenic activities, including industrial, urban, or petroleum transportation and petroleum hydrocarbon production, are responsible for releasing the majority of PAHs into the environment. PAHs can be mixed with foods through food handling, drying, roasting, baking and processing (Ahmad et al., 2019a).

3.1.1.6. Endocrine disrupting compounds (EDCs)

EDCs are defined as “an endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations” (Kabir et al., 2015; WHO, 2017). According to the US Environmental Protection Agency (EPA), EDC is an exogenous compound that can interfere with the synthesis, secretion, transport, metabolism, receptor binding, or elimination of endogenous hormones, altering the endocrine and endothelial systems (De Coster et al., 2012; Mnif et al., 2011; Nohynek et al., 2013; Schug et al., 2011). In determining the EDCs criteria proposed by the European Commission, it became clear that EDCs should represent three actions (Slama et al., 2016): endocrine activity; harmful and/or pathological endocrine-mediated activity; and cause-effect relationship between substance and endocrine activity in exposed subjects.

Furthermore, the European Food Safety Authority (EFSA) considers that most EDCs are manufactured substances that interfere with the endocrine system by binding to hormone receptors and/or regulating gene expression. Indeed, epigenetic changes, such as DNA methylation and/or acetylation and histone modifications, appear to be involved in mechanisms involved in endocrine disruption (Gore et al., 2015).

EDCs are of natural and synthetic origin. Naturally occurring human hormones such as 17β-estradiol (E2) and testosterone (estrogen and androgen) are secreted by humans and enter WWTPs. Synthetic origin such as the estrogen-active oral contraceptive 17α-ethinylestradiol (EE2) (Gadupudi et al., 2021)

EDCs can display different pathways to contaminate the human body. The most common routes of exposure are inhalation, ingestion, and direct contact (Balaguer et al., 2017; Gore et al., 2015; Kabir et al., 2015). Following these pathways, EDCs can enter the food chain and accumulate in tissues from animals to humans (Balaguer et al., 2017). Most EDCs appear to be lipophilic, thus getting in adipose tissue, therefore, their half-lives are usually long (Gore et al., 2015; Sargis, 2015). These two features explain why EDCs can accumulate for years in the fatty tissue of any animal, making co-contamination very frequent (Barouki, 2017). Humans and other large mammals and top carnivores are at the top of the food chain to store larger EDCs by bioaccumulation and bio amplification. These processes can produce a "cocktail" of effects with undetermined consequences (Zhang et al., 2011). Indeed, long-term exposure to many compounds can result in cumulative, resonance, and/or synergistic effects. The dose relationship can be complex for many EDCs causing divergent effects at different concentrations. Furthermore, different EDCs induce unconventional dose responses due to the other effects on binding hormone receptors (Barouki, 2017; Gore et al., 2015; Kabir et al., 2015; Schug et al., 2011; Zoeller et al., 2012). Last but not least, the complexity of the dangerous impact of EDCs includes the concept of vulnerable windows. Indeed, evidence suggests that the timing of exposure is of prime importance in assessing effects on the endocrine system (Barouki, 2017; Kabir et al., 2015; Schug et al., 2011; Zoeller et al., 2012).

Municipal wastewater contains complex mixtures of chemicals that are diverse in their structure and biological activity (Reemtsma et al., 2010). There has been growing concern that some of these chemicals can disrupt the endocrine system of organisms, possibly affecting growth, development, and reproduction (Hecker et al., 2009; Kidd et al., 2007). These EDCs differ from traditional contaminants in that they can be biologically active at low concentrations, creating their unique challenges. For traditional risk assessment methods (Hecker et al., 2009). In addition to some EDCs such as natural or synthetic steroid hormones, plasticizers, etc., municipal wastewater contains a large number of unidentified chemical substances that have the potential to contribute to internal disturbances in the environment.

Natural and synthetic EDC released into the environment by humans, animals, and industry; mainly through the wastewater treatment system, before reaching the receiving agency (soil, surface water, sediment and groundwater) (Abdallah, 2016). EDCs' main distribution in the environment is illustrated in Figure 3.1.

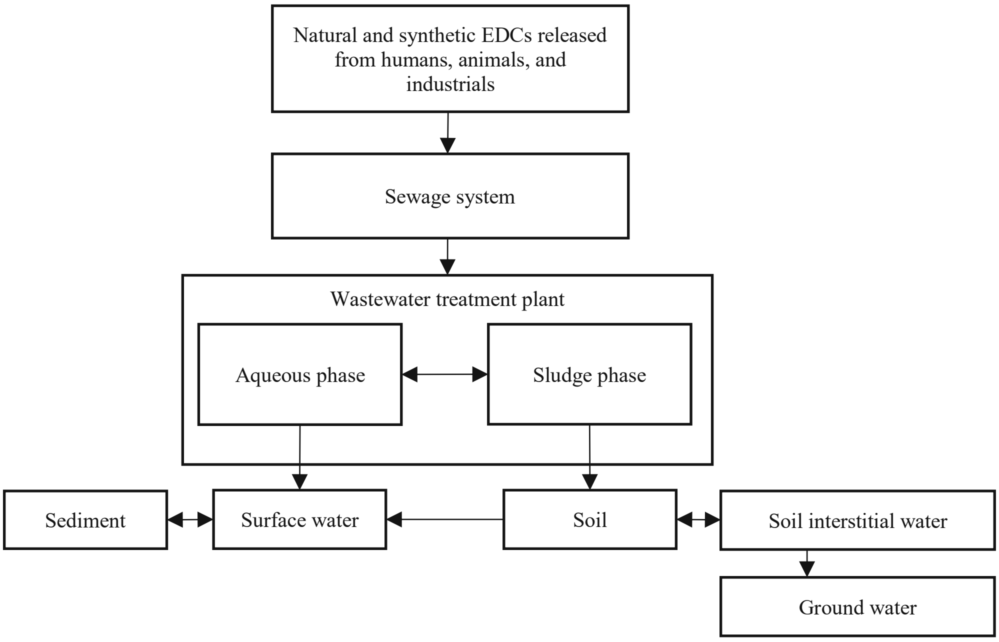

Figure 3.1. EDCs' distribution in the environment (according to Ingerslev et al. (2003), modified)

Observed in the output of WWTPs, endocrine-disrupting compounds (EDCs) are recognized as a group of trace substances that are not fully eliminated by WWTPs. Emissions of EDCs have caused adverse effects on aquatic sensitization categories such as: nervous system damage, feminization and reproductive disruption or inhibition of photosynthesis (Aris et al., 2014; Neale et al., 2017a; Plahuta et al., 2017; Stalter et al., 2013). Peschke et al. (2014) has shown that EDCs cause feminization of male fish and population size changes in female organisms. Furthermore, TrOCs in surface and groundwater can contaminate drinking water sources.

The results of van der Linden et al. (2008), Estrogenic activity was detected in all water samples, were collected from different sources throughout the Netherlands. The estrogenic activity found in the water samples ranged between 0.39 and 1.0 ng/L EEQ (Estrogenic Equivalents) for the municipal WWTPs samples and 0.18-0.50 ng/L EEQ for the surface water samples. Androgenic activity was detected, with again the highest activity in the raw hospital effluent (86 ng/L DHT-EQ (Dihydrotestosterone

Equivalents)), followed by the industrial effluent (81 ng/L DHT-EQ). Activity in the municipal WWTPs effluents was detected at a level of 0.8 ng/L DHT-EQ, but no activity was detected in the surface water. The androgenic activity levels in the WWTPs effluent are consistent with levels reported in effluents elsewhere using YAS. Androgenic activity found in these studies could be almost completely explained by the presence of known (natural) androgens, which are found regularly in effluent and surface waters in the ng/L range. No androgenic activity was detected in the paper mill effluent. Previously, paper mill effluents have been attributed to being a source of environmental androgens in several studies. Possibly, the extensive treatment that is applied at this particular plant decreased the androgenic activity to a level below the AR-Calux of 0.1 ng/L DHT-EQ.

3.1.2. Occurrence of micropollutants in wastewater

Conventional municipal wastewater treatment plants typically remove organic sum parameters (biological oxygen demand (BOD_5) and chemical oxygen demand (COD)), suspended solids, and nutrients (nitrogen and phosphorus) (Ternes et al., 2017). However, most micropollutants cannot be entirely removed and therefore, even treated effluent is considered one of the entry paths into the aquatic environment. The primary sources and their pathways to introduce micropollutants into the environment are represented in Figure 3.2.

Previous studies focusing on the removal of micropollutants from municipal wastewater have shown that the removal efficiency achieved by biological treatment varies widely depending on the physicochemical properties of the compounds in sewage (i.e., adsorption affinity and biodegradability) and the treatments applied (Gros et al., 2010; Jelic et al., 2011). In addition, physicochemical properties such as the n-octanol/water partition coefficient (K_{ow}), the degradation rate, and the organic carbon normalized sediment/water partition coefficient (K_{oc}) (Zhao et al., 2013), as well as other features related to their release such as the waste stream flow or the PCPs usage patterns, that vary by region and season, also determine the fate and concentration of these compounds in the environment (Montes-Grajales et al., 2017). In biological processes, activated sludge removal can be significant (10–80%) for some hydrophobic (e.g., dialkyl dimethyl ammonium chloride, irbesartan, and oxazepam) or charged compounds (e.g., amitriptyline, fluoxetine, and propranolol) (Margot et al., 2015; Rosal et al., 2010). Degradable substances (caffeine, ibuprofen, and paracetamol) can be significantly removed by metabolic reactions on the substrate mixture or co-metabolism (Falås et al., 2016; Velázquez et al., 2017). Table 3.2 summarizes the information of micropollutants in the WWTPs

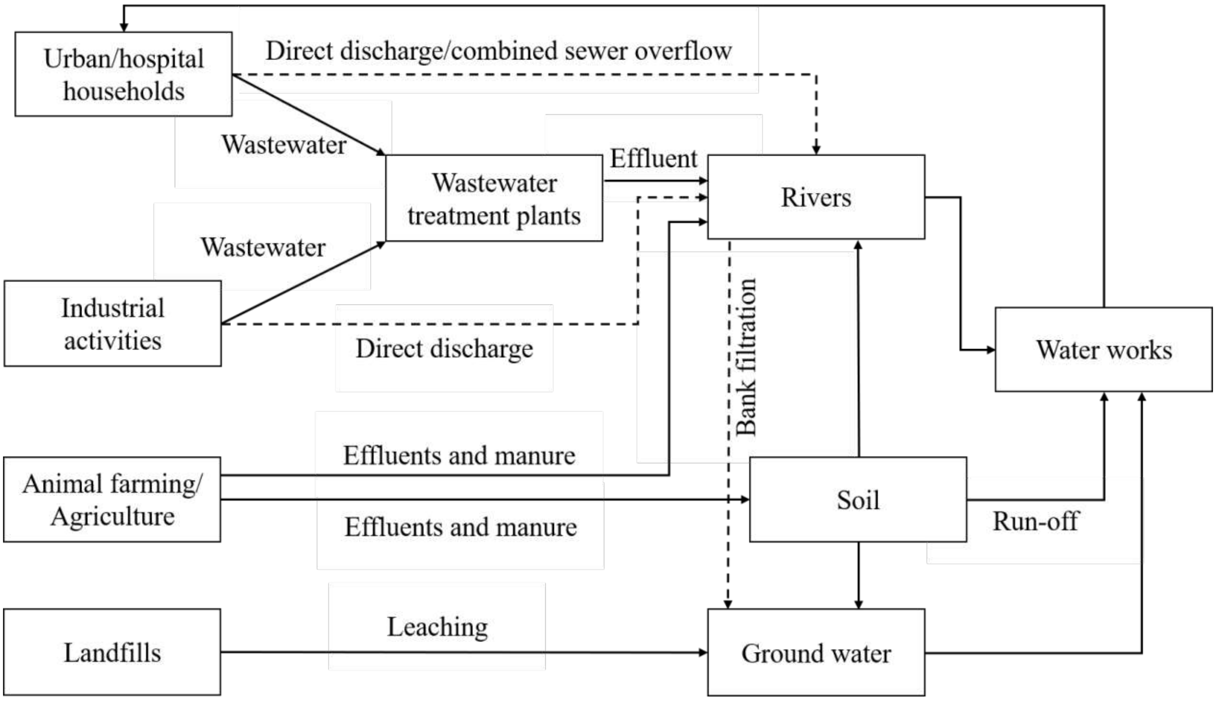

Figure 3.2. Sources and pathways for the release of micropollutants into various environmental compartments (according to Ahmad et al. (2019a), modified)

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Table 3.2. Concentrations and removal efficiency of micropollutants in WWTPs

Category	Important subclasses	Selected compounds	Influent (µg/L)	Effluent (µg/L)	Removal (%)	References
Pharmaceuticals	Analgesic and anti-inflammatory	Acetaminophen	1.57 – 56.9	N.D. – 0.03	98.7 – 100	9, 13, 22, 24, 25, 26, 29, 32, 33
		Diclofenac	< 0.001 – 94.2	< 0.001 – 0.69	< 0 – 81.4	6, 11, 12, 13, 14, 18, 21, 22, 25, 26, 27, 30, 31, 33
		Ibuprofen	< 0.004 – 603	N.D. – 55	72 – 100	10, 11, 12, 15, 18, 21, 22, 23, 26, 31, 33
		Ketoprofen	< 0.004 – 8.56	< 0.003 – 3.92	10.8 – 100	11, 12, 21, 22, 24, 26, 30, 33
		Mefenamic acid	< 0.017 – 1.27	< 0.005 – 0.39	< 0 – 70.2	21, 24, 30, 31, 32
		Naproxen	< 0.002 – 52.9	< 0.002 – 5.09	43.3 – 98.6	7, 11, 12, 13, 21, 22, 23, 24, 25, 26, 33
		Salicylic acid	0.58 – 63.7	N.D. – 0.50	89.6 – 100	22, 32, 34, 35
	Anticonvulsant	Carbamazepine	< 0.04 – 3.78	< 0.005 – 4.60	< 0 – 62.3	2, 5, 11, 12, 21, 22, 23, 24, 25, 26, 27, 31, 33
	Lipid regulator	Bezafibrate	0.05 – 1.39	0.03 – 0.67	9.10 – 70.5	11, 22, 25, 31, 32
		Clofibric acid	0 – 0.74	N.D. – 0.33	< 0 – 93.6	11, 22, 25, 31, 32
		Gemfibrozil	0.10 – 17.1	< 0.0025 – 5.24	< 0 – 92.3	22, 23, 31, 32, 33
	Antibiotic	Erythromycin	0.14 – 10.0	0.02 – 2.84	< 0 – 82.5	1, 21, 26, 32, 33
		Sulfamethoxazole	< 0.003 – 0.98	< 0.003 – 1.15	4 – 88.9	2, 3, 11, 13, 14, 16, 21, 22, 23, 25, 26, 28, 32, 33
		Trimethoprim	0.06 – 6.80	< 0.01 – 3.05	< 0 – 81.6	1, 2, 3, 11, 13, 14, 22, 23, 24, 25, 26, 28, 33
	β-Blocker	Atenolol	0.1 – 33.1	0.13 – 7.60	< 0 – 85.1	11, 13, 22, 23, 25, 26, 32, 33
		Metoprolol	0.002 – 1.52	0.003 – 0.25	3 – 56.4	11, 22, 24, 25, 27
	Nervous stimulant	Caffeine	0.22 – 209	N.D. – 43.50	49.9 – 99.6	12, 22, 23, 24, 32, 33

Category	**Important subclasses**	**Selected compounds**	**Influent (µg/L)**	**Effluent (µg/L)**	**Removal (%)**	**References**
Personal care products	Musk fragrance	Galaxolide	0.03 – 25	< 0.06 – 2.77	87.8	22, 32, 36
		Tonalide	< 0.05 – 1.93	< 0.05 – 0.32	84.7	22, 32, 36
	Disinfectant	Triclosan	0.03 – 23.9	0.01 – 6.88	71.3 – 99.2	10, 11, 12, 19, 20, 22, 23,
	Insect repellant	DEET	2.56 – 3.19	0.61 – 15.8	65.6 – 79.5	11, 25, 32
	UV-filter	Benzophenone-3	< 0.079 – 0.90	< 0.079 – 0.23	63.8 – 98.2	21, 25, 32,
Steroid hormones		Estrone	0.01 – 0.17	< 0.001 – 0.08	74.8 – 90.6	2, 4, 10, 11, 12, 14, 17, 22, 32
		Estradiol	0.002 – 0.05	< 0.001 – 0.007	92.6 – 100	2, 4, 11, 14, 17, 22, 32
		17α-Ethynyl estradiol	0.001 – 0.003	< 0.001 – 0.002	43.8 – 100	2, 4, 5, 11, 17, 32
		Estriol	0.125 – 0.80	N.D.	100	11, 32
Surfactants		Nonylphenol	< 0.03 – 101.6	< 0.03 – 7.8	21.7 – 99	14, 32
		Octylphenol	< 0.2 – 8.7	0.004 – 1.3	< 0 – 96.7	37
Industrial chemicals	Plasticizers	Bisphenol A	< 0.013 – 2.14	< 0.03 – 1.10	62.5 – 99.6	12, 13, 22, 23,
		Di-bytyl phthalate (DBP)	N.D. – 11.8	N.D. – 4.13	73.6 – 75.5	38
		di(2-ehlhexyl) phthalate (DEHP)	0.003 – 70.0	0.0001 – 54.0	25 – 97	10, 32
		di-methyl phthalate (DMP)	N.D. – 6.49	N.D. – 1.52	84.8 – 93.5	32

Category	Important subclasses	Selected compounds	Influent (μg/L)	Effluent (μg/L)	Removal (%)	References
Industrial chemicals	Fire retardant	tris(2-chloroethyl) phosphate (TCEP)	0.06 – 0.50	0.06 – 2.40	< 0	10, 22, 25, 32
		tris(1-chloro-2-propyl) phosphate (TCPP)	0.18 – 4	0.10 – 21	< 0	25
		Benzotriazole	1119 – 44000	2 – 7	80 – 100	8, 22, 27, 32
Pesticides	Herbicide	Atrazine	0.02 – 28	0.004 – 0.73	< 0 – 25	22
		Diuron	0.03 – 1.96	0.002 – 2.53	26.7 – 71.9	22, 24, 26
	Insectcide	Diazinon	< 0.684	0.0007 – 4.16	< 0	22
	Fungicide	Clotrimazole	0.012 – 0.08	N.D. – 0.005	84.5 – 93.6	22

N. D.: not decteed.

1. Botero-Coy et al. (2018), 2. Di Marcantonio et al. (2020), 3. D'Alessio et al. (2018), 4. Zhang et al. (2018), 5. Krzeminski et al. (2019), 6. Escapa et al. (2018), 7. López-Serna et al. (2019), 8. Gatidou et al. (2019), 9. Villar-Navarro et al. (2018), 10. Wang et al. (2019), 11. Gretzschel et al. (2020), 12. Bogunović et al. (2021), 13. Gonzalez-Gil et al. (2019), 14. Kennes-Veiga et al. (2021), 15. Jia et al. (2020), 16. Zhou et al. (2019a), 17. Kennes-Veiga et al. (2022), 18. Granatto et al. (2020), 19. Zhao et al. (2020), 20. Fan et al. (2020), 21. Ma et al. (2020), 22. Liu et al. (2021), 23. Devault et al. (2021), 24. Costa et al. (2021), 25. Ofrydopoulou et al. (2022), 26. Guillossou et al. (2021), 27. Fundneider et al. (2021), 28. Kairigo et al. (2020), 29. Natarajan et al. (2021), 30. Hara-Yamamura et al. (2022), 31. Goswami et al. (2021), 32. Mojiri et al. (2022), 33. Son et al. (2022), 34. Cerqueira et al. (2019), 35. Lopez et al. (2022), 36. Li et al. (2022), 37. Schinkel et al. (2022), 38. Dong et al. (2022)

3.2. Advanced wastewater treatment

As demonstrated in Table 3.2, many TrOCs such as EDCs, PPCPs, pesticides/bactericides, and some household chemicals are not well removed during biodegradation (cf. Margot et al. (2015), Yang et al. (2017)). The appearance of these compounds in biological treatment systems demonstrates the need for additional processes to remove them from wastewater. In this context, one possible solution for wastewater quality improvement is to upgrade existing WWTPs with the advanced treatment processes.

To date, there has been an ongoing effort to identify technically and economically viable advanced wastewater treatment options for minimizing micropollutants from conventional biological treatment (Abtahi et al., 2018; Bonvin et al., 2016). Powdered activated carbon (PAC) adsorption (Boehler et al., 2012; Margot et al., 2013), membrane filtration (Gracia-Lor et al., 2012; Urtiaga et al., 2013), and oxidation (Bourgin et al., 2018; Lee et al., 2013) can be performed as advanced treatment in wastewater treatment plants.

Besides the type and dosage of activated carbon (Kårelid et al., 2017), the extent of adsorption also depends on the operating conditions (Azhar et al., 2016) and the composition of the water matrix (Zietzschmann et al., 2016). Activated carbon allows the removal of a broad spectrum of micropollutants due to its high specific surface area and its unique combination of highly developed porous network and surface chemical properties (Álvarez-Torrellas et al., 2016). Granular activated carbon (GAC) has been studied in several treatment plants, showing a slight decrease in efficiency depending on the compound and the frequency of GAC regeneration/replacement (Grover et al., 2011; Reungoat et al., 2012). Due to its smaller particle size, PAC is generally superior in terms of adsorption kinetics and may be more efficient than GAC (Nowotny et al., 2007). However, the slow reaction rate and problems with separation of PACs from wastewater (Abegglen et al., 2009; Ruhl et al., 2014), competition between micro-contaminants and effluent organic matter low molecular weight compounds onto PACs (Zietzschmann et al., 2016), and the potential need for an additional disinfection step to meet more stringent standards for wastewater reuse (Rizzo et al., 2019) limits its application. Micro-granules of activated carbon (μGAC) have recently emerged as an exciting form of activated carbon used in waste treatment plants due to various advantages over PACs, including reduction of waste solids required treatment, it is not necessary to inject a coagulant such as $FeCl_3$ to prevent leakage of activated carbon and is simpler to operate at a similar cost (Alves et al., 2018; Mailler et al., 2016).

Membrane technology (e.g., high-pressure nanofiltration (NF) and reverse osmosis (RO) membranes) has received much attention due to the removal of many trace substances in wastewater. The effluent wastewater can then be reused in groundwater or agriculture. The modularity and integrability of NF and RO with other systems (such as activated carbon, ozonation) are high (Oller et al., 2018; Taheran et al., 2016). However, the major drawback of NF is the generation of a concentrated stream, which can be up to 10–20% of the original wastewater volume (Kappel et al., 2014). The high energy requirements due to increased pressure operation, as well as the costs associated with clogging problems and membrane replacement, prevent these filtration techniques from being genuinely sustainable (González et al., 2015; Taheran et al., 2016).

Given the limitations of activated carbon and membranes, ozone has been offered as a viable alternative for advanced treatment of municipal wastewater due to its versatility (Prieto-Rodriguez et al., 2013) and potential ability to both reduce the release of micropollutants into water bodies and improve the quality of wastewater for reuse purposes (De la Cruz et al., 2012).

3.3. Ozone and ozonation process

3.3.1. Basics of ozone

In 1785, the Dutch chemist Martinus van Marum was conducting experiments involving electric sparks on the surface of water when he noticed an unusual odor, which he attributed to an electrical reaction, not realizing that he was actually created ozone. Until 1839, the chemist Christian Friedrich Schönbein noticed a similar pungent odor and recognized it as a common odor after a flash of lightning. He called the gas “ozone” because of its strong smell (in Greek ozein). The formula for ozone (O_3) was not determined until 1865 by Jacques-Louis Soret and confirmed by Schönbein in 1867 (von Sonntag et al., 2012).

Ozone is a highly reactive and unstable molecule composed of three oxygen atoms. Ozone formation is endothermic, and ozone is thermodynamically unstable and readily converted to oxygen. Its smell is sensitive for the human nose from an indicative level of 15 μg/m^3 to a clear identification when the ozone concentration is 30-40 μg/m^3. At room temperature, ozone is an unstable gas and it is blue when it is viewed under sufficient thickness (Baig et al., 2010). The following ozone structures can be found at Figure 3.3.

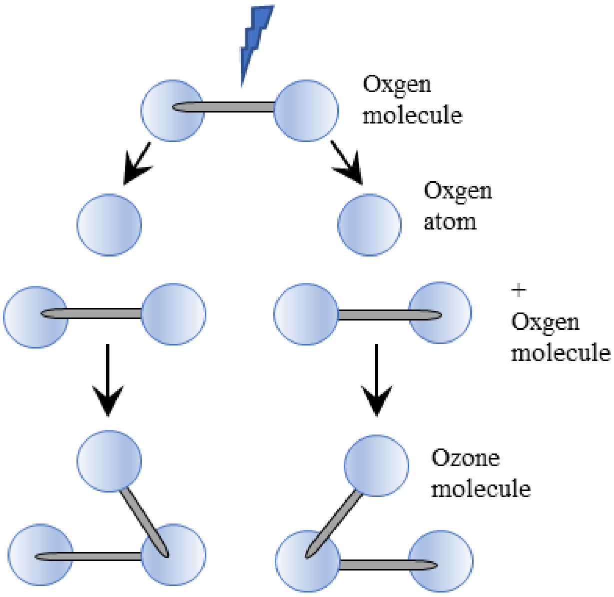


Figure 3.3. The structure of ozone

Moreover, a summary of the physicochemical and thermodynamic properties of ozone is presented in Table 3.3

Table 3.3. Ozone properties

Property	Unit	Value	Reference
Molecular formula		O_3	(Baig et al., 2010; von Sonntag et al., 2012)
Molecular mass	g/mol	47.998	
Melting point	ºC	-192.7	
Boiling point	ºC	-110.5	
Henry constant at 0^0C	atm/M	35	
Henry constant at 20^0C	atm/M	100	
Solubility in water	g/L	0.0105	
Density (at 0^0C, gas)	g/L	2.144	
Free molar formation entalpy	KJ/mole	142.2	

3.3.2. Ozone generation

The ozone storage is a problem; thus, ozone is produced on-site (Baig et al., 2010). Nowadays, the ozone generator (Figure 3.4) used for industrial applications, is based on the improvement of the one invented by Werner von Siemens in 1857 (von Sonntag et al., 2012). From all the techniques of ozone generation: electrolysis of water, high-stress discharge inside an oxygen stream, photolysis of oxygen by UV radiation ($\lambda < 220$ nm), and decomposition of oxygen by constant radiation; only electric discharge (Corona) allows industrial production (> 2 kg/h) as with other systems ozone is rapidly converted to oxygen (Baig et al., 2010).

The corona electric discharge consists of an electrical energy flow passing through a narrow gap filled with oxygen or air. When it happens, the connection between the oxygen molecules is broken up and oxygen radicals are produced, that connect with the oxygen molecule to ozone. The residual heat has to be removed by a cooling system. (Kreuzinger et al., 2011).

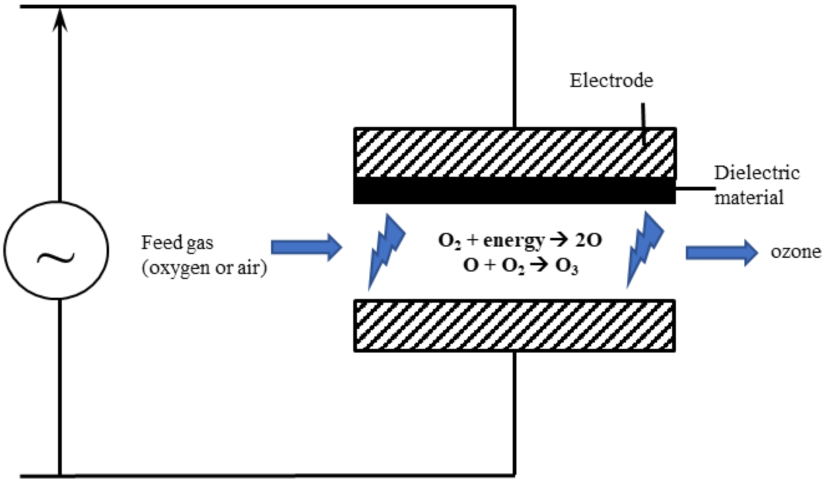


Figure 3.4. Basic principle of an ozone generator

(Adopted from https://www.lenntech.com.pt/library/ozone/generation/ozone-generation.htm)

The produced ozone concentration varies depending on the feed gas, for instance, for oxygen-fed ozone systems, the range is 6-16 % (typically 8-12%), and for air-fed ozone systems the range of 1-4 % (Rakness, 2011).

3.3.3. Advantages and disadvantages of ozone

Advantages of ozone include: (1) it can quickly be produced from air or oxygen by electrical discharge; (2) it reacts readily with organic and inorganic compounds; (3) a wide range of applications such as disinfection, reduction of chemical oxygen demand, color, odor and turbidity of treated water, and (4) any excess of ozone in the water will readily decompose into oxygen, without leaving any residue. Therefore, ozone is used as a chemical reagent in synthesis, water and wastewater treatment such as oxidation of biological pollutants, removal of taste, odor and color and reduction of turbidity. In the EU, ozone is used for disinfection and odor absorption in drinking water since 1906 in France, and then in other countries in the region (Ikehata et al., 2018; von Gunten, 2018; Wu et al., 2018).

However, ozone also has disadvantages such as difficulty in maintaining residual ozone after sterilization, making it difficult to prevent the re-growth of microorganisms. It is therefore necessary to use additional secondary disinfectants (e.g., chlorine) to maintain water quality (Demir et al., 2016). Other disadvantages include: formation of oxidation byproducts, such as bromate, aldehydes, and the difficult, to transfer mass of ozone to wastewater.

Ozone technology developments have been opened new applications for these conventional water treatment technologies. The change of ozone technology has identified unique, more disinfection-resistant microorganisms such as Giardia and Cryptosporidium cysts and governmental regulations designed to protect public health from the hazards of ingestion of these microorganisms.

3.3.4. Application of ozonation process in wastewater treatment

Ozonation has been intensively tested as advanced wastewater treatment in the laboratory- (Chys et al., 2017; Mecha et al., 2016), pilot- (Gerrity et al., 2011; Singh et al., 2015), and a full-scale (Blackbeard et al., 2016; Schollée et al., 2018) has been studied and proven to be one of the most effective and easily implementable techniques to reduce micropollutants in municipal wastewater (Cruz-Alcalde et al., 2019; Gomes et al., 2017). In Switzerland, the process is considered one of the best available technologies to fulfill the requirements of protecting the water resource, which aims to ensure the removal of an average of 12 indication substances (Eggen et al., 2014; Norte et al., 2018). Although the current legislative situation in Germany does not explicitly require the construction of advanced treatment units, several WWTPs have been upgraded with ozonation to reduce micropollutants emissions into the aquatic environment (Rizzo et al., 2019). In Austria, pilot plants (including ozonation and GAC) were operated for application and performance monitoring (Rizzo et al., 2019; Schaar, 2015). Ozonation is also used in full-scale treatment plants in France and Sweden (Östman et al., 2019; Penru et al., 2018). Figure 3.5 shows the increasing trend of articles published in academic journals containing the word "ozonation and wastewater" since 2010.

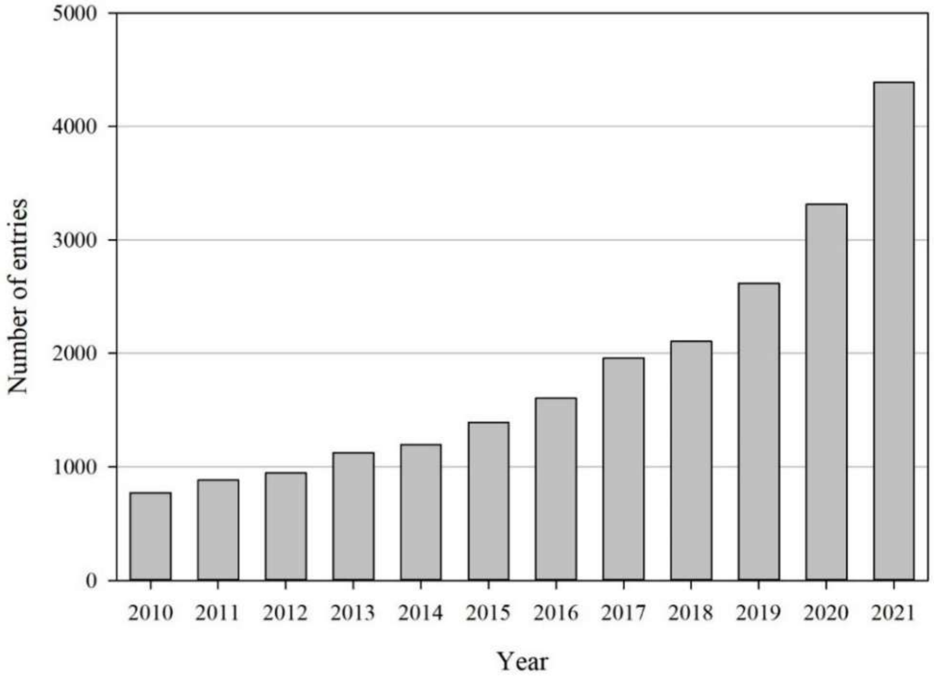


Figure 3.5. Number of entries searching "ozonation and wastewater" in Science Direct (only Research Articles)

During ozonation, two different reaction mechanisms are responsible for the degradation of micropollutants, namely the reaction with molecular ozone and the indirect reaction of hydroxyl radicals ($OH^{\bullet}$) generated by the reaction of ozone with certain electron-rich organic compounds, e.g., phenols and secondary amines (von Sonntag et al., 2012). Ozone reacts selectively with compounds containing electron-rich elements such as olefins, deprotonated amines, or activated aromatics, exhibiting a reaction rate constant (k_{O3}) over several orders of magnitude in ranges from 1 to 10^7 $M^{-1}s^{-1}$ (von Sonntag et al., 2012). With relatively low selectivity, $OH^{\bullet}$ is capable of oxidizing many micropollutants species with extremely high reaction rate constants (k values $OH^\bullet$ in the range 10^8 –10^9 $M^{-1}s^{-1}$, revealing unique differences by at least one order of magnitude), making the indirect reaction mechanism beneficial for the removal of ozone-refractory contaminants (Gligorovski et al., 2015; Lee et al., 2013). Dissolved organics and micropollutants are usually not mineralized, but are converted to smaller and structurally related substances, which are generally more biodegradable and less toxic (Hübner et al., 2015; Völker et al., 2019). Effluent organic matter serves as one of the most important parameters for ozonation as it contains many ozone reactive functional groups, reducing the amount of oxidants available for reaction with TrOCs (Chys et al., 2017; Rizzo et al., 2019). In the ozonation of biologically treated municipal wastewater, the specific ozone dose normalized to dissolved organic carbon concentrations (i.e., g O_3/g DOC) are commonly used as the operating parameter to compare effluents with different DOC concentrations. However, it is unclear whether the same ozone and $OH^\bullet$ exposure is achieved at the same g O_3/g DOC in other substrates. Supposing the effluent organic matter characteristics of different cities are the same. In that case, it can be hypothesized that the same g O_3/g DOC induces similar ozone and $OH^\bullet$ exposure, regardless of the substrate (Lee et al., 2013). Another precondition for comparing removal efficiencies is nitrite compensation of the specific ozone dose. Nitrite reacts quickly with ozone, consuming 3.43 g O_3/g NO_2-N (Lee et al., 2013). To remove micropollutants from WWTPs wastewater, typical ozone dosage ranges from 0.25 to 1.5 g O_3/g DOC (Baresel et al., 2016; Lee et al., 2016; Rizzo et al., 2019). At ozone doses < 0.5 g O_3/g DOC, more than 80% removal is possible for degradable micropollutants such as the pharmaceuticals diclofenac, sulfamethoxazole, and carbamazepine (Bourgin et al., 2018; von Sonntag et al., 2012). However, removal of ozone-resistant micropollutants (e.g., ibuprofen, clofibric acid, p-chlorobenzoic acid, and chloramphenicol) generally requires higher dosages (> 1.0 g O_3/g DOC) to achieve removal efficiency of at least 80% (Yao et al., 2018).

3.3.5. Formation of oxidation byproducts as a result of ozonation

In the ozonation reaction, low mineralization may occur (i.e., the oxidation may be incomplete). It leads to the accumulation of intermediates, which are oxidation byproducts. These oxidation byproducts may under some circumstances, but not systematically, be potentially more toxic than the primary pollutants (Gomes et al., 2017; Luo et al., 2014; Margot et al., 2013; Stalter et al., 2010).

Bromate is a byproduct of oxidation that can be formed when wastewater is ozonized (Figure 3.6). Bromate is potentially carcinogenic and is formed during ozonation in wastewater containing bromide (von Gunten, 2003b; von Sonntag et al., 2012). Bromate formation is also noteworthy because bromate is not degraded in rivers and under aerobic shoreline conditions (Schindler et al., 2015). Therefore, many studies on water ozonation have focused on this compound. WHO and the European Union jointly recommend a limit of 10 µg/L in drinking water, but the European Union recommends lower values for the Member States where possible (WHO, 2017). Hollender et al. (2009) found that the low levels of bromides in the effluents lead to low concentrations of bromates after ozonation (7.5 µg BrO_3^-/L), specifically below the drinking water standard.

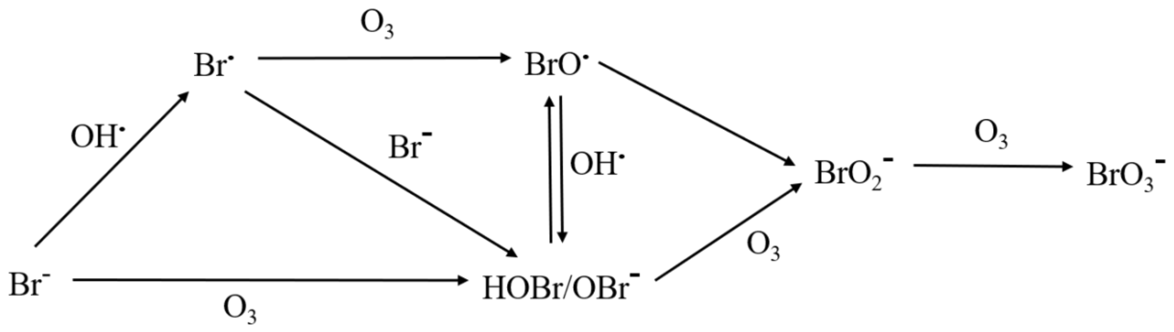


Figure 3.6. Reaction scheme for bromate formation during ozonation of bromide containing waters (according to Ratpukdi et al. (2011), modified).

The study of Schindler et al. (2015) introduced a test procedure to test the case-specific suitability of effluent. The application of this test procedure to seven wastewater treatment plants, with wastewater representing a wide range of biological processes and industrial contribution rates, revealed bromide concentrations (0.034 − 48 mg/L) and bromate formation between < 2 and > 400 µg/L upon ozonation (specific ozone dosage: 0.5, 1 and 1.5 mg O_3/mg DOC). This corresponds to bromate yielding 0.3 − 31.7% mg BrO_3^- /mg Br^- (0.2−19.8% mol BrO_3^- / mol Br^-). Therefore, wastewater ozonation is a potential source of bromate for surface water in the future and should be investigated whether this poses a risk to ecosystems or drinking water sources. Soltermann et al. (2017) showed that bromate was only significantly formed at specific ozone doses ≥ 0.4 - 0.6 g O_3/g DOC. Therefore, it is feasible to remove micropollutants with high ozone reactivity and partially remove others without significant bromate formation.

4. Materials and Methods

This chapter begins with an overview of the experiments. Then, it continues with a detailed explanation of the production of ozone stock solution, the set up at laboratory experiments, and pilot plant. Then, introducing the wastewater sources used in this thesis. Finally, the experimental work presented in this Ph.D. study is described in detail, including analytical parameters and separation methods.

4.1. Experiment overview

Lab-scale experiment 1 is designed to target and test the elimination of micropollutants and the formation of byproducts (bromate) during ozonation. The effluent samples from a WWTP in Austria were used for the investigation. The experiments based on the guideline by the Swiss experts is used in the laboratory to assess and evaluate the processability (Zappatini et al., 2015). The focus of lab-scale experiment 1 has been to investigate the degradation efficiency of micropollutants at the different specific ozone doses, also considering the formation of bromate (BrO_3^-) as an oxidation byproduct. Results are compared with reference data from the literature to provide a follow-up assessment of applicability.

Lab-scale experiment 2 aimed to evaluate the impact of ozonation on the biodegradability change of recalcitrant COD in treated urban wastewater. The effluent samples from four Austrian municipal wastewater treatment plants operating at full nitrification and denitrification (high sludge retention time and low food to microorganism ratio) were investigated. The experiments were similar to lab-scale experiment 1 with three specific ozone doses (low, average, and high). The focus of lab-scale experiment 2 has been to evaluate the correlation between ozonation process/specific ozone dose and biodegradability also the effect on total organic parameters.

The pilot-scale experiments were conducted at a full-scale WWTP. The pilot-scale plant is an advanced wastewater treatment system (ozonation and granular activated carbon). The objective was long-term monitoring of the toxicity of wastewater after passing through an advanced wastewater treatment system with an operating modality based on an in vitro biological assay kit targeting toxicological endpoints under “real life” conditions. Routine sampling for chemical contaminants of emerging concern (CEC) analysis and effect-based method testing (EBM) was efficient control and monitoring.

4.2. Laboratory experiments

4.2.1. Production of ozone stock solution

The ozone stock solution was produced based on the guideline of Zappatini et al. (2015). The experimental setup is illustrated in Figure 4.1.

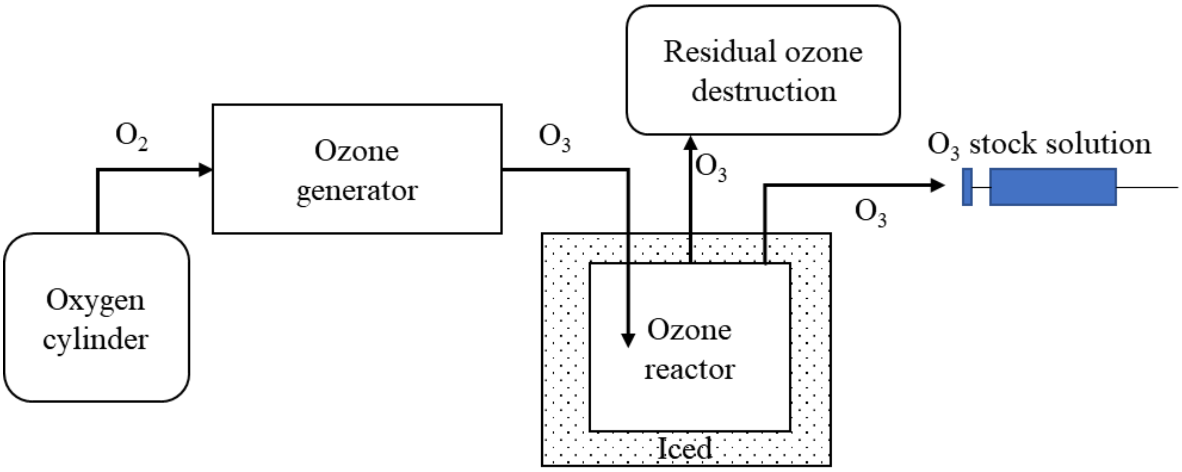


Figure 4.1. The structure of the ozone system

Ozone is unstable and therefore cannot be stored in the same way as oxygen. It is necessary to produce ozone with an ozone generator continuously with the oxygen tank. Oxygen is supplied to the ozone generator (Fischer technology model OZ200/5) that was kept at the power 35 W with a flow rate of 10 L/h. The generated O_3 was fed to the reactor for the O_3 stock solution via ozone-resistant hose material (PTFE). The ozone reactor (glass bottle, 2 liters) was filled with deionized water that was stored in the fridge overnight. Gaseous ozone is introduced into the liquid as fine bubbles through an aeration stone, producing a concentrated O_3 stock solution. The concentration of the O_3 stock solution can vary greatly depending on the temperature. Therefore, the O_3 stock solution was cooled in an ice bath following the procedure by Zappatini et al. (2015). The ozone concentration was determined by the indigo method (see chapter 4.6.1) and photometry ($\varepsilon = 2950$ l/mol.cm and $\lambda = 258$ nm) (Bader et al., 1981; von Sonntag et al., 2012). Ice is added to keep the stored ozone stock solution stable. The ozone concentration in the stock solution varied between 40 and 55 mg O_3/L, depending on the experiments. Because not all gaseous ozone is soluble in water and exits the reactor, a bottle with potassium iodide solution is used to remove residual ozone. In addition, an ozone alarm device was used, which provides audible and visual warnings from a concentration of 0.1 ppm in the ambient air. Since ozone is a toxic gas with irritating effects, it needs to be worked inconspicuously and with special attention to safety. Figure 4.2 shows the ozone system in the laboratory.

Figure 4.2. The ozone system in the laboratory

1. ozone generator, 2. spectrometers, 3. pump, 4. reactor for the O_3 stock solution, 5. ice bath, 6. potassium iodide solution, 7. ozone alarm device

4.2.2. Experimental setup for micropollutant abatement

The batch test was used to determine the degradation of micropollutants. The wastewater (effluent) was mixed with the O_3 stock solution in 50 and 100 mL-Schott bottles. The mixing ratio of wastewater and O_3 stock solution was based on the nitrite compensated targeted D_{spec}, the ozone concentration in the ozone stock solution, and the DOC and nitrite in the wastewater. The number of Schott bottles used per experiment varied and was adapted to the specific ozone doses investigated. For example, with the selected specific ozone doses of 0.2, 0.4, 0.6, 0.8, and 1.0 (g O_3/g DOC), six Schott bottles were required. Five bottles were filled with the wastewater and the O_3 stock solution (adapted to the specific ozone dose), and the sixth bottle only with the wastewater (reference sample). All experiments were carried out in duplicates. The experimental setup is shown in Figure 4.3 and Table 4.1.

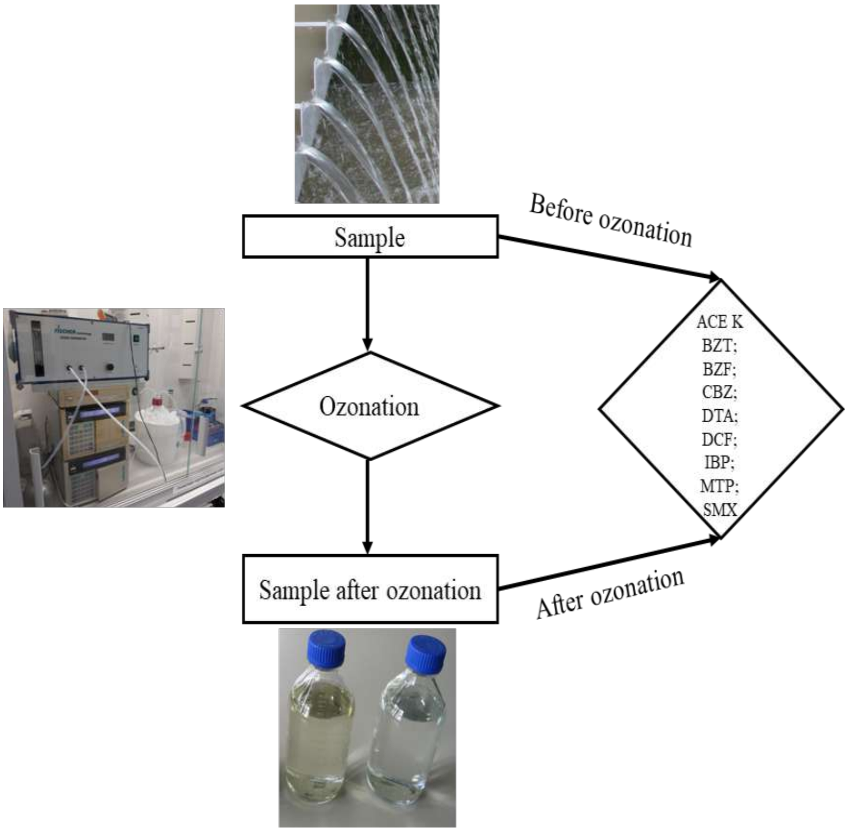


Figure 4.3. The experimental set up, including analyzed parameters

Table 4.1. Schematic ratio of wastewater and ozone stock solution in the ozonation batch tests

Applied volumes / sample	**D_{Spec} (g O_3/g DOC)**					
	0	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Number of Schott bottles	1	2	3	4	5	6
Total volume (mL)	50	100	100	50	50	50
Volume of ozone stock solution (V_O_3)	V_WW	V_O_3	V_O_3	V_O_3	V_O_3	V_O_3
Volume of the investigated wastewater sample (V_WW)		V_WW	V_WW	V_WW	V_WW	V_WW

4.2.3. Experimental setup for the biodegradability study

The experiments in this study were similar to Chapter 4.2.2 and with three targeted D_{spec} (0.4, 0.6 and 0.8 g O_3/g DOC). The wastewater (effluent) was mixed with the O_3 stock solution in 0.5 L-Schott bottles. The mixing ratio of wastewater and O_3 stock solution was based on the targeted D_{spec}, the ozone concentration in the ozone stock solution and the DOC and nitrite in the wastewater (see Table 4.3). To ensure that the volume and dilution of wastewater was the same in every batch for the dose-specific experiments, the sum of the ozone stock solution and the deionized water was kept constant, as shown in Table 4.2. Typically, 450 mL of wastewater was diluted by 50 mL of the ozone stock solution and deionized water. All experiments were carried out in triplicate. After a reaction time of approximately 1 hour, the samples were aerated with a fine ceramic aerator for 15 min to remove possible residual ozone.

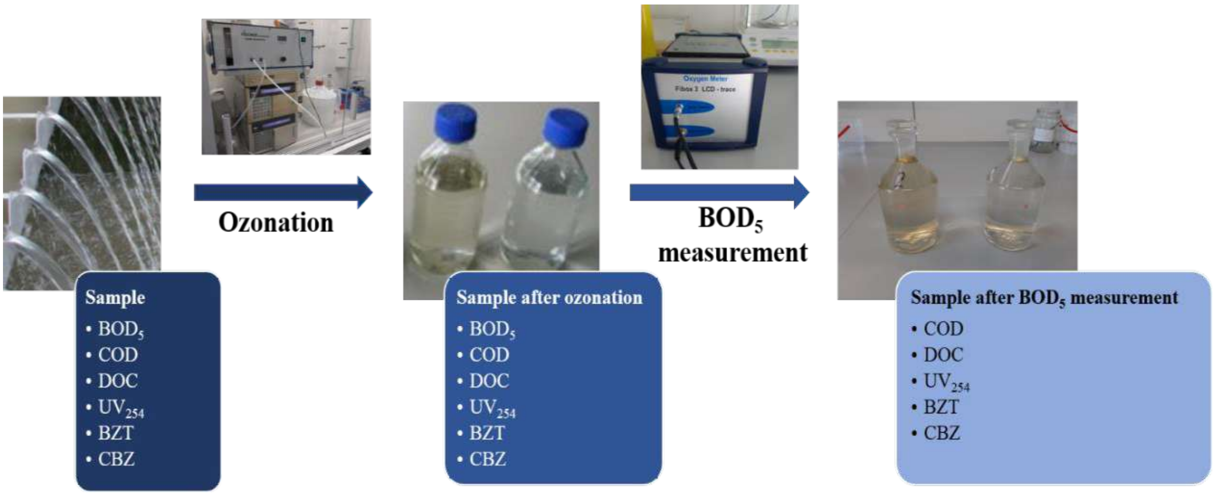


Figure 4.4. The experiment setup, including analyzed parameters

Carbamazepine (CBZ) and benzotriazole (BZT) were analyzed as process control parameters to evaluate the validity of the ozonation experiments. This was done by comparing the observed abatement with values expected from literature and own experiments. In that regard, both substances are recommended as process indicator substances for ozonation by Jekel et al. (2015). The two micropollutants show different reactivity during ozonation: CBZ is an indicator substance for highly reactive compounds, whereas BZT represents moderately reactive compounds, reflected by their second-order rate constants $k_{O3} = 3 \times 10^5$ $M^{-1}s^{-1}$ for CBZ and $k_{O3} = 230$ $M^{-1}s^{-1}$ for BZT (Huber et al., 2003). Based on the high k_{O3} for CBZ an abatement of ≥ 80 – 90% is expected for D_{spec} above 0.4 g O_3/g DOC. A lower abatement can be considered an indication for methodological or experimental shortcomings.

Table 4.2. Schematic ratio of deionized water, wastewater and ozone stock solution in the ozonation batch tests

Applied volumes / sample	WW before ozonation	WW after ozonation (0.4 g O_3/g DOC)	WW after ozonation (0.6 g O_3/g DOC)	WW after ozonation (0.8 g O_3/g DOC)
Volume of deionized water (V_DW)	V_DW	V_DW	V_DW	V_O_3
Volume of ozone stock solution (V_O_3)		V_O_3	V_O_3	
Volume of the investigated wastewater sample (V_WW)	V_WW	V_WW	V_WW	V_WW

Table 4.3. Nitrite compensated specific ozone doses (D_{spec}) and applied volumes in the ozonation experiments

Name	D_{spec} (g O_3/g DOC)	V_Total (mL)	V_Wastewater (mL)	V_DI water (mL)	V_Ozone (mL)
WWTP1a	0.65	500	460	0	40
WWTP1b	0.61	500	455	0	45
WWTP2a	0.69	500	450	0	50
WWTP2b	0.67	500	450	0	50
WWTP2c	0.43	500	450	22	28
	0.62	500	450	10	40
	0.78	500	450	0	50
WWTP3	0.46	500	435	27	38
	0.67	500	435	13	52
	0.87	500	435	0	65
WWTP4	0.45	500	440	26	34
	0.65	500	440	12	48
	0.83	500	440	0	60

4.3. Pilot plant experimental setup

A flow scheme of the pilot plant, following the multibarrier approach combining ozonation and granular activated carbon filtration, is shown in Figure 4.5 - including the sampling points for this study. The three ozone reactors (O_3-R) operated in series had a total volume of 12 m^3 and the hydraulic retention time varied between 9 and 40 min, depending on the inflow dynamics of wastewater. The activated carbon filter was filled with 1.8 m^3 of granular activated carbon (GAC), type Epibon A (Donau Carbon, Frankfurt, Germany), and treated a side stream of 8 m^3/h, which resulted in a hydraulic retention time of 13.5 min. A specific nitrite compensated ozone dose of 0.55 g O_3/g DOC was targeted in the automated process control system based on a UV_{254} – DOC mathematical model and continuous UV_{254} measurement and posteriori ranged between 0.4 and 0.7 g O_3/g DOC in the routine operation and between 0.2 and 0.9 g O_3/g DOC including specific research campaigns. The sampled bed volumes of the granular activated carbon filter ranged from approx. 1,000 (start of monitoring) to 33,100 (final sampling campaign). After approximately 2,000 bed volumes a biological activation of the granulated carbon filter could be observed.

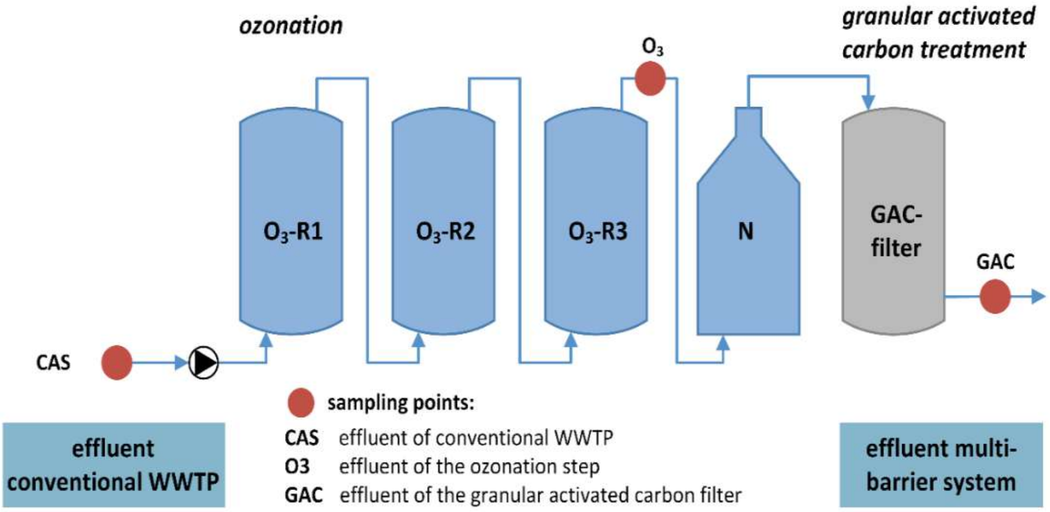


Figure 4.5. Flow scheme of the advanced treatment demonstrator plant with the sampling points (O_3-R…ozone reactor, N…feed tank for GAC-filter, GAC…granular activated carbon)

4.4. Analyzed parameters

4.4.1. Sampling and investigating wastewater characteristics

In lab-scale experiment 1, effluents from a WWTP in Austria were used in this study. The grab sample was collected with a polyethylene tank (20 liters). After collection, the samples were immediately stored in a refrigerator. Wastewater was placed at room temperature (23 ± 2ºC) for at least 3 h to increase the temperature before starting the experiment. The average wastewater parameters are listed in Table 4.4.

Table 4.4. Average wastewater parameters of the effluent samples

Parameters	Unit	Average values ± standard deviation
COD	mg/L	14.3 ± 2.54
DOC	mg/L	4.08 ± 0.39
BOD_5	mg/L	1.04 ± 0.47
TN	mg/L	0.89 ± 0.9
NH_4^+	mg/L	0.47 ± 0.15
NO_2^-	mg/L	0.47 ± 0.15
TP	mg/L	0.24 ± 0.11

In lab-scale experiment 2, effluent samples from four Austrian municipal wastewater treatment plants (WWTPs) operating at full nitrification and denitrification (high sludge retention time and low food to microorganism ratio) were investigated. Samples were collected in a polyethylene tank (20 liters) and filtered with glass fiber filters (0.45 µm) before the experiments for reasons of reproducibility of measurements at the low concentrations expected. Parameter values relevant for this study are listed in Table 4.5. Effluent samples were collected as 24-h volume proportional composite samples (constant volume, variable time) or grab samples. As the goal of this part was to demonstrate the change in biodegradability due to the effects of ozone on the water matrix, representative daily composite samples were not taken for all experiments.

Table 4.5. Average wastewater parameters of the four investigated WWTPs

Name	Type of sample	COD (mg/L)	BOD_5 (mg/L)	DOC (mg/L)	Nitrite (mg/L)
WWTP1a	Grab	15.22 ± 0.00	0.65 ± 0.07	5.29 ± 0.06	0.02 ± 0.00
WWTP1b	Composite	18.32 ± 0.63	0.68 ± 0.12	6.45 ± 0.10	0.05 ± 0.00
WWTP2a	Grab	15.56 ± 0.00	1.42 ± 0.38	5.85 ± 0.06	0.05 ± 0.00
WWTP2b	Composite	14.81 ± 0.64	1.38 ± 0.14	5.81 ± 0.06	0.05 ± 0.00
WWTP2c	Grab	15.56 ± 1.92	1.33 ± 0.10	5.22 ± 0.00	0.03 ± 0.00
WWTP3	Grab	17.24 ± 0.00	1.99 ± 0.14	6.28 ± 0.12	0.2 ± 0.00
WWTP4	Grab	18.18 ± 0.00	1.91 ± 0.04	6.82 ± 0.10	0.1 ± 0.00

In pilot-scale experiment 3, a monthly routine monitoring was performed between May 2018 and May 2019. After evaluation of the sampling type, it was decided to take all samples as grab samples in 1.5 L aluminum bottles, according to the recommendations of BioDetection Systems BV (Amsterdam, the Netherlands). Over the sampling period of 13 month, in total 16 samples were taken and extracted, but not every bioassay was applied to every sample. All dates and operational data are summarized in Table 4.6.

Table 4.6. Summary of sampling campaigns frequency of sampling for each sampling point, sorted by specific ozone dose.

D_{spec} ($g\ O_3/g\ DOC$)	BV* [-]	Routine monitoring	O_3 dose-campaign	Influent WWTP	CAS	O_3	GAC
0.18			x		x	x	
0.27			x		x	x	
0.31	6,764		x		x	x	
0.43	33,098	x			x	x	x
0.47	28,854	x			x	x	x
0.55			x		x	x	
0.57			x	x	x	x	x
0.62	978	x			x	x	x
0.62	16,212	x			x	x	x
0.66	14,991	x		x	x	x	x
0.71		x			x	x	x

Dspec (g O3/g DOC)	BV* [-]	Routine monitoring	O3 dose-campaign	Influent WWTP	CAS	O3	GAC
0.75			x		x	x	
0.78			x		x	x	
0.83	2,591	x			x	x	x
0.89	3,667	x			x	x	x
0.92			x		x	x	

*BV: Bed volumes are only given for routine campaigns

4.4.2. Micropollutants

Nine TrOCs were selected for analysis based on existing and proposed EU legislation, metabolism and excretion from the human body, known environmental occurrence, persistence during wastewater treatment and toxicity to aquatic organisms (Zoumpouli et al., 2020). It included pharmaceuticals, corrosion inhibitors, and sweeteners (Table 4.7)

Table 4.7. Overview of TrOCs analyzed

Substance	Acronym	Substance class	CAS-Number
Acesulfame K	ACE K	Sweetener	5589-62-3
Bezafibrate	BZF	Lipid regulator	41859-67-0
Benzotriazole	BZT	Corrosion inhibitor	95-14-7
Carbamazepine	CBZ	Anti-convulsant	298-46-4
Diatrizoic acid dihydrate	DTA	Iodinated contrast medium	50978-11-5
Diclofenac	DCF	Analgesic/anti-inflammatory	15307-79-6
Ibuprofen	IBP	Analgesic/anti-inflammatory	31121-93-4
Metoprolol	MTP	Beta blocker	37350-58-6
Sulfamethoxazole	SMX	Antibiotic	723-46-6

Metoprolol is a beta-blocker that is mainly used to treat high blood pressure and heart disease, Benzotriazole is a complexing agent and is usually found in the range of several µg/L in treated municipal wastewater. Benzotriazole only reacts moderately with ozone but can be easily eliminated by adsorption on activated carbon. Sulfamethoxazole is an antibiotic from the group of sulfonamides and is used to fight urinary tract infections and pneumonia. Carbamazepine is used to treat epilepsy. It is also known that carbamazepine is hardly eliminated in the activated sludge process. Acesulfame K is a synthetic sweetener that is added to many foods and is considered an anthropogenic tracer due to the high concentrations in the sewage treatment plant effluent. Bezafibrate belongs to the class of lipid-lowering drugs and is used to treat high cholesterol levels. Diclofenac and ibuprofen are analgesics. While ibuprofen is broken down well in the activated sludge process, diclofenac is largely persistent in conventional wastewater treatment. Diatrizoic acid dihydrate is used in the treatment of control, prevention, and improvement of the following health issues, conditions, and symptoms (diagnostic imaging methods, urography, angiography, computed tomography, cholangiography, imaging the gastrointestinal tract in patients allergic to barium and other conditions) (Kreuzinger et al., 2020).

4.4.3. Toxicity parameters

The *in vitro* bioassay test battery was designed to target mode of actions based on well-defined toxic mechanisms that cover relevant steps along the toxicity pathway as recommended by Escher et al. (2012), Escher et al. (2018); Neale et al. (2017b), see Figure 4.6. Even though positive signal responses cannot be directly translated into a higher-order effect, every adverse outcome begins with a molecular initiating event, thus demonstrating the link between biological response at the cellular level with higher-order effects on the organ, followed by the organism and eventually the population level, which is summarized under the concept of adverse outcome pathways, according to Ankley et al. (2010).

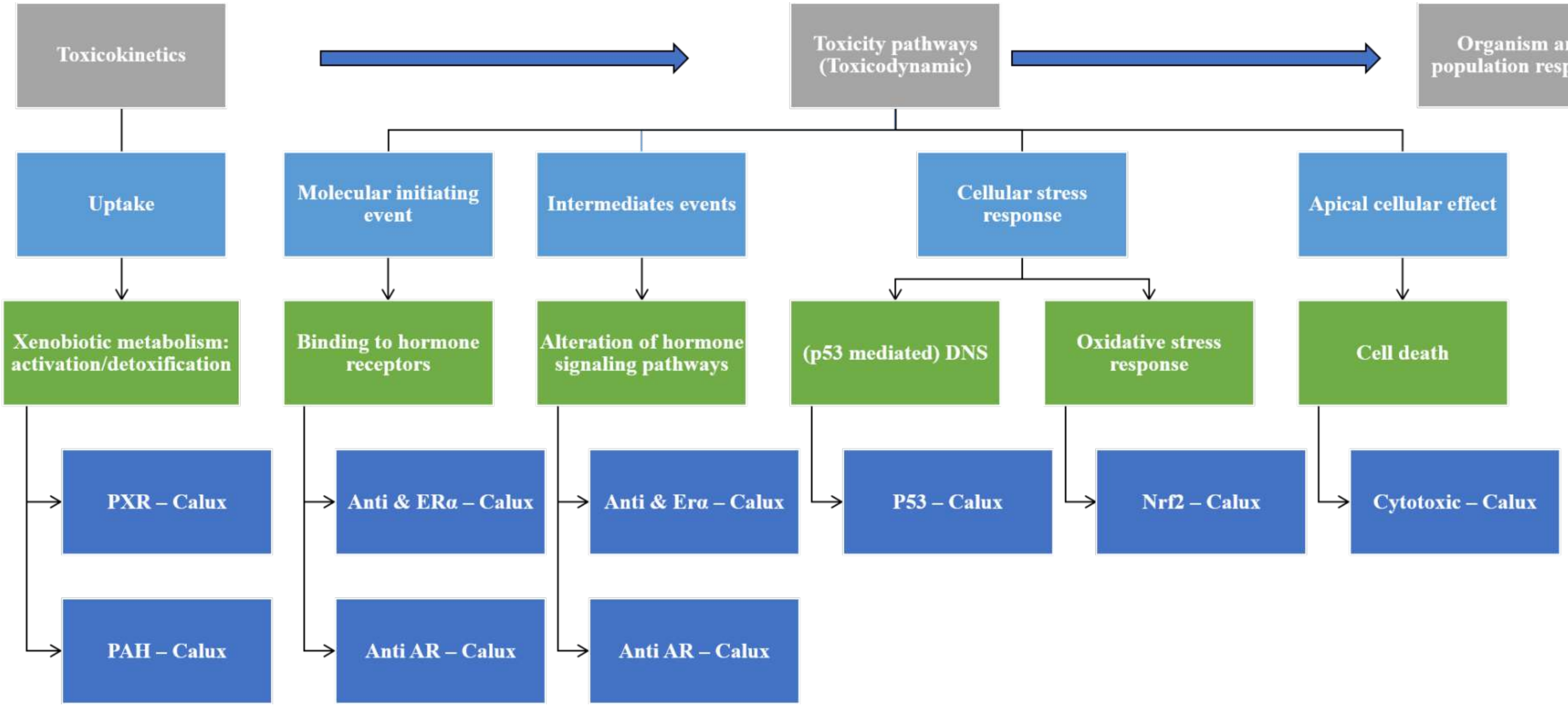


Figure 4.6. *In vitro* bioassay panel allocated to the Toxicity Pathway Classifications (according to Neale et al. (2017a), modified)

The wastewater extracts were analyzed by BioDetection Systems BV (Amsterdam, the Netherlands) with nine CALUX® (Chemical Activated Luciferase eXpression) reporter gene bioassays. Five of the nine modes of action investigated in this long-term monitoring were suggested for WWTP effluent monitoring in the joint NORMAN and Water Europe Position paper (2019) by the NEREUS COST Action ES 1403. Additional three bioassays, which cover typical MOAs first applied for water quality assessment (Escher et al., 2021) included to consider also genotoxicity, cytotoxicity, and anti-estrogenicity as an additional hormone-mediated assay. The principle of the bioassay is described in Alygizakis et al. (2019).

4.5. Analytical methods

4.5.1. Determination of the ozone concentration using the indigo method

The measuring principle is based on the fact that potassium indigotrisulfonate ($C_{16}H_7K_3N_2O_{11}S_3$) is decolorized by ozone in a stoichiometric reaction. The ozone concentration can be calculated from the measured decrease in absorbance at a wavelength of 600 nm (DIN 38408-3, 2011). A UV/VIS spectrometer (Dr. Lange-Cadas 100) with a quartz cuvette (5 cm) was used to measure the spectrophotometer at 600 nm.

4.5.2. Wastewater parameters

DOC was measured with a Total Organic Carbon Analyser TOC-L CPH from Shimadzu using direct method. This method is also known as NPOC (non-purgeable organic carbon), removed, after acidification, TIC from the sample and after thermal-catalytic combustion carbon dioxide was detected with a non-dispersive infrared (NDIR) cell. The measured value of the carbon dioxide concentration corresponded to the DOC.

A Continuous Flow Analyzer (CFA) - SAN Plus System from Skalar company was used to analyze NO_2^-. The concentration was determined based photometric principles.

COD was analyzed with small tube test (STT) (Hach-Lange DR 2800; Hach-Lange COD Test LCK 314).

BOD was measured after 5 days as BOD_5. ATU was added as a nitrification inhibitor to ensure that the consumed oxygen measured as BOD_5 was limited to respiration for organic matter oxidation. Oxygen was measured with luminescence-based measurement (SP-PSt3-NAU-D5-YOP, PreSens Precision Sensing GmbH) to obtain daily results. The sensor (luminophore) was attached to the inner surface of a BOD-bottle (see Figure 4.7) and the signal was measured with electro-optical components without direct contact. To validate this method, parallel measurements for the determination of residual oxygen after 5 days were conducted with an oxygen probe (WTW), see Table 4.8.

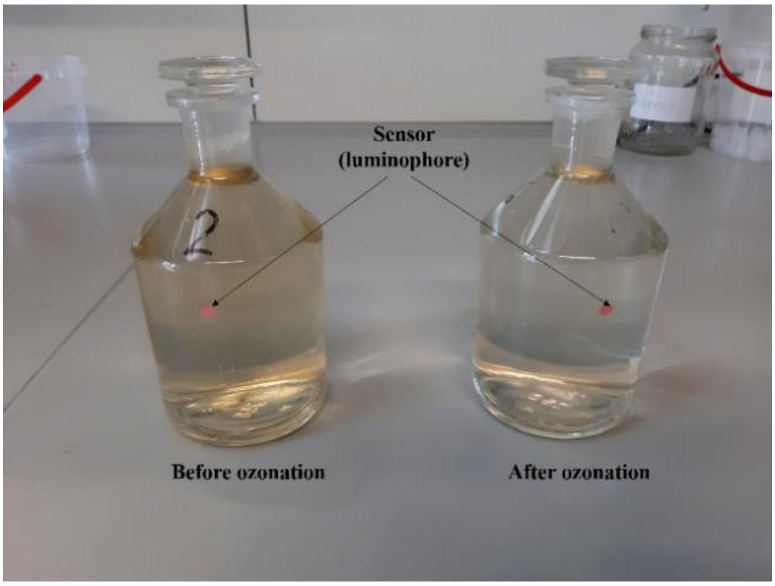


Figure 4.7. BOD luminescence-base measurement

Table 4.8. Comparison of BOD_5 determined with two different oxygen sensors

Sample	**BOD_5 (Luminescence-based measurement), mg/L**	**BOD_5 (WTW probe), mg/L**
WWTP3	1.74	1.85
WWTP4	1.68	1.57

The spectral absorbance coefficient at 254 nm (UV_{254}) was measured with a UV/VIS spectrometer (Dr. Lange – Cadas 100).

All measurements were carried out according to the standardized methods listed in Table 4.9.

Table 4.9. Overview of the analyzed conventional parameters and the applied methodology

Parameter	Abbreviation	Methodology
Chemical oxygen demand	COD	ISO 15705
Biochemical oxygen demand	BOD_5	ISO 5815-1, EN1899-2
Dissolved organic carbon	DOC	EN 1484
Spectral absorption coefficient at 254 nm	UV_{245}	Spectrophotometric Absorption
Ammonium	NH_4^+-N	ISO 11732
Nitrate/Nitrite compounds	NO_3-N / NO_2-N	ISO 13395
Bromide	Br^-	HPLC MS/MS
Bromate	BrO_3	HPLC MS/MS

4.5.3. Micropollutant analysis

The wastewater samples were filtrated with VWR glass fiber filter diameter 45 mm and pore size 1µm. Analytical standard in ethanol concentration of 1mg/mL in ethanol were prepared.

For the analysis of the micropollutants in this work, as well as for the determination of bromide and bromate concentrations, the automated online solid-phase-extraction (SPE) coupled with LC-MS/MS analysis method was used. This is a coupling of two techniques, liquid chromatography (HPLC) with mass spectrometry (MS). A sample in the solution can thus be separated by HPLC, and the individual components directly characterized via the MS (see more in Appendix 1).

Injecting volumes of 10 mL of sample were used for the automated online solid-phase extraction. HPLC separation with eluent 0,1 % acetic acid solution in deionized water (A) and 0,1 acetic acid in Acetonitrile solution (B) were performed in gradient mode. The online SPE and HPLC separation programs can be seen in Table 4.10.

Table 4.10. The gradient program for online SPE and HPLC separation

Time	Online SPE			HPLC		
	Flow	Gradient		Flow	Gradient	
min	mL/min	% A	% B	mL/min	% A	% B
0	1.0	100	0	0.8	100	0
5.6	1.0	100	0	0.8	100	0
0	1.0	100	0	0.8	80	20
5	1.0	100	0	0.8	90	10
8	0.5	0	100	0.8	90	10
8.2	1.0	0	100	0.8	0	100
8.5	1.0	0	100	0.8	60	40
17	1,0	0	100	0.8	60	40
19	1.0	100	0	0.8	5	95
25	1.0	100	0	0.8	80	20

The high-pressure liquid chromatograph (HPLC) used for the elution was an Agilent System consisting of two Binary pumps, a degasser to degas the eluents, CTC PAL autosampler with Peltier-Cooler and Rheodyne 2-position,6-port switching valve. The MS/MS system consisted of a Hybrid triple quadrupole linear trap ion trap tandem mass spectrometer QTrap 3200 from AB Sciex company.

For automated online solid phase extraction (online SPE) a Phenomenex Strata X On-Line extraction cartridge (20 x 2.0 mm; 25µm) was used. The HPLC separation was done via analytical column Phenomenex Luna C-18 (150 x3.0 mm; 5µm) and Phenomenex C18-Security guard cartridges (40 x 3.0 mm). For quantitative analysis the MRM Analysis with electrospray ionization mode (MRM ESI) by 500°C and nitrogen collision gas was used (Table 4.11).

Table 4.11. Parameter MRM Analysis with electro spray ionization mode

Scan type	MRM	MRM
Polarity	Negative	Positive
Dwell (msec)	150	
Collision gas CAD	Medium	
Curtain gas CUR	25	
Ion source gas 1 GS1	50	
Ion source gas 2 GAS2	50	
Ion spray voltage IS	- 4500	
Temperature TEM	500	
Entrance potential EP	-1	

The confirmatory and identifying mass and all other parameters of the MS/MS can be found in Table 4.12.

Table 4.12. Mass properties of all analyzed compounds by HPLC MS/MS

Compound	Polarity	Q1 mass	Q3 mass	Identifying mass			
		m/z	m/z	m/z	DP	CE	CXP
Ace K	negative	161.800	78.111/81.937	81.9	-35	-20	
BZF	negative	359.99	153.9/274.1	274.1	-25	-26	-2
BZT	positive	120.097	65.1/92.2	65.1	46	31	4
CBZ	positive	237.727	193.3/194.3	194.3	51	25	4
DTA	positive	614.713	233.1/361.0	361.0	51	25	6
DCF	negative	293.823	214.1/250.1	250.1	-20	-16	-2
IBP	negative	204.972	159.0/161.0	161.0	-20	-12	0
MTP	positive	267.810	74.0/77.1	77.1	41	75	4
SMX	positive	254.171	156.2/92.25	92.2	41	33	4

The signal to noise ratio (S/N) and lower limit of detection (LOD) are given in Table 4.13.

Table 4.13. Analyzed micropollutants and analytical quality criteria

Substance	CAS	Signal to noise ratio (S/N) and LOD (ng/L) (Standard concentration: 10 ng/L)	
		S/N	LOD
ACE K	5589-62-3	14	2.10
BZF	41859-67-0	123	0.10
BZT	95-14-7	287	0.10
CBZ	298-46-4	610	0.05
DTA	50978-11-5	22	1.36
DCF	15307-79-6	1100	0.03
IBP	31121-93-4	126	0.24
MTP	37350-58-6	7	4.29
SMX	723-46-6	284	0.11

4.5.4. Bioassay

All wastewater samples were filtered through a glass fiber filter (pore size 3 µm) and the maximum volume of a sample after filtration was 1,000 mL. The samples were concentrated by solid-phase-extraction (SPE) with Oasis HLB cartridges (500 mg, 6cc, Waters 186000115) according to the protocol of BDS with slight modifications regarding the final resuspension of the sample that had been evaporated to dryness. A description of the steps in the SPE process is shown in Figure 4.8.

The cartridges were conditioned with 6 mL acetonitrile and 6 mL deionized water, both of which were drawn through the cartridges under a low vacuum with a vacuum manifold to remove residual bonding agents. The filtered samples were loaded onto the cartridge under a slight vacuum; the flow over the cartridge was adjusted to a few drops per second in order to not exceed 10 mL/min. After loading, the cartridges were washed with 6 mL methanol, 5 % in water (w/w), and then dried for 30 minutes under vacuum in order to remove excess water remaining on the cartridge. Subsequently, the adsorbed analytes were eluted from the cartridges to a 20 mL culture tube with 10 mL methanol and 10 mL acetonitrile at a flow rate of approx. 5 mL/min. Afterward, the samples were evaporated to dryness (± 0.5 mL) under a stream of nitrogen at room temperature. This volume was transferred from the culture tube to the vial and rinsed with 0.5 mL methanol and 0.5 mL acetonitrile. The final volume of the 1.5 mL extracted sample was kept in the fridge at 7 °C prior to analysis.

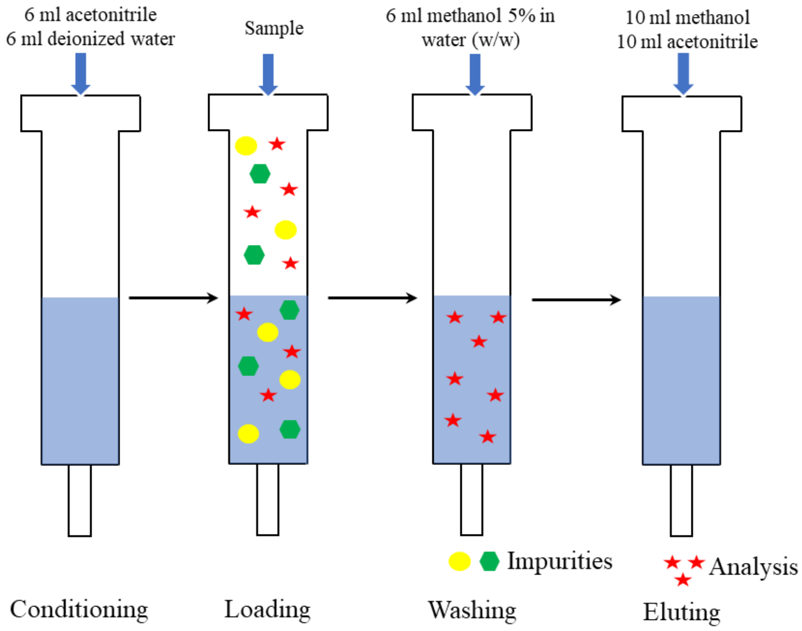


Figure 4.8. The steps in the SPE process

For quantification of the analyzed effect, the results of the CALUX® bioassays are provided as biological equivalent concentrations (BEQs) per liter sample related to reference compounds given in Table 4.14. An individual LOQ is determined for every single analysis. Genotoxicity was analyzed with and without the addition of S9 for metabolic activation. A difference in results from testing with and without S9 addition elucidates if metabolization or detoxification of ingredients occurred (Escher et al., 2012) and helps to differentiate between directly and indirectly acting genotoxic compounds. Not each endpoint was targeted in every sample: while the hormone-mediated MOAs ERα and anti-AR CALUX® were analyzed in all samples, the remaining six endpoints were analyzed alternately according to the frequency depicted in Table 4.14.

If the BEQ was below the LOQ, half the LOQ was used as a result. This approach was applied in order not to exclude results < LOQ from statistical analysis. Due to the sample-specific LOQs, the BEQ derived from results < LOQ can slightly deviate and, in some cases, give the impression of an increased signal along with the treatment steps.

Table 4.14. Information on the CALUX® *in vitro* bioassay panel and frequency of analysis

Bioassay	Measured endpoint	Reference compound	EBT*	Frequency of analysis			Key reference
				CAS	O_3	GAC	
Cytotox	Repression of constitutive transcriptional activation / cytotoxic activity	Tributyltin acetate	-	16	16	7	1
Erα*	Estrogen receptor α-mediated signalling	17β-Estradiol	0.1 ng BEQ/L	16	16	7	2
anti-Erα	Repression of estrogen receptor α-mediated signalling	Tamoxifen	-	2	2	2	3
anti-AR*	Repression androgen receptor activation	Flutamide	14 µg BEQ/L	16	16	7	4
Nrf2*	Activation of the Nrf2 pathway / oxidative stress response	Curcumin	10 µg BEQ/L	13	13	5	1
p53 + S9	p53-dependent pathway activation / genotoxicity response with metabolic activation S9	Cyclophosphamide	-	5	5	5	1
p53 - S9	p53-dependent pathway activation / genotoxicity response without metabolic activation S9	Actinomycin	-	3	3	3	1
PAH*	Aryl-hydrocarbon receptor activation / toxic PAH - xenobiotics metabolism	Benzo[a]pyrene	6.2 ng BEQ/L	8	8	4	5
PXR*	Activation of pregnane X receptor / xenobiotic metabolism and sensing	Nicardipine	3 µg BEQ/L	3	3	3	6

* suggested in the joint NORMAN and Water Europe Position paper (2019);

1. van der Linden et al. (2014), 2. Sonneveld et al. (2004), 3. van der Burg et al. (2010b), 4. van der Burg et al. (2010a), 5. Pieterse et al. (2013), 6. Escher et al. (2018)

*EBTs linked to the MOAs were retrieved from literature (Escher et al., 2018; van der Oost et al., 2017); for endpoints suggested in the joint NORMAN and Water Europe Position paper (2019) the lower EBTs suggested in these two publications were applied.

5. Results and Discussions

5.1. Micropollutant abatement

5.1.1. Results for abatement

An overview of the percentage elimination of TrOCs examined at five specific ozone dose (D_{spec}) is shown in Figure 5.1, whereby the D_{spec} has been rounded to one decimal point for better readability. As expected by the chemical diversity of micropollutants, the results range from 29% to 99%. At the lowest D_{spec} of 0.2 gO_3/g DOC, partial removal occurred for most compounds. The medium D_{spec} of 0.6 - 0.7 g O_3/g DOC, half of all compounds were removed for more than 80%. At the highest D_{spec} of 1.0 g O_3/g DOC all compounds were removed to approx. 100%.

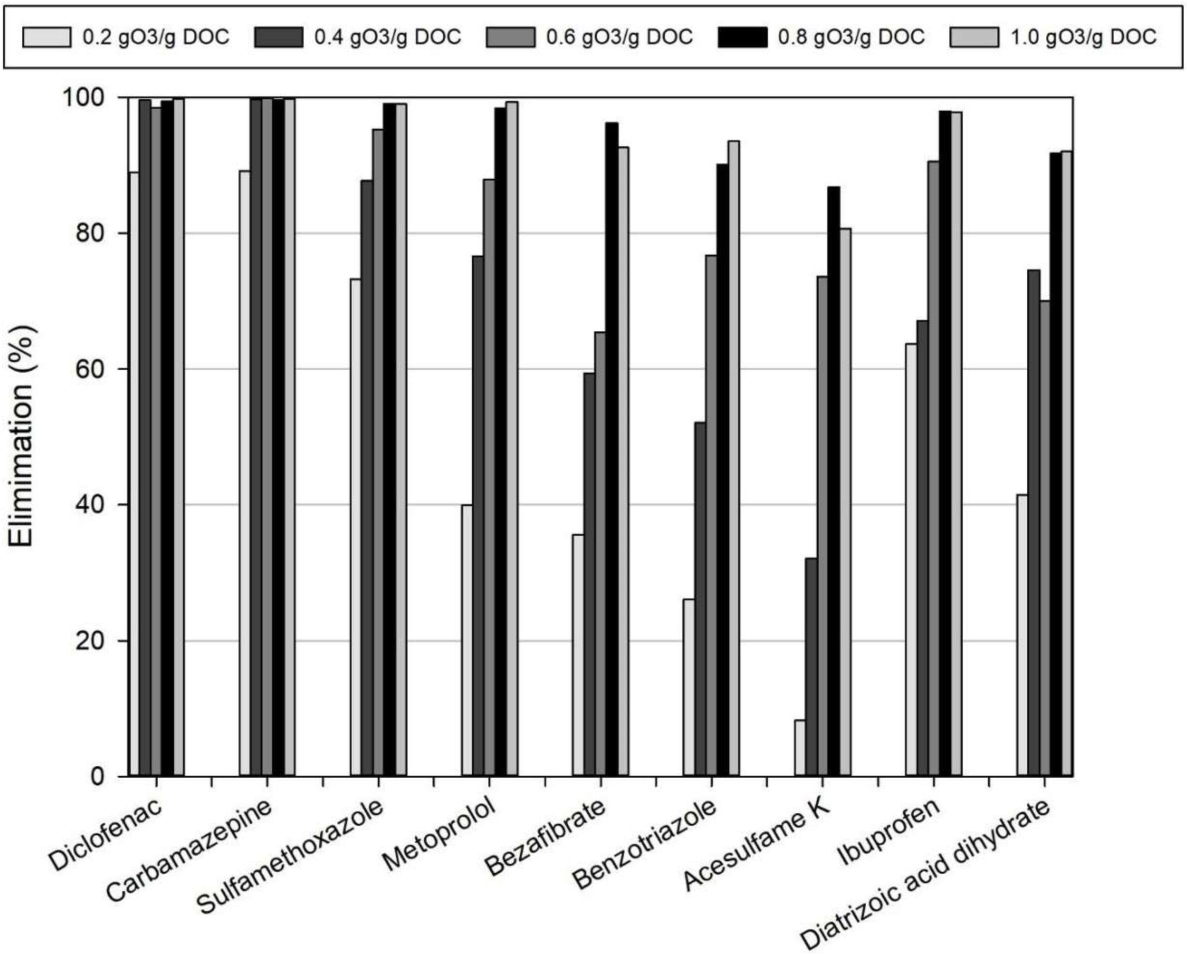


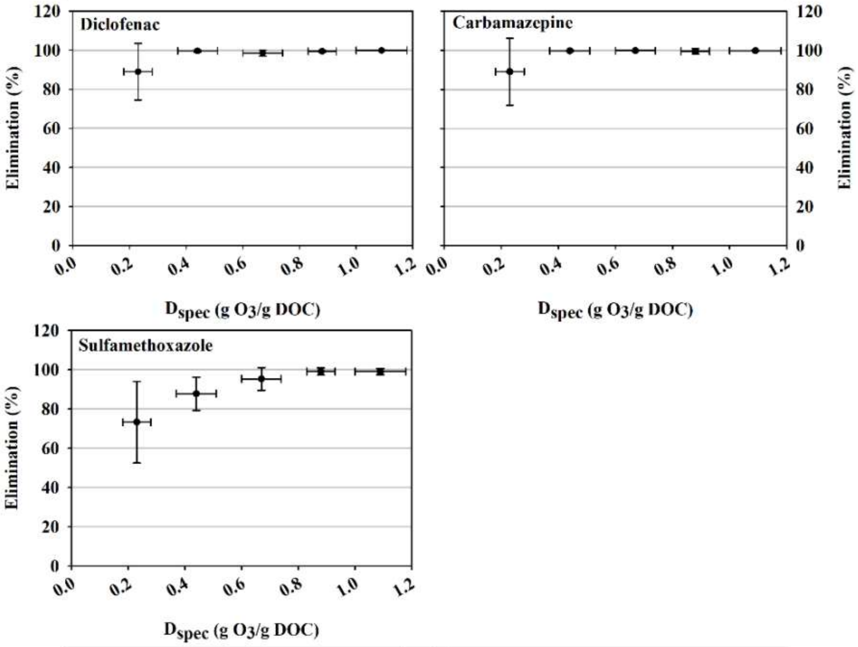
Figure 5.1. The percentage elimination of TrOCs examined for the effluent of WWTP at five D_{spec}

The micropollutants may be classified into three groups (see Figure 5.2) according to Jekel et al. (2015) with three groups, group I: highly reactive compounds ($K_{O3} \geq 10\ M^{-1}s^{-1}$) were readily removed by more than 90%; group II: medium reactive compounds ($10 \leq K_{O3} \leq 10^5\ M^{-1}s^{-1}$) had a moderate removal of 50 to 90%; and group III: low reactive compounds ($K_{OH} \leq 10^9\ M^{-1}s^{-1}$) were hard to remove. Group I consisted of 3 of the tested compounds, 4 compounds were in group II, while 2 compounds were in group III. Similar classifications of compounds have been used in previous studies of Lee et al. (2013) and Stapf et al. (2016).

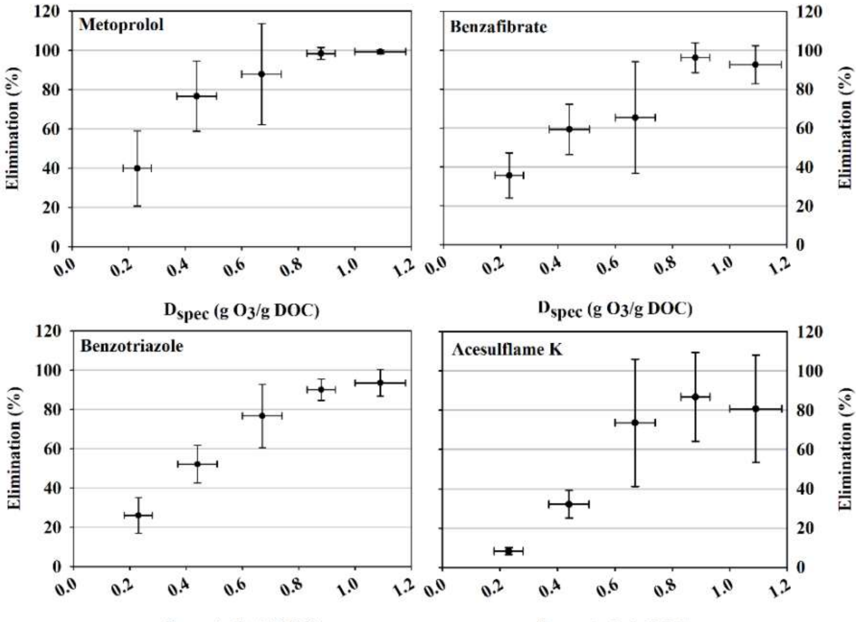
Figure 5.2 shows the percentage removal of TrOCs studied, for which a specific ozone dose has been rounded to one decimal point for better readability. Removal of diclofenac, carbamazepine, and sulfamethoxazole over 90% can be measured with a specific ozone dose of about 0.45 g O_3/g DOC. These results also agree with data from previous studies (Hollender et al., 2009; Lee et al., 2013; Schaar, 2015).

With metoprolol, results showed that >80% removal was achieved from a specific ozone dose of 0.6-0.7 g O_3/g DOC. Bezafibrate reacts similarly to metoprolol. The rate of decomposition increases linearly with the amount of ozone added. From a specific ozone dose of 0.7, about 80% is removed, and these results apply to all four drainage samples. The characteristic degradation performance of the three compounds benzotriazole, acesulfame-K and ibuprofen showed similarities, although ibuprofen had a low O_3 reactivity for the first two mentioned compounds. In the case of the last two micropollutant mentioned, only >80% removal can be measured from a D_{spec} = 1.0 g O_3/g DOC. In summary, it can be found that a very good decomposition rate (i.e. > 80%) is achieved for all micropollutants examined at a specific ozone dose in the range 0.6-1.0 g O_3/ g DOC.

Group I: $K_{O3} \geq 1 \times 10^5\ M^{-1}s^{-1}$ (highly reactive compounds)



Group II: $10 \leq K_{O3} \leq 1 \times 10^5\ M^{-1}s^{-1}$ (medium reactive compounds)



Group III: $K_{OH} \leq 1 \times 10^9\ M^{-1}s^{-1}$ (low reactive compounds)

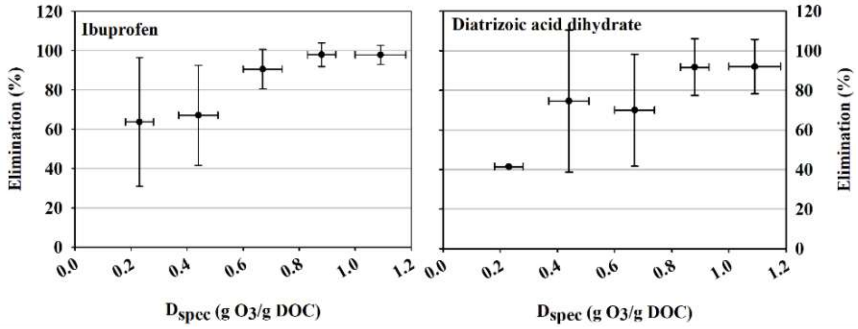


Figure 5.2. Elimination of micropollutants in % at different D_{spec}

Ultraviolet absorbance at 254 nm (UV_{254}) is relatively stable and straightforward. It is a promising parameter for identifying the efficiency and behavior of ozone o biologically treated wastewater and gives a good insight into the correlation between the decrease of UV absorbance, the ozone dosage, and the elimination of micropollutants (Gerrity et al., 2012). Linear regression was used to evaluate the correlation between the relative reduction of UV_{254} (ΔUV_{254}) and the oxidation of micropollutants. Figure 5.3 shows the relative changes of UV_{254} versus D_{spec} for of the investigated micropollutant.

The contaminant degradation profiles with group I (highly reactive compounds) were steep, as illustrated in Figure 5.3. The high slopes indicate rapid reaction rates, as would be expected for these compounds, and the low vertical intercepts indicate that the elimination of these particular compounds started at the same time as the elimination of UV_{254} absorbance. Therefore, the correlation of the elimination and ΔUV_{254} is not appropriate (i.e., $R^2 \leq 0.5$). With the compounds in group 2 (medium reactive compounds), the slopes of the micropollutants had lower because their oxidation started quicker than that of UV_{254} absorbance. This was apparent in the different regression properties in Figure 5.3, but the models still indicated fast reaction rates by either a high slope or vertical intercept. Due to the reduced reactivity, regression analyses for UV_{254} were possible (i.e., $R^2 \geq 0.8$). For group 3 the oxidation starts much later and at a much slower rate than changes in UV_{254} absorbance.

Gerrity et al. (2012) connected the steepness and intercepts of the correlations to the particular reaction rates of the micropollutants: The steeper the slope, the faster the reaction of the particular micropollutants with ozone and •OH, while low vertical intercepts indicate that micropollutants elimination starts at the same time as UV_{254} reduction occurs. A negative intercept of the correlation indicates that a minimal UV_{254} is required before the elimination of these micropollutants can be expected, which mainly occurs for micropollutants with a moderate reactivity with ozone and OH•.

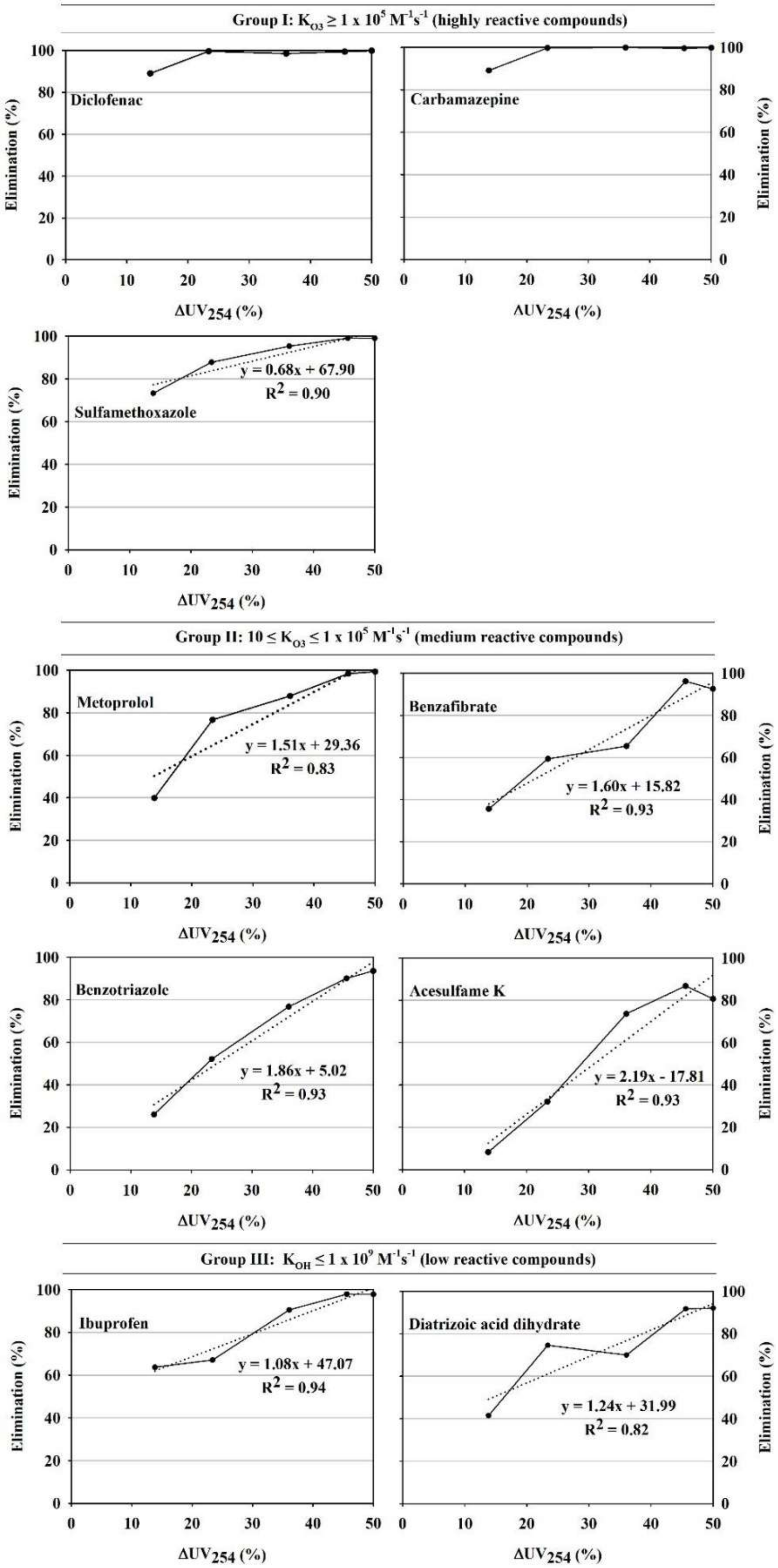


Figure 5.3. Linear correlation between the reduction in UV_{254} absorbance (ΔUV_{254}) with the elimination of TrOCs

5.1.2. Model for the prediction of the elimination of trace substances

The elimination of a micropollutant during ozonation can be expressed by the following Equation 1 (Lee et al., 2013):

$$\ln\frac{C}{C_0} = -k_{O_3}\int[O_3]dt - k_{OH^\cdot}\int[OH^\cdot]dt \qquad (1)$$

c : Concentration of the substance (µg/L)
t : Time (s)
k_{O3} : Reaction constant of substance with ozone ($M^{-1}s^{-1}$)
$k_{OH\cdot}$: Reaction constant of substance with OH radicals ($M^{-1}s^{-1}$)
O_3 : Concentration of ozone (mg O_3/L)
$OH^\cdot$: Concentration of OH radicals (mg O_3 / L)

As can be seen in the equation above, the reaction constants of the respective trace substance and the ozone and hydroxyl radical exposure are necessary for the calculation. The reaction rate constants are already known for many micropollutants and are summarized in Table 5.1 for TrOCs examined in this work.

Table 5.1. Reaction constants of the selected TrOCs

Substance	**Substance class**	**O_3 - Reaction**	**pK_a**	**k_{O3}**	**$k_{\cdot OH}$**	**Key reference**
				(M^{-1} s^{-1})	**(M^{-1} s^{-1})**	
DCF	Analgesic/anti-inflammatory	High	4.2	1×10^6	7.5×10^9	1, 2
CBZ	Anticonvulsant	High	-	3×10^5	8.8×10^9	1
SMX	Antibiotic	High	1.7; 5.6	5.7×10^5	5.5×10^9	1
MTP	Beta blocker	Moderate	9.7	4×10^4	7.3×10^9	3
BZF	Lipid regulator	Moderate	3.6	590	7.4×10^9	1, 4, 5
BZT	Corrosion inhibitor	Moderate	-	230	4.5×10^9	1
ACE K	Sweetener	Moderate		88	4.5×10^9	6
DTA	Iodinated contrast medium	Low	1.2; 7.9; 11.7	18.65	3×10^9	7
IBP	Analgesic/anti-inflammatory	Low	49	9.6	7.4×10^9	1, 5

1. Huber et al. (2003); 2. Sein et al. (2008); 3. Benner et al. (2008); 4. Dantas et al. (2007), 5. Huber et al. (2004); 6. Kaiser et al. (2013); 7. Ning et al. (2008)

For the micropollutants as carbamazepine, diclofenac, sulfamethoxazole, and metoprolol, complete elimination of nearly 100% was calculated with all three D_{spec} (0.6, 0.8 and 1 g O_3/g DOC). The measured values were between 98-100% for carbamazepine, sulfamethoxazole and diclofenac and between 87-99% for metoprolol. According to Lee et al. (2013) and Stapf et al. (2016), the efficient elimination of carbamazepine and diclofenac is due to the high reaction rate constant k_{O3} (see Table 5.1). Metoprolol also reacts quickly with ozone, but the O_3 reactivity is more moderate compared to carbamazepine and diclofenac and depend on the pH value of the wastewater, and thus higher D_{spec} of ozone are necessary for almost complete elimination. For bezafibrate, the elimination was between 87-96%, for benzotriazole between 67-81%, for acesulfame K between 58-71%, and for ibuprofen between 68-79%. The D_{spec} measured values were between 64-92% for bezafibrate, between 75-92% for benzotriazole, 71-85% for acesulfame K and between 90-97% for ibuprofen. Figure 5.4 graphically shows the comparison of the predicted and the actually measured elimination performance.

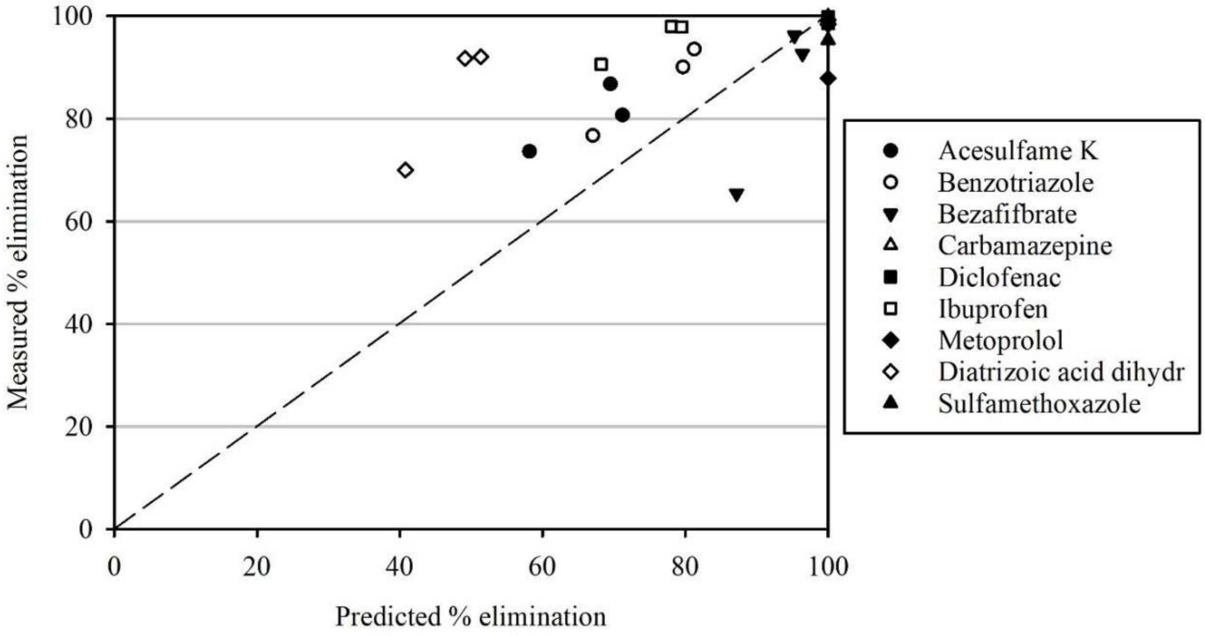


Figure 5.4. Comparison of the measured and predicted elimination performance

The predicted and the measured values were the same for the trace substances, which have high reactivity to ozone (diclofenac and carbamazepine). In the case of the moderately reacting micropollutants with ozone (see Table 5.1), a higher elimination is calculated compared to the measured values at low D_{spec}. As the amount of ozone increases, the results of the calculation approach those of the measured values. The prediction of the elimination of micropollutants could be useful for predictions, especially in the case of substances that react moderately with ozone (e.g., bezafibrate and benzotriazole), since substances that have a high reactivity towards ozone (e.g., diclofenac), as a rule, almost at low ozone quantities be completely dismantled. For the exact results of the elimination, however, the measurement should not be dispensed with. In the studies of Schindler et al. (2015), the predicted and measured eliminations for all selected micropollutants agree well. These excellent parallels could not be achieved in the course of the present work. The results of the present work confirm that the prediction of elimination can be calculated well for certain micropollutants, but the application does not have the same accuracy for all micropollutants. Deviations of 40-60% between forecast and calculation are possible, see benzotriazole, acesulfame K and ibuprofen in Figure 5.4. Bourgin et al. (2018) observed in their study that the prediction of the elimination of micropollutants highly reactive with ozone (carbamazepine, diclofenac) works extremely well. Deviations from the predicted and calculated elimination have been observed primarily in the case of trace substances, which have a low reactivity to ozone. As was also observed in the present work, these are mainly substances that are mainly broken down via hydroxyl radicals, such as ibuprofen. As a possible cause for the deviation of the measured values compared to the calculated values, Hollender et al. (2009) show that hydraulic behavior is not ideal, which means that ozone and hydroxyl radicals do not come into contact with all micropollutants and are therefore not oxidized. Apart from poor mixing, the sorption of micropollutants on other particles and colloids could also prevent oxidation.

5.1.3. Formation of oxidation byproducts during ozonation

Formation of toxic oxidation byproducts can occur during the ozonation of wastewater, such as bromate (Soltermann et al., 2016; Zimmermann et al., 2011). Bromate, a potential human carcinogen, forms during ozonation of bromide-containing waters through a combination of reactions involving ozone and secondary oxidants such as hydroxyl and carbonate radicals (von Gunten, 2003b). Figure 5.5 shows the concentration of bromate in ozonated samples at D_{spec}. The concentration of bromate increases with D_{sepc} and ranges from 0.23 ± 0.05 g O_3/g DOC to 1.09 ± 0.09 g O_3/g DOC. The bromate yield can be defined as the dimensionless ratio of the bromate concentration normalized by the initial bromide concentration (µg BrO_3^- / µg Br^-). Table 5.2 provides a summary of data on bromide, bromate, and bromate yield in this study.

Table 5.2. Bromide and bromate concentration, bromate yield

D_{spec} (g O_3/g DOC)	Bromide (µg/L)	Bromate (µg/L)	Bromate yield* (%)
0.23 ± 0.05	220.00 ± 84.71	0.00 ± 0.00	22.87
0.44 ± 0.07	211.75 ± 73.23	0.65 ± 0.28	25.77
0.66 ± 0.09	210.00 ± 18.74	2.52 ± 2.35	26.38
0.88 ± 0.05	169.25 ± 62.32	5.24 ± 5.38	40.67
1.09 ± 0.09	150.00 ± 86.97	11.22 ± 9.85	47.41

*Bromate yield = ([bromide]/[bromide]$_0$)

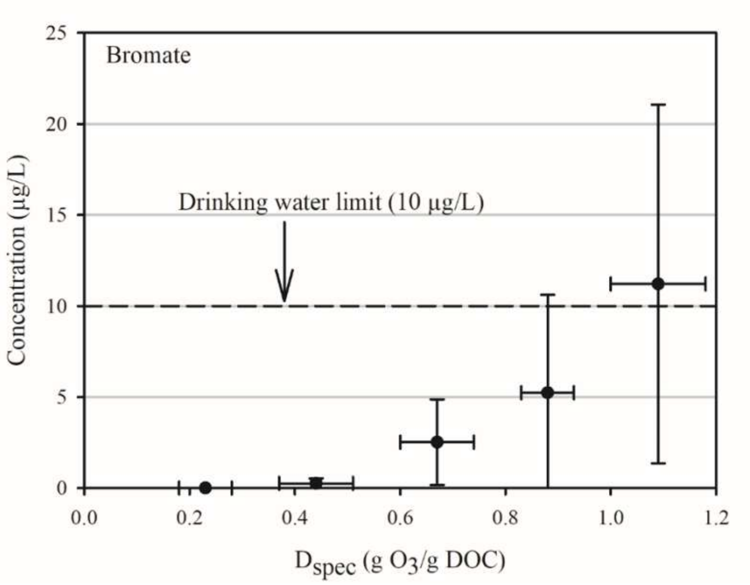


Figure 5.5. Bromate formation at different D_{spec} (g O_3/g DOC)

Ozonation with D_{spec} at 0.44, 0.6, and 0.8 g O_3/g DOC increased bromate concentrations to 0.65 µg/L, 2.52 µg/L, and 5.24 µg/L, respectively (Figure 5.5). However, at 1.09 ± 0.09 g O_3/g DOC, the bromate concentration was 11.22 ± 9.85 µg/L higher than the guideline value of the drinking water standard (10 µg/L) (WHO, 2017). To avoid adverse effects from bromate, the use of D_{spec} at 1.0 g O_3/g DOC is not recommended albeit its performance on the removal of micropollutants was highest.

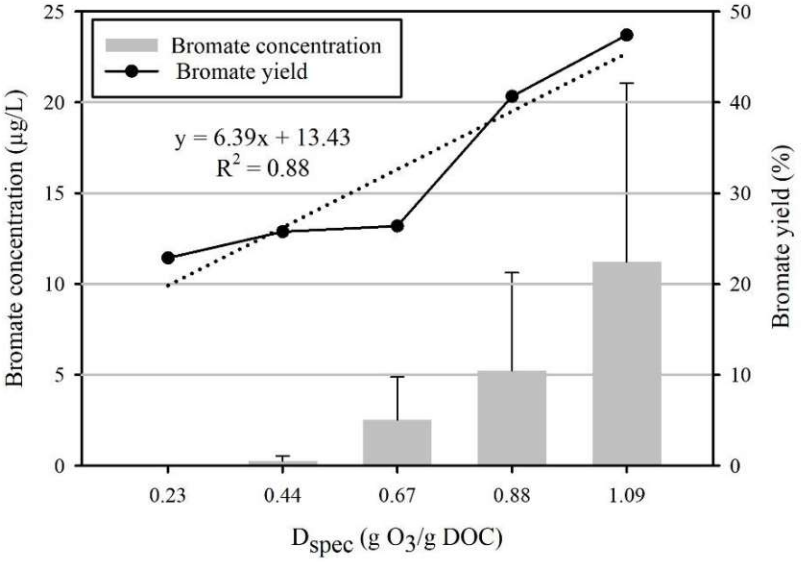


Figure 5.6. Relationship between bromate and bromate yield and D_{spec}

The previous studies (Chon et al., 2015; Soltermann et al., 2017; Wu et al., 2019) demonstrated that bromate production can be described by two stages characterized by D_{spec} range. With $D_{spec} \leq 0.2$ g O_3/g DOC, is characterized by insignificant bromate formation and a small effect of the initial bromide concentration. However, with $D_{spec} \geq 0.4$ g O_3/g DOC, the concentration of bromate increases almost linearly with an increase in D_{spec}. Figure 5.6 showed the relationships between bromate yields and D_{spec}. Thus, a reduction of the bromide concentration typically leads to a proportional reduction of the bromate concentration.

5.2. Effect of ozonation on biodegradability of the effluent of WWTP

Ozonation as an advanced treatment step is applied for the abatement of organic micropollutants. The effect of ozonation on conventional organic sum parameters that are routinely measured for the evaluation of treatment efficiency and legal compliance is not assessed at the same intensity. Based on the obtained results, the question of whether an increase in D_{spec} that is typically applied for micropollutant abatement from urban wastewater affects organic sum parameters is addressed. Thus, the change in organic sum parameters at different D_{specs} is discussed, and a special focus is placed on the subsequent biological activities assessed via the 5-day exposure for conventional BOD_5 measurement. In the comparison of different wastewater matrices, relative rather than absolute changes were determined absolute changes are given in the Appendix 3

Figure 5.7 represents a summary graph for the outcomes after ozonation and exposure time for BOD_5 measurement.

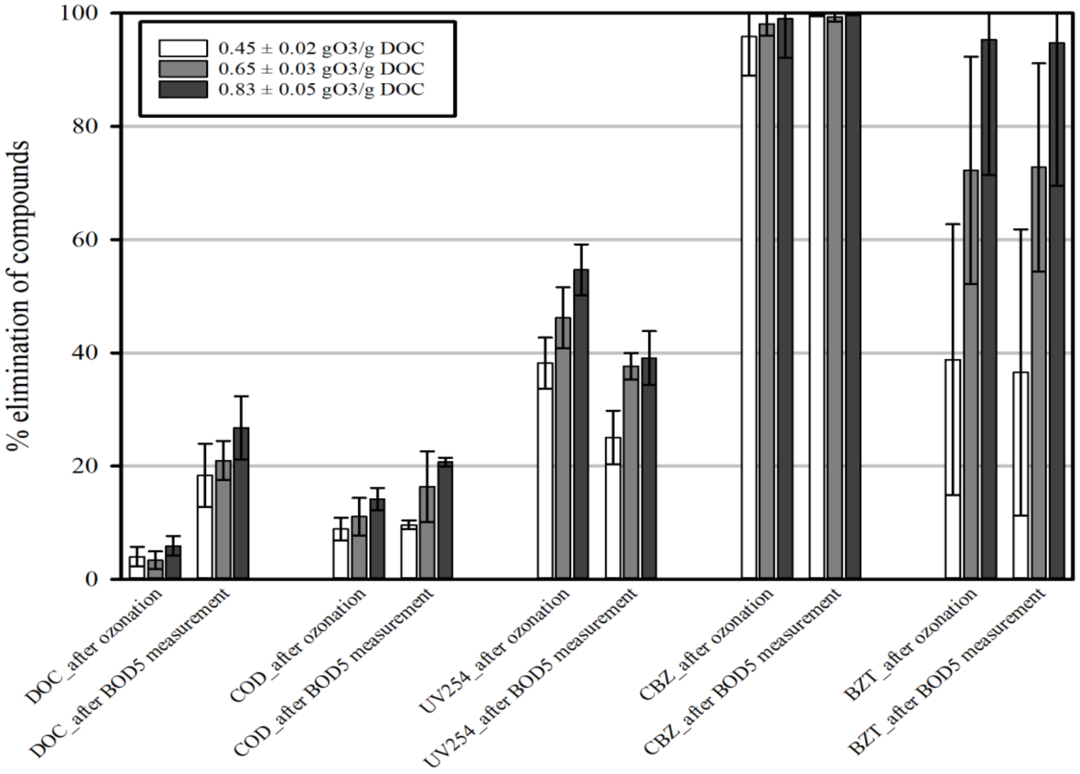


Figure 5.7. Dose-specific elimination of organic sum parameters and micropollutants after ozonation and exposure time for BOD_5 measurement. Average values over all investigated samples (n = 3 for D_{spec} of 0.45 and 0.83 g O_3/g DOC; n = 7 for 0.65 g O_3/g DOC).

5.2.1. Effect of ozonation on micropollutants

The results for the two organic micropollutants investigated primarily for process validation of the experimental ozonation setup are depicted in Figure 5.8 and Figure 5.9 and in the Appendix 3.

Figure 5.8 shows the dose-specific abatement of CBZ for all samples. As expected, a high CBZ abatement was already achieved at the lowest D_{spec} of 0.42 g O_3/g DOC investigated. For WWTP3, the abatement was slightly lower (approximately 90%), and it increased with a D_{spec} up to 98%. After the exposure time for the BOD_5 measurement, even in WWTP3, the CBZ concentration was < LOQ at 0.46 g O_3/g DOC.

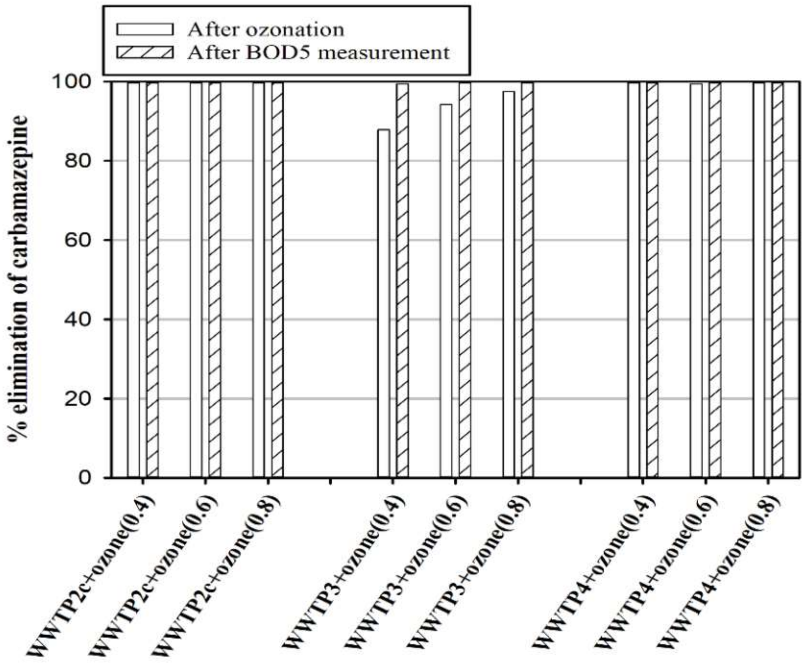


Figure 5.8. Dose-specific abatement of CBZ

Figure 5.9 shows the dose-specific abatement of BZT. It followed a dose-specific pattern, typical for moderately reactive substances. After exposure for BOD_5 measurement, no further removal was observed.

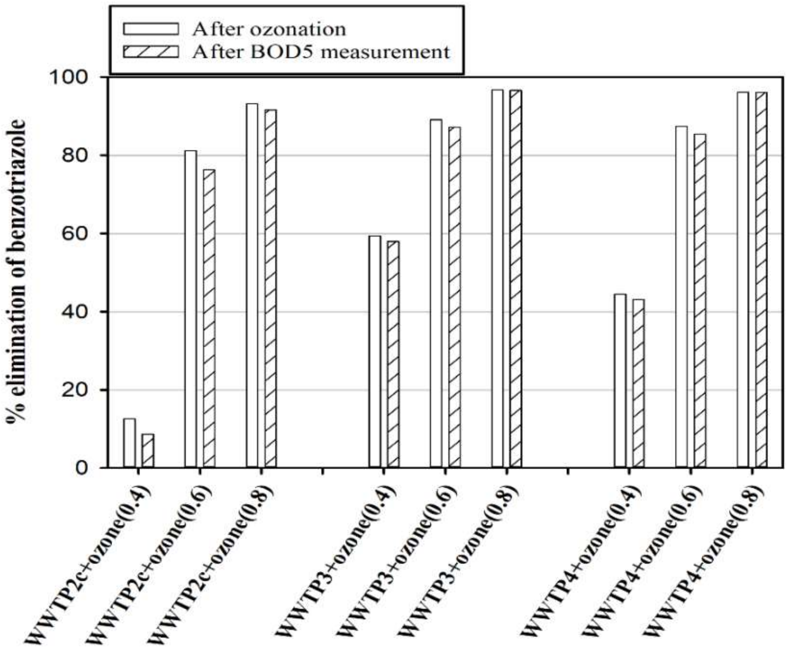


Figure 5.9. Dose-specific abatement of BZT

The CBZ abatement was quite efficient because CBZ is a highly reactive compound (k_{O3} = 3×10^5 $M^{-1}s^{-1}$). In Figure 5.7, the mean decrease was correlated with increasing D_{spec}, and it was higher than 90% for all investigated D_{spec}. This finding coincided with the results of previous studies, which indicated a CBZ decline of at least 90% for an ozone dose of only 0.45 g O_3/g DOC (Bourgin et al., 2018; Lee et al., 2013; Rizzo et al., 2019; Schaar et al., 2010; Zimmermann et al., 2011).

The BZT abatement was lower than that of CBZ, which is in line with its lower k_{O3} value (k_{O3} = 230 $M^{-1}s^{-1}$). BZT showed a moderate average abatement of approximately 60–80% at a targeted D_{spec} of 0.6 g O_3/g DOC, mainly due to reaction with the strong and unselective OH radical originating from the reaction of ozone with the organic wastewater matrix (Rosal et al., 2010). Moreover, OH radical exposure varies with the ozone dose and wastewater quality, such as the presence of ozone and hydroxyl radical scavengers or competitors, pH, and alkalinity (Lee et al., 2013). Such varying exposure resulted in the typical dose-specific elimination pattern for BZT shown in Figure 5.7. The higher standard deviations observed for BZT are in line with results presented in a review paper by Rizzo et al. (2019).

As a consequence of the abatement obtained for the two selected reference substances, the ozonation experimental setup was considered valid.

5.2.2. Effect of ozonation on organic sum parameters

5.2.2.1. BOD_5

To assess the increase in biodegradability, BOD experiments were performed, as increased oxygen consumption indicates higher levels of respiration for the biological oxidation of organic carbon. The results of the BOD measurements over a period of 5 days demonstrated the expected increase due to the oxidation of refractory organic matter present in the ozonated WWTP effluent. The BOD_5 concentrations of the 7 investigated samples originating from 4 different WWTPs before and after ozonation with 0.4 to 0.8 g O_3/g DOC (nitrite compensated D_{spec}) are shown in Table 5.3. BOD concentrations between day 1 and day 5 are listed in Table 5.4. Before ozonation, the BOD_5 ranged between 0.60 and 1.99 mg/L. This is typical for Austrian WWTPs operating at full nitrification and denitrification with a high sludge retention time (SRT) corresponding to a low food to microorganism (F/M) ratio. The oxidative effect of ozone resulted in a higher BOD_5, ranging from 1.46 to 3.40 mg/L.

Table 5.3. BOD_5 concentration of 4 WWTPs before and after ozonation.

Name	D_{spec} (g O_3/g DOC)	BOD_5 (mg/L) before ozonation	BOD_5 (mg/L) after ozonation
WWTP1a	0.65	0.60 ± 0.07	1.46 ± 0.11
WWTP1b	0.61	0.62 ± 0.11	1.83 ± 0.04
WWTP2a	0.69	1.28 ± 0.34	1.83 ± 0.08
WWTP2b	0.67	1.38 ± 0.14	2.18 ± 0.05
WWTP2c	0.43	1.24 ± 0.13	2.52 ± 0.09
	0.62		2.47 ± 0.11
	0.78		2.36 ± 0.04
WWTP3	0.46	1.99 ± 0.14	2.42 ± 0.06
	0.67		2.54 ± 0.09
	0.87		2.29 ± 0.05
WWTP4	0.45	1.91 ± 0.04	3.20 ± 0.18
	0.65		3.26 ± 0.00
	0.83		2.99 ± 0.06

Table 5.4. BOD concentration during five days of measurement (BOD_1 - BOD_5) before ozonation (D_{spec} = 0) and after ozonation

Name	**D_{spec} (g O_3/g DOC)**	**BOD_1 (mg/L)**	**BOD_2 (mg/L)**	**BOD_3 (mg/L)**	**BOD_4 (mg/L)**	**BOD_5 (mg/L)**
WWTP1a	0	0.21 ± 0.06	0.31 ± 0.02	0.29 ± 0.02	0.49 ± 0.04	0.65 ± 0.07
	0.65	0.33 ± 0.02	0.96 ± 0.06	1.17 ± 0.06	1.43 ± 0.08	1.59 ± 0.12
WWTP1b	0	0.25 ± 0.08	0.41 ± 0.09	0.39 ± 0.10	0.57 ± 0.09	0.68 ± 0.12
	0.61	0.59 ± 0.04	1.23 ± 0.03	1.48 ± 0.07	1.82 ± 0.11	2.01 ± 0.05
WWTP2a	0	0.25 ± 0.11	0.83 ± 0.19	0.69 ± 0.19	1.03 ± 0.25	1.42 ± 0.38
	0.69	0.52 ± 0.07	1.09 ± 0.07	1.45 ± 0.09	1.73 ± 0.12	2.03 ± 0.09
WWTP2b	0	0.24 ± 0.02	0.92 ± 0.07	0.71 ± 0.13	1.03 ± 0.13	1.38 ± 0.14
	0.67	0.74 ± 0.18	1.43 ± 0.14	1.72 ± 0.15	2.10 ± 0.08	2.42 ± 0.05
WWTP2c	0	0.40 ± 0.08	0.71 ± 0.08	0.88 ± 0.09	n.a.	1.33 ± 0.10
	0.43	0.84 ± 0.08	1.45 ± 0.09	1.82 ± 0.07	n.a.	2.52 ± 0.09
	0.62	0.93 ± 0.05	1.55 ± 0.09	1.87 ± 0.14	n.a.	2.47 ± 0.11
	0.78	1.06 ± 0.09	1.88 ± 0.34	2.03 ± 0.02	n.a.	2.62 ± 0.04
WWTP3	0	0.38 ± 0.09	0.87 ± 0.08	1.24 ± 0.08	1.52 ± 0.11	1.99 ± 0.14
	0.46	0.40 ± 0.15	1.00 ± 0.22	1.66 ± 0.04	2.07 ± 0.05	2.42 ± 0.06
	0.67	0.54 ± 0.06	1.25 ± 0.05	1.78 ± 0.07	2.16 ± 0.03	2.54 ± 0.09
	0.87	0.43 ± 0.07	1.32 ± 0.11	1.93 ± 0.08	2.29 ± 0.08	2.63 ± 0.06
WWTP4	0	0.49 ± 0.04	0.79 ± 0.03	1.08 ± 0.02	n.a.	1.91 ± 0.04
	0.45	1.02 ± 0.11	1.72 ± 0.06	2.24 ± 0.11	n.a.	3.20 ± 0.18
	0.65	1.12 ± 0.12	1.84 ± 0.13	2.30 ± 0.18	n.a.	3.26 ± 0.00
	0.83	1.20 ± 0.04	2.02 ± 0.04	2.47 ± 0.07	n.a.	3.40 ± 0.07

n.a.: no analyzed

In Figure 5.10, the relative increase in BOD_5 during the dose-specific experiments is depicted as % of the initial concentration in the effluent samples before ozonation. The highest change already occurred between zero and the lowest investigated ozone dose of 0.45 ± 0.02 g O_3/g DOC for all samples (88.75% for WWTP2c, 21.23% for WWTP3, and 67.46% for WWTP4). A further increase in D_{spec} to 0.65 ± 0.03 g O_3/g DOC resulted in a less pronounced increase. This slight increase in BOD_5 further continued at 0.83 ± 0.05 g O_3/g DOC (96.67% for WWTP2c, 31.70% for WWTP3, and 77.98% for WWTP4).

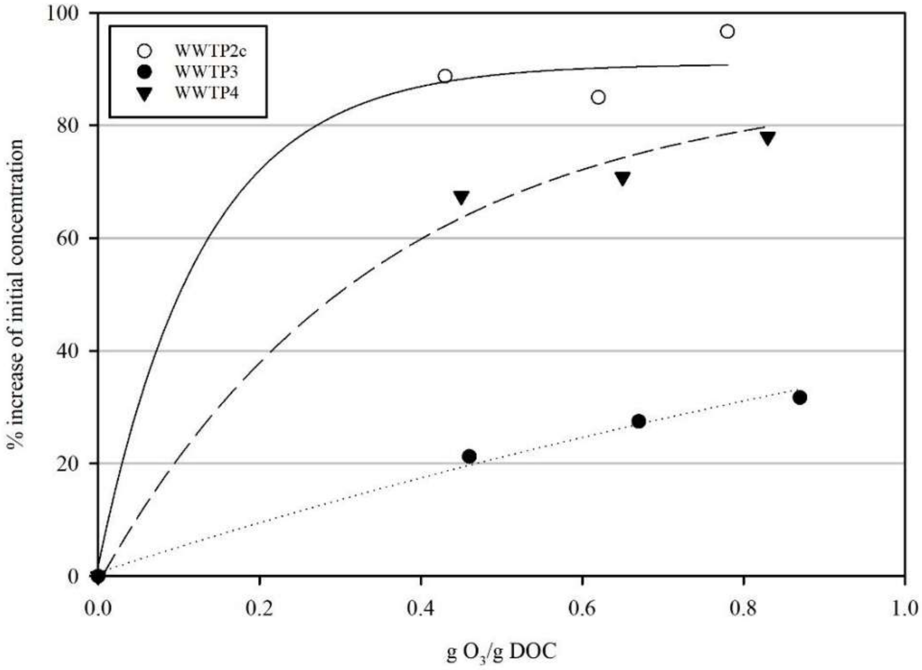


Figure 5.10. Dose-specific increase in BOD_5 in WWTP2c, WWTP3, and WWTP4

Figure 5.11 shows the relative increase in BOD_5 for the targeted D_{spec} of 0.6 g O_3/g DOC (0.65 ± 0.03 g O_3/g DOC), analyzing all samples to obtain a broader view of changes to be expected at a D_{spec} typically applied for organic micropollutant abatement. The average BOD_5 concentration reached 94.44 ± 58.23%, with a minimum of 27.47% (WWTP3) and a maximum of 192.78% (WWTP1b), indicating the significance of the different matrices encountered in treated urban wastewater.

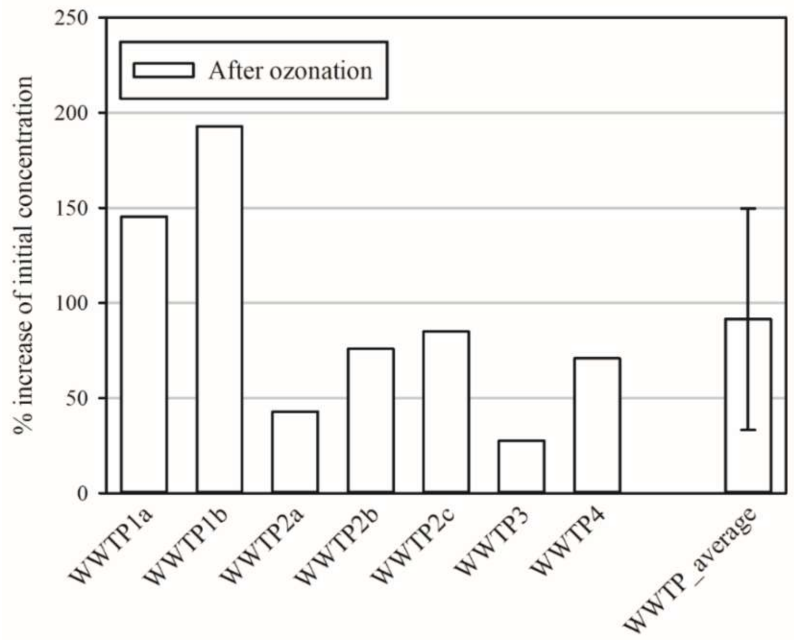


Figure 5.11. Increase in BOD_5 at an average D_{spec} of 0.65 ± 0.03 g O_3/g DOC (n=7)

Over the measurement period of five days, the oxidative effect of ozone resulted in a higher BOD_5 of the ozonated WWTP effluent samples (Figure 5.10 and Figure 5.11). The observed increase in BOD_5 can be attributed to a transformation of organic compounds into less complex species (Siddiqui et al., 1997). Compounds with higher molar masses are considered to be less biodegradable than lighter compounds (Testolin et al., 2020). Ozonation results in a higher availability of these partially oxidized products for further biological processes, e.g., microbial energy sources for aerobic respiration, which is indeed assessed by the parameter BOD. A rise in BOD_5 after ozonation indicates a higher biodegradability of matrix substances that are recalcitrant to biodegradation in conventional activated sludge treatment, as reported in the literature (Nishijima et al., 2003; Nöthe et al., 2009).

Due to the mean increase of approx. 90% at 0.6 g O_3/g DOC, indicating a final value of 190 % or roughly a doubling of the BOD_5, an effluent value that is close to the legal standards before ozonation could result in noncompliance with BOD limits. This result indicates the necessity of a high level of elimination of biodegradable substances in the preceding biological stage, which is typically achieved at low F/M ratios (high-SRT) in activated sludge plants with full nitrification and denitrification. Therefore, implementing ozonation at high-SRT WWTPs with lower effluent organic matter (Khan et al., 1998) is crucial not only for limiting oxidant competition between organic compounds and micropollutants but also to ensure legal compliance of BOD, a conventional wastewater parameter.

5.2.2.2. DOC

The DOC concentrations of the 7 investigated samples originating from 4 different WWTPs before and after ozonation with 0.4 to 0.8 g O_3/g DOC (nitrite compensated D_{spec}) are shown in Table 5.5.

Table 5.5. DOC concentrations (mg/L) before and after ozonation and BOD_5 measurement

Name	DOC concentration (mg/L)				
	D_{spec} (g O_3/g DOC)	WWTP effluent	WWTP effluent after BOD_5 measurement	Ozonated effluent	Ozonated effluent after BOD_5 measurement
WWTP1a	0.65	5.29 ± 0.06	5.14 ± 0.06	5.22 ± 0.11	4.28 ± 0.13
WWTP1b	0.61	6.45 ± 0.06	6.19 ± 0.06	6.26 ± 0.00	5.16 ± 0.00
WWTP2a	0.69	5.85 ± 0.06	5.85 ± 0.06	5.78 ± 0.19	4.89 ± 0.11
WWTP2b	0.67	5.81 ± 0.06	5.78 ± 0.00	5.59 ± 0.06	4.59 ± 0.13
WWTP2c	0.43	5.22 ± 0.00	5.39 ± 0.08	5.11 ± 0.00	4.59 ± 0.06
	0.62			4.96 ± 0.06	4.22 ± 0.11
	0.78			5.00 ± 0.00	4.04 ± 0.06
WWTP3	0.46	6.28 ± 0.13	5.63 ± 0.20	6.02 ± 0.07	5.02 ± 0.07
	0.67			6.02 ± 0.07	4.75 ± 0.07
	0.87			5.86 ± 0.00	4.52 ± 0.07
WWTP4	0.45	6.82 ± 0.11	6.52 ± 0.07	6.64 ± 0.07	5.27 ± 0.07
	0.65			6.48 ± 0.11	5.00 ± 0.11
	0.83			6.36 ± 0.00	4.81 ± 0.07

Figure 5.12 shows the results of the dose-specific investigation for DOC elimination between 0.45 ± 0.02 (minimum) and 0.83 ± 0.05 (maximum) g O_3/g DOC. Immediately after ozonation, the DOC elimination varied between 2.13% (0.45 g O_3/g DOC for WWTP2c) and 6.71% (0.87 g O_3/g DOC for WWTP3), and a correlation with D_{spec} could not be observed.

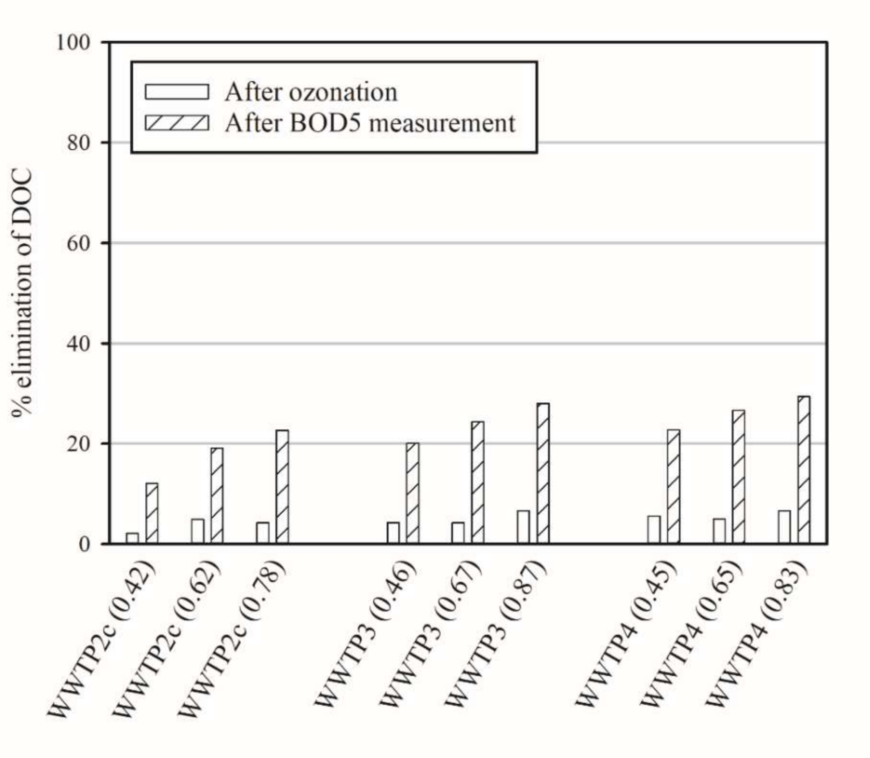


Figure 5.12. Dose -specific elimination of DOC

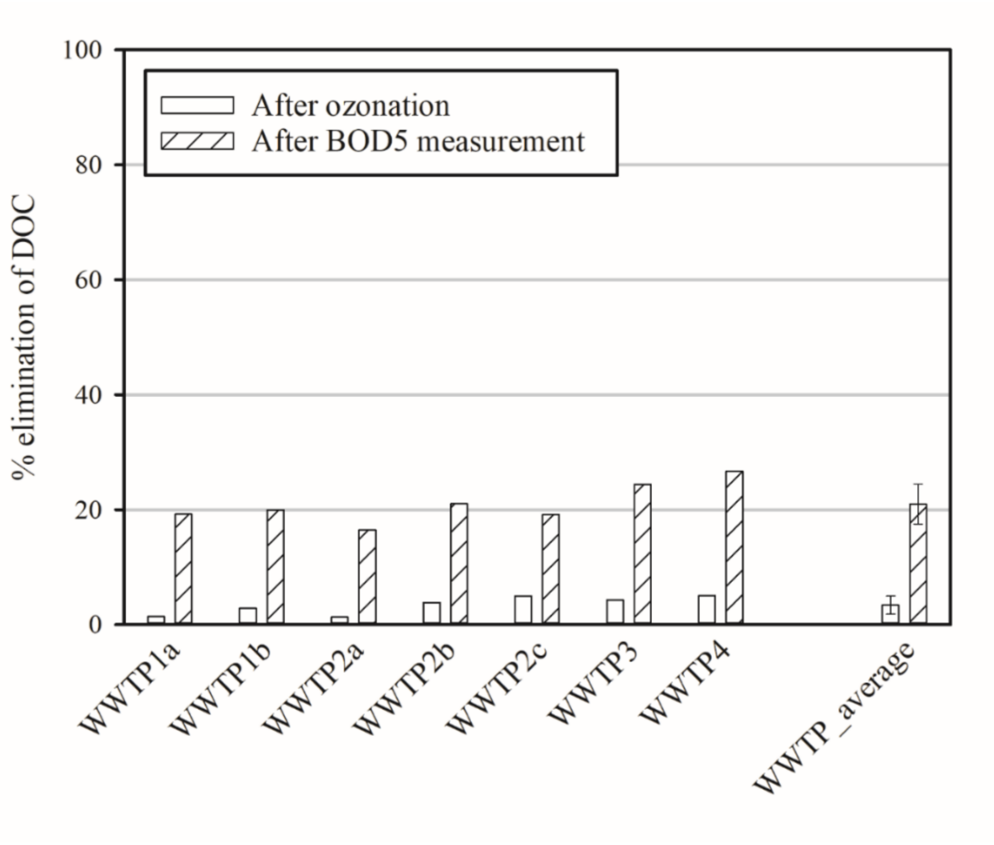


Figure 5.13. Elimination of DOC at 0.65 ± 0.03 g O_3/g DOC (n=7)

Figure 5.13 shows the DOC decrease for the targeted D_{spec} of 0.6 g O_3/g DOC (0.65 ± 0.03), analysing all samples at a D_{spec} typically applied for organic micropollutant abatement. Immediately after ozonation, the mean DOC elimination was 3.36 ± 1.58%, with a minimum at 1.27% (WWTP2a) and a maximum at 6.67% (WWTP4). The decreases of the three samples (WWTP2c, WWTP3, and WWTP4) were on a similar order of magnitude at approximately 4.7%.

In contrast, after the exposure time for the BOD_5 measurement, the elimination of DOC correlated with D_{spec} for all investigated samples (see Figure 5.12). The lowest elimination was 12.06% at 0.43 g O_3/g DOC (WWTP2c), and the highest was 29.44% at 0.83 g O_3/g DOC (WWTP4).

After the exposure time for the BOD_5 measurement, the mean removal of DOC increased to 20.97 ± 3.47%, and in accordance with the results after ozonation, the lowest value was obtained for WWTP2a (16.46%) and the highest for WWTP4 (29.44%).

In general, an observed decrease in DOC during biological wastewater treatment can be considered equivalent to biodegradable DOC (BDOC). In the ozonation stage, there was only a slight, dose-independent decrease in DOC (Figure 5.7), which was in line with the partial oxidation and a degree of mineralization of < 10% at the D_{spec} that is usually applied for micropollutant abatement (Sauter et al., 2021; von Sonntag et al., 2012). During ozonation, molecular structures are attacked, which means that the DOC usually becomes more bioavailable and can be broken down in a subsequent biological process, e.g., in biologically activated carbon (BAC) filters, sand filters or even receiving water bodies. DOC removal and modification of chemical structures by ozonation predominantly depend on the DOC composition, which explains the differences encountered for the various samples investigated. Values obtained in this study are comparable with those in the published literature. The study by Molnar et al. (2012) showed DOC reductions from 5% to 25% at a D_{spec} of 0.4 – 3 g O_3/g DOC. Mao et al. (2014) reported DOC reductions up to 20% achieved by ozonation in conventional treatment plants at D_{spec} of 0.6 – 1.6 g O_3/g DOC. High values were not reproducible in our experiments but can be connected to the higher D_{spec} and higher initial DOC values and DOC composition in these studies, respectively. Our results are in line with Sauter et al. (2021), who reported a DOC decline of 5% at 0.65 ± 0.09 g O_3/g DOC, and Stapf et al. (2017) showed an average DOC decrease of 5% for various WWTPs. Additionally, Nöthe et al. (2009) reported a low impact of ozonation on the DOC (4 – 9% decrease between 0.4 and 0.8 g O_3/g DOC). Nöthe et al. (2009) attribute the low impact to the low degree of decarboxylation, since DOC is only eliminated when decarboxylation reactions occur or when substances that are already substantially oxidized are further oxidized.

The increase in DOC elimination determined after the exposure for the BOD_5 measurement was also observed by Khan et al. (1998), who studied BDOC in high- and low-SRT WWTPs as well as in water reclamation plants using ozonation and subsequent BAC, applying the same methodological approach as in the present paper. Similar results are reported by Nishijima et al. (2003). Moreover, there were able to demonstrate a correlation of BDOC and D_{spec}, which is in accordance with the dose-dependent structural changes determined by delta UV_{254} and specific UV absorbance at 254 nm (SUVA), respectively (see Figure 5.7). The BDOC in the effluent of the conventional WWTPs was calculated as the difference between the initial DOC and the DOC after BOD_5 exposure with an average share of 4% (96% refractory DOC). After ozonation, this share increased to 15, 18 and 22%, corresponding to applied D_{spec} values of 0.4, 0.6 and 0.8 g O_3/g DOC, respectively.

5.2.2.3. COD

The dose-specific elimination of COD is presented in Table 5.6 and Figure 5.14. After ozonation, the lowest elimination was 6.67% (WWTP3), and the highest was 16.67% (WWTP2). The correlation with D_{spec} was not immediately obvious: while the lowest COD elimination was always observed at the minimum applied D_{spec} and the highest elimination at the maximum D_{spec}, varying results were obtained at the medium D_{spec} of 0.6 g O_3/g DOC. For WWTP2c and WWTP4, it corresponded to the results obtained for a D_{spec} of 0.4 g O_3/g DOC and for WWTP3 to the results obtained for a D_{spec} of 0.8 g O_3/g DOC. The most likely reasons for this observation are the small differences in the COD that result from low absolute concentrations and the resolution of the applied method.

Table 5.6. COD concentrations (mg/L) before and after ozonation and BOD_5 measurement

Name	COD concentrations (mg/L)				
	D_{spec} (g O_3/g DOC)	WWTP effluent	WWTP effluent after BOD_5 measurement	Ozonated effluent	Ozonated effluent after BOD_5 measurement
WWTP1a	0.65	15.22 ± 0.00	14.13 ± 0.00	13.04 ± 0.00	11.23 ± 1.26
WWTP1b	0.61	18.32 ± 0.63	16.48 ± 0.00	16.48 ± 0.00	13.92 ± 0.63
WWTP2a	0.69	15.56 ± 0.00	17.04 ± 0.64	14.07 ± 0.64	13.33 ± 0.00
WWTP2b	0.67	14.81 ± 0.64	16.67 ± 0.00	13.33 ± 0.00	13.33 ± 1.92
WWTP2c	0.43	15.56 ± 1.92	15.93 ± 0.64	14.07 ± 1.28	14.07 ± 0.64
	0.62			12.96 ± 0.64	13.33 ± 0.00
	0.78			12.96 ± 0.64	11.85 ± 0.64
WWTP3	0.46	17.24 ± 0.00	18.77 ± 1.76	16.09 ± 0.00	15.71 ± 0.66
	0.67			16.09 ± 0.00	15.33 ± 0.66
	0.87			14.94 ± 0.00	14.94 ± 0.00
WWTP4	0.45	18.18 ± 0.00	20.45 ± 1.97	16.29 ± 0.66	16.29 ± 0.66
	0.65			16.29 ± 0.66	15.53 ± 0.66
	0.83			15.91 ± 1.14	13.64 ± 0.00

In contrast, after five days of exposure for BOD_5 measurement, a dose-dependent increase in COD elimination was detected for all WWTPs, which was more significant for the higher ozone doses. The minimum COD elimination was 9.52% at 0.43 g O_3/g DOC (WWTP2c) and the maximum 25.00% at 0.83 g O_3/g DOC (WWTP4).

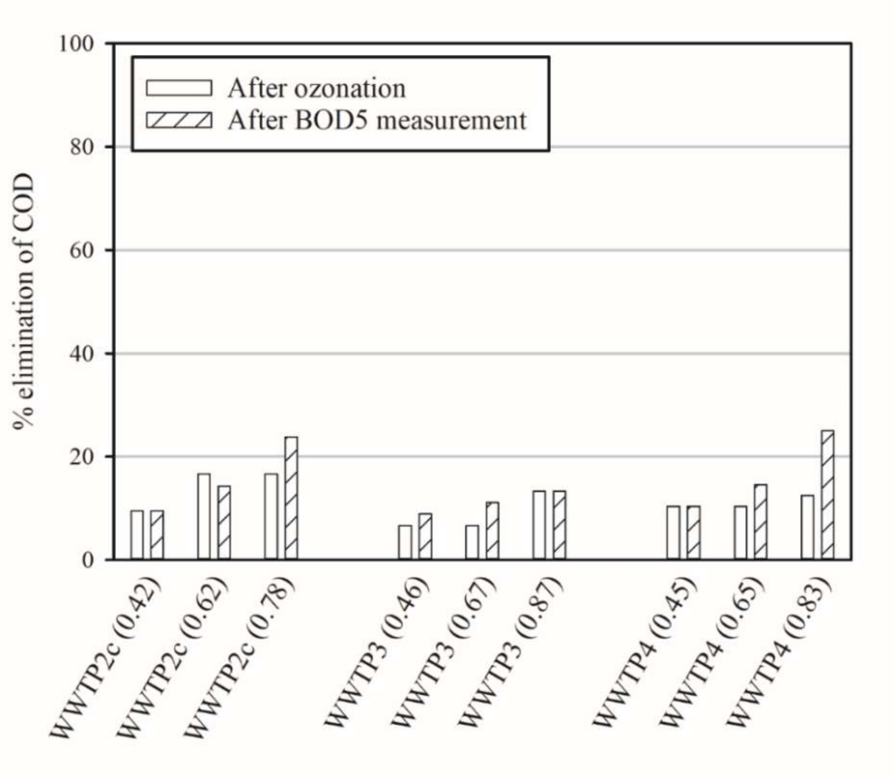


Figure 5.14. Dose -specific elimination of COD

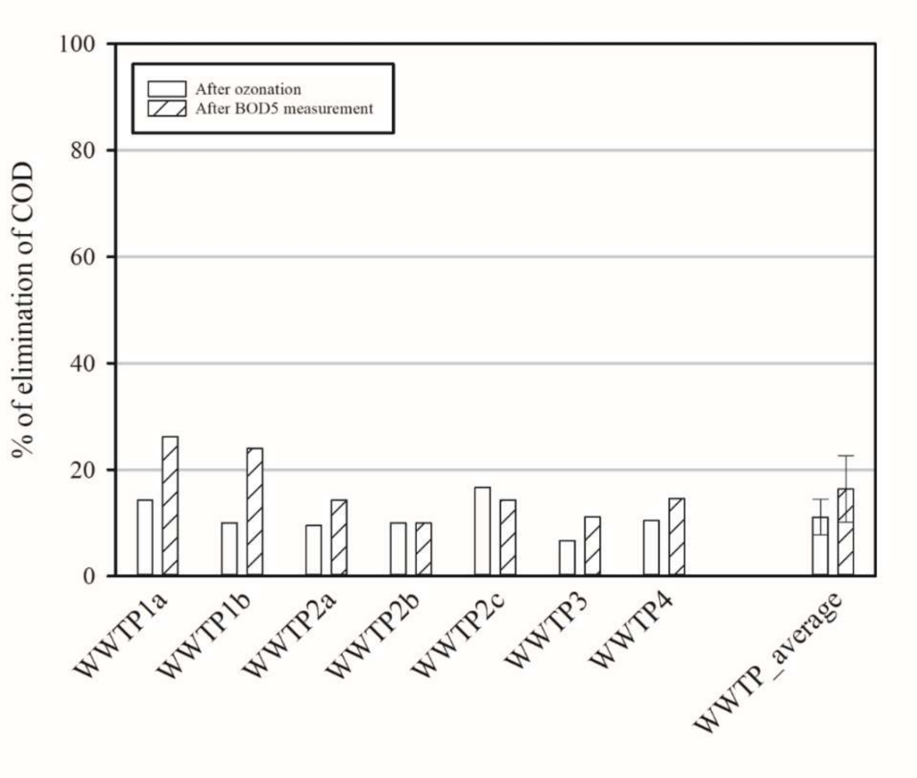


Figure 5.15. Elimination of COD at 0.65 ± 0.03 g O_3/g DOC (n=7)

Figure 5.15 shows the COD abatement for the targeted D_{spec} of 0.6 g O_3/g DOC (0.65 ± 0.03 g O_3/g DOC). After ozonation, the mean removal of COD was 11.08 ± 3.32%, with a minimum of 6.67% (WWTP3) and a maximum of 16.67% (WWTP2c). After the exposure time for the BOD_5 measurement, the mean removal of COD was 16.35 ± 6.25%, with 10.00% as the lowest value (WWTP2b) and 26.19% (WWTP1a) as the maximum.

The COD measurement provides information about the degree of oxidation in organic compounds but also comprises oxygen required for inorganics that are not fully oxidized (e.g., nitrite). In contrast, the DOC measurement only provides information on the amount of carbon that could be mineralized to CO_2. Thus, it appears possible that COD could supply additional information regarding ozone scavenging by the wastewater matrix compared to the information that can be obtained from DOC. Furthermore, COD is a parameter typically used in wastewater treatment for process design and evaluation as well as for regulation. The fact that COD is reduced while DOC only changes slightly during ozonation indicates that ozone treatment transforms the structure of organic matter and mainly forms transformation products via direct oxidation (Ekblad et al., 2019; Liu et al., 2015; Pešoutová et al., 2014). For this reason, dose-dependent elimination can be expected after ozonation, as summarized in Figure 5.7. Exposure to biological processes simulated by BOD measurement only indicated a slightly greater dose-dependent removal.

The COD/DOC ratio is a measure of the average degree of oxidation of organic compounds. For low F/M, WWTPs a COD/DOC ratio of approx. 3 typically can be expected, as reported by Nöthe et al. (2009). In wastewater samples, the average COD/DOC ratio before ozonation was 2.76 ± 0.14 (Table 5.7). After ozonation (0.65 ± 0.03 g O_3/g DOC), the ratio decreased to 2.54 ± 0.10, indicating a higher degree of oxidation and fewer oxidation equivalents required for full oxidation to CO_2. After exposure to the BOD_5 measurement, the ratio increased again to a value of 2.92 ± 0.23, which resulted from a different change in COD compared to DOC. Obviously, the high degree of BDOC disappearance (higher removal of DOC, as shown in Figure 5.7) indicates the predominant removal of higher oxidized carbon species that are characterized by a comparably lower COD, resulting in the low change of COD observed during exposure for BOD_5 measurement. This line of argument results in the expectation of an even lower COD/DOC ratio with increasing D_{spec}. Indeed, data for

0.83 ± 0.05 g O_3/g DOC indicate a lower ratio for the individual wastewater samples and again a substantial increase after BOD measurement.

Table 5.7. COD/DOC ratio of the investigated samples before and after ozonation and BOD_5 measurement

Name	COD/DOC ratio				
	D_{spec} (g O_3/g DOC)	WWTP effluent	WWTP effluent after BOD_5 measurement	Ozonated effluent	Ozonated effluent after BOD_5 measurement
WWTP1a	0.65	2.88	2.75	2.50	2.62
WWTP1b	0.61	2.84	2.66	2.63	2.70
WWTP2a	0.69	2.66	2.91	2.43	2.73
WWTP2b	0.67	2.55	2.88	2.38	2.90
WWTP2c	0.43	2.98	2.96	2.75	3.06
	0.62			2.61	3.16
	0.78			2.59	2.94
WWTP3	0.46	2.75	3.33	2.68	3.13
	0.67			2.68	3.23
	0.87			2.55	3.31
WWTP4	0.45	2.67	3.14	2.53	3.09
	0.65			2.51	3.11
	0.83			2.50	2.83
Average		2.760	2.95	2.57	2.98

5.2.2.4. BOD_5/COD ratio

The BOD_5/COD ratios before and after ozonation are given in Figure 5.15. Ozonation resulted in an increase from 0.08 to 0.17 (mean values over all investigated samples). As shown in Figure 5.16, in the dose-specific investigation, there was a clear increase in the ratio with increasing D_{spec}. This was caused by a dose-specific increase in BOD_5 and a simultaneously occurring decrease in COD. The starting values were typical for WWTPs characterized by a low F/M ratio.

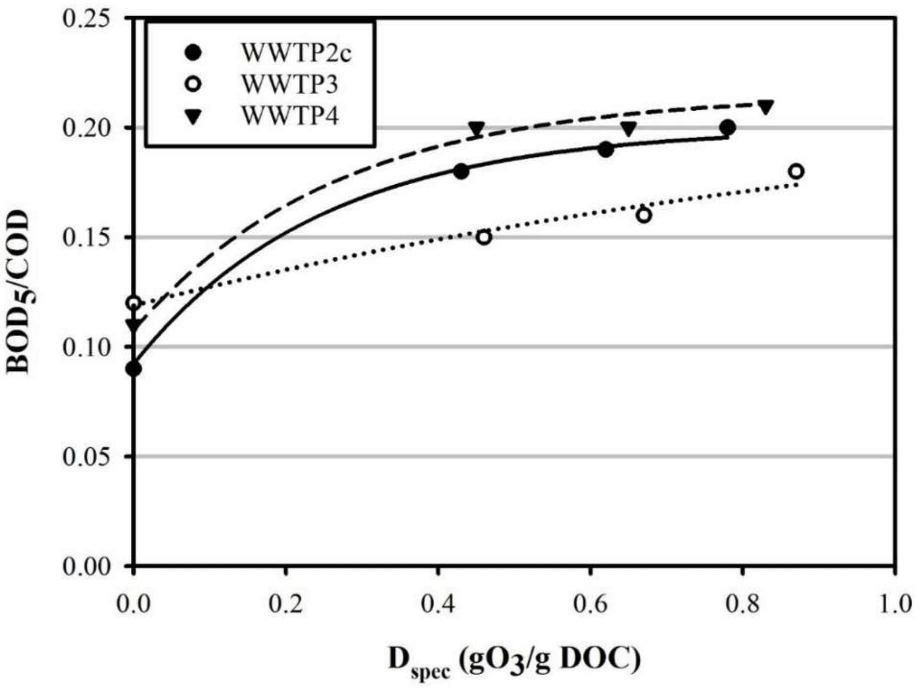


Figure 5.16. Correlation of BOD_5/COD ratio and D_{spec}

The BOD_5/COD ratio can be used as a further indicator for biodegradability. Based on the population-specific organic loads in the inflow of an urban WWTP, a BOD_5/COD ratio of 0.5 can be assumed. Biological treatment results in a decrease in this ratio depending on the SRT. While the BOD_5/COD ratio in the effluent of high F/M (low-SRT) plants ranges between 0.25 and 0.35, high-SRT plants with nutrient removal usually achieve a ratio of approx. 0.1, indicating greater removal of BDOC compared to the removal of refractory organic compounds. In the current study, the BOD_5/COD ratios were calculated for wastewater before and after ozonation (see Table 5.8). There was a steady, dose-dependent increase in the BOD_5/COD quotient from 0.09 (before ozonation) to 0.17 (average value after ozonation), indicating enhanced biodegradability. This finding corroborates the results of Van Aken et al. (2015), who reported that the biodegradable fraction increases during ozone treatment and that the amount of refractory COD decreases because of the partial degradation of the pollutant. The enhanced biodegradability is attributed to the transformation of the effluent organic matter into readily biodegradable compounds by ozonation, such as aldehydes and carboxylic acids (Siddiqui et al., 1997).

Table 5.8. BOD_5/COD ratio before and after ozonation.

Name	D_{spec} (g O_3/g DOC)	BOD_5/COD Before ozonation	BOD_5/COD After ozonation
WWTP1a	0.65	0.043	0.12
WWTP1b	0.61	0.037	0.12
WWTP2a	0.69	0.091	0.14
WWTP2b	0.67	0.093	0.18
WWTP2c	0.43	0.085	0.18
	0.62		0.19
	0.78		0.20
WWTP3	0.46	0.115	0.15
	0.67		0.16
	0.87		0.18
WWTP4	0.45	0.105	0.20
	0.65		0.20
	0.83		0.21
Average		0.081	0.17

5.2.2.5. UV_{254}

The change in UV_{254} for the dose-specific experiments during ozonation and BOD_5 measurement – caused by the oxidative effect of ozone on the aromatic ring structures absorbing at 254 nm and the effects of further biological activities – is shown in Figure 5.17. Corresponding numbers are given in Table 5.9. An increase in delta UV_{254} is a consequence of a higher decrease in UV_{254} during ozonation. The relative decrease in UV_{254} correlated with the increase in the D_{spec}. Immediately after ozonation, the lowest delta UV_{254} was 34.36% (0.43 g O_3/g DOC for WWTP2c), and the maximum delta UV_{254} was 57.42% (0.83 g O_3/g DOC for WWTP4).

Table 5.9. UV absorption at 254 nm (1/cm) before and after ozonation and BOD$_5$ measurement

Name	UV absorption at 254 nm (1/cm)				
	D$_{spec}$ (g O$_3$/g DOC)	WWTP effluent	WWTP effluent after BOD$_5$ measurement	Ozonated effluent	Ozonated effluent after BOD$_5$ measurement
WWTP1a	0.65	0.122 ± 0.00	0.128 ± 0.00	0.074 ± 0.00	0.074 ± 0.00
WWTP1b	0.61	0.138 ± 0.00	0.149 ± 0.00	0.084 ± 0.00	0.085 ± 0.00
WWTP2a	0.69	0.128 ± 0.00	0.141 ± 0.00	0.071 ± 0.00	0.079 ± 0.00
WWTP2b	0.67	0.127 ± 0.00	0.139 ± 0.00	0.067 ± 0.00	0.076 ± 0.00
WWTP2c	0.43	0.108 ± 0.00	0.121 ± 0.00	0.071 ± 0.00	0.085 ± 0.00
	0.62			0.051 ± 0.00	0.069 ± 0.00
	0.78			0.049 ± 0.00	0.066 ± 0.00
WWTP3	0.46	0.114 ± 0.00	0.133 ± 0.00	0.072 ± 0.00	0.087 ± 0.00
	0.67			0.059 ± 0.00	0.076 ± 0.00
	0.87			0.055 ± 0.00	0.072 ± 0.00
WWTP4	0.45	0.135 ± 0.00	0.155 ± 0.00	0.077 ± 0.00	0.094 ± 0.00
	0.65			0.064 ± 0.00	0.084 ± 0.00
	0.83			0.058 ± 0.00	0.078 ± 0.00

After the exposure time for the BOD$_5$ measurement, the delta UV$_{254}$ correlated with D$_{spec}$ as well; however, an increase in UV$_{254}$ resulted in a lower delta UV$_{254}$ than after ozonation. Again, the lowest delta UV$_{254}$ was determined for WWTP2c at 0.43 g O$_3$/g DOC (20.96%), and the highest was determined at 0.87 g O$_3$/g DOC for WWTP4 (42.02%).

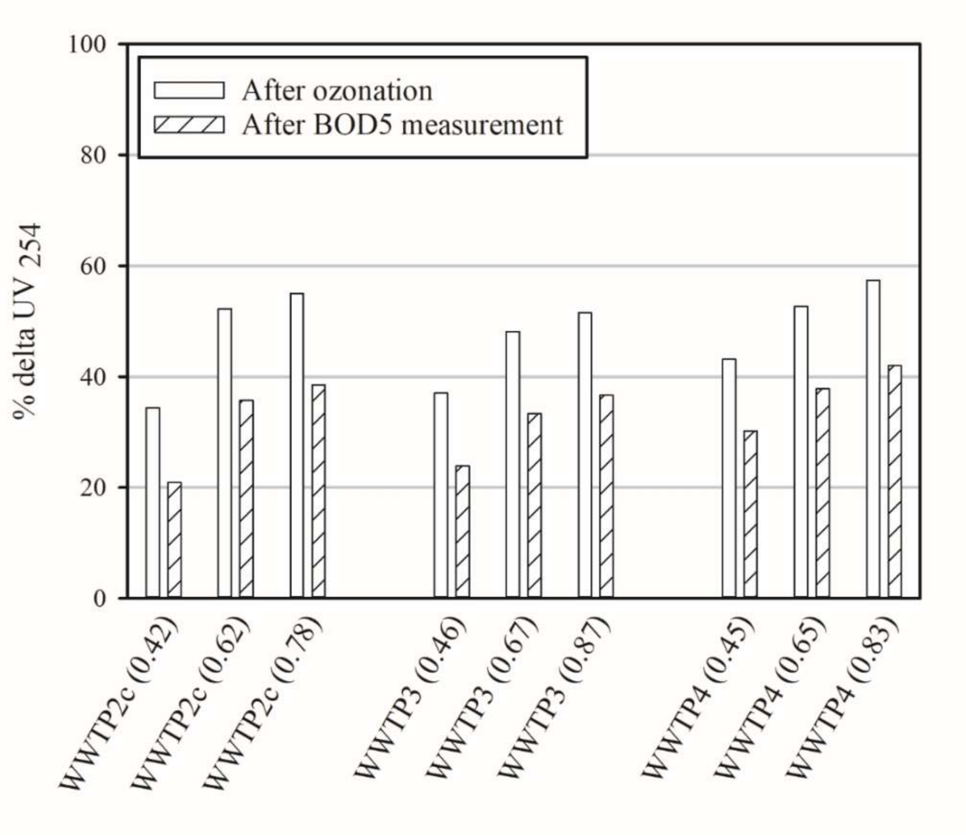


Figure 5.17. Dose-specific decrease of UV_{254}

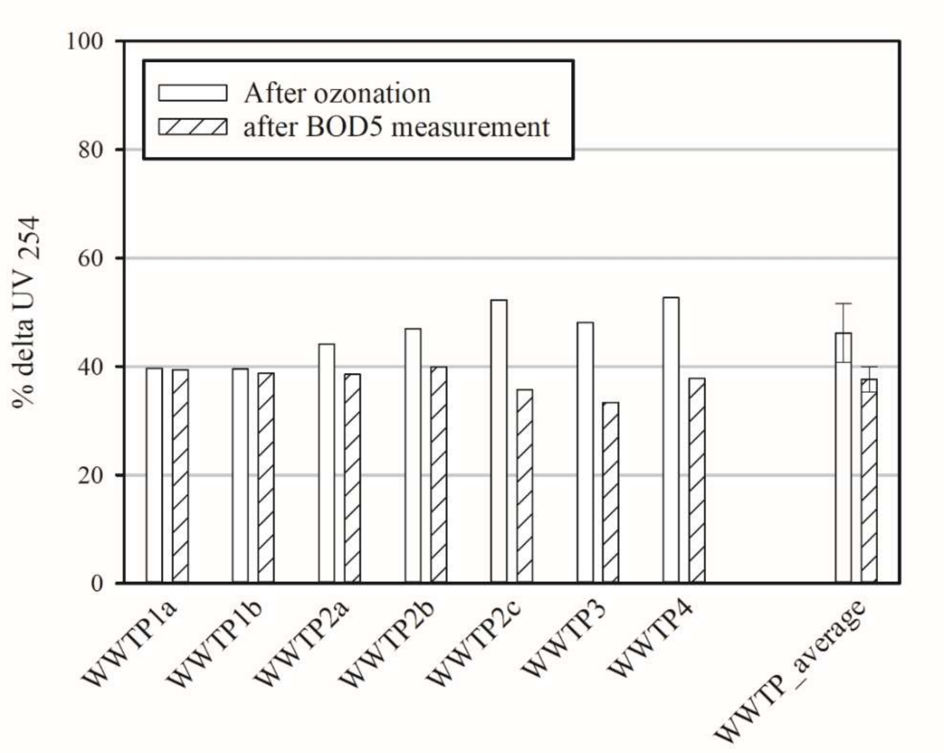


Figure 5.18. Decrease of UV_{254} at 0.65 ± 0.03 g O_3/g DOC (n=7)

The delta UV_{254} for the targeted D_{spec} of 0.6 g O_3/g DOC (0.65 ± 0.03) is presented in Figure 5.17. After ozonation, the mean change in UV_{254} was 46.17%, with a minimum of 39.52% at 0.61 g O_3/g DOC (WWTP1b) and a maximum of 52.66% at 0.65 g O_3/g DOC (WWTP4).

After the exposure time for BOD_5 measurement, the mean decrease in UV_{254} was 37.64%, the lowest value obtained was 33.33% at 0.67 g O_3/g DOC (WWTP3), and the highest was 39.94% at 0.67 g O_3/g DOC (WWTP2b). Except for WWTP1 (similar results as after ozonation), the results from Figure 5.18 can be confirmed, showing a lower delta UV_{254} after BOD_5 measurement.

UV_{254} generally corresponds to the content of organic carbon (DIN 38404-3, 2005). The typical shoulder in the UV absorbance pattern of WWTP effluents at approximately 260 nm is due to the absorbance of aromatic structures or conjugated double bonds, among others present in non-readily biodegradable compounds, e.g., humic substances, or products of microbial metabolism and lysis (Schaar et al., 2010). Because ozone attacks double bonds, active aromatic constituents, and organic functional groups, such as carboxylic groups (von Gunten, 2003a), ozonation effectively reduces the UV_{254}. The dose-specific UV_{254} decline correlates with micropollutant abatement (cf. Figure 6 and Figure 8 of Appendix 3); which makes the delta UV_{254} a suitable surrogate parameter and parameter for process control of ozonation, respectively (Bahr et al., 2007; Chon et al., 2015; Dickenson et al., 2009; Nanaboina et al., 2010; Stapf et al., 2016). The dose-response curve of delta UV_{254} is illustrated in Figure 5.7.

Interestingly, after exposure to BOD_5 measurement, the absolute UV absorption increased again (see Table 5.10), resulting in a delta UV_{254} constantly lower than that directly after ozonation. This phenomenon was independent of the applied D_{spec}. Higher DOC elimination compared to oxidation equivalents (COD) was observed, as discussed above, indicating the potential transformation of organic substances by processes other than biological respiration (oxidation). Transformation can involve the assimilatory formation of complex organic compounds with aromatic structures that increase aromaticity and thus can result in an increase in UV_{254} (Weishaar et al., 2003).

Table 5.10. SUVA (L/(mg·m)) before and after ozonation and BOD_5 measurement

Name	**SUVA (L/(mg·m))**				
	D_{spec} (g O_3/g DOC)	**WWTP effluent**	**WWTP effluent after BOD_5 measurement**	**Ozonated effluent**	**Ozonated effluent after BOD_5 measurement**
WWTP1a	0.65	2.32	2.50	1.42	1.74
WWTP1b	0.61	2.14	2.40	1.33	1.64
WWTP2a	0.69	2.18	2.42	1.24	1.61
WWTP2b	0.67	2.19	2.40	1.21	1.66
WWTP2c	0.43	2.07	2.25	1.38	1.85
	0.62			1.04	1.64
	0.78			0.97	1.64
WWTP3	0.46	1.81	2.36	1.19	1.73
	0.67			0.98	1.60
	0.87			0.94	1.59
WWTP4	0.45	1.98	2.38	1.19	1.79
	0.65			0.99	1.68
	0.83			0.91	1.63
Average		2.10	2.39	1.14	1.68

Following up on the UV measurement, the specific UV absorbance at 254 nm (SUVA) as ratio of the UV_{254} and the DOC is a measure for the aromaticity of a water sample and is considered useful for the estimation of the aromatic carbon content (Weishaar et al., 2003). In the WWTP effluent samples, the SUVA ranged from 1.8 – 2.3 L/(mg·m) and decreased by 44% (arithmetic mean for 0.65 ± 0.03 g O_3/g DOC) after ozonation (Table 5.7). This decrease is a result of the partial oxidation of the UV-active moieties. The constant DOC content and the dose-dependent reduction in SUVA demonstrate the structural alterations of the organic matrix.

After the exposure time for the BOD_5 measurement, a dose-dependent increase in SUVA was observed as a result of both the increase in UV_{254} absorbance and a simultaneous decrease in DOC. In combination with a predominating degradation of nonaromatic organic carbon, this increase in aromaticity strongly supports the hypothesis on the assimilatory formation of complex organic compounds comprising aromatic structures (Hoppe-Jones et al., 2010).

5.3. Toxicological monitoring

The long-term toxicological monitoring during operating conditions offered the valuable chance to encounter realistic conditions comprising fluctuations in wastewater quantity and quality as well as operational problems that have an impact on both conventional and advanced treatment. With regard to process stability of the conventional biological treatment, insufficient nitridation within the two-step nitrification process for example can result in the accumulation and therefore occurrence of nitrite in the effluent, which has a decisive impact on the ozone consumption (3.43 mg O_3/mg NO_2-N) and - depending on the control strategy of an ozonation plant – also on the effective (nitrite compensated) D_{spec}. A model calculation with a D_{spec}-setpoint of 0.55 g O_3/g DOC for a DOC effluent concentration of 4.5 mg/L demonstrates that the occurrence of 0.3 mg NO_2-N/L decreases the effective D_{spec} to 0.3 g O_3/g DOC, representing a decrease by 42%. The campaigns with the lower specific ozone doses tried to mimic these situations and evaluate the decline in micropollutant removal and finally the impact on the toxicity endpoints. Apart from the estrogenic activity which revealed a slightly lower signal reduction during the two lowest ozone doses (< 0.3 g O_3/g DOC) no clear correlation within the whole tested dose range (0.18-0.92 g O_3/g DOC) could be determined.

Specific ozone doses D_{spec} for CEC abatement from tertiary treated wastewater are recommended to range from 0.4 to 0.6 g O_3/g DOC (Rizzo et al., 2019), with a D_{spec} of 0.55 g O_3/g DOC for the first full-scale WWTP upgraded with ozonation at Neugut, Switzerland (Bourgin et al., 2018).

Due to enhanced efforts required for DOC-related process control, most of the ozonation plants apply flow-proportional dose control based on a DOC-default value in the effluent of the conventional stage. Operating the plant with a specific D_{spec}-setpoint without considering potential nitrite occurrence can have a relevant effect as demonstrated by the model calculation with 0.3 mg NO_2-N/L above. Thus, considering fluctuations in the effluent DOC or the occurrence of nitrite of a CAS plant, a deviation from the setpoint of 0.55 g O_3/g DOC easily can result in an actual specific ozone dose ranging between 0.4 and 0.7 g O_3/g DOC.

Figure 5.19, as a summarizing graph, gives an overview of the removal range for the investigated MOA considering all sampling campaigns irrespective of the specific ozone doses. Genotoxicity and anti-estrogenicity were not integrated due to their lack of occurrence. A median removal of > 80% was achieved only for estrogenicity and cytotoxicity. Estrogenicity was the endpoint with the lowest variations. After ozonation, the 25th percentile removal was > 80%, and after GAC, the minimum removal determined was 84%. A removal < 70% can be related to Dspec < 0.3 g O3/g DOC, though. Cytotoxicity seemed to have higher variations, but all results were < LOQ after ozonation and activated carbon treatment, respectively. Thus, the calculated removal based on ½ LOQ can deviate. The same is valid for anti-androgenicity with 100% of the data < LOQ after advanced treatment.

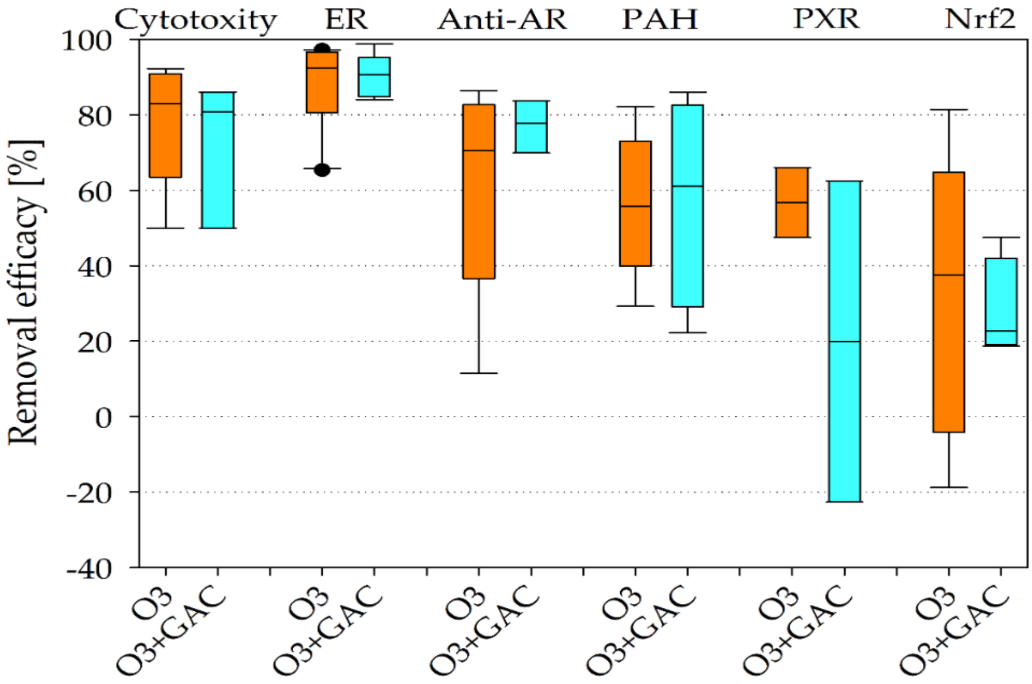


Figure 5.19. Boxplots showing the range of removal for the investigated MOA along the multibarrier treatment system over the one-year monitoring

A comparison of the median for the various bioanalytical equivalent concentrations for this ozone dose range is thus compared with the currently discussed EBTs with currently discussed MOA-specific EBT (cf. Appendix 4). Table 5.11 shows the n-fold exceedance of the median relative to currently discussed EBT values according to the concept suggested by y Alygizakis et al. (2019)

Table 5.11. n-fold EBT – exceedance of the median BEQ for all sampling campaigns*

Bioassay	EBT	Influent WWTP (n=2)	CAS (n=8)	O_3 (n=8)	GAC (n=5)
Cytotox	-	34.35	0.95	< 0.52	< 0.56
ERα	0.1	🟥 21.9	🟨 0.57	🟦 *< 0.05*	🟦 *< 0.06*
anti-AR	14	🟩 < 37.0	🟦 < 3.10	🟦 < 3.65	🟦 < 4.40
Nrf2	10	🟥 < 2820	🟧 135.0 (n = 6)	🟨 66.5 (n = 6)	🟨 92.5 (n = 4)
PAH	6.2	🟧 320	🟧 170.0 (n = 4)	🟧 75.5 (n = 4)	🟨 39.0 (n = 3)
PXR	3	< 12 (n = 1)	🟧 40 (n = 3)	🟨 18 (n = 3)	🟨 15 (n = 3)

🟦 **BEQ/EBT < 1**	🟩 **1 ≤ BEQ/EBT < 3**	🟨 **3 ≤ BEQ/EBT < 10**	🟧 **10 ≤ BEQ/EBT < 100**	🟥 **> 100 EBT**

< … 50% or more of the samples were below the limit of quantification

* The calculation of the median values is based on 2 campaigns for the influent, 8 campaigns for CAS and O_3 and 5 campaigns for GAC.

In order to get a broader picture on the approach, two samplings of the CAS influent are included too, exhibiting the highest values in the inflow of the conventional biological WWTP. A typical pattern for the degree of exceedance could be observed by a decline of the response from left to right, following the treatment train. An increase in treatment steps resulted in an improvement of the water quality even if the BEQ was still exceeded by up to 9-fold for selected endpoints other than hormone-mediated endpoints; the latter decreased below LOQ in the advanced treatment (labelled with "<"). According to van der Oost et al. (2017) bioassay responses of more than 100% of the EBT are indicative for a high risk, however, appropriate measures can be taken if chemicals causing the effects are known. Considering the findings of (Neale et al., 2015; Neale et al., 2017a) who linked *in vitro* effects and detected organic micropollutants in surface water with mixture-toxicity modelling, this will only be applicable for a limited number of endpoints, among them estrogenic effects. The > 100-fold exceedance for the influent of the wastewater treatment plant was significantly reduced by conventional biological wastewater treatment; based on the amount of CECs currently in use and the fact that CEC removal by conventional treatment is limited, the additional barrier of advanced treatment technologies should be taken into account in the future even if bioassay responses after advanced treatment with a multibarrier system comprising O_3 and GAC were still elevated for endpoints like PAH-like activities and oxidative stress activities.

5.3.1. Cytotoxic activity (Cytotox CALUX®)

The non-specific toxicity endpoint cytotoxicity provides an estimate of the overall toxic burden of all chemicals within a mixture. Cytotoxic effects detected in the effluent of the conventional treatment were reduced by ozonation (Figure 5.20) to levels below LOQ (Figure 5.21). Also, after activated carbon the cytotoxicity was below LOQ, a quantification of further removal after ozonation, however, was not possible based on the data < LOQ.

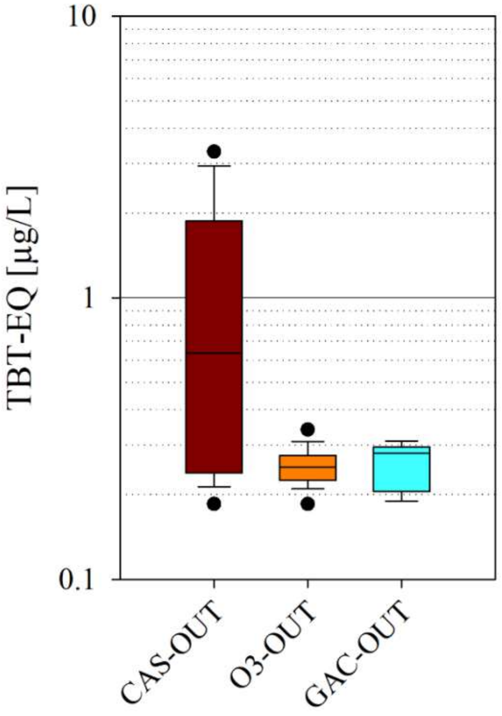


Figure 5.20. Boxplots of cytotoxicity as tributyltin acetate equivalents after the treatment steps of the multibarrier system for advanced treatment.

After the conventional treatment, cytotoxicity was in the range representative for other Austrian CAS-plants with full nitrification and denitrification (Braun et al., 2021). The advanced treatment proved beneficial for baseline toxicity removal, confirming the suitability of the multibarrier system. Since transformation products formed during ozonation are more hydrophilic, they are less cytotoxic, but still contribute to mixture effects (Escher et al., 2011). Biodegradation during biologically activated GAC theoretically offers the potential to reduce these effects, but in the present study it was not possible to prove this due to the non-detects after ozonation. A significant reduction after ozonation was also determined in a study on three German WWTPs (Dopp et al., 2021). In addition, they also revealed the effect reduction potential of biological posttreatment with a fluidized bed reactor. Even though GAC, applied in the present study, differs from the fluidized bed reactor, both systems represent biological posttreatment processes. Thus, it is a strong indication for an additional benefit of GAC and the strength of the multibarrier approach.

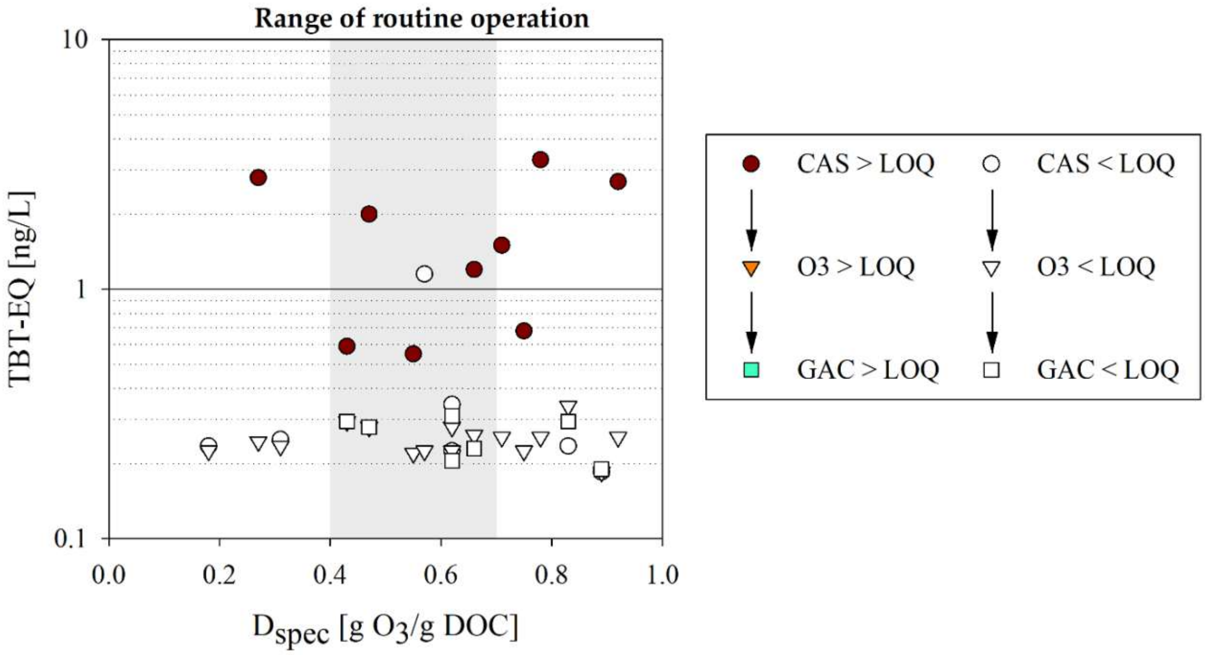


Figure 5.21. Change in cytotoxicity (as tributyltin acetate equivalents) along the treatment train at various nitrite compensated specific ozone doses during the sampling campaigns including the range of routine operation.

As cytotoxicity is a non-specific toxicity endpoint that provides an estimate of the overall toxic burden in a mixture, it is considered important to be investigated (Neale et al., 2020).

5.3.2. Estrogenic activity (ERα CALUX®), Anti-androgenic activity (anti-AR-CALUX®) and anti-estrogenic activity (anti- ERα CALUX®)

Estrogenicity as a specific toxicity endpoint for estrogenic receptor-mediated activity significantly decreased during advanced treatment (Figure 5.22). The reduction of the bioanalytical equivalent concentrations that occurred already during ozonation can be attributed to the high reactivity of high-potency estrogens with ozone (Huber et al., 2005). This conclusion is permitted since estrogenicity is one of the endpoints with a high overlapping of the biological and chemical BEQ. Calculating effects from chemical analysis, Neale et al. (2015) was able to explain up to 80% of the estrogenic receptor activation in surface water by only five chemicals.

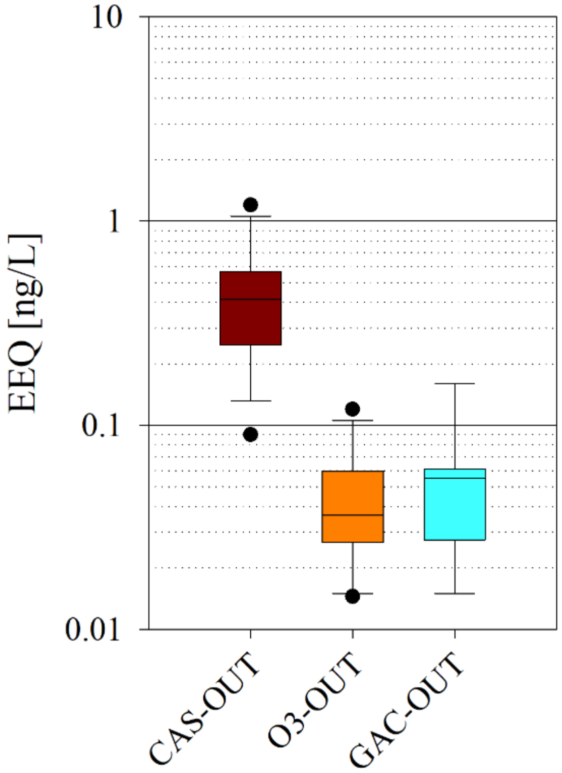


Figure 5.22. Boxplots of estrogenicity as 17β estradiol equivalents after the treatment steps of the multibarrier system for advanced treatment.

The range of 17β estradiol equivalents (EEQs) observed in the effluent of the conventional treatment was in accordance with nine Austrian WWTPs (Braun et al., 2021) and can be considered as representative for WWTPs operated according to the EU requirements for eutrophication sensitive areas (Directive 91/271/EEC, 1991) applying biological nitrogen removal (tertiary treatment). Biological nitrogen removal can only be achieved at low loaded wastewater treatment plants with high solids retention time, a parameter known to correlate well with estrogenicity removal (Clara et al., 2005). This is partly reflected by data for ERα-CALUX determined in the effluent of 12 European WWTPs along the Danube River (Alygizakis et al., 2019). WWTP with secondary treatment (i.e., only BOD removal) are mostly characterized by higher EEQ compared to tertiary treatment.

According to NEREUS Deliverable 13 (2018) an average decrease of estrogenic activity by approx. one order of magnitude was observed during conventional treatment. The results of this part showed that an average decrease by another order of magnitude can be accomplished with advanced treatment. A significant EEQ decrease by ozonation was also observed during other full-scale studies (Dopp et al., 2021; Escher et al., 2009; Wolf et al., 2022). The reduction of the EEQ that occurred during ozonation can be attributed to the high reactivity of high-potency estrogens with ozone (Huber et al., 2005). This conclusion is permitted since estrogenicity is one of the endpoints with a high overlapping of the biological and the chemical BEQ; the latter are calculated by summing up the products of the chemical concentration and the corresponding relative effect potencies (Kase et al., 2018; Neale et al., 2015). Even though estrogenicity decline could not be quantified for GAC, a good EEQ removal potential can be assumed based on a review on toxicity removal by advanced wastewater treatment with ozonation and activated carbon treatment (Völker et al., 2019). According to the published data, the median reduction for AC treatment amounted to 75%.

In Figure 5.23 the EEQs of each sampling campaign along the treatment train were compared to the EBT of 0.1 ng EEQ/L for estrogenic activity which can be considered as fully established (Escher et al., 2018). While the EEQ in the effluent of the CAS plant always exceeded the EBT, ozonation resulted in a decrease below the EBT in most cases. Except for the two lowest nitrite compensated specific ozone doses < 0.3 g O_3/g DOC with EEQ-abatement ranging between 60 and 70%, an average decrease of more than 88% was achieved (n=12). In some cases, EEQs were reduced by ozonation below the limit of quantification (LOQ), which hindered the quantification of the removal by activated carbon. Only during one sampling campaign a further reduction by GAC could be determined. In literature a good EEQ removal potential was also reported for activated carbon (Völker et al., 2019).

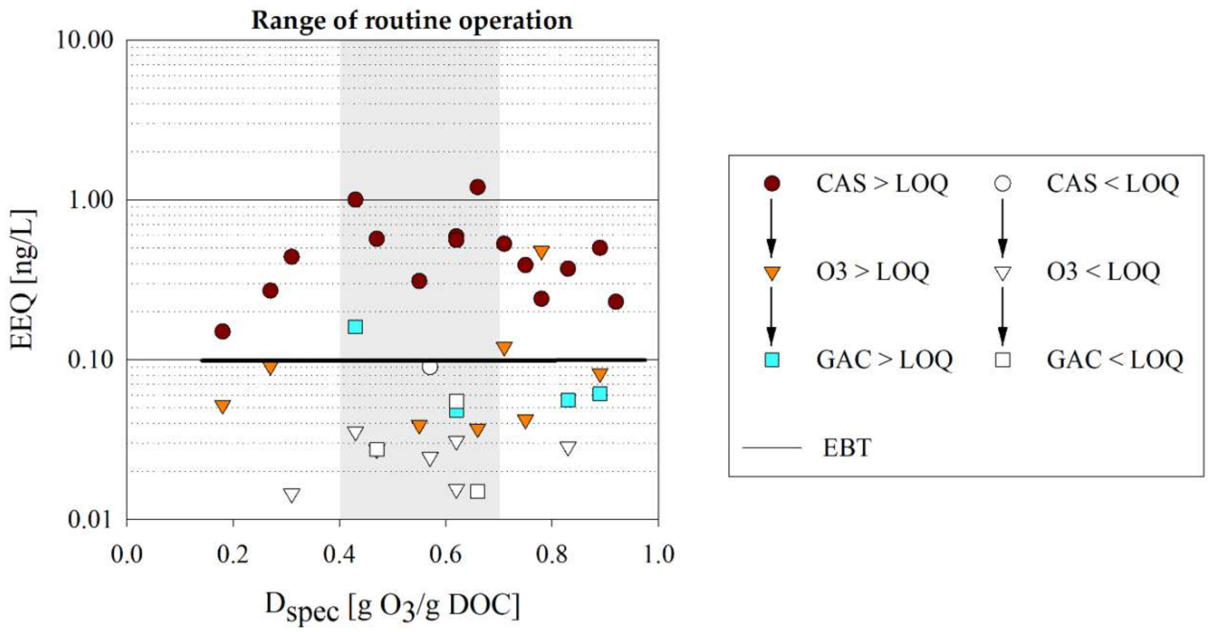


Figure 5.23. Change of estrogenicity as 17β estradiol equivalents along the treatment train at various nitrite compensated specific ozone doses during the sampling campaigns including the range of routine operation.

Results of Maletz et al. (2013) confirmed the necessity of advanced sewage treatment processes to minimize the estrogenic burden of highly charged sewages such as hospital wastewaters. The advantage of membrane bioreactors, as well as the suitability of ozone treatment, could be verified with regard to this specific effect. However, assessment of endocrine activities based on the sole assessment of receptor-based assays would have been insufficient to objectively characterize the overall endocrine potential of the analyzed samples. In fact, advanced treatment of effluents using ozonation appeared to result in greater endogenous estrogen production, potentially due to the generation of reactive metabolites by this treatment step. Therefore, the authors recommend a combinate ion of receptor-mediated assays such as the YES or ER-Calux Assay to enable objective assessment of the endocrine disrupting potential of complex samples. In addition, further expansion of endocrine bioassay batteries is recommended by the inclusion of androgen and thyroid hormone receptor assays are recommended (Maletz et al., 2013). Receptor-mediated estrogenicity is one of the most relevant MOA for endocrine disrupting compounds (Kase et al., 2018). Consequently, the significant reduction (median removal > 90%) can be considered a substantial benefit of the multibarrier system.

Studies on the removal of endocrine effects during advanced treatment put more focus on agonistic activity, even though pharmaceuticals like diclofenac belong to the group of hormone receptor antagonists (Völker et al., 2019). While the difference was less pronounced for the androgenic receptor (eleven vs. nine studies), 22 studies were done on estrogenicity and seven on anti-estrogenicity. Three of them reported tamoxifen equivalent concentrations < LOQ. Similar results were obtained in the presented study with two samplings covering anti-estrogenicity. Anti-estrogenicity was only measured once in the effluent of the conventional treatment and decreased to < LOQ after ozonation. This observed decline contradicts four studies reporting an increase after ozonation, which appeared to correlate with an increasing ozone dose (Völker et al., 2019). Contrary to this, an unclear elimination pattern was found on a full-scale ozonation plant, i.e., independent of the ozone dose, formation and elimination were observed during six monitoring campaigns (Wolf et al., 2022).

Anti-androgenicity as another specific endpoint for hormone receptor-mediated activity was below LOQ in ten out of sixteen samples even before advanced treatment (Figure 5.24). Even though ozonation resulted in a decrease below LOQ for the remaining six samples, the median does not reflect a further removal, which is a result of the sample specific LOQs, partly being lower in CAS than in O_3. Considering the single BEQs during the long-term monitoring depicted in Figure 5.25, however, the further removal potential is demonstrated. None of the effluent samples of both advanced treatment technologies exceeded the EBT of 14 µg Flutamide-EQ/L. According to Völker et al. (2019) the reduction potential was given for both ozonation (81.5%) and activated carbon (62.4%).

Based on the low activity even before advanced treatment the BEQs were in the lower range of effluents of other conventional biological WWTPs in Austria (unpublished data) and in the Danube River Basin (Alygizakis et al., 2019).

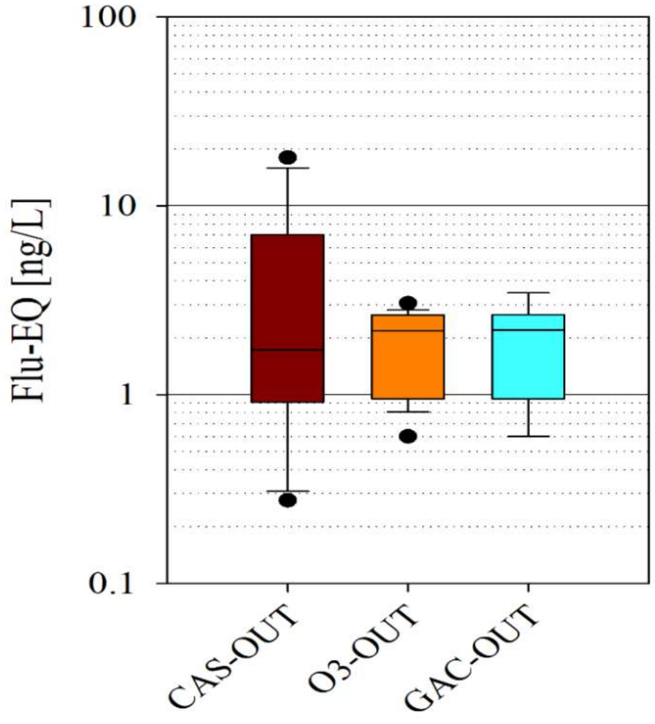


Figure 5.24 Boxplots of anti-androgenicity as flutamide equivalents after the treatment steps of the multibarrier system for advanced treatment.

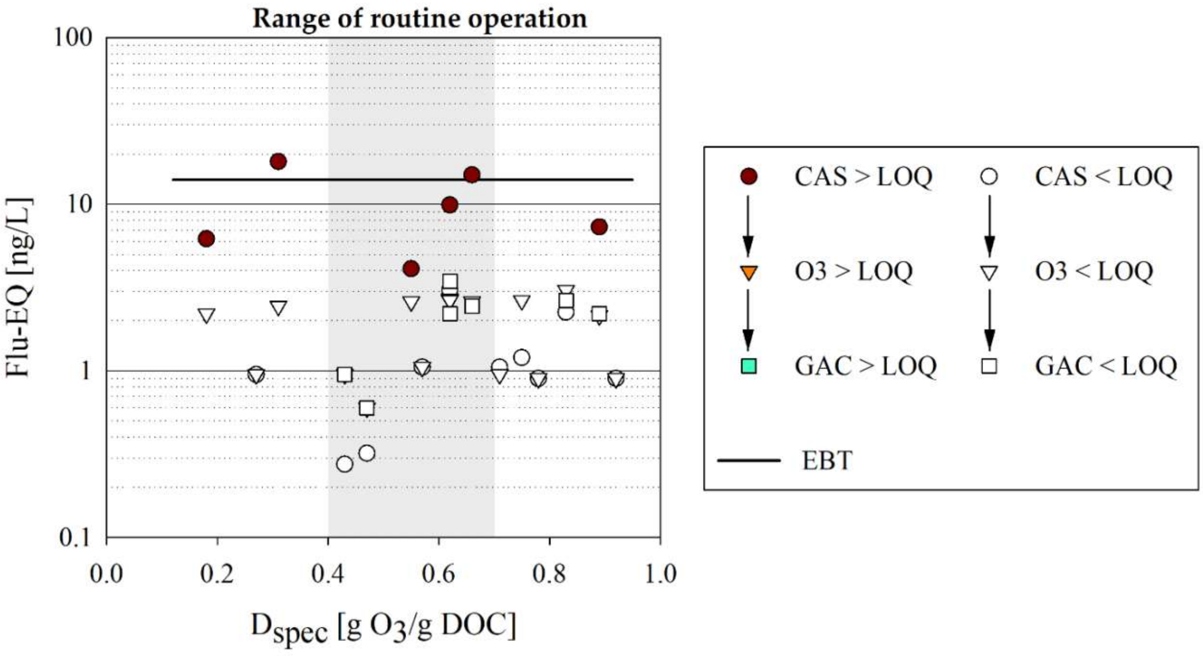


Figure 5.25. Change of anti-androgenicity as flutamide equivalents along the treatment train at various nitrite compensated specific ozone doses during the sampling campaigns including the range of routine operation.

The anti-androgenicity was in the lower range measured for effluents of other conventional biological WWTPs in Austria (Braun et al., 2021) and in the Danube River Basin (Alygizakis et al., 2019). Despite the calculation with ½ LOQ due to 100% non-detects in the effluent of the advanced treatment stages, a clear removal pattern for anti-androgenicity in ozonation and the multibarrier system was detected during the monitoring campaign (69 and 77%, respectively). The removal was in line with published data for ozonation (81.5%) and activated carbon (62.4%) (Völker et al., 2019). In contrast, a current full-scale study with ozonation did not identify a clear removal pattern (Wolf et al., 2022).

Stalter et al. (2011) investigated the effects of O_3 and AC on endocrine activities (estrogenicity, anti-estrogenicity, androgenicity, anti-androgenicity) with bioassays. Wastewater was collected after biodegradation (activated sludge treatment) following the secondary clarifier (SC) and after subsequent advanced treatments (O_3 and AC). Conventional treatment reduced estrogenicity, androgenicity activity by 78-99% compared to the untreated influent wastewater. Anti-androgenicity and anti-estrogenicity were not detectable in the influent but appeared in SC, possibly due to the more effective removal of respective agonists during conventional treatment. EDCs after SC ranged from 2.0 to 2.8 ng E-EQ/L, from 4 to 22 µg OHT-EQ/L, from 1.9 to 2.0 ng T-EQ/L, from 302 to 614 µg F-EQ/L. In particular, estrogenicity and anti-androgenicity occurred in environmentally relevant concentrations. Combination of O_3 and AC further reduced EDCs effectively (estrogenicity: 77 - 99%, anti-androgenicity: 63 - 96%) (Stalter et al., 2011).

The study of Reungoat et al. (2010) shows EDCs removed about 90 to 95% after ozonation. Besides, AC filtration was able to efficiently adsorb residual estrogenic compounds and further reduced the EEQ by another 95% to levels below the detection limit of 0.02 ng E-EQ/L and the final effluent concentration was below the quantification limit of 0.06 ng E-EQ/L. The overall treatment efficiency for the removal of estrogenic activity was greater than 99%.

5.3.3. Toxic PAHs (PAH CALUX®) and Xenobiotic sensing (PXR CALUX®)

The PAH-CALUX® belongs to the specific toxicity endpoints which induces xenobiotic metabolism. This endpoint is characterized by a high frequency of occurrence in municipal wastewater (Völker et al., 2019). Since polycyclic aromatic hydrocarbons (such as the here used standard reference compound Benzo[a]prene) also act as AhR ligands, the PAH-CALUX® can be applied to quantify the AhR receptor activation induction. Positive signals for PAH activity were detected in all samples of 12 WWTPs effluents investigated by Alygizakis et al. (2019) who identified PAH activity as one of the most frequently detected endpoints, together with PXR activity. In the current long-term monitoring, the ozonation resulted in a decrease of the Benzo[a]pyrene equivalents (Figure 5.26). Despite the decline of the signals, Figure 5.27 demonstrates the continuous exceedance of the EBT even after advanced treatment and a median removal of 58 % was quantified (n=8), which is in a similar order of magnitude as reported by Völker et al. (2019), who calculated a removal percentage for both ozonation and activated carbon between 79 and 84%.

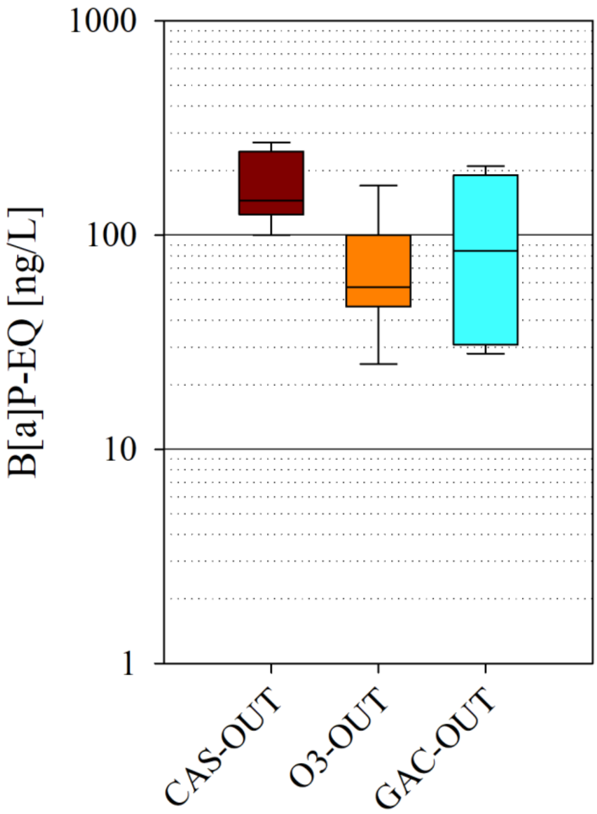


Figure 5.26. Boxplots of toxic PAH-like compounds in benzo[a]pyrene equivalents after the treatment steps of the multibarrier system for advanced treatment.

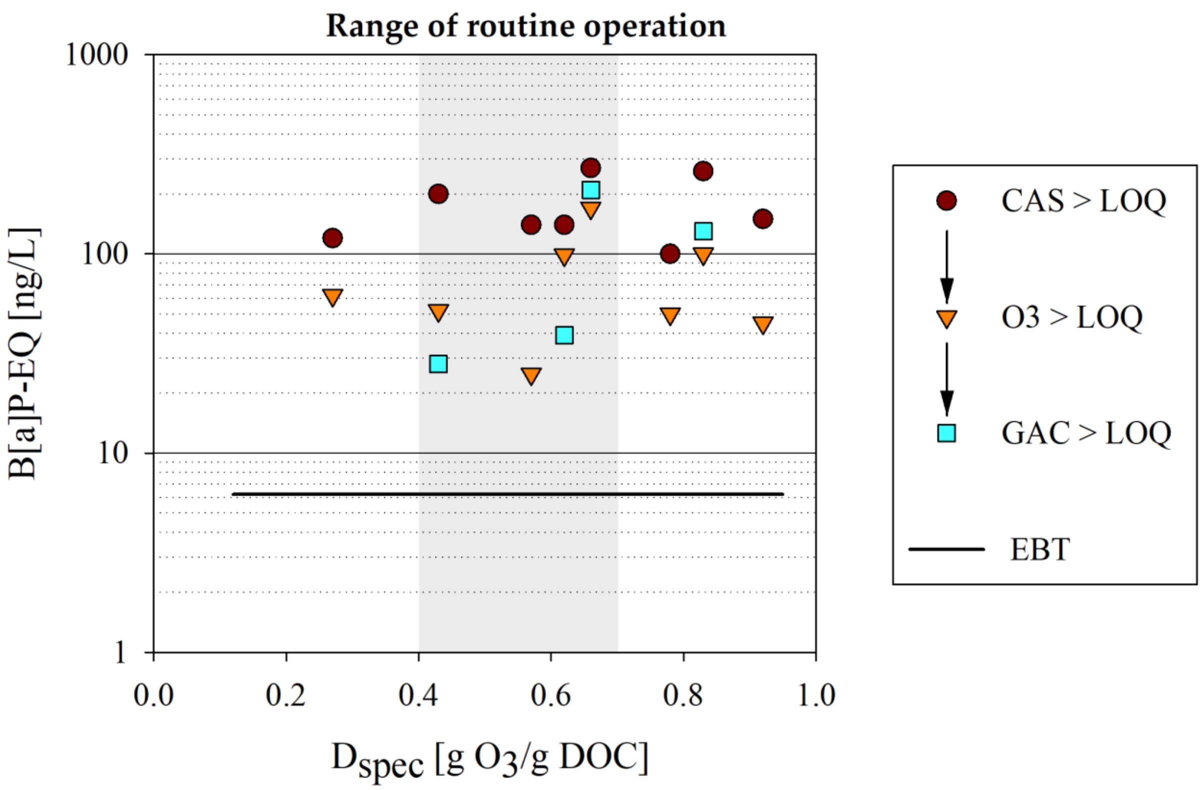


Figure 5.27. Change of toxic PAH-like compounds in benzo[a]pyrene equivalents along the treatment train at various nitrite compensated specific ozone doses during the sampling campaigns including the range of routine operation.

The PAH-CALUX belongs to the specific toxicity endpoints which induce xenobiotic metabolism. This endpoint is characterized by a high frequency of occurrence in municipal wastewater (Völker et al., 2019). Positive signals for PAH activity were detected in all WWTP effluents investigated in Austria (n = 9) and in the Danube River Basin (n = 12) (Alygizakis et al., 2019; Braun et al., 2021). The BEQ in the present paper were in a similar concentration range, thus, representative for urban WWTPs. The removal efficiency for B[a]P-EQ of approx. 60% during advanced treatment was slightly lower than published values of 79 and 84% for ozonation and activated carbon, respectively (Völker et al., 2019). After all, the multibarrier system could not reduce the activity below the discussed EBT of 6.2 ng B[a]P-EQ/L.

In addition to the PAH Calux, the PXR Calux is another bioassay targeting the induction of xenobiotic metabolism. Since the pregnane X receptor is activated by different types of chemically nonrelated compounds, comprising environmental pollutants and pharmaceuticals, this bioassay can be applied for xenobiotic sensing (Lemaire et al., 2006)

Results on the activation of the pregnane X receptor (PXR) as one of the endpoints inducing xenobiotic responses are depicted in Figure 5.28 (n = 3). As shown in Figure 5.29, BEQs for one of the three analyzed campaigns were below the LOQ for all three sampling sites. The remaining two campaigns delivered a 48-66% removal during ozonation, for the GAC filter, however, contradictory results were obtained. While an increase of the PXR activity was observed for the first sampling with approx. 1,000 bed volumes (BV) treated, a further removal after ozonation occurred in a sampling campaign after one year (33,100 bed volumes). Reasons for these divergent results are not clear; on the one hand, the adsorption capacity of the activated carbon is very high at 1,000 BV, on the other hand, the biological activation of the filter can be assumed to be still in the start-up phase. Both processes (adsorption and biological degradation) occur in parallel, with a share depending on the treated wastewater and the BV. Usually, it is not possible to differentiate between the two processes in a GAC filter (Bourgin et al., 2018). Völker et al. (2019) reported a reduction by a combined ozonation – GAC treatment train with a median removal of ≥78 %.

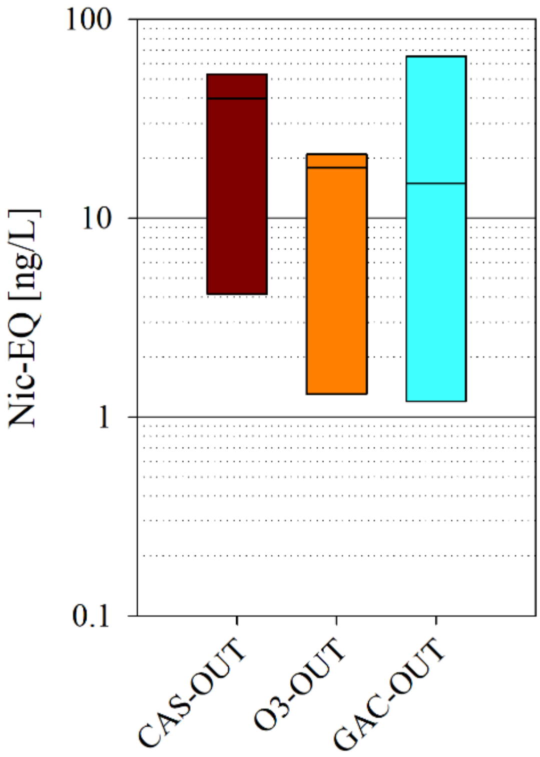


Figure 5.28. Boxplots of xenobiotic sensing by PXR CALUX® in nicardipine equivalents after the treatment steps of the multibarrier system for advanced treatment

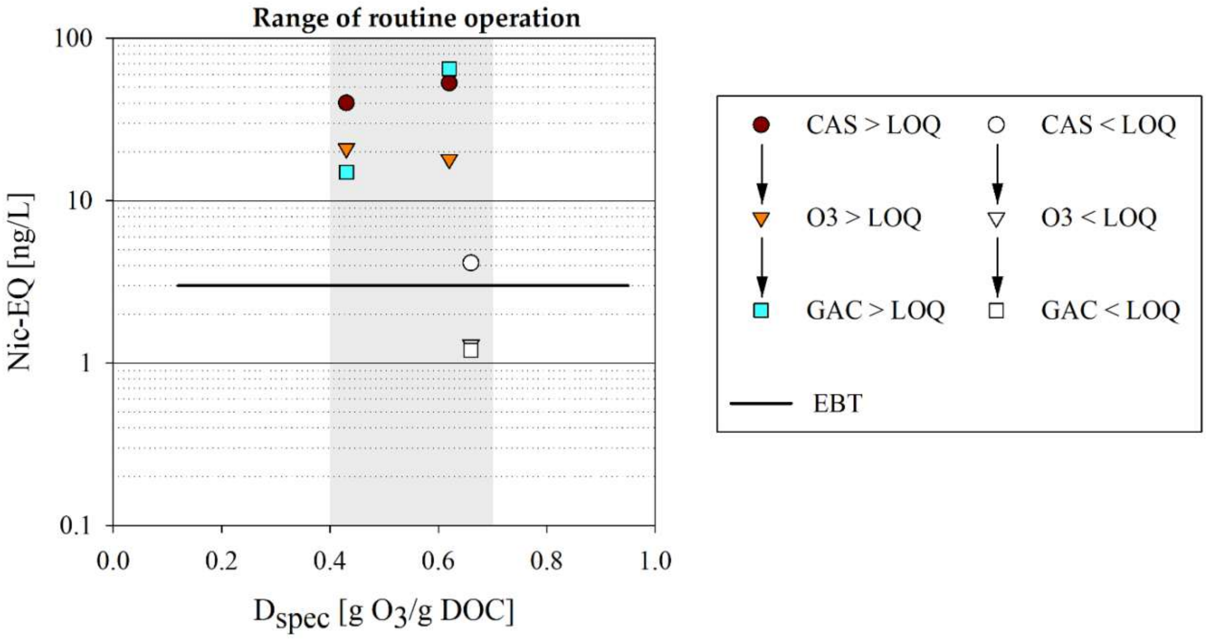


Figure 5.29. Change of xenobiotic sensing by PXR CALUX® in nicardipine equivalents along the treatment train at various nitrite compensated specific ozone doses during the sampling campaigns including the range of routine operation.

Neale et al. (2015) considers bioassays targeting xenobiotic metabolism as well as repair and defence mechanisms as sensitive tools to detect the occurrence of CECs since effects can often be identified at concentrations lower than those resulting in cell death or damage as confirmed by literature (Escher et al., 2012) and the present results. In addition Escher et al. (2018) and Völker et al. (2019) state that the activation of metabolism as a toxicokinetic process cannot be considered an adverse effect per se, but rather an indication for the presence of bioactive chemicals. Thus, wastewater samples can be considered subject to the induction of specific endpoints like the arylhydrocarbon receptor (AhR) targeted by the PAH CALUX® and the pregnane X receptor (PXR) as observed in the long-term monitoring without necessarily being linked to an adverse outcome especially when considering the high level of treatment in the multibarrier system. Also, according to van der Oost et al. (2017) a slight exceedance of EBTs in one or two bioassays is not inherently linked to an increased ecological risk. After all, it has to be considered that not only WWTP discharge as a point source, but also diffuse sources, e.g. from agriculture, show effects (Neale et al., 2017b).

5.3.4. Oxidative stress (Nrf2 CALUX®)

The reactive toxicity endpoint for oxidative stress revealed results above LOQ in all tested samples before and after advanced treatment (Figure 5.30). Despite a reduction during advanced treatment, the samples still had the potency to trigger oxidative stress response mechanisms and the signal was never reduced below the EBT (Figure 5.31). As to conventionally treated wastewater, Alygizakis et al. (2019) identified oxidative stress (Nrf2 CALUX®) in 10 out of 12 conventional WWTP effluent samples and the numbers were in the same order of magnitude as in the presented long-term study, confirming the consistent exceedance of EBTs. For the ozonation process a mean removal of more than 52% was observed. Additional removal in the GAC step was observed during three of five sampling campaign, while the other campaign revealed in increase in the response and no change to the ozonation, respectively. In comparison, Völker et al. (2019) reported a 62% removal by ozonation and a strong variation in removal by a combinative treatment of O_3 and GAC ranging between >25 and >95% (average of 44%).

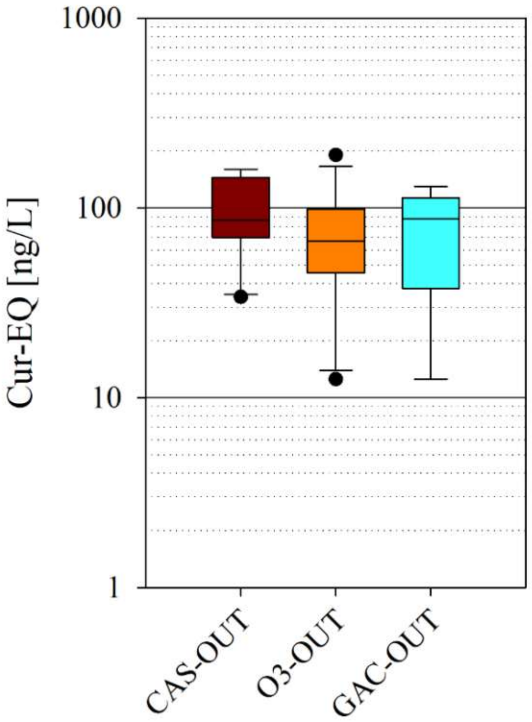


Figure 5.30. Boxplots of oxidative stress in Nrf2 CALUX® in curcumin equivalents after the treatment steps of the multibarrier system for advanced treatment.

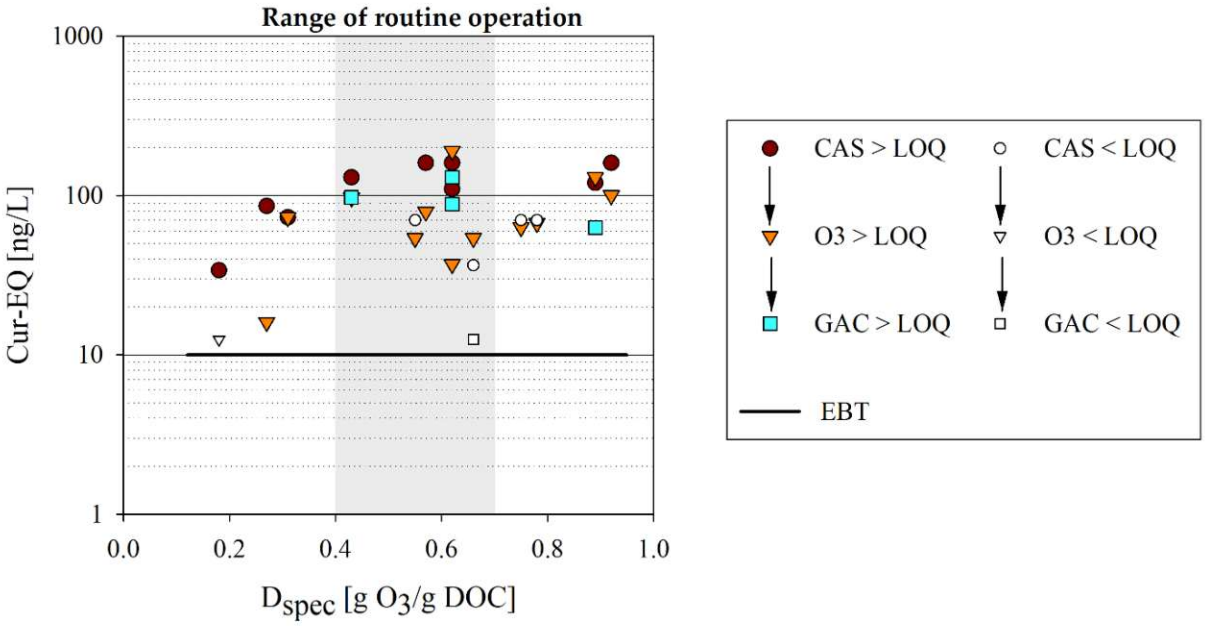


Figure 5.31. Change of oxidative stress in Nrf2 CALUX® in curcumin equivalents along the treatment train at various nitrite compensated specific ozone doses during the sampling campaigns including the range of routine operation.

In the monitoring studies in Austria and the Danube river basin (Alygizakis et al., 2019; Braun et al., 2021), oxidative stress (Nrf2 CALUX®) was identified in 18 out of 21 conventional WWTP effluent samples. The numbers were in the same order of magnitude as in the presented long-term study, confirming the consistent exceedance of EBTs. For the ozonation process, a median removal of 46% was determined. Additional reduction in the GAC step was observed during three of five sampling campaigns. In contrast, the other campaigns revealed an increase in response and no change to the ozonation. Literature results showed 63% removal for an ozonation plant and > 25 to > 95% (median of 44%) for a combined O_3 and GAC treatment (Völker et al., 2019).

Oxidative stress represents a rather general cellular stress response that can often be detected before cytotoxic effects (König et al., 2017; Neale et al., 2020). This is consistent with the present results, with cytotoxic effects more often < LOQ, even before advanced treatment. Like biological treatment, ozonation processes lead to transformation rather than mineralization, which causes the weaker decline observed for oxidative stress response compared to other investigated endpoints, such as estrogenicity or anti-androgenicity.

5.3.5. Genotoxic activity (p53 CALUX®)

The results of the p53 CALUX® assay were below the limit of quantification both with (n=5) and without the addition of the metabolic activation mix S9 (n=3). These results were confirmed by Ames tests conducted in parallel with various bacterial strains (Misik et al., 2020). Irrespective of S9 addition the standard bacterial strains TA98 and TA100 consistently gave negative results in three sampling campaigns.

6. Summary and conclusion

This thesis aims to expand the existing knowledge to support and extend practical applications of the ozonation process in the field of urban wastewater treatment. As a base, the study focused on municipal wastewater treatment plants in Austria, characterized by high treatment efficiency, including biological nutrient removal with complete nitrification and denitrification.

The experiments presented in this thesis were carried out in three main phases. The first two phases were carried out on a laboratory scale, while the third phase was carried out on a pilot scale.

In the first stage of this thesis, the focus has been to investigate the degradation efficiency of micropollutants at the different specific ozone doses (0; 0.2; 0.4; 0.6; 0.8; and 1.0 g O_3/g DOC), with a special focus on considering the formation of bromate as oxidation byproduct. Indicator substances with different reactivity with ozone were applied to monitor the success of ozonation and evaluate the experimental setup. Diclofenac and carbamazepine are the main indicators of the highly reactive group, together with sulfamethoxazole. The representatives of the moderately reactive group are benzotriazole, acesulfame, bezafibrate and metoprolol. Micropollutants categorized as low ozone reactive compounds were ibuprofen and diatrizoic acid dihydrate. Results are compared with reference data from the literature to provide a follow-up assessment of applicability. For the investigated ozone doses, the micropollutant abatement for highly reactive compounds ranged from 73 – 99%. The abatement of indicator substances with moderate reactivity (40 – 99%) showed the typical pattern of increasing removal with increasing D_{spec}, mostly due to the contribution of the indirect reaction pathway and increasing $OH^•$ formation with increasing D_{spec}. For the representatives of the low reactive compounds a similar pattern was observed and an abatement of 41 – 97% was achieved. The predicted removal of micropollutants using ozone and $OH^•$ exposure and the corresponding reaction rate constants was higher than observed for moderately reactive compounds due to mechanistic reasons.

Bromate formation ranged between 0.65 ± 0.28 and 11.22 ± 9.85 µg/L. The guideline value for drinking water (10 µg/L) was only exceeded at > 0.88 ± 0.05 g O_3/g DOC, which is higher than the usually applied doses for micropollutant removal (0.6 - 0.7 g O_3/g DOC). At D_{spec} below 0.8 g O_3/g DOC, the limit was not exceeded despite bromide concentrations of approx. 200 µg/L. Thus, the ozone dose range recommended for micropollutant abatement (0.4 – 0.7 g O_3/g DOC) did not only prove successful with regard to micropollutants, but also appropriate regarding bromate formation at the bromide concentrations investigated.

The second stage of this thesis focused on the effect of ozonation on BOD_5 and other relevant conventional organic sum parameters (BOD_5, COD, DOC, UV_{254}) in order to assess the change in biodegradability of substances previously recalcitrant to biological degradation. Enhanced biodegradability of wastewater by ozonation can be evaluated by an increase in BOD_5 for carbon removal, additional indications can be obtained from parameters such as the BDOC or a change in the BOD_5/COD ratio.

In addition to investigating the change in organic sum parameters by ozonation, there was a special focus on the further changes after exposure to biodegradation during BOD_5 measurement. BOD_5 measurement is a standardized biochemical procedure (in a controlled environment) that can be considered a simulation of biological post treatment. While the changes in traditional organic sum parameters have been reported before, the higher degradation potential for a subsequent biological process due to the increase in biodegradability has not been systematically investigated.

The results showed that there was a dose-dependent BOD_5 increase that varied among the investigated WWTPs (21 –193%). The DOC did not substantially decrease after ozonation, which is consistent with the low reported degree of mineralization, while partial oxidation led to a quantifiable decrease in COD from 7 to 17%. Delta UV_{254} (34 – 57%) and the decline in SUVA (33 – 54%) after ozonation indicated structural changes by oxidation, clearly correlating with D_{spec}. In contrast, for COD and DOC (BDOC), a clear dose-response pattern was observed only after exposure to BOD_5 measurement.

The rise in BDOC from 4 to a maximum of 22% is another indication for the improved biodegradability. This was further supported by the change in the BOD_5/COD ratio from 0.08 to a maximum of 0.21. Summarized, obtained results showed a dose-dependent increase in biological activity after ozonation, which was linked to the enhanced biodegradability of substances that are recalcitrant to biodegradation in conventional activated sludge treatment.

These considerations can be applied to any kind of technical post treatment step involving biological processes, such as sand filtration, BAC filtration, soil aquifer treatment and even processes that promote reuse in agricultural irrigation and receiving waters in general. Further degradation of organic compounds in biological post treatment steps is linked to biomass growth affecting backwashing intervals of filtration steps. Without biological post treatment, the additional degradation of organic matter would be shifted to the downstream environments as soil or receiving water bodies. In the case of technical processes, the further reduction in biodegradable organic carbon emissions is another step towards sustainable water resource management in addition to micropollutant abatement. The enhanced biodegradability after ozonation has to be considered, especially for low-SRT WWTPs that – compared to high-SRT WWTPs – are characterized by higher effluent organic matter (EfOM) concentrations. Higher levels of EfOM result in increased competition with micropollutant abatement processes, namely, oxidation-reactive functional moieties in ozonation or competitive adsorption during activated carbon treatment. Additionally, the rise in BOD due to ozonation can potentially result in exceeding legal effluent standards in wastewater plants. This emphasizes the role of a high treatment standard in conventional biological treatment (i.e., low-SRT plants) as a foundation for advanced treatment steps and, independent of toxicology reduction, the importance of biological post treatment after ozonation.

The overall goal of the third phase was to monitor the long-term toxicity of a multi-barrier advanced wastewater treatment under real-world conditions. The toxicity was assessed by applying a MOA-based in vitro bioassay battery to target the following relevant toxicological endpoints along the toxicity pathway: cytotoxicity, estrogenicity, anti-estrogenicity, anti-androgenicity, toxic PAH, xenobiotic sensing, oxidative stress, and genotoxicity. Toxicological long-term monitoring delivered a valid basis for assessing a multibarrier system's applicability and performance for an advanced treatment.

The combination of the two approaches applied in the present study, namely the quantification of toxicity reduction (expressed as bioanalytical equivalent concentration, BEQ) and comparison of the BEQ with currently discussed EBTs represented a solid means of assessing the final effluent quality of the multibarrier system combining ozonation and granular activated carbon treatment.

Despite natural variations in the wastewater characteristics and other factors influencing conventional biological treatment efficiency over the 13-month monitoring, the overall removal pattern for various MOAs revealed a decrease in BEQs. Even though the positive effect of ozonation resulting in signals below LOQ for some MOAs impeded the toxicity assessment for GAC treatment, a combination of O_3 and GAC is strongly recommended for advanced treatment to follow the multibarrier approach.

Since the presented toxicological results did not reveal significant differences within the ozone dose range of 0.4 - 0.7 g O_3/g DOC, which is recommended for micropollutant abatement, it can be concluded that potential toxicological requirements should not be limiting for the operation of multibarrier systems for advanced treatment in case of ozonation and activated carbon filters. Even though measures like implementing advanced treatment at WWTPs do not result in a complete removal and mitigation in receiving waters, advanced treatment represents a relevant step in reducing the toxicological burden for the aquatic environment.

Effect-based bioassays with their linked EBTs should be used as treatment goals and quality criteria for design, operation, and advanced wastewater treatment evaluation. Thus, long-term monitoring confirmed the positive effects of multibarrier advanced wastewater treatment systems, which are usually only evaluated by micropollutant removal based on chemical analysis. It could be demonstrated that advanced WWTPs designed to eliminate micropollutants are suitable for significantly reducing the toxicity within the framework of experimental studies under real-world conditions.

Based on the results obtained in this thesis, it can be concluded that ozone technology is an advanced technology in treating wastewater and restoring water quality in the future.

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Appendix

Appendix 1

Determination of organic micropollutants in wastewater by fully automated online solid phase extraction coupled with LC-MS/MS analysis

In this study different compounds (Table 1) including pharmaceuticals, hormones, corrosion inhibitors and sweeteners were analyzed. Analytical methods for automated online solid phase extraction coupled with LC-MS/MS analysis were applied.

Experimental

Reagents

Analytical standard, in their original form, either as a solid, fluid or in liquid used for calibration can be found in Table 1.

Table 1. Analytical standard used for Determination of organic micropollutants in wastewater by online SPE coupled with LCMSMS analysis

Compound	CAS	Company	Formula	Molar mass
Acesulfame K	55589-62-3	Supelco	$C_4H_4KNO_4S$	201.2
Bezafifbrate	41859-67-0	Sigma-Aldrich	$C_{19}H_{20}ClNO_4$	361.8
Benzatriazole	95-14-7	Sigma-Aldrich	$C_6H_5N_3$	119.1
Carbamazepine	298-46-4	Sigma-Aldrich	$C_{15}H_{12}N_2O$	236.3
Diatrizoic acid dihydrat	50978-11-5	Sigma-Aldrich	$C_{11}H_9I_3N_2O_4$	649.9
Diclofenac	15307-79-6	Sigma-Aldrich	$C_{14}H_{10}Cl_2NO_2Na$	318.1
Ibuprofen	31121-93-4	Sigma-Aldrich	$C_{13}H_{17}O_2Na$	228.3
Metoprolol	37350-58-6	RTC, Sigma-Aldrich	$C_{15}H_{25}NO_3\cdot C_4H_6O_6$	267.4
Sulfametxoazole	723-46-6	Sigma-Aldrich	$C_{10}H_{11}N_3O_3S$	253

HPLC grade organic solvents: Ethanol (CAS 64-17-5), Acetonitrile (CAS 75-05-8) and Acetic acid (CAS 64-19-17) as well as mobile phase for online solid phase extraction and column chromatography were purchased from Sigma Aldrich.

Instrumental

The high-pressure liquid chromatograph (HPLC) used for the elution was an Agilent System consisting of two Binary pumps, a degasser to degas the eluents, CTC PAL autosampler with Peltier-Cooler and Rheodyne 2-position,6-port switching valve.

The MS/MS system consisted of a Hybrid triple quadrupole linear trap ion trap tandem mass spectrometer Q Trap 3200 from AB Sciex.

For automated online solid phase extraction (online SPE) a Phenomenex Strata X On-Line extraction cartridge (20 x 2.0 mm; 25µm) was used. The HPLC separation was done via analytical column Phenomenex Luna C-18 (150 x3.0 mm; 5µm) and Phenomenex C18-Security guard cartridges (40 x 3.0 mm). For quantitative analysis the MRM Analysis with electrospray ionization mode (MRM ESI) by 500°C and nitrogen collision gas was used.

Procedures

Sample preparation and HPLC

The wastewater samples were filtrated with VWR glass fiber filter diameter 45 mm and pore size 1µm. Analytical standard in ethanol concentration of 1mg/mL in ethanol were prepared.

Injecting volumes of 10 mL of Sample were used for the automated online solid phase extraction. HPLC separation with eluent 0,1 % acetic acid solution in deionized water (**A**) and 0,1 acetic acid in Acetonitrile solution (**B**) were performed in gradient mode. The program for online SPE and HPLC separation can be seen in Table 2.

Table 2. The gradient program for online SPE and HPLC separation

Time	**Online SPE**			**HPLC**		
	Flow	**Gradient**		**Flow**	**Gradient**	
min	mL/min	% A	% B	mL/min	% A	% B
0	1,0	100	0	0,8	100	0
5,6	1,0	100	0	0,8	100	0

0	1,0	100	0	0,8	80	20
5	1,0	100	0	0,8	90	10
8	0,5	0	100	0,8	90	10
8,2	1,0	0	100	0,8	0	100
8,5	1,0	0	100	0,8	60	40
17	1,0	0	100	0,8	60	40
19	1,0	100	0	0,8	5	95
25	1,0	100	0	0,8	80	20

LC-MS/MS

For quantitative analysis was used the MRM Analysis with electrospray ionization mode (MRM ESI) by 500°C and nitrogen collision gas (Table 3).

Table 3. Parameter MRM Analysis with electrospray ionization mode

Scan type	**MRM**	**MRM**
Polarity	Negative	Positive
Dwell (msec)	150	
Collision gas CAD	Medium	
Curtain gas CUR	25	
Ion source gas 1 GS1	50	
Ion source gas 2 GAS2	50	
Ion spray voltage IS	-4500	
Temperature TEM	500	
Entrance potential EP	-1	

The confirmatory and identifying mass and all other parameters of the MS/MS can be found in Table 4.

Table 4. Mass properties of all analyzed compounds by HPLC MS/MS

Compound	**Polarity**	**Q1 mass**	**Q3 mass**	**Identifying mass**			
		m/z	**m/z**	**m/z**	**DP**	**CE**	**CXP**
Ace K	negative	161.800	78.111/81.937	81.9	-35	-20	
BZF	negative	359.99	153.9/274.1	274.1	-25	-26	-2

BZT	positive	120.097	65.1/92.2	65.1	46	31	4
CBZ	positive	237.727	193.3/194.3	194.3	51	25	4
DTA	positive	614.713	233.1/361.0	361.0	51	25	6
DCF	negative	293.823	214.1/250.1	250.1	-20	-16	-2
IBP	negative	204.972	159.0/161.0	161.0	-20	-12	0
MTP	positive	267.810	74.0/77.1	77.1	41	75	4
SMX	positive	254.171	156.2/92.25	92.2	41	33	4

Results

Validation of the method

The chromatograms of Determination of organic micropollutants in wastewater by fully automated online solid phase extraction coupled with LCMSMS analysis are shown Figure 1 and Figure 2.

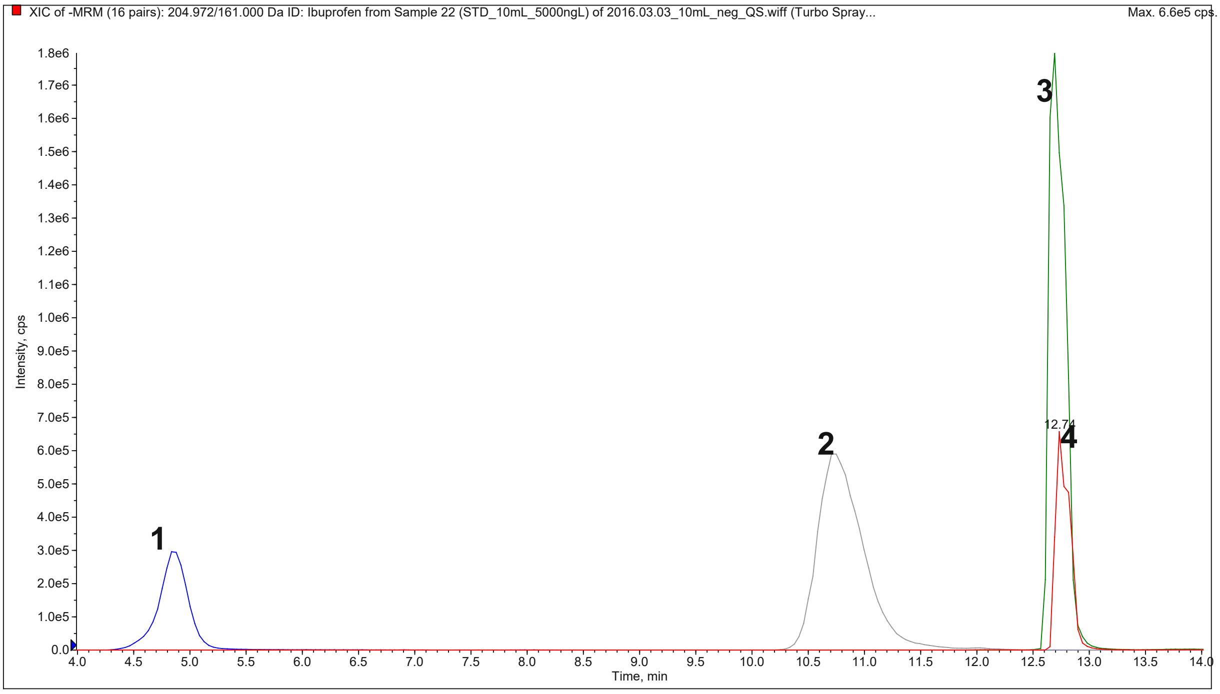

Figure 1. Chromatogram for detection of 5000 ng/L micropolution mixing standard solution; ESI (+) mode

Nr.	1	2	3	4
Compound	Acesulfame K	Bezafibrate	Diclofenac	Ibuprofen

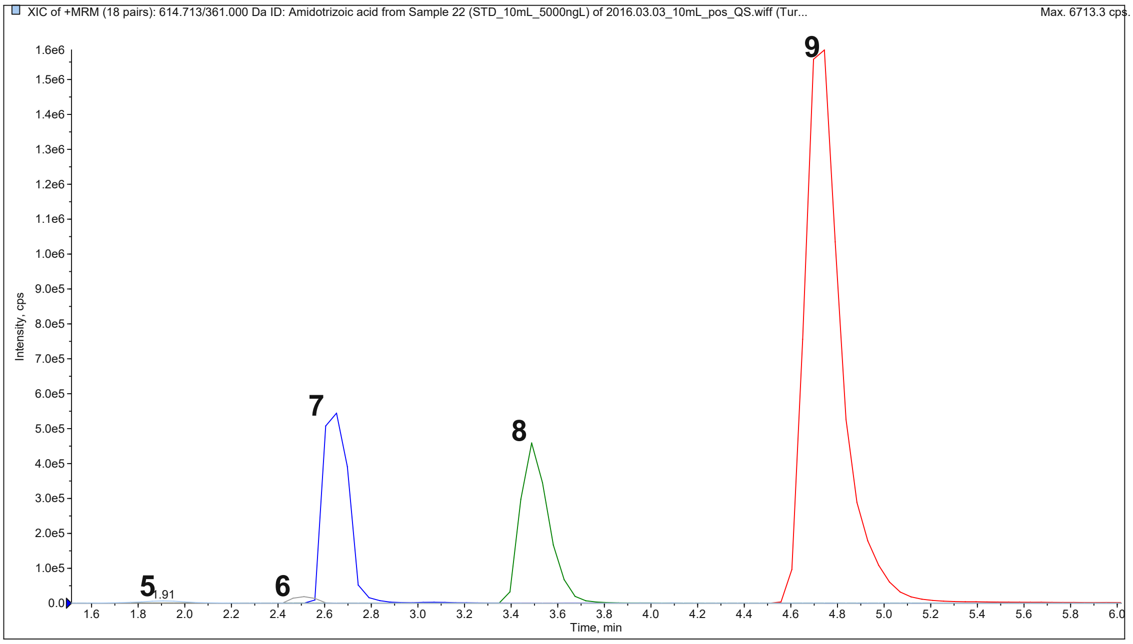

Figure 2. Chromatogram for detection of 5000 ng/L micropolution mixing standard solution; ESI (-) mode;

Nr.	5	6	7	8	9
Compound	Metoprolol	Diatrizoic acid	Benzotriazole	Sulfamethoxazole	Carbamazepine

Different mixing standard from 10 compounds in deionized water (concentrations of 10, 50, 500, 5000 ng/L) were used for extern calibration (Figure 3 and Figure 4).

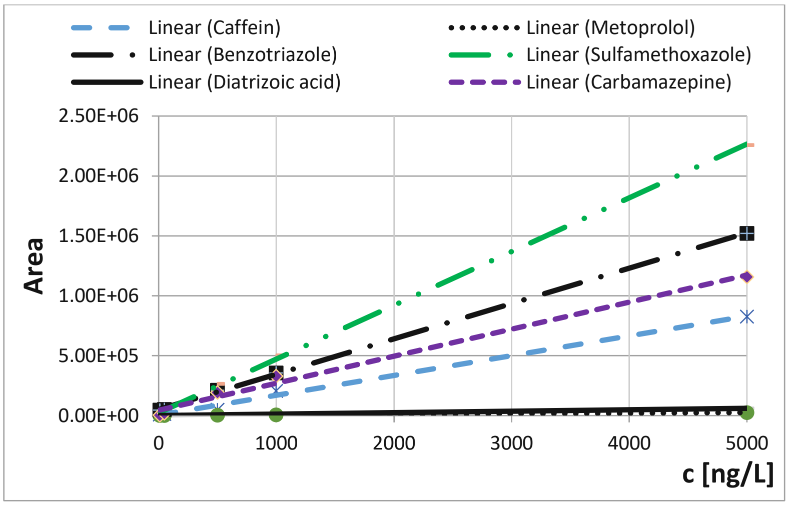

Figure 3. Linearity study results for different compounds, concentrations 10-5000 ng/L; ESI- Mode;

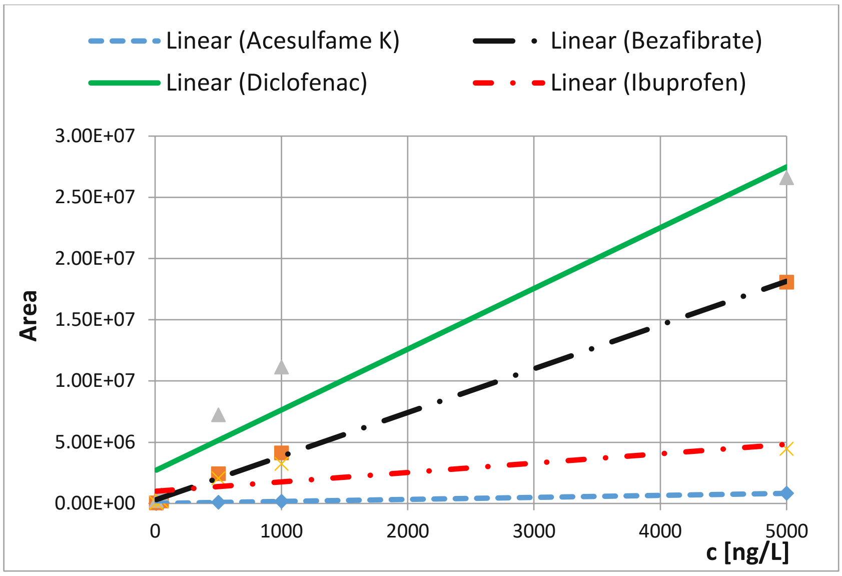

Figure 4. Linearity study results for different compounds, concentrations 10-5000 ng/L; ESI- Mode;

Using the LC-MS/MS chromatogram for Standard solution is possible direct to determinate Quantification and Detection limits LCMS analysis (Figure 5). These results have more practical than scientific relevance but in the chromatographic methods sometimes is using 3σ for LOD and 10σ for LOQ of signal to noise (S/N) is more accurate and significantly than scientific calculation (Kromidas, 2011).

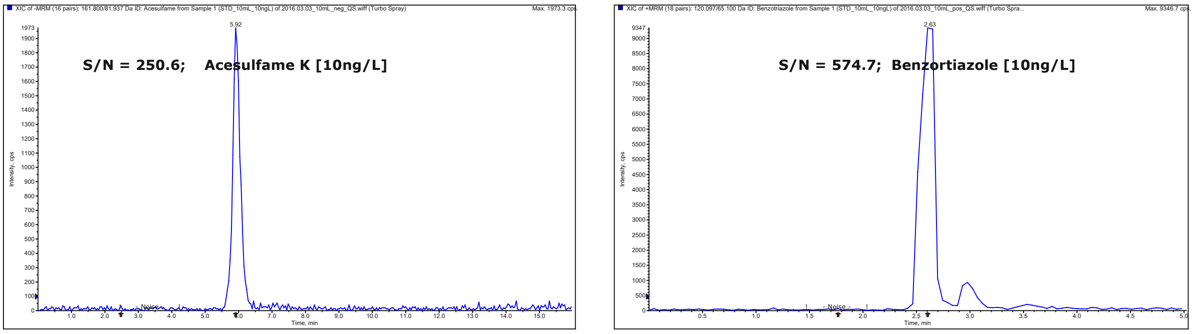

Figure 5. An example calculation of S/N for Acesulfame K and Benzotriazole, concentration 10 ng/L

The results of validation parameter (DIN 32645) for the developed analytical method can be seen in Table 5.

Table 5. The results of validation parameter for the developed analytical method for determination of organic micropollutants in wastewater

Compound	SD	R	DIN 32645		S/N	
			LOD	LOQ	10	LOD
	%		ng/L	ng/L	ng/L	ng/L
Acesulfame K		0.9995	9,4	18,7	14	2,10
Bezafibrate		0,9985	11,4	22,8	123	0,10
Benzotriazole		0.9996	16,2	32,5	287	0,10
Carbamazepine		0,9991	18,9	37,3	610	0,05
Diatrizoic acid		0,9992	16,6	32,8	22	1,36
Diclofenac		0,9383	9,1	18,1	1100	0,03
Ibuprofen		0,7822	9,2	18,5	126	0,24
Metoprolol		0.9994	26,3	53,6	7	4,29
Sulfamethoxazole		0,9993	15,9	31,9	284	0,11

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Appendix 2

Data of the lab-scale experiment 1

1. The number of samples in experiment

g O_3/g DOC	Samples (n)								
	ACE K	BZF	BZT	CBZ	DTA	DCF	IBP	MTP	SMX
0.23 ± 0.05	5 3*	5	5	5 4*	5 1*	5	5 4*	5	5
0.44 ± 0.07	5 4*	5	5	5 4*	5 2*	5	5 4*	5	5
0.66 ± 0.09	10 9*	10 9*	10	10 9*	10 2*	10	10 9*	10	10
0.88 ± 0.05	14 12*	14	14	14 13*	14 6*	14	14 13*	14 10*	14
1.09 ± 0.09	6 5*	6	6	6 5*	6 4*	6	6 5*	6	6

* The samples had the results after analysed.

2. The result of micropollutant abatement in %

Compounds	**Specific ozone doses (g O_3/g DOC)**				
	0.23 ± 0.05	**0.44 ± 0.07**	**0.67 ± 0.07**	**0.88 ± 0.05**	**1.09 ± 0.09**
Acesulfame K	8.22 ± 1.8	32.08 ± 6.99	73.62 ± 32.41	86.77 ± 22.62	80.72 ± 27.36
Bezafibrate	35.61 ± 11.58	59.35 ± 12.94	65.43 ± 28.7	96.21 ± 7.65	92.64 ± 9.78
Benzotriazole	26.04 ± 9.03	52.1 ± 9.6	76.72 ± 16.15	90.07 ± 5.42	93.54 ± 6.78
Carbamazepine	89.11 ± 17.11	99.74 ± 0.27	99.9 ± 0.17	99.58 ± 1.4	99.84 ± 0.16
Diatrizoic acid dihydrate	41.44 ± 0	74.59 ± 35.94	69.99 ± 28.35	91.75 ± 14.29	92.05 ± 13.77
Diclofenac	89.01 ± 14.5	99.61 ± 0.57	98.53 ± 1.53	99.43 ± 0.69	99.85 ± 0.28
Ibuprofen	63.71 ± 32.73	67.11 ± 25.44	90.55 ± 10.09	97.94 ± 5.97	97.85 ± 4.81
Metoprolol	39.89 ± 19.15	76.65 ± 17.86	87.87 ± 25.79	98.37 ± 3.11	99.27 ± 0.95
Sulfamethoxazole	73.27 ± 20.83	87.76 ± 8.48	95.31 ± 5.82	99.08 ± 1.81	99 ± 1.57

Appendix 3

Data of the lab-scale experiment 2

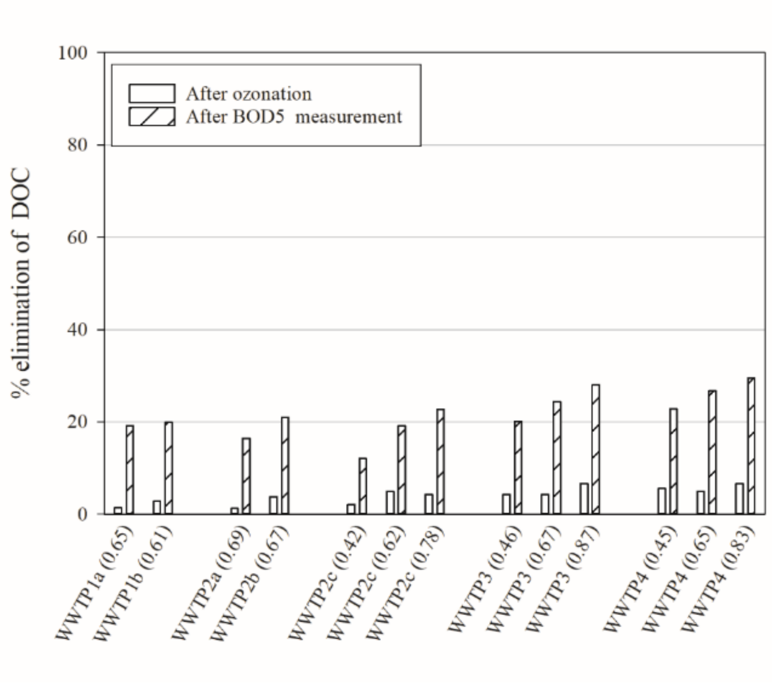

Figure 1. DOC elimination for all investigated D_{spec}

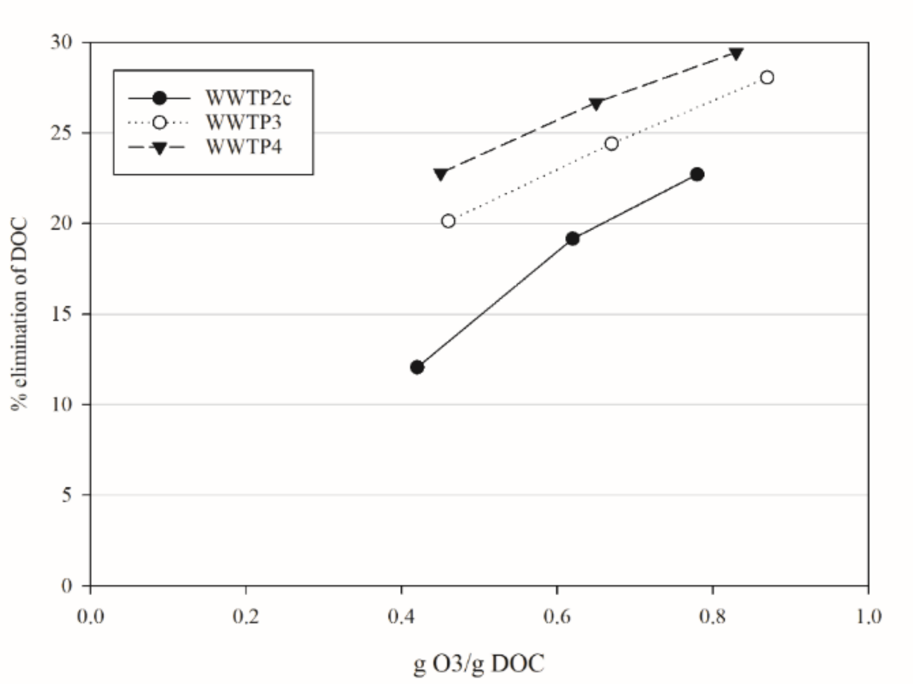

Figure 2. Dose-specific decrease of DOC in WWTP2c, WWTP3, and WWTP4 after ozonation

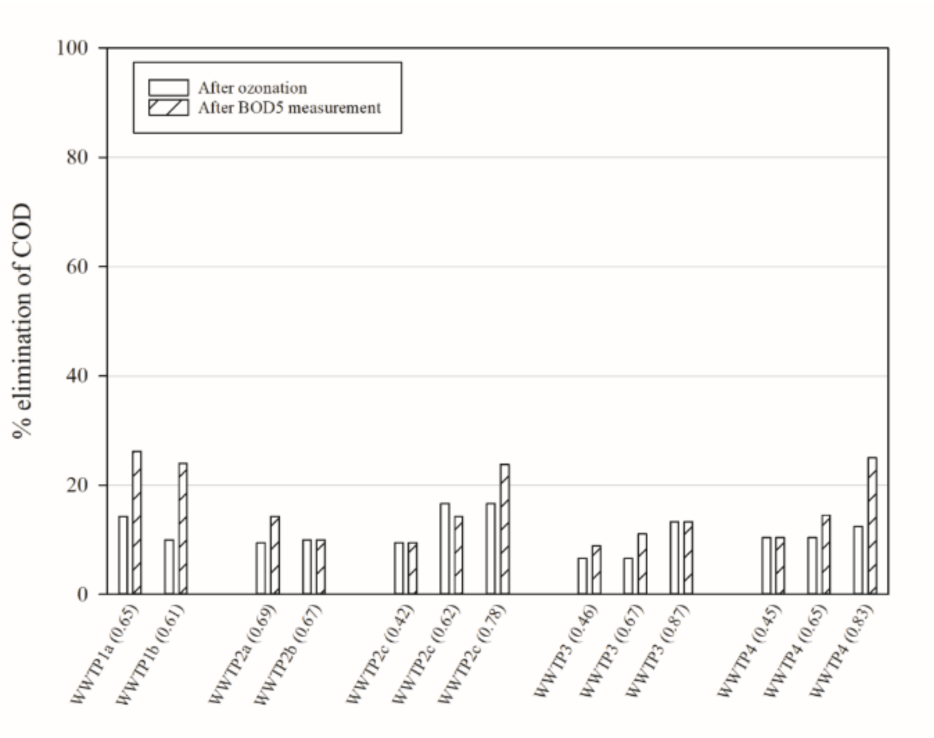

Figure 3. COD elimination for all investigated D_{spec}

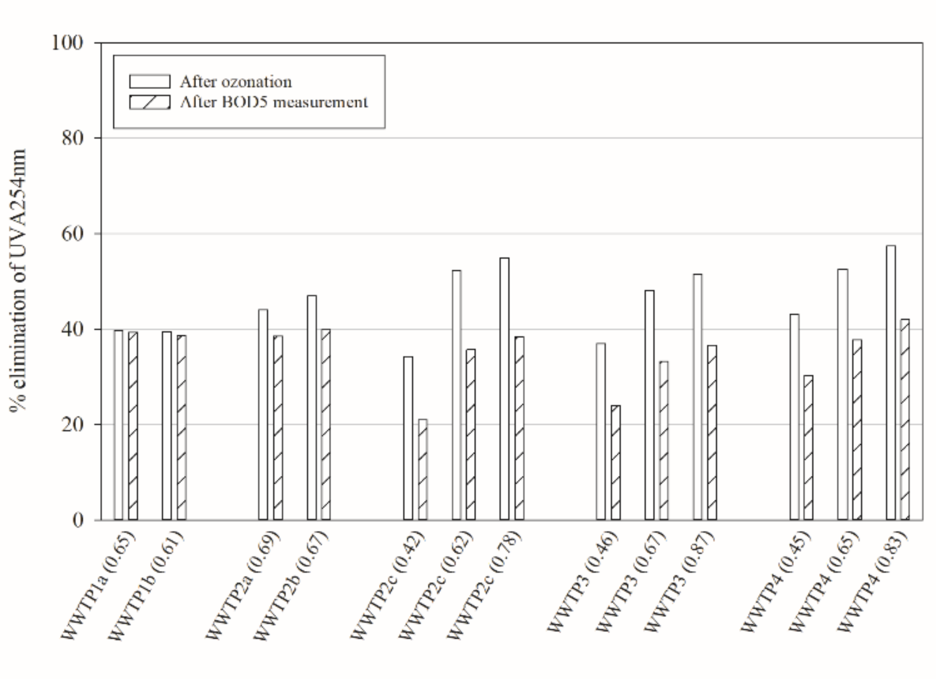

Figure 4. Delta UV_{254} for all investigated D_{spec}

Table 1. CBZ concentrations (ng/L) before and after ozonation and BOD_5 measurement

Name	**CBZ concentrations (ng/L)**				
	D_{spec} (g O_3/g DOC)	**WWTP effluent**	**WWTP effluent after BOD_5 measurement**	**Ozonated effluent**	**Ozonated effluent after BOD_5 measurement**
WWTP1a	0.65	212.69 ± 2.72	201.09 ± 3.26	5.66 ± 0.39	1.81 ± 1.66
WWTP1b	0.61	252.98 ± 5.09	244.32 ± 3.86	7.61 ± 0.83	5.49 ± 1.10
WWTP2a	0.69	110.85 ± 3.18	136.12 ± 1.60	< LOQ	< LOQ
WWTP2b	0.67	120.83 ± 1.49	136.00 ± 3.88	< LOQ	< LOQ
WWTP2c	0.43	98.58 ± 3.77	103.12 ± 3.00	< LOQ	< LOQ
	0.62			< LOQ	< LOQ
	0.78			< LOQ	< LOQ
WWTP3	0.46	104.19 ± 2.00	106.45 ± 2.46	12.62 ± 13.46	< LOQ
	0.67			5.99 ± 8.27	< LOQ
	0.87			2.59 ± 4.26	< LOQ
WWTP4	0.45	200.52 ± 2.12	199.40 ± 2.65	< LOQ	< LOQ
	0.65			< LOQ	< LOQ
	0.83			< LOQ	< LOQ

Table 21. BZT concentrations (ng/L) before and after ozonation and BOD_5 measurement

Name	BZT concentrations (ng/L)				
	D_{spec} ($g\ O_3/g$ DOC)	WWTP effluent	WWTP effluent after BOD_5 measurement	Ozonated effluent	Ozonated effluent after BOD_5 measurement
WWTP1a	0.65	1623.97 ± 10.87	1552.54 ± 5.14	872.52 ± 41.51	816.30 ± 58.79
WWTP1b	0.61	2099.69 ± 27.66	2043.22 ± 38.72	1231.55 ± 96.01	1172.16 ± 68.70
WWTP2a	0.69	1750.38 ± 35.28	1715.30 ± 10.00	451.97 ± 59.78	401.30 ± 67.67
WWTP2b	0.67	1860.38 ± 50.89	1735.30 ± 45.83	231.97 ± 19.49	194.97 ± 22.28
WWTP2c	0.43	973.03 ± 12.62	1047.48 ± 7.70	850.81 ± 15.40	888.59 ± 6.67
	0.62			182.70 ± 12.16	203.59 ± 24.23
	0.78			66.48 ± 18.10	82.03 ± 21.54
WWTP3	0.46	2242.46 ± 25.32	2334.42 ± 13.27	911.43 ± 45.65	942.85 ± 33.50
	0.67			244.76 ± 11.80	288.06 ± 37.39
	0.87			74.26 ± 13.92	76.33 ± 4.55
WWTP4	0.45	1780.00 ± 31.35	1854.24 ± 11.66	989.09 ± 61.50	1014.09 ± 5.25
	0.65			224.85 ± 24.10	260.30 ± 9.09
	0.83			68.26 ± 9.67	70.00 ± 2.06

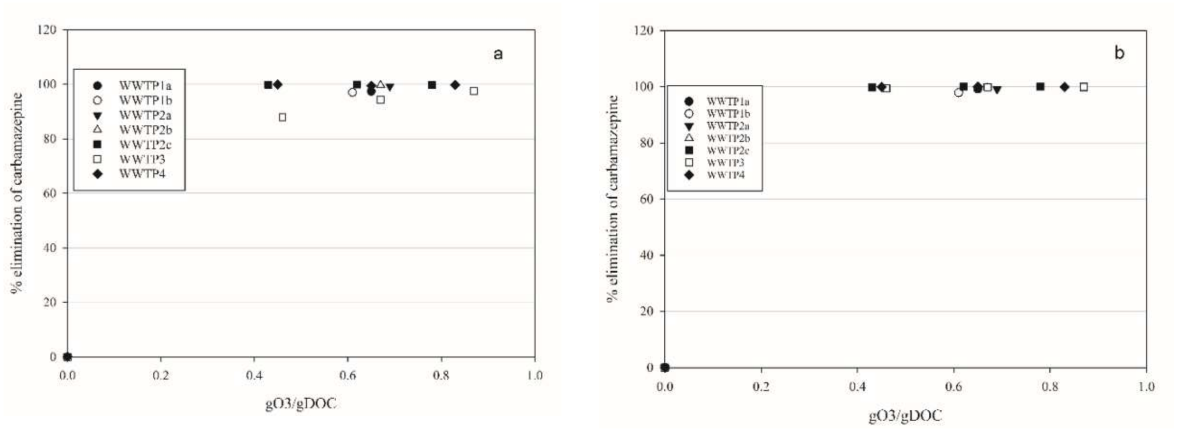

Figure 5. Correlation of CBZ abatement and D_{spec} (a) after ozonation and (b) after BOD_5 measurement

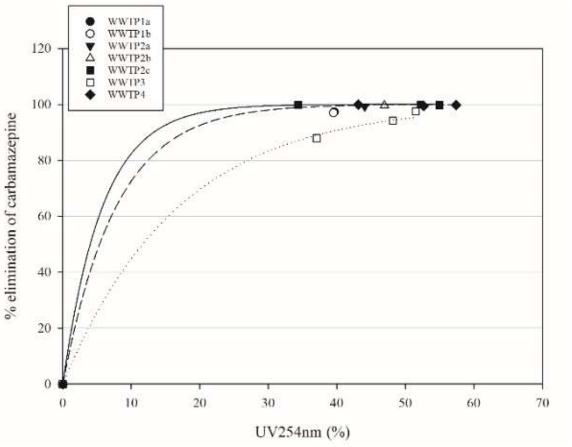

Figure 6. Correlation of CBZ abatement and delta UV_{254} after ozonation. Nonlinear regression fit with exponential rise to maximum ($f = a * (1 - e^{-b*x})$)

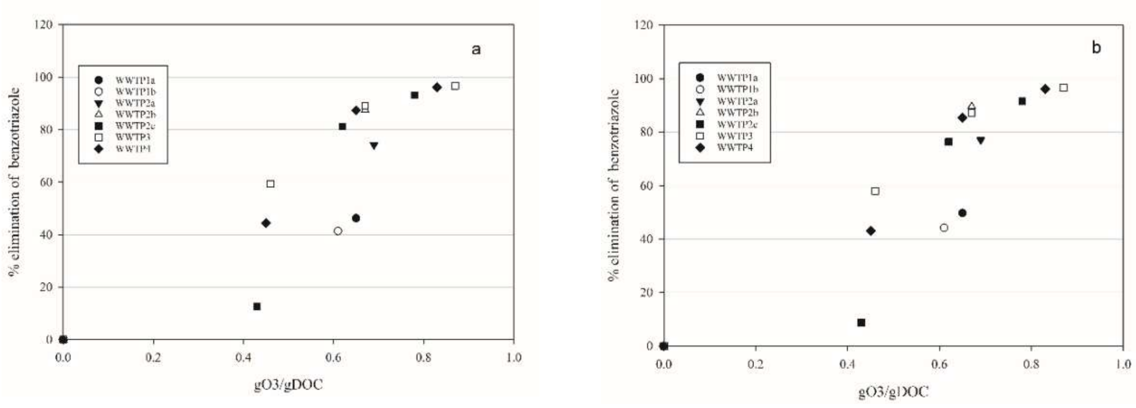

Figure 7. Correlation of BZT abatement and D_{spec} (a) after ozonation and (b) after BOD_5 measurement (b)

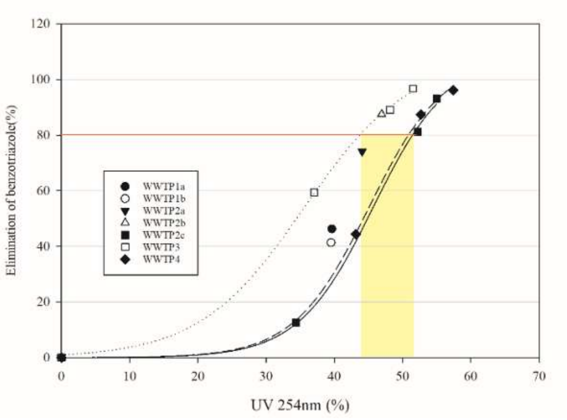

Figure 8. Correlation of BZT abatement and delta UV$_{254}$ ~~(a)~~ after ozonation. Box indicating 80% abatement for the different wastewaters. Nonlinear regression fit with sigmoidal, sigmoid, 3 parameters $\left(f = \frac{a}{1+e^{-\left(\frac{x-x_0}{b}\right)}}\right)$ was applied for the correlation between BZT and UV$_{254}$

Appendix 4

Data of the pilot-scale

Table 1. BEQs for cytotoxicity, estrogenicity and toxic PAH-like activities along the multibarrier system for each sampling campaign

Date	D_{spec}	Cytotox (µg TBT-EQ/L)			ER (ng EEQ/L)			PAH (ng B[a]P-EQ/L)		
	g O$_3$/g DOC	CAS-OUT	O3-OUT	GAC-OUT	CAS-OUT	O3-OUT	GAC-OUT	CAS-OUT	O3-OUT	GAC-OUT
18.09.2018	0.18	**0.24**	**0.23**		0.15	0.05				
19.03.2019	0.27	2.80	**0.25**		0.27	0.09		120	62	
18.09.2018	0.31	**0.25**	**0.24**		0.44	**0.01**				
22.05.2019	0.43	0.59	**0.30**	**0.295**	1	**0.04**	0.16	200	52	28
09.04.2019	0.47	2.00	**0.28**	**0.28**	0.57	**0.03**	**0.028**			
18.09.2018	0.55	0.55	**0.22**		0.31	0.04				
19.03.2019	0.57	**1.15**	**0.23**		**0.09**	**0.02**		140	25	
14.05.2018	0.62	**0.35**	**0.28**	**0.31**	0.59	**0.03**	**0.055**	140	99	39
12.11.2018	0.62	**0.23**	**0.23**	**0.205**	0.56	**0.02**	0.048			
16.10.2018	0.66	1.20	**0.26**	**0.23**	1.2	0.04	**0.015**	270	170	210
19.02.2019	0.71	1.50	**0.26**		0.53	0.12				
18.09.2018	0.75	0.68	**0.23**		0.39	0.04				
19.03.2019	0.78	3.30	**0.26**		0.24			100	50	
13.06.2018	0.83	**0.24**	**0.34**	**0.295**	0.37	**0.03**	0.056	260	100	130
03.07.2018	0.89	**0.19**	**0.19**	**0.19**	0.5	0.08	0.061			
19.03.2019	0.92	2.70	**0.26**		0.23			150	45	
EBT		not available			0.1			6.2		

Numbers in bold: data below the limit of quantification (LOQ) was taken as ½ LOQ

Table 2. BEQs for anti-androgenicity (Anti-AR), xenobiotic sensing (PXR) and oxidative stress (Nf2) response along the multibarrier system for each sampling campaign

Date	D_{spec}	**Anti-AR (µg Flu-EQ/L)**			**Nrf2 (ng Cur-EQ/L)**			**PXR (ng Nic-EQ/L)**		
	g O_3/g DOC	**CAS-OUT**	**O3-OUT**	**GAC-OUT**	**CAS-OUT**	**O3-OUT**	**GAC-OUT**	**CAS-OUT**	**O3-OUT**	**GAC-OUT**
18.09.2018	0.18	6.20	**2.20**		34	**12.5**				
19.03.2019	0.27	**0.95**	**0.95**		86	16				
18.09.2018	0.31	18.00	**2.45**		73	73				
22.05.2019	0.43	**0.28**	**0.95**	**0.95**	130	97	97	40	21	15
09.04.2019	0.47	**0.32**	**0.60**	**0.60**						
18.09.2018	0.55	4.10	**2.60**		**70**	54				
19.03.2019	0.57	**1.05**	**1.05**		160	79				
14.05.2018	0.62	**3.05**	**2.70**	**3.45**	160	190	130	53	18	65
12.11.2018	0.62	9.90	**2.65**	**2.20**	110	37	88			
16.10.2018	0.66	15.00	**2.60**	**2.45**	**36.5**	54	**12.5**	**4.15**	**1.3**	**1.2**
19.02.2019	0.71	**1.05**	**0.95**							
18.09.2018	0.75	**1.20**	**2.65**		**70**	63				
19.03.2019	0.78	**0.90**	**0.90**		**70**	67				
13.06.2018	0.83	**2.25**	**3.05**	**2.65**						
03.07.2018	0.89	7.30	**2.15**	**2.20**	120	130	63			
19.03.2019	0.92	**0.90**	**0.90**		160	100				
EBT		14			10			3		

Numbers in bold: data below the limit of quantification (LOQ) was taken as ½ LOQ

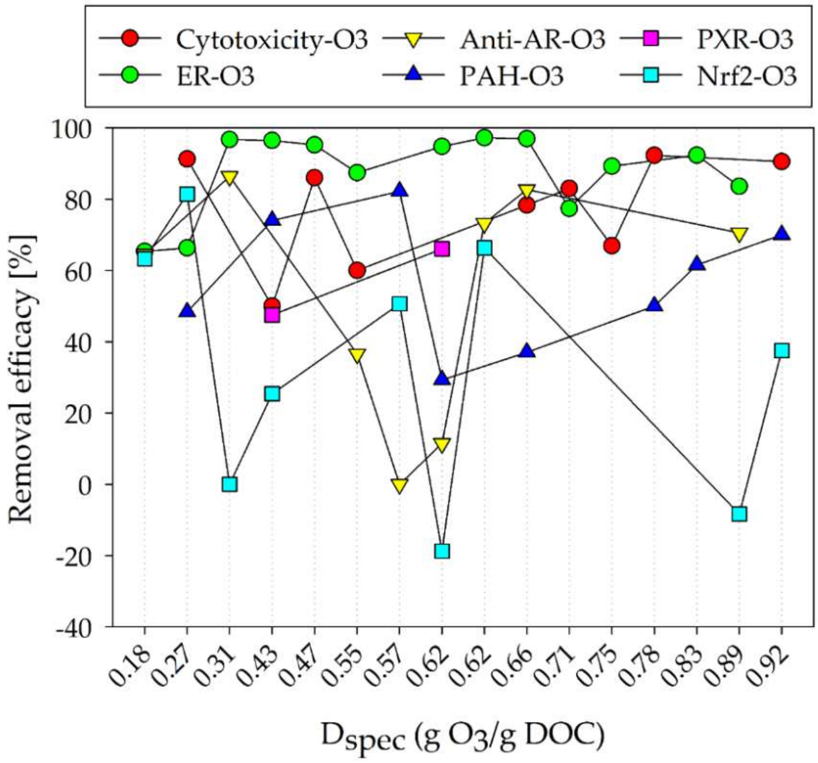

Figure 3. The range of removal for the investigated MOA after ozonation over the one-year monitoring

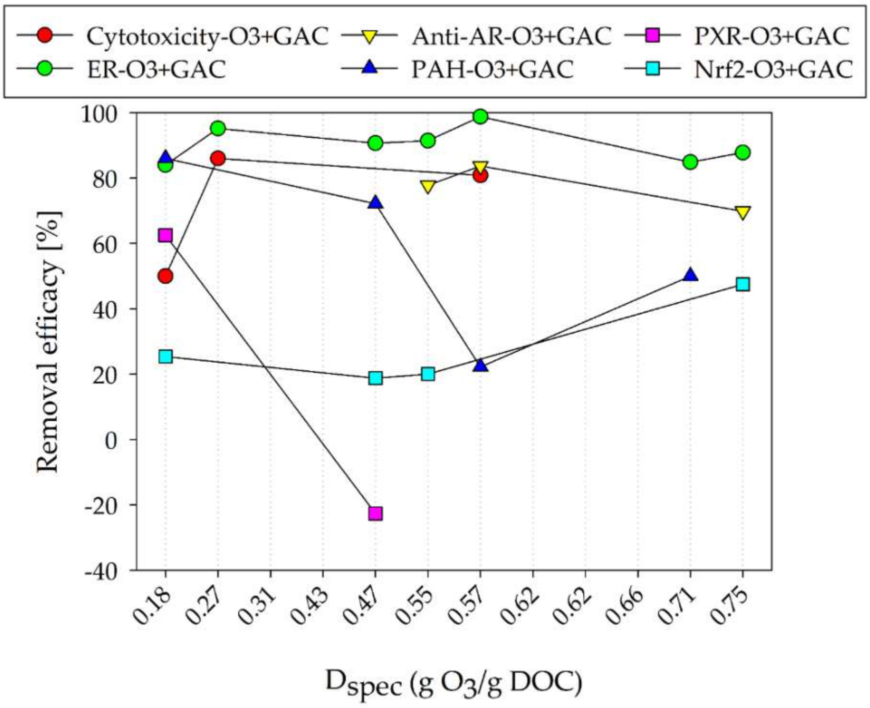

Figure 4. The range of removal for the investigated MOA after the multibarrier system (ozonation and GAC) over the one-year monitoring