

Impact of ozonation on the degradation of organic molecules in aquaculture wastewaters (en ce compris une introduction à la méthodologie de la recherche)

Auteur : Scantamburlo, Carla-Marie

Promoteur(s) : Heinrichs, Benoît

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Impact of ozonation on the degradation of organic molecules in aquaculture wastewaters

Carla-Marie Scantamburlo

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*"Dans la vie, rien est à craindre.
Tout est à comprendre."
- Marie Curie*

Abstract

English version

Vietnam has become the second largest shrimp exporter worldwide. To meet demand and prevent livestock from viral contamination, farmers heavily rely on antibiotics, such as oxytetracycline (OTC). This extensive use leads to water pollution. Treating the wastewater after shrimp harvesting and before its release into the Mekong River is a necessity. To overcome the inability of conventional biological treatment processes to remove micro-pollutants, advanced oxidation processes (AOP) are a promising alternative. This work evaluated the potential of ozonation for the degradation of organic molecules present in aquaculture water.

A preliminary study on para-nitrophenol validated the ozonation protocol. The degradation of OTC was then thoroughly investigated and the impact of OTC concentration, ozone (O_3) dosage, bubbling system and water salinity were assessed. UV-Vis spectrophotometry, Total Organic Carbon (TOC) analysis, and High Performance Liquid Chromatography coupled to Mass Spectrometry (HPLC-MS) were used as complementary characterization techniques. Exploratory experiments were also conducted to broaden the research scope to the rifampicin antibiotic degradation and inactivation of the bacterial strain *Pantoea agglomerans*.

Results highlighted the fast degradation of a 0.1 mmol/l of OTC solution (-90% after 5 minutes in a 400 ml batch reactor) for given ozonation parameters (31 mmol/h). Only a partial mineralization of the by-products was reached (-35% in TOC after 6 hours). A partial reaction order for OTC of 0.57 was determined through a kinetic study, suggesting a complex degradation involving both direct O_3 attack and indirect $\bullet OH$ radical pathways. The bubbling system and the salinity affected the degradation significantly, suggesting mass transfer limitations and the scavenging role of Cl^- as key factors. Coupling O_3 treatment to other AOP processes could enhance the process efficiency to reach complete mineralization of by-products.

Version française

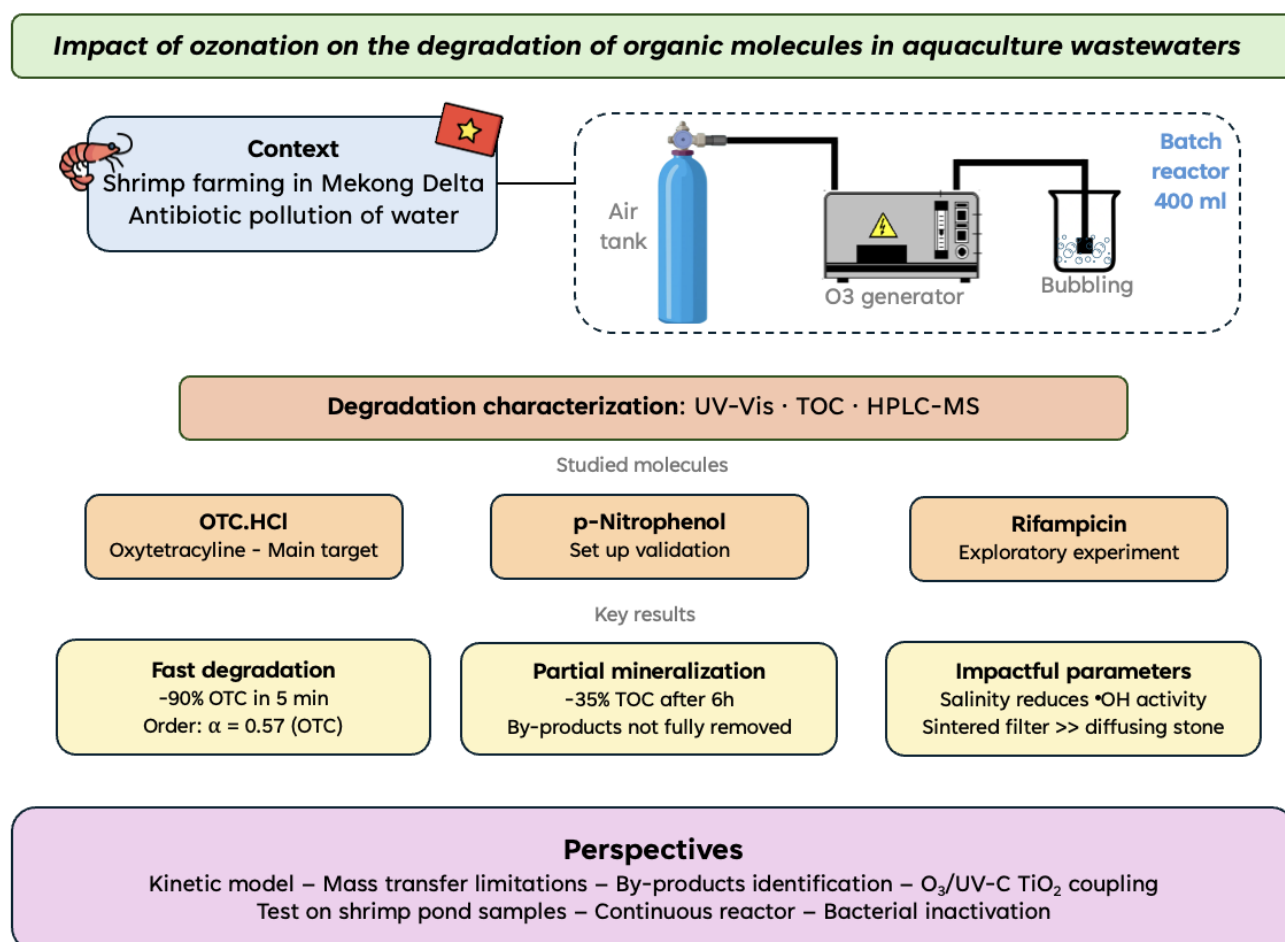
Le Vietnam est devenu le deuxième exportateur mondial de crevettes. Pour répondre à la demande et prévenir la contamination virale de leurs élevages, les producteurs ont largement recours à des antibiotiques, tels que l'oxytétracycline (OTC). Cette utilisation intensive entraîne une pollution de l'eau. Il est indispensable de traiter les eaux usées après la récolte des crevettes et avant leur rejet dans le Mékong. Pour pallier l'incapacité des procédés de traitement biologique conventionnels à éliminer les micropolluants, les procédés d'oxydation avancés (AOP) constituent une alternative prometteuse. Ce travail a évalué le potentiel de l'ozonation pour dégrader des molécules organiques présentes dans les eaux d'aquaculture.

Une étude préliminaire sur le para-nitrophénol a permis de valider le protocole d'ozonation. La dégradation de l'OTC a ensuite été étudiée de manière approfondie et les impacts de la concentration en OTC, du dosage d'ozone (O_3), du système de bullage et de la salinité de l'eau ont été évalués. La spectrophotométrie UV-Vis, l'analyse du carbone organique total (TOC) et la chromatographie en phase liquide à haute performance couplée à la spectrométrie de masse (HPLC-MS) ont été utilisées comme techniques de

caractérisation complémentaires. Des expériences exploratoires ont également été menées afin d'étendre le champ de recherche à la dégradation de la rifampicine (antibiotique) et à l'inactivation de la souche bactérienne *Pantoea agglomerans*.

Les résultats ont mis en évidence la dégradation rapide d'une solution d'OTC à 0.1 mmol/l (-90% après 5 minutes dans un réacteur batch de 400 ml) pour des paramètres d'ozonation donnés (31 mmol/h). Seule une minéralisation partielle des sous-produits a été obtenue (-35% de TOC après 6 heures). Un ordre de réaction partiel de 0.57 pour l'OTC a été déterminé par une étude cinétique, suggérant une dégradation complexe impliquant à la fois une attaque directe par l' O_3 et des voies indirectes par les radicaux $\bullet OH$. Le système de bullage et la salinité ont une influence significative sur la dégradation, suggérant les limitations de transfert de matière et le rôle de piégeage du Cl^- comme facteurs clés. Le couplage du traitement par O_3 à d'autres procédés AOP pourrait améliorer l'efficacité du procédé afin d'atteindre une minéralisation complète des sous-produits.

Graphical abstract



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Introduction

Shrimp farming is a cornerstone of the Vietnamese aquaculture sector with the Mekong Delta yielding roughly 75% of the annual national production. Over the past decades, aquaculture production has expanded rapidly and Vietnam has become the second-largest shrimp exporter worldwide. Farmers in the Mekong Delta region now face increasing pressure to meet this demand. Yet, shrimp farming is extremely vulnerable to the environmental parameters (*i.e.* pH, salinity, dissolved oxygen, temperature) and diseases (Cao *et al.*, 2024). It is therefore essential to keep livestock healthy to avoid losing an entire production and the associated income (Lan, 2013). To tackle this issue, farmers resort to excessive and unregulated use of several antibiotics, leading to the accumulation of antibiotic residues in water and soil.

Among the antibiotics employed, oxytetracycline stands out as one of the most widespread due to its broad spectrum, efficiency and low cost (Z. Li *et al.*, 2019; Luu *et al.*, 2021). It is thus a relevant target for this study. From a wider perspective, the presence of antibiotic residues in shrimp ponds highlights the need to develop technologies able to degrade molecules resistant to traditional biological water treatment processes.

The removal of micro-pollutants such as antibiotics has become a major challenge for conventional wastewater treatment systems. For that purpose, advanced oxidation processes have been of particular interest for several years. These methods rely on the formation of reactive oxidising species to break down complex molecules. Among the different advanced oxidation processes available, ozonation offers several advantages. Used since the 19th century for disinfection or colour removal, ozone can be generated *in situ*, avoiding the storage and transport of hazardous chemicals. It is well-known for its unique ability to generate highly reactive hydroxyl radicals. As some of the most powerful oxidants used in water treatment, these species greatly improve degradation efficiency (Jamali *et al.*, 2024; von Gunten, 2003). These properties make ozone particularly well-suited for application in aquaculture wastewater treatment. Additionally, employing ozone to remove micro-pollutants and antibiotics has shown promising results. Several studies have reported near-complete elimination of oxytetracycline through ozonation, achieving removal rates as high as 98% within one hour (K. Li *et al.*, 2008; Uslu and Balcioglu, 2008).

The objective of this Master's thesis was to evaluate the potential of ozonation as a treatment strategy for the removal of pollutants linked to aquaculture wastewater. A preliminary study was performed on the model pollutant para-nitrophenol to validate the experimental procedure. In a second step, the degradation of oxytetracycline hydrochloride was thoroughly studied to understand the limiting factors of the degradation efficiency, including the pollutant concentration, ozone dosage, pH, bubble generation, and salinity of the medium. Additional experiments were also performed on a second antibiotic, rifampicin, and on the bacterial strain *Pantoea agglomerans* to broaden the scope of the study.

This manuscript was structured around four main parts. A state of the art first introduces the context behind shrimp production and ozonation processes. It is followed by a detailed experimental section. Then, the main results are reported, and observations are discussed in the last section, leading to the conclusion and perspectives.

Objectives

This Master's thesis aimed to assess the effectiveness of an ozonation process on the degradation of organic molecules present in aquaculture wastewater. More specifically, wastewater from shrimp farms located in the Mekong Delta, Vietnam, was targeted. To this end, the work was structured into several phases.

First, a reliable experimental setup and protocol had to be validated. This was done using the model pollutant para-nitrophenol. The general dynamics of the system were identified. This included determining the reproducibility of experiments and the optimal parameters for the ozone generator, and comparing the information given by two analysis techniques: UV-Visible spectrometry and Total Organic Carbon measurement.

Then, the degradation of oxytetracycline hydrochloride (OTC·HCl) was studied. As one of the most widely used antibiotics in Vietnamese aquaculture, OTC·HCl was selected as the primary target compound. The effects of OTC·HCl concentrations and O₃ dose were monitored, along with a first kinetic characterisation of the process. The influence of the bubbling system choice was also assessed by comparing a glass sintered filter and an aquarium diffusing stone. To better reflect Vietnamese water conditions, the impact of an increased salinity of the medium was evaluated.

To broaden the scope of antibiotics studied, the ozonation of rifampicin was investigated, with the same conditions as for OTC·HCl.

Finally, a preliminary assessment on the degradation of the bacteria *Pantoea agglomerans* with ozone was conducted.

Together, these objectives were selected to establish experimental foundations for assessing the effectiveness of ozone in removing organic compounds from aquaculture water.

Chapter 1

State of the art

1.1 Wastewater treatment

1.1.1 Water pollution problematic

Within the last few decades, there has been a significant increase in the interest regarding the study of water treatment and decontamination. Water is a resource of major importance, either for its role in society, industry and life on Earth. In light of the increasing demand due to a growing human population, water has become indispensable for most human activities, agriculture being a prime example. As of today, approximately 50% of the global population still lacks access to water treatment facilities. According to the World Health Organisation, the use of untreated water could lead to the propagation of numerous diseases (Léonard, 2025).

Therefore, fighting against water pollution is imperative. According to Crini and Lichtfouse (2019), *"pollution of water occurs when one or more substances that will modify the water in a negative fashion are discharged into it. These substances can cause problems for people, animals and their habitats and also for the environment."* They also define two main types of pollution: single-source and multiple-source pollution. Single-source pollution may be associated with the extraction of pollutants from industrial sources. As the name suggests, in the case of multi-source pollution, pollutants come from a variety of sources. Overall, water pollution is mainly anthropogenic-based.

1.1.2 Wastewater treatment plants

To tackle this issue, wastewater treatment plants have been developed and consist of four key steps, including physical, chemical and biological treatments. These elements are represented schematically in Figure 1.1.

1. **Pre-treatment:** The main objective is the removal of waste materials, including but not limited to branches, sand, and oil. To do so, three distinct methods must be employed: *screening* (which involves removing items greater than 1 cm), *grit removal* (which eliminates sand through decantation) and *grease removal* (which gathers floating greases).
2. **Primary treatment:** Sedimentation is employed to remove solid suspension matter. When the sludge reaches the bottom of the reactor, it is withdrawn.
3. **Secondary treatment:** Bacteria and other microorganisms are used to assimilate the remaining organic matter, using oxygen. The removal of excess sludge is achieved through a process of clarification that utilises nitrogen and phosphorus.

4. **Tertiary treatment:** It encompasses all the necessary treatment processes following secondary treatment, prior to effluent discharge. Nitrogen and phosphorus are primarily targeted. Sludge is also managed, either by incinerating it or spreading it in fields. Their energy value is optimised.

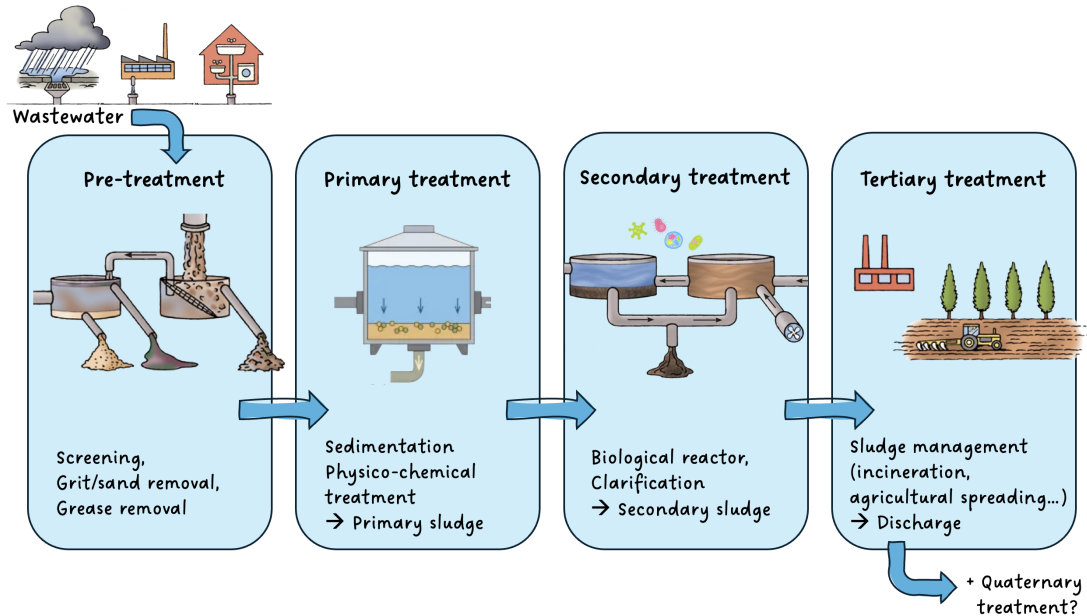


Figure 1.1: Schematic representation of a wastewater treatment plant (Adapted from Léonard, 2025 and IDEA, 2024).

However, recent regulations from the European Union suggest the introduction of an additional treatment step. The purpose of the quaternary treatment is to eliminate micro-pollutants, such as antibiotics, chemical substances, bacteria or viruses. This can be achieved through a variety of decontamination methods, including chlorination, UV irradiation, photocatalysis or ozonation (Lambert, 2025). The framework of this master's thesis falls within this last treatment step.

1.1.3 Water parameters

Before going deeper into the problematic of water treatment, it is worth defining some parameters of great importance:

- **Biological Oxygen Demand (BOD):** It measures the amount of oxygen consumed by a living organism after being incubated in the dark at 20 °C. The total BOD is usually achieved after 28 days (BOD_{28}). Given that this long period is not suitable for everyday practice, the BOD_5 (i.e. after five days) is often measured, as most of the organic carbon is assimilated within three to four days (about 70% of the BOD_{28}).
- **Chemical Oxygen Demand (COD):** It represents the amount of oxygen in water necessary to oxidise organic matter. In contrast to BOD, the measure is faster. It consists of oxidising the organic matter with a powerful oxidant in an acidic medium.

BOD and COD can be linked. The biodegradability index of wastewater can be assessed by taking the ratio BOD_5/COD . The greater the ratio, the more biodegradable the water is.

Other parameters can be monitored, such as the salinity or the nitrogen and phosphorus levels (Léonard, 2025). Following these elements provides information about the eutrophication level. In other words, it corresponds to the amount of excessive nutritive matter in water, which may lead to algae proliferation.

1.2 Shrimp farms in Mekong Delta

1.2.1 Shrimp farming in Mekong Delta

The shrimp farming industry in the Mekong Delta constitutes a key pole of the Vietnamese aquaculture (see [Figure 1.2](#)), as this region accounts for 75% of the national shrimp production (Lan, [2013](#)). This figure is all the more significant given that in 2020, Vietnam was the world's second-largest exporter of shrimp, accounting for nearly 4 billion USD in the country's exports (Phuong, [2023](#); Luu *et al.*, [2021](#)). However, the shrimp farming sector is characterized by a high degree of vulnerability, as the production can be affected by several factors such as the water salinity, meteorological conditions, shrimp diseases, *etc.* In order to keep their shrimps healthy and satisfy the growing demand, farmers frequently resort to the use of several substances (*e.g.* antibiotics or nutriment), resulting in water pollution. Indeed, keeping the livestock in good health is a necessity.

Of the 337 614 families residing in the Mekong Delta region, about 86% of them make a living from shrimp farming. Thus, losing the production would result in the loss of revenue, which could have dramatic consequences (Lan, [2013](#)). To illustrate this point, shrimps are very sensitive to bacterial diseases and viruses. An infection may thus result in the loss of the entire production of a farm (Braun *et al.*, [2019](#)). Farmers, therefore, have recourse to various antibiotics and pesticides to keep their shrimp healthy. Nevertheless, access to antibiotics remains largely unregulated and unsupervised. Indeed, farmers mostly rely on the advices of antibiotic salespeople who have no veterinary expertise and whose sole objective is to maximize production (Pham *et al.*, [2015](#)).



Figure 1.2: Shrimp pond of a farm located in Trà Vinh province (Tran X.N.A, PhD student, personal communication, 2026).

[Figure 1.3](#) shows a shrimp affected by the White Spot Syndrome Virus (WSSV). This virus poses a significant threat to shrimps, with a mortality rate of 3-10 days (Ramachandran *et al.*, [2024](#)). To illustrate the impact of the disease on shrimp farming, in 2024, *"the total area of shrimp farms affected by the disease was over 1,009 ha. Soc Trang was the province with the largest diseased shrimp farming area with nearly 319 ha, accounting for 37.79% of the total diseased area, followed by Trà Vinh and Bạc Liêu"* (Vietnam Agriculture News, [2024](#)).



Figure 1.3: Shrimp affected by the White Spot Syndrom Virus (Ramachandran *et al.*, 2024).

1.2.2 Optimal breeding conditions

To ensure shrimp health, a series of optimal breeding conditions have been established. The main factors impacting the water quality for shrimp farming are the water salinity and temperature, the dissolved oxygen (DO), suspended solids, nitrogen and phosphorus. According to Cao *et al.*, (2024) and Leigh *et al.*, (2020), the optimal breeding conditions are listed in Table 1.1.

Parameter	Value
Temperature	between 26 - 32°C
Salinity	between 10 - 25 g/l
pH	between 6 - 9
DO	> 3.5 mg/l
Nitrogen	< 0.009 mg/l
phosphorus	between 0.001 - 0.1 mg/l
Suspended solid	100 mg/l

Table 1.1: Optimal breeding conditions (Cao *et al.*, 2024; Leigh *et al.*, 2020).

Each of these parameters has its own importance for the health of shrimps. Temperature has a direct impact on the metabolism of crustaceans, their immune response or their oxygen consumption. Keeping the nitrogen levels low allows healthy shrimps to develop. When phosphorus exceeds the recommended range, it can lead to the proliferation of plankton. Finally, the cleanliness of the water is also important. An accumulation of food and faeces may induce a buildup of nutrients, leading to excessive eutrophication (Cao *et al.*, 2024).

Yet, the conditions are often sub-optimal (Leigh *et al.*, 2020), mainly due to the climate change, for which Vietnam is one of the most affected countries. Most particularly, the temperature and salinity levels are the two most affected parameters. Climate change also has an impact on the equilibrium between wet and dry seasons and on the levels of precipitation. As an example, an increase in precipitation induces loud noises of raindrops and stresses shrimps. Thus, they go hide at the bottom of the ponds, which is the most exposed to accumulated pathogens (Cao *et al.*, 2024).

These optimal parameters values were compared to the ones provided by a shrimp farmer from the Trà Vinh University farm. In his farm and at the moment of the interview (*i.e.* December 2025), the water temperature was at 27°C during the day, and could reach 31°C at noon. Regarding the DO, it ranged between 6 to 7 mg/l. This was explained by the fact that the shrimps were still in their early stage of development. Once grown-up, the DO should drop to 4-5 mg/l. Finally, the salinity was of about 7 g/l (Tran X.N.A, PhD student, personal communication, 2026).

1.2.3 Use of antibiotics

The use of antibiotics in the Mekong Delta is widespread and the molecules used are numerous. Antibiotics target bacteria and their role is to prevent excessive bacterial growth in the water. According to shrimp farmers, a high bacterial load increases the risk of viral emergence. Since controlling viruses is key to reducing shrimp mortality, antibiotics are used to keep the bacterial population below a certain threshold, thereby limiting the conditions favourable to viral outbreaks.

As antibiotics are used extensively, they therefore represent one of the major sources of pollution in the Delta, followed by nutrients and agricultural chemicals. It was reported that a shrimp farm uses, on average, three types of antibiotics (Pham *et al.*, 2018), which corresponds to 700 g of antibiotics per ton of fish produced. This value is seven times higher than that of other producing countries (Pham *et al.*, 2021).

Within the framework of my technical internship at the Industrial University of Ho Chi Minh City (Vietnam), focus was made on the photocatalytic degradation of oxytetracycline with titanium dioxide (TiO₂) for the treatment of shrimp farm wastewater. A screening of the antibiotics used in the Mekong Delta was conducted. The results are presented in Table 1.2.

Location	Type of Antibiotic	Year	Reference
Can Gio* & Ca Mau provinces	Trimethoprim (Diaminopyrimidine), Sulfamethoxazole (Sulfonamide), Norfloxacin and Oxolinic acid	2004	(Tuan & Munekage, 2004)
Can Tho city & An Giang province	Enrofloxacin (Fluoroquinolone), Trimethoprim (Diaminopyrimidine), Sulfamethoxazole/Sulfadiazine (Sulfonamide), Florfenicol (Phenicol), Amoxicillin/Ampicillin (Penicillin), and Doxycycline/Oxytetracycline (Tetracycline).	2015	(Giang <i>et al.</i> , 2015).
Can Tho & Dong Thap provinces	Trimethoprim (Diaminopyrimidine), Oxytetracycline (Tetracycline), Sulfamethoxazole/Sulfadiazine (Sulfonamide)	2015	(Pham <i>et al.</i> , 2015)
Soc Trang & Ben Tre provinces	Sulfamethazine/ Sulfadiazine/ Sulfamethoxazole (Sulfonamide), Trimethoprim (Diaminopyrimidine), Ciprofloxacin/Enrofloxacin (Fluoroquinolone)	2016	(Braun <i>et al.</i> , 2019)
An Giang province	Trimethoprim (Diaminopyrimidine), Sulfamethoxazole (Sulfonamide), Amoxicillin (β -lactam), Enrofloxacin (Fluoroquinolone), Doxycycline (Tetracycline), Kanamycin (Aminoglycoside)	2017	(Ström <i>et al.</i> , 2019)
Dong Nai*, Dong Thap provinces	Oxytetracycline (Tetracycline), Sulfonamide, Quinolone, Cephalosporin, Florphenicol (Phenicol), Aminoglycoside	2018	(Luu <i>et al.</i> , 2021)

Table 1.2: Use of antibiotics in the Mekong Delta, based on the year and location. Locations with a "*" are located outside of the Mekong Delta, but the related article did not made more distinction. The main antibiotic classes are sorted by color: (Fluoro)quinolones, Tetracyclines, Sulfonamides and Diaminopyrimidines.

In shrimp farming, the four main types of antibiotics used are sulfamethoxazole, trimethoprim, enrofloxacin, and oxytetracycline. Table 1.2 synthesizes the use of antibiotics depending on the year and location. Based

on color code, four families of antibiotics stand out for their frequent use: (fluoro)quinolones, sulfonamides, diaminopyrimidines, and tetracyclines. Figure 1.4 provides a more visual representation of the distribution of shrimp farm cited in Table 1.2, in the Mekong Delta.

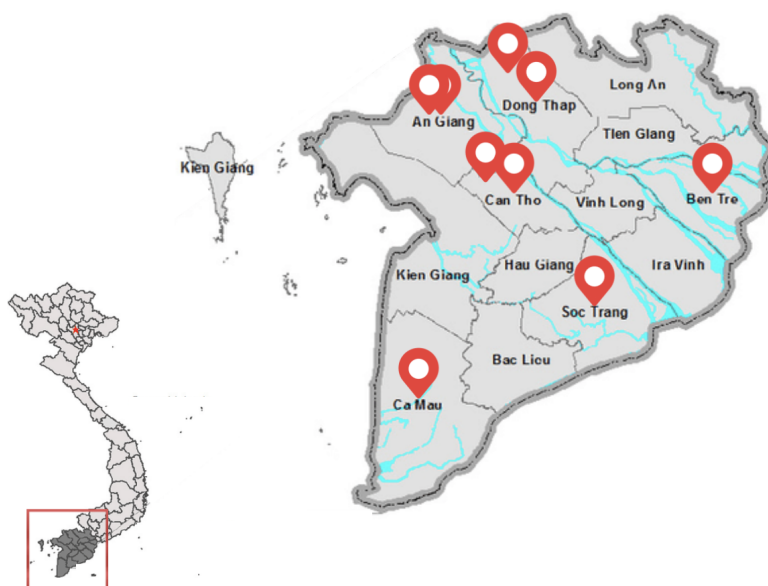


Figure 1.4: Locations of shrimp farms cited in Table 1.2 and located in the Mekong Delta.

Oxytetracycline (OTC - $C_{22}H_{24}N_2O_9$), a type of tetracycline, stood out as one of the most frequently employed antibiotic in the Mekong Delta. The molecular structure of OTC ($M_m = 496.9$ g/mol) is presented in Figure 1.5. It is a four-ring molecule of which three (rings B, C and D) absorb light at wavelengths between 250-300 and 340-380 nm (Z. Li *et al.*, 2019).

Its efficiency and affordability makes it a first-choice antibiotic for shrimp farmers (see Figure 1.6). Thus, the study of the degradation of this antibiotic will be one of the focuses of this master thesis.

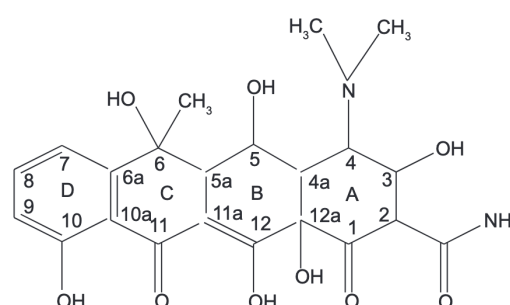


Figure 1.5: Schematic diagram of the molecular structure of OTC (Z. Li *et al.*, 2019).

OTC is not completely soluble in water: 313 mg/l (≈ 0.3 g/l) in water at 25 °C (National Center for Biotechnology Information (a), 2026). This would induce remaining powder traces during the experiments, biasing the actual concentration of OTC to be degraded. To overcome this issue, oxytetracycline hydrochloride (OTC · HCl) was studied. Indeed, its solubility is of about 100 g/l (National Center for Biotechnology Information (b), 2026), enabling total dissolution in water at 25 °C.

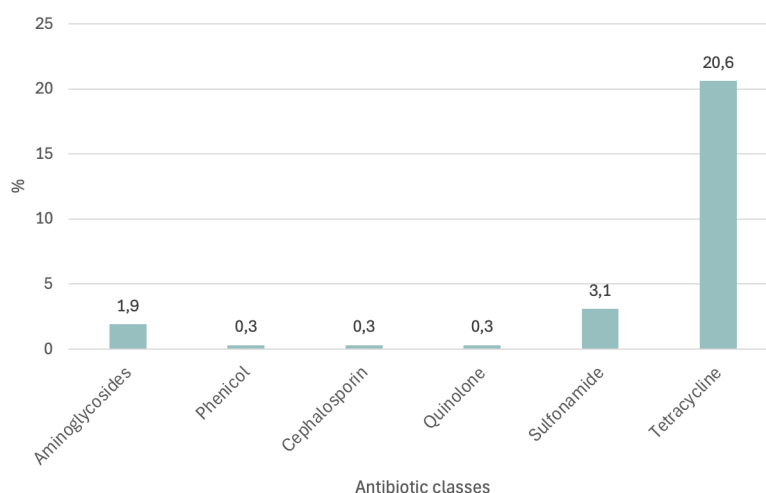


Figure 1.6: Common antibiotics used by shrimp farms (%), adapted from (Luu *et al.*, 2021). The tetracycline family stands out as the most frequently used.

It is worth noting that recent study have shown the resistance of bacteria to some antibiotics. A prime example is the *Vibrio parahaemolyticus* bacteria. It is known to cause liver and pancreas necrosis in shrimp, but it can also affect human health. It was shown that it exhibited strong to moderate resistance to commonly used antibiotics in shrimp farming, such as Amoxicillin, Sulfamethoxazole, Tetracyclines or Quinilones. However, it was highly sensitive to Rifampicine (Huynh *et al.*, 2019; Quach *et al.*, 2025).

1.3 Interest of studying ozone

As mentioned in Section 1.1.2, there are three main water treatment methods: physical, chemical and biological ones. Chemical methods are especially interesting for oxidizing quickly and completely organic pollutants such as antibiotics (Jamali *et al.*, 2024). Chemical disinfection has been common practice since the early 20th century and has evolved alongside the development of advanced oxidation treatments (see Figure 1.7). Disinfection can be defined as the inactivation of pathogenic microorganisms, such as bacteria and viruses, using chemical oxidants to prevent diseases. Oxidation, on the other hand, corresponds to the elimination of organic and inorganic contaminants through an oxidation-reduction reaction. The oxidant is important in these reactions because it can be selective and react only with particular functional groups. Finally, advanced oxidation involves the *in situ* generation of highly reactive radical species (e.g. hydroxyl radicals), which are less selective and can oxidise a wider range of contaminants to treat refractory micro-pollutants (von Gunten, 2024).

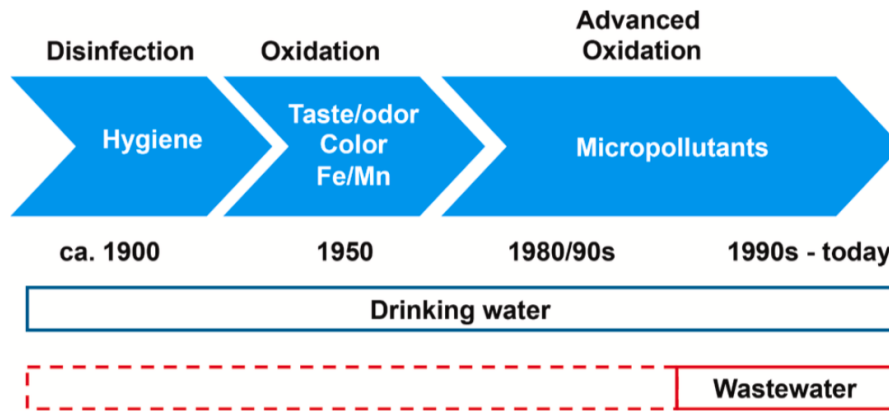


Figure 1.7: Evolution of chemical treatment applications from the 1900s until today. Disinfection = inactivation of pathogenic micro-organism by a chemical oxidant. Oxidation = elimination of contaminants through an oxidation-reduction reaction. Advanced oxidation = oxidation of refractory micro-pollutants via highly reactive radicals produced *in situ* (von Gunten, 2024).

Ozonation is a well-known chemical method. The use of ozone for water treatment dates back to the late 1800s (Jamali *et al.*, 2024). More precisely, it was used for drinking water treatment for disinfection purposes, such as colour and odour control, decolouration, and micropollutant removal, or for oxidation purposes, as described in von Gunten (2003).

1.3.1 Ozone properties

Ozone (O_3) is a toxic blue gas with a sharp smell that contains an unhealthy allotrope of O_2 . The main properties of O_3 are listed in Table 1.3. One of the most unique features of O_3 is its ability to form strong hydroxyl radicals ($\bullet OH$), which are known to be the strongest oxidants in water (von Gunten, 2003).

Table 1.3: O_3 main properties, adapted from Jamali *et al.*, (2024).

Parameter	Value
Molecular mass	48 g/mol
Density (0°C, 1 bar)	2.14 kg/m ³
Solubility in water (15 °C)	0.456 g/l

1.3.2 Ozonation mechanism and kinetics

The general kinetics behind an ozone treatment for the removal a micro-pollutant (MP) can be written as,



Using a first order kinetic model for Equation 1.1, the expression of the rate law can be established, as shown in Equation 1.2 for a constant O_3 concentration (von Gunten, 2003; Jamali *et al.*, 2024).

$$-\frac{d[MP]}{dt} = k[MP][O_3] \quad (1.2)$$

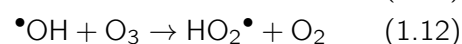
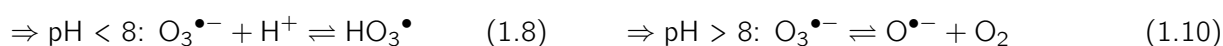
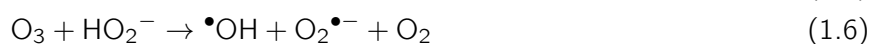
$$\rightarrow \ln \frac{[MP]}{[MP]_0} = -kt[O_3] \quad (1.3)$$

Ozonation is most effective for micro-pollutants containing amino groups, activated aromatic systems or double bonds. It is not effective for protonated amino groups, primarily due to their pKa being close to 10.

The rate constant k decreases for electron-withdrawing groups (such as Cl^-) and increases for electron-donor groups. It can also be affected by the temperature through the Arrhenius law (Equation 1.4). To induce the ozonation reaction, an activation energy (E_a) of 35 to 50 kJ/mol is necessary (von Gunten, 2003).

$$k = Ae^{-\frac{E_a}{RT}} \quad (1.4)$$

Ozone is not stable water. Depending on the pH, the degradation mechanism of ozone in water can change a lot. It can be expressed as,



Equation 1.5, 1.6 and 1.7 correspond to the initiation steps of the mechanism, which can be accelerated depending on the pH of the reaction (von Gunten, 2003).

According to Jamali *et al.*, (2024) and Hoigné *et al.*, (1983; 1985), the action mechanisms of ozone to degrade a micro-pollutant can be divided into two main categories: direct and indirect attacks. They can be sorted as:

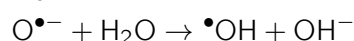
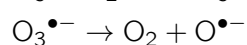
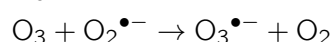
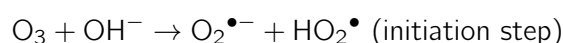
1. **Direct attack of O_3 :** Ozone molecules react selectively with specific electron-rich sites of the target molecule. As an example, for oxytetracycline, these sites include the aromatic rings and the enol groups, which present high electron density. The direct attack can proceed through three main reaction types:

- (a) Cycloaddition (Criegee mechanism): O_3 adds across a $\text{C}=\text{C}$ double bond, forming a molozonide¹ intermediate that decomposes into carbonyl fragments,



- (b) Neutrophilic addition: O_3 attacks electron-poor sites, though this pathway is less dominant for oxytetracycline.
- (c) Electrophilic substitution: O_3 acts as an electrophile and substitutes a hydrogen on an aromatic ring, leading to hydroxylation of the ring.

2. **Indirect attack of the $\bullet\text{OH}$ radicals:** This involves the decomposition of O_3 in water, generating hydroxyl radicals, which then attack the pollutant. The decomposition chain can be summarised as follows (Hoigné *et al.*, 1985),



¹A molozonide is the product of cycloaddition of an alkene to ozone, which is unstable and short-lived. It can be broken and rearranged quickly to form more stable species.

This being known, the real extent of the ozone reaction has been established by von Gunten (2003). It follows both the concentrations of O_3 and $\bullet OH$. The rate law shown in Equation 1.2, can be written as,

$$-\frac{d[MP]}{dt} = k_{O_3}[MP][O_3] + k_{OH}[MP][\bullet OH] \quad (1.13)$$

$$= (k_{O_3} + k_{OH}R_c)[MP][O_3] \quad (1.14)$$

$$\rightarrow R_c = \frac{[\bullet OH]}{[O_3]} \quad (1.15)$$

with R_c the ration between the amounts of O_3 and $\bullet OH$ present.

1.3.3 Advanced Oxidation Processes

The enhancement of ozonation activity can be achieved through its integration with various methodologies. These new proceses are called Advanced Oxidation Processes (AOPs) and are the most efficient alternative to eliminate natural pollutants (see Figure 1.8). Their main advantage is the *in situ* generation of strong oxidants such as $\bullet OH$ radicals. These may cause risks for human beings and the aquatic biota by damaging the aquatic environment. Jamali *et al.*, (2024) defines oxidation as "the transfer of a single or more electrons from a reducing agent to an oxidant, with changes of composition for both". More details into the AOPs related to ozonation are given in Section 1.5.2.

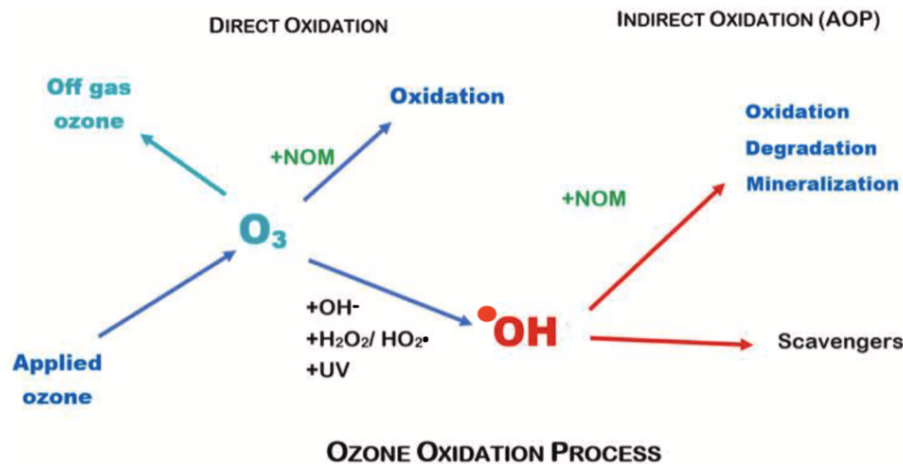


Figure 1.8: A comparison of direct ozonation and advanced oxidation processes (AOPs). For direct oxidation, molecular O_3 selectively reacts with natural organic matter (NOM). In the indirect oxidation pathway (AOP), ozone decomposition is initiated by hydroxide ions (OH^-), hydrogen peroxide ($H_2O_2/HO_2^\bullet$) or UV irradiation. This generates hydroxyl radicals ($\bullet OH$), which are distinct from hydroxide ions (OH^-). The formation of these radicals leads to the oxidation, degradation and mineralisation of pollutants. They can also be consumed by scavengers (Jamali *et al.*, 2024).

1.3.4 Ozonation by-products

Degradation of a micro-pollutant with ozone is often partial. This results in the formation of degradation by-products, *i.e.* electron-poor compounds with smaller molecular mass and high hydrophilic properties, that are only partially modified in comparison to the parent molecule (Jamali *et al.*, 2024). On average, for one parent molecule, six by-products can be identified. The toxicity of the by-products should be investigated as new effects may emerge (von Gunten, 2003).

Another key element regarding by-products is their biodegradability. It appears that on average only 19% of them can be biodegraded. To assess this, Jamali *et al.*, (2024) summarized the biodegradability index of different functional groups after an ozonation treatment (see Figure 1.9).

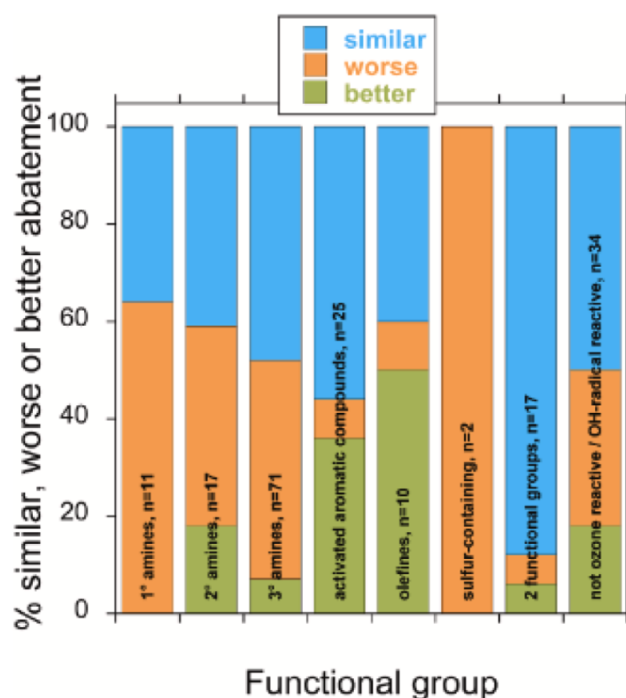


Figure 1.9: Biodegradability of different functional groups after an ozonation treatment (Jamali *et al.*, 2024).

1.3.5 Ozone measurement

Ozone measurement is a tricky aspect of ozonation. Indeed, it can either be measured in the gaseous or liquid form, and each has its own measurement techniques. This sections aims to provide an overview of the methods available.

1.3.5.1 Dissolved ozone

Some well-known techniques to measure the dissolved ozone concentration are the iodometric or the indigo methods.

The indigo method suggests that the concentration of ozone can be followed by the decolorization of an indigo trisulfonate solution. This is done by measuring the absorbance of indigo at 600 nm with a UV-Visible spectrophotometer. By following the absorbance of indigo trisulfonate, the concentration of ozone can be determined. Indeed, the absorbance is proportional to the indigo concentration (*cf.* Beer-Lambert law), and a decrease in indigo concentration corresponds to a decrease in ozone concentration. As stated by Bader & Hoigné (1981), *"one molecule of trisulfonated indigo reacts with one molecule of ozone under proper reaction conditions including high rate of mixing"*. The Beer-Lambert law enables to switch from absorbance values to concentrations. An issue about this method is the need to realize a standard curve.

The iodometric method is based on the titration with thiosulfate of a solution in which ozone has reacted with potassium iodide (KI) to form iodine (I₂). Yet, this technique is less suitable in the practice as it can be time-consuming (CHEMetrics, 2023).

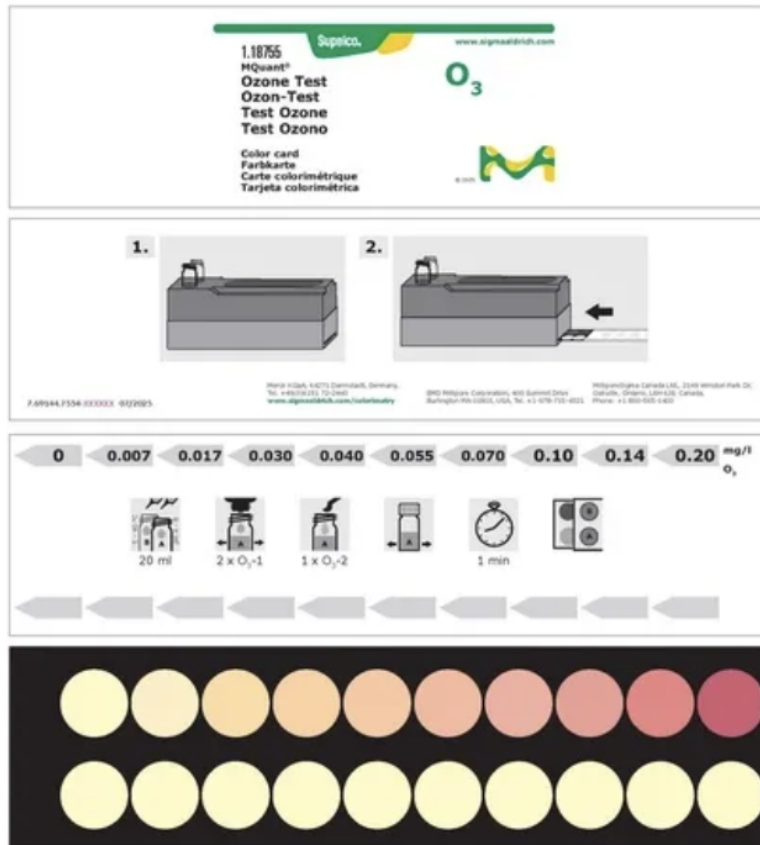


Figure 1.10: Ozone MColortest Disk comparator (VWR).

It is possible to use portable test kits, such as the Ozone MColortest Disk comparator (VWR, see [Figure 1.10](#)) used by Mahy *et al.*, (2020). The principle behind it is similar as the dipsticks used in swimming pools to monitor chlorine. The tested sample changes color according to the ozone concentration. However, this method only provides an average concentration value. For this master's thesis, Julien Mahy kindly offered me to use his test kit, unfortunately, the reactants were out of date.

1.3.5.2 Gaseous ozone

Measuring gaseous ozone is slightly more complex as it requires the use of special devices, such as sensors and analyzers. As an example, Kuosa *et al.*, (2015) used the Orbisphere 3660 ozone analyzer. Sensors can either be electrochemical (EC) or metal-oxide sensors (MOS). EC sensors are based on electrolytes which provide an electrical current proportional to the amount of ozone present. MOS sensors require the contact between an oxidizing agent and a heated metal-oxide plate to generate an electric current (Ellenburg, Jessica, 2021). However, these techniques are usually more costly.

⇒ Given the fact that ozone measurement requires either a specific piece of equipment or tedious supplementary manipulations, the follow-up of the ozone concentration was left aside for this master's thesis. To get an insight of the amount of ozone bubbled in the reaction solution, the reference plots of the ozone generator used were considered sufficiently reliable (see [Appendix B](#)).

1.4 Degradation of a model pollutant: para-Nitrophenol

para-Nitrophenol (PNP, CAS: 100-02-7) is a well-studied model pollutant, namely for photocatalysis and ozonation (Farcy *et al.*, 2024; Wang *et al.*, 2021). The molecular structure of PNP is presented in Figure 1.11, and its molecular weight is 139.11 g/mol. Prior to studying the degradation of OTC with ozone, focus was made on PNP in order to analyze the general dynamics of the system. Indeed, this molecule is already commonly used at the laboratory scale, notably for photocatalyst efficiency tests. As its dosage is well-known, it represents a good basis for preliminary ozonation experiments.

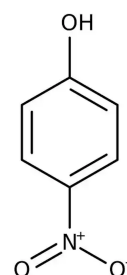


Figure 1.11: Schematic diagram of the molecular structure of PNP (Fischer Scientific, 2026).

PNP is a type of mono-nitrophenol and is commonly used for several applications in the chemical industry such as pharmaceuticals, synthetic dyes, insecticides and explosives. It is known to be one of the most toxic mono-nitrophenols given its high stability and solubility (16 g/l at 25°C) in fresh and marine environments. This can be linked to the nitro group present in the molecular structure, which induces electron withdrawal and leads to an increased resistance to biological and chemical oxidation. Taking all of this into account, the Organization for Economic Co-operation and Development (OECD) recommends PNP as a model compound for the study of the biodegradation of pollutants (Godain *et al.*, 2020; Kanimozhi and Kanthimathi, 2023).

Acute exposure to PNP may cause health issues such as blood disorders, kidney and liver failure, skin irritations, anemia or poisoning. Thus, the Environmental Protection Agency (EPA) of the United States has included PNP in the "*Emergency Planning & Community Right-to-know Act*" and recommended its concentration in drinking water not to 10 µg/l. The monthly mean concentration of PNP in industrial effluents from the "*organic chemicals, plastics, and synthetic fibers sector*" (United States) was of about 162 µg/l when the EPA regulation was written. This emphasizes the importance efficient removal techniques (Yahya *et al.*, 2021).

1.4.1 Current degradation methods

1.4.1.1 Ozone processes

The use of ozone to degrade PNP has already been the subject of extensive research. Among these studies is a series of articles written by the team of Kuosa *et al.*, (2007; 2009; 2015). Ozone was introduced in a bubble column reactor, for which the mass balances in the liquid and gas phases have been established. The dissolution of ozone was modeled using the volumetric mass transfer coefficient $k_L a$ and Henry's law. Axial dispersion in the gas phase and the gas hold-up were also accounted for. The reaction kinetics was assumed second-order overall. Several species were tracked: PNP, hydroquinone, nitrate, and a residual COD grouping the contributions of unknown intermediates. An additional ozone self-decomposition term, attributed to radical chain reactions, was included to close the ozone mass balance.

Their results showed that the decomposition rate of PNP depends on the pH and that the reaction scheme could be represented by two main pathways (see Figure 1.12). In the first pathway, PNP reacts directly with ozone to produce hydroquinone and nitrate ions (NO_3^-). Hydroquinone then further reacts to produce a residual COD. In the second pathway, PNP is directly oxidized into this residual COD (COD_{res}), which can then either be fully mineralized into CO_2 and H_2O , or further oxidized to produce NO_3^- . At pH 5, the hydroquinone pathway was found to be about twice as fast as the second pathway. At pH 2, both pathways had similar rates. Kuosa *et al.*, (2015) also revealed that at acidic pH, the degradation of PNP

was achieved by a direct O_3 attack, whereas at basic or neutral pH, the indirect pathway, which induces the action of $\bullet OH$ radical, was predominant.

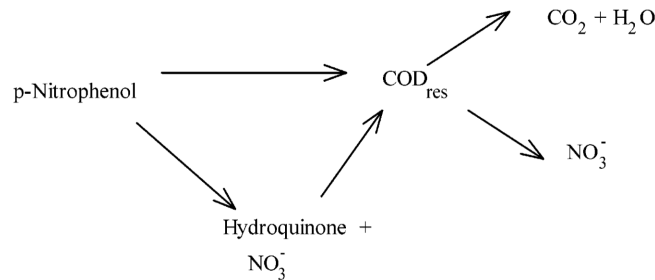


Figure 1.12: Ozone reaction scheme to degrade PNP. Two pathways have been identified: the hydroquinone route and the direct oxidation into residual COD. At pH 5, the hydroquinone pathway is twice as fast as the second pathway (Kuosa *et al.*, 2007).

To enhance the activity of the $\bullet OH$ radicals, Wang *et al.*, (2021) assessed the impact of combining O_3 and UV light. This resulted in a higher degradation efficiency, compared to the condition with ozone alone. Finally, it is also possible to introduce a catalyst in the experimental set up for ozonation (see Figure 1.13). This was done by Singh and Majumder (2025), who coupled ozone with a zinc oxide (ZnO) catalyst. For 1 l of a 50 mg/l PNP solution, 300 mg of ZnO were added. The effect of the O_3 flow rate, bubble size and pH were also analyzed. This study highlighted several interesting facts, namely:

- Introducing ZnO led to an increase of the rate constant.
- Working in the alkaline condition favor the $\bullet OH$ radicals formation and degradation efficiency.
- The rate constant cannot be fully represented by chemical aspects, parameters such as the Reynolds number, bubble diameter or gas holdup must be included.
- The bubble size is inversely proportional to the gas velocity.

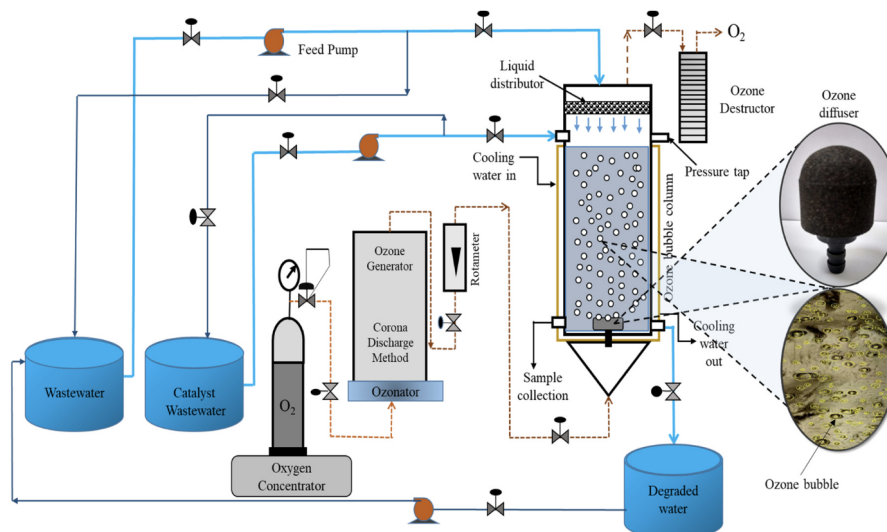


Figure 1.13: Catalytic (ZnO) ozonation plant prototype layout suggested by Singh and Majumder (2025). $[ZnO] = 300 \text{ mg/l}$ and $[PNP] = 50 \text{ mg/l}$.

1.4.1.2 Other processes

Ozonation is not the only efficient technique to remove PNP. On the one hand, Farcy *et al.*, (2024) studied a process based on heterogeneous catalysis to degrade the model pollutant, by working with different ZnO compositions and UV-A light. On the other hand, Yahya *et al.*, (2021) presented a method based on polyphenylsulfone-based blend membranes and reached a 99% removal efficiency.

→ The insights gathered from the literature on the ozonation of PNP will provide a solid basis for designing a series of preliminary experiments, before investigating the degradation of oxytetracycline with ozone.

1.5 Degradation of OTC through ozonation

Ozone may be applied in different ways to degrade OTC. It can be through direct and sole ozonation of the solution, combining it with a catalyst and UV light or other hybrid methods. This section aims to provide the reader a general overview of these techniques.

1.5.1 Direct ozonation

Direct ozonation relates to the only use of O_3 to remove antibiotics. This is done by bubbling ozone in the reaction solution directly, without adding any supplementary degradation methods. The degradation of OTC by direct ozonation has been the subject of numerous studies. From one article to another, operating conditions such as the initial concentrations of OTC and O_3 , the medium or the time of treatment varied. They are summarised in Table 1.4.

It can first be highlighted that O_3 is highly efficient at degrading OTC, achieving near-complete removal after 5 minutes in batch reactors (K. Li *et al.*, 2008; Uslu and Balcioglu, 2008). In the case of a continuous reactor treating real aquaculture wastewater, Gorito *et al.*, (2022) reached 98% of removal with a retention time of 10 minutes. The initial O_3 concentration and flow rate were noted as key parameters to influence the degradation rate. Another crucial parameter is the pH, as already observed for the degradation of PNP. Depending on the pH, the degradation pathway either occurs by direct O_3 attack (acidic conditions) or by the generation of $\bullet OH$ radicals (alkaline conditions). Li *et al.*, (2008) showcased that working at the highest pH does not necessarily provide the best efficiency. From the three pH values tested (3, 7, and 11), pH 7 provided the best results (see Figure 1.14). Indeed, at pH 11, intermediary species appeared and acted as $\bullet OH$ radical scavengers, which slowed down the degradation. This observation was confirmed by Park *et al.*, (2023), who studied the kinetics of OTC when introducing para-chlorobenzoic acid (pCBA) and tert-butyl alcohol (t-BuOH) to follow ozone specifically. At pH 5, direct O_3 attack was the main oxidant of OTC. It can be linked to the reactivity of phenol groups and double bonds with respect to ozone.

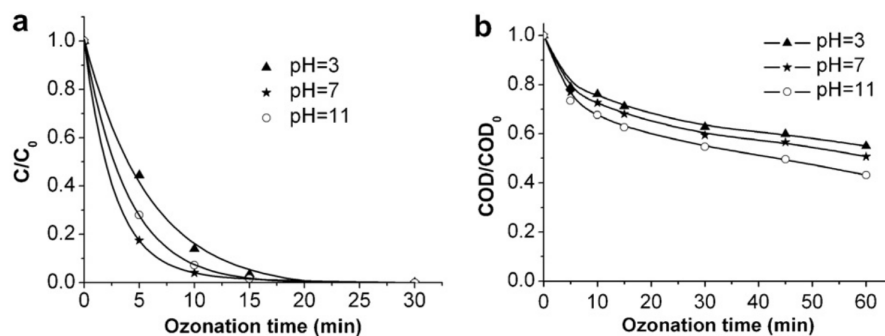


Figure 1.14: (a) Effect of pH (3, 7, 11) on the oxidation of OTC and (b) COD removal during ozonation. Parameters: [OTC] = 100 mg/l; [O_3] = 11 mg/l; 25 °C (K. Li *et al.*, 2008).

Introducing a real medium instead of synthetic water also complicated the degradation and acted as a limiting factor. Two examples can be presented. On the one hand, Uslu and Balcioglu (2008) showed that the degradation of 50 mg/kg of OTC in cow manure was incomplete, even after 90 minutes of treatment. In contrast, only 5 minutes were required to remove 97% of the antibiotic (10 mg/l) in synthetic water. On the other hand, working with aquaculture wastewater provides better results. Gorito *et al.*, (2022) observed a slower removal rate due to the ions present in the medium. Direct ozone attack was still a predominant factor of the degradation mechanism, offering a 98% removal efficiency after 10 minutes in a continuous reactor.

Finally, some toxicity and biodegradability tests were also conducted. The results of the different studies converged to a similar observation: ozone treatment leads to the formation of several by-products (Park *et al.*, 2023), with several being known to be toxic to some aquatic organisms. Li *et al.*, (2008) tested the toxicity of the water after treatment on the marine bacteria *Vibrio fischeri*. They found that for treatment times shorter than 30 minutes, the toxicity of the solution increased after treatment. For prolonged ozonation (*i.e.* more than 30 minutes), the toxicity finally decreased. However, depending on the model species used for the toxicity tests, different results can be observed. For Gorito *et al.*, (2022), no increase in the toxicity was observed for embryos of the zebrafish *Danio rerio*, a common model organism, after continuous treatment. In all the studies mentioned, the BOD₅/COD ratio (*i.e.* the biodegradability index) increased after treatment.

Table 1.4: Experimental conditions and OTC removal efficiency for conventional ozonation studies. TSS = total suspended solid; FF = florfenicol; SDM = sulfadimethoxine; SMX = sulfamethoxazole; TMP = trimethoprim.

Reference	Matrix	[OTC] ₀	[O ₃]	pH	Experimental set-up	Measured parameters	OTC removal
K. Li <i>et al.</i> , 2008	Synthetic water	100–200 mg/l	11 mg/l (gas phase)	3, 7 and 11	Batch (V=1.4 l), 60–120 min	COD, BOD ₅ /COD, toxicity	~90% at pH 7 within 5 min
Uslu and Balcioglu, 2008	Synthetic water + cow manure	5–10 mg/l (water) / 10–50 mg/kg (manure)	11.2–32.7 g/m ³ (gas phase)	5 and 8.5	Semi-batch/ glass bubble column, 5–90 min	COD, BOD ₅ /COD, TOC, toxicity	>97% within 5 min (synthetic water); incomplete after 90 min (manure)
Park <i>et al.</i> , 2023	Synthetic water	5–50 mg/l	4.67–6.29 mg/l (gas phase); flow rate 0.13–0.44 l/min	5.1–5.5	Batch (V=1 l), 60 min	by-products, toxicity	>99%
Gorito <i>et al.</i> , 2022	Synthetic water + real aquaculture effluent (trout farm, Portugal)	10 mg/l (batch) / 100 ng/l (continuous)	~50 g/Nm ³ (batch) / 1.5 mg/l (continuous); flow rate 150–300 Ncm ³ /min	6 - 8	Batch (500 ml) + continuous bubble column, 60 min (batch) / 10 min (continuous)	FF, SDM, SMX, TMP; DOC, COD, BOD, TSS, by-products, toxicity	>98% (continuous, aquaculture effluent); complete <30 min (batch, synthetic water)

1.5.2 Hybrid ozone processes

Aside from direct ozonation, (photo)catalytic ozonation processes have been studied. Their objective is to enhance the efficiency of the degradation of OTC, notably by favorising the generation of radicals and reactive oxygen species (ROS).

1.5.2.1 Catalytic ozonation

In catalytic ozonation processes, a solid catalyst is added to the system. This provides a greater surface area and active sites for the reaction to take place. Solid catalysts also have the advantages of being easily separated from the treated water and can be reused before needing to change them. A type of solid catalyst of great interest are metal oxides. They offer large surface contact areas while maintaining low production costs. In their paper, Mohsin and Mohammed (2021) coupled zinc oxide to the initial sole ozone process (ZnO/O_3). A schematic view of the experimental setup is available in Figure 1.15. Several parameters were recorded, namely the catalyst's impact, the pH, the temperature and the OTC removal.

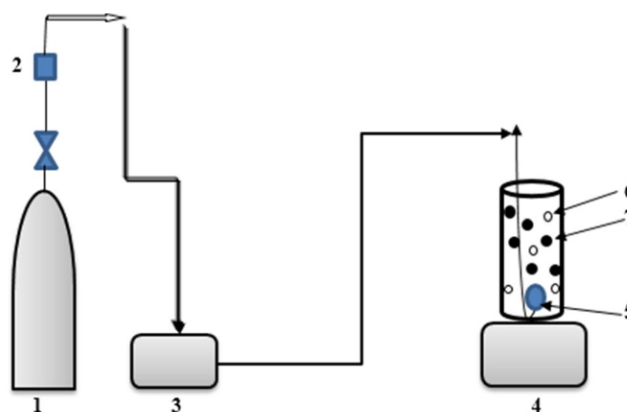


Figure 1.15: Graphic diagram of the experimental set-up for catalytic ozonation: (1) Oxygen source (2) Flow meter (3) Ozone generator (4) Magnetic stirrer (5) Diffuser (6) Ozone bubble (7) Catalyst particles (Mohsin & Mohammed, 2021).

As for direct ozonation, their results showed that the pH was a key factor to the degradation and that alkaline conditions promoted the formation of hydroxyl radicals. The addition of the ZnO catalyst offered better degradation results compared to direct ozonation. This can be assessed in Figure 1.16. Interestingly, the catalyst concentration did not have a significant impact. For a fixed OTC concentration, increasing the amount of ZnO increases the total catalytic surface area without raising the number of pollutant molecules. Consequently, fewer OTC molecules are present per unit of surface exposed to ozone. As a result, a greater proportion of the catalyst's active sites remain underutilised, thereby reducing overall catalytic efficiency.

Tolvanen *et al.*, (2026) realised a similar study to compare catalytic and non-catalytic ozonation. The main difference comes from the pharmaceutical mixture used (*i.e.* five antibiotics with a 40 mg/l concentration each) and the catalysts used: Fe-H-Y and Fe-H-Beta zeolites. It should be noticed that before starting the experiment, the aqueous phase was first saturated with ozone. Ozone was injected until a plateau concentration was reached, which correspond to the gas-liquid mass transfer equilibrium. The antibiotics were then introduced. This procedure enabled easy reproducibility. As for Mohsin and Mohammed (2021), the catalytic ozonation outperformed ozone alone, both for removing the antibiotic and the mineralization by-products. More specifically, the Fe-H-Beta zeolite was identified as the most efficient, with a higher total organic carbon (TOC) removal (82% vs. 63% in the non-catalytic system).

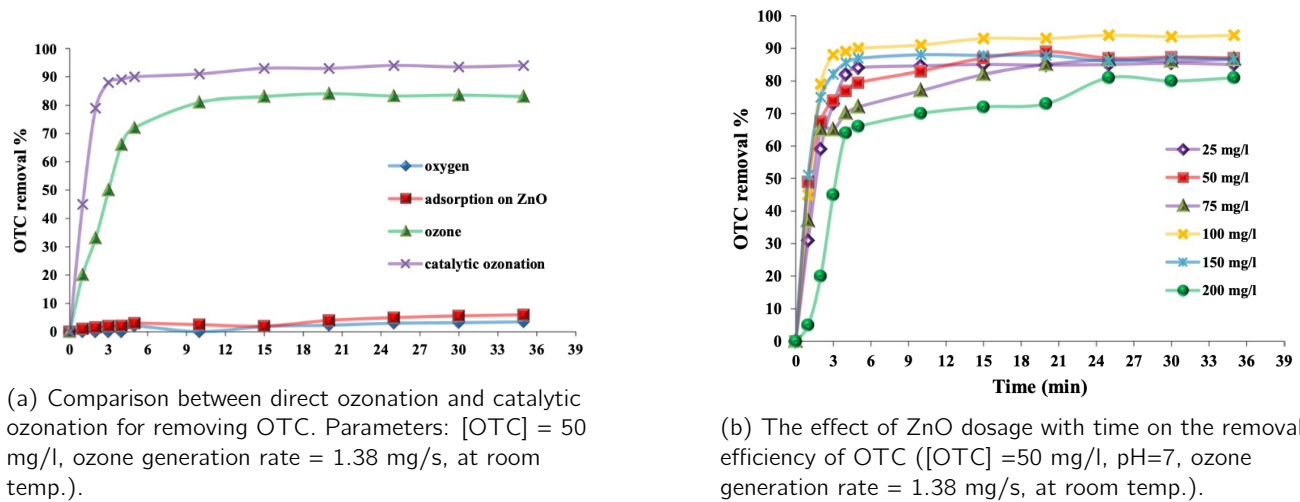


Figure 1.16: Results obtained by Mohsin and Mohammed, 2021.

1.5.2.2 Photocatalytic ozonation

One step further is photocatalytic ozonation. Photocatalysis mainly relies on semi-conductor material which is activated in the presence of light. This results in the production of ROS species that can oxidise antibiotics and micropollutants.

A series of article that are particularly important within the framework of this master's thesis are the one written by Mahy *et al.*, (2019; 2020). An ozonation/UVC photocatalytic reactor was developed at the laboratory and pilot scales in order to remove 22 micro-pollutants from wastewater. Titanium dioxide (TiO_2 , $\epsilon_0 = 3.2\text{eV}$) photocatalytic coating was employed given its stability, efficiency and widespread use. For each pollutant, a $10\mu\text{g/l}$ concentration was selected. The process took place in two steps. First, the solution was ozonated for 30 minutes ($[\text{O}_3]=15\text{ mg/l}$). It was then transferred in a crystallizing lamp and set under UV-C light ($\lambda = 254\text{ nm}$, 20 W/m^2) for six hours, with samples taken after 1, 2, 4 and 6h. A schematic view of the pilot scale reactor is visible in Figure 1.17. The results of this study revealed that after the ozonation step, 5 micro-pollutants were already completely removed, and 8 partially. After the UV-C + TiO_2 treatment, 9 additional antibiotics were totally degraded, which highlights the necessity to combine both techniques. This resulted in the removal of 16 out of the 22 micro-pollutants initially present (see Figure 1.18).

These results are confirmed by the review made by the team of Pelosato *et al.*, 2022. It highlighted that degradation was overall improved when doped photocatalytic materials were placed under UV light. As for catalytic and non-catalytic ozonation, the pH was still a predominant factor in the generation of radical species. The use of UV light enhanced this production. Other photocatalytic substances were investigated such as ZnO or graphitic carbon nitride ($\text{g-C}_3\text{N}_4$). The first exhibited a high photocatalytic activity, yet a low stability in aqueous media.

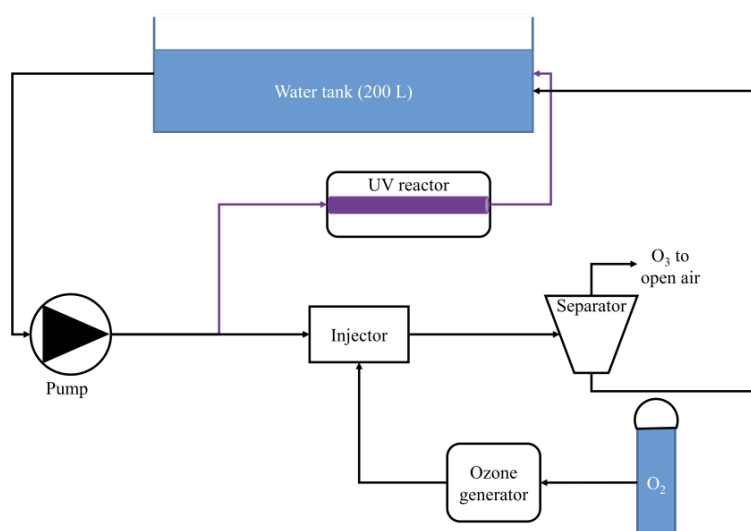


Figure 1.17: Diagram of the pilot scale reactor developed by Mahy *et al.*, 2019.

Table 3. Degradation efficiencies in absolute percentage on the model polluted water for the sprayed sample at laboratory scale.

Pollutant	Degradation with O ₃ (%)	Degradation with UVC (%)	Degradation with UVC - photocatalysis (%)	Total degradation (%)
Lindane	0	0	18	18
Atrazine	0	100	–	100
Brominated diphenyl ether	0	100	–	100
Tributylphosphate	0	0	62	62
Di(2-ethylhexyl)phthalate	0	9	74	83
Metoprolol	50	11	39	100
Carbamazepine	100	–	–	100
Diclofenac	100	–	–	100
Sulfamethoxazole	100	–	–	100
1H-benzotriazole	0	84	10	94
Desethylatrazine	0	100	–	100
2,6-dichlorobenzamide	0	0	32	32
Bromacile	85	15	–	100
Simazine	100	–	–	100
Chlortoluron	100	–	–	100
Isoproturon	100	–	–	100
DDT	9	91	–	100
Acetyl-4-Sulfamethoxazole	43	57	–	100
Iohexole	6	94	–	100
Iopromide	0	100	–	100
Clarithromycin	100	–	–	100
Terbutryn	100	–	–	100

–: already eliminated before this treatment.

Figure 1.18: Degradation results after the ozonation and UV-C+ TiO₂ treatments Mahy *et al.*, 2020.

1.6 Key messages and work plan

This literature review has enabled several key messages to be highlighted. Firstly, shrimp farming in the Mekong Delta relies heavily on the use of antibiotics, which are largely unregulated. One of the most widely used molecules is oxytetracycline, renowned for its effectiveness and affordability. In order to minimise the risk of bacterial overpopulation and viral diseases, the use of antibiotics is one of the only effective solutions for farmers, despite leading to water pollution. Therefore, treating the wastewater after the shrimp harvest and before its release into the Mekong is essential. In the longer term, treatment processes that are sufficiently efficient to eliminate viruses and maintain bacteria within acceptable limits could contribute to the gradual reduction in antibiotic use.

On the other hand, literature confirms the effectiveness of ozonation in removing organic micro-pollutants, such as antibiotics. Two action pathways have been identified: direct ozone attack and indirect attack via the *in situ* formation of highly reactive hydroxyl radicals (under alkaline pH conditions). Sole ozonation

processes have achieved a removal rate of up to 98% for oxytetracycline in just a few minutes. In parallel, hybrid processes have been developed. These aim to combine ozonation with other methods, such as solid catalysts, photocatalysis or H_2O_2 . One of the main limitations identified in the literature is the impact of the water matrix on degradation efficiency, for example, in shrimp pond water. Furthermore, research into by-products and their effects is limited.

This Master's thesis arises in this context. First, an ozonation protocol will be validated using the well-documented model pollutant PNP. The work will then aim to characterize the efficiency of ozonation in degrading $\text{OTC} \cdot \text{HCl}$ under various operating conditions, including antibiotic concentration, ozone dosage, salinity, and the choice of bubbling device. Based on these results, an initial evaluation of the reaction kinetics will be conducted. Finally, exploratory experiments will be performed. The first experiment will expand the scope of the antibiotic to include rifampicin. The second experiment will focus on inactivating the *Pantoea agglomerans* bacterial strain. These results will lay a solid experimental foundation for the future development of a water treatment process adapted to this context.

Chapter 2

Experimental section

2.1 Chemicals

Several chemical compounds were used throughout the experiments. A complete list, including the catalogue and batch number of each chemical, is available in the Appendix [A](#).

2.2 Experimental ozonation set-up

To study the degradation of a chemical compounds by ozonation, an experimental device had to be set up and is presented in [Figure 2.1](#).

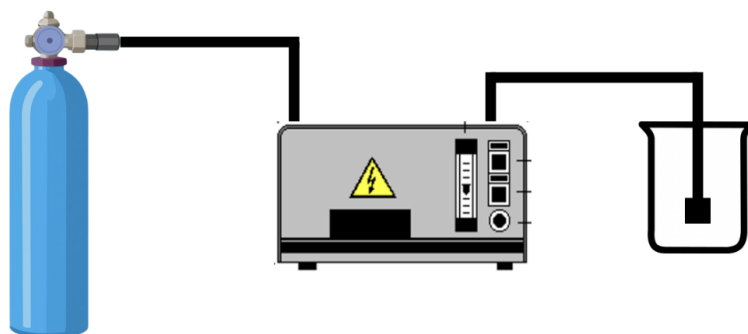


Figure 2.1: Experimental ozonation set up used. From left to right: air tank, ozone generator, bubbler, reactor.

Air from the ALPHAGAZ 1 AIR tank (Air Liquide, Belgium) is sent to an ozone generator (Triogen Lab2B). Since ozone is a very instable gas, it cannot be stored and has to be produced *in situ*. The overall reaction for ozone formation is given by [Equation 2.1](#) and is endothermic ($\Delta H^\circ = +142.2$ kJ/mol).



The air volumetric flow rate is set using the rotameter incorporated on the ozone generator. The oxygen in air is converted into ozone through a corona discharge. The percentage of ozone wanted at the outlet is regulated by a variable control knob ranging between 0 and 100%, which adjusts the frequency of the alternating current applied to the electrodes. As this frequency increases, the number of corona discharge cycles per second increases, which dissociates more O_2 molecules and thus produces more O_3 (Triogen, 2022). The gas containing O_3 (*i.e.* a N_2 , O_2 , O_3 mixture) is then continuously injected into a glass bottle via a bubbler equipped with a glass sintered filter.

Consequently, the set-up can be associated to a semi-batch reactor with continuous ozone feed and batch operation for the pollutant.

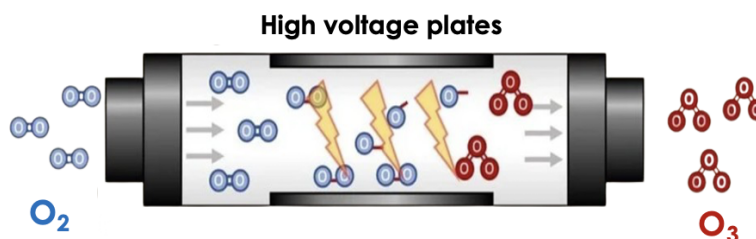
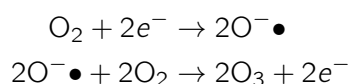


Figure 2.2: Schematic representation of the ozone formation by a corona discharge (A. Roth *et al.*, 2023).

The core concept behind the corona discharge is to force a flow of dry gas containing oxygen (*i.e.* the gas vector) between two metal electrodes with high voltage (see Figure 2.2). One of the electrodes must be covered by a dielectric material. An electric discharge is generated by applying a high-frequency alternating voltage between the electrodes (typically in the kHz range). For the Lab2B ozone generator used, the dielectric material is ceramic and the electrodes are made of 316 stainless steel. These discharges tend to dissociate the gas and form a "cold plasma", which is unstable and highly ionized. A rupture of the bonds between two stable oxygen molecules occurs, leading to single oxygen atoms ($O^{\bullet}$ radicals). The unstable oxygen radicals then interact with stable O_2 , forming an ozone (O_3) molecule (A. Roth *et al.*, 2023; Sarron *et al.*, 2021). The mechanism behind this reaction is,



2.3 Strain of bacteria cultured for the experiments

To study the degradation of bacteria by ozonation, a strain of non-pathogenic bacteria was cultured: *Pantoea agglomerans* (see Figure 2.3). It is an opportunist Gram negative aerobic bacillus from the family *Enterobacteriaceae* (Cruz *et al.*, 2007). The strain was provided and cultured by Pr. Damien Thiry from the Department of Infectious and Parasitic Diseases at the Faculty of Veterinary Medicine and Center for Fundamental and Applied Research for Animals and Health (ULiège). The cell sub-culture was realised under the supervision of Pr. Célia Joaquim-Justo from the Laboratory of Animal Ecology and Ecotoxicology (ULiège).

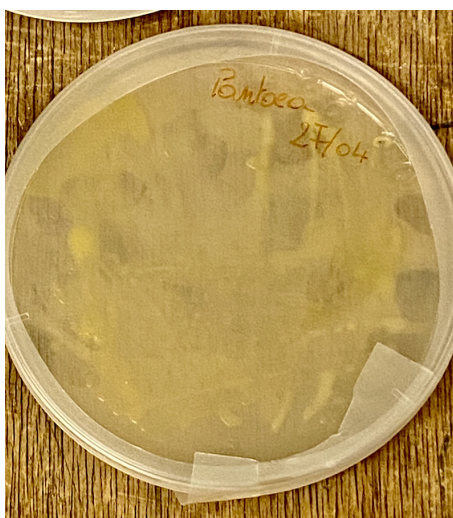


Figure 2.3: *Pantoea agglomerans*

2.4 Sample characterization techniques

2.4.1 UV-visible spectrophotometer

The degradation of a chemical compound can be followed by measuring its absorbance. In other words, the measure consists in *"following the amount of discrete wavelength (λ) of UV ($100 < \lambda < 380$ nm) or visible light ($380 < \lambda < 780$ nm) absorbed by a sample, compared to a reference sample"* (Tom, Justin, 2023). This was done by using the GENESYS UV-visible spectrophotometer manufactured by Thermo Scientific (see Figure 2.4).



Figure 2.4: GENESYS 150 UV-visible spectrophotometer used for the experiments.

The central idea behind this technique is that light has an energy that is inversely proportional to the wavelength. Thus, a short wavelength will generate more energy. For a given substance, a specific amount of energy is needed to promote an electron to a higher energy state which detects absorbance. From this, each substance has its own energy and wavelength.

The function of the UV-vis spectrophotometer (see Figure 2.5) can be explained in five steps¹ (Tom, Justin, 2023):

1. **Choice of the light source:** The instrument requires a stable light source capable of emitting light across a broad range of wavelengths. Most often, a xenon lamp is selected due to its high intensity in both UV and visible light. In this type of lamps, the electric current passes through a xenon atmosphere, inducing a radiation in a continuous spectrum ranging from 200 to 1000 nm. Despite its high intensity, this type of lamp presents some disadvantages compared to tungsten or halogen lamps, notably in terms of cost and stability.
2. **Wavelength selection:** This step is particularly important as a specific wavelength adapted to the sample must be isolated. To do so, a monochromator is employed. It separates the light into several narrow wavelengths. The wanted wavelength is then obtained thanks to diffraction gratings. These rotate to reflect light with the right angle. Other filtering methods exist, such as absorption, interference, cutoff or bandpass filters. They are sometimes combined with a monochromator to improve measurement precision.
3. **Sample analysis:** Before analysing the sample, a blank measurement must be performed using a PMMA cuvette filled with the same solvent as the sample, but without the compound of interest. This reference measurement serves as a basis for comparison. Indeed, the composition of the sample greatly influences the results. During the analysis, light passes through the sample.

¹The steps described correspond to the operating procedure for a dual-beam spectrophotometer equipped with a xenon lamp, such as the GENESYS 150 used in these experiments. Other configurations exist, notably single-beam devices.

4. **Detection:** After passing through the sample, the light reaches a detector that converts it into an electrical signal proportional to the light intensity. Two types of detectors exist: photoelectric coatings and semi conductors. For photoelectric coatings, a negatively charged electron is ejected when exposed to light.
5. **Output signal:** The electrical signal generated by the detector is then processed and displayed on a computer, typically it is shown as an absorbance spectrum being a function of wavelength.

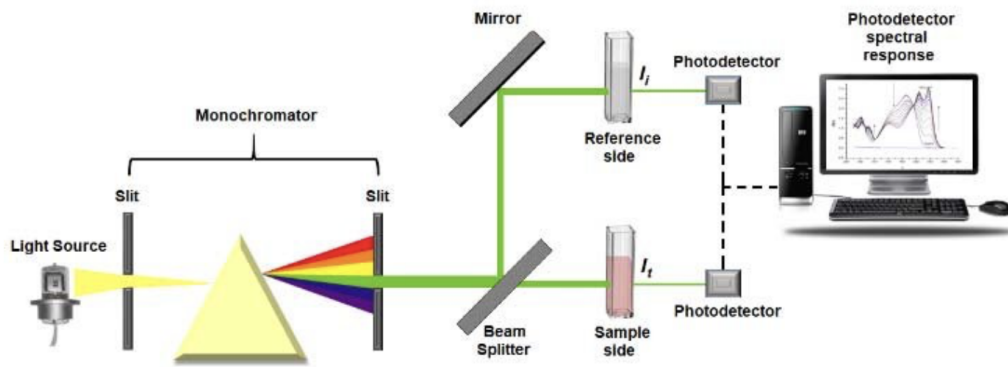


Figure 2.5: Schematic diagram of a dual beam UV-Vis spectrophotometer (Braga *et al.*, 2019).

More specifically, the absorbance is obtained by measuring the light intensity before and during the passage through the sample,

$$A = \log_{10} \left(\frac{I_o}{I} \right) = -\log_{10}(T) \quad (2.2)$$

where I_o is the light intensity before the sample, I is the intensity of the light passing through the sample. $\frac{I}{I_o}$, or the transmittance T , expresses the amount of light that passed through the sample.

UV-Visible (UV-Vis) spectroscopy is commonly used in several sectors such as DNA and pharmaceuticals analysis, quality controls in the food industry and wastewater treatments. This technique has several advantages and drawbacks such as,

ADVANTAGES	DRAWBACKS
Non destructive for the sample	Interference of absorbing species
Quick measures	Light scattering
Easy to use	Stray light

Within the framework of this master thesis, UV spectroscopy was considered as a quantitative method, even though no internal standards were used. This premise has already been discussed in the literature (W. A. Khan *et al.*, 2026; Kaur *et al.*, 2021) and will be demonstrated by the different curves obtained throughout this report.

A calibration curve for the analysis of OTC · HCl with the UV-Vis spectrophotometer was made and is detailed in Appendix C.2.

2.4.2 Measure of the total organic carbon (TOC)

The objective of this characterization technique is to quantify the carbon present in a sample coming from organic matter. The analysis of the Total Organic Carbon (TOC) is a well-known method for wastewater treatment and is frequently used as a global indicator of the water quality. TOC is even considered as the

"most suitable index for the control of civil and industrial wastes". However, it only offers a quantitative view of the carbon in the sample, without detailing the origin of it. Thus, the measure is non-specific. Finally, TOC can be of natural or anthropic source, such as fertilizers or pesticides (Visco *et al.*, 2005). The TOC-L from Shimadzu was used to conduct the experiments (see Figure 2.6).

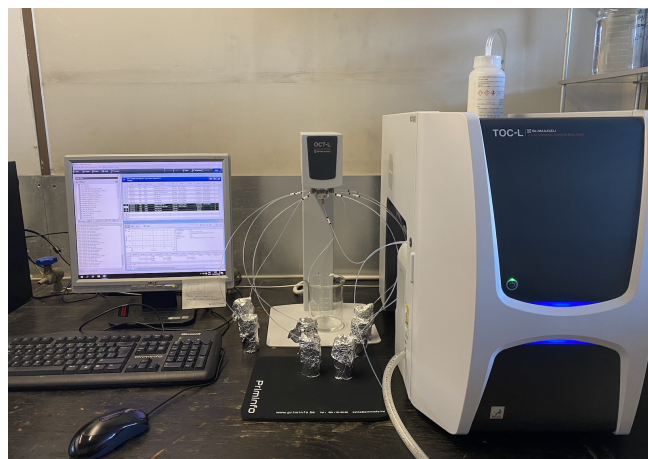


Figure 2.6: Shimadzu TOC-L machine used for the experiments. From left to right: computer for result display, 8-port valve and TOC-L machine.

Before detailing the measurement of TOC, it is worth defining important terms (see Figure 2.7), as stated by the EN 1484 standard (Shimadzu Europe, 2026):

- **Total Carbon (TC):** All carbon species in a sample, both organic and inorganic.
- **(Total) Inorganic Carbon (IC):** It targets more specific compounds such as bicarbonate (HCO_3^-), carbon monoxide (CO), carbonate (CO_3^{2-}) and dissolved carbon dioxide (CO_2). Inorganic carbon compounds can be purged by either gassing them off or decreasing the pH to shift the equilibrium to CO_2 .
- **Total Organic Carbon (TOC):** It represents the carbon that is organically bound and present in water. The inorganic carbon is removed from the total carbon in a sample to determine the total organic content ($\text{TC} - \text{IC} = \text{TOC}$).
- **Non-Purgeable Organic Carbon (NPOC):** It is the fraction of total organic carbon in water that cannot be removed by purging (stripping) with gas after the sample has been acidified.
- **Purgeable Organic Carbon (POC):** It measures organics that are volatile or semi-volatile ($\text{POC} = \text{TOC} - \text{NPOC}$) and that can be purged.

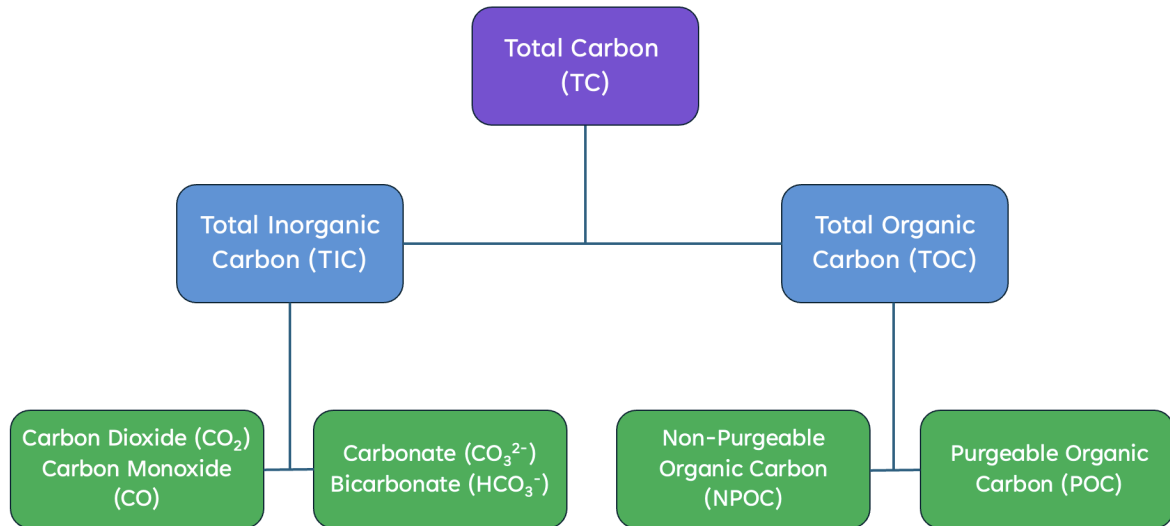


Figure 2.7: Repartition of the different types of carbon. The total carbon can be split into the total inorganic (TIC) and total organic (TOC) carbon. (Veolia, 2023).

Several methods exist to determine the TOC, namely the difference, addition or direct method.

For the **difference method**, the TOC is obtained by measuring the IC and TC. TC is calculated by thermal oxidation of the sample. The sample is injected into a furnace at 680°C and all the organic carbon is incinerated. The evaporated CO₂ is then measured by Non-Dispersive Infrared (NDIR) detection. To find the IC, the sample is acidified to a low pH, which converts all inorganic carbon species (dissolved CO₂, HCO₃⁻ and CO₃²⁻) into CO₂ gas. This CO₂ is then expelled by stripping and measured by NDIR. This can be explained by Figure 2.8. At low pH, the IC is mainly due to dissolved CO₂, which can be easily extracted by stripping and measured. Finally, TOC = TC - IC. The main drawback related to this method is the error propagation. To mitigate the risk, the EN 1484 standard recommends that TOC ≥ IC (Shimadzu Europe, 2026).

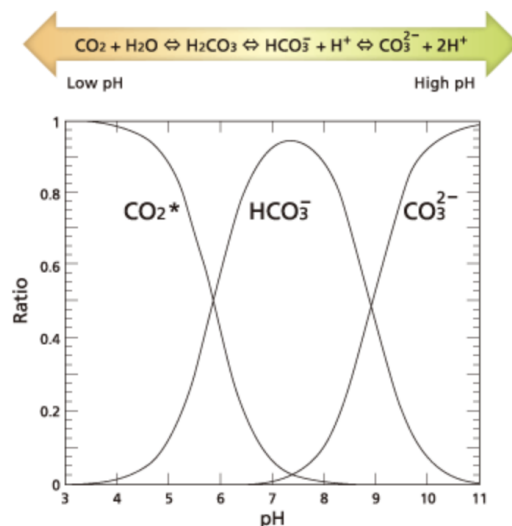


Figure 2.8: Bjerrum plot of carbonate species depending on the pH (Shimadzu United Kingdom, 2026).

The **addition method** calculates the TOC by summing the POC and NPOC values, while the **direct method** suggests that the sample does not contain any volatile compounds and considers that TOC = NPOC.

A calibration curve for the TOC-L machine was made and is detailed in Appendix C.1.

2.4.3 High Performance Liquid Chromatography - Mass Spectrometry (HPLC-MS)

HPLC-MS (see Figure 2.9) is a quantitative analytical method that combines high performance liquid chromatography (HPLC) and mass spectrometry (MS). The objective of HPLC is to separate chemical compounds in a solution, while MS identifies them according to their mass. This coupled method is frequently used in sectors such as pharmacy, food and water analysis, and environmental studies.

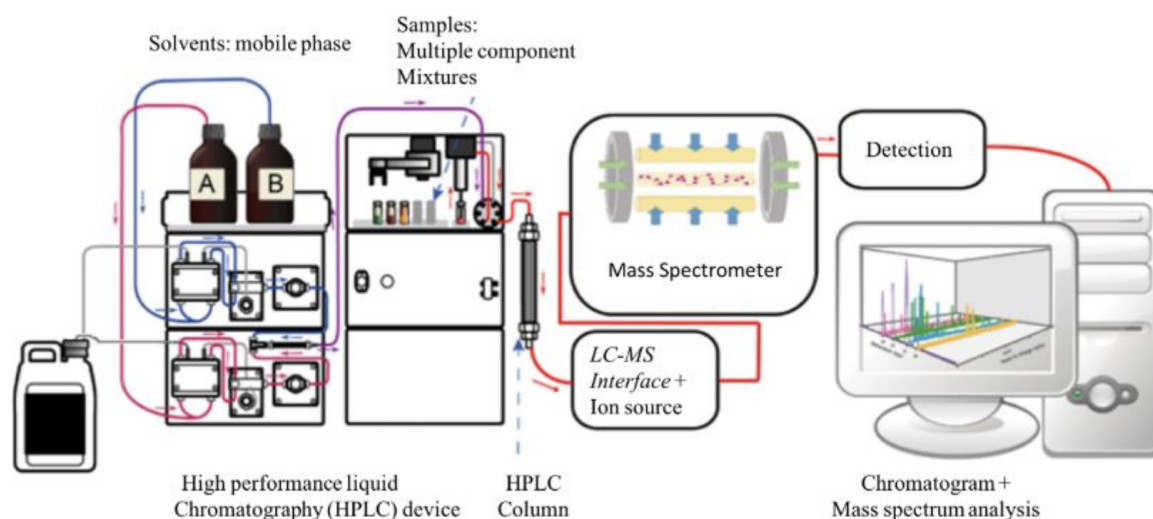


Figure 2.9: Schematic diagram of an HPLC-MS machine (Pankaj, 2023).

2.4.3.1 High Performance Liquid Chromatography

Chromatography comes from the Latin words "*chrôma*", meaning color, and "*gráphô*", meaning writing. The objective of high-performance liquid chromatography (HPLC) is to separate compounds in a mixture. This is achieved by passing a mobile phase through a stationary phase that is packed in a chromatography column. The separation of the products occurs in the stationary phase. The main steps of an HPLC analysis are (Thermo Fisher Scientific, n.d.):

1. A pump pushes the solvents through the system, starting the flow of the mobile phase.
2. The samples are injected into the mobile phase and travel to the head of the column.
3. The physical separation of the components occurs on the stationary phase inside the column.
4. The separated compounds then move to the detector for identification of the analytes. This is made possible by the electrical signals they generate.
5. The electric signal is then translated into a chromatogram of the analyte versus time.

The separation of the components in the column can be achieved based on different key parameters such as the *polarity*, *molecular mass* or *electrical charge*.

Two main techniques exist for polarity-based separation: normal phase and reverse phase chromatography. The main difference lies from the polarity of the stationary phase. In **normal-phase** chromatography, the stationary phase is polar and the mobile phase is non-polar. This technique is primarily used to separate compounds with similar polarities. Polar components bind to stationary phase and non-polar ones elute faster with the mobile phase. For **reverse-phase** chromatography (see Figure 2.10), it is the opposite.

The stationary phase is non-polar, while the mobile phase is polar. Based on hydrophilic interaction, the non-polar compounds take more time to exit the column, while polar molecule travel faster through it. This second method is especially interesting for the analysis of pharmaceuticals. Due to their versatility, the reverse-phase columns are the most frequently used as they are suitable for a wider range of molecules (Hawach Scientific, [n.d.](#)). The column used in the master's thesis, is a reverse phase column.

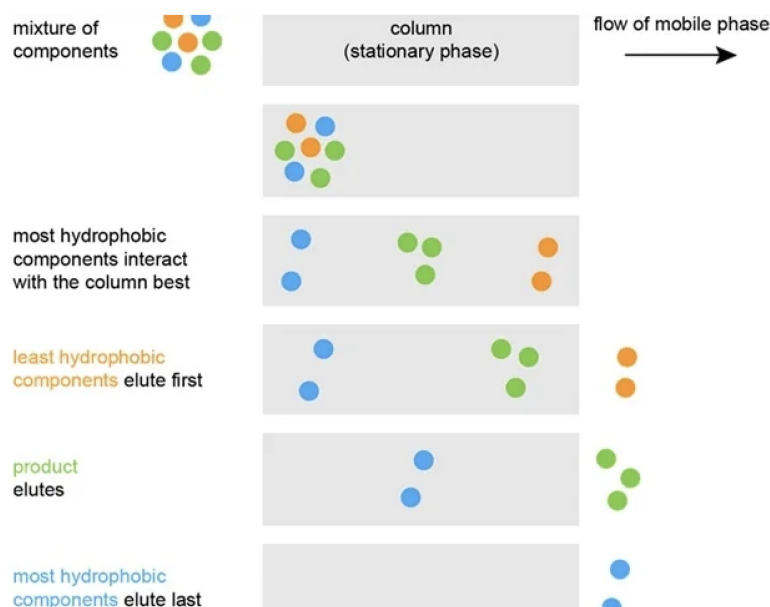


Figure 2.10: Schematic diagram of a reverse phase chromatography column (Shaffer, Catherine, [n.d.](#)).

2.4.3.2 Mass Spectrometry

Once the chemical compounds have been separated in the HPLC column, they are sent to the MS. Its objective is to identify and quantify molecules based on their mass-to-charge ratio (m/z), "where m is the molecular weight of the ion (in daltons), and z is the number of charges present on the measured molecule" (Urban, 2016).

A mass spectrometer (see [Figure 2.11](#)) is composed of three primary components: the ion source, the analyzer, and the detector. The following steps are to be observed in the course of an MS analysis (Henrik's Lab, 2024):

1. **Electrospray ionization (ESI):** Molecules are transferred to the gas phase. In this step, charged ions are generated. To carry out this process, the sample is passed through a capillary under an applied electric field. Electric repulsion leads to the formation of a fine aerosol, which subsequently evaporates, resulting in the generation of charged gas-phase ions. Ions are then accelerated in a focused beam.
2. **Time-of-flight (TOF) analyzer:** Ions are accelerated in an electric field with a known voltage. Atoms with the same charge possess the same kinetic energy. It has been determined that lighter ions travel at a faster rate than heavier ones. The same reasoning applies to more electrically charged ions, which are faster. The time of flight (TOF) is then measured, and the m/z ratio is calculated based on the distance traveled.
3. **Ion identification:** The results are represented in the form of a chart displaying the relative abundance versus m/z ratio. It is a well-established fact that the higher the peak, the greater the abundance of the ion. In order to identify the ion, it is necessary to compare the m/z ratio to the masses listed in the periodic table of elements. Tables are available for more complex molecules.

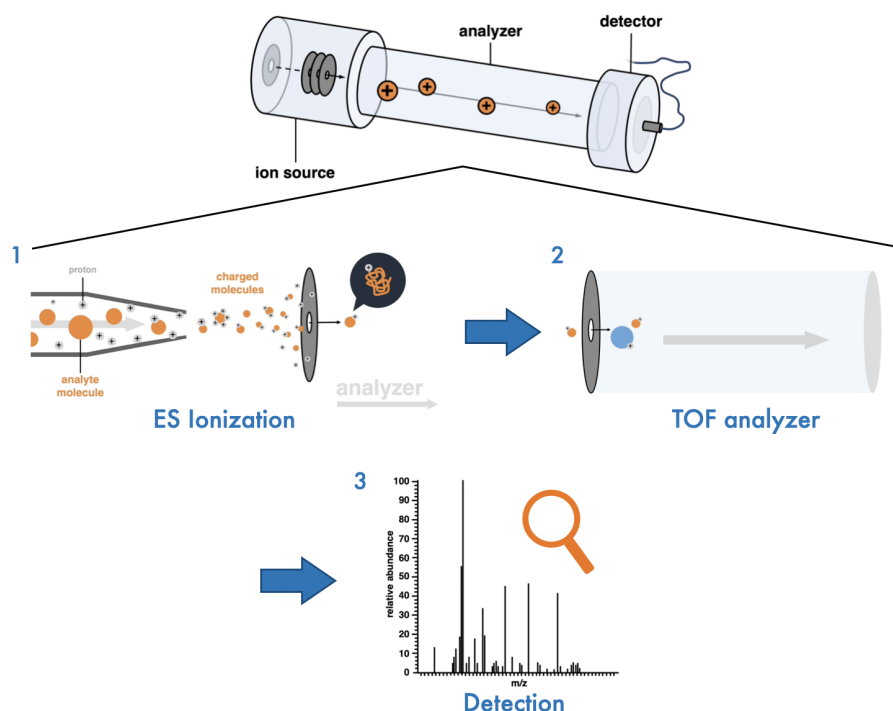


Figure 2.11: Schematic diagram of the main components of a mass spectrometer (Adapted from Henrik's Lab, 2024).

2.4.3.3 Protocol for the analysis

In order to use the HPLC-MS machine, a specific protocol had to be followed for each analysis requiring this equipment. It was kindly provided by Dr. Caroline Douny from Laboratory of Food Analysis, Department of Food Sciences, Faculty of Veterinary Medicine, Fundamental and Applied Research for Animals & Health (ULiège). It is available in Appendix D.

2.5 Preliminary degradation tests on PNP with ozone

As presented in Section 1.4, PNP is a frequently used model pollutant. Thus, before going deeper in the degradation mechanism of antibiotics, some preliminary tests were performed with this molecule. The degradation of PNP was monitored by following its absorbance, using the UV-Vis spectrophotometer. The experimental set up used for this section is presented in Figure 2.12. The sintered filter was fixed on a Drechsel safety tube and was purchased on Carl Roth. It had a diameter of 24 mm and a pore size between 100 and 160 μm .

2.5.1 Blank curves

Prior to performing degradation tests of PNP, some blank curves had to be done. These curves enable to determine whether PNP can degrade by itself (*i.e.* without bubbling ozone in the solution) by contact with air or under stirring. These will serve as a reference to ensure that the degradation observed can be attributed to ozone only.

2.5.1.1 Regulation of the ozone dose injected by the generator

The ozone generator used produces ozone from air and two parameters can control the ozone dose injected in the solution:

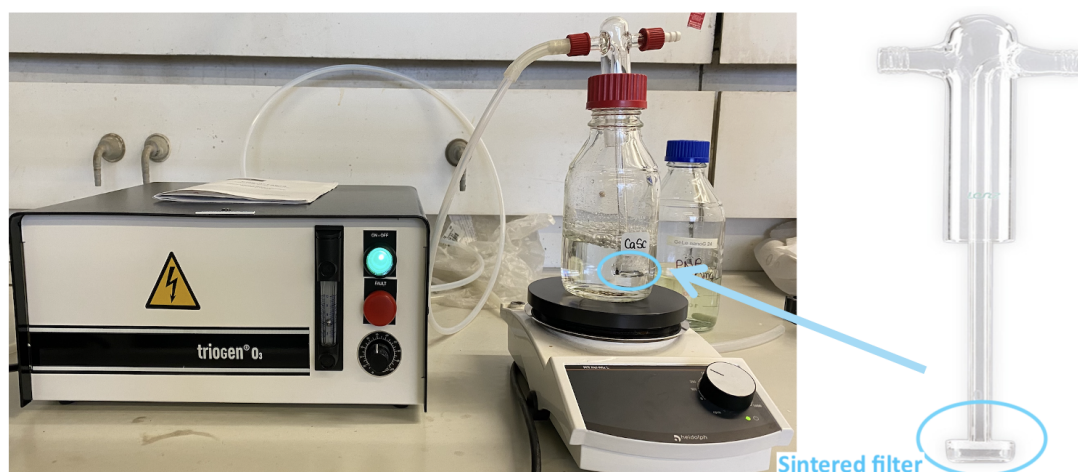


Figure 2.12: Experimental set up used for the experiments with PNP.

1. **The air volumetric flow rate (l/min):** It determines the rate at which air passes through the generator.
2. **The ozone output (%):** The percentage of ozone present in the output gas flow is regulated by a rotating knob ranging between 0 and 100%. The higher the percentage, the greater the O_3 concentration and molar flow rate injected.

To know the ozone concentration and mass flow rate related to a given volumetric flow rate/ ozone output duo, the manufacturer provided two references, presented in Figure 2.13. For this test, the air volumetric flow rate is set to 4 l/min and the Pot Setting is fixed to 5.5 (i.e. 50% in ozone output). This corresponds to an ozone concentration of 6.5 mg/l and an ozone mass flow rate of 1.5 g/h. To ensure unit consistency throughout this report, these values are converted to mmol/l and mmol/h, respectively. To do this, divide the “mass” values by 48 g/mol (the molecular mass of O_3). Thus, an ozone concentration 0.13 mmol/l and an ozone molar flow rate of 31 mmol/h are found.

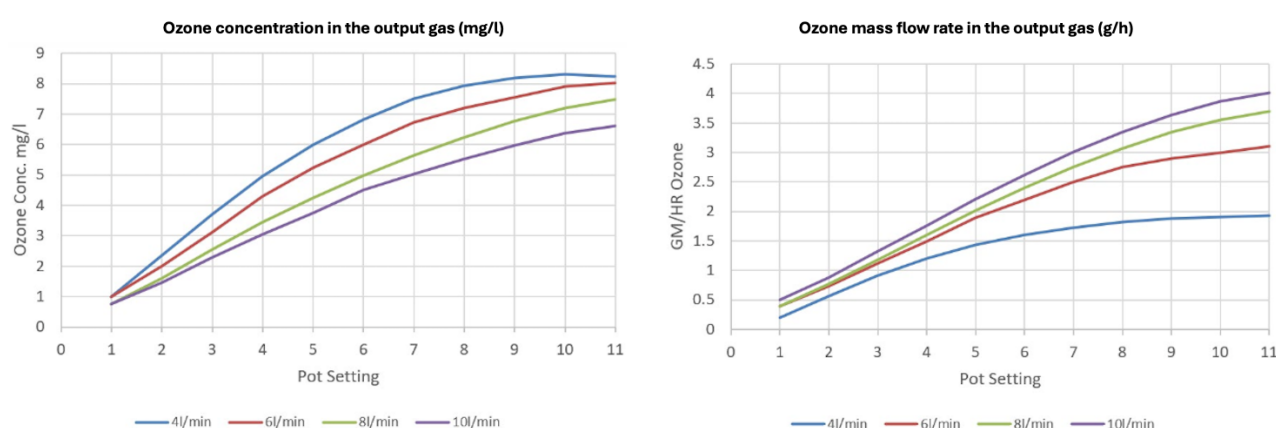


Figure 2.13: Performance curves for the Triogen Lab2B ozone generator. Left: ozone concentration (mg/l). Right: ozone mass flow rate (g/h) as a function of the potentiometer setting (Pot Setting), for different air flow rates (l/min). The potentiometer ranges from 0 to 11. 0 = 0% ozone and 11 = 100% ozone in the output flow (adjusted via the rotating knob).

2.5.1.2 Blank curves conditions

Three conditions were studied, all under stirring:

1. **Ozone generator turned on and 0% in ozone output:** The ozone generator is turned on and fixed to a volumetric flow rate of 4 l/min but 0% in ozone output (Pot Setting = 0). This condition examines the effect of bubbling air to the solution without ozone.
2. **Ozone generator turned off, but air passed through:** Air from the air tank is bubbled into the solution with a volumetric flow rate of 4 l/min. This condition highlights the effect of bubbling.
3. **The solution was left out in the open air:** The solution is simply left in the hood to open air, without bubbling nor passing through the ozone generator. This conditions follows the natural degradation of PNP.

Three blank conditions were analyzed to confirm that no ozone is generated at an ozone output of 0% and to determine whether PNP undergoes degradation when exposed solely to air (with no O₃).

The stirring speed was 250 rpm. A 0.36 mmol/l stock solution of PNP was prepared by weighing 50 mg of chemical and diluting it into 1 l of milliQ water (18.2 mΩ). This stock solution was kept for all further experiments. For this test, a 0.14 mmol/l solution was made by taking 80 ml of stock solution and adding milliQ water to reach a volume of 200 ml. Then, 1 ml samples were taken after 0-15-60-150-240-330-420-510 seconds (8 min 30 sec) for a total of 8 samples. To analyze the samples with the UV-Vis spectrophotometer, they were placed into PMMA cuvettes (VWR) for analysis. The absorbance of PNP was measured at a wavelength of 317 nm.

However, two forms of PNP may co-exist, depending on the pH of the solution: protonated (at acid pH) and deprotonated (at alkaline pH). To ensure that only the protonated form is present, a drop of sulfuric acid (H₂SO₄) was added to the sample before measuring the absorbance. Both forms of PNP are shown in Figure 2.14. Therefore, a drop of H₂SO₄ will be systematically added to sample in all further experiments.

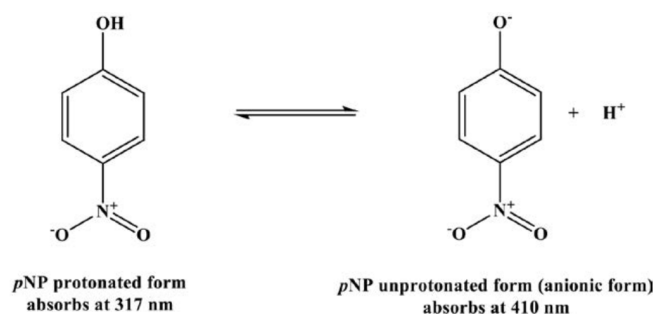


Figure 2.14: Acid dissociation equilibrium of PNP (Syedd-León *et al.*, 2020).

2.5.2 Replicates

A second point of concern was the reproducibility of results when ozone is bubbled into the reactor. To assess reproducibility, five replicates were conducted under the following conditions: initial PNP concentration of 0.11 mmol/l and a ozone flow rate of 31 mmol/h (*i.e.* an air flow rate of 4 l/min and ozone output of 50%, see Figure 2.13). To this end, 60 ml of the 0.36 mmol/l stock solution of PNP was added to 140 ml of milliQ water, reaching a reactor volume of 200 ml. As previously, eight samples were taken over 8 min and 30 seconds and analyzed with the UV-Vis spectrophotometer. The analysis procedure remained the same.

2.5.3 Degradation of PNP over time with various initial concentrations

To assess the degradation of PNP over time, four initial PNP concentrations were selected (0.036-0.07-0.11-0.14 mmol/l) and a constant ozone flow rate of 31 mmol/h was chosen. To obtain these concentrations, the dilutions shown in Table 2.1 were made from the 0.36 mmol/l stock solution.

Table 2.1: Volumes to be taken from the PNP stock solution 0.36 mmol/l to prepare the concentration range 0.036-0.07-0.11-0.36 mg/l for the degradation test. For each concentration 200 ml are prepared.

Concentration (mmol/l)	Stock solution volume (ml)	MilliQ water volume (ml)
0.036	20	180
0.07	40	160
0.11	60	140
0.14	80	120

The absorbance was measured as previously explained, with 8 samples of 1 ml taken over 8 min and 30 seconds and analyzed with the UV-Vis spectrophotometer. The procedure related to the analysis remained unchanged.

2.5.4 Influence of the O₃ flowrate

A first insight of the kinetics of ozonation can be observed by studying the influence of the ozone input flow rate on the degradation of PNP. Four ozone flow rates were selected (17 - 21 - 26 - 31 mmol/h) by setting a constant volumetric flow rate of air of 4 l/min at the input and using the ozone output control of the ozone generator (20 - 30 - 40 - 50%) to change the percentage of ozone in the output feed (0.06 - 0.09 - 0.11 - 0.13 mmol/l). The initial PNP concentration was fixed to 0.11 mmol/l and was obtained by diluting 60 ml of the stock solution into 140 ml of milliQ water. The procedure for the sampling and measurement of the absorbance with the UV-Vis spectrometer remains identical.

The range of ozone flow rate was chosen to match the ones selected by Singh and Majumder (2025) (*i.e.* 0.35 - 0.50 mg/s), enabling easy comparison. The 0.11 mmol/l PNP concentration ensures a good degradation of PNP and absorbance values within the linearity domain of the Beer-Lambert law (*i.e.* for absorbances between [0, 1.2] (Région Académique Île-de-France, 2026)), whose general form is given by,

$$A = \epsilon \cdot l \cdot C \quad (2.3)$$

where A is the absorbance (-), ϵ is the molar extinction coefficient² (l/mol.cm), l is the length of the pathway (cm) and C is the concentration (mol/l).

2.5.5 Use of two complementary analysis techniques: UV-Vis spectrometry and TOC

Analysing the absorbance with a UV-visible spectrophotometer provides valuable insight into the degradation of PNP. However, it does not allow to quantify the amount by-products or the quantity of remaining organic compounds. One way to obtain this information is to perform a total organic carbon (TOC) analysis. This was achieved using the Shimadzu TOC-L Total Organic Carbon Analyser (see Section 2.4.2).

To assess the complementary of the information obtained, a 0.11 mmol/l PNP solution was prepared by diluting 69 ml of the 0.36 mmol/l PNP stock solution with 161 ml of Milli-Q water, resulting in a final volume of 230 ml. A continuous ozone flow rate of 31 mmol/h was injected. Samples were taken for

²This coefficient is dependent of the studied molecule, the solvent and the wavelength.

both the TOC and absorbance measurements at times $t=0$ and 4 minutes. During the TOC experiment, the sample volume was of 30 ml, while 1 ml was taken for the spectrophotometer.

2.6 Study of the degradation of OTC · HCl with ozone

2.6.1 First degradation test

To get a first insight of the degradation of OTC · HCl with ozone, a degradation test over six hours was set up. The evolution of the concentration of OTC was followed using three different analysis techniques: UV-Vis spectrophotometer, TOC and HPLC-MS. The experiment was repeated three times to prove reproducibility, however, only one curve was analyzed with the HPLC machine.

A 0.2 mmol/l stock solution was prepared by weighing 0.0994 g of OTC · HCl, which was kept in a glove-box and under an argon atmosphere. It was then diluted in 1 liter of milliQ water. For the experiment, a total volume of 400 ml was necessary and an initial concentration of OTC · HCl of 0.1 mmol/l (≈ 50 mg/l) was targeted. Thus, 200 ml of stock solution was added to 200 ml of milliQ water. The ozone flow rate was set at a flowrate of 31 mmol/h and an inlet gaseous O_3 concentration of 0.13 mmol/l. The ozone generator was let to stabilize for 10 minutes before starting the experiment. The solution was continuously stirred at 250 rpm and ozone was injected through a bubbler.

20 ml samples were taken after 0-5-15-30-120-240-360 min for the TOC analysis. The total volume taken is 140 ml, representing a volume variation of 35%. Since the solution used only dealt with a powder (*i.e.* OTC · HCl) homogeneously dispersed in the reactive system, it was not necessary to take account of the volume variation. Indeed, Pirard *et al.*, (2014) showed that the volume variation due to sampling did not influence the results. However, sampling volume may have a significant impact, namely when a photocatalytic substance coated on a film is used to degrade a pollutant. For the replicate used for the HPLC-MS analysis, 0.5 ml samples were taken after 0-2-5-15-20-30-60-120-150-180-210-240-270-300-330-360 minutes to get a complete and detailed view of the degradation. For the UV-Vis spectrophotometer, samples of 1 ml were required and taken at the same times mentioned for HPLC-MS.

2.6.2 Influence of the initial OTC concentration

To assess the degradation of OTC over time, seven initial OTC concentrations were selected: 0.004 - 0.01 - 0.02 - 0.03 - 0.04 - 0.07 - 0.1 mmol/l. This range was selected as 0.04 mmol/l (≈ 20 mg/l) is the concentration supposedly used by the shrimp farmers in the Mekong Delta (Tran X.N.A, PhD student, personal communication, 2025). This range was also chosen for degradation tests available in the literature as it enables to follow the evolution of the OTC concentration along the experiment (Park *et al.*, 2023; K. Li *et al.*, 2008). However, other sources stated that the real antibiotic concentration in the ponds should range between the ng/l to μ g/l level (Park *et al.*, 2023; Nwe *et al.*, 2025; Binh *et al.*, 2018). Shimitzu *et al.*, (2013) has recorded a OTC concentration of 18 ng/l in shrimp ponds.

A constant ozone flow rate of 31 mmol/h was selected (*i.e.* air volumetric flow rate = 4 l/min and ozone output = 50%). To obtain the desired concentration range, dilutions of a 0.2 mmol/l stock solution were made, as presented in Table 2.2. This stock solution was prepared by diluting 0.0994 g of OTC · HCl into 1 liter of milliQ water.

For each concentration, 3 replicates were made and 1 ml samples were taken after 0-15sec-1-3-5-7 minutes to be analyzed with the UV spectrometer. The analysis of the absorbance at 355 nm was made directly after the withdrawal.

Table 2.2: Volumes to be taken from the OTC stock solution 0.2 mmol/l to prepare the concentration range 0.004 - 0.01 - 0.02 - 0.03 - 0.04 - 0.07 - 0.1 mmol/l for the degradation test. For each concentration, 200 ml are prepared.

Concentration (mmol/l)	Concentration (mg/l)	Stock solution volume (ml)	MilliQ water volume (ml)
0.004	2	4	196
0.01	5	10	190
0.02	10	20	180
0.03	15	30	170
0.04	20	40	160
0.07	35	70	130
0.1	50	100	100

Yet, the Drechsel tube used until now (see [Figure 2.12](#)) broke down. A diffusing stone (purchased on Amazon.be, supplier: Qwork), as the ones typically employed in fish tanks, replaced it. The height of the stone was 36 mm and its length was 23 mm (see [Figure 2.15](#)). To better understand the hydrodynamic behavior of the diffusing stone, the experiment was performed twice: with and without stirring. When the solution was stirred, the rotation speed of the magnetic bar was set to 100 rpm. Indeed, higher rotation speeds lead to the apparition of vortexes and uncontrollable management of the diffusing stone.

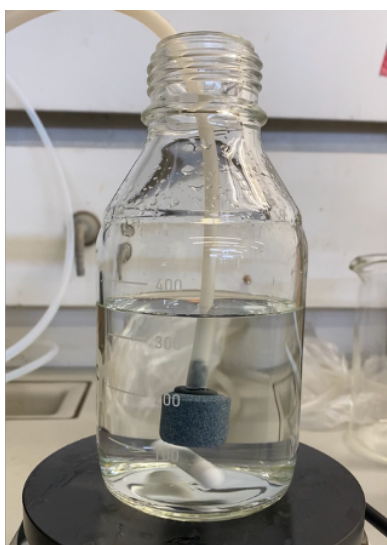


Figure 2.15: Diffusing stone used to replace the sintered filter.

2.6.3 Influence of the ozone generator parameters

In addition to the initial antibiotic concentration, the ozonation parameters, such as the air volumetric flow rate and the molar flow rate of ozone, can be adjusted to improve the degradation of OTC · HCl. To assess that, the degradation of 0.1 mmol/l of OTC · HCl was followed for four ozonation conditions (see [Table 2.3](#)). [Figure 2.16](#) provides a global view of the parameters taken into account. Samples of 1ml were taken after 0-15sec-1-3-5-7 minutes, and they were analyzed with the UV spectrometer. The analysis of the absorbance at 355 nm was made directly after the withdrawal. In addition, 0.5 ml were taken for HPLC-MS at each time and 20 ml samples were taken after 0 and 7 min to be analyzed by TOC. HPLC-MS samples were kept in the fridge and dark, while TOC samples were kept in the dark using aluminum foil and analyzed directly after the experiment.

Table 2.3: Studied conditions depending on the ozonation parameters: volumetric gas flow rate (l/min), ozone output (%), ozone molar flow rate (mmol/h) and gaseous ozone concentration (mmol/l) injected in the solution. The concentration of OTC · HCl was fixed at 0.1 mmol/l and the reaction volume was 250 ml.

Condition	Volumetric flow rate (l/min)	flow Ozone (%)	output Ozone molar flow rate (mmol/h)	Ozone concentration (mmol/l)
Reference	4	50	31	0.13
1	6	10	12.5	0.01
2	6	20	17	0.05
3	10	30	31	0.05
4	4	30	21	0.09
5	8	50	47	0.09
6	6	60	48	0.13
7	4	100	42	0.17

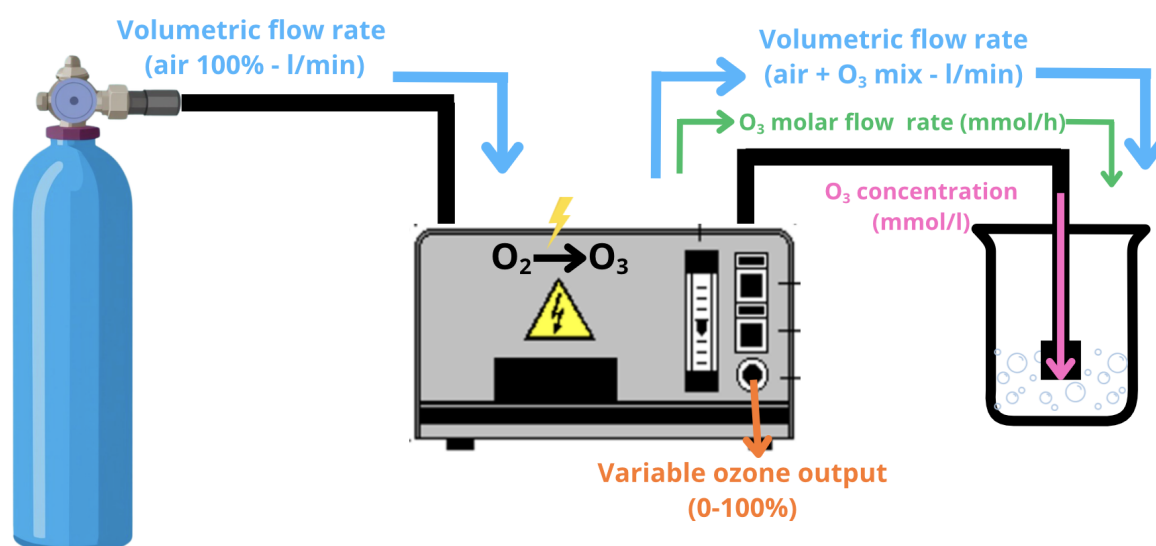


Figure 2.16: Schematic view of the different parameters taken into account to optimize the ozonation process. A fixed air volumetric flow rate (l/min) is sent to the ozone generator. The percentage of ozone wanted in the output gas is set by the rotating knob, ranging from 0 to 100%. A gas mixture (air + O₃) is then injected in the solution through the bubbling system, with a given gaseous ozone molar flow rate (mmol/h) and concentration (mmol/l).

2.6.4 Influence of changing the bubbling system on the 6 h degradation test

As mentioned in Section 2.6.2, another bubbling system had to be introduced due the breakage of the Drechsel tube. Therefore, experiments were continued with a diffusing stone. Before going any further, the efficiency of the diffusing stone to degrade OTC · HCl over six hours was compared to the one of the sintered filter. Thus, the experiment depicted in Section 2.6.1 was replicated.

A 0.1 mmol/l OTC · HCl solution is prepared by diluting 200 ml of the 0.2 mmol/l stock solution into 200 ml of milliQ water. The stock solution was prepared by taking 0.0994 g of OTC · HCl powder and diluting it into 1 liter of milliQ water (at 18 mΩ). Ozone was continuously injected via the diffusing stone at a molar flow rate of 31 mmol/h and a concentration of 0.13 mmol/l (i.e. volumetric flow rate of air of 4 l/min and 50% in variable output). Samples were taken for the TOC and UV-Vis spectrophotometer after 0 - 1 - 3 - 5 - 15 - 30 - 60 - 120 - 240 - 360 minutes. 20 ml samples were taken for the TOC analysis,

while 1 ml was kept for the UV-Vis analysis and measured at 355 nm. This experiment was replicated twice.

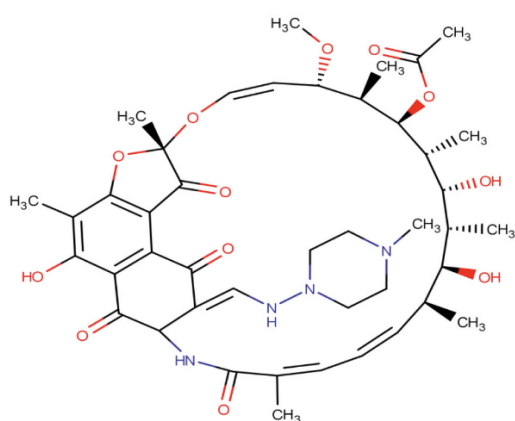
2.6.5 Impact of the water salinity

To simulate more accurately the water conditions in the Mekong Delta, the salinity of the water medium had to be increased. Farmers from the Mekong Delta revealed that the average salinity of shrimp pond is of 7 g/l (Tran X.N.A, PhD student, personal communication, 2026). Nevertheless, this value may vary according to several factors, such as the temperature, rainfall frequency or tides (Nguyen, 2019). This matches with the optimal salinity conditions reported by Leigh *et al.*, (2020): below 10 g/l or above 25 g/l.

From the 0.2 mmol/l stock solution of OTC · HCl, 200 ml were taken. 200 ml of milliQ water was added to reach a reaction volume of 400 ml and a OTC · HCl concentration of 0.1 mmol/l. The ozone parameters were kept unchanged: ozone mass flow rate of 31 mmol/h and an ozone concentration of 0.13 mmol/l. To reach the 7 g/l salinity, 2.8 g of sodium chloride (NaCl) were dissolved into the 400 ml of solution used for the experiment. Samples were taken after 0-1-3-5-15-30-60-120-240-360 min to be analysed by the UV-Vis spectrophotometer and TOC machine.

2.7 Degradation of Rifampicin

Following recent discussions with shrimp farmers, another antibiotic was identified to be used: rifampicin ($C_{43}H_{58}N_4O_{12}$). This molecule has a brownish color (see Figure 2.17) and a molecular weight of 822.9 g/mol. Rifampicin is bactericidal and typically used for the treatment of human tuberculosis (Venkataramana *et al.*, 2016; Tran *et al.*, 2017). The following methodology was inspired by the protocols provided by Benetton, (1998), Tanasă *et al.*, (2022) and Tilinea *et al.*, (2017) to study the degradation of rifampicin by UV-Vis spectrophotometer.



(a) Molecular structure (M. Khan *et al.*, 2017).



(b) Powder appearance.

Figure 2.17: Rifampicin molecular structure and powder appearance.

Rifampicin has two ionizable groups. Especially, at pH 7 (and $pK_a = 7.9$), its heterocyclic nitrogen atom is still protonated and forms a yellow-colored complex (Tanasă *et al.*, 2022). To maintain the pH constant, a phosphate buffer had to be prepared, according to the European Pharmacopoeia (European Directorate for the Quality of Medicines & HealthCare (EDQM), 2010). 10.359 g of Na_2HPO_4 (Sigma-Aldrich) and 8.26 g of $NaH_2PO_4 \cdot 2H_2O$ (Merck) had to be dissolved into two liters of milliQ water (see Figure 2.18). It should be emphasized that the Na_2HPO_4 required prior dissolution in a small quantity of water heated at 60 °C and under stirring.

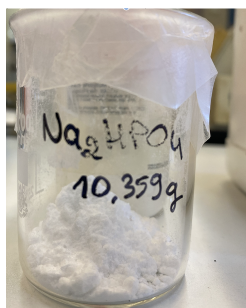
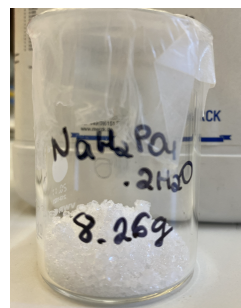
(a) Na_2HPO_4 powder.(b) $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ powder.

Figure 2.18: Composition of the pH 7 phosphate, appearance of the powders.

The solubility of rifampicin in water is low: 1.4 g/l. To obtain a 0.2 mmol/l stock solution, 0.164 g of rifampicin (Thermo Scientific) were dissolved into 4 ml of methanol (Sigma-Aldrich) and 1 l of phosphate buffer. This resulted in an orange solution (see Figure 2.19), that was kept in the dark and the fridge.

For the degradation test, 200 ml of a 0.05 mmol/l solution was prepared by taking 50 ml of rifampicin stock solution and 150 ml of phosphate buffer. The ozone parameters were kept identical as the ones used to degrade OTC · HCl: 31 mmol/h and 0.13 mmol/l. 1 ml samples were taken after 0 - 30 sec - 1 - 2 - 3 minutes and analyzed at 475 nm with the UV-Vis spectrophotometer. This experiment was replicated three times. Prior to using the UV-Vis spectrophotometer, a calibration curve for rifampicin had to be done. It is presented in Appendix C.3. One of the triplicates was also analyzed by TOC, 20 ml samples were taken after 0 - 3 - 15 minutes.

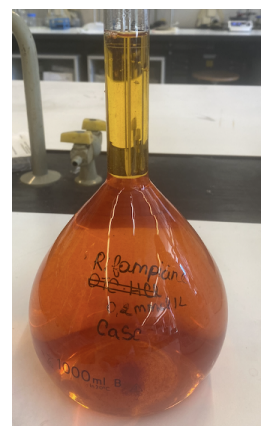


Figure 2.19: Rifampicin stock solution with concentration 0.2 mmol/l.

Chapter 3

Results

3.1 Preliminary degradation tests on PNP with ozone

3.1.1 Blank curves

To get the blank curves for PNP, three conditions were tested:

- **Condition 1:** Ozone generator turned on, with a bubbling flow rate of 4 l/min but 0% in ozone output and under stirring.
- **Condition 2:** Ozone generator turned off, but air passed through to generate bubbles and under stirring.
- **Condition 3:** The solution was left out in the open air under stirring.

To ensure that degradation did not continue once the sample was collected, the absorbance was measured twice: immediately after sampling and again at the end of the experiment (after approximately 10 minutes). It can be highlighted that the values remain overall stable (see [Table 3.1](#) and [Figure 3.1](#)), as predicted.

Table 3.1: Evolution of the absorbance of PNP at a wavelength of 317 nm for three different conditions.

	Absorbance during the test			Absorbance measured after 10 minutes		
Time (sec)	Cond. 1	Cond. 2	Cond. 3	Cond. 1	Cond. 2	Cond. 3
0	1.331	1.352	1.355	1.332	1.353	1.355
15	1.372	1.365	1.362	1.367	1.355	1.362
60	1.337	1.355	1.323	1.336	1.351	1.32
150	1.341	1.38	1.315	1.343	1.377	1.314
240	1.308	1.35	1.318	1.305	1.343	1.316
330	1.315	1.357	1.316	1.318	1.361	1.313
420	1.346	1.332	1.354	1.344	1.33	1.351
510	1.357	1.354	1.357	1.353	1.35	1.352

The dotted lines on both graphs represent the measurements of absorbance made 10 minutes after the sampling time, while the solid lines correspond to the values obtained directly after the sampling. From [Figure 3.2](#), it can be seen that absorbance is relatively constant over time, which was expected. Indeed, if a decrease of absorbance had occur, it would have mean that the degradation of PNP is not only affected by ozone, but also by the ambient air. The test also confirmed the 0% ozone output operating mode of the ozone generator means that no ozone is produced.

These blanks showed that, for future PNP-related experiments, any observed decrease in absorbance is attributable to ozonation and not to other factors, such as the natural degradation of PNP.

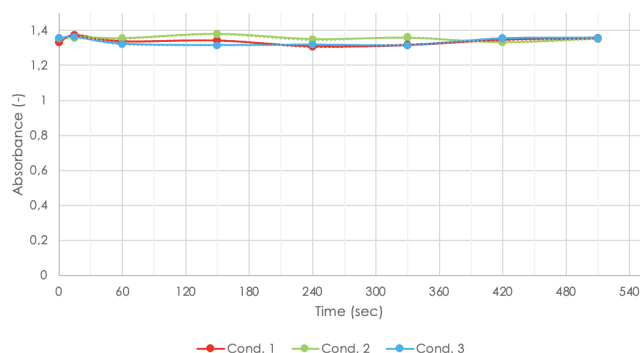


Figure 3.1: Evolution of the absorbance of PNP over time for three blank conditions, **overall view**. Solid lines = absorbance values measured directly after sampling, dotted lines = absorbance values measured 10 min after sampling.

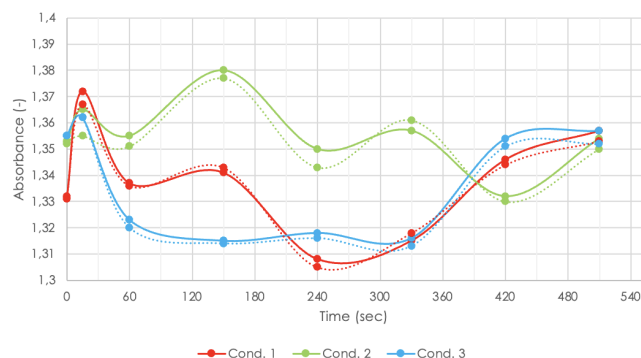


Figure 3.2: Evolution of the absorbance of PNP over time for three blank conditions, **zoom-in**. Solid lines = absorbance values measured directly after sampling, dotted lines = absorbance values measured 10 min after sampling.

3.1.2 Replicates

The replicates overlap adequately from one replication to another (see [Table 3.2](#) and [Figure 3.3](#)). Some differences may come from experimental error, but also from the way the bubbler was placed in the reactor. Indeed, it was observed that the inclination and depth of the bubbler in the reactor tend to impact the rate of the degradation. This issue is supposedly related to mass transfer issues. These aspects will be addressed in future work.

Table 3.2: Evolution of the absorbance over time for five replicates. Operating conditions: $[PNP] = 0.11 \text{ mmol/l}$ and O_3 flow rate = 31 mmol/h . The absorbance was measured at 317 nm .

Time (sec)	Absorbance at 317 nm (-)				
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5
0	0.984	0.988	0.995	1.032	0.993
15	0.892	0.913	0.888	0.925	0.922
60	0.724	0.724	0.694	0.704	0.737
150	0.453	0.423	0.401	0.406	0.431
240	0.212	0.183	0.171	0.161	0.222
330	0.071	0.06	0.052	0.045	0.081
420	0.025	0.021	0.016	0.017	0.025
510	0.012	0.016	0.008	0.011	0.013

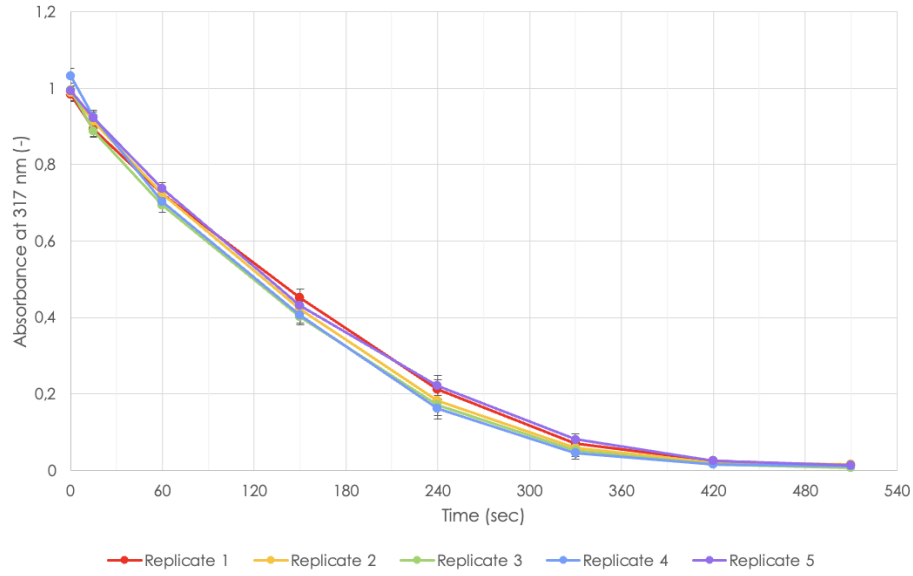


Figure 3.3: Evolution of the absorbance over time for five replicates. The half length of the error bars is σ and contains 68% of the values if the gaussian error is accepted. Operating conditions: [PNP] = 0.11 mmol/l and O_3 flow rate = 31 mmol/h. The absorbance was measured at 317 nm.

For each time, the variance (s^2) over the five replicates and the standard deviation (σ) were calculated using Equation 3.1 and 3.2, respectively.

$$s^2 = \frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2 \quad (3.1)$$

$$\sigma = \sqrt{s^2} \quad (3.2)$$

where n is the number of replicates, x_i is the measured value and $\bar{x}$ is the sample mean, which is calculated as follows,

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i \quad (3.3)$$

The results confirm that the experiment is highly reproducible (see Table 3.3), with a maximum standard deviation of $2.6 \cdot 10^{-2}$ observed at $t=240$ s.

Table 3.3: Values of the sample variance and standard deviation, for each time point ($n=5$). Operating conditions: [PNP] = 0.11 mmol/l and O_3 flow rate = 31 mmol/h.

Time (sec)	Variance (s^2)	Standard-deviation (σ)
0	3.71E-04	1.93E-02
15	2.92E-04	1.71E-02
60	2.99E-04	1.73E-02
150	4.34E-04	2.08E-02
240	6.90E-04	2.63E-02
330	2.09E-04	1.44E-02
420	1.82E-05	4.27E-03
510	8.50E-06	2.92E-03

3.1.3 Degradation of PNP with ozone

3.1.3.1 Variation of the initial PNP concentration

Degradation tests with ozone were performed for four initial PNP concentrations: 0.036-0.07-0.11-0.14 mmol/l (see Table 3.4). The link between the PNP absorbance measured by the UV-Vis spectrometer and the related concentration was made through the Beer-Lambert law and the calibration is curve available in Appendix E.

Table 3.4: Evolution of the concentration (mmol/l) over time for the PNP concentration range 0.036-0.07-0.11-0.14 mmol/l, and an O₃ flow rate of 31 mmol/h. The absorbance was measured at 317 nm.

	Remaining PNP concentration (mmol/l)			
Time (sec)	[PNP]=0.14 mmol/l	[PNP]=0.11 mmol/l	[PNP]=0.07 mmol/l	[PNP]=0.036 mmol/l
0	0.1450	0.1090	0.0761	0.0362
15	0.1340	0.0979	0.0611	0.0288
60	0.1120	0.0782	0.0421	0.0126
150	0.0836	0.0488	0.0172	0.0018
240	0.0568	0.0231	0.0029	0.0004
330	0.0337	0.0084	0.0013	0.0004
420	0.0186	0.0030	0.0008	0.0004
510	0.0087	0.0015	0.0008	0.0004

This experiment showed that, across all initial concentrations tested, near-complete degradation of PNP was achieved within 510 seconds (see Figure 3.4), with lower initial concentrations reaching negligible absorbance values sooner.

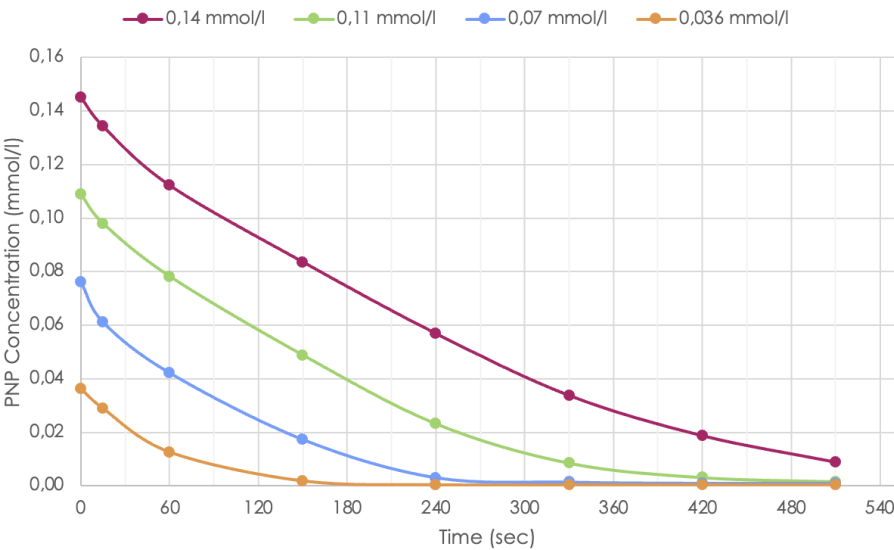


Figure 3.4: Evolution of the concentration (mol/l) over time for the PNP concentration range 0.036-0.07-0.11-0.14 mmol/l, and an O₃ flow rate of 31 mmol/h. The absorbance was measured at 317 nm.

3.1.3.2 Influence of the ozone flow rate on the kinetics

The flow rate at which ozone is injected in the reactor may also impact the degradation rate of PNP. To assess this, a range of four O₃ flow rates (17-21-26-31 mmol/h) was studied for a given initial PNP concentration of 0.11 mmol/l.

The remaining PNP concentrations for each flow rate are listed in [Table 3.5](#) and presented [Figure 3.5](#). The fastest degradation occurs for the highest ozone flow rate (31 mmol/h). To compare with the other flow rates, it can be seen that after 330 seconds, the remaining PNP concentration is of 0.04 mmol/l, which is about 10 times smaller than at 17 mmol/h (0.0046 mmol/l).

Table 3.5: Evolution of the concentration (mmol/l) over time for the O_3 flow rate range 17-21-26-31 mmol/h, and a fixed PNP concentration of 0.11 mmol/l. The absorbance was measured at 317 nm.

Remaining PNP concentration (mmol/l) depending on the O_3 flowrate				
Time (sec)	17 mmol/h	21 mmol/h	26 mmol/h	31 mmol/h
0	0.1080	0.1110	0.1060	0.1060
15	0.1030	0.0956	0.0954	0.0973
60	0.0913	0.0875	0.0817	0.0752
150	0.0721	0.0615	0.0521	0.0428
240	0.0547	0.0400	0.0274	0.0175
330	0.0400	0.0208	0.0117	0.0046
420	0.0274	0.0102	0.00371	0
510	0.0173	0.00415	0.00175	0

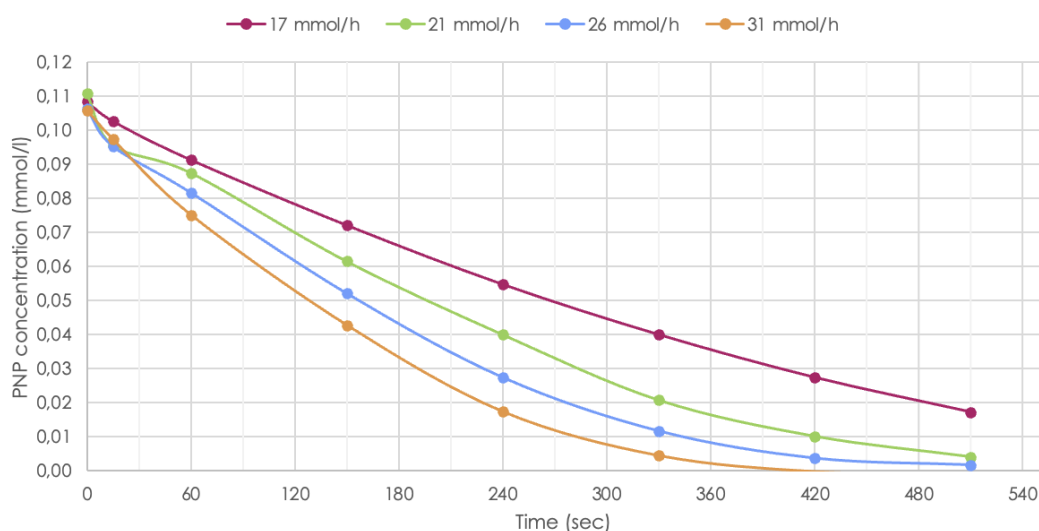


Figure 3.5: Evolution of the PNP concentration (mmol/l) over time for the O_3 flow rate range 17-21-26-31 mmol/h, and a fixed initial PNP concentration of 0.11 mmol/l.

Not only the molar flow rate of ozone has been increased, but also the ozone concentration injected in the reactor. Indeed, for a constant volumetric flow rate (4 l/min), the value of the ozone output (*i.e.* the percentage of ozone in the feed) has been gradually increased. It leads to a higher ozone molar flow rate and concentration entering the reactor. The O_3 concentration related to the molar flow rate selected is shown in [Table 3.6](#).

Table 3.6: Ozone molar flow rate and concentration obtained when keeping the volumetric flow rate of air constant and increasing the variable output percentage.

Volumetric flow rate (l/min)	Variable output (%)	O ₃ mass flow rate (mmol/h)	O ₃ concentration (mmol/l)
4	20	17	0.06
4	30	21	0.09
4	40	26	0.11
4	50	31	0.13

3.1.3.3 Use of two complementary analysis techniques: UV-Vis spectrometry and TOC

Table 3.7 compares the results obtained simultaneously with the UV-Vis spectrophotometer and the TOC machine, allowing to assess the degradation of PNP both at the molecular level and in terms of total organic carbon content. It is noteworthy that the results found by both analysis technique provided different information. With the UV-Vis spectrophotometer, a decrease of 86.9% of the PNP concentration is visible after four minutes. This indicates that the majority of the studied compound was degraded. However, the TOC only decreased by 11% over the same period. This result shows that the carbon present in the sample is not only due to the PNP, but also to other degradation by-products, which are still present after the four minutes of ozonation. These observations are consistent with the ones of Singh *et al.*, (2025). The authors attributed the slight decrease in TOC to carboxylic acids and aldehydes that resisted to further oxidation. Therefore, the UV-Vis spectrophotometer and TOC are complementary techniques that, combined, provide a general view of the state of the sample. The UV-Vis spectrophotometer focuses on following the studied molecule itself, while TOC analyses both the compound and the degradation by-products.

Table 3.7: Comparison of the results obtained with the TOC and the UV-Vis spectrophotometer. The initial PNP concentration was 0.1 mmol/l and the ozone flow rate was 31 mmol/h.

Time (min)	PNP concentration (mmol/l)	TOC (mg _C /l)
0	0.1103	6.336
4	0.0143	5.608

It is also possible to compare the theoretical initial TOC to the one measured by the TOC-L analyzer. The theoretical TOC at time zero can be calculated knowing the chemical formula of PNP (C₆H₅NO₃), its molar mass (139.11 g/mol) and the concentration of the PNP (0.11 mmol/l),

$$\begin{aligned}
 \text{TOC}_{\text{PNP},0} &= \frac{\text{PNP concentration (mg/l)}}{\text{Molar mass of PNP (g/mol)}} \times (\text{Number of Carbons} \times \text{Molar mass of Carbon (g/mol)}) \\
 &= \frac{15 \text{ mg/l}}{139.11 \text{ g/mol}} \times (6 \times 12 \text{ g/mol}) = 7.76 \text{ mg}_C/\text{l}
 \end{aligned}$$

There is a 1.4 mg_C/l¹ difference between the theoretical and measured TOC values. This could be associated to minor impurities present in the PNP powder itself, or to inaccuracies in the dilutions performed to prepare the 0.11 mmol/l PNP solution that was analyzed.

¹Throughout this manuscript, it was decided to keep the units of TOC values in mg_C/l, as is done in the literature. To compare this value with the evolution of the concentration (mol/l) of the degraded molecule, it would need to be divided by 12 g/mol (*i.e.* the molecular mass of carbon).

3.2 Degradation of OTC with ozone

3.2.1 First degradation test

The degradation of OTC·HCl was monitored using three analytical techniques (UV-Vis spectrophotometry, TOC analysis, and HPLC-MS) and the results for each are presented below.

For the analysis with the **UV spectrophotometer**, the concentration values are shown in [Table 3.8](#). The equations of the calibrations curves presented in [Appendix C.2](#) are employed to obtain the OTC concentrations. For Replicates 1 and 3, [Equation C.4](#) is used as the samples were not acidified. For Replicate 2, the analysis was made by adding 0.5 ml of HCl 1M, thus [Equation C.3](#) was taken into account. As mentioned in [Appendix C.2](#), it is considered that the 0.5 ml of HCl does not dilute the concentration of OTC present in the sample.

Table 3.8: Evolution of the concentration (mmol/l) values over 6 hours time for an initial OTC concentration of 0.1 mmol/l and an ozone flow rate of 31 mmol/h. The absorbance was measured at 355 nm.

Time (sec)	Concentration (mmol/l)		
	Rep. 1	Rep. 2	Rep. 3
0	0.1024	0.1027	0.1033
2			0.0230
5	0.0101		0.0113
15	0.0008	0.0035	0.0025
20			0.0024
30	0.0005	0.0030	0.0014
60	0.0004		0.0008
120	0.0005	0.0020	0.0007
150			0.0006
180			0.0005
210			0.0006
240	0.0005	0.0020	0.0004
270			0.0004
300			0.0007
330			0.0005
360	0.0004	0.0020	0.0003

As in [Section 3.1.2](#), the variance and standard deviation were calculated (see [Table 3.9](#)). These parameters were calculated only for times where three replicates values were recorded.

Table 3.9: Values of the sample variance (s^2) and standard deviation (σ), for time points where 3 replicates were made ($n=3$). Operating conditions: [OTC] = 0.1 mmol/l and O_3 flow rate = 31 mmol/h.

Time (min)	Variance (s^2)	Standard-deviation (σ)
0	5.63E-08	2.37E-04
15	3.64E-06	1.91E-03
30	3.14E-06	1.77E-03
120	1.13E-06	1.06E-03
240	1.13E-06	1.06E-03
360	1.25E-06	1.12E-03

From Figure 3.6, it can first be observed that the experiment can be easily reproduced with similar results and performances. This graph also illustrates the quick degradation of OTC when ozone is employed. Indeed, the concentration reach a near zero after only 15 minutes of ozonation.

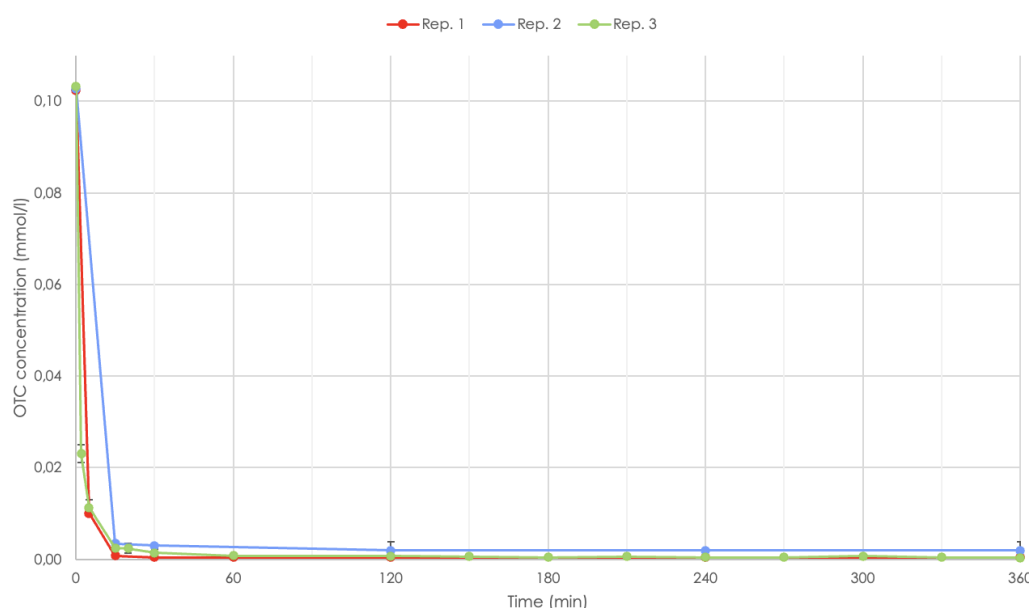


Figure 3.6: Evolution of the OTC concentration (mmol/l) over time for three replicates. The half length of the error bars is σ and contains 68% of the values if the gaussian error is accepted. Initial OTC·HCl concentration = 0.1 mmol/l and O_3 flow rate = 31 mmol/h.

Moving to the **TOC** analysis, the results of the amounts of organic carbon in the sample, as well as the variance and standard-deviation are presented in Table 3.10. The results have a similar trend from one replicate to another (see Figure 3.7). Yet, for Replicate 2, the values are slightly lower. This may be linked to the age of the OTC·HCl solution used, which might affect the initial carbon content of the sample. For Replicates 1 and 3, the solution was prepared on the same day (but the experiments were conducted on two different dates). For Replicate 2, the solution was 1 day old. Indeed, OTC·HCl is known to be relatively unstable in aqueous solution, with a degradation occurring within a few days after the preparation of the stock solution. It was not possible to remove all the organic carbon present in the sample during the six hours of the treatment.

Table 3.10: Values of the TOC (mg C/l), sample variance (s^2) and standard deviation (σ) for time points where 3 replicates were made ($n=3$). Operating conditions: [OTC] = 0.1 mmol/l and O_3 flow rate = 31 mmol/h.

Time (min)	TOC (mg C/l)			Statistical parameters (-)	
	Replicate 1	Replicate 2	Replicate 3	Variance (s^2)	Standard-deviation (σ)
0	23.49	24.6	24.24	3.21E-01	5.66E-01
2			23.77		
5	22.1		22.86		
15	21.2	21.03	21.42	3.82E-02	1.96E-01
30	20.16	20.58	20.56	5.61E-02	2.37E-01
60	19.33				
120	18.7	17.59	18.49	3.48E-01	5.90E-01
240	17.16	15.57	16.95	7.46E-01	8.64E-01
360	16.47	14.77	16.15	8.16E-01	9.03E-01

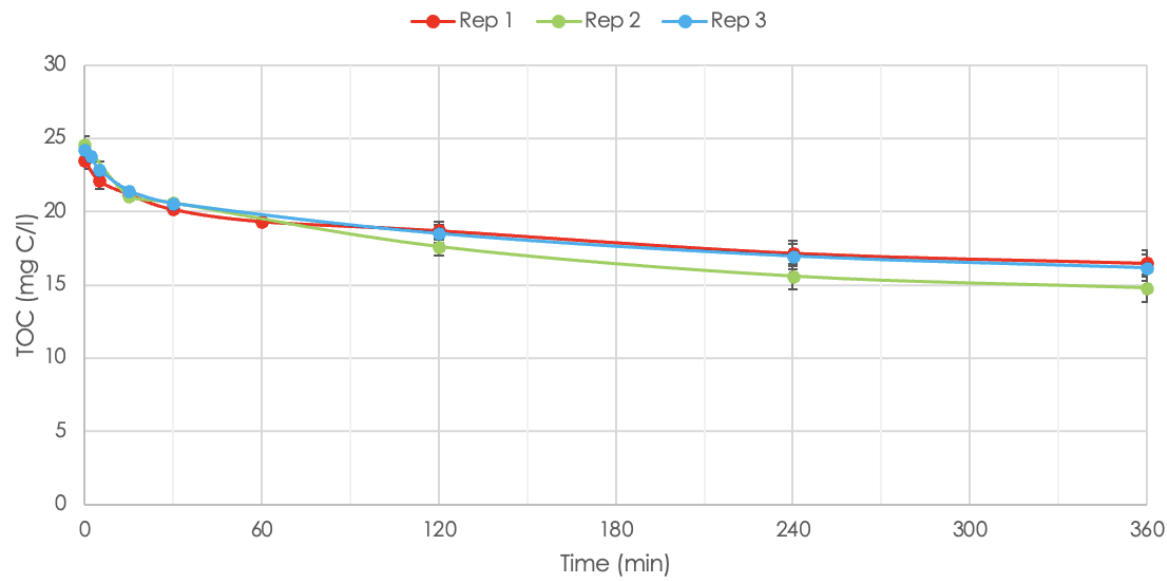
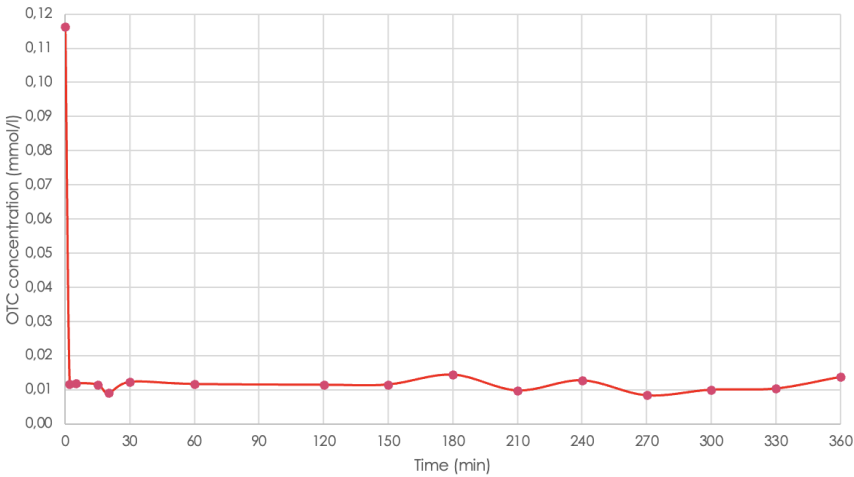


Figure 3.7: Evolution of the TOC (mg C/l) over 360 min. The half length of the error bars is σ and contains 68% of the values if the gaussian error is accepted.

Finally, the evolution of the OTC · HCl concentration during the six hours of treatment was monitored with the **HPLC-MS machine**. For this method, only one test was made (see Figure 3.8a). The HPLC-MS concentration profile reveals two distinct phases (see Figure 3.8b). During the first two minutes, a sharp decrease from 0.1163 to 0.0118 mmol/l is observed, corresponding to the rapid degradation of OTC. After that, the concentration of OTC remains mostly stable for the remaining time of the experiment. The shape of the curve is similar to the one obtained with the UV-Vis spectrophotometer, highlighting the suitability of both methods for monitoring the degradation of the OTC and confirming the findings.

Time (min)	Concentration (mmol/l)
0	0.1163
2	0.0118
5	0.0120
15	0.0116
20	0.0091
30	0.0124
60	0.0118
120	0.0115
150	0.0116
180	0.0145
210	0.0099
240	0.0128
270	0.0085
300	0.0101
330	0.0104
360	0.0138

(a) Experimental data



(b) Concentration profile over time

Figure 3.8: Evolution of the concentration (mmol/l) over 6 hours for an initial OTC concentration of 0.1 mmol/l and an ozone flow rate of 31 mmol/h.

Yet, the results for HPLC-MS are much more sensitive than with the UV-Vis spectrophotometer. Indeed,

they fluctuate more and do not reach zero concentration. Two explanations can be suggested for this observation. First, it may be explained by the presence of degradation by-products sharing a molar mass close to the one of the parent OTC molecule (460.4 g/mol). As an example, α -apo-oxytetracycline and β -apo-oxytetracycline ($C_{22}H_{22}N_2O_8$) both have a molar mass of 442.2 g/mol. Thus, their chromatographic peaks may overlap with the one of OTC, leading to an overestimation of the remaining OTC concentration. Alternatively, the plateau observed in the curve may simply reflect a complete degradation of OTC, with a residual signal attributed to a baseline offset of the HPLC-MS detector.

For all analysis methods used, the efficiency can be calculated for each replicates, using [Equation 3.4](#).

$$\text{Efficiency} = \frac{\text{Initial value} - \text{Final value}}{\text{Initial value}} \times 100 \quad (3.4)$$

For the HPLC-MS and the UV-Vis spectrophotometer, the efficiency value correspond to the removal of OTC·HCl over time (see [Table 3.11](#)). However, for the TOC, the efficiency is related to the remaining amount of organic carbon in the sample. It does not only accounts for OTC·HCl disappearance, but also for the degradation of by-products, giving an overview of the sample carbon content.

Table 3.11: Efficiency of degradation based on each analysis method and replicate. The initial OTC·HCl concentration was 0.1 mmol/l and the ozone flow rate was 31 mmol/h.

Efficiency of the degradation depending on the analysis method (%)			
Replicate	UV-Vis spectrophotometer	TOC	HPLC-MS
1	99.63%	29.88%	
2	98.01%	39.95%	
3	99.71%	33.37%	92.73%

3.2.2 Influence of the initial OTC concentration, stirring and bubbling

3.2.2.1 First trial

The results of the experiments, for each initial concentration, replicate and whether stirring was applied or not, are presented in [Table 3.12](#). The values for the remaining concentration of OTC·HCl were found using the calibration curve for non-acidified samples presented in [Appendix C.2](#).

Table 3.12: Evolution of the concentration (mmol/l) of OTC · HCl measured over time for several initial concentrations (0.01 - 0.04 - 0.01 mmol/l) with and without stirring at 100 rpm. The absorbance was recorded at 355 nm and the ozone settings were 31 mmol/h and 0.13 mmol/l. The diffusing stone was used to introduce the ozone bubbles in the solution.

[OTC.HCl] = 0.1 mmol/l						
	Stirring			No Stirring		
	Concentration (mmol/l)			Concentration (mmol/l)		
Time (min)	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	0.0985			0.0995		
0.25	0.0691			0.0600		
1	0.0222			0.0187		
3	0.0081			0.0039		
5	0.0022			0.0015		
7	0.0011			0.0008		

[OTC.HCl] = 0.04 mmol/l						
	Stirring			No Stirring		
	Concentration (mmol/l)			Concentration (mmol/l)		
Time (min)	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	0.0405	0.0406	0.0406	0.0406	0.0404	0.0395
0.25	0.0177	0.0214	0.0161	0.0143	0.0143	0.0151
1	0.0058	0.0060	0.0055	0.0057	0.0050	0.0053
3	0.0013	0.0008	0.0006	0.0011	0.0012	0.0018
5	0.0007	0.0004	0.0003	0.0006	0.0006	0.0010
7	0.0005	0.0003	0.0002	0.0005	0.0006	0.0007

[OTC.HCl] = 0.01 mmol/l						
	Stirring			No Stirring		
	Concentration (mmol/l)			Concentration (mmol/l)		
Time (min)	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	0.0103	0.0110		0.0106	0.0105	0.0104
0.25	0.0015	0.0012		0.0015	0.0009	0.0014
1	0.0005	0.0003		0.0003	0.0003	0.0003
3	0.0003	0.0003		0.0002	0.0002	0.0002
5						
7						

For each concentration, the experiment can be easily replicated, no matter the initial OTC · HCl concentration (see [Figure 3.9](#)). For the 0.01 mmol/l condition with stirring (see [Figure 3.9f](#)), only two replicates were done. From [Figure 3.9c](#) and [3.9d](#), it can be noticed that in the case on stirring, the results are slightly less reproducible than in the non-stirred condition. This may be explained by the fact that, in the stirred condition, the diffusion stone frequently hit the magnetic bar. Furthermore, it was more difficult to collect the samples. Since the stone slipped and did not stayed in place as easily, sampling is more difficult, leading to delays of a few seconds during pipetting.

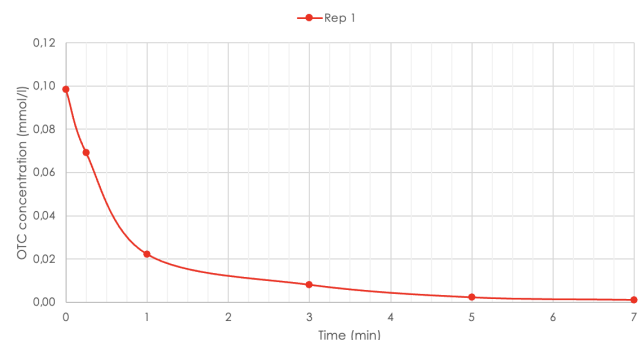
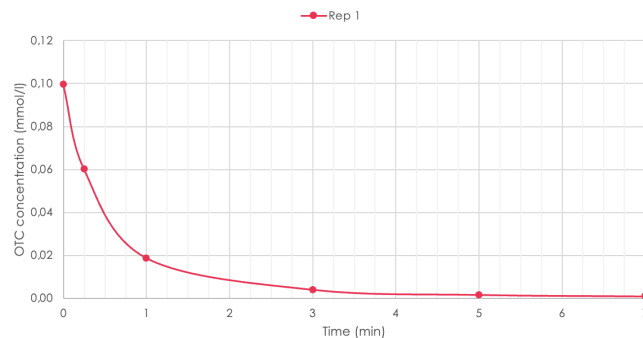
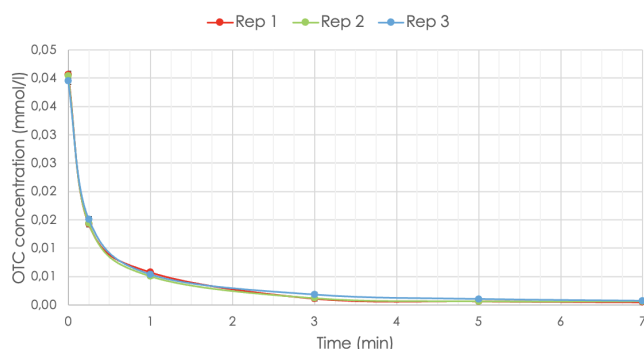
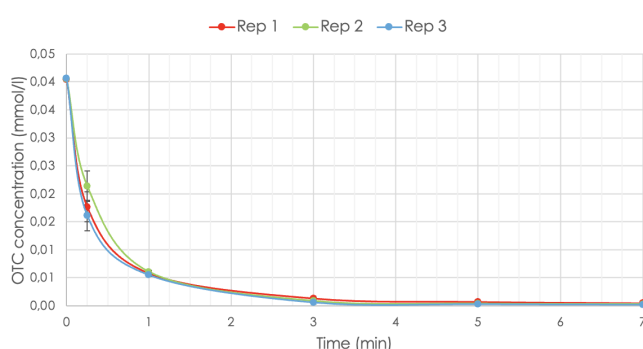
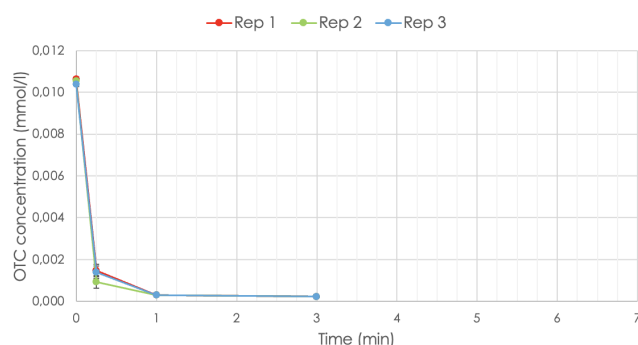
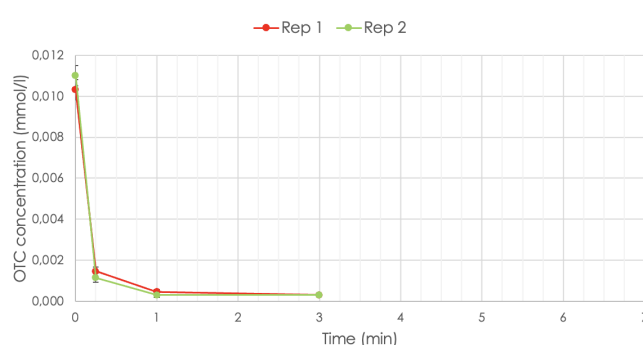
(a) $[\text{OTC} \cdot \text{HCl}] = 0.1 \text{ mmol/l}$ - No stirring(b) $[\text{OTC} \cdot \text{HCl}] = 0.1 \text{ mmol/l}$ - Stirring(c) $[\text{OTC} \cdot \text{HCl}] = 0.04 \text{ mmol/l}$ - No stirring(d) $[\text{OTC} \cdot \text{HCl}] = 0.04 \text{ mmol/l}$ - Stirring(e) $[\text{OTC} \cdot \text{HCl}] = 0.01 \text{ mmol/l}$ - No stirring(f) $[\text{OTC} \cdot \text{HCl}] = 0.01 \text{ mmol/l}$ - Stirring

Figure 3.9: Degradation of $\text{OTC} \cdot \text{HCl}$ over 7 minutes, depending on the initial antibiotic concentration (0.01 - 0.04 - 0.1 mmol/l) and stirring (or not) of the solution at 100 rpm. The half length of the error bars is σ and contains 68% of the values if the gaussian error is accepted.

3.2.2.2 Influence of stirring and the selected bubbling system

To decide whether stirring has a significant impact on the degradation of $\text{OTC} \cdot \text{HCl}$, Figure 3.10 can be observed. Those curves were constructed by taking the mean values of the three replicates in each condition. It can be noticed that the stirring only slightly impacted the degradation results, which could suggest that the bubbles generated are sufficient to homogenize the solution.

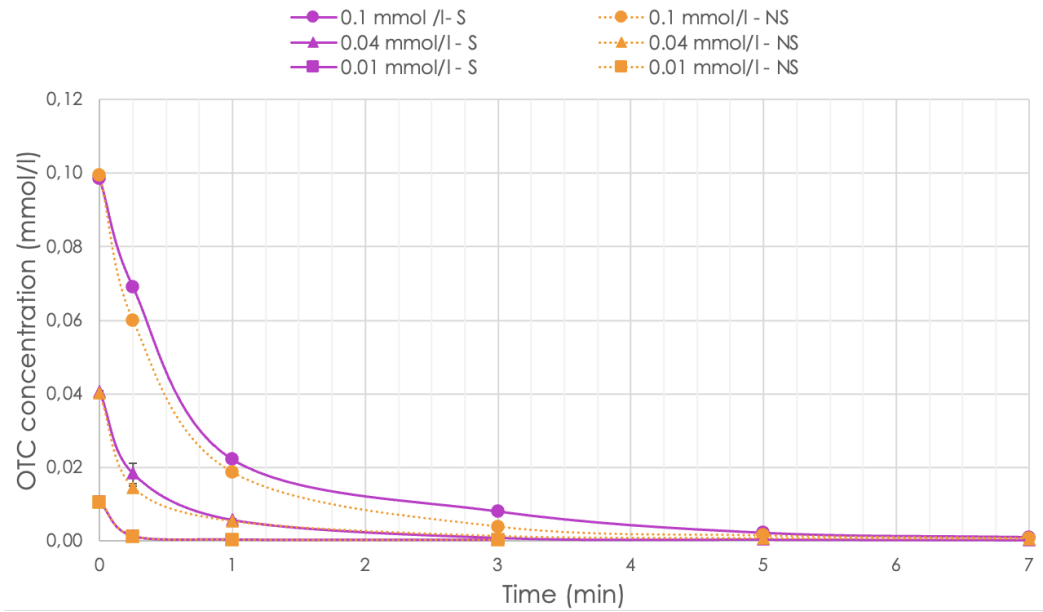
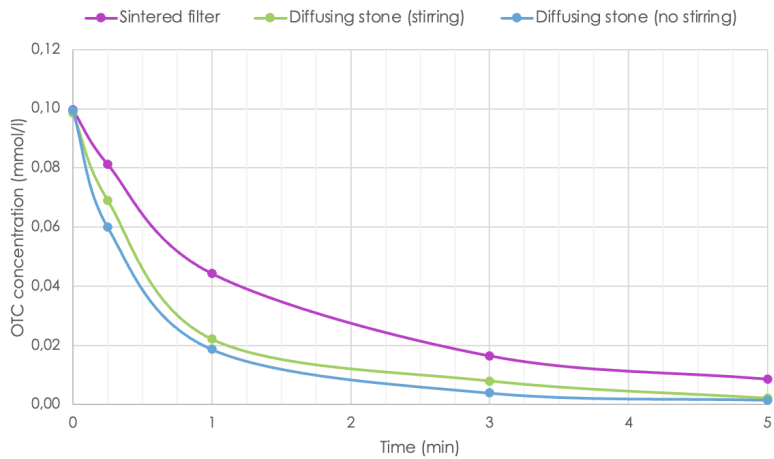


Figure 3.10: Comparison between the stirring (S) and no stirring (NS) cases depending on the initial OTC·HCl concentration: $\circ$ = 0.1 mmol/l, $\triangle$ = 0.04 mmol/l and $\square$ = 0.01 mmol/l. The half length of the error bars is σ and contains 68% of the values if the gaussian error is accepted.

For the 0.1 mmol/l condition, only one replicate was made either for stirring or no stirring. The reason of this is that the tests were initially conducted using the first sintered filter used (see Figure 2.12). The bubbler broke after the second replicate of the 0.1 mmol/l condition. This allows us to compare the performance of the two bubblers at the same initial OTC concentration. The diffusing stone is more efficient to degrade OTC·HCl than the sintered filter previously used (see Figure 3.11b). Indeed, there is a 10% difference in efficiency between the two bubbling systems: 91% for the sintered filter and around 98% for the diffusing stone (see Figure 3.11a). It also highlights that the effect of stirring is not significant when using the diffusing stone, as suggested by Figure 3.10.

	OTC·HCl (mmol/l)		
Time (min)	SF	DS (S)	DS (NS)
0	0.100	0.099	0.100
0.25	0.081	0.069	0.060
1	0.044	0.022	0.019
3	0.017	0.008	0.004
5	0.009	0.002	0.002
Efficiency	91.3%	97.7%	98.45%

(a) Experimental data. SF = sintered filter + stirring (250 rpm), DS (S) = diffusing stone + stirring (100 rpm), DS (NS) = diffusing stone + no stirring.



(b) Concentration profile over time

Figure 3.11: Comparison on the degradation efficiency based on the bubbler used for an initial OTC·HCl concentration of 0.1 mmol/l and an ozone flow rate of 31 mmol/h.

Overall, these results demonstrate that ozonation is highly effective at degrading OTC·HCl regardless of the initial concentration tested, reaching a nearly complete degradation after 7 minutes under all

conditions. From these experiments, it was decided to use the diffusing stone, without stirring, for upcoming experiments given its higher efficiency compared to the sintered filter.

3.2.2.3 Broader concentration range

To get an insight of the kinetics of OTC · HCl during the degradation test, additional concentrations had to be recorded. Four new concentrations (0.004 - 0.02 - 0.03 - 0.07 mmol/l) were studied under the same conditions as previously (see [Table 3.13](#)).

Table 3.13: Evolution of the concentration (mmol/l) of OTC · HCl measured over time for several initial concentrations: 0.004 - 0.02 - 0.03 - 0.07 mmol/l. The absorbance was recorded at 355 nm and the ozone settings were 31 mmol/h and 0.13 mmol/l. The diffusing stone was used to introduce the ozone bubbles in the solution.

Time (min)	Initial OTC · HCl concentration			
	0.004 mmol/l	0.02 mmol/l	0.03 mmol/l	0.07 mmol/l
0	0.0048	0.0203	0.0318	0.0742
0.25	0.0016	0.0049	0.0072	0.0415
1	0.0012	0.0010	0.0016	0.0127
3	0.0009	0.0007	0.0008	0.0018
5	0.0006	0.0009	0.0009	0.0016
7	0.0009	0.0007	0.0009	0.0012

The tests were done without stirring, as [Figure 3.9](#) revealed that it did not influenced the results significantly when the diffusing stone was used. For this experiment, no replicates were done as the reproducibility of the results was proven in [Table 3.12](#).

From these results and the ones obtained for the concentrations 0.01 - 0.04 - 0.1 mmol/l, a complete view of the degradation of OTC · HCl according to time and the initial concentration can be visualised (see [Figure 3.12](#)). As expected, the greater the OTC · HCl, the longer it takes to reach a near-zero concentration. However, by looking at the slope for each curves between 0 and 15 seconds, it can be observed that the initial the degradation rate is faster for higher OTC · HCl concentration (the slope is steeper).

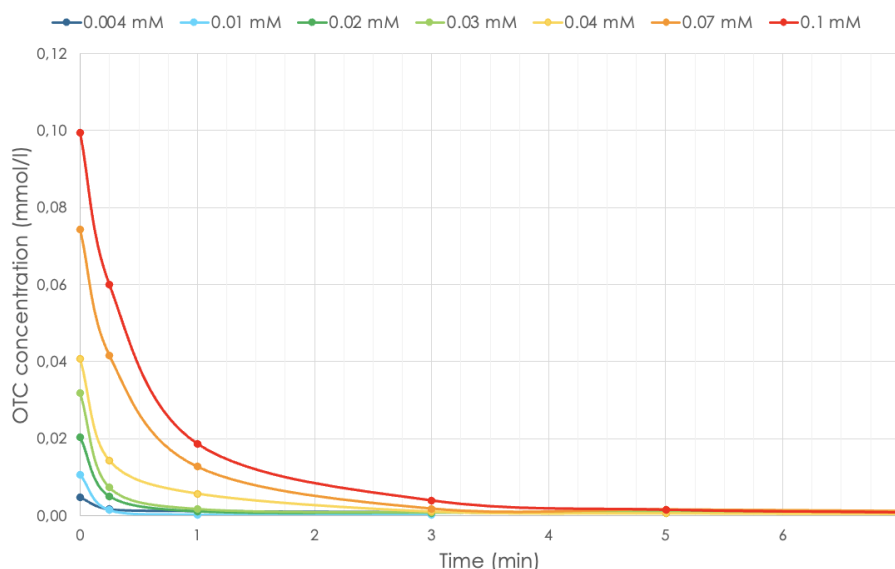


Figure 3.12: Evolution of the concentration (mmol/l) of OTC·HCl measured over time for several initial concentrations: 0.004 - 0.01 - 0.02 - 0.03 - 0.04 - 0.07 - 0.1 mmol/l. The absorbance was recorded at 355 nm and the ozone settings were 31 mmol/h and 0.13 mmol/l. The diffusing stone was used to introduce the ozone bubbles in the solution.

3.2.3 Influence of the ozone generator parameters

As achieved for PNP, the impact of the ozone flow rate and concentration on the degradation of OTC·HCl can be studied. This was done by selecting a range of ozone parameter to remove a given OTC·HCl concentration of 0.1 mmol/l. The treatment time was of seven minutes and the samples were analyzed by UV-Vis spectrophotometer, TOC and HPLC-MS.

3.2.3.1 First trial

The impact of changing the ozone generator parameters was first investigated across four conditions at the outlet of the ozone generator:

- **Condition 1:** Ozone molar flow rate of 31 mmol/h and an ozone concentration of 0.13 mmol/l (reference case).
- **Condition 2:** Ozone molar flow rate of 31 mmol/h and an ozone concentration of 0.05 mmol/l.
- **Condition 3:** Ozone molar flow rate of 48 mmol/h and an ozone concentration of 0.13 mmol/l.
- **Condition 4:** Ozone molar flow rate of 42 mmol/h and an ozone concentration of 0.17 mmol/l.

The results of the degradation tests over seven minutes were analyzed with the UV-Vis spectrophotometer, TOC and HPLC-MS (see Table 3.14, Figure 3.14a, and 3.15a, respectively).

Table 3.14 and Figure 3.13 present the results from the **UV-Vis spectrophotometer**. Comparing Conditions 1 and 2, it appears that an increase of the O_3 flow rate while keeping the O_3 concentration constant (*i.e.* Condition 2) does not improve the degradation kinetics. On the other hand, for Condition 3, a constant O_3 flow rate and a O_3 concentration reduced to 0.05 mmol/l were selected and slowed down the ozonation process. This could suggest that the O_3 concentration, rather than the molar flow rate, is the limiting factor of the reaction. Beyond a given concentration reached, it acts as a saturation concentration. Thus, supplying ozone at a higher flow rate would not provide additional benefit. Finally, in Condition 4, where both the ozone molar flow rate and concentration are increased (*i.e.* the generator

is set to its maximum capacity), the degradation of OTC · HCl is slightly improved over the reference condition.

Table 3.14: Evolution of the OTC · HCl and concentration (mmol/l) depending on the parameters of the ozone generator selected. Operating conditions: [OTC · HCl] = 0.1 mmol/l

Time (min)	Concentration (mmol/l)			
	Cond. 1	Cond. 2	Cond. 3	Cond. 4
0	0.1066	0.1062	0.1046	0.1079
0.25	0.0724	0.0674	0.0864	0.0536
1	0.0213	0.0212	0.0461	0.0186
3	0.0082	0.0080	0.0176	0.0034
5	0.0017	0.0021	0.0110	0.0023
7	0.0008	0.0007	0.0064	0.0003

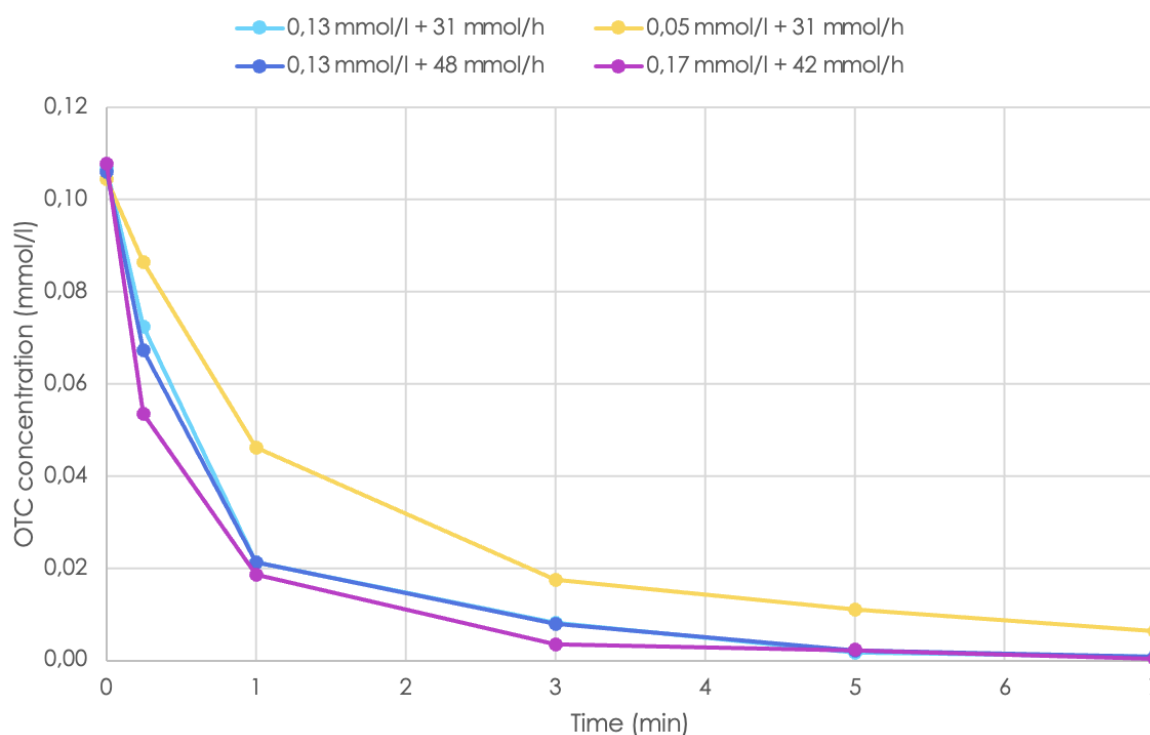
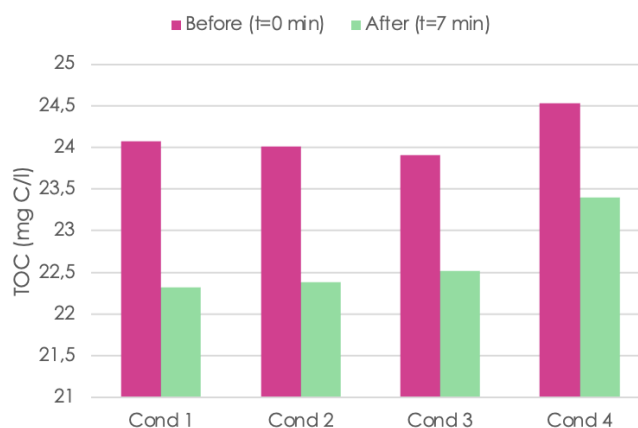


Figure 3.13: Evolution of the OTC · HCl concentration (mmol/l), measured by the UV-Vis spectrophotometer, depending on the parameters of the ozone generator selected. Operating conditions: [OTC · HCl] = 0.1 mmol/l

In parallel to the UV-Vis spectrophotometer, the **TOC** was measured at the beginning and at the end of the experiment (see [Figure 3.14a](#) and [3.14b](#)). It appears that the condition selected barely has an impact on the TOC after seven minutes. Even more, it showcases the fact that in the early stages of the ozonation process, OTC · HCl is rapidly degraded by ozone, but the degradation by-products accumulate simultaneously in the solution. These by-products, which still contain organic carbon, cannot be fully mineralized within such a short treatment time, explaining why the TOC remains relatively high regardless of the ozone generator parameters selected.

	TOC (mg C/l)			
Time (min)	1	2	3	4
0	24.07	24.01	23.91	24.53
7	22.32	22.38	22.52	23.4
Efficiency	7.27%	6.79%	5.81%	4.61%

(a) Experimental data



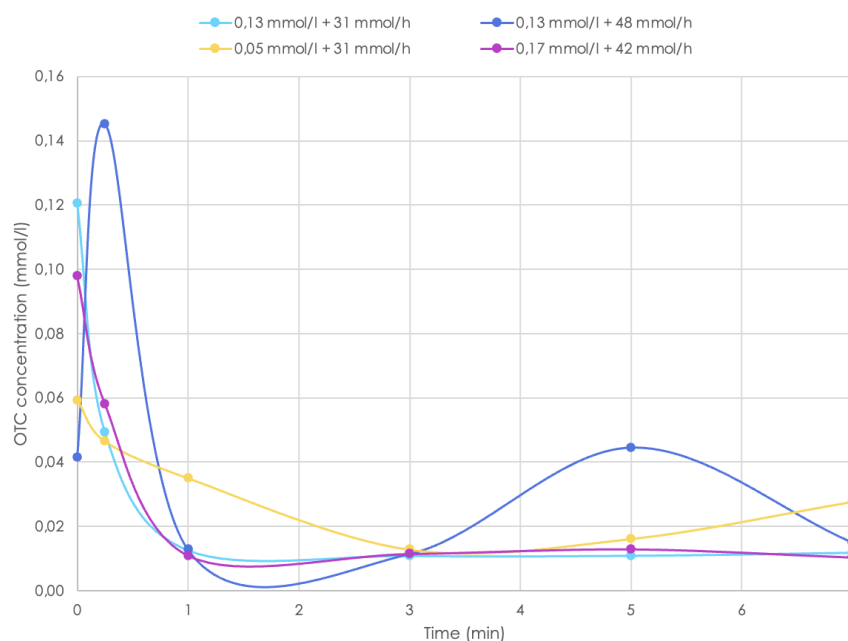
(b) Bar chart of the evolution of the TOC (mg C/l)

Figure 3.14: Evolution of the TOC (mg C/l) over 7 minutes before and after treatment with ozone, depending on the ozone generator parameter. The initial OTC · HCl concentration was 0.1 mmol/l.

Finally, each condition was analyzed with the HPLC-MS machine (see Figure 3.15). Unfortunately, the results are incoherent, as they fluctuate a lot and even the initial concentration differs greatly from one condition to another. There are several possible explanations, including the use of old micropipettes, whose calibration are no longer guaranteed, and sample degradation between collection and analysis (approximately 36 hours) due to the instability of OTC · HCl. Consequently, the HPLC-MS results for this section were considered unreliable and are therefore not further interpreted.

	Concentration (mmol/l)			
Time (min)	1	2	3	4
0	0.1205	0.0415	0.0593	0.0979
0.25	0.0493	0.1452	0.0465	0.0581
1	0.0125	0.0128	0.0350	0.0110
3	0.0107	0.0115	0.0128	0.0115
5	0.0108	0.0446	0.0162	0.0129
7	0.0118	0.0150	0.0277	0.0104

(a) Experimental data



(b) Degradation curves

Figure 3.15: Evolution of the OTC · HCl concentration (mmol/l) measured by HPLC-MS over 7 minutes of treatment with ozone, depending on the ozone generator parameters. The initial OTC · HCl concentration was 0.1 mmol/l.

3.2.3.2 Broader condition range

To get a complete view of the dynamics of the system when the ozone parameters are changed, four additional conditions (0.01 mmol/l + 12.5 mmol/h, 0.05 mmol/l + 17 mmol/h, 0.09 mmol/l + 21 mmol/h

and 0.09 mmol/l + 47 mmol/h) were added to the ones previously studied. This resulted in a total of 8 conditions, which are summarized in Table 2.3. For this extended test, the samples were taken at the same time periods (0 - 15sec - 1 - 3 - 5 - 7 minutes) and were only analyzed by the UV-Vis spectrophotometer. Table 3.15 provides the concentration results for all 8 conditions tested.

Table 3.15: Evolution of the OTC · HCl concentration (mmol/l) depending on the parameters of the ozone generator selected and over 7 minutes. Operating conditions: [OTC · HCl] = 0.1 mmol/l. The bubbling system used is the diffusing stone.

Remaining OTC · HCl concentration (mmol/l)				
Time (min)	0.01 mmol/l + 12.5 mmol/h	0.05 mmol/l + 17 mmol/h	0.05 mmol/l + 31 mmol/h	0.09 mmol/l + 21 mmol/h
0	0.1073	0.1054	0.1046	0.1021
0.25	0.0978	0.0825	0.0864	0.0745
1	0.0723	0.0370	0.0461	0.0237
3	0.0273	0.0165	0.0176	0.0095
5	0.0190	0.0094	0.0110	0.0031
7	0.0153	0.0046	0.0064	0.0009

Remaining OTC · HCl concentration (mmol/l)				
Time (min)	0.09 mmol/l + 47 mmol/h	0.13 mmol/l + 31 mmol/h	0.13 mmol/l + 48 mmol/h	0.17 mmol/l + 42 mmol/h
0	0.1045	0.1066	0.1062	0.1079
0.25	0.0673	0.0724	0.0674	0.0536
1	0.0233	0.0213	0.0212	0.0186
3	0.0079	0.0082	0.0080	0.0034
5	0.0019	0.0017	0.0021	0.0023
7	0.0008	0.0008	0.0007	0.0003

Several observations can be made from the curves obtained (see Figure 3.16). From a general point of view, the greater the ozone flow rate and concentration, the more efficient the removal of OTC · HCl. By looking closer at the curve, it can be observed that the ones for 0.09 - 0.13 - 0.17 mmol/l tend to overlap, no matter the ozone molar flow rate selected. This may suggest that saturation concentration of ozone is reached. Beyond this concentration, the efficiency of the degradation cannot be improved. For lower concentrations (*i.e.* the curves for 0.01 and 0.05 mmol/l), the removal of OTC · HCl is linked to the amount of ozone brought to the system. The analysis of this graph could also underline that at higher flow rates, ozone may not have time to interact with the liquid solution. Indeed, in the velocity at which it is injected in the liquid is too high, ozone could be directly ejected in the outlet gas, without having time to dissolve and react.

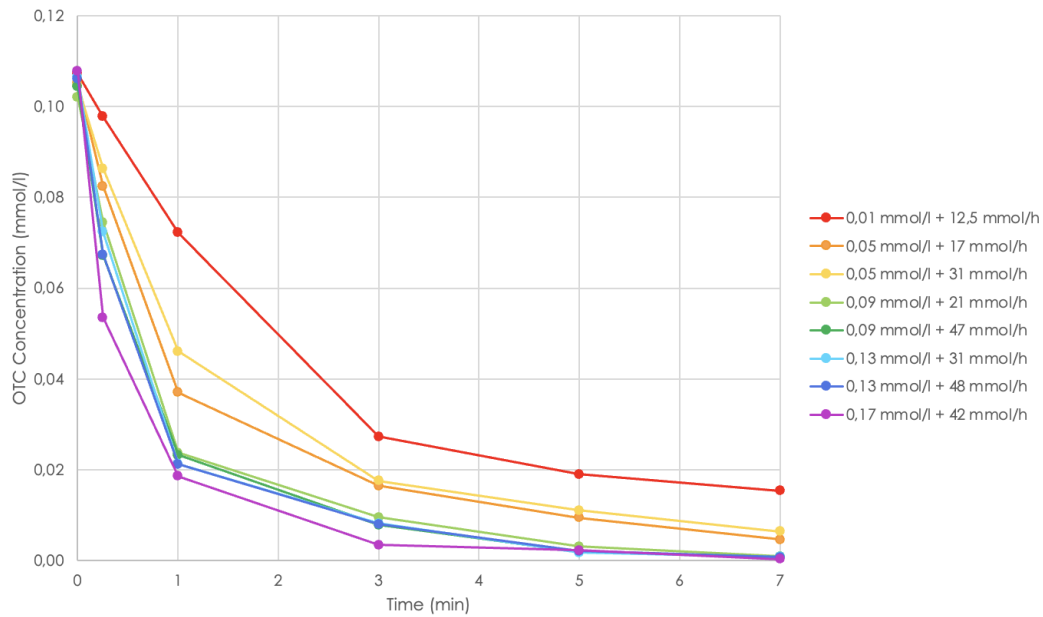
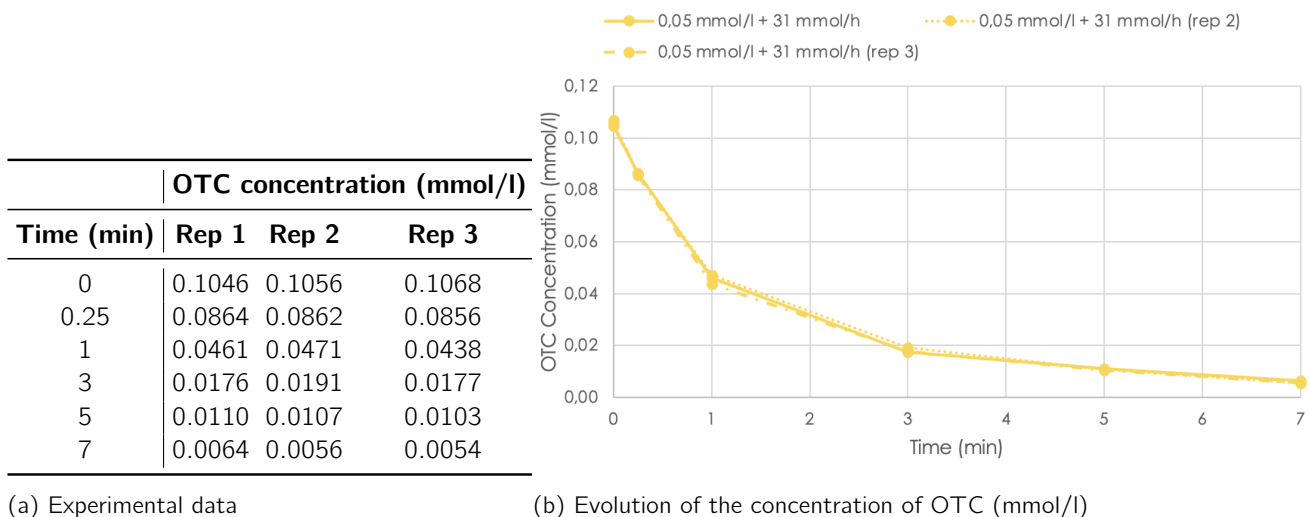


Figure 3.16: Evolution of the remaining OTC·HCl concentration (mmol/l) depending on the parameters of the ozone generator selected and over 7 minutes. Operating conditions: [OTC·HCl] = 0.1 mmol/l. The bubbling system used is the diffusing stone.

3.2.3.3 Triplicates and estimation of the error

Strangely, the curve for 0.05 mmol/l + 17 mmol/h is below the one for 0.05 mmol/l + 31 mmol/h. Intuitively, the opposite behavior should have occurred. To confirm this result, triplicates were made for the case 0.05 mmol/l + 31 mmol/h. As illustrated in Figure 3.17a and Figure 3.17b, the curves overlap nicely, highlighting the reproducibility of the experiment.



(a) Experimental data

(b) Evolution of the concentration of OTC (mmol/l)

Figure 3.17: Evolution of the concentration (mmol/l) over 7 minutes and three replicates with the ozone parameters 0.05 mmol/l + 31 mmol/h. The initial OTC·HCl concentration was 0.1 mmol/l.

From this results, the error on the results can be estimated. Given that the curves obtained overlap and are close one to another (see Figure 3.16), knowing whether the error is significant would allow one to assess the reliability of the results. Therefore, the σ should be calculated. In statistics, this value is of great importance as one can confirm that 68% of the results for within a range of values with and error

of $\pm\sigma$.

$$\sigma = \sqrt{s^2} = \sqrt{\left(\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2\right)} \quad (3.5)$$

In Equation 3.5, n corresponds to the number of replicates, s is the variance and σ is the standard-error. The calculation has been done over all replicates and time points (see Table 3.16). When reporting the σ on the degradation curve as error bars, the plot reveals that the error on the experimental data is small, and will therefore not affect the accuracy of the results for the other curves (see Figure 3.18).

Table 3.16: Estimation of the variance and standard-deviation value for the triplicates performed with the ozone parameters 0.05 mmol/l + 31 mmol/h and an [OTC·HCl]=0.1 mmol/l.

Time (min)	Mean	Variance	Standard-deviation (σ)
0	0.106	1.19E-06	0.0011
0.25	0.086	1.96E-07	0.0004
1	0.046	2.83E-06	0.0017
3	0.018	6.92E-07	0.0008
5	0.011	1.23E-07	0.0004
7	0.006	2.80E-07	0.0005

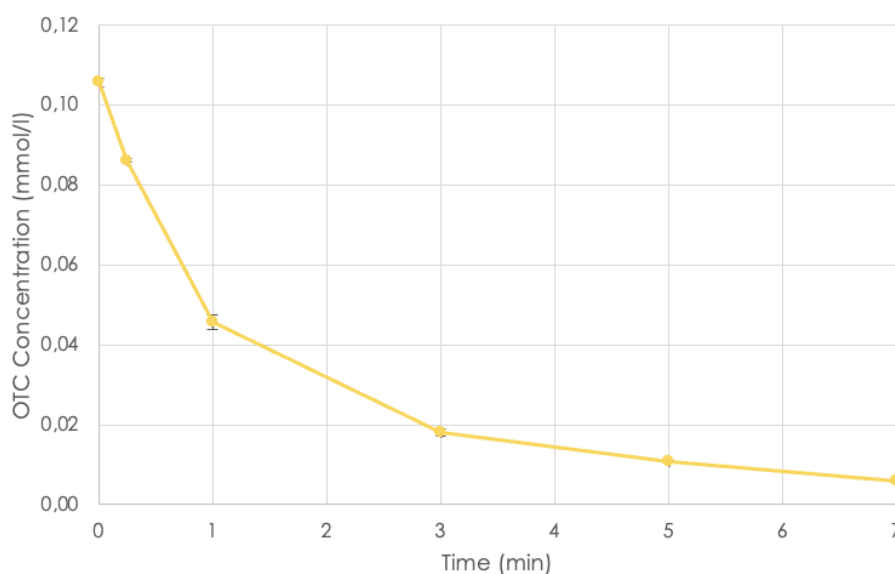


Figure 3.18: Representation of the error bars ($y \pm \sigma$) on the degradation curve of OTC·HCl with the ozone parameters 0.05 mmol/l + 31 mmol/h. The absorbance was measured at 355 nm and the mean values for the triplicates were taken.

Finally, it is possible conclude on whether the 0.05 mmol/l + 17 mmol/h and 0.05 mmol/l + 31 mmol/h curves in Figure 3.16 are significantly different or not. To do that, the error bars calculated in Table 3.16, are added in Figure 3.16, leading to Figure 3.19.

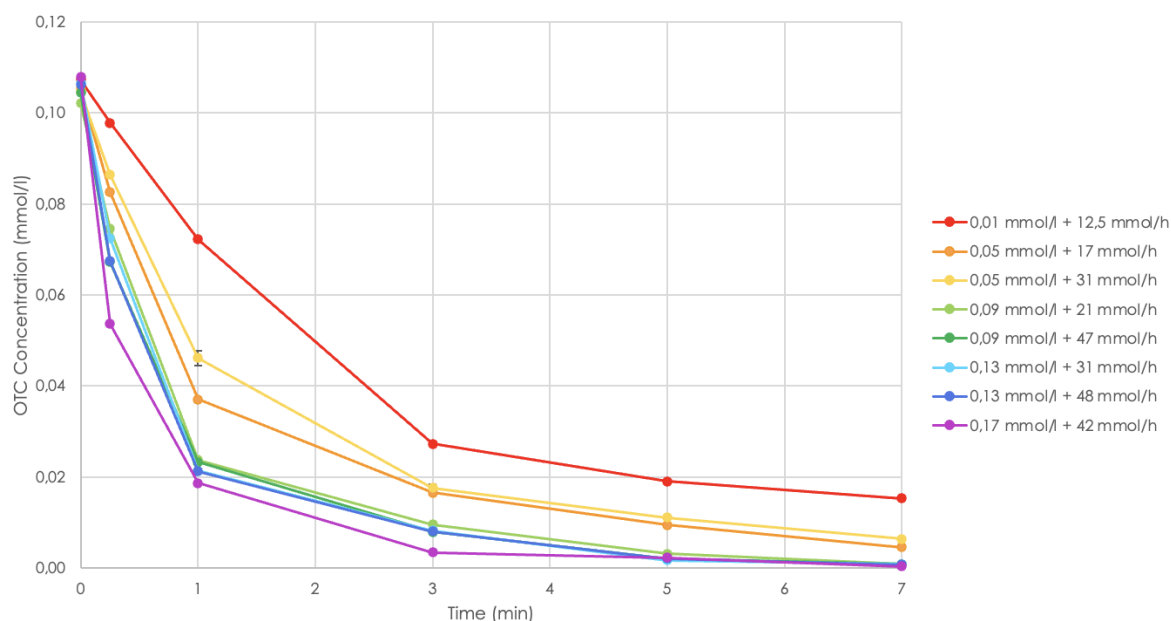
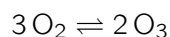


Figure 3.19: Evolution of the remaining OTC·HCl concentration (mmol/l) depending on the parameters of the ozone generator selected and over 7 minutes. Operating conditions: [OTC·HCl] = 0.1 mmol/l. The bubbling system used is the diffusing stone. The error bars ($y \pm \sigma$) for the curve 0.05 mmol/l + 31 mmol/h are added to determine whether this curve is significantly different from the 0.05 mmol/l + 17 mmol/h curve.

The two curves follow similar behaviors and their values are close, especially from $t = 3$ minutes. Moreover the error bar at $t = 1$ minute is larger, and could include the error of the 0.05 mmol/l + 17 mmol/h curve. To conclude firmly on whether the difference between these two curves is significant, the error bars for the orange curve should also have been calculated. However, replicates were not performed for this condition. The two curves corresponding to 0.05 mmol/l show very similar degradation profiles. This suggests that increasing the ozone flow rate from 17 to 31 mmol/h does not significantly enhance the degradation rate. Therefore, the difference between these two curves could be a visual artefact.

Another factor could be taken into account to explain the difference between the two curves. The volumetric flow rate fixed with the rotameter differs between the two conditions: 10 l/min for 0.05 mmol/l + 31 mmol/h and 6 l/min for 0.05 mmol/l + 17 mmol/h. A higher volumetric flow rate should lead to a more vigorous agitation in the liquid, which enhances the mass transfer and degradation.

Given the reaction,



the total volumetric flow rate slightly decreases between the inlet and the outlet of the generator, as three moles of oxygen yield only two moles of ozone. Nevertheless, this difference should be very small.

Therefore, the higher volumetric flow rate of the 0.05 mmol/l + 31 mmol/h curve should further enhance its degradation efficiency compared to the 0.05 mmol/l + 17 mmol/h one. The opposite behavior is observed. This reinforces the idea that the difference between the two curves is most likely a visual artefact linked to experimental variability, as highlighted by the error bar analysis.

3.2.3.4 Impact of the selected bubbling system

As all tests regarding this experiment were performed with the diffusing stone, one may wonder about the impact of changing the bubbling system. This was discovered somewhat by accident. During the experiments, the sintered filter was inadvertently connected to the ozone generator instead of the diffusing

stone. Measurements were taken with this setup under three conditions: $0.01 \text{ mmol/l} + 12.5 \text{ mmol/h}$, $0.05 \text{ mmol/l} + 17 \text{ mmol/h}$ and $0.05 \text{ mmol/l} + 31 \text{ mmol/h}$. Once the mistake was noticed, the procedure was repeated, this time using the diffusing stone (see Figure 3.20). It appears that the diffusing stone achieves a faster degradation of OTC·HCl than the sintered filter, suggesting that the diffusing stone could be more interesting when coming to the degradation of the antibiotic. This result is consistent with the trends observed in Figure 3.11b.

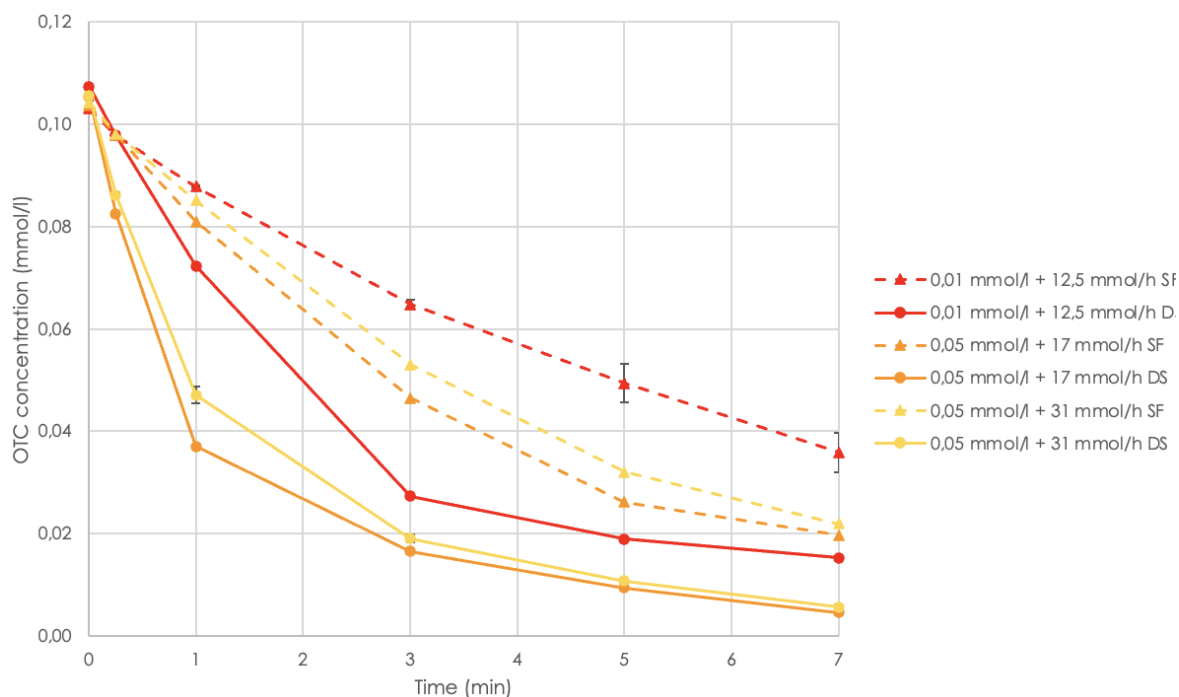


Figure 3.20: Comparison between the efficiency of sintered filter (SF) and the diffusing stone (DS) on the removal of OTC·HCl (initial concentration of 0.1 mmol/l) for three ozone parameters: $0.01 \text{ mmol/l} + 12.5 \text{ mmol/h}$, $0.05 \text{ mmol/l} + 17 \text{ mmol/h}$ and $0.05 \text{ mmol/l} + 31 \text{ mmol/h}$. For $0.01 \text{ mmol/l} + 12.5 \text{ mmol/h}$ SF and $0.05 \text{ mmol/l} + 31 \text{ mmol/h}$ DS replicates were available and error bars were added ($y \pm \sigma$).

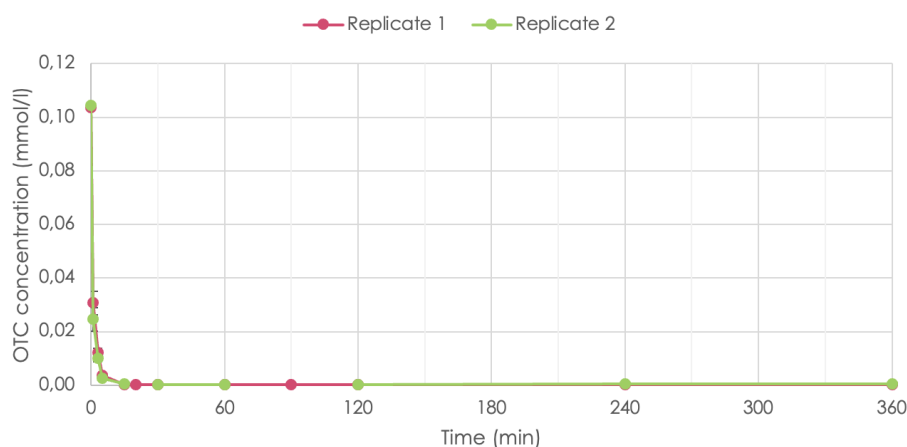
3.2.4 Influence of changing the bubbling system on the 6 h degradation test

The previous section showed that the choice of the selected bubbling system (sintered filter >< diffusing stone) has an impact on the degradation efficiency on a short time scale (7 min). It appeared that the diffusing stone was a more suitable option. To confirm this insight, the 6 hour degradation test performed in Section 3.2.1 was repeated and the diffusing stone replaced the sintered filter. The samples were then analysed with the TOC and the UV-Vis spectrophotometer (see Table 3.17, Figure 3.21a and 3.21b).

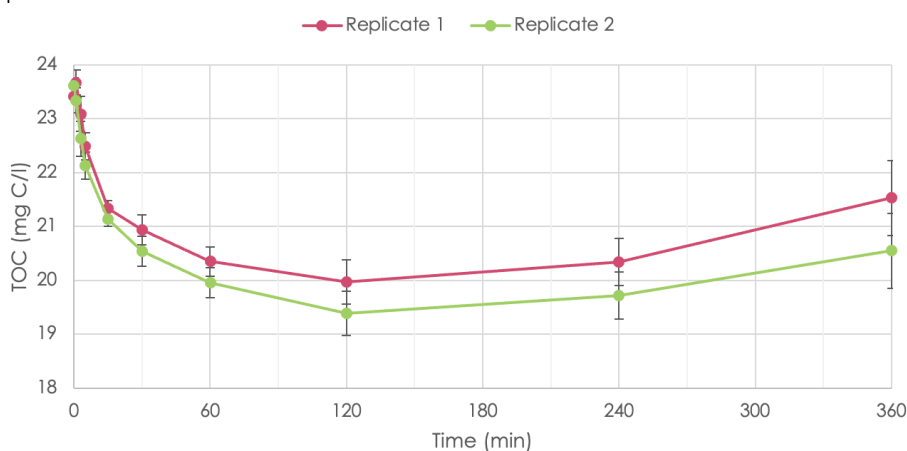
For the concentration values obtained with the UV-Vis spectrophotometer, the trends are coherent with those obtained previously when the sintered filter was used. However, intriguing results are found for the TOC analysis. It appears that after 120 minutes, the TOC values start to increase again. Nevertheless, lower TOC values are found for the second replicate. This difference between the two replicates can be explained by fact that, for the first replicate, the tube connecting the stone to the tube of the ozone generator developed a leak at around 90 minutes. Parafilm was applied to seal the hole immediately. This could have impacted the degradation of the by-products and thus the TOC, as the amount of ozone being released was significantly lowered during that period.

Table 3.17: Evolution of the OTC·HCl concentration (mmol/l) and TOC (mg C/l) when the diffusing stone is used. Operating conditions: [OTC·HCl] = 0.1 mmol/l, O₃ concentration = 0.13 mmol/l and O₃ molar flow rate = 31 mmol/h.

Time (min)	Concentration (mmol/l)			TOC (mg C/l)		
	Replicate 1	Replicate 2	Standard-deviation (σ)	Replicate 1	Replicate 2	Standard-deviation (σ)
0	0.1037	0.1045	5.49E-04	23.42	23.62	1.41E-01
1	0.0307	0.0246	4.33E-03	23.67	23.34	2.33E-01
3	0.0122	0.0101	1.48E-03	23.09	22.63	3.25E-01
5	0.0036	0.0027	6.58E-04	22.49	22.13	2.55E-01
15	0.0003	0.0005	1.10E-04	21.34	21.14	1.41E-01
30	0.0002	0.0003	5.49E-05	20.94	20.54	2.83E-01
60	0.0002	0.0003	5.49E-05	20.35	19.96	2.76E-01
120	0.0002	0.0002	0.00E+00	19.97	19.39	4.10E-01
240	0.0002	0.0004	1.10E-04	20.34	19.72	4.38E-01
360	0.0002	0.0004	1.10E-04	21.53	20.55	6.93E-01



(a) Concentration profile over time



(b) TOC profile over time

Figure 3.21: Evolution of the concentration (mmol/l) of OTC·HCl and TOC (mg C/l) over 6 hours when the diffusing stone is used. The half length of the error bars is σ and contains 68% of the values if the gaussian error is accepted. The OTC·HCl concentration was of 0.1 mmol/l and an ozone flow rate of 1.5 gO₃/h.

3.2.5 Impact of the salinity

The impact of increasing the salinity of the medium by adding 2.8 g of NaCl into 400 ml of solution (7 g/l) was assessed. Visually, it can first be observed that the colour of the solution evolved during the first hour of the experiment (see [Figure 3.22](#)). The samples shifted from transparent to yellow after only one minute. The yellow colour reached a peak intensity after 3 minutes, before gradually turning transparent again. For the five remaining hours, the solution stayed colourless. This could be linked to the apparition and reaction of by-products with the salty medium.



Figure 3.22: Evolution of the colour of the samples over the first hour of the experiment.

The results of the degradation test across six hours are listed in [Table 3.18](#), for both the TOC and UV-Vis spectrophotometer.

Table 3.18: Evolution of the OTC · HCl concentration (mmol/l) and TOC (mg_C/l) when the salinity of the medium is 7 g/l. Operating conditions: [OTC · HCl] = 0.1 mmol/l, O₃ concentration = 0.13 mmol/l and O₃ molar flow rate = 31 mmol/h.

Time (min)	UV-Vis spectrophotometer Concentration (mmol/l)	TOC TOC (mg _C /l)
0	0.10432	24.12
1	0.04056	24.03
3	0.01737	23.37
5	0.00976	22.9
15	0.00139	22.05
30	0.00092	21.88
60	0.00100	21.68
120	0.00061	21.75
240	0.00030	22.36
360	0.00030	24.24

When looking at the degradation curve (see [Figure 3.23](#)) obtained with the UV-Vis spectrophotometer, it can be seen that the results with salty water completely overlap the ones found previously for the synthetic water with a salinity of 0 g/l. This could suggest that the salinity has no impact on the degradation mechanism of OTC · HCl.

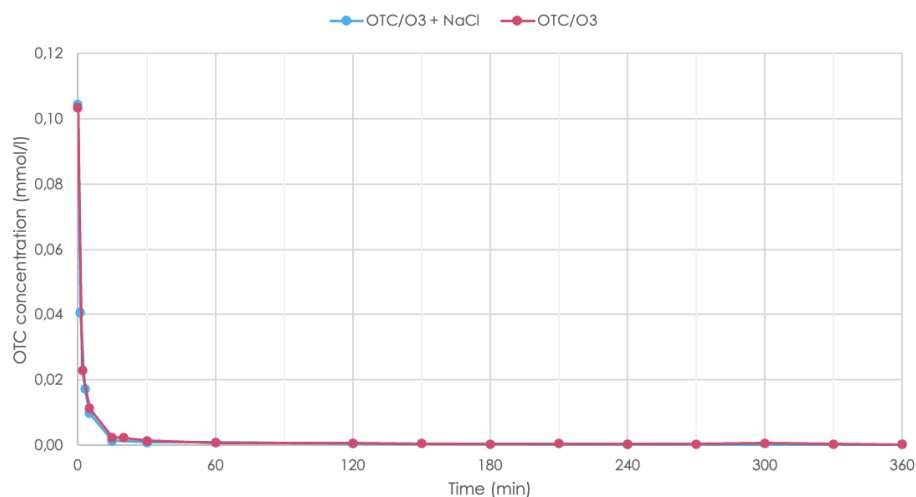


Figure 3.23: Evolution of the concentration (mmol/l) of OTC · HCl across 6h with and without NaCl.

When comparing the TOC plots (see [Figure 3.24](#)), the curve for NaCl exhibits some rather surprising behavior. Instead of decreasing, it reaches a plateau, before increasing again. A detailed discussion about the impact of NaCl is undertaken in [Section 4.2.4](#). Unfortunately, only one replicate was made for this experiment. Therefore, this trend cannot be confirmed. Moreover, the stirring of the solution has to be stopped after 3 hours due to an insufficient level of solution remaining. Yet, stirring should only have a minute impact on the results as motion induced by the sintered filter should homogenize the solution correctly (Dr. Calvo S., Leading research logistics provider, personal communication, 2026). This matches with the observations made in [Figure 3.9](#), which highlighted that the effect of stirring was not significant when using the diffusing stone.

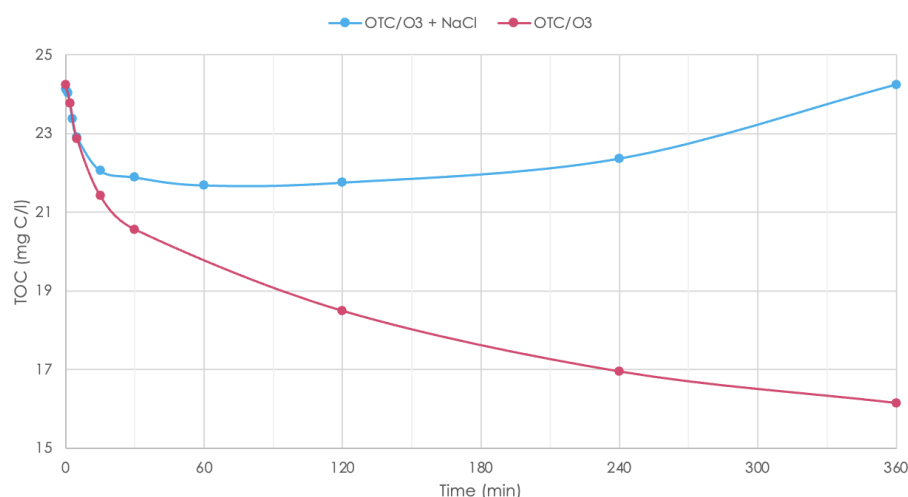


Figure 3.24: Evolution of the TOC (mg_C/l), of OTC · HCl across 6h with and without NaCl.

3.3 Degradation of Rifampicin

The degradation of rifampicin by ozone was done in triplicates. The results for the evolution of the absorbance at 475 nm and the corresponding remaining concentration of antibiotic are shown in [Table 3.19](#). The Beer-Lambert equation obtained from the calibration curve in [Appendix C.3](#) is used to convert absorbances into concentrations.

Table 3.19: Evolution of the rifampicin concentration (mmol/l). Operating conditions: [Rifa] = 0.05 mmol/l, O₃ concentration = 0.13 mmol/l and O₃ molar flow rate = 31 mmol/h.

Time (min)	Concentration (mmol/l)			
	Replicate 1	Replicate 2	Replicate 3	Standard-deviation (σ)
0	0.0510	0.0505	0.0523	9.02E-04
0.5		0.0190	0.0214	1.68E-03
1	0.0073	0.0075	0.0074	1.25E-04
2	0.0019	0.0013	0.0014	3.31E-04
3	0.0010	0.0008	0.0006	1.91E-04

One can see that the trend of the degradation curve (see [Figure 3.25](#)) is similar to the ones found previously for PNP and OTC. Nearly complete removal of rifampicin is observed after 3 minutes, highlighting the efficiency of ozone to break down this molecule.

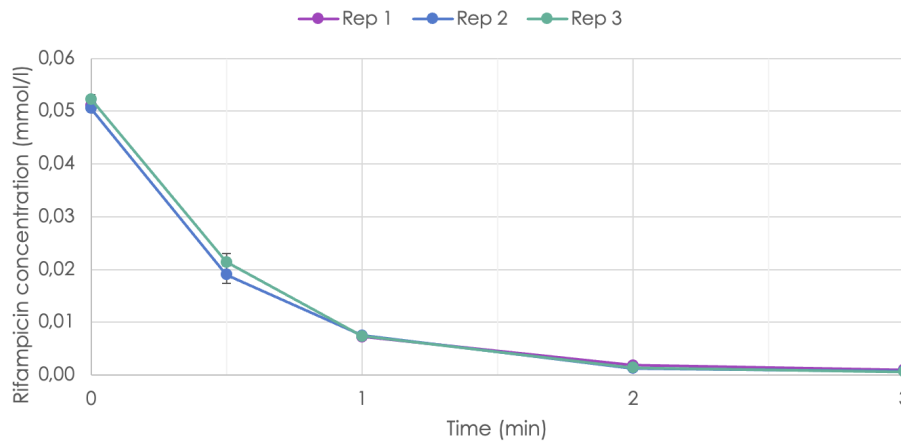


Figure 3.25: Evolution of the concentration (mmol/l) over time for the O₃ flow rate 31 mmol/h and an O₃ concentration of 0.13 mmol/l. The half length of the error bars is σ and contains 68% of the values if the gaussian error is accepted. The initial rifampicin concentration was 0.05 mmol/l.

For one on the replicates, samples were taken after 0 - 3 - 15 minutes to be analyzed by TOC (see [Table 3.20](#)).

Table 3.20: Evolution of the TOC over time for the O₃ flow rate 31 mmol/h and an O₃ concentration of 0.13 mmol/l. The initial rifampicin concentration was 0.05 mmol/l.

Time (min)	TOC (mg _C /l)
0	260.1
3	256.8
15	245.4

These values can be compared to the theoretical TOC at time zero for rifampicin,

$$\begin{aligned}
 \text{TOC}_{\text{Rifa},0} &= \frac{\text{Rifampicin concentration (mmol/l)}}{\text{Molar mass of rifampicin (g/mol)}} \times (\text{Number of Carbons} \times \text{Molar mass of Carbon (g/mol)}) \\
 &= \frac{0.05 \text{ mmol/l}}{822.9 \text{ g/mol}} \times (43 \times 12 \text{ g/mol}) = 25.7 \text{ mg C/l}
 \end{aligned}$$

The results differ by a factor 10, which is intriguing. An explanation could be a saturation of the NDIR detector of the TOC machine due to a too concentrated sample, even though it seems unlikely. It could also be due to a mistake in the setting up of the measurement. Anyway, the results were not considered reliable for a comparison with the ones obtained for OTC · HCl.

Even the test was made on exploratory purposes only, it would have been relevant to replicate the experiment to assess if a similar behavior occurred once the sample diluted.

Chapter 4

Discussion

4.1 Preliminary tests with PNP

4.1.1 Influence of the PNP concentration on the kinetics

The objective of this experiment was to monitor the kinetics of the degradation and to determine wherever the rate law could be associated to a pseudo-first order for PNP, with a constant ozone concentration. As suggested by Singh and Majumder (2025), the kinetics of the degradation could be the following,

$$\frac{d[PNP]}{dt} = -k_{O_3}[O_3][PNP] \quad (4.1)$$

$$= -k_{app}[PNP] \quad (4.2)$$

with the apparent constant rate $k_{app} = k_{O_3}[O_3]$.

If the concentration of ozone is assumed constant, *i.e.* ozone is continuously fed to reactor and instantly consumed, and if the reaction can be considered as a pseudo-first order for PNP and can be derived from the integration of Equation 4.3,

$$\ln \left(\frac{[PNP]_0}{[PNP]} \right) = k_{app}t \quad (4.3)$$

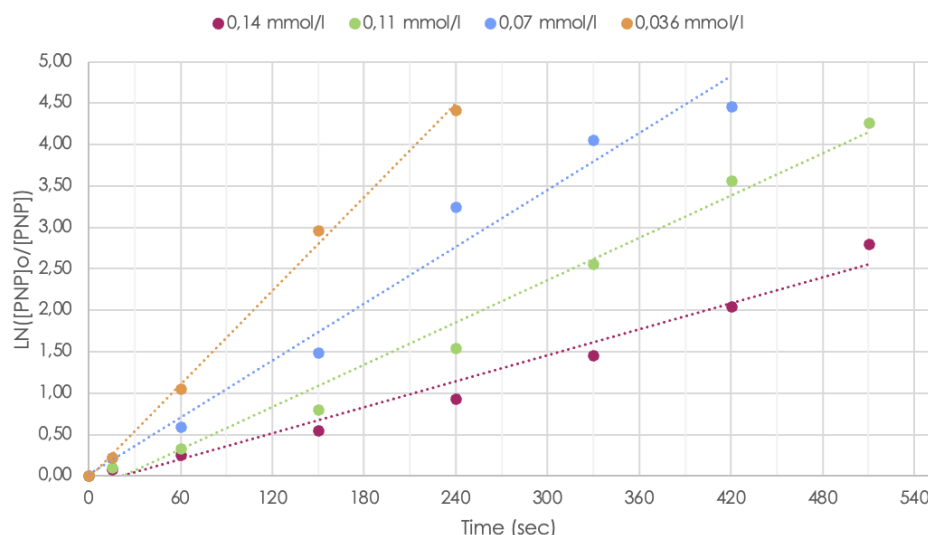
where $[PNP]_0$ is the initial PNP concentration and $[PNP]$ the concentration after a given time.

Thus, the rate constant can be found from the slope the curve $\ln \left(\frac{[PNP]_0}{[PNP]} \right)$ with time (see Figure 4.1).

As illustrated in Table 4.1, the slope increases with decreasing initial PNP concentrations.

This observation raises several questions. Although the concentration and flow rate of gaseous ozone injected into the solution is constant, the values of the kinetic constants do not follow a pseudo-first-order model, which should give the same k_{app} value for the four series of points corresponding to the four different initial PNP concentrations. In fact, the experimental data points do not align perfectly with the linear approximation. Instead, they follow a curved trajectory, with concave or convex sections.

The results of this experiment indicate that chemical kinetics alone do not fully govern the degradation of PNP by ozone. In further investigation, the impact of bubble size, gas-liquid mass transfer should be studied to obtain a deeper understanding of the mechanism of ozonation. Future research should prioritize evaluating the gaseous-to-liquid ozone transfer rate and measuring the precise fraction of ozone that actively participates in the reaction.



Initial [PNP] (mmol/l)	k_{app}
0.14	0.0052
0.11	0.0085
0.07	0.0115
0.036	0.0188

Table 4.1: Value of the slope k_{app} for the initial PNP concentration range: 0.036-0.07-0.11-0.14 mmol/l.

Figure 4.1: Approximation of k_{app} from the plot slope of $\ln \left(\frac{[PNP]_0}{[PNP]} \right)$ over time for a constant ozone flow rate of 31 mmol/h.

4.2 Study of OTC · HCl

4.2.1 First degradation test and comparison of ozonation with UVC-photocatalyst

This first degradation test of OTC · HCl revealed that ozone can effectively degrade the antibiotic within the six hours period studied. More specifically, the results of the HPLC-MS and UV-Vis spectrophotometer showed that about 90% of the OTC · HCl present initially was degraded after five minutes only. However, when focusing on the total organic carbon present in the sample, the ozonation process achieved only a 35% degradation. This suggests that the degradation by-products could not be removed efficiently. When comparing Figure 3.6 and 3.7, it appears that the degradation of the OTC · HCl occurs rapidly in the first moments of the treatment, followed by a slower and incomplete degradation of the by-products. To improve the by-products removal efficiency, an idea could be to combine ozone to another process.

To illustrate this assumption, one can compare the performances of ozone to degrade 0.1 mmol/l of OTC · HCl, to the ones of a photocatalytic test (UVC-PC). It consisted in exposing the same OTC · HCl solution to UV-C light and using titanium dioxide (TiO_2) as a photocatalyst with a concentration on 200 mg/l (Tran X.N.A, PhD student, personal communication, 2026).

Figure 4.2 enables a rapid comparison of both processes. Figure 4.2b and 4.2c demonstrate that ozone is quicker to degrade OTC · HCl than the UVC-PC process. To achieve 90% of degradation, it only takes 5 minutes for the O_3 process, while 60 minutes in the case the UVC-PC (according to the HPLC-MS results). As mentioned previously, the HPLC analysis is more sensitive. Thus, the curves are less smooth than for the UV-Vis spectrophotometer, even though they exhibit similar trends. However, UVC-PC is much more efficient to reduce the TOC ($\text{O}_3 = -35\% > \text{UVC-PC} = -60\%$, see Figure 4.2a).

The results suggest that both techniques have their strengths and are complementary. While O_3 enables a fast degradation of the parent compound (*i.e.* OTC · HCl), the UVC-PC system is more effective at removing degradation by-products. As previously reported in the literature, combining UVC-PC and O_3 could be an effective approach to degrade both the antibiotic and its by-products.

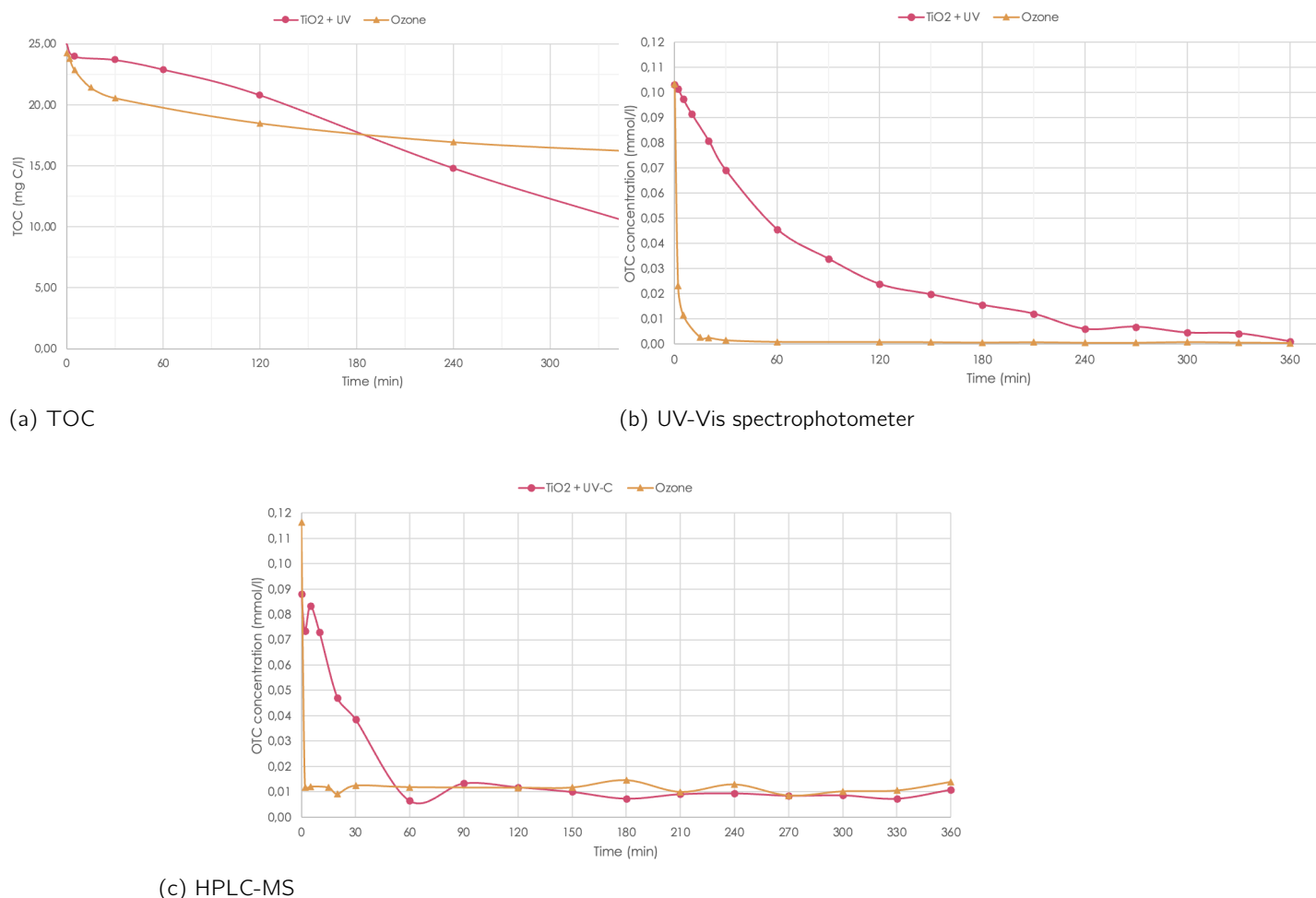


Figure 4.2: Comparison of the performances of the UVC-PC and O₃ processes on a 0.1 mmol/l OTC · HCl solution. Ozone process: O₃ flow rate = 31 mmol/h. UVC-PC process: UV-C light intensity = 4 uW/cm² and [TiO₂]=200 mg/l

4.2.2 Influence of the OTC concentration

4.2.2.1 Impact of the bubbling system on the gas-liquid mass transfer

An almost 10% difference in the degradation efficiency was observed between the sintered filter and the diffusing stone. It may be explained by gas-liquid mass transfer limitations. In this regard, the size of the bubbles is a key parameter. Smaller bubbles offer a larger surface, which maximises the contact between gaseous ozone and the liquid solution. A greater contact leads to an improved degradation efficiency. Several factors affect the bubble size, such as the porosity of the material or the area of the porous surface.

A comparison between the bubble size of the two bubbling system employed was undertaken through an X-ray tomography experiment. The core concept of this technique is to produce a 3D model of a porous material using X-rays, enabling the determination of the pore size. This analysis done by Dr Sébastien Calvo and Dr. Alexandre Léonard from the CARPORVISU platform (ULiège). However, the results of this analysis are not yet available at the time of writing. Once obtained, they would provide a great insight into the bubble size distribution of sintered filter and diffusing stone. From that, a more in depth discussion on the ozonation efficiency could be made.

Based on the current results, it can nevertheless be assumed that the diffusing stone generates smaller bubbles than the sintered filter, resulting in a larger gas-liquid interface, leading to a more efficient mass

transfer. This would support the selection of the most effective device maximizing OTC · HCl degradation, although other criteria must also be considered, as discussed in Section 4.2.3.

4.2.2.2 Determination of the kinetics

The results indicate the initial concentration of OTC · HCl has an impact on the rate of its degradation. It could be worthwhile to investigate the kinetics of the antibiotic regarding its concentration. To this end, the flow rate and concentration of gaseous O₃ injected in the solution was considered as a constant.

Therefore, the rate law can be written using the empirical model and simplified,

$$r = k[\text{OTC}]^\alpha [\text{O}_3]^\beta \quad (4.4)$$

$$r_{\text{OTC}} = k'[\text{OTC}]^\alpha \quad (4.5)$$

where $k' = k[\text{O}_3]^\beta$, α and β are the orders of the reaction for OTC and O₃, respectively.

It is worth noting that the concentration of dissolved ozone should be considered when calculating the reaction rate. Unfortunately, this concentration could not be measured, and only the concentration of gaseous O₃ was available. As this concentration is considered constant and included in the apparent rate constant k' , the following equations remain acceptable.

For each concentration, the initial rate can be calculated using,

$$r_{\text{initial}} = -\frac{dC}{dt} = -\frac{C_{t_1} - C_{t_0}}{t_1 - t_0} \quad (4.6)$$

with $t_1 = 15$ seconds and $t_0 = 0$ seconds.

By doing so for each concentration tested, Table 4.2 is obtained,

Table 4.2: Initial rate calculated for different initial OTC · HCl concentration tested.

[OTC] (mmol/l)	0.004E-03	0.01E-03	0.02E-03	0.03E-03	0.04E-03	0.07E-03	0.1E-03
Initial rate (mol/l.s)	2.120E-07	6.171E-07	1.024E-06	1.639E-06	1.707E-06	2.177E-06	2.632E-06

Looking back at Equation 4.5, k' and α still need to be determined. This can be carried out by performing an optimization with the Python tool. The curve.fit function of the SciPy library was used. The Python program is available in Appendix G. By running the simulation, the optimization results are $k' = 0.0005$ and $\alpha = 0.5736$. The fitted data can be visualized in Figure 4.3.

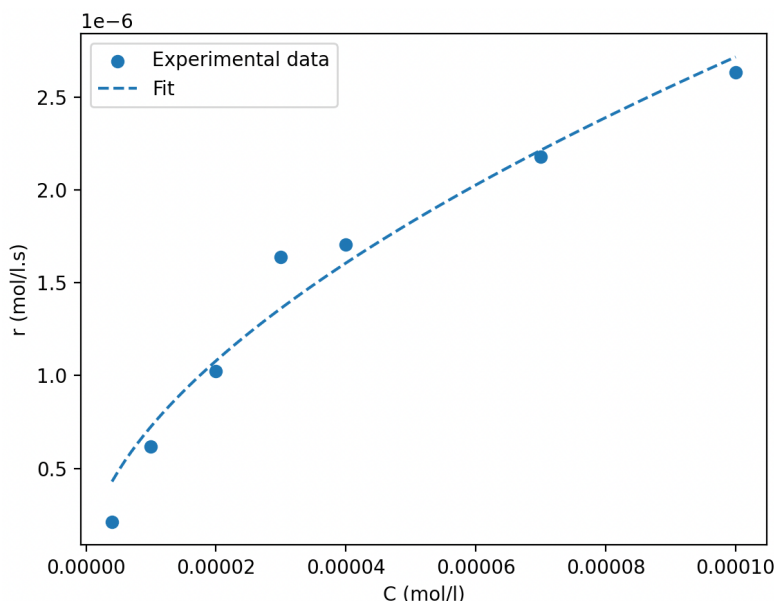


Figure 4.3: Data fit for OTC

The units of the apparent constant k' can be determined by,

$$r = k \cdot C^\alpha$$

$$\frac{\text{mol}}{\text{l} \cdot \text{s}} = k \left(\frac{\text{mol}}{\text{l}} \right)^\alpha$$

$$\rightarrow k = \left(\frac{\text{mol}}{\text{l}} \right)^{1-\alpha} \cdot \text{s}^{-1} = \left(\frac{\text{mol}}{\text{l}} \right)^{0.43} \cdot \text{s}^{-1}$$

If $\alpha = 1$ (first order), then k' would be s^{-1} , which is consistent with the standard unit for a first-order rate constant.

To find the kinetics that most closely match the actual reaction, a Langmuir-type model could be considered and expressed as,

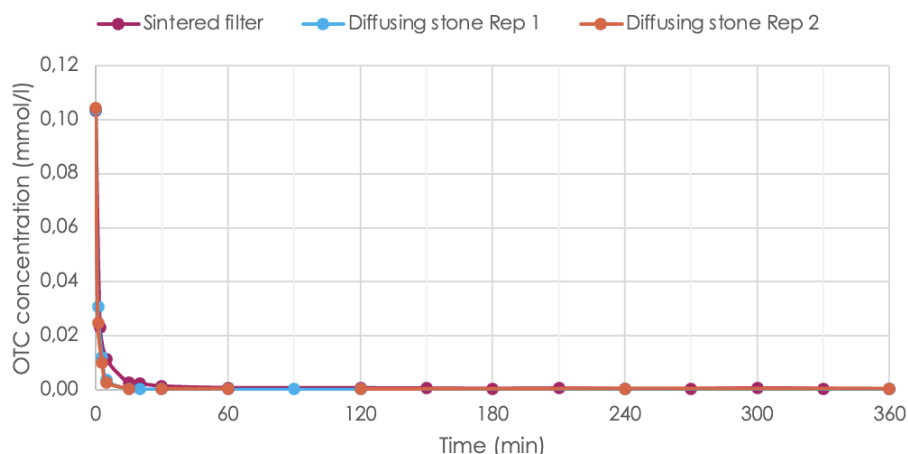
$$r = \frac{k[\text{OTC}]^m}{(1 + K[\text{OTC}])^n}$$

where K is the equilibrium constant.

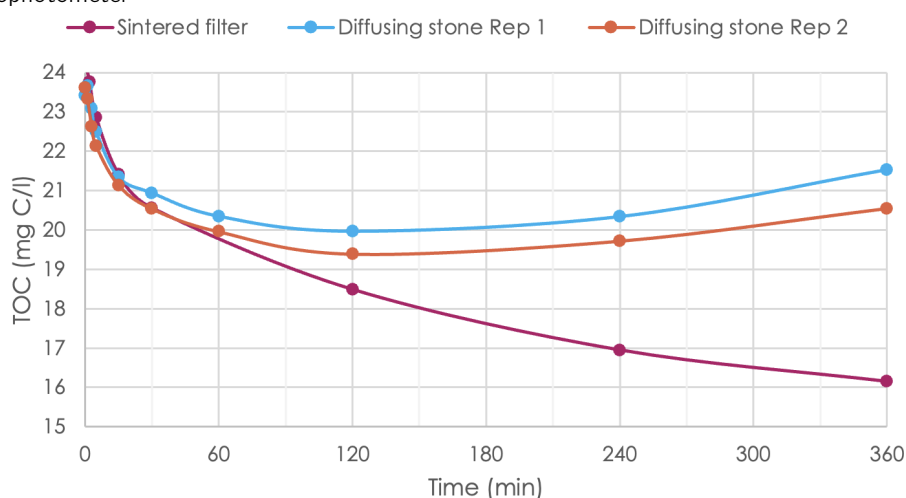
The next step could be to adjust the values of the exponents m and n to determine which values best match the experimental data.

4.2.3 Influence of changing the bubbling system on the 6 h degradation test

The results obtained for the diffusing stone can be compared to those found with the sintered filter (see Figure 4.4). For the UV-Vis analysis, similar results are obtained for either bubbling system (see Figure 4.4a). This illustrates their ability to degrade the OTC · HCl molecule efficiently. Figure 4.4b shows that the diffusing stone is not suitable when it comes to eliminating the degradation by-products.



(a) UV-Vis spectrophotometer



(b) Total organic carbon

Figure 4.4: Evolution of the concentration (mmol/l) of OTC · HCl and TOC (mg C/l) over 6 hours depending on the bubbling system used. The OTC · HCl concentration was of 0.1 mmol/l and an ozone flow rate of 31 mmol/h.

An explanation to the difference may be directly related to the intrinsic behavior of the diffusing stone. Experiments performed in previous studies have revealed the stone's habit to absorb some of the OTC · HCl or the by-products present at higher concentration in the reactor at earlier stage of process, and then releases them over time (Pr. Lambert S. and Dr. Mahy J., Professor and Post-doctoral researcher, personal communications, 2026). If the stone is of mediocre quality, it may also contain dust or calcium carbonate (CaCO_3) that could end up in the sample.

It is noteworthy that an increase in TOC cannot be attributed to the degradation process itself. The organic carbon present in solution exclusively originates from the OTC · HCl molecule and its derived by-products. Therefore, its value can only decrease or remain stable if the organic by-products appear at the same rate as OTC is degraded. Any observed rise in TOC must necessarily come from an external carbon source, such as organic compounds released by the diffusing stone, the tubing, or other components of the experimental setup.

Another possible explanation to this phenomenon could be the corrosion of the plastic pipe connecting the stone to the generator, which led to the hole in the pipe. Indeed, some materials are known to deteriorate due to the oxidative properties of ozone. This happens when the concentration of ozone exceeds 50 mg/l (i.e. 50 ppm or 1.04 mmol/l). The Oxytrading (2026) website provides a ranking of the materials most

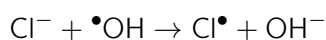
likely to degrade. However, silicone, which is the material of which the plastic tube is made, appears to have an excellent resistance to ozone. Thus, the pipe failure may be attributed to the stress applied to the pipe of connected to the stone at the junction with generator's output.

From these observations, the use of the diffusing stone should be avoided.

4.2.4 Impact of the salinity

As shown in [Figure 3.24](#), the curve for the OTC solution with a salinity of 7 g/l has a surprising trend. The literature offers some possible explanations.

The water matrix is an important factor in an ozonation process. Impurities in the water can affect the efficiency of the degradation ([Liao et al., 2001](#)). Chloride ions (Cl^-) are part of the inorganic ions which are the most common in wastewater effluents. They are known for their scavenging role of hydroxyl radicals ([Qiu et al., 2023](#)). For a sufficiently high Cl^- concentration, the concentration of $\bullet\text{OH}$ radicals decreases, which reduce their activity ([Liao et al., 2001](#)). [Asghar et al., \(2022\)](#) explained that the reaction between chloride ions and hydroxyl radical forms chloride radicals that could form HOCl^- species.



These chlorine radicals may react with organic intermediates, forming chloro-organic compounds more resistant to further oxidation. This may explain the plateau found in the curve.

Yet, the scavenging effect of chloride is minimal compared to the significant impact of pH on radical formation. It also does not explain why the TOC values rise again. One possible explanation lies in the volume decrease of the sample. At the end on the the experiment, the remaining volume was of about 150 ml (out of the 400 ml initially present). Thus, the evolution of the $\text{OTC} \cdot \text{HCl}$ concentration has been affected, resulting in an altered value of the concentration measured by the TOC machine. As previously stated, an increase of the TOC results cannot be linked to the degradation process.

To confirm these hypothesis, it would be interesting to reproduce this experiment taking more precautions during the sample procedure to limit the margin of error associated with the decreasing volume.

4.3 Bacterial testing

Degradation of the bacteria *Pantoea agglomerans* with ozone had to be dropped. Several issues were faced before and during the experiment, such as excessive sample dilution, incorrect dye choice for bacteria staining, difficulties to count the bacteria with the microscope or death of the culture. Unfortunately, a lack of time made it impossible to carry out the experiment completely.

While refining the experimental protocol, it was nevertheless possible to slightly assess the bactericidal potential of ozone. In a preliminary test, ozone was bubbled (31 mmol/h) for 2 hours into a solution containing *Pantoea* in their culture medium. As issues were encountered with the dye used to count individual bacteria, colony counting was considered as an alternative approach. The test revealed that, after treatment, no bacterial colonies were able to form. However, it was not possible to quantify the degradation precisely, as the beaker overflowed during the experiment. This was due to the fact that no opening had been left for the gas to escape. In the final protocol, individual bacterial counting using the dye will be used instead.

Nevertheless, assessing the efficiency of ozone regarding bacteria degradation remains of primary focus. The experimental protocol finally selected for this experiment is available in [Appendix H](#) for future work.

Chapter 5

Conclusion and perspectives

5.1 Conclusion

The core objective of this Master's thesis was to evaluate the efficiency of an ozonation process to degrade organic molecules such as the ones present in aquaculture wastewater from the Mekong Delta.

Preliminary tests on para-nitrophenol validated the experimental setup and confirmed the reproducibility of the measurements. The reference ozone parameters (i.e. 31 mmol/h and a concentration in the feed gas of 0.13 mmol/l) were selected and kept throughout the study. From this test, it was also possible to perform a consistent comparison between the information given by the UV-Vis spectrophotometer and the TOC analyser.

Next, the degradation of the antibiotic OTC·HCl demonstrated the strong oxidative power of ozone. The UV-Vis analysis showed a complete disappearance of the absorption peaks of OTC ($\lambda=355$ nm) after 5 minutes. The HPLC-MS analysis, which is more sensitive, provided a more nuanced answer: 90% of the parent compound could be degraded within the same time frame. Yet, the organic content of the sample was only reduced by 35% after the 6 hours of continuous ozone treatment. This indicates that the by-products formed during degradation were not fully mineralized. When comparing these results to the ones found in the case of a UV-C photocatalytic treatment (TiO_2), it was found that ozone is faster to degrade OTC, but photocatalysis reaches a 60% reduction in TOC. This highlights the complementarity of both treatment processes and the interest in coupling them.

The kinetic study performed for the OTC/ O_3 system revealed that the reaction does not follow a pseudo first order model. Indeed, partial order of 0.57 for OTC suggests a more complex mechanism. This could be associated to the competition between direct O_3 attack and indirect $\bullet\text{OH}$ radicals pathway, or to diffusional and mass transfer issues.

The assumption of gas-liquid transfer limitation was supported by the comparison of two bubbling systems: a sintered glass filter and an aquarium diffusing stone. Results suggested that the choice of the bubbling system is of great importance. Even though the aquarium stone lead to a faster removal rate, it introduced absorption and leaching issues, skewing the TOC values. It was concluded that the sintered filter outperformed the aquarium stone.

Furthermore, the salinity of the water matrix also altered the degradation of OTC. When increasing it to 7 g/l, as in shrimp ponds in the Mekong Delta, the TOC values exhibited an intriguing behavior. They first decreased before remaining stationary and finally rising slightly. This is partly due to the scavenging role of the Cl^- ions on $\bullet\text{OH}$ radicals. These could slow down the degradation process by creating chloro-organic by-products due to the formation of HOCl^- species. Nevertheless, this experiment requires replication to draw firm conclusions.

An exploratory investigation was done on rifampicin, a second antibiotic actively used by shrimp farmers,

to confirm and extend the knowledge acquired with OTC. The UV-Vis results indicated similar trends to OTC: a rapid and complete degradation of the parent compound under identical ozone conditions. However, the abnormal TOC values made it impossible to conclude on the mineralization of the sample. This highlights the need to repeat and broaden the test under more controlled condition.

Finally, the bacterial degradation tests on *Pantoea agglomerans* could not be completed within the time-frame of this work due to protocol refinement challenges. However, preliminary results seemed to confirm the high potential of ozone to degrade bacteria rapidly.

Overall, this study constituted a first characterization of the efficiency of ozonation processes to remove organic compounds. It highlighted the fast and efficient effect of ozone on degrading parent molecules. Yet, it showed some limitations related to the incomplete mineralization of by-products. It also revealed the complex mechanism behind ozonation. To enhance the overall degradation efficiency on real water samples, coupling this method with other advance oxidation processes could be interesting.

5.2 Perspectives

This work was a first step towards a more in-depth study, for which several approaches can be considered.

An important aspect to study is determining a complete reaction mechanism for ozonation. This includes investigating more complex kinetic models and clarifying the role of $\bullet\text{OH}$ radicals. To do so, an experiment with an $\bullet\text{OH}$ scavenger (e.g., tert-butanol) may be set up. This experiment would provide information on whether degradation is dictated by direct O_3 attack or by the indirect hydroxyl radical pathway. In addition, the impact of temperature, pH and salinity on the degradation rate merits further investigation and replication. To obtain an overall view of the parameters affecting the kinetics, focus should be placed on physical factors such as bubble size and gas-liquid transfer limitations. To achieve this, X-ray tomography, high-speed imaging or measurements of oxygenation levels may be employed. Likewise, identifying an effective technique for measuring gaseous and dissolved ozone concentrations remains necessary.

As stated, the ozonation process alone is only partially efficient, as it cannot achieve complete mineralization of the by-products. Therefore, combining this method with other AOPs appears promising. As a first step, a simultaneous $\text{O}_3/\text{UV-C TiO}_2$ treatment may be implemented to assess the performance of such a process. Based on the observations made, this hybrid method is expected to combine the rapid degradation of OTC by O_3 with the superior mineralization of by-products achieved by the UV-C TiO_2 treatment. In future work, other hybrid processes may also be investigated, such as the $\text{O}_3/\text{H}_2\text{O}_2$ process, which has already been widely studied in the literature.

The exploratory experiments on rifampicin and bacteria should also be pursued. Investigating the degradation of this molecule would broaden knowledge of antibiotic degradation and may serve as a starting point for the treatment of antibiotic mixtures, which are more representative of real wastewater. The control of bacteria concentration is also a major concern. The bacterial inactivation experiments should be completed following the protocol established in this work and extended to other pathogenic strains relevant to shrimp farming. Additionally, the degradation of viruses by ozonation requires investigation.

Attention should be paid to the by-products formed during the ozonation process. Indeed, identifying these by-products would provide information on their toxicity. Toxicity tests with model organisms such as *Daphnia magna* or *Brachionus plicatilis* would provide valuable insight into water toxicity before and after treatment. Determining other important parameters, such as suspended solids, dissolved oxygen, BOD and COD, would also provide key information on water quality and biodegradability.

Working on water samples collected from shrimp ponds in the Mekong Delta would be highly beneficial for this study. First, it would allow a comprehensive characterization of the biological and organic content of the water. Second, the efficiency of the treatment process could be assessed under conditions beyond those of synthetic water experiments.

Ultimately, these steps would pave the way for transitioning from the batch reactor used in this study to a continuous-flow reactor. Moving towards a more realistic and scalable process would enable the assessment of the treatment efficiency under conditions that more closely reflect the constraints of shrimp aquaculture in the Mekong Delta.

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Appendix A

Compound inventory

This Appendix is a summary of all the compounds used during the experiments. Chemicals are sorted alphabetically.

Compound	Supplier	CAS number	Catalogue number	Batch number
4-Nitrophenol (PNP)	Sigma-Aldrich	100-02-7	100297 4867	MKCG8809
Acetonitrile (ACN) for HPLC-MS grade	VWR Chemicals	75-05-8	83640.400	25H064011
Acetic acid (glacial) 100%	Merck	64-19-7	1.00063.0510	K50416262 832
DAPI for nucleic acid staining	Sigma-Aldrich	28718-90-3	D9542-5MG	0000104230
Hydrochloric acid 37% (HCl)	VWR Chemicals	7647-01-0	20252.420	193244017
LB-Medium (Luria/Muller)	Carl Roth		X968.1	
Oxytetracycline hydrochloride (OTC.HCl)	Thermo Scientific	2058-46-0	J62489.14	U21L013
Potassium hydrogen phthalate (KHP)	Sigma-Aldrich	877-24-7	100335 8966	MKCP8539
Rifampicin 95%	Thermo Scientific	13292-46-1	455620050	A0475E
Sodium carbonate (Na_2CO_3)				
Sodium chloride (NaCl), anhydrous, ACS reagent >99%	Sigma-Aldrich	7647-14-5	746398	MKBR0024V
Sodium dihydrogen phosphate dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$)	Merck	13472-35-0	1.06342.1000	K91235542 434
Sodium hydrogen carbonate (NaHCO_3)	Alfa Aesar	144-55-8	14707	S07E001
Sodium phosphate dibasic (Na_2HPO_4)	Sigma-Aldrich	7558-79-4	S0876	BCCD2696

Appendix B

Reference curves provided by Triogen Lab2B

Along with the Triogen Lab2B ozone generator, reference curves were provided to provide the user an insight about the ozone flow rate and concentration injected. These curves are presented in [Figure B.1](#).

However, the curves are plotted in mg/l and g/h. To ensure unit consistency throughout this report, ozone concentration and flow rate values are converted to mol/l and mol/h, respectively. To do this, divide the “mass” values by 48 g/mol (*i.e.* the molecular mass of ozone).

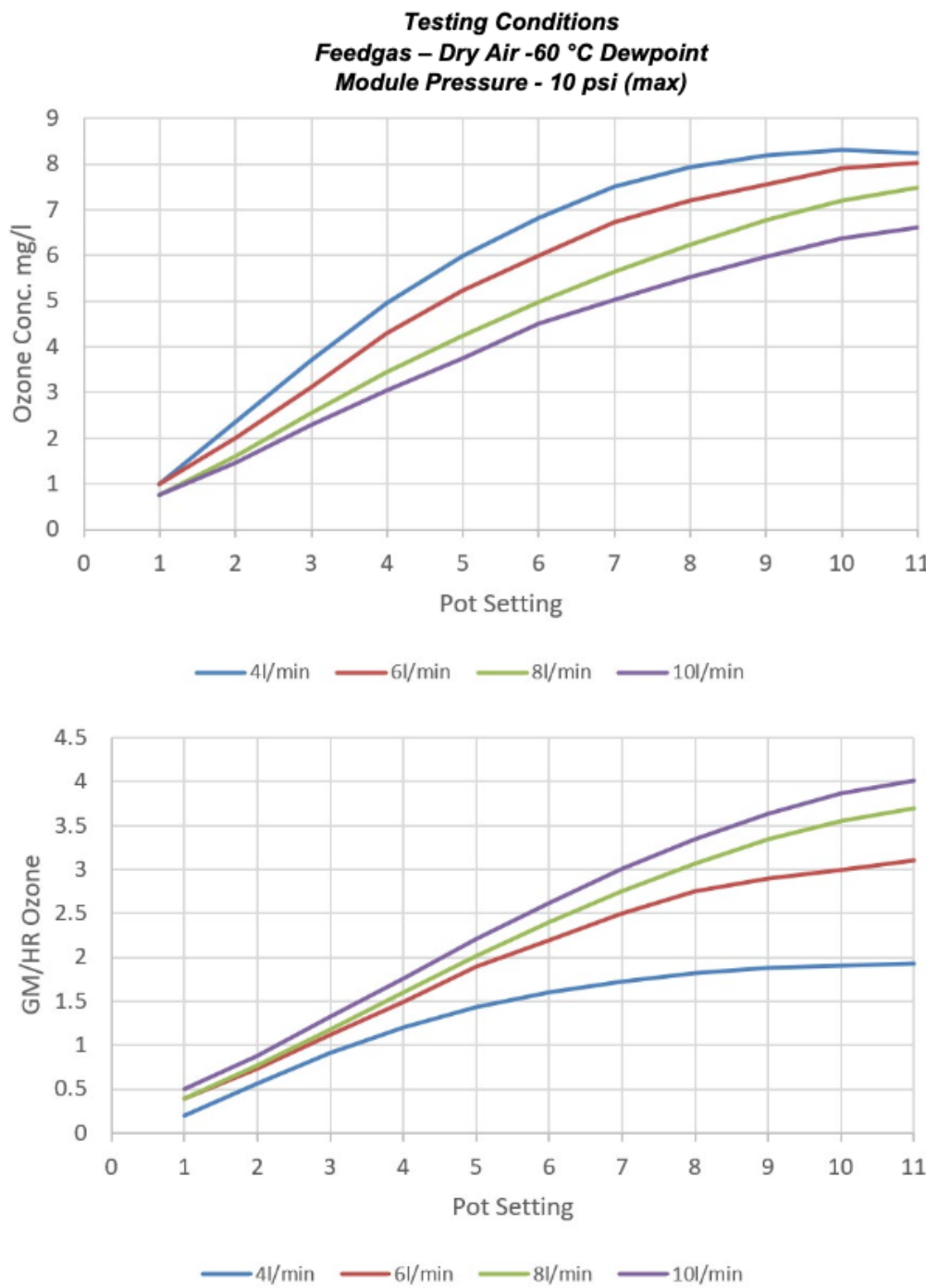


Figure B.1: Reference curves for the ozone concentration (mg/l) and flow rate (g/h) obtained based on the air volumetric flow rate and ozone percentage selected (Triogen, 2022).

Appendix C

Calibration of the different analysis techniques used

C.1 Total Organic Carbon (TOC)

Within this section the detailed procedure regarding the calibration of the TOC-L machine is presented, when using the difference method to calculate TOC. It consists of two calibration curves, one for the Total Carbon (TC), and one for the Inorganic Carbon (IC). To do this, the protocol recommended by Shimadzu, [n.d.](#) was followed. These calibrations curves were then used as a reference for all experiments requiring a TOC analysis.

C.1.1 TC calibration curve

The calibration curve for TC was made from a potassium hydrogen phthalate (KHP) solution. To do so, KHP powder had first to be dried (see [Figure C.1](#)) in an oven to remove all the water contained in it. To prepare 100 ml of a 1000 mg_C/l stock solution, 0.213 g of KHP were weighted and diluted into milliQ water. It was then diluted to obtain 50 ml of a 100 mg_C/l, by taking 5 ml of the stock solution and adding milliQ water.

$$V_{taken} = \frac{100mg_C/l \times 50ml}{1000mg_C/l} = 5ml$$

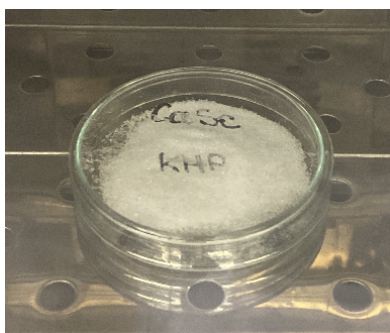


Figure C.1: Dried KHP powder used for the TC curve.

This was done as the concentration of the stock solution was too high for the auto dilution feature of the TOC-L machine to perform the different dilutions correctly. Auto dilution was used in order to minimize human mistake and increase precision of the results. The selected concentration range was: 2.5, 5, 10, 20, 25, 30, 35, 40, 50 mg_C/l.

The TC calibration curve is visible in [Figure C.2](#) and the mean area calculated at for each concentration is presented in [Table C.1](#). As can be seen the R^2 value is high (0.9999), meaning high accuracy. The calibration curve is given by

$$y = 2.2248x - 0.5024$$

(C.1)

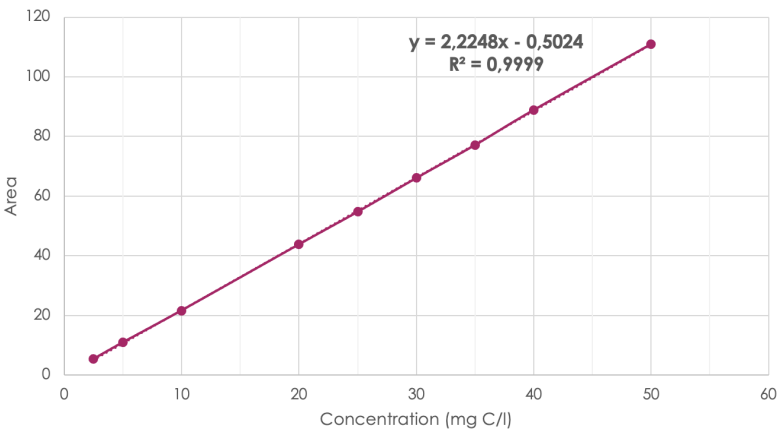


Figure C.2: TC calibration curve.

Concentration (mg _C /l)	Mean Area
2.5	5.337
5	10.9
10	21.62
20	43.78
25	54.7
30	66.05
35	77.11
40	88.88
50	111

Table C.1: Value of the mean area for the selected concentration range.

C.1.2 IC calibration curve

For the IC calibration curve, another stock solution (100 ml) had to be prepared, with the same carbon concentration (1000 mg_C/l). This stock solution is a combination of two carbonates: sodium hydrogen carbonate (NaHCO₃) and sodium carbonate (Na₂CO₃). 0.3497 g of NaHCO₃ and 0.4412 g of Na₂CO₃ were diluted into 100 ml of milliQ water.

As for the TC curve, the solution was then diluted to obtain 50 ml of a 100 mg_C/l, by taking 5 ml of the stock solution and adding milliQ water. The same concentration range was selected. The IC calibration curve is visible in [Figure C.3](#) and the mean area calculated at for each concentration is presented in [Table C.2](#). The equation of the calibration curve is given by

$$y = 225.867x - 12.598$$

(C.2)

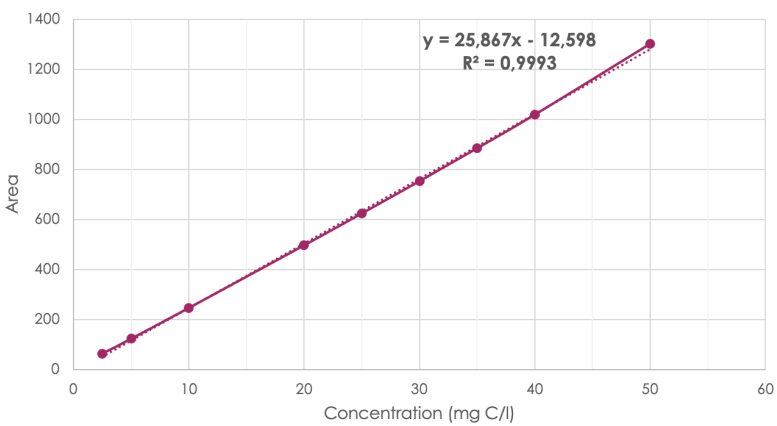


Figure C.3: IC calibration curve.

Concentration (mg _C /l)	Mean Area
2.5	63.43
5	123.4
10	246.3
20	496.7
25	624.2
30	753
35	884.7
40	1019
50	1302

Table C.2: Value of the mean area for the selected concentration range.

C.2 UV-Visible Spectrophotometer for OTC

To obtain a calibration curve of OTC · HCl on the UV-Vis spectrometer, the following procedure, adapted from Sharkawi *et al.*, (2024), was applied.

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0.0994 g of OTC · HCl were weighted on the analytical scale and diluted into 1 l of milliQ water to obtain a 0.2 mmol/l stock solution. From this solution, several dilution are made to get the concentration range: 0.004 - 0.02 - 0.04 - 0.06 - 0.08 - 0.1 - 0.12 - 0.14 - 0.16 mmol/l (see Table C.3 and Figure C.4).

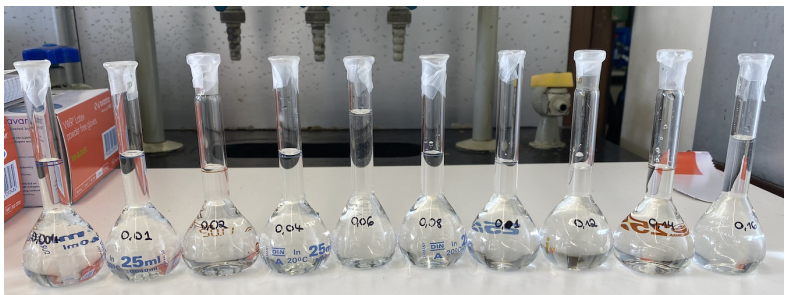


Figure C.4: Concentration range for the calibration of the UV-Vis spectrophotometer.

Table C.3: Volumes of the 0.2 mmol/l OTC · HCl stock solution to be taken to reach the concentration range wanted for the calibration curve.

Concentration (mmol/l)	Concentration (mg/l)	Volume taken (ml)
0	0	0
0.004	2	0.5
0.02	10	2.5
0.04	20	5
0.06	30	7.5
0.08	40	10
0.1	50	12.5
0.12	60	15
0.14	70	17.5
0.16	80	20

For each concentration, a 1 ml sample was taken for analysis. Before placing the sample into the UV-vis spectrophotometer, it was acidified by adding 0.5 ml of HCl 1 M. Indeed, OTC has three pKa values (3.27, 7.32, and 9.11) and at neutral pH, different forms of OTC coexists, as presented in Figure C.5. Thus, the literature suggests that, at low pH, only the protonated form of OTC should be present. At neutral pH (*i.e.* 4 < pH < 6) OTC is present in its zwitterion form, and for pH values above 8, OTC is negatively charged (Andrade *et al.*, 2020; Jia *et al.*, 2025).

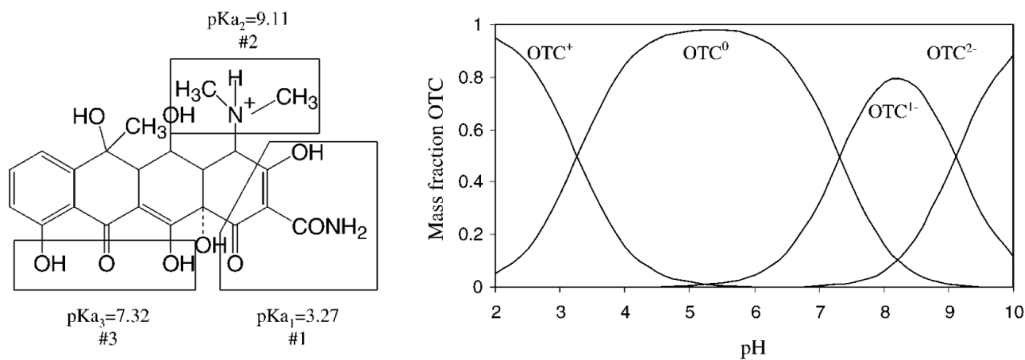


Figure C.5: OTC structure and speciation as a function of pH (Jones *et al.*, 2005).

100 ml of the 1 M HCl solution was prepared by diluting 8.3 ml of HCl concentrated at 37% (VWR Chemicals) into milliQ water. The acidification was systematically done just before analyzing the sample. The absorbance of each sample was then recorded at 268 and 355 nm, and is shown in Figure C.6. From the absorbance values collected at each concentration, the calibration curve presented in Figure C.7 is obtained. It is worth noting that adding a significant amount of HCl in the sample (0.5 ml $\gg$ 1 ml of sample) influence its real concentration by diluting it. However, it is considered that the 0.5 ml of HCl added act as a "standard" and thus will not be considered as a dilution of the concentration of OTC present in the sample. If the same amount of HCl is systemically added, the sample will always be diluted by the same amount which can be considered as a constant.

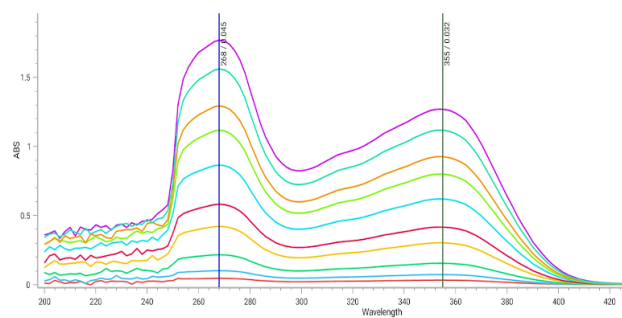


Figure C.6: Absorbance spectrum of OTC · HCl measured at 268 and 355 nm. Samples were acidified with HCl 1M before analysis (pH=2).

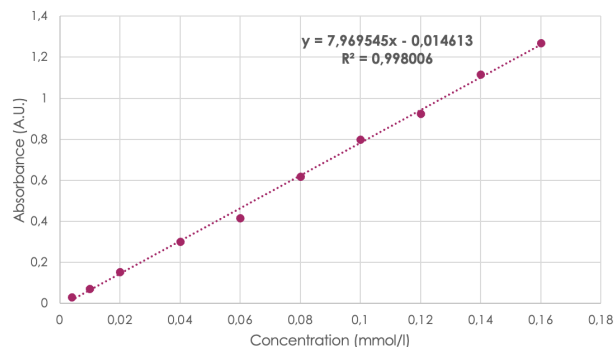


Figure C.7: Calibration curve obtained from the measured absorbances when the sample is acidified.

The calibration equation obtained is,

$$y = 7.969545x - 0.014613 \quad (\text{C.3})$$

However, it is also possible to analyse the absorbance without acidifying the sample with HCl. Several observations can be made from the absorbance spectrum in Figure C.8. It can be noticed that the peak previously recorded at 268 nm has shifted slightly to the higher wavelengths (around 275 nm). This is due to the change in the protonation state of OTC. At pH 5.5, OTC is in its zwitterionic form (OTC^0 , cf. Figure C.5).

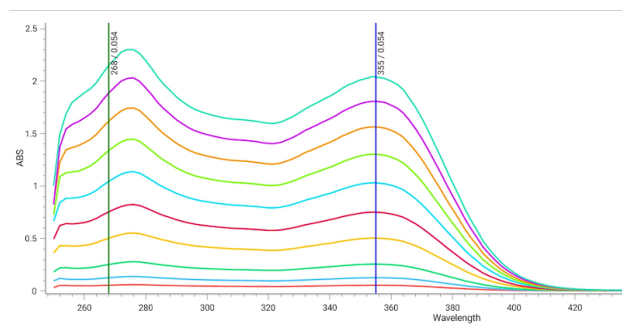


Figure C.8: Absorbance spectrum of OTC · HCl measured at 268 and 355 nm. Samples were not acidified before analysis (pH=5.5).

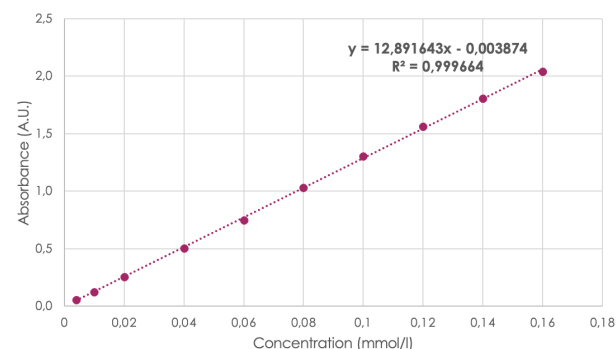


Figure C.9: Calibration curve obtained from the measured absorbances when the sample is not acidified.

The calibration curve is presented in Figure C.9 and the equation obtained in this case is,

$$y = 12.891643x - 0.003874 \quad (\text{C.4})$$

Therefore, to the question "Which protocol should be followed ?", the answer is either. Indeed, when using either calibration equation, similar concentrations are obtained.

C.3 UV-Visible Spectrophotometer for Rifampicin

In a similar way as for OTC · HCl, a calibration curve for rifampicin had to be made to analyse it by UV-Vis spectrophotometer. The protocol suggested by Benetton, (1998), was adapted. From a 0.2 mmol/l stock solution of rifampicin, several dilutions were made to get the concentration range: 0 - 0.01 - 0.02 - 0.04 - 0.06 - 0.08 - 0.1 - 0.12 - 0.16 mmol/l (see Table C.4 and Figure C.10). Dedicated volumes of stock solution were taken and phosphate buffer solution was added to reach 25 ml.

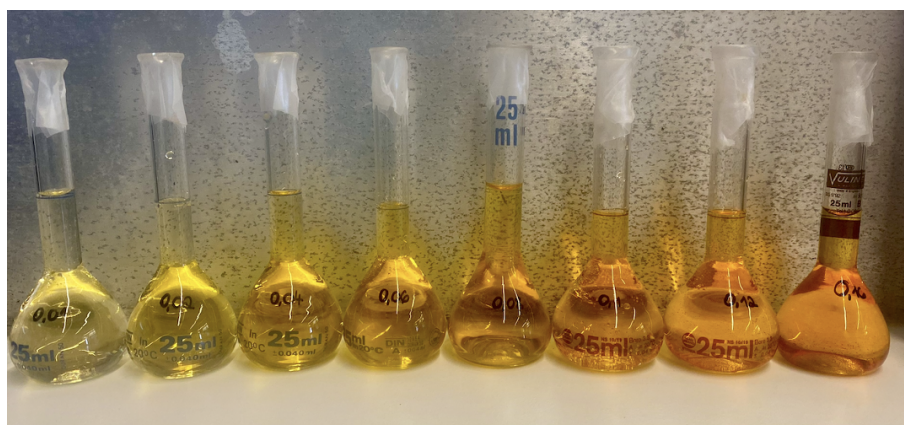


Figure C.10: Concentration range of rifampicin for the calibration of the UV-Vis spectrophotometer.

Table C.4: Volumes of the 0.2 mmol/l rifampicin stock solution to be taken to reach the concentration range wanted for the calibration curve.

Concentration (mmol/l)	Concentration (mg/l)	Volume taken (ml)
0	0	0
0.01	8.2	1.25
0.02	16.5	2.5
0.04	32.9	5.0
0.06	49.4	7.5
0.08	65.8	10.0
0.10	82.3	12.5
0.12	98.8	15.0
0.16	131.7	20.0

For each concentration, a 1 ml sample was taken for analysis and the absorbance was measured at 475 nm. The absorbance spectrum of rifampicin is shown in Figure C.11.

From the values recorded, the calibration equation can be drawn by matching each concentration to its corresponding absorbance value,

$$y = 7.9979x \quad (\text{C.5})$$

The calibration curve is presented in Figure C.12. Given the high R^2 value (0.9994), this curve can be used with confidence.

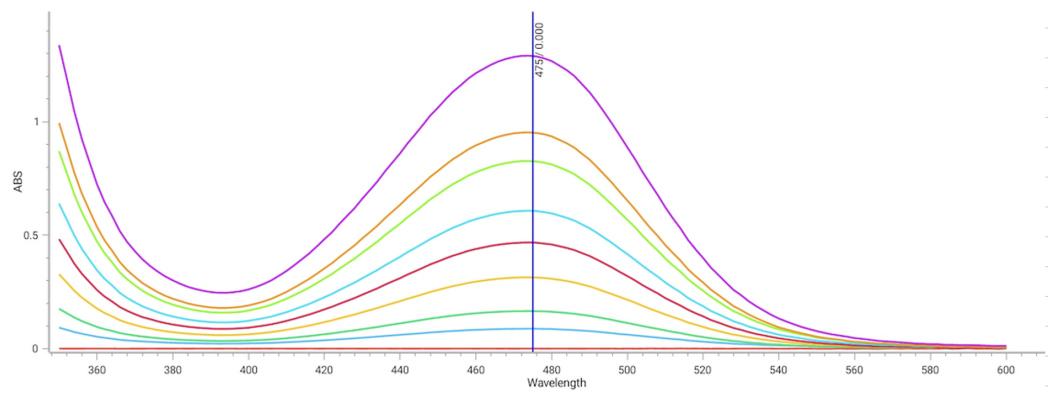


Figure C.11: Absorbance spectrum of rifampicin measured at 475 nm.

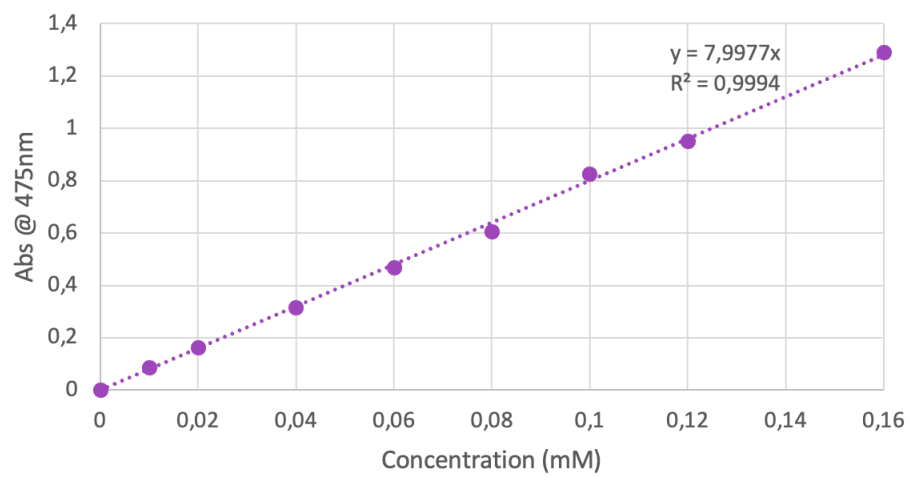


Figure C.12: Calibration curve obtained for rifampicin.

Appendix D

Standard Operation Procedure for the HPLC-MS

Fatty acid analysis Standard Operation Procedure (SOP)

Key data

Code (as appearing in SOP_Overview)	Oxytetracycline
Title (full text)	Oxytetracycline analysis in water by LC-MS
Version (major change/minor change)	V1.0
Effective date (not earlier than final signature)	

Signatures

Prof ML. Scippo

Head of Group

Signature

Date

1st Author: Dr C. Douny

Signature

Date

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Fatty acid analysis
Standard Operation Procedure (SOP)

1. Update History

This is the first version.

2. Purpose

Description of the process of analysis of oxytetracycline (OTC) with LC-MS in water samples. Demeclocycline is used as internal standard.

3. Resources (in alphabetical order)

- A. Screw neck vial, clear, 20 ml (548-0248, VWR)
- B. Combination seal magnetic 18 mm (VWRI548-0811)
- C. Water for HPLC (23595.328, VWR Prolabo)
- D. Trichloroacetic acid (TCA) (8.22342.0250, Sigma-Aldrich)
- E. Acetic acid (Normapur, 20104.298, VWR)
- F. Acetonitrile absolute LC-MS (001207802BS, Biosolve)
- G. Injection vial (LP 11090356)
- H. Insert for injection vial (LP 06090357)
- I. Cap for injection vial (LP 11030209)

Fatty acid analysis
Standard Operation Procedure (SOP)

Table 1: OTC standard information.

Name	Oxytetracycline . HCl
CAS number	2058-46-0
Supplier	Sigma-Aldrich
Supplier reference	O5875-10G
Batch number	0000239017
Date of purchase	02/2024
MW	496.89 g/mol
Purity	97.3 %
Storage conditions	Store under inert gas. Light sensitive. Air sensitive.

Table 2: IS standard information.

Name	Demeclocycline . HCl
CAS number	64-73-3
Supplier	Sigma-Aldrich
Batch number	LRAD0977
Supplier reference	PHR1735-1G
Date of purchase	02/2024
MW	501.31 g/mol
Purity	89.9 %
Storage conditions	-20°C

4. Risks and Responsibilities

- Read MSDS on compound

Fatty acid analysis Standard Operation Procedure (SOP)

5. Solutions

- TCA 5 % : Weigh 50 g of TCA (D) and transfer them quantitatively into a 1 L volumetric flask containing distilled water (C). Dissolve and bring to the mark with the same solvent. Store the solution at + 4°C.
- Acetic acid solution at pH=3.55 (0.03 %): In a beaker, pour 0.5 L water (C) and add 150 µl acetic acid (E). Homogenize.
- OTC stock solution (0.3 mg/ml):
Weight approximately but precisely 5 -10 mg of OTC.HCl standard in a 20 ml vial (A), add 3 times the volume of TCA 5% (D), cap the vial (B) and vortex. Store the solution at + 4°C.
- OTC working solutions:
 - o Solution 50 ng/µl OTC: Take 333 µl OTC stock solution and add 1667 µl water pH 3.55/acetonitrile 90/10. Vortex.
 - o Solution 0.2 ng/µl OTC: Take 10 µl of 50 ng/µl OTC solution in a 20 ml vial (A), and add 2490 µl water pH 3.55/acetonitrile 90/10, cap the vial (B) and vortex. Store the solution at + 4°C.
- IS stock solution (0.278 mg/ml):
Weight approximately but precisely 5-10 mg of Demeclocycline.HCl standard in 20 ml vial (A), add 3 times the volume of TCA 5% (D), cap the vial (B) and vortex. Store the solution at + 4°C.
- IS working solutions:
 - o Solution 50 ng/µl IS: Take 363 µl IS stock solution and add 1637 µl water pH 3.55/acetonitrile 90/10. Vortex.
 - o Solution 1 ng/µl OTC: Take 20 µl of 50 ng/µl IS solution in a 20 ml vial (A), and add 980 µl water pH 3.55/acetonitrile 90/10, cap the vial (B) and vortex. Store the solution at + 4°C.

6. Calibration curve

A calibration curve is prepared in injection vials (G), with insert (H) and cap (I):

	vol oxy 0.2 ng/ul (ul)	conc oxy (ng/ul)	vol IS 1 ng/ul (ul)	vol water 0.03 % acetic acid/ACN 90/10 (ul)	Total (ul)
C0	0	0,0000	5	195	200
C1	2,5	0,0025	5	192,5	200
C2	5	0,0050	5	190	200
C3	10	0,0100	5	185	200
C4	25	0,0250	5	170	200
C5	50	0,0500	5	145	200
C6	100	0,1000	5	95	200

Seven solutions are used to construct the calibration curves: the response (ratio between OTC and the IS peak area) is plotted versus standard concentrations. Calibration points are injected before each series of samples and the extract spiked at a concentration corresponding to the central point of the calibration curve is injected one more time after all the samples.

A linear regression is used, and no "fit weighting" is applied. The correlation coefficients R^2 associated with those curves must be higher than 0.98. It is also established that only one point of the curve can deviate from the curve by more than 20 % of the corresponding calculated value.

Fatty acid analysis
Standard Operation Procedure (SOP)

7. Water samples

Water samples are diluted 1000 times:

- 25 x dilution in Eppendorf: 40 µl water sample + 960 µl water pH 3.55/acetonitrile 90/10.
- 40 x dilution in injection vials (G), with insert (H) and cap (I): 5 µl of 40x diluted sample, add 5 µl IS 1 ng/µl and 190 µl water pH 3.55/acetonitrile 90/10.

8. Analytical parameters

OTC was analyzed on a UPLC I Class (Waters) coupled to a Xevo TQS mass spectrometer (Waters), using a Polaris 3 C18-A (150 mm × 2.0 mm, 3 µm), with a MetaGuard Polaris 3 C18-A (10 x 2.0mm) (Agilent).

The UPLC file was: **260204_OTC.ftn**

The UPLC conditions were:

- Column temperature: 40°C
- Sample temperature: 10°C
- Injection volume: 1 µl
- Flow: 0.25 ml/min
- Gradient program:

Time (min)	H2O pH = 3.55 (%)	Acetonitrile (%)	Curve
0	90	10	
1	90	10	6
7.5	50	50	6
7.7	90	10	6
10	90	10	6

Tune page: **260206_Oxytetracycline.ipr**

The MS conditions were:

- ESI ionization: positive mode
- Source temperature: 125 °C
- Voltages:
 - o Capillary (kV): 2.75
 - o Cone (V): 10
 - o Source offset (V): 70
- Desolvation temperature (°C): 350 °C
- Gas flows:
 - o Desolvation (L/h): 550 L/H
 - o Cone (L/h): 150 L/H
 - o Nebuliser (bar): 7
- Analyser:
 - o LM resolution 1: 3
 - o HM resolution 1: 15

Fatty acid analysis Standard Operation Procedure (SOP)

- Ion energy 1: 0.5
- LM resolution 2: 3
- HM resolution 2: 15
- Ion energy 2: 0.5
- Collision gas flow (ml/min): 0.11
- SIR/Multiple reaction monitoring (MRM) mode (**260206_OTC_FS_MRM.exp**):
 - Window 1 (1 to 10 min): SIR of alpha/beta-apo OTC: 443.2
 - Window 2 (1 to 5.15 min): MRM of OTC: 461.2 @15 : 426.2 + 443.2
 - Window 3 (4.9 to 10 min): MRM of IS: 465.2 @ 20 : 448.2 + 430.2

Results were calculated using TargetLynx v4.1 Software (Waters).

9. Chromatogram

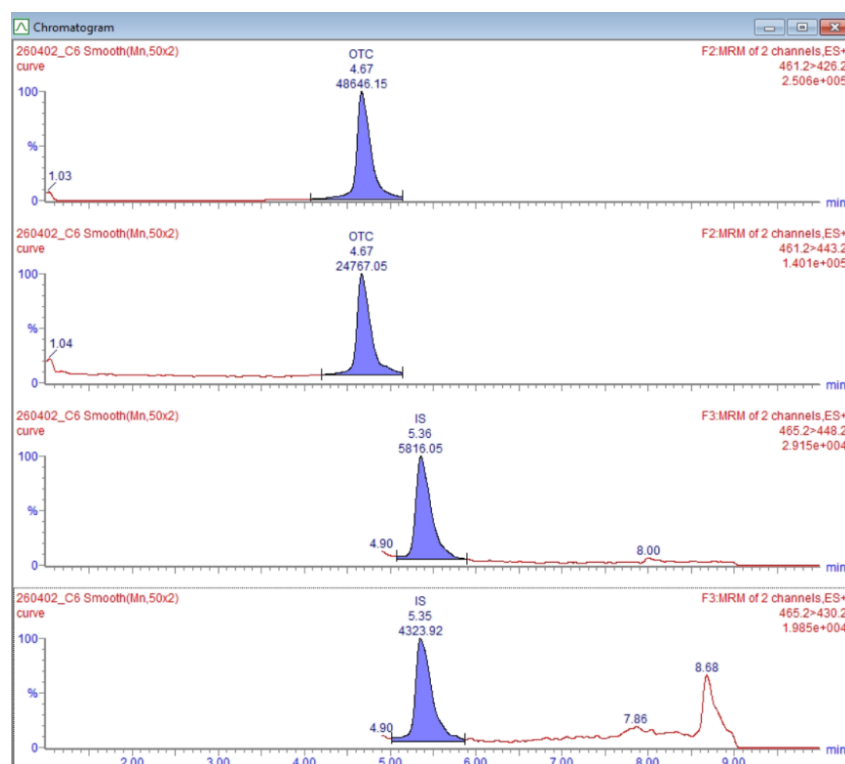


Figure 1: Chromatogram of C6 of calibration curve containing 0.1 ng/μl OTC and 0.025 ng/μl IS.

11. Method performances

- LOD: the limit of detection (LOD) was set as the first point of the calibration curve/2 = 0.0025 ng/μl OTC.
- LOQ: the limit of quantification (LOQ) was set as the first point of the calibration curve = 0.005 ng/μl OTC.

Appendix E

Supplementary data - absorbance values

This appendix summarises all the raw absorbance measured with the UV-Vis spectrometer for the different experiments. In the main text, these values were presented solely in terms of concentration. This conversion was performed using the calibration curves obtained and Beer-Lambert's law.

E.1 Degradation of PNP

E.1.1 Degradation of PNP over time

Table E.1: Evolution of the absorbance values over time for the PNP concentration range 5-10-15-20 mg/l, and an O3 flow rate of 31 mmol/h. The absorbance was measured at 317 nm.

Time (sec)	Absorbance (-)			
	[PNP]=20 mg/l	[PNP]=15 mg/l	[PNP]=10 mg/l	[PNP]=5 mg/l
0	1.323	0.992	0.694	0.33
15	1.224	0.893	0.557	0.263
60	1.023	0.713	0.384	0.115
150	0.762	0.445	0.157	0.017
240	0.518	0.211	0.027	0.004
330	0.307	0.077	0.012	0.004
420	0.17	0.028	0.008	0.004
510	0.08	0.014	0.008	0.004

One might wonder which concentrations relate to the measured absorbance values. The link between the PNP absorbance and concentration is made through the Beer-Lambert law,

$$Abs = k' \times C \Leftrightarrow C = \frac{Abs}{k'} \quad (E.1)$$

where Abs is the absorbance (-) of PNP at 317 nm, C is the concentration of PNP (mol/l) and k' is the Beer-Lambert constant (l/mol) which can be calculated by slope of the curve,

$$Abs_0 = k' \times C_0 \quad (E.2)$$

with Abs₀ and C₀ being the absorbance and concentration of PNP at the initial time. It is worth noting that the linearity domain of this law is valid for the absorbance range [0, 1.2] (Région Académique Île-de-France, 2026).

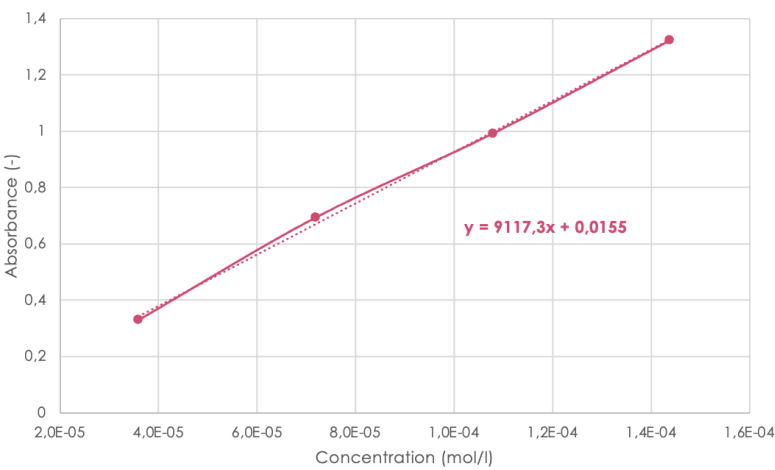


Figure E.1: Graphical determination of the Beer-Lambert constant for PNP. Concentration range: 5-10-15-20 mg/l (expressed in mol/l in the graph). The absorbance is measured at 317 nm.

From the slope of the plot in [Figure E.1](#), the constant k' is obtained ($k'=9117.3$). Using [Equation E.1](#), the remaining concentration of PNP can be calculated at each time.

E.1.2 Influence of the ozone flow rate on the kinetics

Table E.2: Evolution of the absorbance over time for the O₃ flow rate range 17-21-26-31 mmol/h, and a fixed PNP concentration of 15 mg/l. The absorbance was measured at 317 nm.

	Measured absorbance value depending on the O ₃ flowrate (-)			
Time (sec)	0.75 g _{O₃} /h	1 g _{O₃} /h	1.25 g _{O₃} /h	1.5 g _{O₃} /h
0	0.992	1.014	0.973	0.969
15	0.940	0.875	0.873	0.891
60	0.836	0.801	0.748	0.688
150	0.660	0.563	0.477	0.392
240	0.501	0.366	0.251	0.160
330	0.366	0.190	0.107	0.042
420	0.251	0.093	0.034	-0.001
510	0.158	0.038	0.016	-0.007

E.2 Degradation of OTC

E.2.1 First degradation test

Table E.3: Evolution of the absorbance (-) values over 6 hours time for an initial OTC concentration of 0.1 mmol/l and an ozone flowrate of 31 mmol/h. The absorbance was measured at 355 nm.

Time (sec)	Absorbance (-)		
	Rep. 1	Rep. 2	Rep. 3
0	1.316	0.804	1.328
2			0.293
5	0.126		0.142
15	0.006	0.013	0.028
20			0.027
30	0.002	0.009	0.014
60	0.001		0.006
120	0.002	0.001	0.005
150			0.004
180			0.002
210			0.004
240	0.002	0.001	0.001
270			0.001
300			0.005
330			0.002
360	0.001	0.001	0

E.2.2 Influence of the initial OTC concentration, stirring and bubbling

E.2.2.1 First trial

Table E.4: Evolution of the absorbance (-) of OTC·HCl measured over time for several initial concentrations (0.01 - 0.04 - 0.01 mmol/l) with and without stirring at 100 rpm. The absorbance was recorded at 355 nm and the ozone settings were 31 mmol/h and 0.13 mmol/l. The diffusing stone was used to introduce the ozone bubbles in the solution.

[OTC.HCl] = 0.1 mmol/l						
	Stirring			No Stirring		
	Absorbance			Absorbance		
Time (min)	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	1.266			1.279		
0.25	0.887			0.77		
1	0.282			0.237		
3	0.1			0.047		
5	0.025			0.016		
7	0.01			0.007		

[OTC.HCl] = 0.04 mmol/l						
	Stirring			No Stirring		
	Absorbance			Absorbance		
Time (min)	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	0.518	0.519	0.52	0.52	0.517	0.505
0.25	0.224	0.272	0.204	0.18	0.181	0.191
1	0.071	0.074	0.067	0.07	0.061	0.065
3	0.013	0.007	0.004	0.01	0.011	0.019
5	0.005	0.001	0	0.004	0.004	0.009
7	0.002	0	-0.001	0.002	0.004	0.005

[OTC.HCl] = 0.01 mmol/l						
	Stirring			No Stirring		
	Absorbance			Absorbance		
Time (min)	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	0.129	0.138		0.133	0.132	0.13
0.25	0.015	0.011		0.015	0.008	0.014
1	0.002	0		0	0	0
3	0	0		-0.001	-0.001	-0.001
5						
7						

E.2.2.2 Broader concentration range

Table E.5: Evolution of the absorbance (-) of OTC · HCl measured over time for several initial concentrations: 0.004 - 0.02 - 0.03 - 0.07 mmol/l. The absorbance was recorded at 355 nm and the ozone settings were 31 mmol/h and 0.13 mmol/l. The diffusing stone was used to introduce the ozone bubbles in the solution.

Time (min)	Initial OTC · HCl concentration			
	0.004 mmol/l	0.02 mmol/l	0.03 mmol/l	0.07 mmol/l
0	0.059	0.258	0.407	0.953
0.25	0.018	0.06	0.09	0.532
1	0.012	0.01	0.018	0.16
3	0.008	0.006	0.007	0.02
5	0.005	0.008	0.008	0.017
7	0.009	0.006	0.009	0.012

E.2.3 Influence of the ozone generator parameters

E.2.3.1 First trial

Table E.6: Evolution of the OTC · HCl absorbance (-) depending on the parameters of the ozone generator selected. Operating conditions: [OTC · HCl] = 0.1 mmol/l.

Time (min)	Absorbance at 355 nm (-)			
	Cond. 1	Cond. 2	Cond. 3	Cond. 4
0	1.371	1.365	1.345	1.387
0.25	0.931	0.867	1.111	0.689
1	0.274	0.273	0.593	0.239
3	0.105	0.103	0.226	0.044
5	0.022	0.027	0.142	0.029
7	0.01	0.009	0.082	0.004

E.2.3.2 Broader range

Table E.7: Evolution of the OTC · HCl absorbance (-) depending on the parameters of the ozone generator selected and over 7 minutes. Operating conditions: [OTC · HCl] = 0.1 mmol/l. The bubbling system used is the diffusing stone.

Absorbance at 355 nm (-)				
Time (min)	0.01 mmol/l + 12.5 mmol/h	0.05 mmol/l + 17 mmol/h	0.05 mmol/l + 31 mmol/h	0.09 mmol/l + 21 mmol/h
0	1.38	1.355	1.345	1.313
0.25	1.258	1.061	1.111	0.958
1	0.929	0.476	0.593	0.305
3	0.351	0.212	0.226	0.122
5	0.244	0.121	0.142	0.040
7	0.197	0.059	0.082	0.011

Absorbance at 355 nm (-)				
Time (min)	0.09 mmol/l + 47 mmol/h	0.13 mmol/l + 31 mmol/h	0.13 mmol/l + 48 mmol/h	0.17 mmol/l + 42 mmol/h
0	1.343	1.371	1.365	1.387
0.25	0.865	0.931	0.867	0.689
1	0.299	0.274	0.273	0.239
3	0.101	0.105	0.103	0.044
5	0.025	0.022	0.027	0.029
7	0.010	0.010	0.009	0.004

E.2.4 Impact of changing the bubbling system

Table E.8: Evolution of the OTC · HCl absorbance (-) when the diffusing stone is used. Operating conditions: [OTC · HCl] = 0.1 mmol/l, O₃ concentration = 0.13 mmol/l and O₃ molar flow rate = 31 mmol/h.

Absorbance at 355 nm (-)		
Time (min)	Replicate 1	Replicate 2
0	1.333	1.343
1	0.392	0.313
3	0.153	0.126
5	0.043	0.031
15	0	0.002
30	0	0
60	0	0
120	0	0
240	0	0.001
360	0	0.001

E.2.5 Impact of the salinity

Table E.9: Evolution of the OTC · HCl absorbance (-) when the salinity of the medium is 7 g/l. Operating conditions: [OTC · HCl] = 0.1 mmol/l, O₃ concentration = 0.13 mmol/l and O₃ molar flow rate = 31 mmol/h. The absorbance was measured at 355 nm.

Time (min)	Absorbance (-)
0	1.341
1	0.519
3	0.22
5	0.122
15	0.014
30	0.008
60	0.009
120	0.004
240	0
360	0

E.3 Degradation of Rifampicin

Table E.10: Evolution of the rifampicin absorbance (-). Operating conditions: [Rifa] = 0.05 mmol/l, O₃ concentration = 0.13 mmol/l and O₃ molar flow rate = 31 mmol/h.

Time (min)	Absorbance at 475 nm (-)		
	Replicate 1	Replicate 2	Replicate 3
0	0.408	0.404	0.418
0.5		0.152	0.171
1	0.058	0.06	0.059
2	0.015	0.01	0.011
3	0.008	0.006	0.005

Appendix F

Ozone toxicity

F.1 General information on ozone

The ozone molecule (O_3) is naturally present in small traces in the atmosphere (about 0.00006%). Two types of ozone can be distinguished and sorted as "good" and "bad" ozone. [Figure F.1](#) provides an overall view of the ozone repartition in the atmosphere.

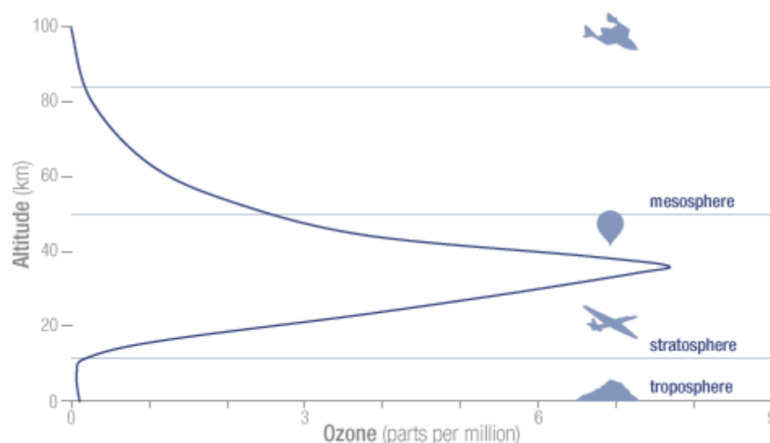


Figure F.1: Repartition of O_3 between the different atmospheric layers (NASA, [2024](#)).

First, the stratospheric ozone, which represents about 90% of the ozone present in the atmosphere. Its objective is to form a protective layer about the surface of Earth to prevent solar UV radiation. It is thus necessary for life on Earth, as it absorbs the energy of UV A, B and C, which can lead to sunburns, skin cancer and blindness if a person is too much irradiated. Stratospheric ozone is created by the reaction of O_2 and the UV rays of the Sun. The amount of O_3 present in the stratosphere is balanced, as it is destroyed by species containing molecules such as nitrogen, bromine or chlorine. Unfortunately, extensive human activities and production of chlorofluorocarbons has disrupted the formation-destruction balance of O_3 . This increases the ozone destruction rate, leading to the ozone hole phenomenon (*i.e.* the thinning of the stratospheric ozone layer) and global warming (NASA, [2024](#); Royal Belgian Institute for Space Aeronomy, [2026](#)).

In contrast to stratospheric ozone, tropospheric ozone is seen as "bad" ozone and accounts for 10% of the ozone present. The troposphere is the lowest layer in the atmosphere (0-15 km). It is a result of nitrogen oxides (NO_x) emissions from vehicles and other volatile organic compounds (VOCs), all contributing to air pollution. This ground-level ozone can cause several health issues to humans, animals and plants (NASA, [2024](#); Royal Belgian Institute for Space Aeronomy, [2026](#)). As for Belgium, it has been observed that the

concentration of tropospheric ozone in Wallonia recorded have increased over the last two decades, from $47.3 \mu\text{g}/\text{m}^3$ in 2007-2017, to $53.8 \mu\text{g}/\text{m}^3$ in 2018-2023 (État de l'environnement wallon, 2024). This can be explained by the especially hot and sunny summers during this second period. The rest of this Appendix is dedicated to the toxicity of tropospheric ozone.

F.2 Health effects & recommendations

Tropospheric ozone is known to cause several health effects, both on short and long term. On the short term (*i.e.* periods shorter than 8 hours and occasionally) such as breath shortness, headaches, coughing, asthma, chronic obstructive pulmonary disease (COPD) and other lung diseases. Long term exposure to ozone (*i.e.* periods longer than 8 hours and for several days or months) can even induce respiratory disease, neurologic disorders, reproductive issues and cardiac effects (American Lung Association, 2025).

To mitigate the risks, several norms and regulations have been emitted by different organisms. Theses values are listed in Table F.1.

Table F.1: Norms and regulations about ozone.

Organism	O ₃ limit	Comment	Source
Walloon region	$120 \mu\text{g}/\text{m}^3$	Maximum daily concentration over 8h.	État de l'environnement wallon, 2024
World Health Organization (WHO)	$100 \mu\text{g}/\text{m}^3$	Maximum daily concentration over 8h.	Organisation Mondiale de la Santé, 2021
Food and Drug Administration (FDA)	$0.05 \text{ ppm } (\approx 100 \mu\text{g}/\text{m}^3)$	For medical devices working indoors.	Environmental Protection Agency (US), 2026
Occupational Safety and Health Administration (OSHA)	$0.10 \text{ ppm } (\approx 200 \mu\text{g}/\text{m}^3)$	Maximum daily concentration for laboratory workers over 8h.	Environmental Protection Agency (US), 2026

F.3 Importance of the fume hood

Knowing the recommendation about the maximum accepted value of O₃ at which some can be exposed over a daily basis, it is possible to calculate a worse case scenario. Let's imagine that the laboratory in which the experiments with ozone are done is not equipped with an aspirating fume hood, and that the ozone flow rate is $1.5 \text{ g}_{\text{O}_3}/\text{h}$ ($31 \text{ mmol}/\text{h}$). The goal would be to determine the time after which the ozone limit has been exceeded in the laboratory. It is assumed that the laboratory has the dimension shown in Figure F.2 ($5 \times 5 \times 3 \text{ m}$).

The volume of the room is given by,

$$V = 5 \times 5 \times 3 = 75 \text{ m}^3$$

If the maximum amount of ozone is 0.1 ppm. Thus, the time after which this value can be calculated

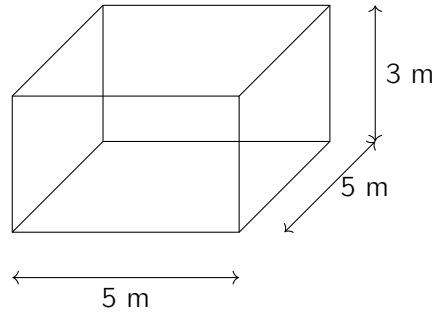


Figure F.2: Schematic representation of the laboratory dimensions: length = 5 m, width = 5 m and height = 3 m.

from

$$\text{Ozone limit } (\mu\text{g}/\text{m}^3) = \frac{\text{Ozone flow rate}(\mu\text{g}/\text{h}) \times \text{Time (h)}}{\text{Volume of the lab (m}^3\text{)}} \quad (\text{F.1})$$

$$\Leftrightarrow \text{Time (h)} = \frac{\text{Ozone limit } (\mu\text{g}/\text{m}^3) \times \text{Volume of the lab (m}^3\text{)}}{\text{Ozone flow rate } (\mu\text{g}/\text{h})} \quad (\text{F.2})$$

$$= \frac{100\mu\text{g}/\text{m}^3 \times 75\text{m}^3}{1.5 \cdot 10^6 \mu\text{g}/\text{h}} = 0.005\text{h} \quad (\text{F.3})$$

It therefore find that it takes only 0.005 hours, or 18 seconds, for the acceptable ozone threshold in the room to be exceeded. This rapid rate highlights the importance of using a fume hood during experiments. In the laboratory used for the tests with ozone, the exhaust airflow rate is 9 m/s, which is even higher than the safety recommendation of 5 m/s (Geens J., laboratory technician, personal communication, 2026).

Appendix G

Python code for data optimization

```
import numpy as np
import matplotlib.pyplot as plt
from scipy.optimize import curve_fit

# Experimental data
C = np.array(..., dtype=float)
r = np.array(..., dtype=float)

# Model
def model(C, k, alpha):
    return k * C**alpha

# Calculation
k0 = 1
alpha0 = 1
p0 = [k0, alpha0]

params, covariance = curve_fit(model, C, r, p0=p0)
k, alpha = params
errors = np.sqrt(np.diag(covariance))

# Results
print(f"k = {k:.4f} ± {errors[0]:.4f}")
print(f"alpha = {alpha:.4f} ± {errors[1]:.4f}")

# Graph
C_fit = np.linspace(min(C), max(C), 100)
r_fit = model(C_fit, k, alpha)

plt.scatter(C, r, label="Experimental data")
plt.plot(C_fit, r_fit, '--', label="Fit")
plt.xlabel("C (mol/l)")
plt.ylabel("r (mol/l.s)")
plt.legend()
plt.show()
```

Appendix H

Experimental protocol for bacteria

This Appendix aims to provide an experimental protocol associated to the degradation of bacteria using ozone. For this test, the bacterial strain *Pantoea agglomerans* is used and subcultured from liquid to liquid. The bacteria are cultured in LB medium at 29 °C. For 500 ml, 12.5 g of LB medium powder (Luria/Muller, Carl Roth) are diluted in milliQ water. It is autoclaved at 120 °C before use.



Figure H.1: Subculturing of the bacterial strain *Pantoea agglomerans* into the LB medium.

Prior to use, some bacteria solution is placed into Falcon tubes and centrifugated at 3000 rpm for 10 minutes (Sigma 3-16KL). The aim of this step is to separate the bacteria from their medium, which limits the risks of bacterial contamination during the test. The pellet (see [Figure H.2](#)) containing the concentrated bacteria is then recovered and diluted into *Daphnia magna* medium¹.

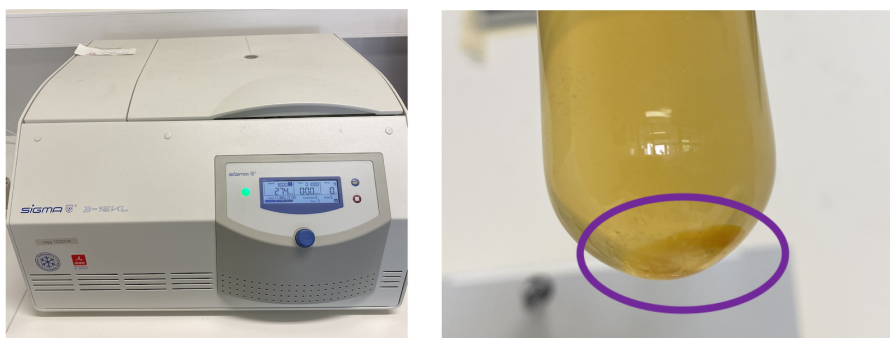


Figure H.2: Centrifugation of the bacteria solution to separate the bacteria pellet from their growth medium.

¹As a reminder, this medium is made from the combinaison of four salts. For 1 liter of distilled water: 0.23 g of KCl, 2.59 g of NaHCO₃, 4.93 g of MgSO₄ · 7 H₂O and 11.76 g of CaCl₂ · 2 H₂O.

The samples (*i.e.* the bacterial suspension) are then placed into two quartz beakers: one for the experiment, under stirring and with an ozone flow rate of 31 mmol/h and concentration of 0.13 mmol/l, and one for the control. Samples are taken after 0-2-5-7-10-15-30-60-90 minutes, given the rapid action of ozone. Prior to launching the experiment, enumeration of cells in the initial bacterial suspension is determined using microscopic counting of strained bacteria under epifluorescent light. To do that, the bacteria must be stained.

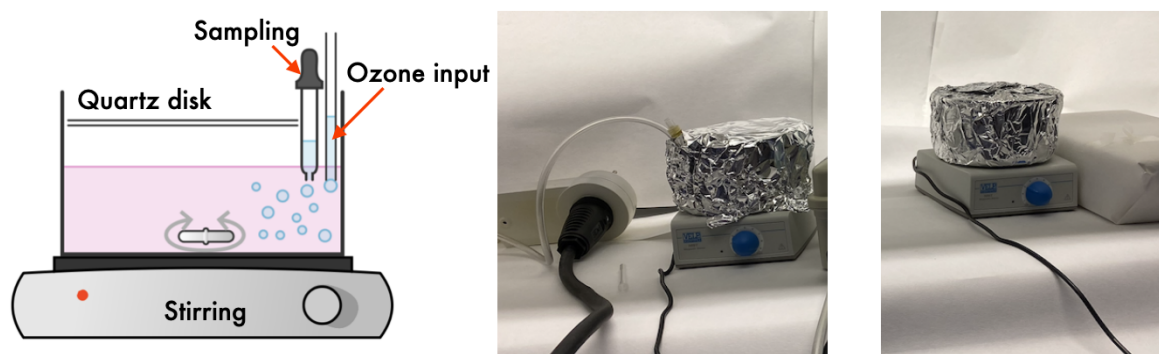


Figure H.3: Setup of the bacterial degradation experiment. Left: beaker into which O_3 is bubbled with a concentration of 6.5 mg O_3 /l and a flowrate of 1.5 g O_3 /h and under stirring. Right: control beaker under stirring.

For the staining procedure, two dyes are used: DAPI (4',6-diamidino-2-phenylindole) and Sytox green. DAPI binds to the DNA of a cell, regardless of the membrane integrity. It allows counting all the bacteria present. Sytox green is used as a dead-cell indicator, as it penetrates only cells with compromised plasma membranes (B. L. Roth *et al.*, 1997). The combination of DAPI and Sytox green allows to obtain the number of remaining alive bacteria (Count with DAPI - Count with Sytox green = number of alive bacteria). Thus, 900 μ l of sample is taken, to which 100 μ l of DAPI (10 mg/ml in milliQ water) and 100 μ l of Sytox green (50 μ M in DMSO) are added. The mixture is shaken briefly and left for 10 minutes in the dark. 5 ml of Daphnia medium are then added.

Before filtering the sample, the filter has to be treated. The filter used is a black isopore PC Membrane of 0.2 μ m (Merck, ref: GTBP 02500). It is placed in a 1% polyvinylpyrrolidone (PVP) solution to erode its surface, reducing the hydrophobicity of the filter and making the distribution of bacteria on the filter more homogeneous. The microscope slides are also prepared by placing one drop of immersion oil (Olympus, type-F) at their center (1). A second slide is placed on top to spread the oil (2). The filter is then placed on the filtration device and the vacuum pump is turned on (3). Once the filtration is complete, the filter is placed on the slide and five drops of immersion oil are added (4). Finally, a cover slip is placed on top (5). A schematic representation of these steps is presented in [Figure H.4](#).

Once the slide is ready, it is observed with a fluorescence microscope (Olympus BX50). This device uses a mercury lamp and the WU cube is selected. For each sample, ten pictures are taken at random locations across the filter surface. This allows a representative bacterial count to be obtained.

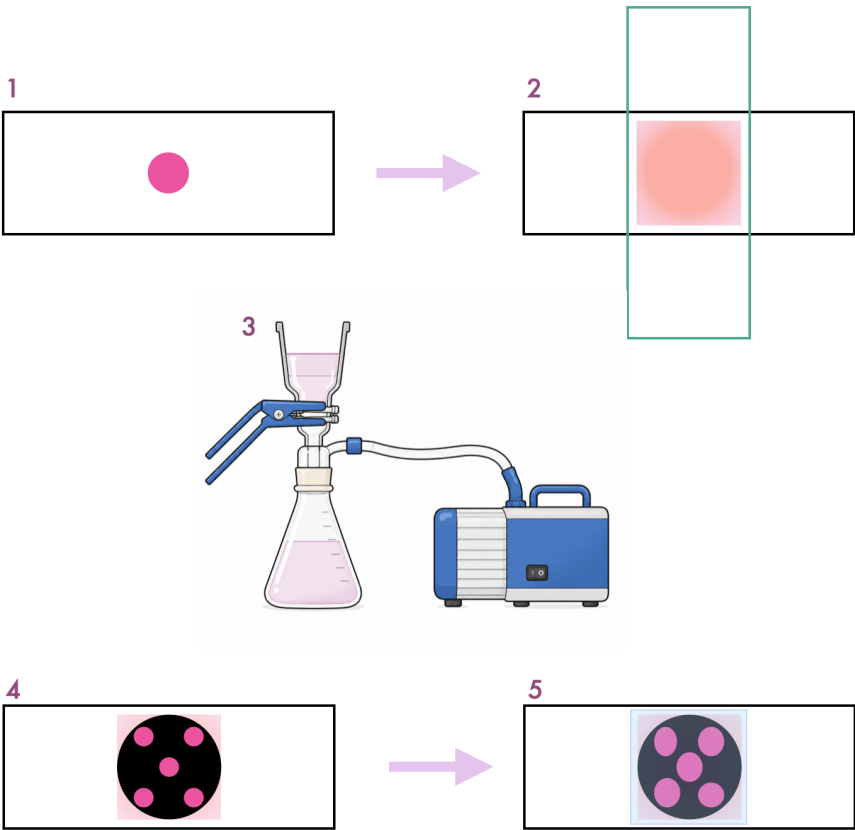


Figure H.4: Schematic representation of the slide preparation and filtration step.