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POST-TREATMENT OF MUNICIPAL WASTEWATER EFFLUENT: EFFECT ON ORGANIC MATTER AND MICROPOLLUTANT REMOVAL

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DEDICATION

To my late parents

ACKNOWLEDGEMENT

First and foremost, I thank God for his strength, care and companionship in all my life.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS

AOPs: Advanced Oxidation Processes

BDOC: Biodegradable Dissolved Organic Carbon

BGAC: Biological Granular Activated Carbon

BOD: Biological Oxygen Demand

COD: Chemical Oxygen Demand

CW: Constructed Wetland

DOC: Dissolved Organic Carbon

DOM: Dissolved Organic Matter

DWWTP: Domestic WasteWater Treatment Plant

Ec: electrical conductivity

EC_{50}: half maximal (50%) Effective Concentration

ECWFD: European Community Water Framework Directive

EfOM: Effluent Organic Matter

EPA: Environmental Protection Agency

EQS: Environmental Quality Standards

ESI: Electro-Spray Ionization

GAC: Granular Activated Carbon

GC: Gas Chromatography

HPLC: High Performance Liquid Chromatography

HRMS: High Resolution Mass Spectometry

LC: Liquid Chromatography

LC_{50}: half maximal (50%) Lethal Concentration

LLE: Liquid-Liquid Extraction

MASE: Membrane-Assisted Solvent Exraction

MS: Mass Spectrometry

MW: Molecular Weight

MWWE: Municipal WasteWater Effluent

MWWTPs: Municipal WasteWater Treatment Plants

NDMA: N-nitrosodimethylamine

NF: Nanofiltration

NH_3-N: ammoniac-nitrogen

NO_3-N: nitrate-nitrogen

NV: Naamloze Vennootschap

Oasis HLB: oasis Hydrophilic-Hydrophobic Balance

Oasis MCX: Oasis Mixed-mode Cation exchange

$HO^{\bullet}$: Hydroxyl radical

ORP: Oxidation-Reduction Potential

PAC: Powdered Activated Carbon

pH: Potential Hydrogen

PLE: Pressurized Liquid Extraction

PO_4^{3-}: ortho-phosphate

Redox: reduction-oxidation potential

SF: Surface Flow

SFE: Supercritical Fluid Extraction

SFCW: Surface Flow Constructed Wetlands

SPE: Solid-Phase Extraction

SPME: Solid-Phase Micro Extraction

SSF: Slow Sand Filtration

SSF: Subsurface Flow

STP: Sewage Treatment Plant

SUVA: Specific Ultra-Violet Absorption

TF: Trickling Filter

TN: Total nitrogen

TOC: Total Organic Carbon

TOF: Time of Flight

UF:Ultrafiltration

UV: Ultra-Violet

UV 254: ultraviolet absorbance at 254 nm

UVA: Ultra-Violet Absorption

WAO: Wet Air Oxidation

WHO: World Health Organization

XAD : Adsorbent resins

ABSTRACT

Dissolved organic matter and organic micropollutants are still found in municipal wastewater treatment plant effluent up to µg/L levels. Although their concentrations are very low in absolute numbers, they should not be considered negligible as they affect aquatic life in receiving water bodies. This study aimed at assessing the removal of dissolved organic matter and micropollutants in the municipal wastewater treatment plant of Aquafin in Harelbeke by post-treatment methods. The treatment consisted of the ozone oxidation process followed by biological and physical-chemical post-treatment. Three different techniques were used in post-treatment. They included trickling filtration, slow sand filtration and biological granular activated carbon filtration. It was aimed to find out the potential of ozone oxidation influence on post-treatment techniques to eliminate organic pollutants in municipal wastewater effluent. On the other hand, physical-chemical parameters were monitored as well in the purpose to assess the quality of the raw effluent. Among all the post-treatment techniques used, the biological granular activated carbon either alone or in combination with ozonation was found the most effective in removal of organic pollutants with a removal efficiency of higher than 95% in average, but this method is mostly limited in use due to the less cost-effectiveness in view of its capital cost. Therefore slow sand filtration in combination with ozone oxidation was revealed as the optimal technology in terms of removal efficiency (higher than 70% in average) of all targeted compounds and considering the cost-effectiveness. The trickling filtration method combined with ozonation also provided a slight improvement in removal of the majority of the compounds studied. Therefore, a combination of ozonation and biological/physical-chemical filtration process was suggested effective to reduce the majority of targeted pollutants at significant level.

CHAPTER ONE

INTRODUCTION

Ongoing processes of industrialization and urbanization due to the population growth are exerting pressure on depleting aqueous resources in different parts of the world. World's fresh water is not being used sustainably, thus a radical amendement of policies to manage competing demands is required. The long-term sustainability of clean water is governed by source water protection, management of water resources and the efficiency of water reclamation from various effluents.

In 1980s, scientists discovered the presence of emerging organic micropollutants in the aquatic environment. "Emerging micropollutants" is a term used to specify chemical compounds that have only been recently analyzed at trace levels in the environment and they are suspected to cause adverse effects on the ecosystem and human (Grassi et al., 2012). Emerging organic micropollutants consist of endocrine disrupting compounds, diagnostic contrast media, musk fragrances, pharmaceuticals, household and personal care products, flame retardants and pesticides in the aquatic environment.

Neverthless, they are officially unregulated or they are still in process to be regulated. Recently to date, a lot of efforts has been done by the European Commission to set the most priority substances and their guideline standards values. The EU Directive on Environmental Quality Standards (Directive 2008/105/EC) defines a list of 33 priority substances and 8 other pollutants. Article 16 of the WFD sets out "Strategies against pollution of water", including the setting of environmental quality standards (EQS) for these priority substances.

Recently, the EU announced that it would add 12 new substances to this priority list. The maximum concentration levels in water of the substances added to the priority list will be set and enforced by 2018. Further, 3 pharmaceuticals were added to a 'watch list' of emerging pollutants and may be added to the priority list at a later date. These 3 chemicals are diclofenac, a commonly-used generic painkiller and the hormones 17 alpha-ethinylestradiol (EE2) and 17 beta-estradiol (E2).

The main source of these pollutants was found in municipal wastewater treatment plants (MWWTPs) as their entry point in natural aquatic systems (surface water, ground water and lakes) (Petrović et al., 2003). MWWTPs were mainly developed to protect the natural aquatic environment by removing dissolved organic matter (DOM), nutrients and pathogens. They were not specifically designed to eliminate the trace levels of emerging compounds found in wastewater. Consequently, the fact that micropollutants can still be detected in the municipal wastewater effluent (MWWE) demonstrates that conventional wastewater treatment does not lead to a complete removal of all these compounds (Vieno et al., 2005; Suárez et al., 2008; Hollender et al., 2009).

Various studies have noticed the associated threat to the MWWE. This is due to the continuous discharge of emerging compounds in the environment even though their concentrations are at trace level. Two major problems for water resource management were raised: the threat on aquatic life and the need for protection for reuse potential of municipal and industrial wastewater effluent. Consequently, all these concerns have led to a significant increase in the research of occurrence, fate, transport and removal of those emerging contaminants from wasterwater to better understand and possibly identify some mitigation opportunities.

Accordingly, new treatment approaches aimed at improving the process efficiency of wastewater treatment need to be employed. Diverse methods are available to eliminate these compounds before their discharge in the environment. They include physical-chemical techniques, such as sorption methods and advanced oxidation processes (AOPs), and biological treatment methods.

Advanced chemical oxidation methods such as ozonation has been found to be an appropriate technology to eliminate at large extent micropollutants of ecotoxicological concern and reducing the probability of their occurrence in the environment. Therefore, this technique has been applied in this work to eliminate or reduce the possible compounds which are persistent in MWWTPs. Furthermore, a post-treatment approach after ozonation of the effluent can be applied as a polishing way for persistent chemical compounds to the ozonation treatment.

However, the cost and complexity of sample analysis to determine the organic micropollutants are taken into account. It is not easily practicable to study all types of organic micropollutants in wastewater at the same time. For that reason, pharmaceuticals were decided to be the purpose subject of this study due to their broadly relevance and occurrence.

CHAPTER TWO

LITERATURE REVIEW

2.1 Organic characteristics of municipal wastewater effluent

Discharge of a secondary effluent from a municipal wastewater treatment plant degrades water quality and inhibits aquatic life in receiving waters. This is due to the discharge of chemical oxygen demand (COD), nitrogen (N) and phosphorus (P) but mostly by discharge of emerging micropollutants persistent in conventional wastewater treatment plants. Indeed, the occurrence of DOM and micropollutants in secondary effluent has raised a concern to public health and the environment. Most of the micropollutants including pharmaceutical compounds are not well degradable in WWTP, some are persistent and recalcitrant and need further treatment. The wastewater effluent should therefore first be well characterized for optimum treatment before being discharged to receiving waters or being reused for other purposes (Shon et al., 2006).

Several studies already worked on effluent organic matter (EfOM) removal using different ways for treatment. EfOM has different characteristics and varies by place and season. Based on the sources, it consists of 3 classes, i.e. (i) natural organic matter coming from drinking water and deteriorated materials (e.g. humic acids), (ii) soluble microbial pollutants (e.g. extracellular polymeric substances) coming from biological processes of wastewater treatment, and (iii) synthetic organic compounds derived from industrial production and domestic use (e.g. pharmaceuticals, pesticides) (Drewes & Fox, 1999). Figure 1 presents different types of DOM found in the MWWE.

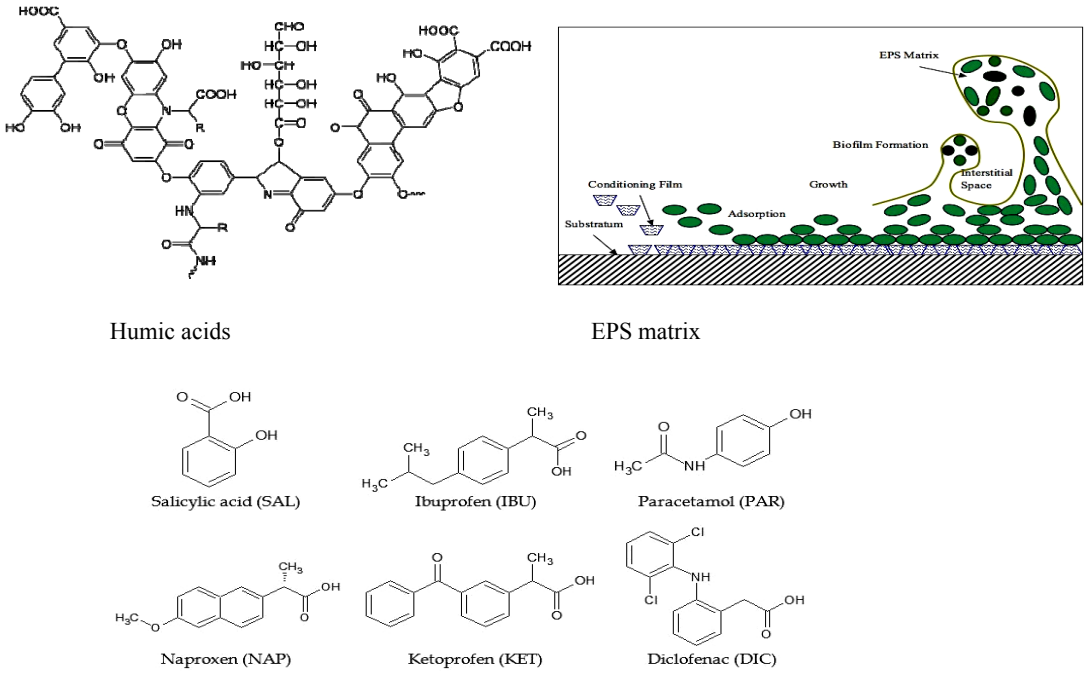


Figure 1: **Diffferent types of DOM found in the MWWE (Wikipedia).**

2.1.1 Surrogate parameters of EfOM

It is difficult to analyze effluent organic matter due to its complexity and heterogeneity. Hence, some surrogate parameter methods are developed as a measure representing EfOM in wastewater effluent. Some conventional parameters are e.g. BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), TOC (Total Organic Carbon) and DOC (Dissolved Organic Carbon). Nevertheless, it has been shown that also fluorescence spectroscopy, (specific) UV absorption ((S) UVA), XAD (adsorbent resin) fractionation and molecular weight (MW) are used to characterize EfOM (Gong et al., 2008). Hudson et al. (2007) identified 5 distinct peak areas in the fluorescence excitation emission matrix (EEMs) to determine different regions where organic materials can be found in aquatic environment. Peaks A and C represented broad fulvic and humic like fluorescence, peak B was similar to the fluorescence of tyrosine, whereas peaks T1 and T2 were similar to the fluorescence of tryptophan as illustrated in Figure 2. The presence of these five peaks provided insight to the types of fluorophores representing the organic matter.

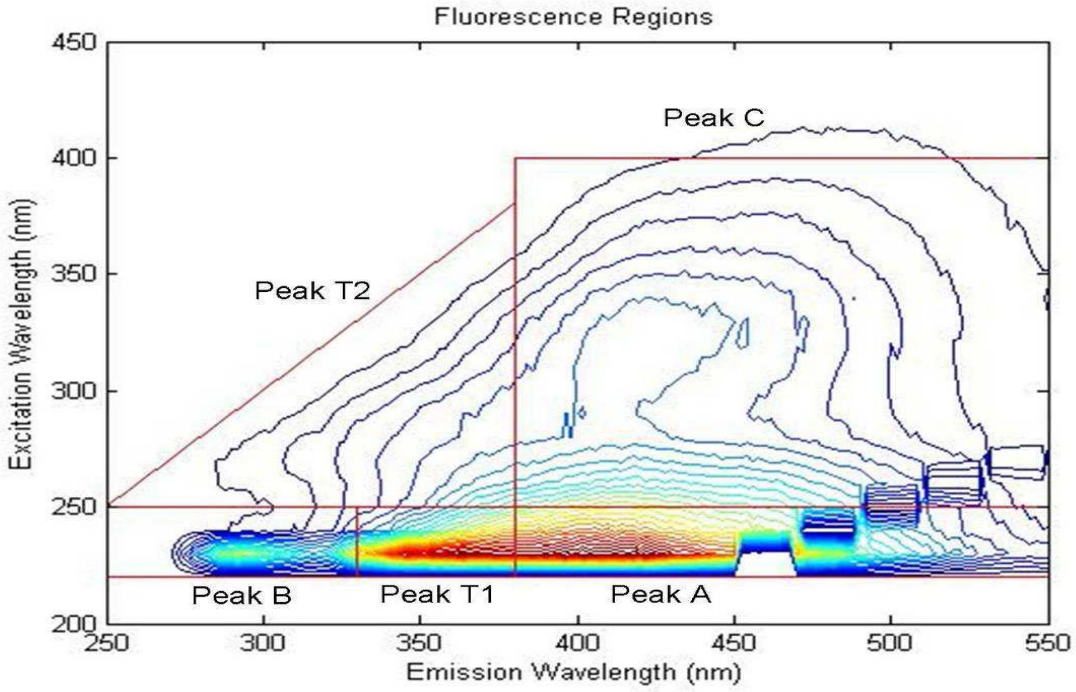


Figure 2: Peak locations representing the organic matter in excitation emission matrix (EEMs) (Hudson et al., 2007)

2.1.2 Micropollutants in MWWE

Organic micropollutants which have been frequently studied in MWWTP's effluent are endocrine disrupting compounds, household and personal care products, pesticides, pharmaceutical compounds, musk fragrances, etc. (Rattier et al., 2014) but as mentioned in chapter one, pharmaceutical compounds were of concern in this thesis.

Over the years, pharmaceuticals were not known to be present in MWWE. It was only in early 80s' when the first analytical methods were developed that their existence in aqueous

environments such as, surface water, wastewater, groundwater and drinking water were detected. Due to their potential biological activity, pharmaceuticals have been paid attention in the environment (Kummerer, 2009). Diverse groups of pharmaceutical residues are elaborated by many researchers depending on their mode of action. Gros et al. (2009) classified pharmaceuticals according to their therapeutic activity. The most discussed pharmaceutical substances include analgesics and anti-inflammatories (e.g. diclofenac, ibuprofen, paracetamol), lipid regulator agents (e.g. emfibrozil, clofibric acid), beta- blockers (e.g. atenolol, metoprolol), antibiotics (e.g. ciprofloxacin, sulfamethazine), steroids and related hormones (e.g. 17 α-ethinylestradiol, 17 β-estradiol). Even though their environmental concentration is generally low (ng/L-µg/L), it can be sufficient to stimulate toxic effects (Stalter et al., 2010; Margot et al., 2013)

The major source of pharmaceuticals in aquatic environments is the discharge of wastewater treatment plants (WWTPs). These compounds mainly stem from their use in households, hospitals and from discharges of wastewater from drug producers. In Figure 3, the different discharge routes of pharmaceuticals are depicted.

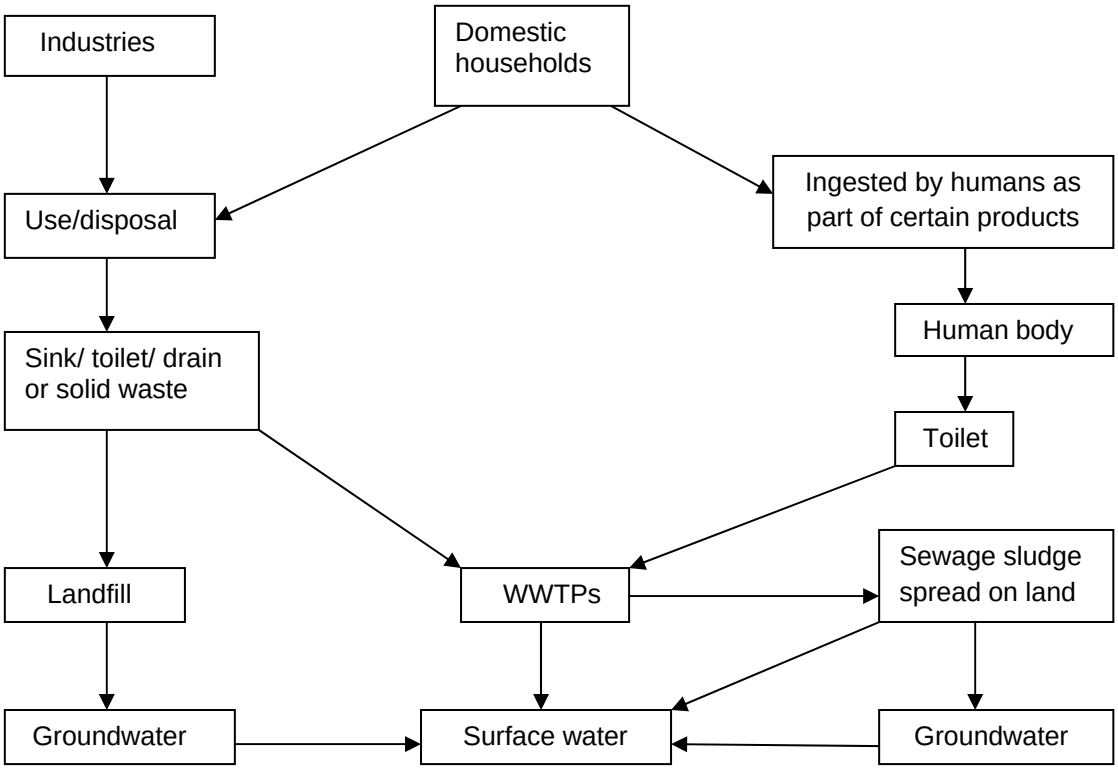


Figure 3: Discharge routes of pharmaceuticals (Bound & Voulvoulis, 2005)

2.1.2.1 Occurrence of pharmaceuticals in MWWE

Pharmaceutical concentrations of ng/L to several µg/L have been globally detected in MWWE (Batt et al., 2006; Hernando et al., 2006; Vieno et al., 2007; Hollender et al., 2009; Reungoat et al., 2010; Falas et al., 2012; Margot et al., 2013). Depending on the study area,

pharmaceutical residuals were reported in various concentrations. Table 1 presents the occurrence of some most investigated compounds.

Table 1: Occurrence of pharmaceutical compounds in MWWE

Compound	Theurapeutic class	LOD (ng/L)	Concentration range (ng/L)	Country studied	Reference
Paracetamol	Analgesic/anti-inflammatory drugs	5	48-418	Spain	Radjénovic et al. (2007)
		10	-	Australia	Reungoat et al. (2010)
Diclofenac	Analgesic/anti-inflammatory drugs	40	786-1991	Spain	Radjénovic et al. (2007)
		1	6-431	Spain	Rosal et al. (2010)
		-	500-1250	Switzerland	Joss et al. (2005)
		50	501-1731	Switzerland	Hollender et al. (2009)
		10	20-210	Canada	Lee et al. (2003)
Ibuprofen	Analgesic/anti-inflammatory drugs	10	40-970	Canada	Lee et al. (2003)
		5	<5 -3910	Finland	Vieno et al. (2007)
		4	< 4- 653	Spain	Rosal et al. (2010)
		20	336-6268	Spain	Radjénovic et al. (2007)
		20	910-2100	Spain	Carballa et al. (2004)
		80	56-86	Switzerland	Hollender et al. (2009)
Ciprofloxacin	Antibiotics	50	70-240	USA	Yang et al. (2011)
		29	30-130	Finland	Vieno et al. (2007)
		10	< 10-5692	Spain	Rosal et al. (2010)
		10	< 10- 30	Australia	Reungoat et al. (2010)
		1	4-407	Korea	Kim et al. (2007)
Sulfamethazine	Antibiotics	5	7-20	Switzerland	Hollender et al. (2009)
Trimethoprim	Antibiotics	0.3	550-720	USA	Gerrity et al. (2011)
		29	99	Spain	Rosal et al. (2010)
		30	71-234	Switzerland	Hollender et al. (2009)
		-	27-141	Australia	Reungoat et al. (2012)
		1	58	Korea	Kim et al. (2007)
Carbamazepine	Antiepileptics	20	24-240	Canada	Brun et al. (2006)
		1	139-210	USA	Synder et al. (2006)
		1	290-2440	Finland	Vieno et al. (2007)

LOD: Level of detection - : not provided

Diclofenac has been detected in MWWE in the range of 6 ng/L to 1991 ng/L (Joss et al., 2005; Radjénovic et al., 2007; Hollender et al., 2009; Rosal et al., 2010). Sulfamethazine and trimethoprim have also been reported by many authors to be in the range of 7 ng/L to 20 ng/L, and 58 ng/L to 720 ng/L, respectively. Ketoprofen and ibuprofen have been reported in the range of 318 ng/l to 2666 ng/l, and < 4 ng/L to 6268 ng/l in MWWE, respectively (Ollers et al., 2001; Lee et al., 2003; Petrovic et al., 2005; Araujo et al., 2008). These findings are quite expected as analgesics and anti-inflammatory drugs are highly consumed all over the world due to their easy accessibility.

In domestic wastewater effluent, Alder et al. (2010) detected a concentration of 1330, 330, 240 and 70 ng /l for atenolol, sotalol, metoprolol and propranolol respectively. Similary, Bueno et al. (2007) reported atenolol, sotalol and propranolol with maximum concentration levels of 4850, 155 and 100 ng/l, respectively, in domestic wastewater effluent. Various concentrations of carbamazepine were detected by different authors in the range of 24 ng/L to 2440 ng/L (Brun et al., 2006; Synder et al., 2006; Vieno et al., 2007) in different places.

2.1.2.2 Fate and pathways of pharmaceuticals in MWWE

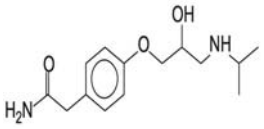
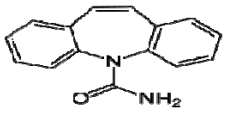
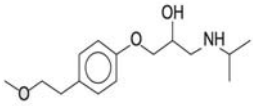
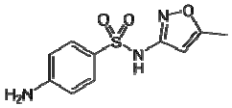
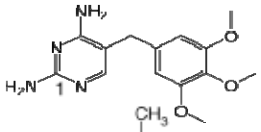
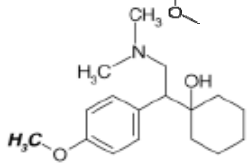
Effluent from MWWTPs containing pharmaceutical residues is discharged into receiving waters. These compounds can enter the aquatic environment as parent compounds or metabolites. The result of interactions between a compound and its environment over a sequence of events is called “fate”. The fate of pharmaceuticals in the aquatic environment depends on diverse factors including physical-chemical properties of the compounds, water matrix, and biotic (e.g. biodegradation) and abiotic (e.g. adsorption, complexation, photodegradation and hydrolysis) factors. Based on mentioned factors and the reactivity levels, pharmaceutical residues are capable to interact with solid particles and microorganisms living in substrate materials (Nakada et al., 2007).

The common physico-chemical properties of pharmaceuticals are the partitioning and sorption coefficients. Partitioning coefficient refers to the tendency of a chemical to concentrate in one phase (solid, liquid or gaseous) of a two phase mixture at equilibrium. The octanol-water partitioning coefficient (Kow mostly expressed in log Kow) is a measure of the partitioning between octanol and water, which describes the hydrophobicity of a compound and is inversely related to the solubility of a compound in water (Nakada et al., 2007; Kasprzyk-Hordern et al., 2008). Compounds with a high Kow have been known to preferentially adsorb to soil and sediment particles in water. Similarly, a sludge adsorption coefficient (Kd) is a ratio of the amount of compound adsorbed to sludge compared to the amount present in aqueous solution.

However, not only hydrophobicity properties can influence the sorption of pharmaceuticals but also other factors such as hydrogen bonding, ionic interactions and complexation can have an important effect on sorption of these compounds. For instance, the acid dissociation constant of the compounds (Ka) can affect the fate of pharmaceuticals. Ka is a measure of the strength of an acidic compound in solution and is the concentration ratio of ionized to unionized species of a compound at equilibrium. The Ka of a compound enables the concentration of the ionized or unionized chemical to be calculated at a provided pH. Most of these substances display different charges at different pH which influences their adsorption and photodegradation processes (Nakada et al., 2007). E.g. adsorption is known as the dominant mechanism for removal of fluoroquinolones (> 50 %) despite of their low kow values but rather due to their zwitterionic characteristics with their high pKa values ranging from 5.9 to 6.4 (Jelic´ et al., 2012). Furthermore, fluoroquinolones can complex divalent cation compounds which can be an indication of degradation of these compounds through

complexation process. Table 2 outlines some pharmaceutical properties.

Table 2: Pharmaceutical properties (Vieno et al., 2007; Rosal et al., 2009)

Compound	MW (g/mol)	Theurapeutic	log Kow (-)	Structure
Atenolol	266	Beta-blocker	0.16	
Carbamazepine	236	Antiepileptics	2.45	
Metoprolol	267	Beta-blocker	1.69	
Sulfamethoxazole	253	Antibiotics	0.89	
Trimethoprim	290	Antibiotics	0.91	
Venlafaxine	277	Antidepressants	0.43	

2.1.2.3 Toxicological and ecological impacts of pharmaceuticals

The potential significance of pharmaceuticals in the environment is defined in the context of bioaccumulation, persistence and toxicity (Abelkop et al., 2013). The existing ecotoxicity data reported in various studies are often complex due to the variability arising from the differences in experimental procedures and conditions (Hernando et al., 2006).

Referring to EU Directive 93/67/EEC, the toxicity data are interpreted and ranked in different levels of toxicity according to the measured half maximal effective (or lethal) concentrations (EC_{50} or LC_{50} value). Three different levels have been distinguished: level one, which is very toxic level comprises an EC_{50} below 1 mg/l; level two, a toxic class with an EC_{50} from 1 to 10 mg/l, and lastly, and a harmful class from 10 to 100 mg/l (EU Directive 93/67/EEC).

Various studies reported the toxicity and ecological impacts of pharmaceuticals (Hernando et al., 2006; Quinn et al., 2008; Stalter et al., 2010). According to Quinn et al. (2008), pharmaceutical compounds may induce unexpected effects in aquatic organisms which may lead to disturbance or change in morphology substrate attachment and feeding behavior. Toxicity effects have been reported in fish as well (Stalter et al., 2010; Li et al., 2011a; Li et al., 2011b; Margot et al., 2013). For e.g. rainbow trout exposed to 270 µg/L of verapamil has revealed an oxidative stress that implies a decrease in lymphocyte numbers and the elevated levels of plasma ammonia concentration, implying impairement of the detoxification process of ammonia (Li et al., 2011b, K'oreje, 2012).

2.1.2.4 Analysis of pharmaceutical residues in the aquatic environment

Due to evolution in analytical methods in time, pharmaceutical compounds have been able to be analzed and quantified in the environment. This has made an increased scientific interest in the pharmaceutical field. Pharmaceutical compounds in wastewater effluents are determined in a combination of different processes involving sampling, sample preparation, extraction and instrumental analysis. All samples to be analyzed should be well representative of the sample area. Sampling techniques and materials that are used must protect target compounds from removal or degradation through processes such as adsorption and photolysis. Sample preparation is the most challenging and time consuming step due to diverse properties of the sample and complex matrix. This process is based on conversion of the matrix into a suitable form for analysis (Pavlovic´et al., 2007). Figure 4 illustrates different processes involved in pharmaceutical analysis.

Sample extraction

After sampling, pharmaceutical compounds are normally extracted from wastewater using various techniques before instrumental analysis. The extraction step is very crucial for the pre-concentration and clean up processes. It improves the sensitivity and selectivity towards the target analytes (Kostopoulou & Nikolaou, 2008). The most generally applied technique for liquid samples is sorptive extraction, mainly solid-phase extraction (SPE). Techniques such as liquid-liquid extraction (LLE) and solid-phase micro extraction (SPME) are used as well.

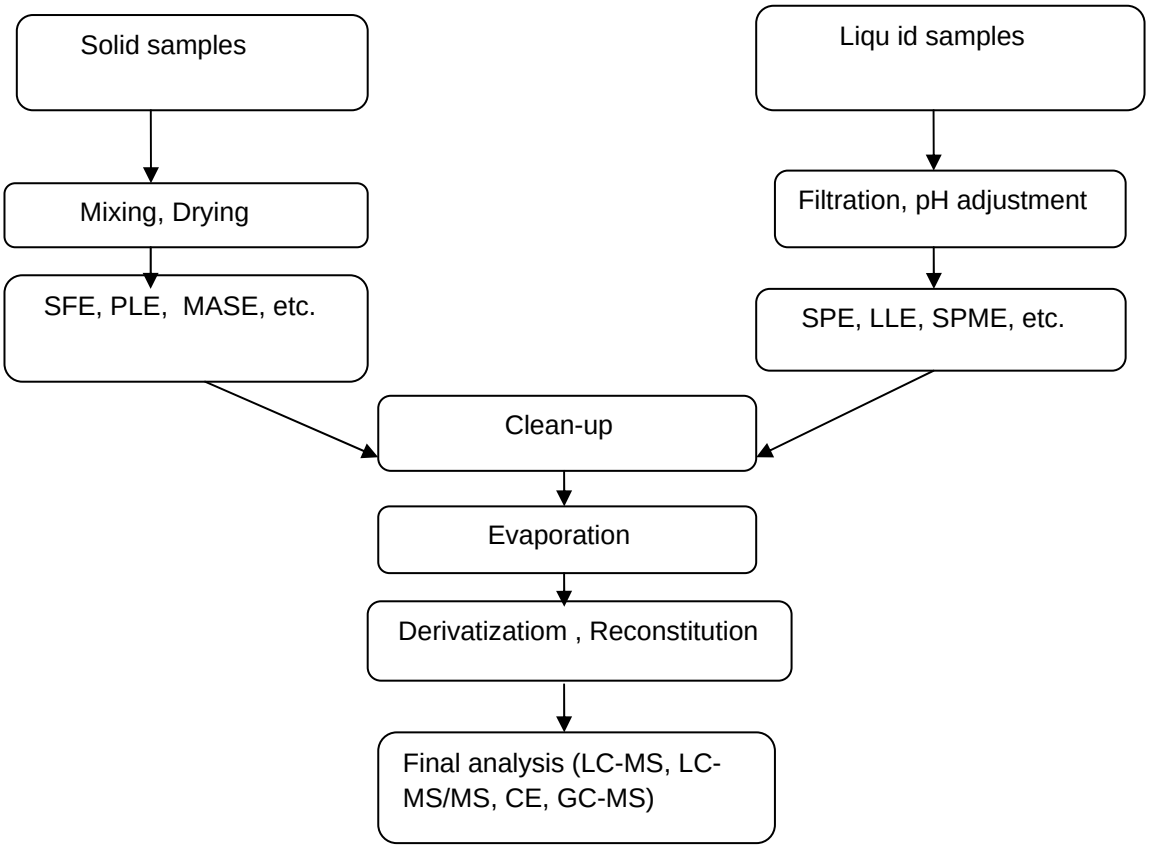


Figure 4: Sample preparation and instrumental analysis of pharmaceuticals in the environment (Pavlovic´et al., 2007).

SFE: Supercritical Fluid Extraction; PLE: Pressurized Liquid Extraction; SPE: Solid-Phase Extraction; SPME: Solid-Phase Micro Extraction; LLE: Liquid-Liquid Extraction; LC-MS: Liquid Chromatography-Mass Spectrometry; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry; GC-MS: Gas Chromatography-Mass Spectrometry.

Solid-phase extraction (SPE)

The basic concept of solid-phase extraction is the sorption of the target analytes onto a sorbent material due to their hydrophobic properties. The analytes should have higher affinity towards the solid phase than the liquid phase (sample matrix). Silica materials (C_8-C_{18} alkyl groups) and polymeric sorbents including Oasis hydrophilic-hydrophobic balance (Oasis HLB) cartridges have been employed for the extraction process (Pavlovic´et al., 2007). Hydrophilic-hydrophobic balance (HLB) sorbents have been developed to improve the pharmaceutical residues extraction. Nowadays, they are the most common used due to their capability to extract the acidic, neutral and basic polar analytes at various pH ranges. They are known to have better recoveries for a numerous compounds compared to silica material sorbents (Gros et al., 2006; Pavlovic´et al., 2007). Oasis MCX mixed mode sorbents with reversed phase and cation- exchange characteristics are used as well but for acidic substances

with good recoveries, whereas for basic and neutral compounds the recoveries are poor (Gros et al., 2006). Solid-phase extraction is also used as automated or online method. Online SPE has advantages over such as solvent consumption, cost and time for extraction (Trenholm et al., 2009). Another type of SPE is used, solid-phase micro extraction (SPME). It is a fast and simple method based on the equilibrium partitioning of the substance between sorbent and sample. However, it is not commonly used for pharmaceuticals extraction.

Liquid-liquid extraction (LLE)

Liquid-liquid extraction is one of the oldest and most widely used techniques in the preparation of samples for qualitative and quantitative analysis. According to Jjemba (2008), liquid-liquid extraction principle is based on the partitioning of sample components between the two immiscible liquid phases (aqueous and organic solvent). The use of this technique in environmental sample analysis is limited due to various disadvantages including use of huge amount of solvents and loss of target analytes due to multistage operations, and also it is time consuming.

Separation

Separation techniques for pharmaceutical compounds is based on chromatography. Although it is an old technique, it finds a lot of applications in the field of pharmaceutical analysis. Gas chromatography (GC) and liquid chromatography (LC) are the main methods utilized. GC is a powerful separation technique for several volatile organic compounds. The target compounds are vaporized and eluted in a gas flow through the column in which they are separated in two different phases such as a liquid stationary phase and a gaseous mobile phase. The GC technique has been applied in various studies (Miège et al., 2006; Sebök et al., 2008, Togola et al., 2007). However, this method implies a compulsory derivatization of rather polar compounds to be analyzed and it is time consuming. Consequently, this may lead to possibility of getting wrong results (Fatta-Kassinos et al., 2011). Contrary to GC, the LC technique has found an increased use with time due to its non-selectivity properties. LC is generally an appropriate method for the determination of compounds that are highly polar, non-volatile or thermo-degradable, which cannot be analyzed by GC (Diaz-Cruz & Barcelo, 2005; Baugros et al., 2008).

Detection

The complex matrix of wastewater samples requires highly sensitive and selective equipments for detection of pharmaceutical trace compounds. Generally, fluorescence and UV-spectroscopy are the most used methods in the past. However, these techniques were found inappropriate for multi-residue analysis. In order to overcome the inadequacy of those techniques, mass spectrometry (MS) technique has become an important one with time for this kind of analysis. The potential of MS is due to its high selectivity and sensitivity as well as confirmation properties (Grujic et al., 2009). MS can be coupled with LC although the combination is prone to matrix effects which may result in ion suppression that lead to reduced selectivity of MS.

The basic principle of mass spectrometry (MS) involves the ionization of a gaseous transformed sample. The ions are passed into a mass analyzer for separation based on their mass to charge (m/z) ratio. The ions heat the sensor of detector and a spectrum of signals is produced, then it amplifies the weak ionic current and it displays on the computer screen (Hoffman et al., 2002). Different ionization techniques are used in mass spectrometry including electrospray ionization, chemical ionization, thermospray ionization, atmospheric pressure chemical ionization and desorption techniques. Diverse types of analyzers are used to filter the ions according to their mass to charge ratio before they are directed towards the detector. Quadrupole (Q) and ion trap analyzers are generally used. These both mass analyzers have however, low mass resolution and mass range. A quadrupole analyzer consists of four rods with circular sections arranged in parallel. It uses stable trajectories to oscillate the electric fields to separate ions according to their mass to charge ratio (m/z) (Hoffmann et al., 2002). In the ion trap analyzer, the ions are trapped inside the volume between the electrodes from the place where they are expelled to the detector according to their mass to charge ratio.

In order to achieve the required high selectivity and sensitivity for the analysis of pharmaceutical compounds in wastewater, new approaches have been introduced such as tandem mass spectrometry and high resolution mass spectrometry. Tandem mass spectrometry (MS/MS) is a combination of multiple mass analyzers with some forms of fragmentation occurring in between the consecutive stages. High resolution mass spectrometry (HRMS) is a kind of innovative alternative used in full scan for screening purposes. Examples of modern HRMS techniques are time-of-flight (TOF), magnetic sector, and orbitrap analyzers. TOF involves the relationship between the mass of ions and their velocity at a given kinetic energy. It has high transmission efficiency and sensitivity. The magnetic sector analyser is based on the principle that ions enter a magnetic field and are separated as result both, centripetal and centrifugal forces. Orbitrap analyzer involves the ions trap due to their electrostatic attraction to the inner electrode in balance with centrifugal forces.

2.2. Legislation on MWWE

Legal and institutional procedure requirements are a basic conceptual tool for environmental management. The legislation is basically assumed to be enacted laws that forbid any kind of pollution that may be caused by human activities unless a permit is granted by competent authority. These legislations may be classified in the form of directives, regulations, guidelines or green taxes.

2.2.1. Legislation on surrogate parameters

Good sanitation is vital to a healthy society. According to the EU Directive 91/271/EEC there exists 2 different types of municipal effluent standards. One type consists of the technology-based standards (effluent discharge standards) which are defined as type level of technology and pollution control performance. The second type involves water quality based limits (in-stream standards) which is applied for the receiving waters. Water-quality based standards are more stringent than technology-based standards. The European Community has

developed a directive to regulate the discharges to surface water. Although the effluent disposal standards may be different from country to country, basic standards for pollutants such as suspended solids, organic matter and nutrient are established (Table 3). Their amount in water-bodies indicates the degree of pollution.

Table 3: Basic conventional wastewater effluent standards (EU, 91/271/EEC)

Parameters	Effluent concentration (mg/l)	Minimum % of reduction (Reduction in relation to the load of influent)
BOD_5	25	70-90
COD	125	75
TSS	35	90

2.2.2. Legislation on micropollutants

The effect of the mixture of thousands of organic micropollutants in wastewater effluent on the aquatic environment is not easily assessable mainly due to the low concentrations, the multiplicity of the chemical structures, and the formation of metabolites. Not all micropollutants are yet regulated in terms of their occurrence in water bodies and wastewater effluents. The EU Directive on Environmental Quality Standards (Directive 2008/105/EC) defines a list of 33 priority substances and 8 other pollutants as explained in chapter one. Moreover, 12 substances would be added to the priority list ('watch list') including 3 pharmaceutical compounds (diclofenac, and the hormones 17 alpha-ethinylestradiol (EE2) and 17 beta-estradiol (E2), and their maximum concentration levels in water will be set and enforced by 2018 (see Chapter one). Table 4 illustrates some chemical substances defined in the watch list including the 3 added pharmaceutical compounds.

Table 4: Some micropollutant guidelines in the watch list of the European directive Directive 2008/105/EC (EC, 2012)

No.	Name of a substance	Inland surface water concentration (µg/l)
1	Anthracene	0.1
2	Atrazine	0.6
3	17-beta estradiol (E2)	4×10^{-4}
4	Diclofenac	0.1
5	Nonylphenols	0.3
6	Octylphenol	0.1
7	Brominated diphenyether	4.9×10^{-8}
8	Aclonifen	0.12
9	Diuron	0.2
10	Quinoxyfen	0.15
11	17-alpha-ethinylestradiol (EE2)	3.5×10^{-5}

2.3. Treatment of MWWE

The wastewater in MWWTPs consists of a complex mixture of micropollutants including pharmaceutical residues. Generally in most MWWTPs, wastewater is treated through conventional treatment systems involving two stages such as primary and secondary treatments. Primary treatment involves physical removal of large particles from wastewater before secondary treatment. Secondary treatment consists of mainly biological treatment where dissolved particles are degraded by microorganisms present in the plant. Mostly, in the activated sludge process. In latter process, pharmaceuticals are removed mainly due to sorption and biodegradation. Although these systems have shown good removal efficiencies for some organic pollutants, others are not adequately removed. For example, Heberer, (2002) reported 17 % reduction in the concentration of diclofenac in the MWWE. This has been confirmed by Joss et al., (2005) and Radjénovic et al., (2009) where they have reported a reduction of 25% of diclofenac after secondary treatment. Advanced treatment technologies have been thought to be an alternative solution that can further improve the effluent quality. These techniques are considered as tertiary treatment processes. Main treatment methods are physical, chemical and biological techniques. They include coagulation and flocculation, adsorption, rapid sand filtration, membrane filtration and advanced oxidation processes.

2.3.1. Coagulation and flocculation

Coagulation and flocculation is one of the oldest treatment methods commonly used to remove suspended solids in water and wastewater by addition of coagulants. During coagulation, the coagulant neutralizes the electrical charges of the particles in water allowing the particles to come close together forming larger particles to be able to settle down. Enhanced coagulation using inorganic or polymeric coagulants has been applied for removal of micropollutants. Alum ($KAl(SO_4)_2 \cdot 12H_2O$) and ferric chloride ($FeCl_3$) are the most common employed coagulants (Lee et al., 2009). Coagulation and flocculation method has

shown a poor reduction (< 50 %) of several micropollutants compounds including pharmaceuticals (Adams et al., 2002; Ternes *et al*., 2002; Vieno et al., 2007).

According to Westerhoff et al. (2005) and Shon et al. (2006), only compounds that have a strong affinity for adsorbed EfOM (with log K_{OW} > 3) can be well removed in the effluent by coagulation and flocculation method. Carballa et al. (2005) reported that diclofenac with log Kow of 4.15 was significantly removed during coagulation-flocculation with efficiency of 70% while compounds with lower Kow values such as carbamazepine, diazepam and ibuprofen were poorly reduced up to the removal efficiencies of 25%. Alum coagulants have resulted in slightly better removal than ferric chloride coagulants (Table 5). Furthermore, treatment with $FeCl_3$ can remove 40% to 70% of BOD_5 and 30% to 60% of COD (Shon et al., 2006).

2.3.2. Adsorption

Adsorption is a physical and surface phenomenon by which organic molecules are attracted to the surface of adsorbent by intermolecular forces of attraction. According to Ternes et al. (2002), adsorption of micropollutants can be done on activated carbon by bed filtration (GAC) or by adding powdered activated carbon (PAC). Activated carbon can effectively remove the majority of the micropollutants. However, competition for adsorption by the organic matter present in MWWE and blockage of the pores of carbon can decrease the adsorption capacity of the target compounds (micropollutants) (Koh et al., 2008). When the adsorption capacity is exhausted, the activated carbon in the GAC bed is regenerated, which is an expensive process (Koh et al., 2008). In comparison, the adsorption process has a better removal of pharmaceuticals than coagulation and flocculation method (Table 5).

2.3.3. Membrane filtration

Membrane technology is a separation technique that uses selective permeable barriers with pore sized to allow the passage of water molecules, but small enough to retain a wide range of particulate and dissolved compounds depending on their nature. A membrane can be considered as a thin film interposed between two fluid phases. Selective permeation through the membrane is governed by the particle/molecular size, the chemical affinity of the particle molecule towards the membrane material and/or the mobility of the particle/molecule. Membrane filtration processes such as nanofiltration and ultrafiltration are able to remove micropollutants including pharmaceuticals in MWWE at effective level (Table 5). It does not produce byproducts or metabolites but operational and maintenance cost can limit their potential use (Petala et al., 2006).

Table 5: Removal (%) of pharmaceutical residues by tertiary treatments methods (Westerhoff et al., 2005 ; Snyder et al., 2007)

Compounds	Coagulation and & Flocculation		PAC adsorption	Membrane iltration	
	$FeCl_3$	Alum		NF	UF
Paracetamol	0	0	87	82	63
Carbamazepine	0	7	55	61	0
Diazepam	0	5	53	75	7
Diclofenac	0	0	64	74	50
Genfibrozil	2	20	0	15	0
Ibuprofen	0	0	48	78	30
Sulfamethoxazole	0	0	43	72	23
Trimethoprim	0	3	40	43	0

2.3.4. Rapid sand filtration

Rapid sand filtration is a technique generally used to remove flocs and organic particles. This system is compact; it has high flow rates (generally between 5 to 15 m/h) and requires a small land area. In rapid filtration, the contaminant particles are removed in or on the media, thus causing the filter to clog after a period. Clogged filters are cleaned by backwashing. This treatment has a poor removal of pharmaceuticals in wastewater effluent (only less than 10 %). It has been reported that bezafibrate is the only compound that shows a slightly high removal (> 10%) than others (Vieno et al., 2007).

2.3.5. Advanced oxidation processes (AOPs)

During the last three decades, the potential of advanced chemical oxidation to remove organic micropollutants in wastewater effluents has been widely recognized (Buffle et al., 2006; Benitez et al., 2009). Advanced oxidation processes (AOPs) refer to a set of chemical treatment measures used to remove organic materials in water and wastewater by oxidation through reactions with hydroxyl radicals ($HO^{\bullet}$). They are capable of destroying completely the target compound but it is not necessarily accompanied by total mineralization. In several cases, degradation byproducts are more easily biodegradable and less toxic than the original compounds, thus implying that a biological post- treatment may be feasible.

The major AOPs include fenton's reaction, ozonation, heterogeneous and homogeneous photocatalysis which are based on near ultraviolet (UV) or solar visible irradiation, electrolysis, ultra-sound and wet air oxidation (Klavarioti et al., 2009) . These processes have shown a considerable potential in the treatment of a number of recalcitrant organic pollutants including pharmaceutical residues. AOPs are considered as a highly competitive water treatment technology for removal of organic pollutants not treatable by conventional techniques due to their high chemical stability or low biodegradability.

Various studies have been done to investigate the performance of AOPs to remove pharmaceutical compounds in aquatic environment (see Table 6). In addition, these

technologies are able to reduce the COD concentration at significant level. Several authors reported different values of COD depending on the technology used); Paraskeva and Graham (2005) reported 34% reduction of COD by ozonation of secondary wastewater effluent. 65 % COD removal was reported by Rosal et al., (2009) using O_3/H_2O_2 technology. In this work particularly, typical ozonation process is applied as a tertiary treatment in order to eliminate to large extent such persistent organic pollutants.

Fenton oxidation

Fenton oxidation is based on the principle that the ferric ions react with hydrogen peroxide through a free radical chain reaction to produce hydroxyl radicals.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + HO\cdot$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + H^+ + HOO\cdot$$

This method may easily be handled and operated to remove pharmaceutical residues in surface water and wastewater effluents as well (Klariovati et al., 2009). However, two potential drawbacks were reported such as narrow pH range of operation and the need of further treatment to recover the ions dissolved during operation. Additionally, a combination method of fenton oxidation with UV radiation provided a complete removal of compound such as amoxicillin in spiked effluent and diclofenac in fresh water (Table 6)

Photolysis

The basic principle of photolysis is the interaction of natural or artificial radiation at 254 nm with the target byproducts (Pereira et al., 2007).

UV/H_2O_2

In combination with hydrogen peroxide the removal efficiency of this method should be improved (Klavarioti et al., 2009).

$$H_2O_2 + UV \longrightarrow 2\ HO^{\bullet}$$

$$HO^{\bullet} + H_2O_2 \longrightarrow HO^{\bullet} + H_2O$$

$$HO_2^{\bullet} + HO_2^{\bullet} \longrightarrow H_2O_2 + O_2$$

It has been shown that the pharmaceutical compounds are poorly eliminated by UV radiation alone in wastewater effluent. Therefore, a combination of the UV radiation together with hydrogen peroxide (UV/H_2O_2) was used in order to improve the removal efficiency of those compounds. Adams et al. (2002) and Pereira et al. (2007) reported a removal of greater than 90 % of carbamazepine (Table 6) by this technology.

TiO_2/UV photocatalysis

This method is heterogenous reaction involving TiO_2 as a photocatalyst (Klariovati et al., 2009). The catalyst is cheap and operation conditions favor a good reaction of target molecule with produced irradiation electrons

$TiO_2 + hw \longrightarrow TiO_2 (e- + h^+)$

$h^+ + OH^- \longrightarrow HO^\bullet$

$e^- + O_2 \longrightarrow O_2$

Electrochemical oxidation

Electrochemical oxidation uses several Ti-based alloys, Pt, PbO_2, TiO_2 and graphite anodes. Two mechanisms are involved such as direct anodic oxidation in which the target pollutant is degraded by anodic electron transfer reaction. Indirect oxidation mechanism involves the liquid bulk solution in which oxidants are formed (Klariovati et al., 2009).

Ultrasound irradiation (sonolysis)

Ultrasound method is based on the induction of sonochemical reaction upon high intensity acoustic irradiation of liquids at frequencies of 20-1000 kHz (Klariovati et al., 2009). However, it is a new technology in water and wastewater treatment, and has been paid less attention in this field.

Sub-critical wet air oxidation (WAO)

The technique involves a thermochemical reaction where hydroxyl radicals and active oxygen species are formed at high temperatures and pressures (Klariovati et al., 2009). It is not economically feasible method due to higher energy requirement.

Ozonation

Ozone is recognized as a powerful oxidizing agent (E° = 2.07 V) (Alvares et al., 2001), which is able to participate in a high number of reactions in wastewater treatments with organic and inorganic compounds. Among the most common oxidizing agents, ozone is only exceeded in oxidation power by fluorine, hydroxyl radicals and atomic oxygen (Bahr et al., 2005).

$$\mathbf{O_2 + ½\ O_2 \longrightarrow 2\ O_3}$$

Ozone decomposes spontaneously during water treatment by a complex mechanism. It can oxidize and transform a substrate in direct or indirect pathways. In direct pathway, the ozone reacts directly with substrate forming products while in indirect pathway, ozone reacts with hydroxide ions (^-OH) or radicals ($R^\bullet$) and decomposes generating oxidants such as hydroxyl radicals ($HO^\bullet$) which then react with the substrate. Due to this strong oxidative properties of ozone and hydroxyl radicals formed, ozonation was found to break down efficiently several

micropollutants including pharmaceuticals found in wastewater effluent at different ozone doses (Nakada et al., 2007; Hollender et al., 2009; Klavarioti et al., 2009; Reungoat et al., 2010; Reungoat et al., 2012; Margot et al., 2013; Michael et al., 2013).

The removal efficiency during ozonation depends on compound reactivity with ozone radical or hydroxide radical ($HO^{\bullet}$) but also with operation conditions such as ozone doses, contact time and the quality of wastewater. Therefore a pharmaceutical compounds should be distinguished in groups at 3 different levels. The first group consists of the compounds with high ozone reactivity (> 90% removal, see Figure 5) consisting of substances which contain electron-rich moieties such as amines, anilines, aromatic systems and double bond structures which can highly react with ozone due to their kinetic properties with second order rate constant ko_3 greater than 10^4 $M^{-1}s^1$ (Hollender et al., 2009; Zimmermann et al., 2011). For e.g. compounds such as carbamazepine, clarithromycin, clindamycin, diclofenac, sulfamethoxazole and trimethoprim belong in mentioned above group.

The second group consists of substances with low ozone but high radical $HO^{\bullet}$ reactivity. Compounds such as ibuprofen, ketoprofen and metronidazole whose the second order rate is lower than 350 $M^{-1}s^{-1}$ and strongly react with unselective $HO^{\bullet}$ radicals with removals of around 60 % with residence time of 5 min (Rosal et al., 2010; Zimmermann et al., 2011). However, a complete degradation of ibuprofen after 20 minutes (Table 6) was reported by Zwiener & Frimmel. (2000) and Vieno et al., (2007).

The third group consists of compounds of low reactivity with either ozone or $OH^{\bullet}$ radicals. According to Hollender et al. (2009), compounds such as atrazine and iopromide have shown low removals, 49% and 62% respectively. This poor degradation was due to low reactivity of these chemicals with ozone and $HO^{\bullet}$ radicals (Figure 5).

However, the higher ozone doses may lead to the formation of potentially harmful byproducts such as N-nitrosodimethylamine (NDMA) and bromate compounds that can be a high risk to aquatic life (Hernando et al., 2006; Hollender et al., 2009 ; Margot et al., 2013). In addition, the high ozone oxidant might be expensive and high maintenance demanding. Many researches proposed a combination of ozone together with other AOP's in order to improve the higher removal efficiency of these techniques.

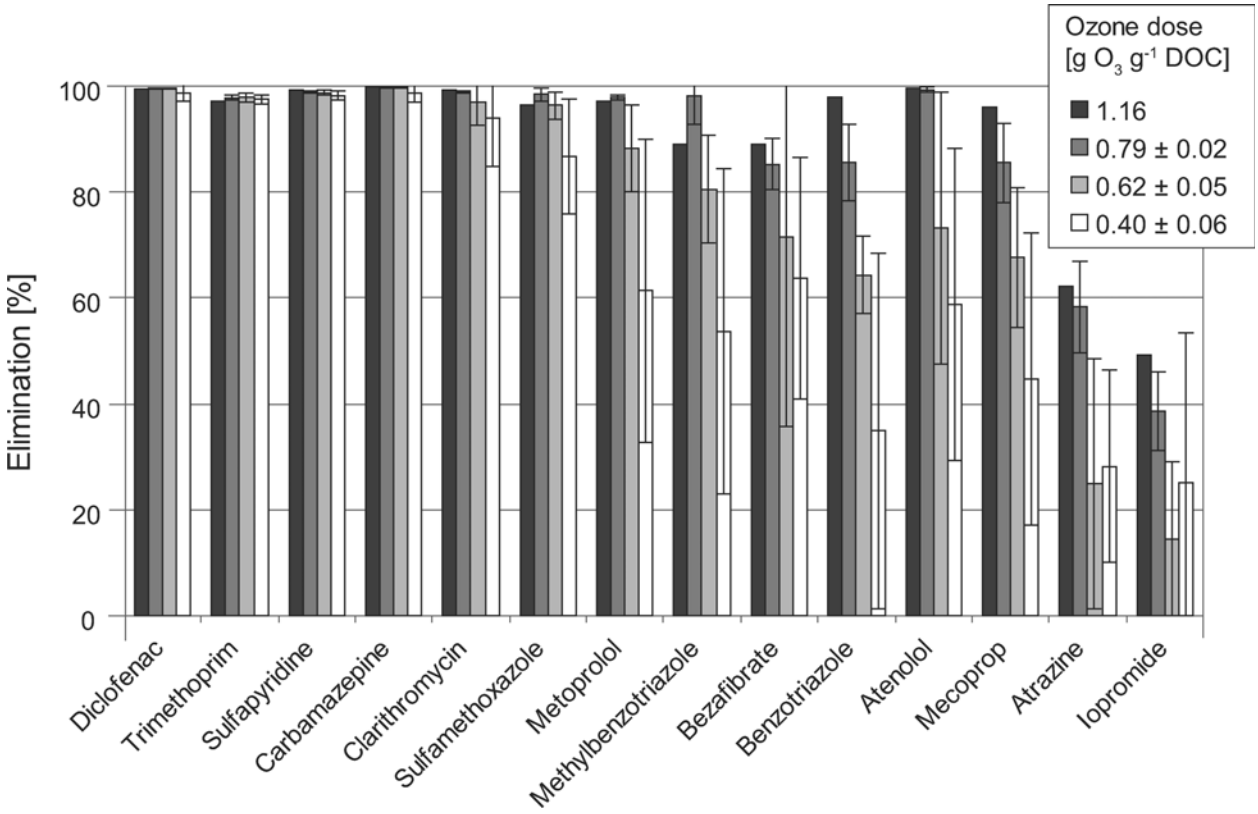


Figure 5: Effect of ozone dose on different pharmaceutical compounds (Hollender et al., 2009)

Table 6: Example of pharmaceuticals treated by AOPs in aquatic environment.

Name of compound	Therapeutic class	Water matrix	Type of treatment	Operating conditions	Removal (%) & remarks
Amoxicillin[g]	Antibiotics	CAS effluent	Photo-fenton	Black light at 365 nm; $[H_2O_2]$ = 2 Mm; pH= 2.5 [Ferrioxalate] = 0.20 mM	89 in 1 min
Azithromycin[c]	Antibiotics	Spiked effluent	Ozonation	0.5-5mg/l O_3; pH (7)	90-99 at O3 dose > 2mg/l
Carbamazepine[d,i]	Analgesics	Drinking water;	Ozonation	0.3 mg/L O_3; pH = 7.5; Tr = 0-1.5 min	95
		Surface water	UV/H_2O_2	10 mg/L H_2O_2/UV (200-300 nm)	90
Clarithromycin[c]	Antibiotics	Spiked effluent	Ozonation	5-5mg/L O_3; pH = 7	90-99 at O_3 dose > 2mg/L
Clofibric acid[a]	Analgesic	Drinking water	O_3/H_2O_2 (2:1)	5 mg/L O_3; Tr = 10 min	98
Diclofenac[a,c,h]	Analgesic	Drinking water	O_3/H_2O_2 (2:1)	5 mg/L O_3, Tr = 10 min	Complete in 100 min
		Fresh water	Photo-fenton	Fe^{2+}/H_2O_2/sunlight; pH = 7.2	Complete in 100 min
		CAS effluent	ozonation	0.5-5mg/L O_3; pH = 7	90-99% at ozone dose of > 2 mg/L

Ibuprofen[a,j]	Analgesic	drinking water	O_3/H_2O_2 (2:1)	5 mg/L O_3, Tr = 10 min	99
		surface river	Ozonation	1-1.3 mg/L O_3; pH = 7.5	complete after 20 min
Sulfadiazine[e]	antibiotic	Spiked effluent	Ozonation	0.5-5mg/L O_3, pH= 7	90-99
Sulfamethoxazole[b,e]	antibiotic	Spiked WWTP and DWTP effulent	Ozonation	0.5-5mg/L O_3, pH= 7	92
sulfapyridine[e]	antibiotic	Spiked effluent	Ozonation	0.5-5mg/L O_3, pH= 7	90-99
Tetracycline[f]	antibiotic	Spiked effluent	Photo- fenton	Solar irradiation; pH= 2.5 $[H_2O_2]$ = 1- 10 mM [Ferrioxalate]= 0.20 Mm	complete after 1 min
Trimethoprim[e]	antibiotic	Spiked effluent	Ozonation	5-15 mg/L O_3	85

[a]Zwiener and Frimmel (2000)
[b]Trovó et al. (2009)
[c]Huber et al. (2005)
[d]Adams et al. (2002)
[e]Ternes et al. (2003)
[f]Bautitz and Nogueira (2007)
[g]Trovó et al. (2008)
[h]Perez-Estrada et al. 2005
[i]Pereira et al. (2007)
[j]Vieno et al. (2007)

2.4. Post-ozonation treatment techniques

During ozonation treatment, reactive byproducts might be formed but also persistent substances against oxidation treatment may occur such as atenolol and benzotriazole (Hollender et al., 2009). Consequently, additional treatment should be considered after advanced treatment of WWTP effluent in order to reduce the discharge load of micropollutants into sensitive receiving waters. It is in that way a post-treatment implying biological and physical-chemical processes has been developed as a suitable technique that could be used to obtain an effective removal of EfOM and trace compounds such as pharmaceuticals. Biological process implies the growth of microorganisms on bed surface media filters forming layers called biofilm. Biofilm growing in biologically active filters are diverse microbial communities; they feed on complex material present in wastewater and transform it into simpler materials. On the other hand, physical-chemical process including sorption mechanism involves the attachment of organic pollutants on the filter surface and then be removed through the absorption or adsorption phenomenon from the filter media surface. Post-ozonation treatment techniques include biological sand filtration (slow sand filtration), biological/physical-chemical granular activated carbon, biological trickling filters and constructed wetlands.

2.4.1 Biological slow sand filtration

Biological slow sand filtration is a technique used for water treatment using a complex biological film that grows naturally on the surface of the sand. Water percolates slowly through the sand with a grain size of 0.15 to 0.30 mm in general at a rate of 0.1 to 0.3 m/h (Grunenfelder et al., 2003). This technique can be used as as a post-ozonation treatment to remove possible byproducts (eg. NDMA) after ozonation (Hollender et al., 2009). It consists of different components such as the housing (tanks), the supernatant, the schmutzdecke which is a biological active layer formed on the surface of slow sand filter, the filter sand and the under-drain medium. According to Hollender et al. (2009), the biological sand filter following ozonation has shown the additional removal capacity range between 15% and 20% for several compounds (diclofenac, atenolol, naproxen, trimethoprim). Naproxen exhibited additional removal of 30 % after ozonation. These findings were latter confirmed by Margot et al. (2013) reporting a slight increase of average removal improvement from 73 % with ozone alone to 76% by slow sand filtration after ozonation for several pharmaceuticals. However, the research on slow sand filtration after ozonation process is still in shortage to have a clear idea on its removal performance.

2.4.2 Biological granular activated carbon

The high competition of organic matter for adsorption and blockage of pores of activated carbon can decrease the adsorption capacity of micropollutants and requires regular regeneration (Koh et al., 2008). Biological processes offer a potentially useful alternative because frequent regeneration of the media is not required and biodegradable dissolved organic carbon (BDOC) is preferentially removed. According to Reungoat et al. (2012) and Rattier et al. (2012), biodegradation and adsorption are complementary mechanisms that offer the extended life of the granular activated carbon and delay organic breakthrough. Activated carbon is also a commonly used media to support biological activity but has received minor

attention so far for the advanced treatment of wastewater although the fact that it is potentially more efficient than biological sand filtration (Gerrity et al., 2011). Reungoat et al. (2012) reported a DOC removal variation of 20% to 50% of the DOC depending on the plant configuration within different treatment plants investigated. This was likely due to biodegradation of organic matter. Filtration process with activated carbon after ozonation has been able to reduce the concentrations of several compounds including diclofenac, sulfamethoxazole, trimethoprim, propranolol, naproxen and carbamazepine over 90%, and reduces non-specific toxicity and oestrogenecity by 70% and 95% respectively (Reungoat et al., 2010). The potential of BAC has been reported over slow sand filtration in removal of DOC (35 to 60%), pharmaceuticals (> 90%) and toxicity (28 to 68%) even without pre-ozonation due to its simultaneous functional mechanisms: adsorption and biodegradation (Reungoat et al., 2011).

2.4.3 Trickling filters

Trickling filter is a simple technique designed in cylinder with open lava rocks media filters. Water percolates through a column filled with a carrier matrix at slow rates to allow microbial growth on the surface creating a layer of film. This technique is generally applied as a conventional wastewater treatment and it is considered to be less effective in removal of DOC and micropollutants. Based on the author's knowledge, there is no study that has been published specifically on treatment wastewater effluents by trickling filters for pharmaceutical compounds.

2.4.4 Constructed wetlands

Various technologies have been employed to reduce the organic pollutants from wastewater effluent before the discharge into receiving waters. However, the potential of low cost technologies like constructed wetlands has only been partially considered. Constructed wetlands are artificial wetlands built as a new or restored habitat after ecological disturbance of natural appearance. Constructed wetlands (CW) can be classified as surface flow (SF) or subsurface flow (SSF) wetlands. They have been introduced as an alternative to wastewater treatment for micropollutants removal (Matamoros and Bayona, 2006). The removal principle of this technique is based on microbiological degradation of biodegradable compounds in anoxic conditions. Wastewater effluents with pharmaceuticals substances have been investigated, using constructed wetlands. Matamoros et al. (2008) noted a wide range of removal behaviors among different substances; for example, in SFCW, a removal efficiency of ibuprofen and ketoprofen substances was higher than 95%. However this was different for compounds carbamazepine and clofibric acid which seem to be persistent with removal efficiency of lower than 50%. However, research on the fate and behavior of organic micropollutants in constructed wetlands is still limited.

2.5. Comparison of post-treatment techniques

From a practical point of view, it is necessary to study the process integration to maximize the treatment performance in removing organic micropollutants and for degradation of organic matter. However, economic feasibility in terms of capital and operational cost should

be considered in the framework of application. Table 7 describes the estimated costs for different post-tretatment techniques.

Table 7: Cost-estimation of post-treatment techniques

Treatment technique	Capacity (m^3/d)	Capital cost (€ millions)	Treatment cost (€) /m^3	Reference
BGAC	64345	49	0.23	http://www.usbr.gov/pmts/water/publications/reportpdfs/Appendix%20C.pdf
	128690	59	0.16	"
	193035	79	0.15	"
CW	-	233877 (SSF)	-	EPA, 2000
SSF	small	0.28	-	http://www.ces.uoguelph./water/pathogenSlowSand.pdf
	medium	0.56	-	"
	large	0.64	-	"
TF	378500	63,4	-	EPA, 2000

(-): not provided

CHAPTER THREE

SCOPE OF THE STUDY

The presence of micropollutants in wastewater treatment effluent has been shown to impose a risk to the aquatic life when they are discharged to the aquatic systems (rivers, lake, etc.). Based on the literature review, conventional treatment is not capable to eliminate many of these compounds at significant level.

Considerable advances in the ozone technology and the experience gained by its treatment of water and wastewater have led to a huge boost in the research related to ozone treatment of secondary treated municipal wastewater in recent years. Based on this consideration, a number of objectives have been elaborated for this study:

The **general objective** of this study was to investigate the effect of ozone on post-treatment methods such as trickling filtration, slow sand filtration and enhanced biologically granular activated carbon adsorption by comparing the difference in treatment of an ozonated and non-ozonated MWWE stream. More **specific**, the objectives could be described as follows:

The first objective was to evaluate the effect of ozone on the degradation of dissolved organic matter and micropollutants with the purpose to assess the potential influence of ozonation to enhance the capacity of post-treatment for removal of poorly degradable compounds in conventional biological treatment.

The second objective was to examine the individual performance of different post-treatment techniques for the removal of dissolved organic matter and micropollutants based on the difference between ozonated and non-ozonated effluent.

The study of micropollutants in MWWE is a rather new topic in the research field nowadays. Therefore, the third objective was to get insight on which pharmaceutical residues could be found in the MWWE of Harelbeke.

Lastly, the fourth objective was to compare the removal efficiency of used techniques in post-treatment in terms of COD and BOD_5, biodegradability, UV254 absorbance, and pharmaceutical residues.

CHAPTER FOUR

MATERIALS AND METHODS

4.1 Sampling area and sample preparation of MWWE

Secondary treated wastewater effluent was collected from the municipal wastewater treatment plant in Harelbeke, Belgium, operated by Aquafin NV. It has a treatment capacity of 116,100 population equivalents. The wastewater is treated by a conventional activated sludge process with an average sludge residence time of 21 days. The plant is equipped with screen filtration, sand and oil trap, primary settling, nitrification and denitrification and secondary clarification. The sampling was done on a fixed base once a week within a period of four months (February to May 2014). Each time, a volume of around 100 liters was collected in previously cleaned (with demineralized water) and rinsed (with effluent) polyethylene containers, transported to the laboratory and stored at 4 °C as soon as possible. Before further analysis, the collected samples were filtered through a rapid sand filter of 0.60 m height with a diameter of 0.065 m containing sand with a grain size of 0.4-0.8 mm. The effluent was fed with a peristaltic pump at 100 rpm resulting in a filtration rate of 5.4 m/h.

4.2 Experimental plan and procedure

In the beginning of this study, an experimental plan was made to look for the possibilities in respect with the removal of organic micropollutants and dissolved organic matter in order to improve the secondary wastewater effluent quality from the WWTP. Two technical processes were applied. The first process was the ozone treatment of secondary biological treated effluent. Thereafter, a biological filtration process was practiced to evaluate the difference between ozonated and non-ozonated effluent for the post-treatment technique.

The entire study period was planned for four months (February-May 2014). During this study, the effluent was treated without spiking of micropollutants (see section 4.3) for the first six weeks, and then subsequently the effluent was spiked with 10 µg/L of micropollutants until the end of the study. The purpose of spiking was to compare both effluents, spiked and unspiked, in order to ensure the presence of adequate micropollutants in the studied effluent.

Sampling for analytical measurements was planned to be done every 3 days (Tuesday, Thursday and Friday) in a week for physical parameters (pH, conductivity and redox potential) and 4 days (Monday, Tuesday, Thursday and Friday) for UV254 measurement. BOD_5 samples were taken up once in a week (every Thursday) whereas the samples for the rest of chemical parameters (COD, NO_3-N, NH_3-N and ortho-PO_4^{3-}) analysis were collected every 2 weeks (on Thursday). The sampling for micropollutants analysis was planned on the 6^{th}, 10^{th} and 12^{th} weeks..

4.2.1 Ozonation of MWWE

Filtered effluent was transferred in a cylindrical batch reactor made of glass containing 11 L of the effluent and approxiametly 4 L of headspace (Figure 6) whereas the fittings and tubings were made of Teflon (Audenaert et al., 2013). During the experiment, a magnetic

stirrer and a circulation pump were used for proper mixing of the effluent in the reactor. A constant gas flow rate of 600 L min^{-1} was maintained through the process. Figure 6 provides a description of the operating reactor set-up. The working conditions during the entire process were at room temperature and a natural occurring pH.

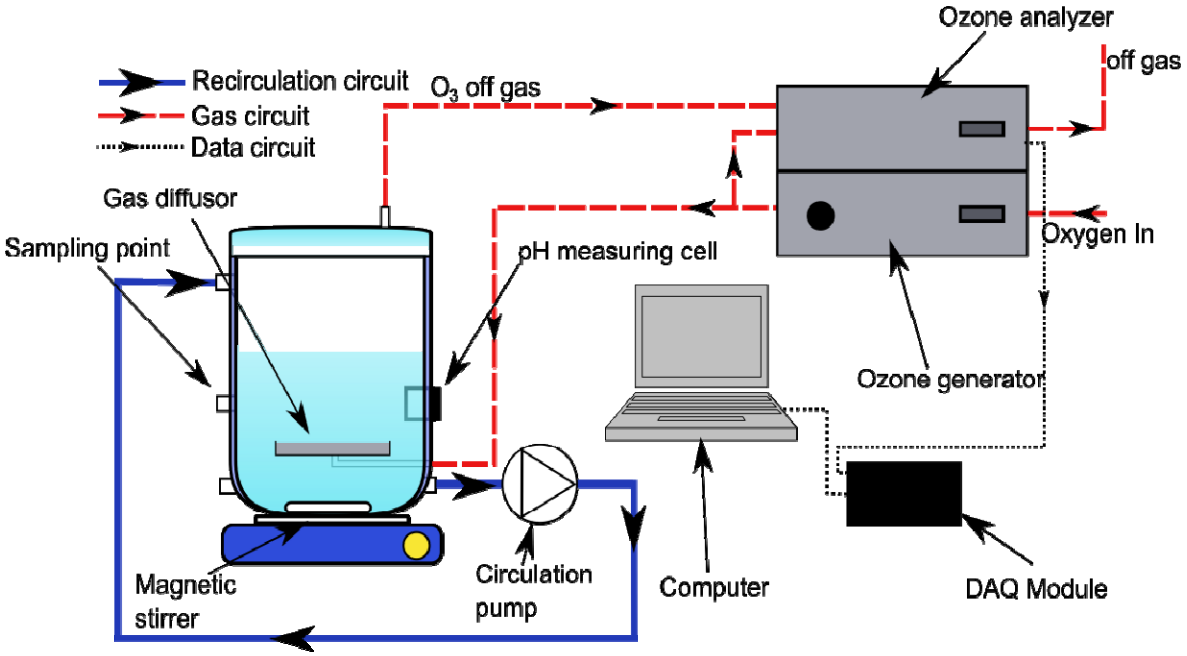


Figure 6: Ozonation experimental set-up diagram (Audenaert et al., 2013)

Every week, 44 L of the effluent was successively ozonated in four subsequent batches. A continuous inflow of oxygen and ozone mixture was provided. The ozone was generated from pure oxygen by a corona discharge ozone generator (Ozomat COM-AD-02, Anseros). The inlet gaseous ozone concentration was set at 8.0 ± 0.1 mg/L for all experiments (calculated based on all gas measurements during the experimental period). During ozonation, the ozone gas was bubbled through a ceramic porous diffuser at the bottom of the reactor. The inlet ozone gas concentration was measured for 5 to 10 minutes, after which the off-gas was measured during the reaction time (20 minutes) using a GM-OEM Anseros gas analyzer based on UV measurements. The gaseous ozone concentration was logged every second and monitored/stored on a computer.

4.2.2 Post-treatment process

Post-treatment of the effluent was conducted through biological and physical-chemical filtration using three different techniques/types of filtration media. A trickling filter (TF) was made of lava rock stones, the slow sand filter (SSF) contained porous fine sand, and a (biological) granular activated carbon (BGAC) filter was used as a third post-treatment technique during this study. Six columns representing biological systems were employed, i.e two replicates for each technique. One replicate received only filtered water (TF, SSF or

BGAC), the other one received both filtered and ozonated effluent TF-O_3, SSF-O_3 or BGAC-O_3) as described in Figure 7.

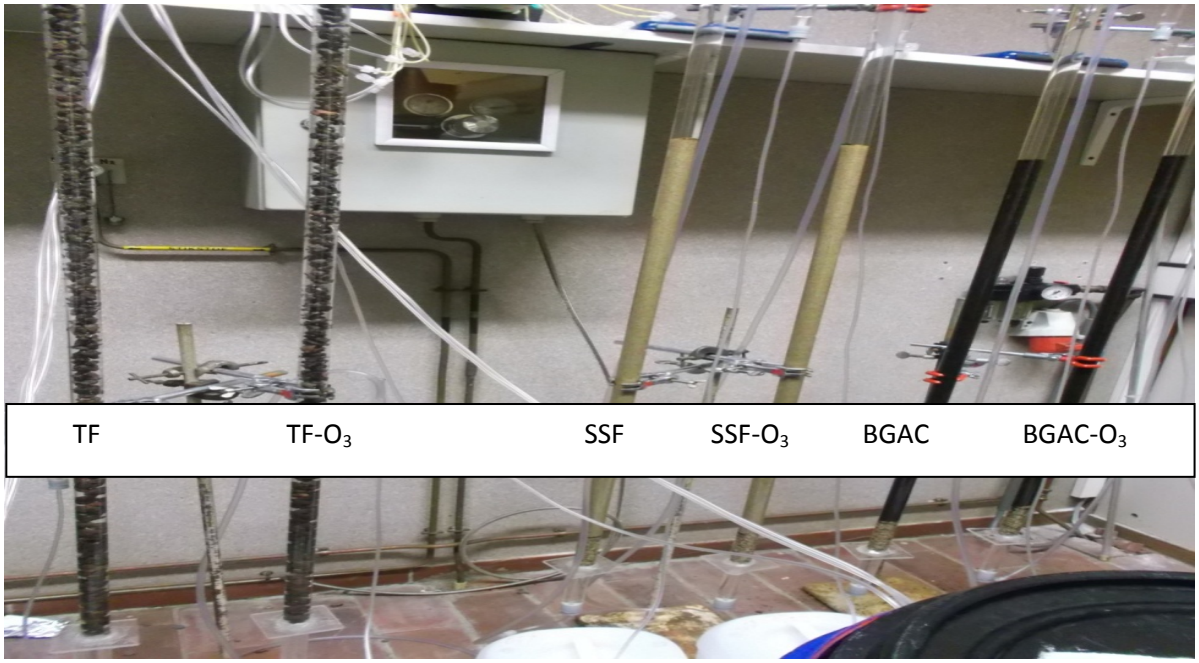


Figure 7: Post-treatment set-up

Depending on the technique, different design parameters were utilized (Table 8) such as the level of effluent to be filtered in the column, the height and diameter of filter materials. The flow rate of the effluent in each column was also characterized.

Table 8: Design parameters of the post-treatment set-up

Design parameter	TF & TF-O3	SSF& SSF-O3	BGAC & BGAC-O3
Filter height (m)	1.0	1.0	1.0
Submerged filter material (m)	0.50	n/a	n/a
Filter material diameter (mm)	not specified	0.6 (average)	1.1 (average)
Water level (m)	n/a	0.4	0.4
Gravel stones (m)	n/a	0.1	0.1
Flow rate (m/h)	0.11	0.11	0.2 9
Bed volume (Bv/d)	5.1	2.5	7.1

n/a: not applicable

The different height of the submerged material for the trickling filters, and the height of the gravel stones which were used to support the sand materials and activated carbon for the slow sand filters and (biological) activated carbon filters were indicated (Table 8). The quantity of water through the volume of filter materials in one day expressed in bed volumes per day was determined as well.

Both ozonated and unozonated effluent was placed in clean containers. Then, water flowed into the column through the plastic tubes under a peristaltic pump pressure at 40 rpm to increase the speed of effluent in the tubes and columns. Then, the water was filtered by lava rock stones for trickling filters, sand granules in slow sand filters, and granular activated carbon for biological granular activate carbon filters techniques, respectively (Figure 8). Thereafter, the samples from the columns were collected at the bottom together with ones from ozonated and unozonated containers for further analytical measurements.

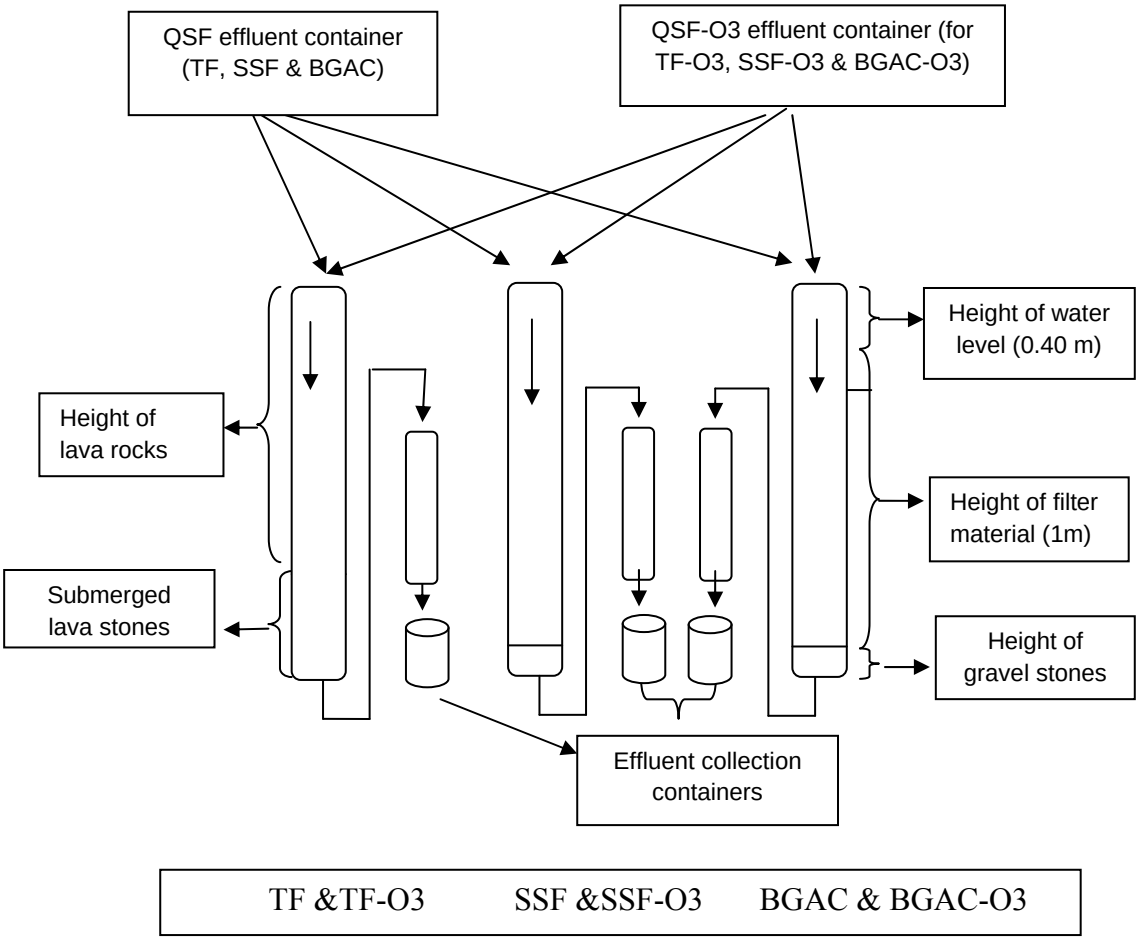


Figure 8: Schematic diagram of the water flow in a filter column during post-treatment

The effluent was collected in pre-cleaned (with demineralized water) 100 mL polyethylene bottles for measurement of physical parameters such as pH, conductivity and redox potential and UV254 nm absorbance. BOD_5 samples and the samples for the rest of chemical parameters (COD, NO_3-N, NH_3-N and ortho-PO_4^{3-}) were collected in pre-cleaned (with demineralized water) 500 mL bottles .

However, a special strategy for sampling micropollutants (pharmaceuticals) was adapted in order to avoid extra contamination and improve the accuracy of analysis. A filtered effluent sample of 200 mL (with 1 µm Whatmann glass fibre filter) was collected in a clean and dry glass bottle, pre-cleaned with 0.1 % ammonium hydroxide (NH_4OH), formic acid, demineralized water and methanol (MeOH). Then, 0.2 g of Na_2EDTA was added to the sample to complex any metal element that could be present. Afterwards, the pH of the sample was adjusted to 3.0±0.1 using 0.1 % formic acid and stored in a freezer at -20.0 °C to prevent any form of degradation that might occur.

4.2.3 Analysis of physical-chemical parameters

Physical-chemical parameters of the collected samples (raw secondary effluent, ozonated effluent and all treated samples in columns) were determined after the post-treatment process. The analyzed parameters include pH, electrical conductivity, and oxidation-reduction potential (ORP). They were analyzed through the Hach HQ 40d multi-meter every week. NO_3-N, NH_3-N, TN, ortho-PO_4^{3-} and COD were also measured with a Hach-Lange DR 2800 spectrophotometer two times a month. Additionally, the UV254 absorbance was measured every week using a Shimadzu 1600 UV-visible spectrophotometer from 200 to 800 nanometers with 0.5 mm increments to characterize the degradation behavior of DOM for treated samples. BOD_5 measurements were weekly recorded using an oxygen meter probe (mettle Toledo inpro 6860i) in triplicate measurement of a blank and all samples collected in in 250 mL glass bottles for each triplicate measurement. All bottles (27 bottles in total) were placed in a water bath operating in a dark room at 20 ^{0}C for a period of 5 days. The oxygen levels at days 0 and 5 were measured to see the difference (oxygen depletion) during 5 days period of measurement. The oxygen depletion in mg/L caused by biodegradation was calculated by substracting the average oxygen level of the blank from the average oxygen level of the samples.

4.3 Analysis of pharmaceutical micropollutants

During this study a set of 40 selected pharmaceuticals was analyzed qualitatively (screening) and/or quantitatively. Referring to the literature (Vergeynst et al. 2014 in press), the selection of pharmaceuticals based on a prioritization taking into account the environmental occurrence, fate, behavior and anticipated ecological effects. In the first measurement, on unspiked samples (week 6), analysis for all 40 substances was conducted. However, a limited number of substances (20 compounds in total) were detected (Table 9). Furthermore, the list of 20 pharmaceuticals detected in unspiked samples (week 6) was narrowed to 9 compounds (Table 10) based on the SPE efficiency, cost, water solubility and their relevance. They were considered as the target pharmaceuticals for spiking at 10 µg/L in the later phase of this research. Analysis of these spiked samples was done twice (on weeks 10 & 12). All samples that have been collected from the post-treatment filters together with ozonated and unozonated effluent were first extracted using a SPE extraction method. The chemicals as well as analytical standards of pharmaceuticals were of high analytical grade.

4.3.1 Solid-phase extraction (SPE)

One day before the SPE extraction, the samples were taken out of the freezer and kept in the fridge to defrost. The pH was adjusted to 7.0 ± 0.1 using 5 M NaOH and 10 % formic acid, then the samples were filtered through 0.45 µm Whatmann nylon filters under vacuum. The extraction was done under vacuum with via Oasis HLB (200 mg, 6 cc) cartridges, conditioned by 6 mL of methanol followed by 6 mL of HPLC water. The samples (100 mL) were subsequently loaded through the cartridges at a constant flow rate of 5 mL min^{-1}. Sample extraction was followed by a washing process using 6 mL of HPLC water after which the cartridge was dried for ± 5 minutes. Later, the analytes were eluted under vacuum with 5 mL of methanol. The eluted samples were then dried through evaporation with a gentle

nitrogen gas stream using Turbovap equipment. The dried samples were reconstituted in 1 mL 10:90 (v/v) MeOH/H_2O with 0.1 % formic acid, then vortexed for 20 seconds and centrifuged for 5 minutes at 3000 rpm. Finally, the reconstituted samples were transferred into 1 mL vials and stored at -10^{0}C for further analysis.

Table 9: List of detected pharmaceuticals during the first measurement (week 6)

Compounds	Formula	MW (g/mol)	Therapeutic class
Acyclovir	$C_8H_{11}N_5O_3$	225	Antivirals
Alprazolam	$C_{17}H_{13}ClN_4$	308	Antiepileptics
Amantadine	$C_{10}H_{17}N$	151	Antivirals
Amitriptyline	$C_{20}H_{23}N$	277	Antidepressants
Amoxicillin	$C_{16}H_{19}N_3O_5S$	365	Antibiotics
Carbamazepine	$C_{15}H_{12}N_2O$	236	Antiepileptics
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331	Antibiotics
Diazepam	$C_{16}H_{13}ClN_2O$	284	Antiepileptics
Diclofenac	$C_{14}H_{11}C_{l2}NO_2$	295	Analgesics and anti-inflammatory drugs
Flumequine	$C_{14}H_{12}FNO_3$	261	Antibiotics
Indomethacin	$C_{19}H_{16}ClNO_4$	357	Analgesics and anti-inflammatory drugs
Lamivudine	$C_8H_{11}N_3O_3S$	229	Antivirals
Levofloxacin	$C_{18}H_{20}FN_3O_4$	361	Antibiotics
Metronidazole	$C_6H_9N_3O_3$	171	Antibiotics
Nalidixic acid	$C_{12}H_{12}N_2O_3$	232	Antibiotics
Nevirapine	$C_{15}H_{14}N_4O$	266	Antivirals
Temazepam	$C_{16}H_{13}ClN_2O_2$	300	Antiepileptics
Tetracycline	$C_{22}H_{24}N_2O_8$	444	Antibiotics
Trimethoprim	$C_{14}H_{18}N_4O_3$	290	Antibiotics
Venlafaxine	$C17H_{27}NO_2$	277	Antidepressants

4.3.2 Analysis of the pharmaceutical target compounds

Both SPE extracts of the collected samples and analytical standards at concentrations 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1000 µg/l of all the 40 pharmaceuticals were injected into a HPLC system (Thermo Finnigan) equipped with a Phenomenex Luna C_{18} column (150 mm × 2.0 mm and 3 µm particle diameter) for chromatography analysis. The separation was done with a binary mobile phase gradient with a solvent A consisting of a mixture of MeOH + 0.1 % formic acid, and a solvent B composed of water + 0.1 % formic acid. For all analysis, the operating column temperature was set at 35^{0}C.

After chromatographic separation, mass spectrometry analysis of the target compounds was performed using an Orbitrap-based Q-Exactive^{TM} mass spectrometer (Thermo Scientific) operating at a high resolution power of 70000 after electrospray ionization (positive mode.). Extracted ion chromatograms were constructed with a width of 5 ppm around the theoretical

exact mass of the analytes and quantification was done using external calibration. In order to take into account both the extraction efficiency (SPE) and matrix effects (ion suppression or enhancement) in the MS instrument, the analytical process efficiency (PE) was determined through the analysis of both non-spiked and prespiked samples and was taken into consideration during analyte quantification.

Table 10: List of targeted pharmaceutical compounds

Compounds	Formula	MW (g/mol)	Therapeutic class
Amantadine	$C_{10}H_{17}N$	151	Antivirals
Amitriptyline	$C_{20}H_{23}N$	277	Antidepressants
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331	Antibiotics
Diclofenac	$C_{14}H_{11}C_{l2}NO_2$	295	Analgesics and anti-inflammatory drugs
Flumequine	$C_{14}H_{12}FNO_3$	261	Antibiotics
Levofloxacin	$C_{18}H_{20}FN_3O_4$	361	Antibiotics
Metronidazole	$C_6H_9N_3O_3$	171	Antibiotics
Trimethoprim	$C_{14}H_{18}N_4O_3$	290	Antibiotics
Venlafaxine	$C_{17}H_{27}NO_2$	277	Antidepressants

CHAPTER FIVE

RESULTS & DISCUSSION

5.1 Characterization of municipal wastewater effluent quality

5.1.1 Physical-chemical characteristics of the raw effluent

Physical-chemical parameters are known as important attributes of water quality assessment. Acquiring knowledge of those parameters can help to be aware of behavior for different components in the aqueous environment. The results obtained in this study are summarized in Table 11.

Table 11: Physical-chemical characterization of the raw effluent

Parameter	Units	Raw effluent	Effluent guidelines
pH	(-)	7.5-8.5	6.5-8.5 (WHO)
Ec	(µS/cm)	750-1315	-
Redox	(mV)	255-388	-
BOD_5	(mg/L)	0.8 ± 0.1 to 1.9 ± 0.3	25 (EU)
COD	(mg/L)	15.5 ± 0.4 to 27.8 ± 0.0	125 (EU)
NO_3- N	(mg/L)	1.3 ± 0.0 to 2.9 ± 0.2	50 (WHO)
NH_3-N	(mg/L)	BDL to 0.06 ± 0.00	0.5 (WHO)
TNb	(mg/l)	1.6 ± 0.0 to 13.4 ± 0.0	-
Ortho-PO_4^{3-}	(mg/L)	1.4 ± 0.1 to 2.6 ± 0.0	0.5 (WHO)
UV 254	(cm^{-1})	0.13-0.22	-

BDL: below detection limit

The pH values varied between 7.5-8.5. The reason for this variation was mainly explained by different effluent matrix due to the fact that the effluent was perhaps different in composition but also sampled at different time. Nevertheless, the obtained values were comparable to natural water systems (6.5-8.5, WHO guidelines) and could not be harmful to aquatic life.

The conductivity values varied significantly and ranged from 750 to 1315 µS/cm throughout the period of study. This showed that the effluent might contain higher concentrations of ions such as chlorides, surphates, calcium, magnesium etc. originating from industrial and households discharges. Surprisingly, higher conductivities (> 1000 µS/cm) were observed from the 5th week on. In consultance with meteorological data (not shown here), the reason for that sudden change could probably due to the increase in ambient temperature and

decrease of precipitations from week 5 through the entire experiment period. From the author's knowledge, the temperature, precipitations and the ionic concentration are the major factors that can affect the conductivity.

Redox potential is a measure of the cleanness and the ability of water to degrade pollutants. The redox potential values were 255 mV to 388 mV. This variation could be due to the change in temperature that could affect the effluent and the lower precipitations (from meteo data) during the experimental running time.

Discharges from MWWTPs still contain a certain amount of nutrients. When these are not in conformity with quality standards or guidelines they can cause eutrophication phenomena of receiving water bodies. In this study, the concentrations of the nutrients (NO_3-N, NH_3-N, TN & ortho-PO_4^{3-}) were determined. The variations of those parameters were generally low and complied with WHO standards for water quality guidelines (as seen in Table 11).

Biological oxygen demand (BOD_5) and chemical oxygen demand (COD) values were found in the range of 0.8 ± 0.1 mg/L to 1.9 ± 0.3 mg/L and 15.0 ± 0.4 to 27.8 ± 0.0 mg/L, respectively. This allowed us to notice that the BOD_5 and COD concentrations were much lower than the required EU guidelines values which are generally in order of 25 mg BOD_5/L and 125 mg COD/L, respectively.

UV absorbance was also measured as a representation for the organic matrix but also as an alternative to assess the removal of organic micropollutants at a wavelength of 254 nanometers (nm). It is a measure of the aromaticity of compounds having a structure consisting of aromatic rings or carbon–carbon double bonds. The UV reduction corresponds to the oxidation of micropollutants. The results showed a variation of 0.13 cm^{-1} to 0.22 cm^{-1} . These values proved the presence of organic matter content in the effluent.

5.1.2 Occurrence of micropollutants in the raw effluent

Micropollutants in aquatic systems are one of the major environmental concerns nowadays. It is not easy to remove them completely or achieving minimal ng/L levels of detection in MWWE. In this study, the effluent taken from Harelbeke was characterized in order to get knowledge about the present micropollutant compounds. The micropollutants analyzed were pharmaceutical compounds as described in chapter one. In unspiked effluent, a limited number of compounds were detected. Only 20 out of the 40 targeted substances were detected in the raw effluent as mentioned in section 4.3. Among the reasons that could explain this limitation include the process efficiency of extraction process or even of the analysis itself that probably inhibited the detection of some compounds which were expected to be identified in the effluent. Also there is a possibility that the chemicals were not present in the sample. The concentrations of detected pharmaceuticals together with their analytical process efficiencies are presented in Table 12. Concentrations of most of the compounds in the raw effluent were noticed in tens to hundreds of ng/L. This is similar to the ranges reported in previous studies (Miao et al., 2004; Gobel et al., 2005; Nakada et al., 2006; Vergeynst et al., 2014 in press). Diclofenac, carbamazepine, and venlafaxine showed the highest concentrations; 367 ng/L, 498 ng/L and 508 ng/L, respectively. These values were in

the same order of magnitude as the ones previously reported in the literature (Vieno et al., 2007; Al-Odaini et al., 2010; Rosal et al., 2010).

The occurrence of diclofenac and carbamazepine was not surprising since they are among the most used and detected drugs in the WWTP effluent (Rosal et al., 2010; Jelic´ et al., 2012). Acyclovir, ciprofloxacin, tetracycline and trimethoprim were detected in a concentrations range between 50 ng/L and 100 ng/L. The remaining compounds were identified below 50 ng/L. However, alprazolam, diazepam, flumequine, lamivudine, nalidixic acid and nevirapine were in lowest concentrations (< 10 ng/L). The low concentrations of these compounds in the raw effluent could be attributed to the high biodegradation rate and maybe sorption to the sludge in secondary treatment. Moreover, possible lower influent concentration could be the reason for these results. This was comparable to the findings of Verlicchi et al. (2012) who found a WWTP biodegradation of 96% for amitriptyline, and of Mascolo et al. (2010) for nalidixic acid (70%).

Table 12: Occurrence of pharmaceutical residuals in the raw effluent

Compounds	PE % (process efficiency)	Concentration (ng/L)	Typical concentrations from literature (Vergeynst et al. 2014 in press)
Acyclovir	11±2	84	-
Alprazolam	66±1	3	-
Amantadine	69±1	44	54 ± 5
Amitriptyline	43±6	34	-
Amoxicillin	48±5	21	
Carbamazepine	53±1	498	460 ± 32
Ciprofloxacin	51±3	74	120
Diazepam	64±2	2	-
Diclofenac	29±11	367	623 ± 59
Flumequine	49±1	6	-
Indomethacin	29±11	42	-
Lamivudine	81±6	2	-
Levofloxacin	66±3	30	-
Metronidazole	81±1	18	-
Nalidixic acid	56±1	5	-
Nevirapine	46±0	9	-
Temazepam	71±1	41	-
Tetracycline	26±3	52	-
Trimethoprim	71±0	65	-
Venlafaxine	65±1	508	208 ± 22

5.2 Ozonation of MWWE

5.2.1 Physical-chemical characteristics of ozonated effluent

Ozonation experiments were performed under batch operation in a glass reactor. Characteristics of the effluent after ozonation were determined. The physical-chemical

parameter variations of ozonated effluent are summarized in table 13. It was observed that the pH variation values were more alkaline after ozone oxidation than before. The increase of pH could be attributed to the reduced amount of carbon dioxide in the reactor. This showed important signification of ozone decomposition in $HO^{\bullet}$ radicals that could also react with pollutants as reported in literature (Nakada et al., 2007; Hollender et al., 2009; Zimmermann et al., 2011).

Redox potential values also increased slightly which was quite logical as the dissolved oxygen levels were increased in the effluent. BOD_5 variations slightly increased compared to ones of raw effluent due to possibly formation of more biodegradable products while the COD variations reduced due to the decrease of chemical pollutants in the effluent.

Table 13: Physical-chemical characterization of ozonated effluent

Parameter	Units	Unozonated effluent	Ozonated effluent
pH	(-)	7.5-8.5	7.9-8.4
Ec	(µS/cm)	750-1315	751-1301
Redox	(mV)	255-388	272-381
BOD_5	(mg/L)	0.8 ± 0.1 to 1. 9 ± 0.3	1.2 ± 0.1 to 2.8 ± 0.3
COD	(mg/L)	15.5 ± 0.4 to 27.8 ± 0.0	12.9 ± 0.8 to 22.4 ± 0.3
NO_3- N	(mg/L)	1.3 ± 0.0 to 2.9 ± 0.2	1.4 ± 0.0 to 4.6 ± 0.1
NH_3-N	(mg/L)	BDL to 0.06 ± 0.00	BDL to 0.50 ± 0.01
TNb	(mg/l)	1.6 ± 0.0 to 13.4 ± 0.0	2.5 ± 0.1 to 12.5 ± 0.1
Ortho-PO_4^{3-}	(mg/L)	1.4 ± 0.1 to 2.6 ± 0.0	1.4 ± 0.0 to 2.3 ± 0.0
UV 254	(cm^{-1})	0.13-0.22	0.07-0.15

BDL : Below detection limit

5.2.2 Effect of ozone on dissolved organic matter in the raw effluent

5.2.2.1 Effect on the COD removal

Ozone oxidation occurs either in a direct reaction with ozone molecule or through the indirect reaction of secondary oxidants such as hydroxyl radicals produced from ozone in aqueous medium. During ozonation of the raw effluent, the concentrations of COD were reduced in range of 15 mg/L-28 mg/ L to 12 mg/L -22 mg/L. It was noticed that the ozone oxidation provided low removal efficiencies ranged from 7% to 20%. Similarly, Rosal et al. (2009) reported the same results demonstrating that low COD concentrations (COD residuals) are difficult to treat in the effluent. The reason for that could be a low concentration of dissolved organic matter that was already detected in the raw effluent.

5.2.2.2 Effect of ozone on the biodegradability of raw effluent

Regardless of low BOD_5 and COD values, the ratio of those 2 parameters was calculated to assess the degree of biodegradability in ozonated effluent. The results of BOD_5/COD ratio variations were low (0.09-0.16) compared to normal characteristics of biodegradability ($BOD_5/COD \geq 2$) but they were higher than ones obtained in the raw effluent (0.05-0.09) due to the increase of BOD_5 but the decrease of COD in ozonated effluent. It was indicated that even though the BOD_5/COD ratios were low, the effluent was still containing biodegradable

materials that could easily be removed in the further treatment than before ozonation (see Figure 9). Several authors reported the same ranges of low BOD_5/COD ratios in oxidized effluent (Gonzalez et al., 2007; Melero et al., 2009)

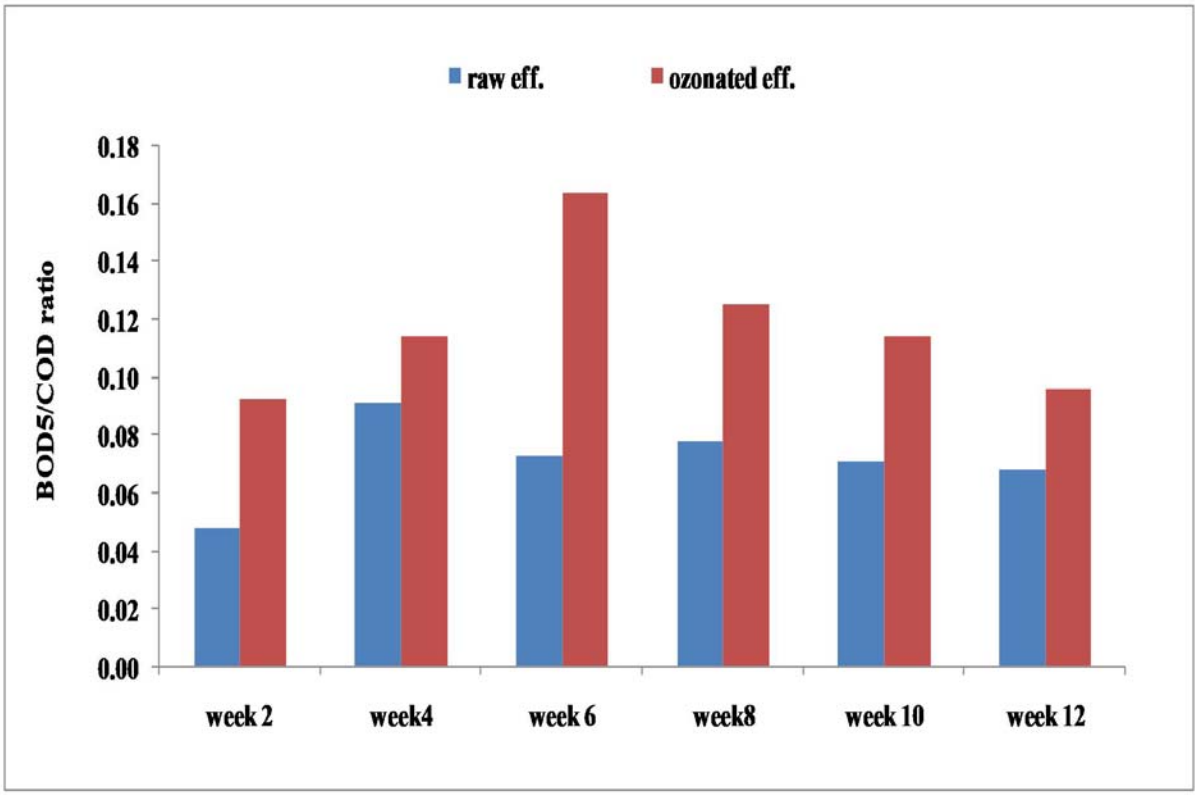


Figure 9: BOD_5/COD ratio increase in ozonated effluent

5.2.2.3. Effect of ozone on the UV 254 absorbance

The reduction of UV absorbance at 254 nm was also studied. The results showed a decrease in UV 254 variations from 0.13 cm^{-1}-0.22 cm^{-1} to 0.07 cm^{-1} to 0.15 cm^{-1}. This reduction could be attributed to the reaction of ozone with aromatic compounds or other compounds with double bond structure that could lead to the splitting of the double bonds thus dissociation of the rings as explained in the Criegee mechanism (Jiang et al., 2013). The highest reduction was observed at 51% (see Figure 10).

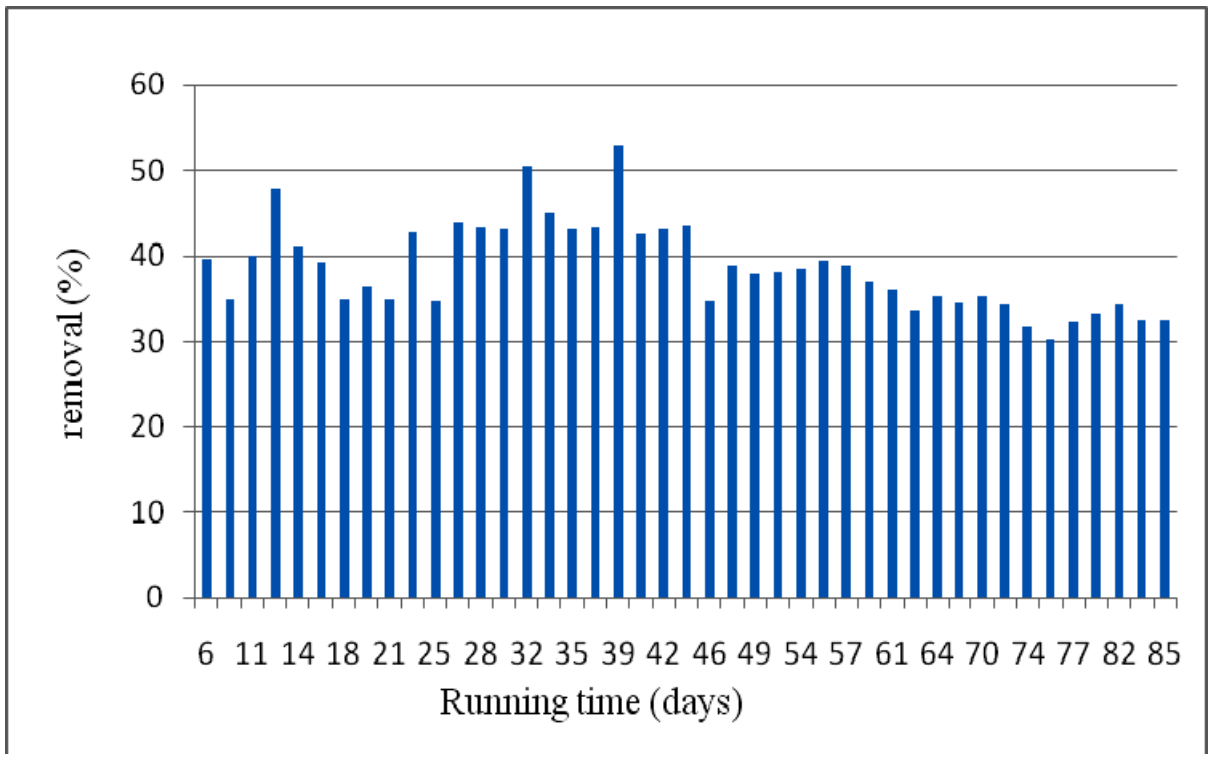


Figure 10: Effect of ozone on UV 254 absorbance

5.2.2.4 Effect of ozone on MWWE micropollutants removal

Since the WWTPs are not able to completely degrade all recalcitrant organic pollutants, advanced treatments have been introduced to overcome this problem. Ozone oxidation was used in this work due to its various advantages such as the high oxidation potential toward persistent compounds, quick reactivity, and also no remaining ozone residuals after ozonation. The results obtained showed that ozonation was effective to oxidize the majority of detected compounds in the raw effluent. Figure 11 provides the removal efficiencies of pharmaceuticals of unspiked effluent in the batch reactor.

The individual concentrations of all compounds were lower than 30 ng/L after ozonation. According to the literature, compounds with amino groups or aniline moieties as well as compounds with aromatic and double bonds are known be eliminated significantly during ozonation (Nakada et al., 2007; Vieno et al., 2007; Michael et al., 2013). The same results were obtained in this work where compounds including acyclovir, indomethacin, lamivudine, nevirapine, tetracycline, diclofenac, amoxicillin, and carbamazepine were reduced to below detection limit with excellent removal efficiencies (≈ 100%). These high efficiencies were most probably attributed to the high second order rate constants for the reaction of those compounds with ozone ($> 10^4$ $M^{-1}s^{-1}$) (Hollender et al., 2009; Zimmermann et al., 2011). The high removal of diclofenac, carbamazepine and indomethacin was also reported by Hollender et al. (2009); Rosal et al. (2010) and Reungoat et al. (2010).

Transformation of amitriptyline, levofloxacin, temazepam, trimethoprim and venlafaxine was in the range of 70% to 97% to below detection limit (see Fgure 11). The findings for

trimethoprim were in the same order of magnitude as reported by Ternes et al. (2003) in spiked STP effluent. Trimethoprim has been also reported as an easily oxidized compound (at 95%) from river water at an ozone concentration of 7.1 mg/L (Adams et al., 2002).

Amantadine, ciprofloxacin, diazepam and metronidazole were slightly removed at an efficiencies of 57-65%. The incomplete elimination of these compounds was possibly due to their low reactivity towards ozone (Rosal et al., 2010). The rate constant for metronidazole was found to be small during ozone oxidation ($ko_3 < 350\ M^{-1}s^{-1}$). However, according to Vieno et al. (2007), the insignificant removal of ciprofloxacin by ozone was not yet quite understood.

Alprazolam, flumequine and nalidixic acid were poorly reduced in this study with lower than 50% removal efficiencies. Surprisingly, nalidixic acid displayed a negative removal. De-conjugation phenomenon of the amide structure of nalidixic acid compound could be the reason for the negative value obtained. Negative removal efficiencies were also reported in other studies such as the one of Nakada et al. (2007).

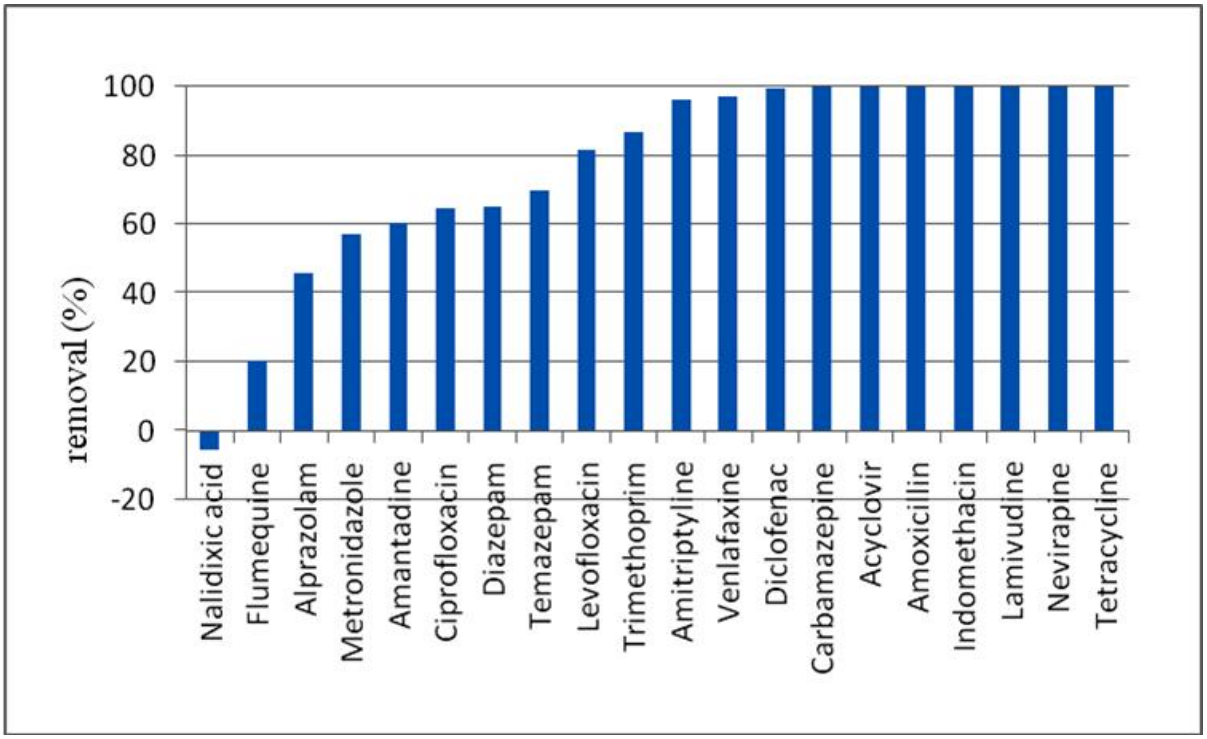


Figure 11: Pharmaceuticals removal efficiency during ozonation of unspiked MWWE

5.3 Post-treatment of municipal wastewater effluent

During ozonation, organic pollutants are probably not fully mineralized but rather partly degraded and transformed into byproduct compounds. These transformation products are expected to be more easily degradable than their parents. Consequently, an additional treatment step can be used as a polishing step or a post-treatment step. Post-treatment techniques applied during this work were trickling filter, slow sand filtration and biological granular activated carbon filtration as mentioned in section 4.2.2.

During the post-treatment processes, three different experiments were performed, one without micropollutants spiking and two with micropollutants spiking. This was done in order to see the difference regarding the pharmaceuticals behavior in MWWE. Raw effluent and ozonated effluent were considered as the influent in the post-treatment filter columns; the raw effluent as influent of filters receiving unozonated effluent, and ozonated effluent was considered as the influent of filters receiving ozonated water. Due to a couple of factors already mentioned in chapter 4.4, a set of 9 pharmaceuticals were considered for further analysis.

5.3.1 Trickling filter

5.3.1.1. Physical-chemical characteristics of the trickling filter effluent

The effluent from trickling filters, both unozonated and ozonated were characterized from physical-chemical point of view. The results are presented in Table 14. A statistical analysis based on T-test at 95% confidence interval showed a small difference between both effluents for.eg. (with p-value of 0.08 for pH measurement). The observed difference could explain a little improvement in physical-chemical parameters that was observed between two effluents but also in comparison with the raw effluent. pH variations ranged between 8.0 to 8.6 in unozonated effluent whereas in ozonated effluent, it was reported in the range of 8.2 and 8.8. This could be due probably to the more alkaline ozonated effluent compared to unozonated effluent. Trickling filter is generally used as a conventional treatment method in the same line as the activated sludge. Probably, this could be the reason of not too much difference that was observed between the effluent in trickling filter and the raw effluent.

Table 14: Physical-chemical characterization of the trickling filter effluent

Parameter	Units	Unozonated effluent	Ozonated effluent
pH	(-)	8.0- 8.6	8.2- 8.8
Ec	(µS/cm)	755-1297	754-1301
Redox	(mV)	280-391	281-386
BOD_5	(mg/L)	0.6 ± 0.1 to 2.3 ± 0.1	BDL to 1.8 ± 0.2
COD	(mg/L)	16.8 ± 0.4 to 25.5 ± 0.4	11.8 ± 1.6 to 18.4 ± 0.2
NO_3- N	(mg/L)	0.3 ± 0.0 to 1.9 ± 0.4	0.5 ± 0.0 to 3.0 ± 0.1
NH_3-N	(mg/L)	BDL to 0.05 ± 0.01	BDL to 0.03 ± 0.01
TNb	(mg/l)	BDL to 8.2 ± 0.0	0.7 ± 0.0 to 12.5 ± 0.2
PO_4^{3-}	(mg/L)	1.4 ± 0.0 to 2.1 ± 0.1	1.6 ± 0.1 to 2.1 ± 0.2
UV 254	(cm^{-1})	0.13-0.20	0.07-0.14

BDL: Below detection Limit

5.3.1.2. Removal of target pharmaceuticals in trickling filter

Unspiked effluent

Concentrations of target pharmaceuticals in trickling filter containing unspiked effluent did not show a significant difference from the raw effluent (see Table 15). Consequently, this implies poor removal efficiencies (< 50%) of chemicals from the filter except flumequine which exhibited a slight removal efficiency of 69%. Furthermore, the negative removals were obtained for amantadine, levofloxacin and metronidazole. The reason for negative removals seemed to be unknown but probably they could be due to the transformations and deconjugation processes in the influent and effluent which might enhance the increase of the concentrations of these compounds. Moreover, the measuring error that could occur during analysis could be the reason for the obtained negative removals.

Table 15: Concentrations of the target pharmaceuticals and their removal efficiencies of unspiked effluent in trickling filter

Compounds	Influent (ng/L)	Effluent (ng/L)	Removal (%)
Amantadine	41	42	-2
Amitriptyline	22	16	27
Ciprofloxacin	34	20	41
Diclofenac	1180	1053	11
Flumequine	16	5	69
Levofloxacin	33	35	-6
Metronidazole	11	14	-27
Trimethoprim	73	72	1
Venlafaxine	701	495	29

Spiked effluent

The results obtained for the spiked effluent in both performed measurements (on week 10 and 12) were relatively comparable. They are presented in Table 16. The removal efficiencies of the target pharmaceuticals was generally low in the trickling filter except for amitriptyline and metronidazole (61, 75% & 97, 99%). The removal of amitriptyline was most probably due to high biodegradability as confirmed by Kasprzyk-Horden et al. (2009) reporting a biodegradation removal of 99% for amitriptyline. Despite of the small log Kow and pKa (see Table 2); metronidazole was highly removed biologically in trickling filter. This was in conflict of what was expected. However, a similar situation was reported by K'oreje (2012) and Gros et al. (2009). The reason for this phenomenon is still unknown. Trimethoprim also has shown partial removal in the first measurement but great removal in the second one (43% and 99%, respectively). This variation was most likely due to the increased number of microorganisms during the second measurement that were maybe able to biodegrade the compound at significant level.

Table 16: Concentrations and removal of target pharmaceuticals in spiked unozonated influent and effluent of the trickling filter

Compound	Influent (ng/L)		Effluent (ng/L)		Removal (%)	
	Week 10	Week 12	Week 10	Week 12	Week 10	Week 12
Amantadine	8776	9620	6616	8190	25	15
Amitriptyline	3811	3616	1502	900	61	75
Ciprofloxacin	5383	5281	3872	3897	28	26
Diclofenac	12172	11616	12854	8677	-6	25
Flumequine	8403	9785	8119	9459	3	3
Levofloxacin	7668	8987	7411	8181	3	9
Metronidazole	7127	5238	215	42	97	99
Trimethoprim	6161	2297	3512	15	43	99
Venlafaxine	11266	12703	11301	11987	0	6

In comparison to the unspiked effluent, the spiked effluent in trickling filter showed slightly higher removal efficiencies for the targeted pharmaceuticals especially for amitriptyline, metronidazole and trimethoprim (75%, 99% and 99%, respectively). The difference that was observed between both effluents could be due to the fact that the pharmaceutical compounds in unspiked effluent had very low concentrations. However, this was expected as trickling filter is known to have a limited removal of micropollutants in wastewaters, although the research on pharmaceutical compounds removal in trickling filter used as a post-treatment technique is still scarce.

Effect of ozonation on the removal of pharmaceuticals in trickling filter

Biological filtration of ozonated effluent in trickling filter clearly improved the removal of pharmaceutical compounds studied in this work compared to the filtration of unozonation effluent (Table 17). More considerable removals were observed in the spiked effluent than in the unspiked effluent. In the spiked effluent, the majority of compounds were reasonably removed with removal efficiencies greater than 65%, except flumequine which showed a removal efficiency below 50% (Table 17). Also amantadine exhibited a lower elimination during the first measurement although it was slightly increased till 72% during the second measurement. The removal improvement could most probably be due to the reason that ozonated effluent contained more easily biodegradable components in comparison with unozonated effluent. Additionally, the increase of oxygen levels in ozonated effluent could more sustain the microorganisms' growth and thus enhance their biological degradation of the pharmaceuticals. On the other hand, the unspiked effluent exhibited a higher removal efficiency (> 60%) for certain compounds such as metronidazole, levofloxacin, trimethoprim and venlafaxine. The low removal efficiencies recorded in unspiked effluent was most probably due to the fact that the compounds were already removed to great extent during ozonation process which might lead to very low concentrations and the increase in analytical uncertainty.

Table 17: Effect of ozonation on the removal efficiency (%) of the target pharmaceuticals in trickling filters

	Unspiked effluent		Spiked effluent			
	Unozonated effluent	Ozonated effluent	Unozonated effluent		Ozonated effluent	
	Removal	Removal	Removal (%)		Removal (%)	
Compound	(%)	(%)	Week 10	Week 12	Week 10	Week 12
Amantadine	-2	-6	25	15	40	72
Amitriptyline	27	-1500	61	75	99	97
Ciprofloxacin	41	50	28	26	99	98
Diclofenac	11	-100	-6	25	100	100
Flumequine	69	-40	3	3	39	42
Levofloxacin	-6	65	3	9	100	99
Metronidazole	-27	55	97	99	74	79
Trimethoprim	1	80	43	99	99	100
Venlafaxine	29	86	0	6	86	74

5.3.2. Slow sand filter

5.3.2.1 Physical-chemical characterization of the slow sand filter

The results of physical-chemical parameters of both effluent, unonated and ozonated effluent treated in slow sand filter are presented in Table 18. Generally, there was not a huge difference in variation between both effluents but also in comparison with the raw effluent. pH variation values were in the same range of 8.1 and 8.6 for both effluent but slight higher than the raw effluent (7.5-8.5). Based on one tail T-test, a p value of 0.03 for pH was obtained which confirmed the similar values recorded for both effluent. It was observed that the pH in ozonated effluent was not affected by slow sand filtration. However, the significant change was observed on UV 254 reduction which exhibited a significant difference between both effluents (see Table 18) and also the raw effluent. This was probably due to high biodegradation rate of organic materials in the ozonated effluent. It was mainly because of the presence of more easily biodegradable organic compounds transformed during ozonation but also due to higher microorganisms' activity in slow sand filter effluent than in the raw effluent.

Table 18: Physical-chemical parameters characterization of effluent in slow sand filter

Parameter	Units	Unozonated effluent	ozonated effluent
pH	(-)	8.1-8.6	8.1-8.6
Ec	(µS/cm)	744-1298	716-1291
Redox	(mV)	291-374	287-373
BOD_5	(mg/L)	0.6 ± 0.0 to 1.8 ± 0.1	0.5 ± 0.3 to 2.1 ± 0.1
COD	(mg/L)	11.8 ± 1.2 to 22.9 ± 0.3	12.4 ± 0.2 to 19.6 ± 0.1
NO_3- N	(mg/L)	0.4 ± 0.0 to 3.1 ± 0.2	0.3 ± 0.1 to 4.2 ± 0.1
NH_3-N	(mg/L)	BDL to 0.16 ± 0.01	BDL to 0.11 ± 0.00
TNb	(mg/l)	BDL to 7.6 ± 0.4	BDL to 10.8 ± 0.1
PO_4^{3-}	(mg/L)	1.3 ± 0.1 to 2.1 ± 0.0	1.5 ± 0.0 to 2.0 ± 0.1
UV 254	(cm^{-1})	0.12-0.22	0.06-0.14

BDL: below detection limit

5.3.2.2. Removal of target pharmaceuticals removal in slow sand filter

Unspiked effluent

Pharmaceutical residuals in unspiked effluent were not significantly removed as was expected (Table 19). A limited number of compounds showed removal efficiencies greater than 50%. They included diclofenac, flumequine, levofloxacin and metronidazole. Only two compounds (ciprofloxacin and levofloxacin) were highly eliminated at removal efficiencies greater than 80%. However, the negative removals were observed for amantadine and trimethoprim due to probably to deconjugation and transformation of those compounds in the influent and the effluent. Moreover, the measuring error that could occur during analysis could be the reason for the obtained negative removals.

Table 19: Concentrations of target pharmaceuticals and their removal efficiencies of unspiked effluent in slow sand filter without ozonation.

Compound	Influent (ng/L)	Effluent (ng/L)	Removal (%)
Amantadine	41	43	-5
Amitriptyline	22	18	18
Ciprofloxacin	34	6	82
Diclofenac	1180	557	53
Flumequine	16	5	69
Levofloxacin	33	5	85
Metronidazole	11	4	64
Trimethoprim	73	155	-112
Venlafaxine	701	453	35

Spiked effluent

In spiked effluent, four out of nine compounds had significant removal efficiencies from 69-99% in both measurements. They include amitriptyline, ciprofloxacin, levofloxacin, and metronidazole. Trimethoprim had a poor elimination efficiency of 10% during the first experiment while in the second it became 88% (Table 20). The limited elimination of trimethoprim during the first experiment could be attributed to non-biodegradability due to the fact that by the time of the first measurement, micropollutants were still not able to degrade it, but also its low Kd (0.3) was suggested as the reason that could make it flowing in the effluent and then still be detected (Verlicchi et al., 2012).

Amantadine and flumequine had low removal efficiencies. This could be attributed to the low biodegradability. It could be suggested that adsorption was the main removal mechanism of these chemicals. They could prefer to sorb to the solid particles than being degraded by microorganisms due to their low hydrophobicity (1.7 and 2.4, respectively). The low hydrophobicities have been also reported in findings of Nakada et al. (2007) as the main reason of low biodegradability.

Table 20: Concentrations and removal of target pharmaceuticals in spiked unozonated influent and effluent of the slow sand filter

	Influent (ng/L)		Effluent (ng/L)		Removal (%)	
Compound	**Week 10**	**Week 12**	**Week 10**	**Week 12**	**Week 10**	**Week 12**
Amantadine	8776	9620	8713	7826	1	19
Amitriptyline	3811	3616	930	1124	76	69
Ciprofloxacin	5383	5281	182	497	97	91
Diclofenac	12172	11616	9448	10311	22	11
Flumequine	8403	9785	8231	9002	2	8
Levofloxacin	7668	8987	43	1396	99	84
Metronidazole	7127	5238	133	58	98	99
Trimethoprim	6161	2297	5553	279	10	88
Venlafaxine	11266	12703	10474	10708	7	16

Effect of ozonation on the removal of pharmaceuticals in slow sand filter

The important effect of ozone in slow sand filtration was noticed, but mostly in spiked effluent (Table 21). Spiked ozonated effluent exhibited considerable removal efficiencies (85%) for the majority of compounds with in both measurements. It was observed that the microorganisms activity was cleary enhanced in ozonated effluent due to the increase of oxygen levels that maintained the growth and well-being of microorganisms. However, amantadine was slightly removed up to 73% during the second measurement. Flumequine was still persistent with poor removal efficiencies (< 50%). It was shown less biodegradable. This could confirm the idea that the main removal mechanism in slow sand filtration is biological degradation rather than sorption mechanism. However, there was no study found in literature that could support these findings. The unspiked effluent reported lower removal efficiencies compared to the spiked ones, due to very low concentrations that were in the ozonated effluent. This could also result to the lower concentrations and the increased uncertainty. The higher removal efficiencies were only reported for levofloxacin, metronidazole, trimethoprim and venlafaxine with removal efficiencies between 70% and 96 % .

Table 21: Effect of ozonation on the removal efficiency (%) of the target pharmaceuticals in slow sand filters.

	Unspiked effluent		Spiked effluent			
	Unozonated effluent	Ozonated effluent	Unozonated effluent		Ozonated effluent	
			Removal (%)		Removal (%)	
Compound	Removal (%)	Removal (%)	Week 10	Week 12	Week 10	Week 12
Amantadine	-5	-12	1	19	59	73
Amitriptyline	18	-1100	76	69	98	95
Ciprofloxacin	82	57	97	91	100	99
Diclofenac	53	-50	22	11	98	100
Flumequine	69	0	2	8	48	43
Levofloxacin	85	71	99	84	100	99
Metronidazole	64	82	98	99	84	89
Trimethoprim	-112	96	10	88	98	100
Venlafaxine	35	90	7	16	85	74

5.3.3 Biological granular activated carbon filtration

Biological granular activated carbon is defined as a combination of granular activated carbon particles with biotic components in which micropollutants removal occurs by adsorption and biodegradation mechanisms simultaneously.

5.3.3.1 Physical-chemical parameters characterization of effluent in biological granular activated carbon filter

Physical-chemical characteristics of unozonated and ozonated effluent in biological granular activated carbon showed a slight change between both effluents. This was confirmed by a one tail T-test that exhibited, for e.g, a p-value of 0.09 for pH. Both unozonated or ozonated effluents showed nearly similar characteristics. However, a huge difference in UV 254 absorbance was noticed when compared to the raw effluent (see Table 22). The reason for this huge difference was probably due to the combined mechanisms that govern the removal of organic pollutants in biological activated carbon. However the other parameters did not change significantly as what could be expected.

Table 22: Physical-chemical characterization of effluent in biological granular activated carbon filter

Parameter	Units	Unozonated effluent	Ozonated effluent
pH	(-)	7.9-8.6	8.0- 8.7
Ec	(µS/cm)	740-1300	730-1319
Redox	(mV)	293-398	298-391
BOD_5	(mg/L)	0.04 ± 0.10 to 0.70 ± 0.20	0.1 ± 0.1 to 1.5 ± 0.2
COD	(mg/L)	BDL to 8.1 ± 0.0	BDL to 6.3 ± 0.4
NO_3- N	(mg/L)	0.4 ± 0.1 to 2.3 ± 0.3	0.8 ± 0.0 to 3.0 ± 0.0
NH_3-N	(mg/L)	BDL to 0.13 ± 0.02	0.02 ± 0.00 to 0.15 ± 0.01
TNb	(mg/l)	BDL to 8.0 ± 0.0	1.0 ± 0.0 to 11.1± 0.2
PO_4^{3-}	(mg/L)	1.4 ± 0.1 to 2.2 ± 0.0	1.4 ± 0.0 to 2.4 ± 0.2
UV 254	(cm^{-1})	< 0.0	< 0.0

BLD: below detection limit

5.3.3.2 Removal of target pharmaceuticals in biological granular activated carbon filter

Unspiked effluent

The removal of target pharmaceuticals in unspiked effluent revealed to be very significant in the biological granular activated carbon filter (Table 23). All compounds that exhibited low removal efficiencies during conventional treatment were able to be highly eliminated (> 80%) in biological granular activated carbon. This could probably result from a combination of two mechanisms (biodegradation and adsortion) working in the filter. The exception was observed for metronidazole which was removed at 73%. This low elimination of metronidazole was due to unkown reason as previously reported in literature (Gros et al., 2009). Due to its low log Kow and pKa, metronidazole could be expected to be eliminated to greater extent (> 73%) by adsorption mechanism, which was not the case in unspiked effluent.

Table 23: Concentrations of target pharmaceuticals and their removal efficiencies of unspiked effluent in biological granular activated filter

Compound	Influent (ng/L)	Effluent (ng/L)	Removal (%)
Amantadine	41	1	98
Amitriptyline	22	2	91
Ciprofloxacin	34	6	82
Diclofenac	1180	7	99
Flumequine	16	2	88
Levofloxacin	33	4	88
Metronidazole	11	3	73
Trimethoprim	73	10	86
Venlafaxine	701	11	98

Spiked effluent

In spiked effluent, the biological granular activated carbon filter exhibited very significant removal of all targeted pharmaceutical components at nearly 100% in both measurements (Table 24). All compounds in the effluent of biological granular activated carbon were below detection limit. These high removal efficiencies of pharmaceuticals in this technique were due to a combination of two parallel mechanisms that occur in the filter, i.e. adsorption and biodegradation. Flumequine and amantadine which showed persistence in the other techniques (trickling and slow sand filters) were considerably removed. The removal of these compounds was most probably enhanced by the adsorption mechanism since these two compounds normally appear in the environment in the charged form that could interact electrostatically with the charged surface of activated carbon. Nevertheless, the effectiveness of those mechanisms depends on the properties of individual compound (log Kow, Kd or pKa) but also the empty bed contact time.

Table 24: Concentrations and removal of target pharmaceuticals in spiked unozonated influent and effluent of the biological granular activated carbon filter

	Influent (ng/L)		Effluent (ng/L)		Removal (%)	
Compound	**Week 10**	**Week 12**	**Week 10**	**Week 12**	**Week 10**	**Week 12**
Amantadine	8776	9620	3	3	100	100
Amitriptyline	3811	3616	5	4	99.9	99.9
Ciprofloxacin	5383	5281	1	21	100	99.6
Diclofenac	12172	11616	0	1	100	100
Flumequine	8403	9785	11	18	99.9	99.8
Levofloxacin	7668	8987	5	14	99.9	99.8
Metronidazole	7127	5238	4	2	99.9	100
Trimethoprim	6161	2297	26	1	99.9	99.9
Venlafaxine	11266	12703	7	8	99.9	99.9

Effect of ozonation on the removal of pharmaceuticals in biological granular activated carbon

Ozonation did not affect the good performance of biological granular activated carbon filter toward the removal of target compounds (Table 25). All substances presented removal efficiencies greater than 99% in spiked effluent. No significant differences were observed between two activated carbon filters, one for ozonated and the other one for unozonated effluent. Therefore, it was observed that the ozone did not show a significant influence on the removal of target pharmaceuticals in biological granular activated carbon filter. The minor impact of ozone on biological granular activated carbon was also reported by Reungoat et al. (2011).

Table 25: Effect of ozonation on the removal efficiency (%) of the target pharmaceuticals in biological granular activated carbon

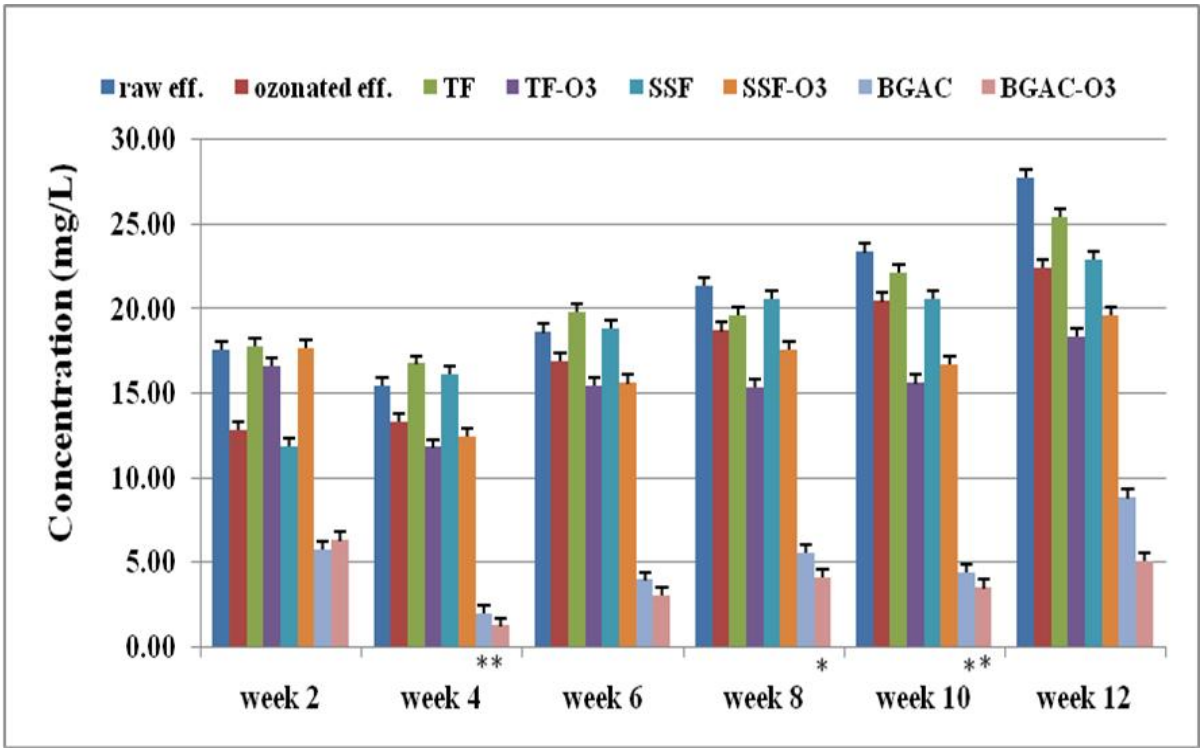
	Unspiked effluent		Spiked effluent			
	Unozonated effluent	Ozonated effluent	Unozonated effluent		Ozonated effluent	
			Removal (%)		Removal (%)	
Compound	Removal (%)	Removal (%)	Week 10	Week 12	Week 10	Week12
Amantadine	98	94	100	100	100	100
Amitriptyline	91	-100	99.9	99.9	99.9	99.8
Ciprofloxacin	82	57	100	99.6	99.6	99.7
Diclofenac	99	50	100	100	100	100
Flumequine	88	0	99.9	99.8	99.8	99.9
Levofloxacin	88	82	99.9	99.8	99.8	99.8
Metronidazole	73	64	99.9	100	100	99.9
Trimethoprim	86	95	99.9	99.9	99.9	99.9
Venlafaxine	98	61	99.9	99.9	99.9	99.9

5.4. Comparison of post treatment techniques

Integrated treatment processes for organic micropollutants usually exhibit optimal operating conditions in different aspects such as removal efficiency and cost-effectiveness. Ozone was found able to oxidize the majority of micropollutants into more biodegradable products. In combination with biological and physical-chemical filtration, those more biodegradable products are very efficiently eliminated out of the effluent.

Comparison in terms of COD and BOD removals

Despite of different effluent (i.e, different water) sampled at different time, a similar trend of chemical oxygen demand and biological oxygen demand reduction was noticed at each time the effluent were sampled (Figures 12 & 13). The results showed lower concentrations in both effluents, unozonated and ozonated in biological granular activated carbon filters compared to other filters, i.e. slow sand filter and trickling filter. Contrary, on week 2 and week 4 measurements, BOD_5 showed different results to what was expected. This could be attributed to the fact that it was during the first days of experiment running, the microorganisms were still growing and adapting to the media conditions as it was only after 14 days the filter columns were started working. In this study, trickling filter containing ozonated effluent was found more efficient than slow sand filtration with ozonated effluent which was not expected. The probable reason for that might be the optimal growth of microorganisms due to highest oxygen levels that could biodegrade effectively a higher number of compounds in trickling filter than in slow sand filter.



*: below detection limit

Figure 12: Chemical oxygen demand concentration in post-treatment techniques

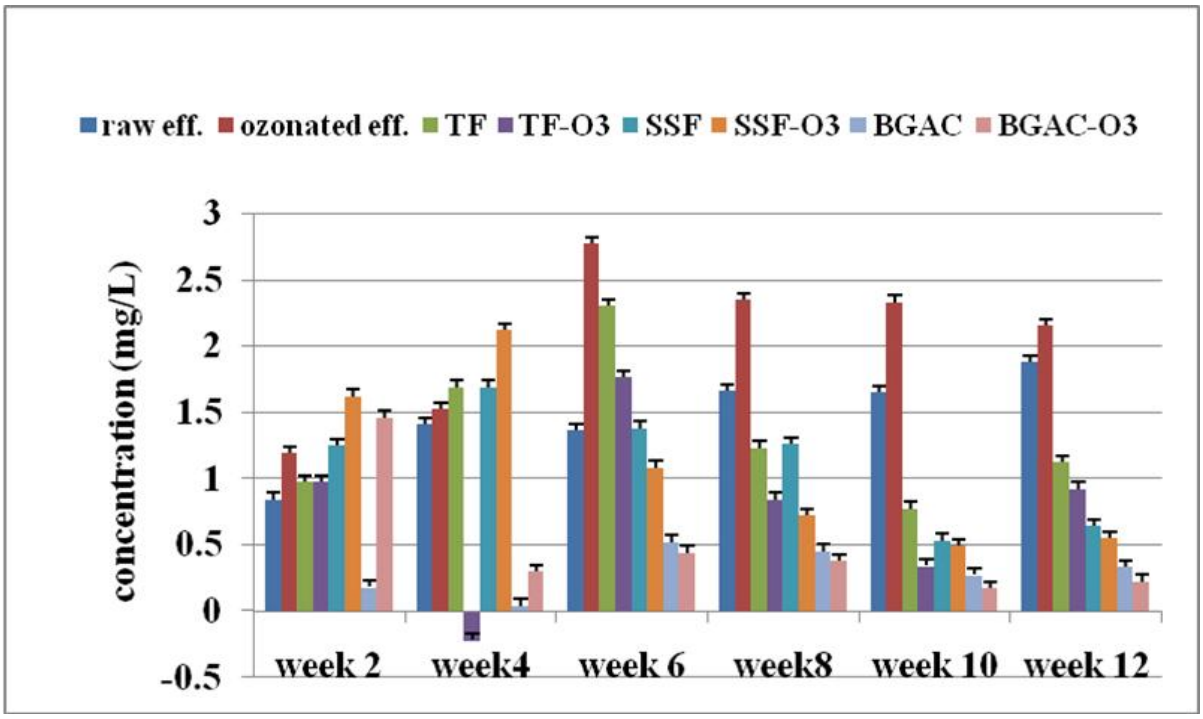


Figure 13: Biological oxygen demand concentration in post-treatment techniques

The BOD_5/COD ratios were calculated for all treatment techniques. The results provided in Figure 14 showed that the BOD5/COD ratios were generally lower than the normal degree of

biodegradabilty (ratio of 2). This was likely because of very minimal concentrations already found in the raw effluent so that the concentrations of BOD_5 and COD in the filters were nearly insignificant to prove higher degree of biodegradability.

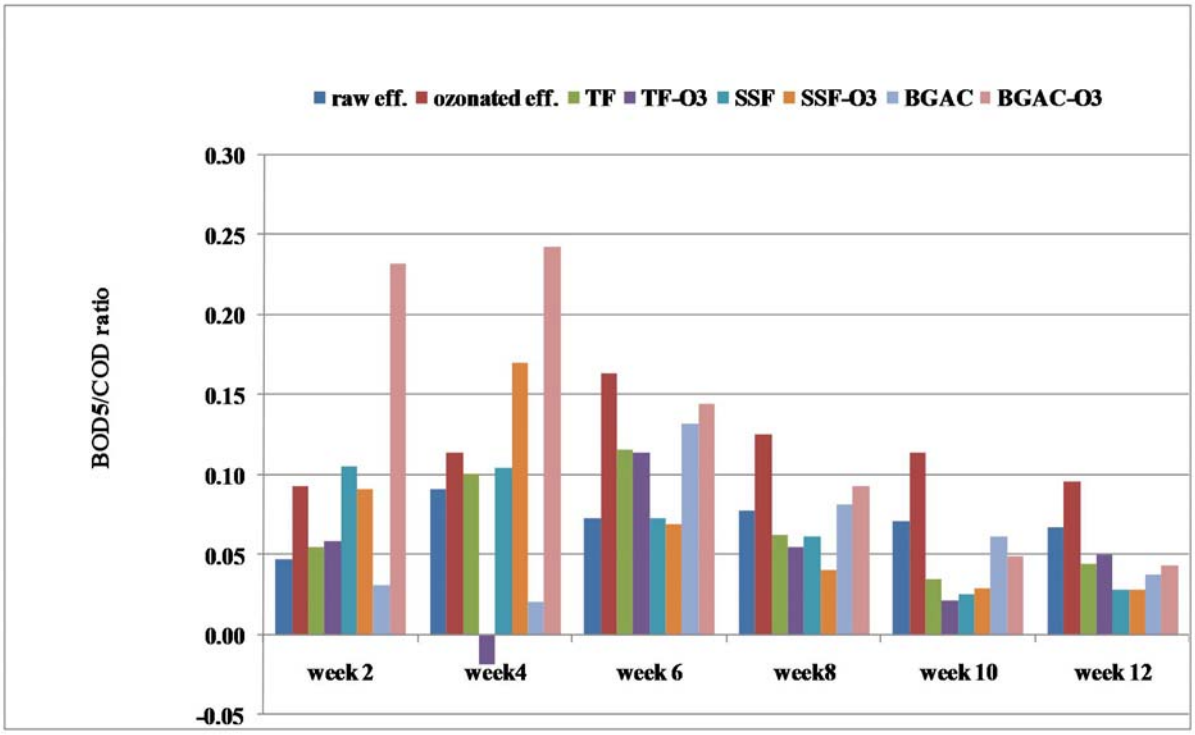


Figure 14: Degree of biodegradability throughout the post-treatment methods.

Comparison in terms of UV 254 absorbance reduction

Figure 15 shows that biological granular activated carbon filters containing ozonated and unozonated effluent were the best options to remove the UV254 absorbance levels at significant levels. The UV 254 values varied between below detection limit level and 0.02 cm^{-1} in both techniques. This finding provides a clear image of how a large number of aromatic compounds were greatly reduced during biological activated carbon filtration.

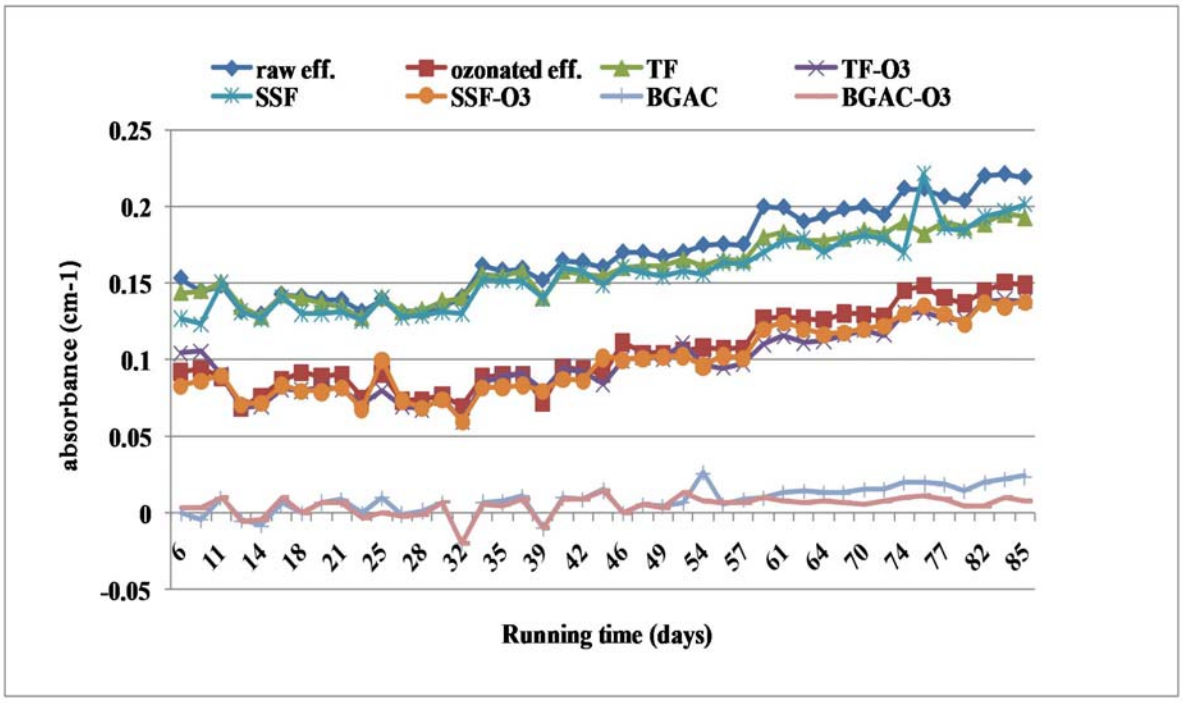


Figure 15: UV 254 absorbance throughout all treatments

A comparison of UV absorbance at different wavelengths (200 nm to 800 nm) for all post-treatments techniques together with raw and ozonated effluents was depicted as well (Tigure 16). The important range of absorbance was observed between 200 nm and 400 nm wavelengths. However, biological activated carbon filters showed less UV absorption followed by ozonated slow sand and ozonated trickling filters, and lastly the slow sand and trickling filters. The cuvette of 4 cm was additionally used to more accurately assess the change in UV absorbance trend for all treatment methods (Figure 17).

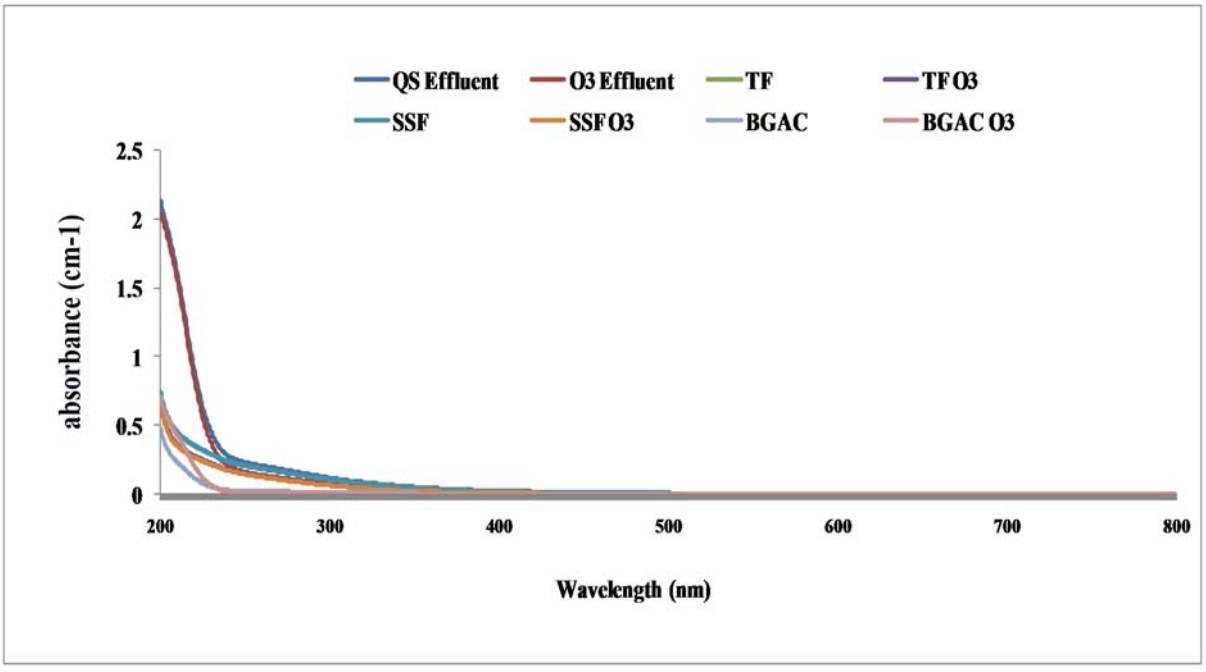


Figure 16: UV absorbance at different wavelength (200nm-800nm) measured with 1 cm cuvette

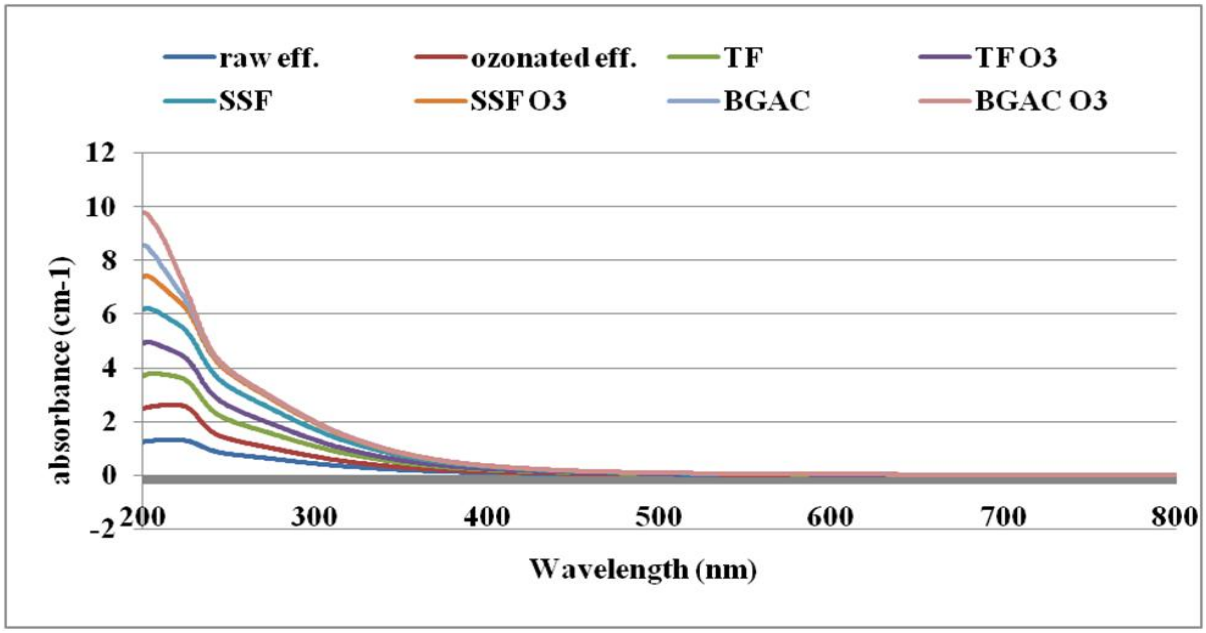


Figure 17: UV absorbance at different wavelength (200nm-800nm) measured with 4 cm cuvette

Comparison in terms of cost-estimation

Next to the performance of each technique, also the economic feasibility of each post-treatment needs to be taken into account to ensure a proper implementation for future full-scale applications. Therefore, a comparison was made based on the operational cost which covered the cost of filling materials (lava rocks, sand grains and granular activated carbon) used and the cost of ozonation per volume of treated effluent. Certain factors were considered for calculations. They included: the flow rate of the effluent through the columns, diameter of filter columns, the height of filter media (see Table 8), density of filter media (500 kg/m^3 for granular activated carbon, 2650 kg/m^3 for fine sand grains and 2600 kg/m^3 for trickling filter). The population equivalence (PE) of the WWTP in Harelbeke is 116,100 PE as previously mentioned in section 4.1.

The calculations considered the entire experimental filter running period of 94 days. Based on literature data (Chys et al., 2014), the cost of ozonation was calculated at 0.02 €/ m^3 treated effluent. Table 26 summarizes the investment cost of filling materials and ozonation at both scales; lab-scale and WWTP scale. However, power consumption and labor costs were not included in the total operational cost since the comparison of the cost between all techniques was mainly based on cost of filling materials and ozonation.

From the results presented in Table 26, it is observed that the biological granular activated carbon material is more expensive (24547 €) than the other techniques, i.e. slow sand and trickling filters. It is followed by trickling filter (11678 €) and finally the slow sand filtration (8069 €). However, despite of the highly significant removal of organic pollutants, the use of

biological granular activated carbon could be limited by its filter material cost that making it less cost-effective technique. Slow sand filtration can be considered a cost-effective technique due to the fact that its filter material cost was found very cheap compared to the other techniques (granular activated carbon and trickling filter).

Table 26: Cost-estimation of treated mass of filter material per day and operational cost in used post-treatment techniques

Parameters	Units	Trickling filter	Slow sand filter	Biological granular activated carbon filter
Flow rate in lab-scale	m^3/h	3.67×10^{-5}	3.67×10^{-5}	1.02×10^{-4}
Total volume treated in lab-scale	m^3	0.08	0.08	0.23
Filling material height	m	1.00	1.00	1.00
Filling material density	kg/m^3	2600	2650	500
Column diameter	m	0.02	0.02	0.02
Mass needed of filling material in lab	kg	0.90	0.92	0.17
Flow rate in WWTP	m^3/d	17415	17415	17415
Mass needed of filling material in WWTP	kg/d	188837	193033	12871
Cost/ kg filling material	€/kg	0.06	0.04	1.88
Total cost of filling material	€	11330	7721	24199
OperationOzonation	$€/m^3$	0.02	0.02	0.02
Lab-scale	€/d	0.002	0.002	0.005
Harelbeke WWTP	€/d	348	348	348
Tot. operational cost (WWTP)	**€/d**	**11678**	**8069**	**24547**

CHAPTER SIX

CONCLUSIONS & RECOMMENDATIONS

6.1 Conclusions

This study aimed to investigate the effect of ozonation on different post-treatment techniques to remove dissolved organic matter and micropollutants from MWWE. The omnipresence of micropollutants in MWWE would raise problems to aquatic life in receiving water bodies but also for human health when the effluent is taken for other purposes reuse.

Physical-chemical parameters were determined to characterize the quality of the effluent studied from the Harelbeke WWTP. It was observed that the physical-chemical parameters were generally in low concentrations complying with the guidelines. This has indicated the potentiality of the Harelbeke MWWTP to treat the raw municipal wastewater complying with EU and WHO water quality standards. From this reason they were limitedly affected by the post-treatment done in this study.

Nevertheless, the results obtained exhibited a certain reduction of DOM (in terms of COD removal, biodegradability and UV254 absorbance) during ozonation, but also biological activated carbon exhibited a considerable reduction compared to the other techniques used for post-treatment.

The presence of pharmaceutical compounds in the MWWE of Harelbeke revealed to be in ng/L to few µg/L concentrations which can not be negligible since those concentrations can induce severe effects on aquatic life.

Ozone oxidation was confirmed a promising method that can achieve higher removal of several pharmaceutical compounds, which will facilitate their further treatment in order to improve the quality of the effluent from MWWTPs.

Among all the post-treatment techniques used, biological activated carbon (either alone or in combination with ozonation) was shown a very effective process for the removal of targeted micropollutants with high removal efficiencies (> 90%). Nevertheless, the high cost of activated carbon materials would be the major limitation of use for this technique.

Slow sand filtration combined with ozone oxidation revealed to be optimal technique as targeted compounds were removed up to 70%. This has shown the power of combining these two techniques since the slow sand filtration alone showed less effectiveness in comparison with their combination. Moreover, this technique could be more interestingly used because it is a cheap technique compared to activated carbon.

Trickling filter alone was shown to be not effective for removal of micropollutants. However, in combination with ozonation it had a slight removal for a majority of compounds. Even if it is not an expensive method, it is not a good option to eliminate pharmaceuticals in MWWE unless it is combined with ozone.

From the statements said above, this can confirm the strong potential of the ozone influence on the post-treatment techniques investigated during this study concluding that ozone is a promising technology that can be applied for the removal of persistent organic pollutants including dissolved organic matter and micropollutants. Moreover, the application of combined technologies should not only allow a better achievement of elimination efficiency but also should contribute towards reduced treatment costs.

6.2 Recommendations

Research on removal of pharmaceutical residues in MWWE is still scarce specifically using the post-treatment techniques that have been used in this work. It is in that way a number of recommendations are provided for future studies:

- Further research is required for confirmation of the findings observed during this study using biological filtration methods in combination with ozone oxidation to assess their effectiveness in removal of pharmaceuticals in municipal wastewater effluent as this research field is still limited all over the world.

- More studies should be conducted in the effluents of different MWWTPs to confirm the findings of this study in Belgium.

- Development of surrogate parameters for degradation of pharmaceutical residues during ozonation is needed to facilitate the discovery of their behaviors under oxidation.

- A model should be developed to predict the impacts of pharmaceutical residues on aquatic life in receiving water.

- Toxicity study of transformation byproduct components formed during ozonation should be conducted to have insight in the types of byproducts released, and be able to assess which post-treatment method can be used for a complete or a significant removal of them.

- The last but not the least, more information on the presence, quantity, and toxicity of pharmaceuticals and their metabolites should be clearly defined and standardized especially when attempting to reuse potential of municipal wastewater effluent.

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