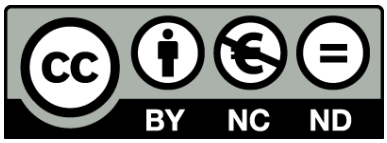
UNIVERSITAT DE BARCELONA

Advanced technologies applied to wastewater treatment plant effluents

Ana Justo Llopis



Doctoral Thesis

ADVANCED TECHNOLOGIES APPLIED TO WASTEWATER TREATMENT PLANT EFFLUENTS

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Universitat de Barcelona

Programa de doctorat d'*Enginyeria i Tecnologies Avançades*

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La Dra. **CARME SANS MAZON**, professora titular del Departament d'Enginyeria Química de la Universitat de Barcelona i el Dr. **ÓSCAR GONZÁLEZ ÁLVAREZ**, professor associat del mateix departament,

CERTIFIQUEN QUE:

El treball d'investigació titulat "**ADVANCED TECHNOLOGIES APPLIED TO WASTEWATER TREATMENT PLANT EFFLUENTS**" constitueix la memòria que presenta la Enginyera Química Ana Justo Llopis per a aspirar al grau de Doctor per la Universitat de Barcelona. Aquesta tesi doctoral ha estat realitzada dins del programa de doctorat "Enginyeria i Tecnologies Avançades", en el Departament d'Enginyeria Química de la Universitat de Barcelona.

I perquè així consti als efectes oportuns, signen el present certificat a Barcelona, 1 de Setembre de 2015.

Dra. Carme Sans Mazón i Dr. Óscar González Álvarez

Directors de la tesi doctoral

"A person who never made a mistake never tried anything new"

Albert Einstein (1879 – 1955)

AGRADECIMIENTOS

Quisiera expresar mi más sincero agradecimiento a los directores de esta tesis, la Dra. Carme Sans y el Dr. Óscar González, tanto por haberme dado la oportunidad de realizar este trabajo bajo su dirección como por el interés que han mostrado en él. Así mismo, dar las gracias al Dr. Santiago Esplugas, director de nuestro grupo de investigación Ingeniería de Procesos de Oxidación Avanzada, por este tiempo que he pasado trabajando en su grupo.

Me gustaría mostrar también mi agradecimiento a los otros grupos de investigación que han colaborado en esta tesis con sus análisis: ACCIONA AGUA S.A.U., Department of Environmental Chemistry (IDAEA-CSIC) y Water and Soil Quality Research Group (IDAEA-CSIC).

Sería complicado nombrar a todos los compañeros que han compartido experiencias conmigo a lo largo de estos años… Muchas gracias a todos y cada uno de vosotros por todos esos momentos dentro y fuera del laboratorio!

Por último, y no menos importante, también me gustaría agradecer a mi familia el apoyo recibido durante estos años. Especialmente a Víctor, mi marido, por su paciencia, y a Nil, nuestro hijo, que nacerá este mismo setiembre.

TABLE OF CONTENTS

ABSTRACT

This thesis is formed as a summary of publications developed in the Chemical Engineering Department from the University of Barcelona. The six publications of this thesis are focused on the application of advanced technologies to Wastewater Treatment Plant (WWTP) effluents that are usually discharged to the aquatic environment.

Water is an essential natural resource for the development of life and for human activities. Over the last few decades, water scarcity and water quality have become issues of major concern. Large amounts of water have been continuously contaminated, especially in developed countries. The restoration of water quality is essential to avoiding higher levels of contamination dealing with the "zero discharge" idea, and enabling water reuse. The implementation of tertiary treatments is necessary to reach the appropriate quality of water from effluents of WWTPs.

It is generally assumed that not all polluting agents are removed through conventional WWTPs. These persistent compounds include the emerging pollutants group, constituted by chemicals of high diverse origin. They are characterized by their high production and consumption volumes, which entails their continuous presence in the environment even at low concentrations. Whereas their occurrence is fairly well-established, their long-term effects and environmental consequences are not clearly identified. Thus, additional advanced treatment steps should be considered to reduce their discharge into receiving waters.

In this work, two groups of effluents that are usually discharge into water bodies without any extra treatment were treated: two types of secondary effluents and Reverse Osmosis (RO) brine effluent. Biologically treated sewage effluent contains a complex matrix of organic materials-Effluent Organic Matter (EfOM). This EfOM consisted of: refractory Natural Organic Matter (NOM), trace levels of synthetic organic compounds and soluble microbial products. Regarding RO, despite the high quality effluent generated, salts, biological constituents and organics, including micropollutants, are concentrated in the rejected effluent. Although their discharge is currently not regulated, safe environmental practices would suggest their treatment before its release and dilution into the environment.

Advanced Oxidation Processes (AOPs) appear to be appropriate for the treatment of waste streams that contains recalcitrant organic matter. These AOPs involves the *in situ* generation of highly reactive hydroxyl radicals ($HO^{\cdot}$). This work is focused in the UV/H_2O_2 and ozonation treatments. On the other hand, taking advantage of the biodegradability enhancement achieved by AOPs, the use of subsequent biological step has been also integrated in order to minimize even further the organic load of the target effluent. The selected biological process was the Biological Activated Carbon (BAC) filter, where microbial communities were established on the exhausted porous of Granular Activated Carbon (GAC) surface.

The main objective of this thesis is the assessment of some advanced processes applied to different WWTPs effluents which are usually discharged to surface waters, sea and oceans. The final objective is the water reclamation and/or the minimization of the

contamination to the aquatic medium. This main objective has been divided in four specific research works: (1) to study the fate of the EfOM present in secondary effluents during oxidation by UV/H_2O_2 and ozonation using LC-OCD technique (Appendix I); (2) to evaluate the performance of treating reclamation RO brines by AOPs and to monitor pharmaceuticals oxidation (Appendix II and III); (3) to combine chemical analyses and bioassays in order to characterize the removal of pharmaceuticals from RO brine by AOPs (Appendix IV); and (4) to assess the integration of BAC filters (and a Moving-Bed Biofilm Reactor (MBBR)) with AOPs when treating RO brines (Appendix V and VI).

In general both AOPs studied performed well in the treatment of secondary effluents and RO brines. Some differences were observed between them due to different mechanisms involved in each AOP leading to a different Dissolved Organic Matter (DOM) composition in the secondary effluents, and micropollutants preferential degradation in RO brine effluents. Besides, some differing results with the bibliography and between the different RO brines studied were obtained. That evidence the considerable complexity inherent in this type of treatment when applied to practical wastewaters due to the high number of factors involved, such as the reactivity of EfOM and variable water quality, which can affect the observed removals. The combination of chemical analyses and bioassays allows complete characterization of the efficiency of advanced water treatment processes to remove recalcitrant pollutants. Finally, the integration of a pre-oxidation stage using UV/H_2O_2 or ozone with a BAC filtration was necessary to completely remove the high concentration of micropollutants present in the municipal RO brines and to reduce the water quality parameters close to values typically found in a conventional secondary effluent.

THESIS DIRECTOR'S REPORT AND LIST OF PUBLICATIONS

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Dra. CARME SANS MAZÓN, Professor from the Chemical Engineering Department of the University of Barcelona, and Dr. ÓSCAR GONZÁLEZ ÁLVAREZ, Associate Professor from the same department, and both directors of the PhD thesis of Ana Justo Llopis, issues the following report related with her participation in the publications derived from this PhD thesis and included in this report:

All the major experimental work and results discussion included in the publications and Congresses communications included in this thesis was entirely developed in the Chemical Engineering Department of the University of Barcelona by Ana Justo Llopis, under the supervision of her PhD directors. The publications included in this thesis were not presented in other doctoral thesis or a thesis presented as a summary of publications.

Other institutions and collaborations, if any, are detailed below each publication (referenced in the manuscript as Appendixes from I to VI):

I. González, O., Justo, A., Bacardit, J., Ferrero, E., Malfeito, J.J., Sans, C. *Characterization and Fate of Effluent Organic Matter Treated with UV/H_2O_2 and Ozonation*. Chemical Engineering Journal (2013), 226 (402–408). doi: http://dx.doi.org/10.1016/j.cej.2013.04.066

   LC-OCD analyses were performed in collaboration with ACCIONA AGUA S.A.U. although the results discussion was carried out by the author of this thesis.

II. Justo, A., González, O., Sans, C., Esplugas, S. *"Regeneración de aguas de rechazo de Osmosis Inversa (OI) mediante la combinación del Proceso de Oxidación Avanzada (POA) UV/H_2O_2 con tratamiento biológico"*. Published in the Book of abstracts as oral communication in the "VII Congreso, La investigación ante la sociedad del conocimiento. Sostenibilidad y Medioambiente" (9-11 de noviembre de 2011, Alcoy). Edited by "Escuela Politécnica Superior de Alcoy" (ISBN: 978-84-694-9814-9)

III. Justo, A., González, O., Aceña, J., Pérez, S., Barceló, D., Sans, C., Esplugas, S. *Pharmaceuticals and Organic Pollution Mitigation in Reclamation Osmosis Brines by*

UV/H_2O_2 and Ozone. Journal of Hazardous Materials (2013), 263 (268-274). doi: http://dx.doi.org/doi:10.1016/j.jhazmat.2013.05.030

LC–MS/MS analyses were performed in collaboration with the Department of Environmental Chemistry (IDAEA-CSIC) although the results discussion was carried out by the author of this thesis.

IV. Justo, A., González, O., Aceña, J., Mita, L., Casado, M., Pérez, S., Piña, B., Sans, C., Barceló, D., Esplugas, S. *Application of bioassay panel for assessing the impact of advanced oxidation processes on the treatment of reverse osmosis brine.* Journal of Chemical Technology and Biotechnology (2014), 89 (1168-1174). doi: 10.1002/jctb.4389

LC–MS/MS analyses were performed in collaboration with the Water and Soil Quality Research Group (IDAEA-CSIC) although the results discussion was carried out by the author of this thesis. Bioassays (not including Microtox®) and results discussion were performed in collaboration with the Department of Environmental Chemistry (IDAEA-CSIC).

V. Justo, A., González, Sans, C., Esplugas, S. *BAC filtration to mitigate micropollutants and EfOM content in reclamation reverse osmosis brines*. Chemical Engineering Journal (2015), 279 (589-596). doi: http://dx.doi.org/10.1016/j.cej.2015.05.018

The author of this thesis and Dr. Óscar González, took under their supervision three degree final project students (Ms. Anna Barba Riba, Ms. Raquel Santos Royo and Ms. Aixa Rodríguez Hilario), who collaborated in some of the experimental part of the work, mainly related with the analytical monitoring of the processes. LC–MS/MS analyses were performed in collaboration with the Water and Soil Quality Research Group (IDAEA-CSIC) although the results discussion was carried out by the author of this thesis.

VI. Vendramel, S. M. R., Justo, A., González, O., Sans, C., Esplugas, S. *Reverse osmosis concentrate treatment by chemical oxidation and moving bed biofilm processes*. Water Science and Technology (2013), 68 (11) (2421-2426). doi: 10.2166/wst.2013.510

The author of this thesis collaborated with the start-up and the experimentation period with Dra. Vendramel, S. M. R.

Below, a detailed list including the impact factor (IF) and subject categories of the journals were the author of this thesis published her works is presented (data obtained from *Journal Citation Report®*, 2014).

- Chemical Engineering Journal (IF: 4.321)

Category Name	Quartile in Category
Engineering, Environmental	Q1
Engineering, Chemical	Q1

- Journal of Hazardous Materials (IF: 4.529)

Category Name	Quartile in Category
Engineering, Civil	Q1
Engineering, Environmental	Q1
Environmental Sciences	Q1

- Journal of Chemical Technology and Biotechnology (IF: 2.349)

Category Name	Quartile in Category
Biotechnology & Applied Microbiology	Q2
Chemistry, Multidisciplinary	Q2
Engineering, Chemical	Q2

- Water Science and Technology (IF: 1.106)

Category Name	Quartile in Category
Engineering, Environmental	Q3
Environmental Sciences	Q3
Water Resources	Q3

Dra. Carme Sans Mazón and Dr. Óscar González Álvarez

Barcelona, 1st of September 2015

NOMENCLATURE

AC	Activated Carbon
AhR-RYA	Aryl hydrocarbon Receptor – Recombinant Yeast Assay
AOPs	Advanced Oxidation Processes
ATP	Adenosine Tri-Phosphate
BAC	Biological Activated Carbon
BB	Building Blocks
BOD_5	Biochemical Oxygen Demand at 5 days
BP	Biopolymers
CAS	Conventional Activated Sludge
COD	Chemical Oxygen Demand
DAPI	4',6-diamidino-2-phenylindole
DNA	Deoxyribonucleic Acid
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EBCT	Empty Bed Contact Time
EfOM	Effluent Organic Matter
ER-RYA	Estrogen Receptor – Recombinant Yeast Assay
FISH	Fluorescence in Situ Hybridization
GAC	Granular Activated Carbon
HS	Humic Substances
LC–MS/MS	Liquid Chromatography coupled to tandem Mass Spectrometry
LC-OCD	Liquid Chromatography – Organic Carbon Detection
LMM	Low Molar Mass
LMMA	LMM organic Acids
LMMN	LMM Neutrals and amphiphilics
MBBR	Moving-Bed Biofilm Reactor
MBR	Membrane Biological Reactor
NOM	Natural Organic Matter
PAC	Powdered Activated Carbon
RO	Reverse Osmosis
rRNA	ribosomal Ribonucleic Acid
RYA	Recombinant Yeast Assay
SBR	Sequencing Batch Reactor
SUVA	Specific UV Absorbance
TOC	Total Organic Carbon
TOD	Transferred Ozone Dose
TRO	Total Residual Oxidants
UV_{254}	UV spectrophotometry at 254 nm
WWTPs	Wastewater Treatment Plants

1. INTRODUCTION

1.1. Water resources

1.1.1. Global water situation

"There is a water crisis today. But the crisis is not about having too little water to satisfy our needs. It is a crisis of managing water so badly that billions of people - and the environment - suffer badly". (World Water Vision, 2000) [1].

Water is an essential natural resource for the development of life and for human activities. Currently, its scarcity is one problem that causes great concern in our society. The growing water shortage problem in arid and semi-arid areas unavoidably leads to more efficient management schemes for water resources.

Today, nearly 1 billion people in the developing world don't have access to it [2]. Yet, we take it for granted, we waste it, and we even pay too much to drink it from little plastic bottles. Water is the foundation of life. And still today, all around the world, far too many people spend their entire day searching for it. In places like sub-Saharan Africa, time lost gathering water and suffering from water-borne diseases is limiting people's true potential [3].

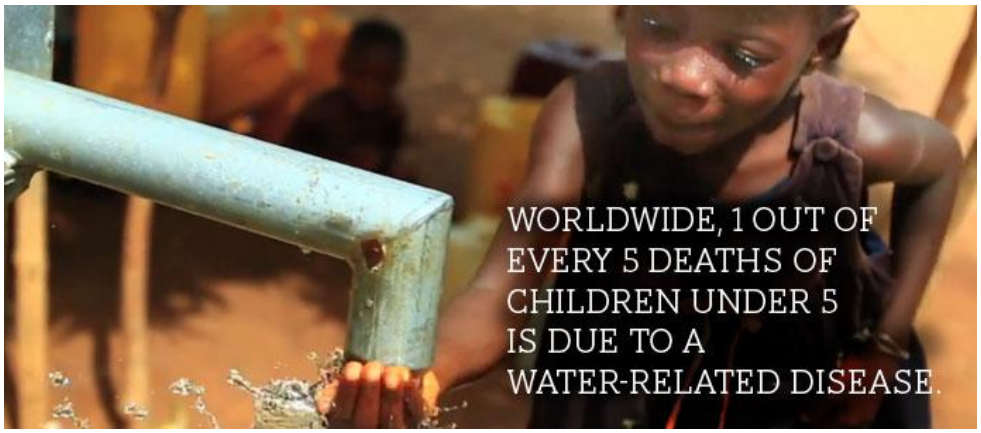

Figure 1. African child and water. Source: "The Water Project".

Good health begins with access to clean water. In developing countries, about 80% of illnesses are linked to poor water and sanitation conditions. One out of every five deaths under the age of five worldwide is due to a water-related disease such as such as diarrhea, cholera, typhoid fever and dysentery among others [2,3].

Without having access to clean water the developing countries cannot relieve hunger neither progress. It should be considered that without access to a reliable source of water, food is hard to grow and even more difficult to preserve and prepare. Globally we use 74% of our water sources for agriculture and irrigation, 18% for industrial uses and only 8% on domestic uses [4]. The 84% of people who don't have access to improved water, also live in rural areas, where they live principally through subsistence agriculture. Sometimes, areas that experience a lack of water suffer because of poor water management, but more often it is a

relatively simple economic issue that can be addressed. This is the difference between physical and economic scarcity.

According to Population Action International, based upon the UN Medium Population Projections of 1998, more than 2.8 billion people in 48 countries will face water stress, both in terms of water scarcity and quality deterioration [5], by 2025. Of these countries, 40 are in West Asia, North Africa or sub-Saharan Africa. Over the next two decades, population increases and growing demands are projected to push all the West Asian countries into water scarcity conditions. By 2050, the number of countries facing water stress or scarcity could rise to 54, with a combined population of four billion people - about 40% of the projected global population of 9.4 billion [6].

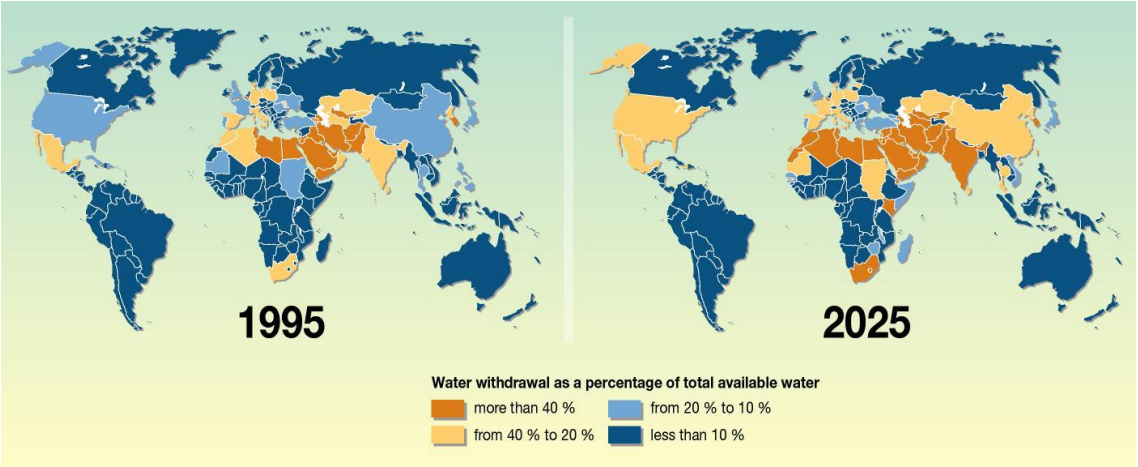

Figure 2. Water stress situation from 1995 to 2025. Source: United Nations Environment Programme (UNEP, 2008).

1.1.2. Water pollution and reuse

Additionally to the global water scarcity, the release of pollutants with potential to harm both humans and the environment into water bodies is the biggest threat to the world's freshwater supplies. Pollution can be defined as *"the introduction by man in the environment of substances or energy liable to cause hazards to human health, harm for living resources and ecological system, damages to structures or amenity, or interference with legitimate uses of the environment"* [7].

The rapid industrial and technological development, as well as the growing of human population, seriously affects the quality of the aquatic medium producing large amounts of wastewater. For this reason, the reclamation of these contaminated waters is currently seen as alternative water resource [5,8–10]. The implementation of tertiary treatments is necessary to reach the appropriate quality of water from effluents of Wastewater Treatment Plants (WWTPs). Most methods of wastewater treatment limit themselves to reproducing the natural process of self-purification which for centuries has suffered water after discharge into water bodies. However, the huge volumes and pollutants to be treated should be accelerated by artificial means. Different degrees of treatment will be necessary depending on the final use of this reclaimed water.

Wastewater treatment is closely related to the standards and/or expectations set for the effluent quality. The main function of a WWTP is to minimize the environmental impact of discharging untreated water into natural water systems [11]. The conventional WWTPs consist of two levels of treatment: preliminary and primary (physical and chemical), and secondary (biological) treatment. These treatments may reduce: suspended solids, biodegradable organics, pathogenic bacteria and nutrients. Besides, as sustainability is promoted within the water cycle, the functions of WWTPs also should be to recover: water resources (reclaimed water), energy (methane from anaerobic digestion) and materials (biosolids and nutrients) [11]. This treated wastewater is usually discharge into the sea or rivers but not used for reuse purposes. Also a WWTP may get a resource from wastewater carrying out a tertiary treatment on the treated wastewater which can be reused [11].

The main objective of wastewater reclamation and reuse projects is to produce water of sufficient quality for all non-potable uses (uses that do not require drinking water quality standards). Using reclaimed water for these applications would save significant volumes of freshwater that would otherwise be wasted [11,12]. Reclaimed water can replace freshwater in traditional practices such as agricultural and landscape irrigation, industrial applications, environmental applications (surface water replenishment, and groundwater recharge), recreational activities, urban cleaning, firefighting, construction, etc. [8,9,11].

In Spain, the current most important uses of freshwater are irrigation (79%), municipal uses (8%), recreational use and golf courses (6%), ecological uses (6%) and industrial applications (1%) (last data available: 2008) [12]. The amount of wastewater that is being reclaimed has increased in recent years (until the economic crisis). For example, in Catalonia, the amount of reclaimed water has doubled during the period from 2005 to 2008. This water is mainly used for environmental applications (79%), such as aquifer replenishment, salt water intrusion control, stream flow enhancement, etc. The remaining water is used for irrigation (9%), municipal uses (1%) and recreational activities (11%), according to last data available at “Agència Catalana de l’Aigua” (2008) [13].

Royal Decree 1620/2007 is nowadays the principal water reuse regulation for Spain. It established the legal regime for the reuse of treated water, including the basic conditions for reuse of treated water, quality standards and the proceedings for initiatives or plans from public authorities’ initiatives. It represented an important advance to standardize water reuse practices despite the large cost of implementation. It contributed to the consolidation of water reuse inside the global water resources management [14].

Central and autonomic governments have been doing noticeably efforts to spread the reuse of reclaimed water for the last two decades. These initiatives were finally recognized, reunited and organized in 2009 under a nationwide project entitled: “Plan Nacional de Reutilización de Aguas”. The objectives of the plan were both strategic and environmental. The main strategic aims were: achieve "zero discharge" objective in coastal areas; replace inland areas pre-potable water concessions by reclaimed water for uses where feasible; promote good practices of reuse of reclaimed water, estimate future reusability, etc. As environmental objectives, the plan envisaged the change from the traditional approach of "supply", founded on the basis of large hydraulic infrastructures, to new strategies of water resources "on-

demand management" and the protection of inland and coastal estuaries ecosystems [15]. The plan covered the entire Spanish territory and its application reached horizon until 2015. However, after the change of government in late 2011 and the economic crisis, the plan seems to have stopped.

Emerging contaminants

It has generally been assumed that possible polluting agents are eliminated through sewage treatment. However, not all polluting agents are removed through standard treatments [16,17]. A number of compounds are known to persist through WWTPs in an unaltered form. Between these persistent compounds is the emergent polluting agent group, constituted by chemicals of a highly diverse origin, characterized by its high production and consumption entailing its continuous presence in the environment [16,17]. Among them, drugs, diagnosis products, steroids and hormones, antiseptics, personal care products, petrol additives, heavy metals and metalloids, surfactants endocrine disruptors, etc. are covered by this term. Table 1 summarizes the sources of the major categories of micropollutants in the aquatic environment [18].

Table 1. Sources of micropollutants in the aquatic environment [18].

Category	Important subclasses	Major sources	
		Distinct	**Nonexclusive**
Pharmaceuticals	NSAIDs*, lipid regulator, anticonvulsants, antibiotics, β-blockers, and stimulants	Domestic wastewater (from excretion) Hospital effluents Run-off from CAFOs** and aquaculture	Sources that are not exclusive to individual categories include: Industrial wastewater (from product manufacturing discharges) Landfill leachate (from improper disposal of used, defective or expired items)
Personal care products	Fragrances, disinfectants, UV filters, and insect repellents	Domestic wastewater (from bathing, shaving, spraying, swimming and etc.)	
Steroid hormones	Estrogens	Domestic wastewater (from excretion) Run-off from CAFOs and aquaculture	
Surfactants	Non-ionic surfactants	Domestic wastewater (from bathing, laundry, dishwashing and etc.) Industrial wastewater (from industrial cleaning discharges)	
Industrial chemicals	Plasticizers, fire retardants	Domestic wastewater (by leaching out of the material)	
Pesticides	Insecticides, insecticides, herbicides and fungicides	Domestic wastewater (from improper cleaning, run-off from gardens, lawns and roadways and etc.) Agricultural run-off	

* NSAIDs: Non-steroidal Anti-inflammatory Drugs
**CAFOs: Concentrated Animal Feeding Operations

The progress on the development of new and more powerful analytical multi-residue chromatographic methods has made available their detection and quantification in natural water bodies and wastewater, at concentrations in the range of ng L^{-1}. Whereas their occurrence is fairly well-established, their long-term effects and environmental consequences are not clearly identified [17]. The World Health Organization says that: *"Although current*

published risk assessments indicate that trace concentrations of pharmaceuticals in drinking-water are very unlikely to pose risks to human health, knowledge gaps exist in terms of assessing risks associated with long-term exposure to low concentrations of pharmaceuticals and the combined effects of mixtures of pharmaceuticals" [2]. Thus, additional advanced treatment steps should be considered to reduce their discharge into receiving waters since its presence in water is undesirable even if compounds have low or acute toxicity [16].

At present, they are not routinely monitored by water treatment companies due to the lack of regulatory requirements, as standards do not exist for most of the compounds. Furthermore, there is an extreme cost involved for monitoring thousands of potential contaminants that are expected to be removed after treatment. Attention has been especially directed towards the fate of compounds in sewage treatment facilities and surface waters in many parts of the world [16].

Although there are no discharge limits for most micropollutants, some regulations have been published. Early in 2000, a strategy was defined by the Directive 2000/60/EC with the aim of identifying priority substances with high risk to the aquatic ecosystems. In 2008, a list of 33 priority substances/groups of substances was established at Union level by the Directive 2008/105/EC, in the field of water policy. Environmental Quality Standards were defined for these 33 priority substances/groups of substances and for other eight pollutants, based on available data of acute and chronic effects to aquatic environment and human health, being expressed as an annual average value (level providing protection against long-term exposure) and/or maximum allowable concentrations (level providing protection against short-term exposure). The recent Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending the Directives 2000/60/EC and 2008/105/EC, updated the water framework policy. The Directive 2013/39/EU promotes the preventive action and the polluter pays principle, the identification of pollution causes, dealing with emissions of pollutants at the source, and finally the development of innovative water/wastewater treatment technologies, avoiding expensive solutions [19,20].

1.1.3. Effluents discharged to aquatic environment

In this section, the two effluents treated in this thesis (secondary effluents and Reverse Osmosis (RO) brine effluents) will be introduced. These two effluents are usually discharge into water bodies without any extra treatment.

Secondary effluents

Biologically treated sewage effluent contains a complex matrix of organic materials-Effluent Organic Matter (EfOM). EfOM can be summarized into three general classes based on their origins [21–23]:

- Refractory Natural Organic Matter (NOM) derived from drinking water;

- Trace levels of synthetic organic compounds produced during domestic use and disinfection by-products generated during the disinfection processes of water and wastewater treatment; and,
- Soluble microbial products derived during the biological treatment process of wastewater.

Similar to the situation that occurs in drinking water treatment due to the presence of NOM, the EfOM present in secondary effluents can cause many technical and environmental problems when the effluents are treated for reclamation purposes. This EfOM is responsible for the majority of the coagulant and disinfectant demand (which results in increased sludge and the formation of potentially harmful disinfection by-product). Moreover, EfOM can interfere with the removal of other contaminants (e.g., competition of adsorption sites in Activated Carbon (AC)). Namely, EfOM is responsible for membrane fouling, contributes to corrosion, acts as a substrate for bacterial growth in distribution systems and can cause eutrophication in receiving water bodies. In addition, EfOM contributes to undesirable color and odor problems and the formation of disinfection by-products and acts as a carrier for metals and hydrophobic organic chemicals [22–24]. Although EfOM is not usually considered a pollutant, it may limit reuse applications or it may be associated with other undesirable organic compounds.

The Advanced Oxidation Processes (AOPs) appear appropriate for the treatment of secondary effluents because of its biorecalcitrant nature. In addition, the efficient removal of EfOM by methods such as AOPs will result in secondary benefits (i.e., water disinfection). It is still commonly perceived that the sustainability of AOPs or other waste water treatment methods should be determined eventually by economics factors. However, given the growing shortage of high quality water, which is expected to worsen with global changes in climate, the water industry and policy makers may have to reconsider the importance of economic issues. Besides, one of the objectives of some water reuse strategies deal with the "zero discharge" where can be included the treatment of secondary effluents.

Although many previous researchers have worked extensively on NOM in surface waters, there have been few studies related to EfOM in wastewater. This is probably due to the diverse characteristics of wastewater, which vary by place and season. However, as concern related to water reuse increases, an interest in characterizing the EfOM has become more important [23].

Reverse Osmosis (RO) brine effluents

Membrane separation processes are becoming quite popular in wastewater treatment and reclamation, since they combine process stability with an excellent quality. RO is one of the most effective treatments for removing a wide range of micropollutants, ions and also organic matter during water recycling [25,26]. RO technology is a pressure-driven membrane process that separates a feed stream into a purified permeate fraction (usually the desired product), which is generally 75-85% of the feed water, and a concentrated retentate fraction,

containing all the constituents rejected by the membranes (commonly named as RO brine) [27]. The membrane retentates include [26,28]:

- Inorganic compounds (salts);
- Organic matter (EfOM) including refractory chemicals added by the public into wastewater (e.g., pesticides, personal care products, pharmaceutical products, endocrine disrruptors); and
- Biological materials (e.g., bacteria, viruses, oocysts, and cell fragments).

Two issues can be identified as major drawbacks for the implementation of this membrane process: the risk of membrane fouling (which may require extensive pretreatment or chemical cleaning of the membranes and may possibly result in a short lifetime of the membranes), and the need of further treatment of the concentrate fraction, since these effluents are usually discharged to surface waters, oceans or groundwaters. While the salt content of RO retentates may pose an environmental risk, endocrine disrupting compounds, pharmaceuticals, pesticides, etc., that are highly rejected by the membranes and which are concentrated in the RO retentate, are of increasing concern. Although discharge of such constituents is not currently regulated, prudent environmental practices would suggest treatment of this concentrated waste stream before its release and dilution in the environment [28].

Treatment and recovery of RO brines will provide a two-way solution to water reclamation plant, mainly increasing the efficiency of reclaimed water and providing a solution to brine handling and disposal according to “zero discharge” idea [27].

Concentrates often contain high amount of low-biodegradable Chemical Oxygen Demand (COD) and inorganic compounds, which limit the use of conventional wastewater treatment processes. AOPs are capable of low-biodegradable COD degradation and wastewater detoxification, being therefore appropriate for degrading the organics present in RO concentrate.

1.2. Advanced Oxidation Processes (AOPs)

In the last decades of XX century some developments in the domain of chemical water treatment led to an improvement in AOPs for organic compounds dissolved or dispersed in aquatic media [29]. AOPs can be defined as those chemical treatment procedures capable of generate *in situ* hydroxyl radicals ($HO^{\cdot}$), a chemical species with high oxidation potential able of removing organic and inorganic compounds by means of oxidation.

AOPs can be classified depending on the source of the oxidizing species or the method employed for its production. A commonly used classification is shown in Table 2 [30,31], where it distinguishes from AOPs (1) based on photolysis, (2) based on ozone, (3) based on hydrogen peroxide, (4) thermal AOPs, (5) high energy AOPs and (6) photocatalysis. This work is focused in the ozonation and UV/H_2O_2.

Table 2. AOPs classification.

Photolysis	UV Photolysis V-UV Photolysis
Ozone based AOPs	Ozonation (alkaline conditions) Ozonation + UV and/or H_2O_2 Ozonation + catalyst
H_2O_2 based AOPs	Fenton Fenton-like Photo-Fenton UV/H_2O_2 Electro-Fenton
Thermal AOPs	Supercritical wet oxidation Wet oxidation Wet oxidation + H_2O_2
High energy AOPs	Ultrasound technologies Electrochemical oxidation Electron beam oxidation Microwaves enhances processes
Photocatalysis	

Some of these types of AOPs result more efficient depending on the target solution. Figure 3 shows the application range of AOPs depending on the organic matter content and the flow rate of the effluent to be treated. As it can be observed in the figure, UV/H_2O_2 and ozonation, are recommended to treat solutions at low flow rates and low organic carbon content. On the other hand, biological treatments are suggested when effluents with high flow rate are used. Finally, the combination of an AOP with biological treatment is recommended for a medium organic content and an intermediate flow rate [30]. AOPs could be incorporated as a pretreatment process for subsequent biological treatment because they can break down the refractory organics into simpler and biodegradable molecular forms [32]. The WWTP effluents treated in this thesis by UV/H_2O_2 and ozonation integrated in some works with a biological treatment were in accordance to the Figure 3.

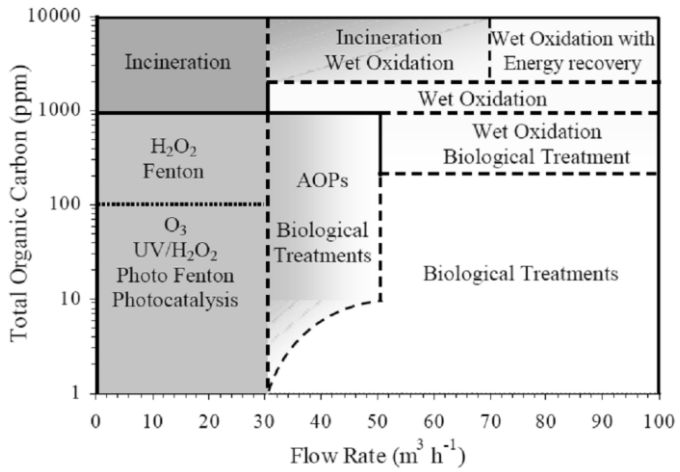

Figure 3. Recommended AOPs treatments depending of organic carbon content and flow rate (adapted from Hancock et al. [30]).

1.2.1. Hydroxyl radical

Many strong oxidants are "free radicals", of which hydroxyl radical is the most powerful oxidizing species after fluorine [33]. A list with the most oxidizing potential species is shown in Table 3.

Table 3. Oxidation potential of common species [33].

Species	Oxidation potential (V)
Fluorine	3.03
Hydroxyl radical	*2.80*
Atomic oxygen	2.42
Ozone	2.07
Hydrogen peroxide	1.78
Perhydroxyl radical	1.70
Permanganate	1.68
Hypobromous acid	1.59
Chlorine dioxide	1.57
Hypochlorous acid	1.49
Chlorine	1.36

Unlike many other radicals, hydroxyl radical is non-selective and thus readily attacks a large group of organic chemicals to convert them to less complex and less harmful intermediate products. At sufficient contact time and proper operation conditions, it is practically possible to mineralize the target pollutant to CO_2, which is the most stable end product of chemical oxidation. The remarkable advantage of AOPs over all chemical and biological processes is that they are totally ''environmental-friendly'' as they neither transfer pollutants from one phase to the other (as in chemical precipitation, adsorption and volatilization) nor produce massive amounts of hazardous sludge (as in activated sludge processes) [34].

According to Pignatello et al. [29,35], the hydroxyl radical promotes oxidation through three different pathways:

- Hydrogen atom abstraction from C-H, N-H or O-H bonds obtaining water as product. This reaction takes place typical in the presence of alkanes and alcohols.

$$HO^{\cdot} + R - H \rightarrow H_2O + R^{\cdot} \quad (1)$$

- Electrophilic addition of $HO^{\cdot}$ radical to a C=C double bond, or to an aromatic ring, hydroxylation. It takes places in the presence of alkenes and aromatic compounds.

$$HO^{\cdot} + = \rightarrow HO - C^{\cdot} \quad (2)$$

$$HO^{\cdot} + PhX \rightarrow HOPhX^{\cdot} \quad (3)$$

- Electronic transference to $HO^{\cdot}$ radical, although this path happens rarely with organic compounds.

$$HO \cdot + R - X \rightarrow RX^{\cdot +} + HO^{-} \quad (4)$$

The way that hydroxyl radical oxidize organic matter consists on a series of chain reactions that generated organics radicals that keep their degradation process until it ultimately became CO_2 and H_2O.

Aromatic molecules with electron donor groups (-OH, -NH_2, etc.) react faster than those with electron acceptor groups (-NO_2, -COOH, etc.) [36]. There are certain organic substances that cannot be oxidized by hydroxyl radical, mainly short chain molecules such as acetic, maleic, and oxalic acid, acetone or simple organochlorine species, i.e. chloroform or tetrachloroethane [37].

One of the main disadvantages of all oxidative degradation processes based on the reactivity of hydroxyl radicals, apart from the cost involved in these technologies, is the scavenging of $HO^{\cdot}$ radicals (consumption of radicals to the detriment of the organic load) by HCO_3^-, CO_3^- ions and other salts [29]. Even an excess of H_2O_2 could also act as hydroxyl scavenger, producing the formation of hydroperoxyl radical ($HO_2^{\cdot}$), that has a much lower reactivity than $HO^{\cdot}$.

$$HO^{\cdot} + HCO_3^- \rightarrow H_2O + CO_3^{\cdot -} \quad (5)$$

$$HO^{\cdot} + CO_3^- \rightarrow HO^- + CO_3^{\cdot -} \quad (6)$$

1.2.2. UV/H_2O_2

The most commonly accepted mechanism for the photolysis of H_2O_2 is the cleavage of the peroxide molecules into hydroxyl radicals with a quantum yield of two $HO^{\cdot}$ radicals formed per quantum of radiation absorbed as can be seen in Equation 7. The dissociation energy to break a O-O bond is 213 kJ mol^{-1}, which implies the use of low wavelength radiation (200-280 nm) to be an efficient process [29].

$$H_2O_2 + h\nu \rightarrow 2HO^{\cdot} \quad (7)$$

The rate of photolysis of aqueous H_2O_2 has been found to be pH dependent and increases when more alkaline conditions are used, probably due to the higher molar absorption coefficient of the peroxide anion respect to the hydrogen peroxide at 253.7 nm. Nevertheless, hydrogen peroxide is known to decompose by a dismutation reaction (Equation 8) with a maximum rate at the pH of its pK_a value (11.6).

$$H_2O_2 + HO_2^- \rightarrow H_2O + O_2 + HO^{\cdot} \quad (8)$$

Reactions of hydroxyl radicals generated in presence of an organic substrate may be differentiated by their mechanisms into the three mentioned classes (section 1.2.1): hydrogen abstraction, electrophilic addition and electron transfer. Moreover, radical-radical recombination must also be taken into account [29].

$$2HO^{\cdot} \rightarrow H_2O_2 \quad (9)$$

The sequence of reactions occurring during the UV/H_2O_2 process used for the oxidation of organic substrates is shown in Figure 4.

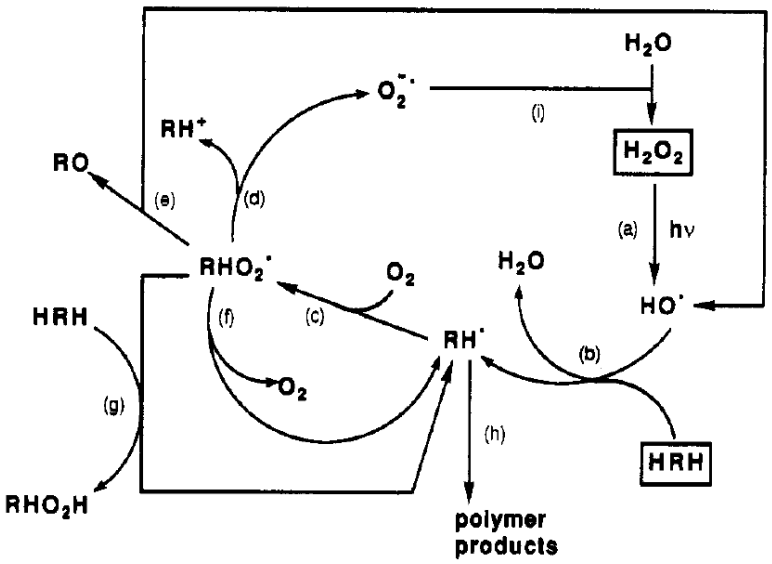

Figure 4. Reaction system for the UV/H_2O_2 process [29].

- **Hydrogen abstraction**

  Hydroxyl radicals generated by hydrogen peroxide photolysis (*a* from Figure 4) react with organic compounds (HRH) primarily by hydrogen abstraction to produce an organic radical ($RH^{\cdot}$) (*b* from Figure 4 and Equation 1). This radical reacts quickly with dissolved oxygen to yield an organic peroxyl radical ($RHO_2^{\cdot}$) (*c* from Figure 4), initiating subsequent thermal oxidation reactions. Peyton et al. [38] proposed three different reaction paths to be followed by either peroxyl radicals or their tetraoxide dimers: (1) heterolysis and generation of organic cations as well as superoxide anion (*d* from Figure 4), (2) 1,3-hydrogen shift and homolysis into hydroxyl radicals and carbonyl compounds (*e* from Figure 4), and (3) back reaction to $RH^{\cdot}$ and O_2 (*f* from Figure 4).

  Nevertheless, hydrogen abstraction by $RHO_2^{\cdot}$ should not be discarded as a process of initiating a chain of thermal oxidation reactions (*g* from Figure 4). In aqueous systems, cations will further react by solvolysis, and superoxide anion will readily disproportionate to yield H_2O_2 (*i* from Figure 4). This is in contrast to the fate of superoxide anion in AOPs utilizing ozone where it reacts primarily with ozone to produce hydroxyl radical.

- **Radical-radical reactions**

  Generated at high concentration, hydroxyl radicals will readily dimerize to H_2O_2 (Equation 10). If an excess of H_2O_2 is used, $HO^{\cdot}$ radicals will produce hydroperoxyl radicals (Equation 11) which are much less reactive and do not appear to contribute to the oxidative degradation of organic substrates.

$$HO^{\cdot} + HO^{\cdot} \rightarrow H_2O_2 \quad (10)$$

$$H_2O_2 + HO^{\cdot} \rightarrow H_2O + HO_2^{\cdot} \quad (11)$$

- **Electrophilic addition**
  Electophilic addition of $HO^{\cdot}$ radicals to organic π-systems will lead to organic radicals (Equation 3) the subsequent reactions of which are quite similar to those mentioned in Figure 4.

- **Electron-transfer reactions**
  Reduction of hydroxyl radicals to hydroxide anions by an organic substrate (Equation 4) is of particular interest in the case where hydrogen abstraction or electrophilic addition reactions may be disfavored by multiple halogen substitution or steric hindrance.

The use of hydrogen peroxide as an oxidant provides a number of advantages in comparison with other methods of chemical or photochemical water treatment: commercial availability of the oxidant, thermal stability and storage *in situ*, infinite solubility in water, lack of mass transfer problems associated with gases, two hydroxyl radicals are formed for each molecule of H_2O_2 photolyzed, peroxyl radicals are generated after HO· attack on most organic substrates leading to subsequent thermal oxidation reactions, minimal capital investment, very cost-effective source of hydroxyl radicals, and simple operation procedure [29].

There are, however, obstacles encountered with the UV/H_2O_2 process. In fact, the rate of chemical oxidation of the contaminant is limited by the rate of formation of hydroxyl radicals, and the rather small absorption cross section of H_2O_2 at 254 nm is a real disadvantage, particularly, when organic substrates will act as inner filters. Special care in process and reactor design must be taken in order to ensure optimal oxygen concentration in and near the irradiated reactor volume [29].

1.2.3. Ozonation

Ozone is a strong oxidant (see Table 3) able to take part in many chemical reactions with organic and inorganic substances through its *in situ* generation. The ozone molecule is an oxygen triatomic molecule that is commonly represented with two Lewis resonance forms (Figure 5).

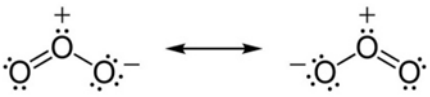

Figure 5. Lewis resonant representation of the molecule of ozone [39].

At room temperature, ozone is an unstable blue gas. It has a characteristic odor that can be detected by human at very low concentration. Its main physical and chemical characteristics are detailed in Table 4.

Table 4. Molecular properties of ozone.

Molecular formula	O_3
Molar mass (g mol^{-1})	47.998
Density (g L^{-1})	2.144 (at 0 °C, gas)
Melting Point (°C)	-192.5
Boiling Point (°C)	-111.9
Solubility in water (g L^{-1})	0.0105
Standard enthalpy of formation (kJ mol^{-1})	142.3
Standard molar entropy (J $K^{-1}mol^{-1}$)	237.7

The ozone transfer efficiency from gas to liquid phase is one of the most critical aspects during ozonation. This transfer is mainly controlled by physical parameters such as temperature, gas flow rate, bubble size, ozone partial pressure and reactor geometry, and also chemical factors like pH, ionic strength and composition of aqueous solutions, etc. [40]. A correct monitoring and control of these parameters is said to be fundamental to determine and achieve high reaction kinetics rates between ozone and organic compounds.

Once dissolved in water, ozone may react with pollutants according to two pathways: by direct reaction as molecular ozone or by indirect reaction through formation of secondary oxidants such as radical species. In practice, both direct and indirect oxidation reactions will take place at the same time. However, one type of reaction will dominate depending on various factors, such as temperature, pH and chemical composition of the water. For example, acidic conditions promote the ozone to react directly with certain functional groups from organic compounds through selective reactions. Meanwhile, in basic medium predominates the decomposition of ozone into hydroxyl radicals even more oxidant species that react unselectively with organic matter [39]. A scheme of these mechanisms and their interaction can be observed in Figure 6 (Adapted from Gottschalk et al. [41]).

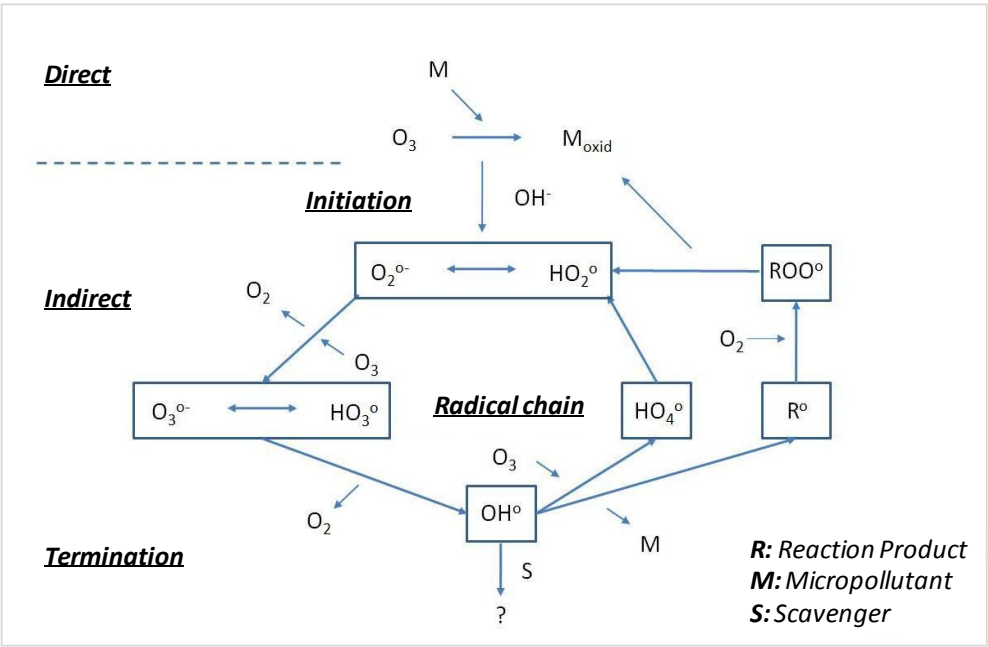

Figure 6. Ozone Reaction Pathways (Adapted from Gottschalk et al. [41]).

O_3 direct reaction

The direct oxidation of organic compounds by ozone is a selective reaction with slow reaction rate constants [41–44]. The selective reactions were ozone act as a dipole, electrophile or nucleophile is due to its resonant structure. Generally, organic compounds degradation takes place over unsaturated groups such as aromatic ring, alkenes, alkynes, etc. [39] and over aliphatic compounds with specific functional groups (i.e. amines and sulfides [45]).

- **Dipole attack: cycle-addition, Criegee mechanism**
  The ozone molecule reacts with the unsaturated bond due to its dipolar structure and leads to a splitting of the bond (1-3 dipolar cycle addition), leading to the formation of compounds named ozonide (Figure 7).

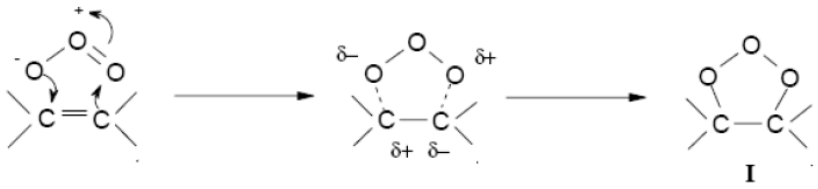

Figure 7. Ozone cycle addition reaction. 1st stage of Criegee mechanism.

In a polar solvent, such as water, the ozonide decomposes into a molecule substituted with a carbonyl group (aldehyde and a ketone) and other species that decomposes resulting in a hydroxyhydroperoxide that ultimately results in the formation of a carbonium compound and hydrogen peroxide (Figure 8).

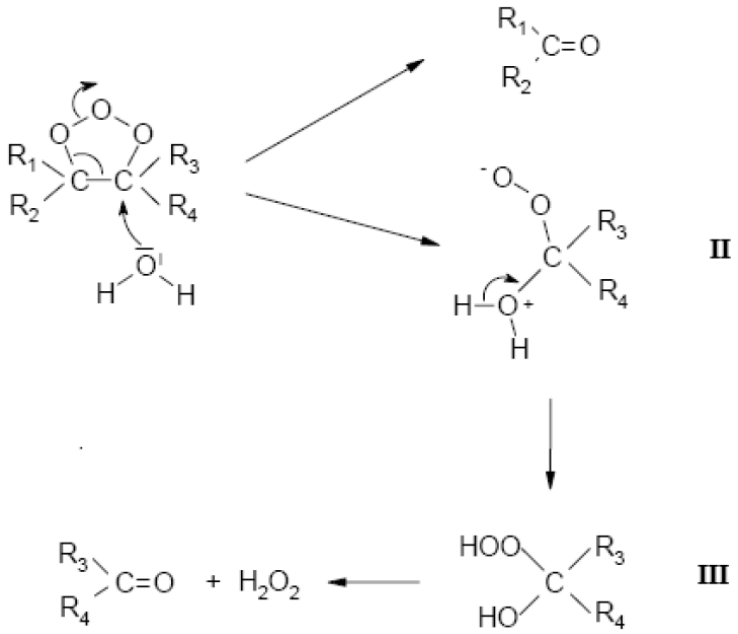

Figure 8. Decomposition of ozodine. 2nd stage of Criegee mechanism.

- **Electrophile attack**

  Electrophilic reactions occur in molecular solutions that have a high electronic density (Figure 9). Aromatics with electron-donating substituents are particularly prone to this type of reactions. Carbons located at positions *ortho*- and *para*- with respect to groups such as -OH, -NH_2, etc., have high charge density and are susceptible to ozone attack.

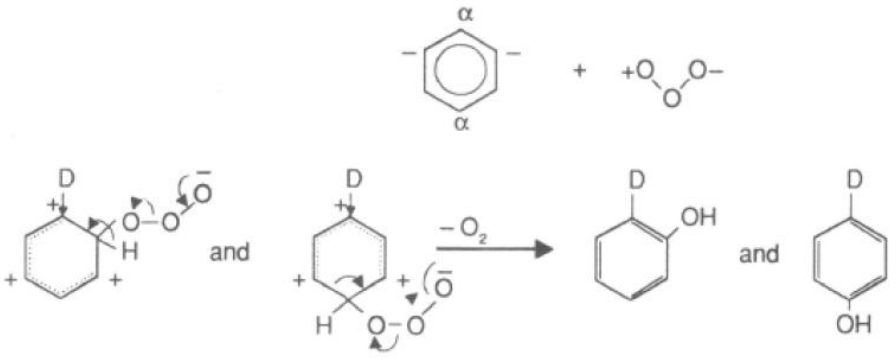

Figure 9. Electrophile reaction of ozone with aromatic compounds.

- **Nucleophile attack**

  Nucleophilic reactions take place mainly where there is a deficit of electrons and particularly at carbon compounds that contain electron-retreating groups, such as –COOH and –NO_2. For these electron-retreating groups, the reaction kinetics are much lower.

O_3 indirect reaction

The indirect reaction pathway involves radicals such as hydroxyl radical, which are unselective. In aqueous medium, OH^- promotes the decomposition of ozone with a subsequent series of chin reactions that lead the formation of hydroxyl radicals, among other radical species. The radical pathway is very complex and is influenced by many substances [41]. The mechanism can be divided in three steps: initiation, radical chain and termination [41].

- **Initiation step**

  The reaction between hydroxide ions and ozone leads to the formation of one superoxide anion radical $O_2^{\cdot-}$ and one hydroperoxyl radical $HO_2^{\cdot}$:

$$O_3 + OH^- \rightarrow O_2^{\cdot-} + HO_2^{\cdot} \qquad k_1 = 70\ M^{-1}s^{-1} \qquad (12)$$

  This hydroperoxyl radical is in an acid-base equilibrium:

$$HO_2^{\cdot} \rightleftharpoons O_2^{\cdot-} + H^+ \qquad pK_a = 4.8 \qquad (13)$$

- **Radical chain**

  The ozonide anion radical ($O_3^{\cdot-}$) formed by the reaction between ozone and the superoxide anion radical ($O_2^{\cdot-}$) decomposes immediately into a hydroxyl radical.

$$O_3 + O_2^{\cdot-} \rightarrow O_3^{\cdot-} + O_2 \qquad k_2 = 1.6 \cdot 10^9\ M^{-1}s^{-1} \qquad (14)$$

From this point, $O_3^{\cdot-}$ decomposes to different species depending on the pH of the media [45].

- For pH ≤ 8:

$$O_3^{\cdot-} + H^+ \rightleftharpoons HO_3^{\cdot} \qquad K_{3+} = 5 \cdot 10^{10}\ M^{-1}s^{-1};\ K_{3-} = 3.3 \cdot 10^2\ s^{-1} \qquad (15)$$

$$HO_3^{\cdot} \rightarrow HO^{\cdot} + O_2 \qquad K_4 = 1.4 \cdot 10^5\ s^{-1} \qquad (16)$$

- For pH ≥ 8:

$$O_3^{\cdot-} \rightleftharpoons O^{\cdot-} + O_2 \qquad K_{5+} = 2.1 \cdot 10^3\ s^{-1};\ K_{5-} = 3.3 \cdot 10^9\ s^{-1} \qquad (17)$$

$$O^{\cdot-} + H_2O \rightarrow HO^{\cdot} + OH^- \qquad k_6 = 10^8\text{-}2 \cdot 10^9\ M^{-1}s^{-1} \qquad (18)$$

Furthermore, ozone itself is susceptible to react with hydroxyl radicals leading to the formation of oxygen and hydroperoxide radical that can start the cycle all over again (Equations 19 and 20). Substances which convert $HO^{\cdot}$ into superoxide radicals $O_2^{\cdot-}/HO_2^{\cdot}$ promote the chain reaction; they act as chain carriers, the so-called promoters [41].

$$O_3 + HO^{\cdot} \rightarrow HO_4^{\cdot} \qquad k_7 = 2.0 \cdot 10^9\ M^{-1}s^{-1} \qquad (19)$$

$$HO_4^{\cdot} \rightarrow HO_2^{\cdot} + O_2 \qquad k_8 = 2.8 \cdot 10^4\ s^{-1} \qquad (20)$$

Organic molecules, R, can also act as promoters. Some of them contain functional groups which react with $OH^{\cdot}$ and form organic radicals $R^{\cdot}$.

$$H_2R + HO^{\cdot} + O_2 \rightarrow R + HO_2^{\cdot} + H_2O \qquad (21)$$

- **Termination step**

Some organic and inorganic substances react with the $OH^{\cdot}$ to form secondary radicals which do not produce superoxide radicals $HO_2^{\cdot}/O_2^{\cdot-}$ (Equations 22 and 23). These scavengers (or inhibitors) generally terminate the chain reaction and inhibit ozone decay.

$$HO^{\cdot} + {CO_3}^{2-} \rightarrow OH^- + {CO_3}^{\cdot-} \qquad k_9 = 4.2 \cdot 10^8\ M^{-1}s^{-1} \qquad (22)$$

$$HO^{\cdot} + {HCO_3}^- \rightarrow OH^- + {HCO_3}^{\cdot} \qquad k_{10} = 1.5 \cdot 10^7\ M^{-1}s^{-1} \qquad (23)$$

Another possibility to terminate the chain reaction is the reaction of two radicals:

$$HO^{\cdot} + HO_2^{\cdot} \rightarrow O_2 + H_2O \qquad (24)$$

1.2.4. AOPs application

Secondary effluents. Monitoring of Effluent Organic Matter (EfOM)

To date, most of the investigations dealing with the use of AOPs in water reclamation focus on the variation of overall water quality parameters, such as Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), COD, Biochemical Oxygen Demand (BOD) or turbidity. Related works that use UV/H_2O_2 and ozonation processes can be found in the literature [8,10,46,47].

To characterize the fate of NOM content, High-Performance Size Exclusion Chromatography has been used more widely than Liquid Chromatography – Organic Carbon Detection (LC-OCD) in studies that use ozone and $HO^{\cdot}$ radicals for the treatment of synthetic solutions containing Humic Substances (HS) [48], natural waters [49–51] and secondary effluents [52]. The use of LC-OCD seems to be more aimed at studies dealing with the largest NOM fractions which are responsible for membrane fouling. Only few literature related to the use of LC-OCD to detect or monitor NOM-constituents can be found (i.e., studies dealing with drinking water treatments [24,53], tertiary treatment for aquifer recharge [21] and photocatalytic oxidation of surface waters [54,55]). Although some authors have already studied the oxidation of NOM present in biotreated effluents by AOP [23,26,28,52], there is still a lack of information about the fate of LC-OCD organic matter fractions during advanced oxidation of municipal biotreated wastewaters.

RO brine effluents

Since there is an urgent need for environmentally friendly management options for RO brines, diverse technologies for the treatment of RO brines have been investigated, including coagulation/flocculation, AC adsorption, ozonation, UV/H_2O_2, Fenton, photocatalysis, sonolysis, electrochemical oxidation and river bank filtration, among others [26,56–59]. However, these studies focused on very diverse aspects of the technologies and there is still a lack of information about the efficiency of AOP to remove micropollutants from these complex matrices and to improve the quality of the effluents. Besides, there is also absence of data about the toxicity assessment of the treated RO brines.

AOP appear to be appropriate for the treatment of waste streams that are highly concentrated in recalcitrant micropollutants (i.e., streams that correspond to rejections higher than 98%).

As above mentioned, the sustainability of wastewater treatment methods should be eventually determined by economic factors. Regarding the RO treatment, the energy inputs that are expended in this technology and the fact that only around 25% of the RO inlet flowrate is treated should also be taken into account. Therefore, the UV/H_2O_2 process and ozonation, which are investigated in this study, appear to be good treatment options for minimizing the potential negative effect of this problematic effluent upon the environment and public health.

1.3. Biological treatment

Biological treatments of wastewaters are those processes in which microbial organisms are used to remove or reduce organic contaminants by breaking them down to simpler products. Bacteria are the most common organism in charge of the biodegradation in the biological treatments and their capability of treatment to biodegrade chemical compounds depends on a variety of factors such as product concentration, chemical structure, pH, Temperature, nutrients availability, Dissolved Oxygen, mixing, etc. Their good performance, together with their economical operability to decontaminate wastewater compared with other treatments, are the reasons why WWTP are using bioreactors as a part of their operation [60].

Biological processes can be classified into five groups depending on oxygen availability and consequently depending on which is the electron acceptor involved in the oxidation process of organic matter: aerobic, anaerobic, anoxic, a combination of this three in the same reactor but at different times, and pond processes (lagoon processes, in which the first three conditions are taking place simultaneously in different zones of the lagoon). Each group of biological process can be further subdivided in: suspended-growth processes, attached-growth processes, or a combination of both.

In this thesis, an aerobic and attached-growth biological process was performed to treat the RO brine with a preliminary advanced oxidation step (Appendix V).

1.3.1. Coupling AOP/Biological treatment

Nowadays there is a continuously increasing worldwide concern for development of alternative wastewater treatments and water reuse technologies. In this context, AOPs are considered a highly competitive water treatment technology for the removal of persistent organic pollutants, although chemical oxidation for the complete mineralization is usually expensive. On the other hand, it is generally assumed that not all polluting agents are removed through conventional biological treatments (more economics than advanced treatments) due to their high chemical stability and/or low biodegradability [61]. As a solution to these two issues, one attractive alternative would be to integrate both strategies. Chemical oxidation processes could be applied as a pre-treatment to convert the initially persistent organic compounds into more biodegradable intermediates, which would then be treated in a biological oxidation process with a considerably lower cost.

1.3.2. Activated Carbon (AC)

Carbon has been used as an adsorbent for centuries. Early uses of carbon were reported for water filtration and for sugar solution purification [62]. AC ability to remove a large variety of compounds from contaminated waters has led to its increased use in the last thirty years. AC has been recognized as one of the most popular and widely used adsorbent in water and wastewater treatment throughout the world [62].

The adsorption process uses forces of molecular attraction to bind soluble and gaseous chemicals to a surface. The process retains and accumulates toxic chemicals present in wastes, yet does not chemically alter them. Carbon used for adsorption is usually treated (activated) to make it very porous. It can be prepared from almost any carbonaceous material (such as wood, coal, lignite and coconut shell) by heating it with or without addition of dehydrating chemicals in the absence of air [62]. This causes the formation of tiny fissures or pores [63], creating a large surface area, where it can adsorb relatively large quantities of material per unit weight of carbon. The AC surface is non-polar which results in an affinity for non-polar adsorbates such as organics. The high surface area, large porosity, well developed internal pore structure consisting of micro-, meso- and macropores (Figure 10) as well as a wide spectrum of functional groups present on the surface of AC make it suitable for the removal of numerous contaminants [62].

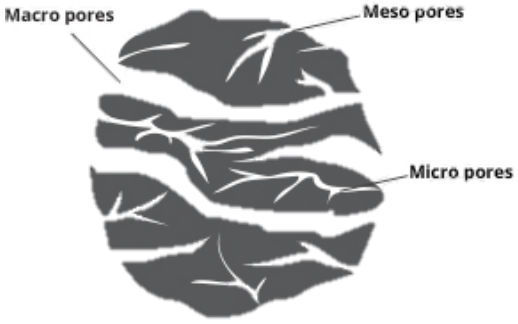

Figure 10. Schematic structure of AC.

AC is a material mostly derived from charcoal. There are two types of AC: Powdered Activated Carbon (PAC) and Granular Activated Carbon (GAC) [64]. PAC is added to industrial wastewater sludge systems to adsorb toxics and in some cases, as a secondary effect, biodegrade compounds. Spent PAC is difficult to regenerate and usually must be disposed. GAC is generally placed in vessels that form a filter “bed”. A GAC bed treats a liquid or aqueous solution/mixture by passing it through the bed. GAC can generally be regenerated.

In fixed bed adsorption, at any given time the bed can be divided into three approximate zones, i.e., the saturated zone (containing carbon nearly saturated with the pollutants), followed by the adsorption zone (were adsorption actually takes place), followed by zone in which the carbon contain little or no adsorbed pollutant. As more wastewater pass through the bed, the adsorption zone expands progressively through the AC as it is becoming saturated. Figure 11 shows the concentration profile and the expansion of saturated zone of a fixed-bed AC absorber.

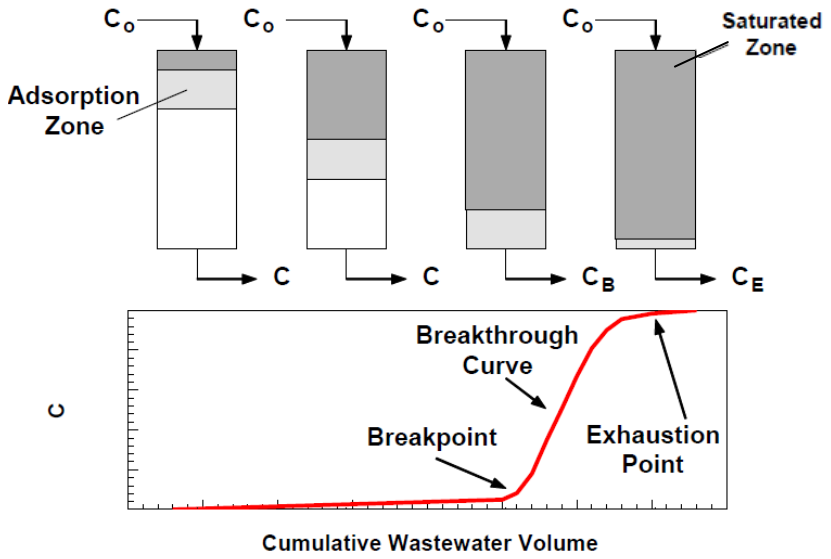

Figure 11. Concentration profile and the expansion of saturated zone of a fixed-bed AC absorber.

Biological Activated Carbon (BAC)

GAC filters have been used for long time to remove by adsorption undesirable organic compounds including biodegradable organic matter, micropollutants, halogenated hydrocarbons and taste and odor compounds [65]. GAC offers an effective mean to remove organic compounds due to its irregular creviced, porous particle shape and affinity for attaching to itself most organics even at low concentrations [66,67]. However, one of the major limitations of GAC is saturation which implies the need to regenerate it, with the economic costs that it entails. On the other hand, crevices and macropores of AC are also an excellent support material for the development of microbial biofilms as they provide protection from shear stress to microorganisms colonies [65–68]. Such colonized filters are referred in the literature as Biological Activated Carbon (BAC) filters. When the GAC media particles start becoming exhausted for adsorption, the rough porous surfaces are amenable to indigenous microbial communities establishment. This transition from GAC to BAC filter is a time-dependent process where simultaneous adsorption and biodegradation processes can coexist [65,66,69]. Precisely, biodegradation mechanism consists on a first adsorption of organic matter, removed from water into macropores, where it is detained long enough to promote its slow biodegradation by attached bacteria [66]. This process extends GAC service life and decrease backwash frequency being the main benefits of BAC filters [67]. Pre-oxidation of high recalcitrant effluents prior to BAC filtration is a commonly used combination. It results in an increase of the biodegradability of the inlet effluent, therefore promotes biological activity of the biofilm and consequently extends GAC media life [27,67,70,71]. The previous reasons leaded to the selection of BAC filtration technology integrated with AOPs for the treatment of RO brines (Appendix V).

Active biomass characterization is important during BAC filter processes in order to establish connections between the degradation process and the biomass involved. Various methods have been used by different authors to assess the biomass activity. These include heterotrophic plate counts [72], phospholipid extraction method [73], Adenosine Tri-

Phosphate (ATP) analyses [65,70,74] and respirometric measurements [69,75] among others. Likewise, determination of microbial communities is also essential information for a better understanding of BAC filters performance. Few studies have been conducted using culture-dependent methods on drinking water BAC applications [74,76,77]. However, only a very small fraction of microorganisms in the environment is cultivable on the commonly applied media. Culture-independent molecular methods are therefore preferred above culture-dependent in most sorts of microbiological investigations in wastewater treatments [77,78]. Within them, 16S ribosomal Ribonucleic Acid (rRNA) gene clone library analysis [74,77,79,80] and Fluorescence in Situ Hybridization (FISH) [81,82], with rRNA-targeted, probes are known to be very powerful tools for the identification of microorganisms in microbial biofilms.

2. OBJECTIVES

Water scarcity and water quality are one of the problems that cause greater concern in our society. For this reason, wastewater reclamation and reuse may play an important role in the development of strategies for the utilization of water resources. Different degrees of treatment will be necessary depending on the final use of this reclaimed water.

The main objective of this thesis is the assessment of some advanced processes applied to different WWTPs effluents which are usually discharged to surface waters, sea and oceans. The final objective is the water reclamation and/or the minimization of the contamination to the aquatic medium. The effluents from WWTPs are usually characterized for its low biodegradability ratio making appropriate the use of AOPs. The AOPs employed were UV/H_2O_2 and ozone.

This main objective has been divided in four specific research works:

1) *To study the fate of the EfOM present in secondary effluents during oxidation by UV/H_2O_2 and ozonation using LC-OCD technique (Appendix I):*

   LC-OCD technique leads to obtain specific quantitative and qualitative information about Dissolved Organic Matter (DOM) fractions during the oxidation process. Two different effluent waters were used and compared in these experiments, including (i) secondary effluent from a Conventional Activated Sludge (CAS) process, and (ii) effluent from a Membrane Biological Reactor (MBR) (ultrafiltration).

2) *To evaluate the performance of treating reclamation RO brines by AOPs and to monitor pharmaceuticals oxidation (Appendix II and III):*

   In a preliminary study, the performance of UV/H_2O_2 process and the biocompatibility of the resultant effluent (using Sequencing Batch Reactor, SBR) were evaluated when treating a RO brine from a municipal WWTP. The optimal operational conditions for UV/H_2O_2 process and the viability of the combination were determined (Appendix II).

   In the next work, the same effluent was subjected to ozonation and UV/H_2O_2 treatments. Different pharmaceuticals were identified in the RO retentate and also the decrease in the concentration of the pharmaceuticals was determined for different oxidant doses. Samples were analyzed using high performance Liquid Chromatography coupled to tandem Mass Spectrometry (LC–MS/MS) (Appendix III).

3) *To combine chemical analyses and bioassays in order to characterize the removal of pharmaceuticals from RO brine by AOPs (Appendix IV):*

   In parallel with the study in Appendix III, chemical analyses and bioassays were combined to characterize the removal of contaminants from RO brine using UV radiation and the two AOPs selected. Bioassays provide the unique possibility to monitor water (and other matrices) quality based on the biological activity of the putatively present pollutants,

rather than depending on their specific structural nature. The bioassays used were: Microtox®, antimicrobial activity test and Recombinant Yeast Assays (RYA).

4) *To assess the integration of BAC filters (and a Moving-Bed Biofilm Reactor (MBBR)) with AOPs when treating RO brines (Appendix V and VI):*

Finally, taking advantage of the biodegradability enhancement achieved by AOPs, the integration of BAC filters with an AOP pre-oxidation step was performed in order to minimize the environmental impacts associated with the direct discharge of reclamation RO brine. The performance on the removal of micropollutants and DOC using exclusively a BAC filter was compared with the performance of two integrated systems which consist of UV/H_2O_2 or ozonation coupled with a BAC filtration. ATP and FISH were applied to ensure and assess the biological activity in GAC filters (Appendix V).

In addition, the performance of a MBBR fed with RO concentrate, previously subjected to other chemical oxidation processes (O_3, O_3/UV, H_2O_2/O_3, O_3/H_2O_2/UV), was also investigated in order to obtain a regenerated effluent (Appendix VI).

3. MATERIALS AND METHODS

3.1. Experimental devices

3.1.1. UV/H_2O_2 experiments

The UV/H_2O_2 experiments were performed in a jacketed 2-L glass reactor, equipped with three mercury low pressure lamps placed inside (*Philips TUV 8W, G8T5*). A magnetic stirrer provided homogeneous conditions inside the tank. The photon flow was measured by an uranyl-oxalate actinometry and the results were expressed in Einstein s^{-1} (see sections of materials and methods of each paper) [83]. The reactor was always covered with foil paper to avoid sunlight interference in the reactions. The following figure (Figure 12) shows a simplified scheme of the UV/H_2O_2 installation [84].

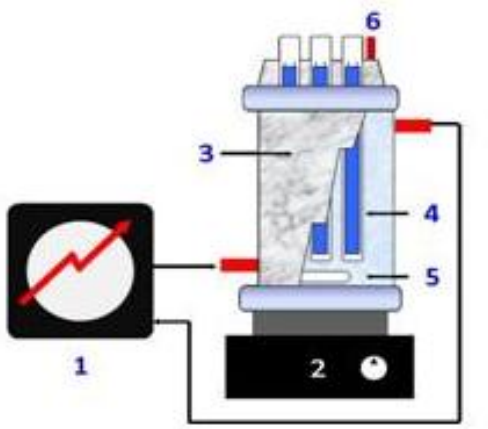

Figure 12. Scheme of the UV/H_2O_2 installation: (1) thermostatic bath, (2) magnetic stirrer, (3) alumina foil, (4) Hg low pressure lamps, (5) reactor, (6) sampling orifice. [84]

Hydrogen peroxide concentration (Panreac 30% w/v) was determined through the metavanadate spectrophotometric procedure at 450nm [85].

3.1.2. Ozonation experiments

Ozonation experiments were performed in a 2-L glass reactor operated in semi-batch mode equipped with a thermostatic jacket. Homogeneous concentrations and temperature inside the reactor were achieved by magnetic stirring. The complete ozonation set-up is described elsewhere [86]. Ozone was produced from pure dry oxygen by electrical discharges (Sander Labor Ozonizator, Sander, GERMANY). Two stainless steel microporous diffusers injected the gas mixture at the bottom of the reactor. Inlet gas pressure and temperature were continuously measured to standardize the ozone doses. Ozone monitoring at the reactor inlet and outlet in the gas phase was achieved using UV analyzers (BMT 963 and BMT 964, BMT Messtechnik Gmbh, GERMANY). Dissolved ozone concentration was measured in some experiments by means of a selective polarographic membraned sensor (Q45H/64, Analytical Technology Inc., UK) located on a recirculation loop with flow rate of 50 L h^{-1}. Liquid and gas phase ozone concentrations were recorded each 10 seconds using a data logger.

Finally, residual ozone was destructed on the catalyst analyzers and or in a solution of acidified potassium iodide in order to avoid its rejection to the atmosphere. The schematic diagram of the lab-scale ozonation system used to carry out the study is shown Figure 13 [86].

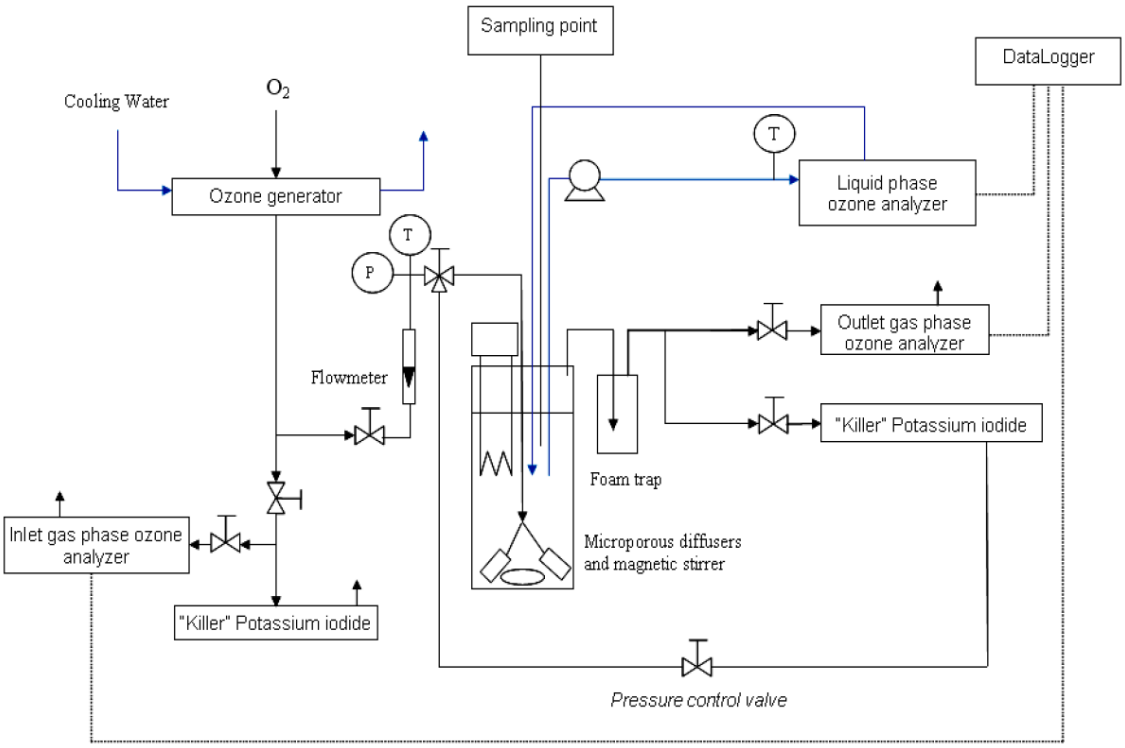

Figure 13. Scheme of the experimental installation for the ozonation experiments [86].

In order to know the oxidant doses applied, the Transferred Ozone Dose (TOD) was calculated. The TOD is the accumulated ozone transferred to the water sample per unit of sample volume. It corresponds to the sum of ozone consumed during the treatment with the residual dissolved ozone at the end of the reaction. It is defined by the following equation and it was determined with the trapezoidal method of numerical integration (Equation 25):

$$TOD = \int_0^t \frac{Q_{gas}}{V_{liq}} \cdot \left([O_3]_{gas\ in} - [O_3]_{gas\ out}\right) \cdot dt_r \qquad (25)$$

where Q_{gas}, V_{liq} and t_r are the gas flow rate, the effluent volume and the reaction time. $[O_3]_{gas}$ in and $[O_3]_{gas}$ out are the ozone gas phase concentrations at the inlet and outlet of the reactor, respectively.

3.1.3. BAC filters

Three biological filters were operated in order to treat the RO brine. Two of them were fed with the resultant effluent from each assayed AOP (UV/H_2O_2 and ozonation) and the other one was fed with RO raw brine. Filtrasorb® 400 agglomerated coal based GAC (Chemviron Carbon, Belgium) was used as a filter media. Its effective size was between 0.6-0.7 mm and its mean particle diameter was 1.0 mm. The set-up consisted of 3 cm inner-diameter glass columns packed with approximately 5 cm of GAC. All columns were protected from the light to

minimize the potential effects of photodegradation and were run under aerobic conditions by aerating the feeding solution (until saturation) just before its entry into the columns. The columns were fed at an average flow rate of 0.79 mL min^{-1}. Contact time resulted 13.4 min (Empty Bed Contact Time (EBCT): 44.7 min). Since this study was focused on biodegradation as a removal mechanism, the GAC was soaked during few days in the WWTP RO brine tank to be close to its saturation state before packaging columns. After that, columns were initially inoculated with secondary sewage sludge in order to accelerate the colonization process. The startup strategy is detailed elsewhere [87].

Figure 14 shows the experimental setup of the BAC filters, where only operated the first, third and fifth columns.

Figure 14. Experimental setup of BAC filters.

3.2. Analytical methods

The water quality parameters used and compared in all the works done in this thesis were described below. The specific central techniques performed in each study were also described.

3.2.1. Determination of Total Organic Carbon (TOC)

The TOC content of samples was measured with a Shimadzu 5055 TOC-VCSN analyzer by means of catalytic combustion at 680 ^{o}C. The device was equipped with an ASI-V Autosampler (Shimadzu). TOC was measured as mg CO_2 L^{-1}. For this reason inorganic carbon had to be previously removed bubbling air throughout the sample after acidification with HCl.

DOC is also measured in this device. The difference is the previous filtration of the sample through 0.45 μm PES filters.

3.2.2. Determination of Chemical Oxygen Demand (COD)

COD analyses were performed to determine the amount of oxygen required to oxidize organic matter of a solution by means of strong oxidant agents. These tests were carried out following the Standard Method 5220 D: closed reflux and colorimetric method [88]. The method consists of heating at high temperature (150 ^{o}C) a known volume of sample with an excess of potassium dichromate in presence of H_2SO_4 over a period of 2 hours in a hermetically sealed glass tube. The dichromate was in excess, and thus, the organic matter was oxidized and dichromate was reduced to Cr^{3+}. Furthermore, to avoid possible interference of chloride in the sample, silver sulfate was also added. The residual chrome IV was then measured by colorimetry in a spectrophotometer (Hach Odyssey DR/2500) at 420 nm (low COD method).

3.2.3. UV Spectrophotometry (UV_{254})

UV spectrophotometry is a technique to measure the absorbance of a solution exposed to UV to one or more than one wavelengths (UV_{254}). Each component in a solution shows a characteristic absorbance that makes it possible to determine the presence of certain compounds. The spectrophotometer used was *Perkin Elmer UV/Vis*, controlled by *Lambda 20* software. The UV_{254} parameter consists of measure the absorbance at 254 nm wavelength, usually an indicator of the level of unsaturated carbon bonds (aromaticity) of organic compounds such as aromatics, phenols, poliaromatic hydrocarbons, ketones, aromatic aldehydes, etc. [89].

3.2.4. Determination of Biochemical Oxygen Demand at 5 days (BOD_5)

The test performed to quantify the biodegradability of the samples was the BOD at 5 days (BOD_5), using Oxitop® manometric system bottles and following the procedures described in the Standard Methods 5210D for respirometry analysis [88]. This method measures the consumed oxygen by the microorganism per water volume unit during 5 days at a fixed temperature of 20 ^{o}C. The Oxitop® analysis uses the cap of the bottle as a manometer, which relates the oxygen consumption with the pressure changes at the constant volume of the flask. During the microbial activity, the CO_2 produced by the bacteria is eliminated by adding two pellets of NaOH as a strong alkaline agent. This pressure drop is monitored by the manometer.

3.3. Liquid Chromatography - Organic Carbon Detector (LC-OCD) technique

The typical water quality parameters that are used to characterize DOM in water, such as TOC, DOC, COD, BOD, and Ultraviolet-visible spectroscopy, are not always sufficient or satisfactory. Therefore, samples treated in the first work in this thesis (Appendix I), secondary effluent, were also analyzed with the LC-OCD technique to obtain specific quantitative and qualitative information about DOM fractions during the oxidation process. The quantitative information is based on a continuous carbon mass determination that is similar to TOC analysis. This measurement is performed with an Organic Carbon Detector. The qualitative analysis consists of a gel chromatographic separation of DOM prior to analysis. This separation takes place with Size Exclusion Chromatography, in which substances with smaller molecular sizes can access more of the internal pore spaces than those with larger sizes. LC-OCD uses UV detection, a Spectral Absorption Coefficient determination at 254 nm and Organic Nitrogen Detection. Combined, these analyses are used to identify the organic fractions that are summarized in Table 5 [90]. Figure 15 shows a typical chromatogram of surface water.

Table 5. Characteristics of DOM fractions in water (Appendix I).

DOM Fraction	Molecular weight	Characteristics
Polysaccharides (Biopolymers (BP))	> 50000 – 2 Mio. g mol^{-1}	Hydrophilic and not UV-absorbing; may be associated with amino acids and proteins.
Humic Substances (HS)	100 – 100000 g mol^{-1}	Consists of humins (non-soluble), humic acids (insoluble in acids) and fulvic acids (soluble in acids) in varying amounts.
Building Blocks (BB) (HS-Hydrolysates)	350 – 500 g mol^{-1}	Intermediates in the degradation process fulvic acids – BB – low molecular weight organic acids.
LMM* organic acids (LMMA)	< 350 g mol^{-1}	Final degradation product of organics, but also released by algae and bacteria.
LMM neutrals and Amphiphilics (LMMN)	< 350 g mol^{-1}	Slightly hydrophobic (amphiphilic), such as alcohols, aldehydes, ketones and amino acids.

*LMM: Low Molar Mass

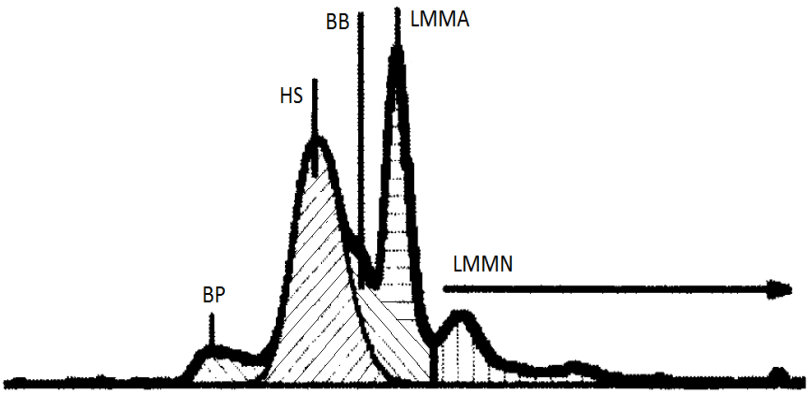

Figure 15. Typical chromatogram of surface water (adapted from Batscher et al. [91]).

3.4. Pharmaceutical analysis

Some pharmaceuticals were analyzed in the RO brine (Appendix III, IV and V). Samples were vacuum filtered through 0.7 μm glass fiber filter by 0.45 μm nylon membrane filters. An aqueous solution of 5% Na_2EDTA was added to achieve a final concentration of 0.1%. Then, aliquots of water were spiked with surrogates, and pre-concentrated onto lipophilic-hydrophilic balanced Oasis HLB (200 mg, 6 mL) cartridges from Waters Corporation (MA, U.S.A.). Pre-concentration was carried out by automatized solid phase extraction system (ASPEC GX-271, Gilson). The cartridges were rinsed with 5mL of HPLC grade water and dried under vacuum for 20 min. The elution was carried out with 8 mL of methanol. Extracts were evaporated to dryness and reconstituted with 1mL of methanol/water, 1/9. For the internal standard calibration, 10 μL of a 1 mg L^{-1} standard mixture of isotopically labeled compounds was added to the prepared samples, and analyzed using high performance LC-MS/MS. In appendix III, LC analysis was performed using an Agilent HP 1100 HPLC (Palo Alto, CA, USA), which was equipped with an autosampler and connected in series to a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer, equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved using a Purospher StarRP-18 endcapped column (125 mm × 2.0 mm, particle size 5 μm), preceded by a C_{18} guard column (4 × 4, 5 μm), both supplied by Merck (Darmstadt, Germany). On the other hand, LC-MS/MS analysis in Appendix IV and V, were performed using a Transcend LC system coupled to triple quadrupole TSQ Vantage, both from Thermo Fisher Scientific (CA, U.S.A.). Analytes were separated on a Halo C-18 endcapped column (50 mm × 2.1 mm, particle size 2.7 μm) preceded by a Halo C-18 guard column (5 mm × 2.1 mm, 2.7 μm). Tandem mass spectrometry detection was performed in multiple reactions monitoring mode using heated electrospray ionization.

3.5. Biological assays

3.5.1. Microtox

Acute toxicity of the different effluents was measured by Microtox® toxicity test, using luminescent *Vibrio fischeri* bacteria. Analysis was conducted according to the standard Microtox® test procedures recommended by the manufacturer (Azur Environmental, Delaware, USA). Toxicity is expressed as Effective Concentration that reduces the bioluminescence to 50 % (EC_{50}) value, the concentration of sample that causes a 50% reduction in light emission after 15 min of contact.

3.5.2. Recombinant Yeast Assays (RYA)

The human Estrogen Receptor – RYA (ER-RYA) (Appendix IV) was performed using the yeast strain BY4741 (MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0) from EUROSCARF (Frankfurt, Germany) transformed with plasmids pH5HE0 (hER) and pVitBX2 (ERE-LacZ) [92]. For the

human Aryl hydrocarbon Receptor - RYA (AhR-RYA), we used the YCM4 yeast strain [93], harboring a chromosomally integrated construct that co-expresses the hAHR and ARNT genes under the Gal1-10 promoter and the pDRE23-Z (XRE5-CYC1-LacZ) plasmid [94,95]. These two RYA assays were used to quantify the presence of estrogens and dioxin-like activity in samples, using already described protocols [94]. Briefly, a grown yeast culture was adjusted to OD of 0.1 and split into 100 µL aliquots in silylated 96-well polypropylene microtiter plates (NUNC™, Roskilde, Denmark) [92]. Sample extracts solved in methanol were applied to the wells in serial dilution scheme based on 1:3 dilution steps. Plates were incubated for 4 h at 30 °C under mild shaking. After incubation, 100 µL YPER™(PIERCE™, Rockford, IL, USA) was added to each well and further incubated at 30 °C for 30 min. Afterwards, 100 µL of assay buffer supplemented with 0.1% 2-mercaptoethanol and 0.5% of 4-methylumbelliferone β-D-galactopyranoside solution (Bio-Rad Laboratories, Hercules, CA, USA) was added to the lysed cells. Plates were read at 355 nm excitation and 460 nm emission wavelengths. Fluorescence was recorded for 11 min (one measurement per 42 s); β-galactosidase activity values were calculated as rates of the increment of arbitrary fluorescence units with time, using standard linear regression methods. To test possible inhibitory activity, yeast cultures were incubated for 6 h with 10 nM estradiol (ER-RYA) or 1 µM β-naphthoflavone (AhR-RYA), added to a 1:60 dilution of each sample, and processed as before. Samples were tested in duplicate. Estrogenic or dioxin-like activities were calculated as estradiol equivalents (E2Eq., ER-RYA) or β-naphthoflavone equivalents (BNFEq., AhR-RYA), by adjusting the data to a first-order Hill equation, as described [95].

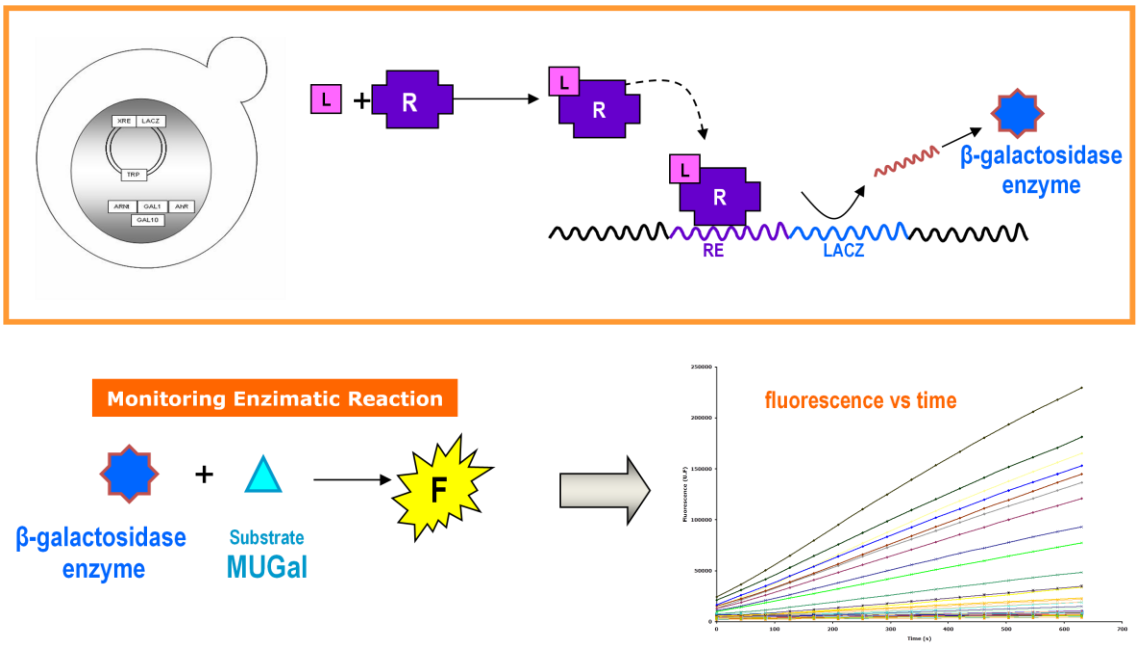

Figure 16. Scheme of the steps performed in a RYA.

Figure 16 was shown in order to illustrate the mechanism that takes place in RYA, based on a typical vertebrate response mechanism that has been introduced into a yeast cell. Once a compound (L) gets inside the cell, it is complexed by a particular Receptor (R). This complex is able to interact with Deoxyribonucleic Acid (DNA) at a specific site, the Response Element (RE). This union activates the expression of the LacZ gene that encodes for the β-galactosidase enzyme. As mentioned before, the presence of this enzyme in solution can be

monitored by adding a substrate and reading the fluorescence emitted by the enzymatic reaction. The slope of the function fluorescence vs time corresponds to the enzymatic activity of β-galactosidase. Activity is proportional to the amount of enzime produced due to the interaction of the compound L and the Receptor (ER or AhR).

3.6. Biofilm assessment

In Appendix V, measurement of ATP was used to validate the presence of biological activity in the GAC filters. ATP is used as primary energy currency by all organisms. Therefore, it can be used as an indicator of biomass content [70]. The procedure followed for liquid phase analysis was BacTiter-Glo™ Microbial Cell Viability Assay (Promega Biotech Iberica, Spain) using a GloMax® 20/20 luminometer (Promega Biotech Iberica, Spain). In short, BacTiter-Glo™ reagent and an equal volume of sample (100 μL : 100 μL) were transferred to an Eppendorf tube. Then, the content was mix on an orbital shaker for 3 min at room temperature, and finally the luminescence was recorded after 30 s and expressed as Relative Light Units (RLU). ATP was measured before and after sample filtration through 0.22 μm filter of polyethersulfone to quantify total and free ATP respectively; ATP present from living cells was determined by calculation [96]. The methodology followed to estimate the ATP contained on cells attached to GAC was based on that developed by Velten et al. [70]: 200 mg of GAC and 100μL of phosphate buffer were added to an Eppendorf tube. After 3 min orbital shaking at room temperature, 300 μL of BacTiter-Glo™ were added. After 3 more minutes of orbital shaking, 200 μL of the supernatant were transferred into a new Eppendorf tube and luminescence was measured after 30 s. ATP measurements were performed in triplicate.

Bacterial population phylum in backwashing waste of the three BAC filters during the last stage of the experimentation (Appendix V) was identified by FISH technique [78,97] followed by Scanning Confocal Laser Microscopy. Prior to hybridization, the biomass was fixed using 4% paraformaldehyde. The specific oligonucleotide probes (Thermo Fisher Scientific, Germany) used are defined in Table 1 (from Appendix V) and more details are available at probeBase [98]. Hybridizations were performed at 46 ^{o}C for 90min with different percentage of formamide (F) (Appendix V). The probes used for in situ hybridization were labeled with the dyes FLUOS (green) or Cy3 (red), and also 4',6-diamidino-2-phenylindole (DAPI) (blue) was added in order to fluorescence DNA. Fluorescence signals were recorded with an Axioskop 2 epifluorescence microscope (Zeiss, Germany). The different steps of the typical FISH protocol where summarized in Figure 17.

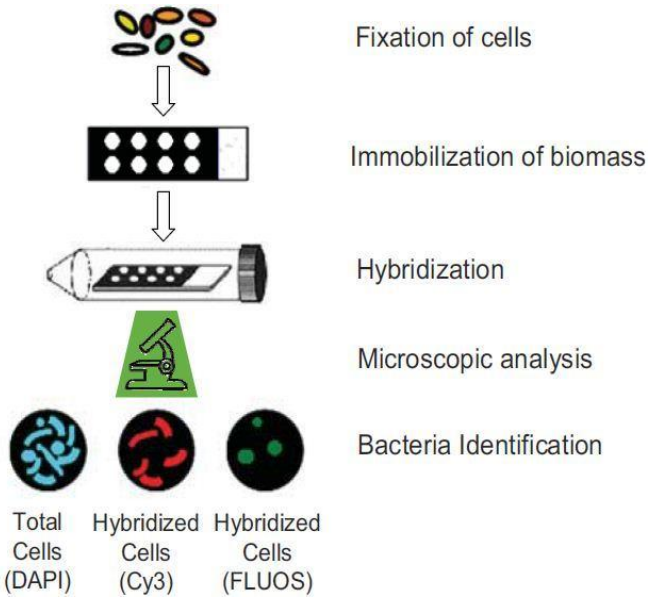

Figure 17. Different steps of the typical FISH protocol [99].

3.7. Studied WWTP effluents

3.7.1. Conventional Activated Sludge (CAS) effluent

In Appendix I, two different secondary effluent waters were used for the characterization and fate of EfOM when treated with UV/H_2O_2 and ozonation. The secondary effluent from a CAS process was from the WWTP of Gavà (Barcelona). The raw CAS effluent was filtered through a conventional filter paper to remove the largest particles.

3.7.2. Membrane Bioreactor (MBR) effluent

The second effluent treated in Appendix I, was from a MBR located in the Vallvidrera WWTP (Barcelona). The MBR combines the biological process of activated sludge with a physic separation, ultrafiltration.

The average physicochemical parameters of both secondary effluents used in the study of Appendix I are summarized in Table 6. The organic load of the CAS effluent was more than two times higher of the MBR effluent.

The LC-OCD characterization were also done for both secondary effluents and shown in Figure 1 of Appendix I. Significant differences in DOM composition were also observed (lower biopolymer and humic content in the MBR effluent).

Table 6. Average physicochemical parameters of the two secondary effluents assayed.

	CAS	MBR
TOC (mg C L^{-1})	17.4 ± 3.4	6.8 ± 1.5
DOC (mg C L^{-1})	15.2 ± 3.8	6.3 ± 0.9
COD (mgO_2 L^{-1})	49.7 ± 3.3	10.9 ± 0.01
pH	7.8 ± 0.1	7.4 ± 0.1
UV_{254} (m^{-1})	30.3 ± 0.9	16.3 ± 1.3
Turbidity (NTU)	2.1 ± 0.56	0.31 ± 0.20
Alkalinity (mg $CaCO_3$ L^{-1})	599 ± 24	432.00 ± 15
Cl^- ($mgCl^-$ L^{-1})	488 ± 30	275 ± 24

3.7.3. RO brine effluent

Two RO brines originate from two different WWTPs were treated in this experimental thesis. In Appendix II, III and VI, the RO brine was from a tertiary treatment in El Prat del Llobregat WWTP, Barcelona (Spain). The secondary effluent from the CAS treatment is subjected to coagulation/flocculation, lamellar settling, disinfection and ultrafiltration, before finally passing through an RO unit to produce indirect potable water quality. This water is reused to mitigate saline intrusion and to supplement groundwater supplies. The RO retentate is currently being discharged into coastal surface waters. On the other hand, the other two works were RO brine was also treated (Appendix IV and V), came from a tertiary treatment in a WWTP located in Tarragona (Catalonia, Spain). This secondary effluent from a CAS treatment is subjected to sand filtration, then microfiltration and finally part of this water fed a two stage RO system. A mixture of sand filtration effluent and RO permeate plus chlorination was suitable for golf court irrigation.

According to the different treatments applied in the two WWTPs, the origin of these wastewaters and the different seasons when the effluents were collected, some differences can be appreciated. Table 7 shows a summary of the typical water parameters of the RO brines treated. The organic load and alkalinity was slightly higher in the RO brine coming from the WWTP of El Prat de Llobregat.

Table 7. Summary of the water quality parameters of RO brines treated.

	RO brine Barcelona	RO brine Tarragona	
	Appendix III	Appendix IV	Appendix V
TOC (mg C L^{-1})	27.6	24.4	23.9
DOC (mg C L^{-1})	27.3	24.2	23.7
COD (mgO_2 L^{-1})	77.0	76.9	61.5
BOD_5 (mgO_2 L^{-1})	2.2	2.3	5.5
pH	8.3	7.4	6.9
UV_{254} (m^{-1})	59.5	46.4	40.5
Turbidity (NTU)	1.07	0.53	0.37
Alkalinity (mg $CaCO_3$ L^{-1})	914	583	308
Cl^- ($mgCl^-$ L^{-1})	1540	1511	1627

Regarding the pharmaceuticals concentrations, these were also higher in the RO brine coming from the WWTP located in Barcelona. The initial concentrations of the eleven pharmaceuticals analyzed ranged from 0.2 to 1.6 μg L^{-1} (Appendix III) and include anti-inflammatory drugs, analgesics, antidepressants, sulphonamides and beta blockers among others. The concentrations of the eleven pharmaceuticals (not all were the same) identified in the other RO brine treated form Tarragona ranged from 17 to 1275 ng L^{-1} (Appendix IV) and from 20 to 3443 ng L^{-1} in the work of Appendix V. The pharmaceuticals subgroups were similar.

4. RESULTS AND DISCUSION

Results and discussion section is divided in two parts. The first part corresponds to the results obtained in Appendix I, where the EfOM of a secondary effluent was characterized when treated by UV/H_2O_2 and ozonation. In the other part, the results concerning to the treatment of the RO brine effluent were summarized (Appendix II-VI). This second part was also divided in two sections, one regarding to the AOPs applied and the other one regarding to the integration of these with biological treatments performed.

4.1. Characterization and fate of EfOM treated with UV/H_2O_2 and ozonation (Appendix I)

During the oxidation processes, EfOM is modified and broken down into smaller compounds, which affect the characteristics of the treated effluent. In this study, DOM present in two secondary effluents from a CAS and a MBR system, was characterized and monitored during UV/H_2O_2 and ozonation reactions with the LC-OCD technique. The following DOM fractions were quantified: BP, HS, BB, LMMN and LMMA. Although both technologies were efficient for nearly eliminating the entire DOM at extended oxidation conditions, some differences were observed between them.

CAS effluent

- In both processes, a large reduction of BP occurred when treating CAS effluent. However, the fate of the different DOM fractions and their contribution to the final effluent composition differ between treatments.

- At the beginning of the UV/H_2O_2 treatment, the greatest decrease was observed in BP fraction, possibly due to their bigger size. Meanwhile, the LMMN, LMMA and BB concentration increased (the BB came exclusively from the break-down of HS). HS was the subsequent fraction that decreased in concentration after this first oxidation stage. The decrease in concentration of the smallest fractions did not begin until the BP and HS in the effluent were reduced. The LMM acid fraction was the last to decrease. At the end of the treatment (H_2O_2 consumption: 2.90 mM; 150 min of irradiation), the overall TOC removal was approximately 47.9%.

- Regarding the ozonation process, removal of BP but also HS and LMMN occurred from the first oxidation steps. LMMA accumulation also took place from the beginning of the ozonation. As the ozonation extended, the accumulation of LMMA increased to become the main fraction. At the end of the treatment (ozone consumption: 6.0 mM), the overall TOC removal was 59.9%.

- Certain observations were drawn from the fate of the DOM fractions during the advanced oxidation treatments. The UV/H_2O_2 oxidation process occurred through hydroxyl radicals, which are non-selective. Thus, the largest and most abundant organic fractions were

attacked to a greater extend. Following the non-selective oxidation mechanism, the final DOM contained all type of LMM compounds and HS.

- In contrast, oxidation was concentrated on the largest fractions (BP and HS) but also on the LMMN during ozonation from the beginning. However, the treatment efficiency and the removal rate of LMMA were clearly lower, which lead to their accumulation. The high alkalinity and the high chloride concentration in the medium would minimize the indirect ozone attack due to scavenging effects. Therefore, the molecular attack of ozone would govern the reaction and increase the selectivity of the oxidation process [39]. In conclusion, very severe oxidation of BP, HS and neutrals is necessary to begin the degradation of the less aromatic and unsaturated fractions in the ozonation process, while simultaneous oxidation of all fractions takes place during the non-selective hydroxyl radical oxidation.

- Although biodegradability increased during both treatments, it reached its highest value at the end of the ozone treatment. The higher biodegradability of the ozonated effluent occurred because 90.3% of the total DOM was LMM compounds and about 40% of them were LMMA, which are readily biodegradable compounds. The parameter biodegradability ratio is consistent with the LC-OCD results.

- As some authors found that Specific UV Absorbance (SUVA) can be used to indicate the effluent aromaticity and also as an indicator of the fraction of humic substances present in different water matrices [87,100,101]. For both treatments, the SUVA decreased during the first 30 min, resulting in poorly aromatic effluents, and then remained nearly constant until the end of the oxidation process. At that point, nearly all of the BP were very reduced in both effluents. However, the humic contents were not significantly reduced and accounted for the predominant organic fraction in both effluents. The treatments provided enough oxidation of the HS to achieve high aromaticity depletion, but were not able to induce sufficient cleavage to attain characteristic structure loss. Therefore, the complete depletion of HS and low SUVA depletion should not be directly correlated. Other authors also obtained the same results [102,103].

MBR effluent

- The evolution of the different DOM fractions in the MBR effluent during UV/H_2O_2 and ozone treatment was marked by the absence of BP, the attack at the beginning of the reaction was mainly directed at HS, which was the most abundant and aromatic fraction in the MBR effluent. However, some differences were observed between both oxidation treatments.

- In the UV/H_2O_2 treatment, the LMM neutral concentration decreased continuously throughout the reaction because the hydroxyl radical availability was larger and the concentration of the other larger organic fractions was smaller. Further oxidation led to the progressive degradation of small neutral compounds and, to a greater extent, BB.

- Regarding ozonation, no BB fraction increments were observed and a significant increase in LMM acid concentration occurred during the removal of the HS fraction. The higher

oxidant to carbon concentration ratio and the reaction selectivity for unsaturated compounds could explain the lack of BB accumulation in this effluent. Further ozonation led to the progressive and exclusive degradation of the LMMN fraction. This degradation resulted in a final DOM that was composed solely of LMMA, which proves their recalcitrance and accumulation during the ozonation of secondary effluents.

- The difference between the biodegradability ratios in the final MBR effluents that were obtained by ozonation and UV/H_2O_2 was even higher than in the CAS case due to the greater accumulation of LMMA during the ozonation of the MBR effluent.

- Again, the SUVA parameter decreased for both processes during the first 30 min. The decrease in SUVA corresponded to humic content removal, but not to the complete elimination of this fraction, which confirms the findings from the CAS case.

4.2. Treatment of reclamation RO brine

4.2.1. AOPs applied to reclamation RO brine

A preliminary study was performed in order to identify the importance of the HO· scavenging effect by H_2O_2 in the UV/H_2O_2 process when treating the reclamation RO brine (Appendix II). If an excess of H_2O_2 is used, HO· radicals will produce hydroperoxyl radicals (Equation 11) which are much less reactive and do not appear to contribute to the oxidative degradation of organic substrates. Generated at high concentration, hydroxyl radicals will readily dimerize to H_2O_2 (Equation 9).

Progressive dosage of H_2O_2 and the addition of the whole amount of H_2O_2 at the beginning of the reaction were compared. In addition, the contribution of UV radiation was evaluated since different time reactions for the consumption of the same amount of H_2O_2 were expected. The results suggested that adding the total amount of H_2O_2 at the beginning of the reaction was more appropriated since although a lower mineralization was observed, both the oxidation of the matrix and the biodegradability ratio were higher for the treatment without spikes, which means best conditions for biological combination. Overall eliminations obtained and necessary irradiation times also suggested that the non-spiked was the best option to carry out an AOP process combined with a biological one.

Pharmaceuticals and organic pollution mitigation in reclamation osmosis brines by UV/H_2O_2 and ozone (Appendix III)

In this study, reclamation RO concentrates from a municipal WWTP were subjected to ozonation and UV/H_2O_2 treatments. The eleven target pharmaceuticals were monitored by LC–MS/MS throughout the oxidation processes. The initial k_{obs} values, which provide information on pharmaceutical removal at the lowest initial oxidant doses, and the percentage removal of the pharmaceuticals at the different oxidant doses were calculated. Additionally the study was

completed with the measurement of the typical water quality parameters that enable the post-treatment effluent quality to be assessed to evaluate the suitability of implementing a biological step after chemical oxidation and to develop correlations between micropollutant removal and typical organic parameters.

- The range for k_{obs} was found to be 0.8-12.8 L mmol O_3^{-1} and 9.7-29.9 L mmol $H_2O_2^{-1}$ for the ozonation and UV/ H_2O_2 process.

- The indirect pathway was not the dominant mechanism in the ozonation of these RO brines due to the high alkalinity and chloride concentration. Sulfamethoxazole, Sulfamethazine and Naproxen, among others, showed high k_{obs} values and good percentage removals even at low ozone doses according to Reungoat et al. [71] because these pharmaceuticals have electron-rich functional groups, that are highly reactive with molecular ozone. Atenolol, Carbamazepine, Codeine, Trimethoprim and Diclofenac showed the lowest initial k_{obs} values (in the order mentioned). Atenolol and Carbamazepine showed the lowest percentage of removal at low ozone dosages. In addition, Carbamazepine, Atenolol and Diclofenac were the only drugs that did not show higher than 80% removal when an ozone dose of 0.82 mg O_3 mg TOC^{-1} was used.

- Some differing results were also observed and evidence the considerable complexity inherent in this type of treatment when applied to practical wastewaters due to the high number of factors involved, such as the reactivity of EfOM and variable water quality, that can affect the observed removals [104].

- Hydroxyl radicals degrade compounds in UV/H_2O_2 treatment. Trimethoprim, Paroxetine and Sulfamethoxazole exhibited the lowest k_{obs} values (in the order mentioned). In addition, Trimethoprim and Paroxetine exhibited the lowest percentage removals when low H_2O_2 doses were assayed. A removal higher than 80% was obtained for all of the drugs, with the exception of Trimethoprim, Paroxetine and Codeine, when a dosage of 0.11 mgH_2O_2 mgTOC^{-1} was used. The UV/H_2O_2 process showed higher performance for removing compounds that were problematic in ozonation, such as Atenolol, Diclofenac and Carbamazepine. In contrast, Paroxetine and Trimethoprim required high H_2O_2 doses to achieve acceptable percentage removals. The poor removal exhibited by the pharmaceutical Trimethoprim was unexpected because most of pharmaceuticals show similar $HO^{\bullet}$ reaction rate constants [56,104]. The contribution of direct UV photolysis could be partially responsible for the better removal of some pharmaceuticals.

- The results showed that UV/H_2O_2 was more efficient than ozone at removing the pharmaceuticals from the RO brines in most cases, with the exception of Trimethoprim, Paroxetine and Sulfamethazine. The amount of H_2O_2 needed to remove the sum of the analyzed pharmaceuticals can be observed to be significantly lower compared to the amount of ozone required.

- Regarding the COD, TOC, aromaticity and turbidity, although the final degradation achieved with both AOPs was different due to the different reaction conditions assayed, the processes were compared by choosing as a reference the oxidant dose that provided complete elimination of most of the pharmaceuticals (1.38 mgO_3 mgTOC^{-1} and 0.54

mgH_2O_2 $mgTOC^{-1}$). The results showed that ozonation led to negligible mineralization and lower removal of COD and aromaticity than the UV/H_2O_2 treatment.

- The biodegradability ratio of the resulting effluents reached satisfactory and similar values for both processes. Thus, implementing a biological step after chemical oxidation could reduce the organic load of the effluents in a cost-effective way, improving the quality of the effluent for a more environmentally friendly discharge. The resulting effluents from the oxidation processes did not show any non-specific toxicity.

- The effluent exhibited very similar SUVA values independent of the oxidation process that was used. Thus, pharmaceutical removal appeared to be correlated with the SUVA parameter. Further research in this direction is required to corroborate whether this behavior can be extended to other oxidation treatments, such that SUVA can be considered as a surrogate for assessing contaminant removal efficiency.

- While the pH and turbidity levels remained constant during the UV/H_2O_2 process, these levels increased during ozonation up to maximum values of 8.76 and 332 NTU, respectively. Ozonation stripped the CO_2 and volatile fatty acids [27] from the wastewater, slowly increasing the pH during the experiments and leading to the formation of a white precipitate (calcite precipitate).

Application of bioassay panel for assessing the impact of AOPs on the treatment of RO brine (Appendix IV)

In this work, chemical analyses (addressed to defined sets of compounds, pharmaceuticals in this work) and bioassays (targeting biological activities rather than specific compounds) were combined to characterize the removal of eleven pharmaceuticals from tertiary RO brine by UV radiation and the two AOPs selected.

Effect of AOPs in water quality

- Only Ketoprofen and Diclofenac were readily decomposed by UV, suggesting that additional/alternative oxidative treatments are required for the elimination of a significant fraction of pharmaceuticals.

- In contrast, ozonation and UV/H_2O_2 process showed much higher removal rates for most of the pharmaceuticals, although some of them, such as Atenolol, Clarithromycin and Trimethoprim, were relatively recalcitrant to one or both treatments. For most cases, high oxidant doses (2 mgO_3 $mgTOC^{-1}$ in the ozonation case and 0.5 mgH_2O_2 $mgTOC^{-1}$ in the UV/H_2O_2 process) were necessary to ensure significant removal (higher than 80%) for most of the contaminants present in the matrix.

- Pharmaceutical removal pseudo-first-order kinetic constants (*ks*) were determined for both AOPs using removal values up to approximately 80%. The calculated values ranged from 0.04 to 0.37 min^{-1} and 0.01 to 2.57 min^{-1} for ozonation and UV/H_2O_2 processes respectively.

- The observed differences could be related to the different mechanism pathways involved in the two treatments, as observed and commented on in previous work [104,105]. However, it is difficult to predict the kinetics of microcontaminants in this kind of matrix due to the high number of factors involved, such as the reactivity of the EfOM and variable water quality, that can affect the observed removals [28,106].

Measurement of biological activity of water samples through the reclamation treatment

- The *E. coli* antibiotic activity bioassay detected very low antimicrobial activity in brine samples, and only after an important pre-concentration step (100 000 fold). A residual antibiotic concentration equivalent to 15 µg of Ampicillin per L of brine was estimated.

- Data from the Microtox® acute toxicity test performed with raw water samples from the main steps of the tertiary treatment process showed no acute bacterial toxicity for any of them as other authors obtained [105,107,108]. The level of antibacterial activity of the pre-concentrated RO brine was consistent with the negative results obtained with Microtox®.

- ER-RYA analyses revealed no significant estrogenic activity in any of the water samples, a result consistent with the relatively low levels of biological activity observed in the toxic tests. In contrast, AhR-RYA revealed a substantial dioxin-like activity in water samples previous to the RO process and also in the RO brine, probably reflecting the presence of organic anthropogenic compounds. The distribution of dioxin-like activity through the different steps of the treatment was very similar to the one observed for the total pharmaceuticals and was essentially removed by the RO process.

Removal of dioxin-like activity by oxidation processes

- AhR-RYA was used to assess the evolution of dioxin-like activity through the two AOPs performed, and also with UV radiation itself. Although the most drastic removal of dioxin-like activity was observed during the first seconds (<20 s) of each treatment applied (58%, 88% and 69% for UV, UV/H_2O_2 and ozonation, respectively), a particular behavior was observed for all processes from this point on.

- UV radiation did not decrease significantly the total dioxin-like residual activity of the RO brine after more than an hour of treatment. Besides, an increase of the activity was observed, probably due to the formation of by-products that exhibited high response to dioxin-like assay.

- UV combined with H_2O_2 resulted in a negligible decrease for the dioxin-like response during several minutes of treatment (more than 15 min), although a final reduction of 97% of the initial activity response was accomplished after 109.1 min of treatment.

- On the contrary, ozonation resulted in a continuous and rapid decrease of dioxin-like activity, achieving almost total response removal in 10 min, with an apparent half-life of only 50 s. This value is comparable with the ones calculated for the different pharmaceuticals (2–20 s^{-1}), and underscores the efficiency of the ozonation method.

- There was a pseudo-linear relationship between dioxin-like activity and the total amount of pharmaceuticals for all treated samples. This indicates that the non-characterized dioxin-like components present in brine showed a susceptibility to the different treatments similar to the pharmaceuticals analyzed in this work.

4.2.2. Biological treatment applied to reclamation RO brine

As a second step of the preliminary study conducted in Appendix II, a SBR system was performed after the UV/H_2O_2 treatment. This biological process consisted of 1-L SBR continuously stirred and aerated to promote aerobic conditions. Series of 3 SBR were performed during the tests. The SBR were loaded with muds collected from the activated sludge system of El Prat de Llobregat WWTP (Total Volatile Suspended Solids of 100 mg L^{-1}). Each set of SBR experiments was loaded simultaneously in cycles of 24 hours. The SBRs were fed with three different solutions: the RO retentate without treatment (R1), the effluent treated by means UV/H_2O_2 with 20 mgH_2O_2 L^{-1} (R2) and the effluent treated also through UV/H_2O_2 with 40 mgH_2O_2 L^{-1} (R3).

Organic matter did not experimented changes in R1, which was fed with RO brine without treatment. On the contrary, biomass was able to degrade part of the organic load in R2 and R3. This behaviour was in accordance with the low biodegradability ratio of the RO retentate (0.06). As expected, the fact that AOPs can break down organic molecules and turn them into more biodegradable compounds was confirmed by the results obtained when exposing the same bacterial population to pre-treated effluents.

In conclusion, the combination of an AOP with a biological treatment was appropriate for treating the RO brine, removing almost 60% of the initial TOC. For this reason, a more accurate work was performed in Appendix V.

In addition of the study showed in Appendix V, which is commented below, another work was developed combining several oxidation techniques (O_3, O_3/UV, H_2O_2/O_3, O_3/H_2O_2/UV) with a MBBR (Appendix VI). In particular, the combination of ozone, peroxide and UV (O_3/H_2O_2/UV) led to the highest removals of COD (36%) and DOC (53%), in contrast with the result attained in the control experiment (without pretreatment), which presented lower removals (18% of COD and 14% of DOC).

Attached biomass was carefully scraped from the carriers and used for microscopic observation. In general, carriers were recovered by thin biofilms presenting an important microbial diversity. A higher diversity was observed in the MBBR fed with the RO retentate (control reactor). In this case, protozoan and filaments were observed. Examination of the attached biomass collected from the MBBR operating with pre-oxidized wastewater revealed a lower diversity, in terms of protozoan population and the absence of filaments.

Oxidation of the retentate contributed to obtain good results in terms of organic matter removal and reduction of organic matter aromaticity. The effluent of the combined

process (oxidation + MBBR) presented low DOC levels, in the range of 6 to 12 mgC L^{-1} (initial value of DOC 24 ± 6.0 mgC L^{-1}).

BAC filtration to mitigate micropollutants and EfOM content in reclamation RO brines (Appendix V)

The suitability of a BAC process to treat municipal wastewater RO concentrate was evaluated at lab scale during 320 days of operation. BAC alone and combined UV/H_2O_2–BAC and ozone–BAC were performed. ATP analyses and FISH technique were applied to ensure and assess the biological activity in GAC filters.

Pretreatment of RO brine

- The tertiary RO brine effluent was treated by UV/H_2O_2 (0.82 mgH_2O_2 mgDOC^{-1}) and ozonation (2.2 mgO_3 mgDOC^{-1}) in order to ensure almost total elimination of micropollutants. Then, the two pretreated solutions were used to feed the corresponding biofilter.

- The resulting COD, DOC and UV_{254} average values from UV/H_2O_2 and ozonation treatments are summarized in Table 8.

- In this study, Total Residual Oxidants (TRO) were analyzed because of the biofilters placed after the AOPs. Bromide has also shown to be highly reactive with molecular ozone. As expected, after UV/H_2O_2 treatment, TRO were reduced about 87% because the presence of hydrogen peroxide reduces the formation of bromide oxidation products. On the contrary, when ozone was used the TRO increased from 0.74 to 3.21 mgBr_2 L^{-1}.

Effect of pretreatment on overall water quality

- The inlet and outlet DOC and COD values for the BAC filters fed with raw RO brine, UV/H_2O_2 pre-oxidized and pre-ozonated brines were shown in Figures 1 to 3 (Appendix V), respectively, during 318 days operating in a continuous-flow mode.

- To analyze the results obtained, three different operational periods were established (black continuous lines delimit the different operational periods) mainly based on the profile obtained for the eliminations of DOC and COD achieved with BAC filter fed with raw RO brine [67].

- During the first period of operation (until day 130) DOC and COD eliminations were similar for the three BAC columns During that period adsorption seemed to be the dominant DOC and COD removal mechanism, the adsorption capacity of the GAC was still high while the colonization process seemed negligible.

- In a second stage (until day 240), GAC absorption capacity was decreasing, the biofilm was growing up and biodegradation mechanism started growing in importance. From this point on, the nature of the matrix, in particular the biodegradation degree of the effluent to be treated, gained significance. The ozonated effluent was slightly more biodegradable

and therefore the removals achieved by the corresponding BAC filter were higher in comparison to the UV/H_2O_2 case.

- Finally, the dominant degradation mechanism in the last period was biodegradation since GAC was almost saturated after 240 days of operation. The average percentage removals were again higher for the more biodegradable pretreated effluents.

- Similarly to DOC and COD, the highest removal of UV_{254} took place during the pure absorption period. On the other hand, no significant differences between the established periods were observed regarding pH and turbidity.

- In general, anions and cations concentration remained practically inalterable except phosphate and ammonium concentration that slightly decreased in the three effluents.

- Since the presence of TRO could limit bacterial growth, these were also measured at the outlet of the biofilter to guarantee that the biomass present in the biofilters was able to endure the presence of TRO. The TRO values resulted practically negligible after the columns fed with RO pretreated effluents (0.02 and 0.04 $mgBr_2\ L^{-1}$ for UV/H_2O_2 and ozonation, respectively). A slight TRO signal was detected (0.10 $mgBr_2\ L^{-1}$) when RO brine was biofiltered. The TRO reductions achieved in the biofilter fed with ozonated effluent and the biofilter fed with RO brine and also the good performance of both biofilters, ensure that the biomass present was able to assume the presence of these TRO.

Micropollutants removal

- The occurrence of 11 pharmaceuticals measured in the raw RO brine was assessed after BAC biofiltration. Percentage removal achieved through the biofilter vary from 12% (Sulfamethoxazole – antibiotic) to 92% (Gemfibrozil – lipid regulator).

- Different removal efficiencies were observed for selected pharmaceuticals. Complexity of the treated matrix, properties of the support used in biofilters, inicial concentrations, EBCT and the operational time influence the removal of pharmaceuticals [71,87,109,110].

- Other authors suggest that even though it is hypothesized that adsorption capacity of the AC in the BAC filters is largely exhausted, the removal of specific organic micropollutants may occur by a combination of adsorption and biodegradation [71].

Integrated pre-oxidation and BAC performance

- The results exposed in this work demonstrated that UV/H_2O_2 or ozonation integration with BAC filtration was able to improve overall removals of the analyzed parameters compared to RO brine biofiltration without pretreatment.

- Table 8 shows the average effluent concentrations of the analyzed parameters (DOC, COD and UV_{254}) at the end of the combined treatment. These values correspond to the final operational period (from day 241 to 318) where biological removal was the dominant process in organics removal. Combined treatments provided at least 2 times higher removals. The combined ozone–BAC process performed higher removals than the combination of UV/H_2O_2–BAC process.

Table 8. Average influent and effluent concentrations of DOC, COD and UV_{254} for the three BAC filters.

	RO brine BAC		UV/H_2O_2 – BAC		Ozonation – BAC	
	Influent	*Effluent*	*Influent*	*Effluent*	*Influent*	*Effluent*
DOC (mgC L^{-1})	24 ± 4	17 ± 3	20 ± 3	12 ± 2	19 ± 3	8 ± 2
DQO (mgO_2 L^{-1})	62 ± 8	50 ± 10	44 ± 6	32 ± 7	37 ± 5	21 ± 6
UV_{254} (m^{-1})	41 ± 7	26 ± 6	20 ± 3	11 ± 2	14 ± 4	5 ± 1

Results from biofilm assessment

- ATP from the three influents and effluents was measured after few months of biofilters operation (day 90). The cellular ATP content was very low in the influents. The one in the raw RO brine because the secondary WWTP effluent is subjected to sand filtration and microfiltration before RO, and the other of AOPs pre-treated effluents because AOPs are able to damage and inactivate suspended bacteria [65,96].

- It stands to reason that after a GAC filter with biological activity, total and cellular ATP should increase because of the GAC biomass colonization [65]. The greater increase of ATP content after going through the corresponding BAC filters was for raw RO brine effluent, followed by UV/H_2O_2 and ozone effluents in this order. This confirms that they were biologically active, as other authors obtained [71].

- Regarding ATP contained on cells attached into GAC, it was measured twice. ATP slightly increased for both BAC filters fed with pretreated effluent indicating that the average biomass growth rate was very low and the BAC filters already attained steady biological performance. The ATP of the biofilter fed with raw RO brine increased 40% which means that the biomass concentration had not reached yet a steady stage by day 105. These differences between pretreated and non-pretreated RO brine were possibly due to the ease of bacterial colonization of the more biodegradable pre-oxidized effluents.

- In order to identify the main bacteria phylum present in the three BAC filters, FISH technique was applied to biomass samples from backwashing water (day 311) using the 16S rRNA probes shown in Table 1 of Appendix V. The probe EUB338, specific for the domain Bacteria, was used to evaluate the bacterial presence in the microbial community. Additional probes for α- (ALF1B), β- (BET42a) and γ- (GAM42a) proteobacteria were also employed for a more accurate characterization.

- Hybridization process showed that bacterial strains of BAC filters fed with RO brine and RO brine pretreated with UV/H_2O_2 mainly consisted of β-proteobacteria (Figure 18a and 18b). In the case of the BAC filter fed with pre-ozonated RO brine β-proteobacteria, which were also the predominant (Figure 18c), coexisted with γ-proteobacteria (Figure 18d). No α-proteobacteria could be detected by FISH technique in backwashing waters.

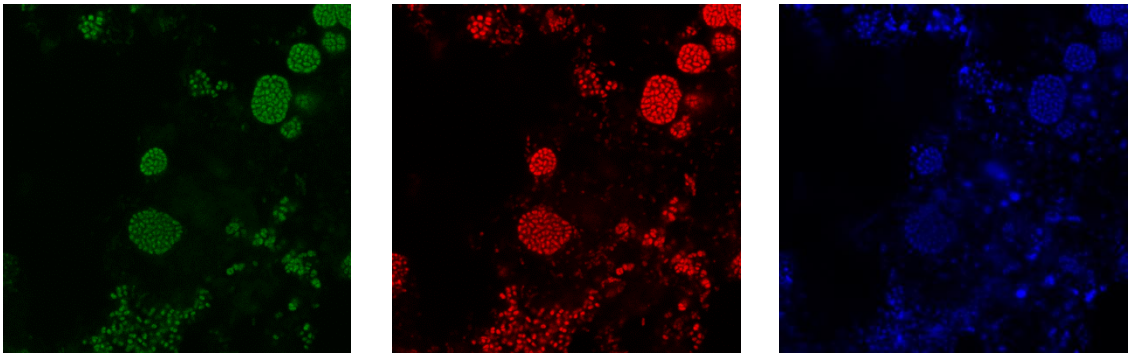

Figure 18a. FISH analysis images of samples collected on day 311 from the BAC filter fed with RO brine. Green: β-proteobacteria; Red: bacteria; Blue: DNA.

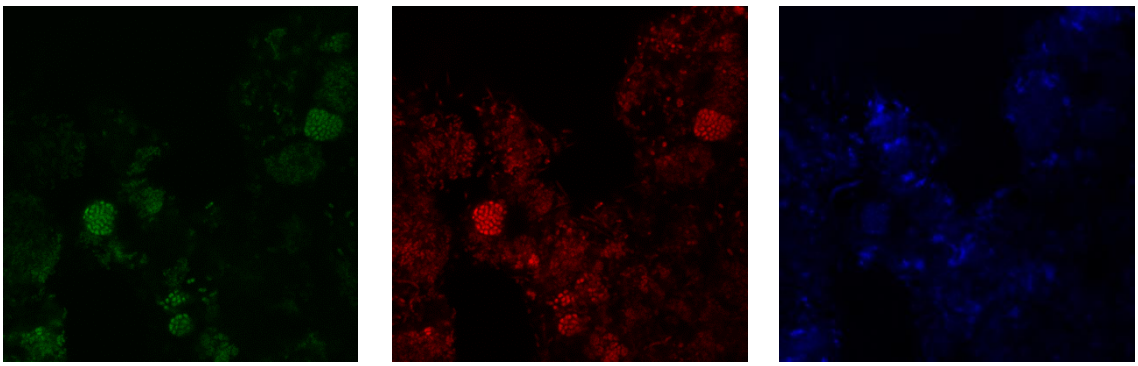

Figure 18b. FISH analysis images of samples collected on day 311 from the BAC filter fed with UV/H2O2 treated RO brine. Green: β-proteobacteria; Red: bacteria; Blue: DNA.

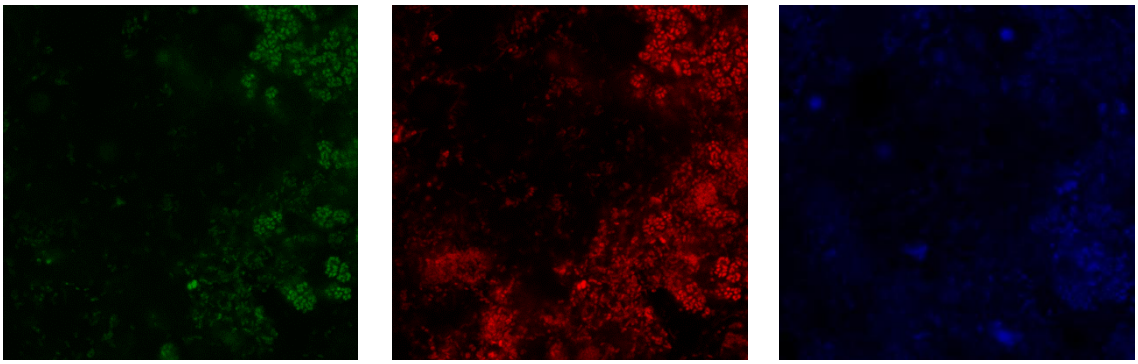

Figure 18c. FISH analysis images of samples collected on day 311 from the BAC filter fed with ozonated RO brine. Green: β-proteobacteria; Red: bacteria; Blue: DNA.

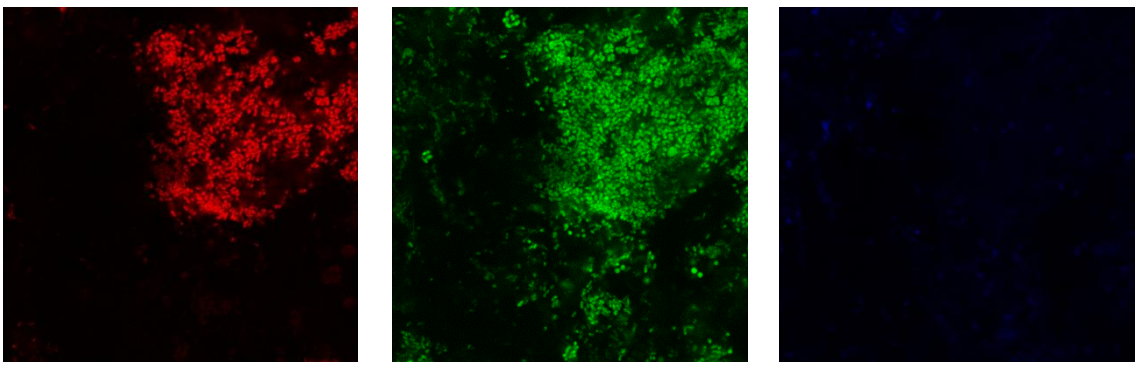

Figure 18d. FISH analysis images of samples collected on day 311 from the BAC filter fed with ozonated RO brine. Red: β-proteobacteria; Green: bacteria; Blue: DNA.

Figure 18. FISH analysis images of samples collected on day 311 from the BAC filters.

- On the other hand, at the end of 318 days of experimentation approximately 0.5g of GAC from each biofilter were collected and grinded for DNA extraction. DNA was extracted using a PowerSoil® DNA Isolation Kit (MO BIO®). The presence of DNA in the three was confirmed by electrophoresis on an agarose gel (1% w/v) containing SYBR® Safe DNA Gel Stain (InvitrogenTM), which allows DNA visualization under UV light (UVItec, UK). Figure 19 shows the advance of the different DNA strains contained in the ladder mix depending on their sizes at the right side of the images and at the other side there is the DNA strain of the three BAC filters. Lighter DNA strains will move forward than heavier strains.

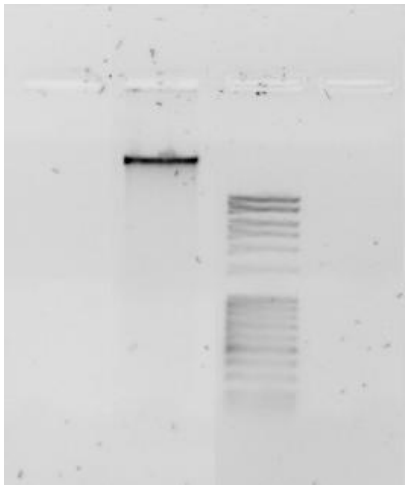

Figure 19a. DNA strains of RO brine BAC filter.

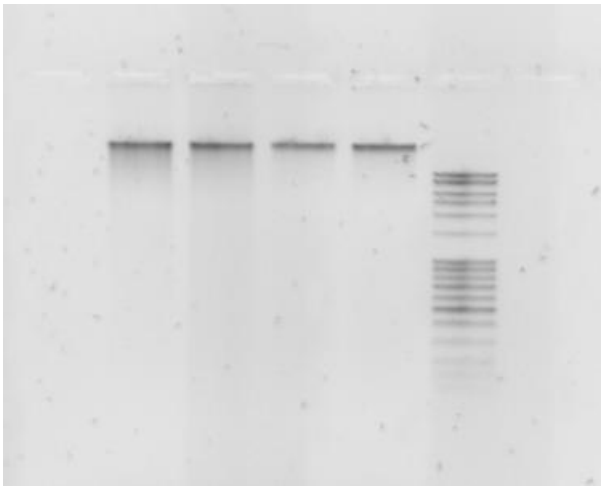

Figure 19b. DNA strains of UV/H_2O_2 (two first replicas) and ozonation (next two replicas) BAC filter respectively.

Figure 19. DNA strains of BAC filters.

5. CONCLUSIONS

Parallel to the results and discussion, conclusions are divided in the same parts.

Characterization and fate of EfOM treated with UV/H_2O_2 and ozonation (Appendix I)

In general, the ozonation and UV/H_2O_2 technologies were effective in degrading BP since the early stage of oxidation. In addition, ozonation, by dominant direct attack, was effective in eliminating HS and the other oxidation byproducts, with the exception of LMMA, which were accumulated from the beginning of the reaction. For MBR effluent and high doses of oxidant, the exclusive presence of LMMA confirmed their recalcitrance to ozonation. On the contrary, the radical non-selective oxidation mechanism of UV/H_2O_2 resulted in final CAS and MBR effluents in which the HS and all of the LMM compounds were present.

Furthermore, monitoring of the organic matter fractions with LC-OCD demonstrated that the reduction of effluent aromaticity (decreasing in SUVA) was not strictly correlated with the complete depletion of HS in the effluents for both advanced treatments.

In summary, although ozonation and UV/H_2O_2 efficiently removed the different DOM fractions, the final composition of the treated effluents was significantly different between the involved oxidation processes.

Treatment of reclamation RO brine

- **AOPs applied to reclamation RO brine**

Pharmaceuticals and organic pollution mitigation in reclamation osmosis brines by UV/H_2O_2 and ozone (Appendix III)

Both ozonation and UV/H_2O_2 technologies performed well in the removal of various analyzed pharmaceuticals from the RO brine of a reclamation plant. However, the UV/H_2O_2 process appeared to be a more promising and efficient tool for treating these concentrates. The UV/H_2O_2 process removed the pharmaceuticals and improved the effluent quality, while using significantly less oxidant compared to ozonation.

The main mechanism in ozonation is the attack of contaminants by molecular ozone. However, the predominant mechanism in the UV/H_2O_2 process involves the reaction of hydroxyl radicals. The different mechanisms produced significant differences in the order of priority with which pharmaceuticals were removed. The UV/H_2O_2 process exhibited a higher performance than ozone, in being able to remove compounds that were problematic for ozone, such as Atenolol, Diclofenac and Carbamazepine. In contrast, Paroxetineand Trimethoprim required high H_2O_2 doses for acceptable levels of pharmaceutical elimination, whereas ozonation produced a good level of removal from the first stages of oxidation. Further research should be made in order to deeply assess the responsibility of these complex matrices on deviations from expected kinetics of pharmaceuticals during their removal by AOPs.

Application of bioassay panel for assessing the impact of AOPs on the treatment of RO brine (Appendix IV)

The results show the efficiency of AOPs for the removal of micropollutants in RO brines, both measured as the degradation of specific pharmaceuticals or by the combined dioxin-like activity of the samples. The nearly complete removal of pharmaceuticals by UV/H_2O_2 and ozone can be achieved only by relatively long treatments and under severe oxidative conditions (0.5 mgH_2O_2 mgTOC^{-1} and 2 mgO_3 mgTOC^{-1}). UV itself is only able to eliminate a reduced subset of compounds. Although little differences were observed between both AOPs considering only the chemical data, the dioxin-like activity test showed significant response variances among treatments after the first 20 s of treatment. Ozonation resulted in a continuous and rapid decrease of dioxin-like activity, with an apparent half-life of only 50 s whereas, for the UV/H_2O_2 process, dioxin-like activity response remained constant for several minutes of reaction (more than 15 min). Notwithstanding these considerations, our data demonstrates that the AOPs for the inactivation of brine represent an integral solution of wastewater treatment with a potential application even for highly polluted inputs. Furthermore, the reported results, combined with other previous reports, confirmed the utility of combining chemical analysis together with bioassays to assess the fate of micropollutants through advanced procedures of waste water treatment.

- **Biological treatment applied to reclamation RO brine**

BAC filtration to mitigate micropollutants and EfOM content in reclamation RO brines (Appendix V)

The results presented in this work show that BAC filtration performed some improvements on water quality parameters of RO brines. Average DOC removal was 28%, and approximately 60% of the pharmaceuticals content was depleted in the biological treatment. Nevertheless, the integration of a pre-oxidation stage using UV/H_2O_2 or ozone with a biological filtration was necessary to completely remove the high concentration of micropollutants present in the municipal RO brines and to reduce the DOC, COD and UV_{254} parameters close to values typically found in a conventional secondary effluent. The integration allows minimizing the environmental impact of their direct discharge. The combination of ozone and BAC filter led to the highest removals of DOC, COD and UV_{254} (66%, 66% and 87% of DOC, COD and UV_{254} removals, respectively).

The presence of biological activity in the three BAC filters studied was confirmed by ATP measurements. Moreover, ATP study showed earlier steady performance in the pre-oxidized fed biofilters. β-Proteobacteria was the main bacteria phylum identified in the three biofilters by FISH technique.

APPENDIX I

Characterization and fate of effluent organic matter treated with UV/H_2O_2 and ozonation

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Published in *Chemical Engineering Journal* 226 (2013) 402-408

Chemical Engineering Journal 226 (2013) 402–408

Contents lists available at SciVerse ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Characterization and fate of effluent organic matter treated with UV/H_2O_2 and ozonation

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HIGHLIGHTS

- Good performance for the elimination of the different DOM fractions.
- The oxidation mechanisms play an important role in the elimination of DOM fractions.
- The molecular mechanism in ozonation leads to the accumulation of LMM acids.
- UV/H_2O_2 results in an effluent composed of all low molar mass fractions and humics.
- Interpretation of SUVA data fails to diagnose the depletion of humic substances.

ARTICLE INFO

Article history:
Received 22 October 2012
Received in revised form 12 April 2013
Accepted 15 April 2013
Available online 25 April 2013

Keywords:
AOPs
DOM
EfOM
NOM
LC-OCD
MBR
Reuse
Wastewater

ABSTRACT

The Advanced Oxidation Processes (AOPs) UV/H_2O_2 and ozonation are widely recognized reclamation treatments. During the oxidation processes, effluent organic matter is modified and broken down into smaller compounds, which affect the characteristics of the treated effluent. In this study, Dissolved Organic Matter (DOM) present in two secondary effluents from a Conventional Activated Sludge (CAS) and a Membrane Biological Reactor (MBR) system, was characterized and monitored during UV/H_2O_2 and ozonation reactions with the Liquid Chromatography – Organic Carbon Detector (LC-OCD) technique. The following DOM fractions were quantified: biopolymers, humic substances, building blocks, Low Molar Mass (LMM) neutrals and LMM acids. Although both technologies were efficient for nearly eliminating the entire DOM at extended oxidation conditions, some differences were observed between them. The two processes were effective in degrading biopolymers since the early stage of oxidation. In addition, ozonation, by dominant direct attack, was effective in eliminating humics and the other oxidation byproducts, with the exception of LMM acids, which were accumulated from the beginning of the reaction. For MBR effluent and high doses of oxidant, the exclusive presence of LMM acids confirmed their recalcitrance to ozonation. On the contrary, the radical non-selective oxidation mechanism of UV/H_2O_2 resulted in final CAS and MBR effluents in which the humic substances and all of the LMM compounds were present.

Furthermore, monitoring of the organic matter fractions with LC-OCD demonstrated that the reduction of effluent aromaticity (decreasing in Specific UV Absorbance (SUVA)) was not strictly correlated with the complete depletion of humic substances in the effluents for both advanced treatments.

1. Introduction

Water is an essential natural resource for the development of life and for human activities. Currently, its scarcity is one problem that causes great concern in our society. The growing water shortage problem in arid and semi-arid areas unavoidably leads to more efficient management schemes for water resources. Part of this management leads to the production of "clean" water in Wastewater Treatment Plants (WWTPs). Currently, the reclamation of these contaminated waters is considered to be an alternative water resource [1–4].

Although reclaimed wastewaters are mainly used for agricultural irrigation, they may also be used in urban areas (lawn watering, recreational amenities, road cleaning, car washing, toilet flushing, etc.), in industry (cooling water), and for the enrichment of groundwater bodies [2,3]. This reuse permits the recovery of effluents that would have been discharged, saving, therefore,

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http://dx.doi.org/10.1016/j.cej.2013.04.066

Nomenclature

AOPs	Advanced Oxidation Processes
BOD_5	Biochemical Oxygen Demand at 5 days (mg O_2 L^{-1})
CAS	Conventional Activated Sludge
COD	Chemical Oxygen Demand (mg O_2 L^{-1})
DOC	Dissolved Organic Carbon (mg C L^{-1})
DOM	Dissolved Organic Matter
EfOM	Effluent Organic Matter
EPS	Extracellular Polymeric Substances
HPSEC	High-Performance Size Exclusion Chromatography
LC-OCD	Liquid Chromatography – Organic Carbon Detection
LMM	Low Molar Mass
MBR	Membrane Biological Reactor
NOM	Natural Organic Matter
OCD	Organic Carbon Detector
OND	Organic Nitrogen Detector
PES	Polyethersulfone
SAC	Spectral Absorption Coefficient (m^{-1})
SEC	Size Exclusion Chromatography
SUVA	Specific UV Absorbance (L mg^{-1} m^{-1})
TOC	Total Organic Carbon (mg C L^{-1})
UV	Ultraviolet
UV_{254}	UV Absorbance at 254 nm (m^{-1})
WWTPs	Wastewater Treatment Plants

drinking water and reducing the amount of pollutants that are released into watercourses [5].

Currently, the implementation of tertiary treatments is needed for reaching appropriate water quality in reclaimed water from secondary WWTP effluents. Different degrees of treatment are necessary depending on the final use of this reclaimed water. Many authors agree that complete oxidation of all of the organics (including biological organisms) to carbon dioxide would result in the highest organic control level [6,7].

The tertiary treatments that were applied in this study (UV/H_2O_2 and ozonation) were selected according to recently and commonly used practices. The UV/H_2O_2 treatment is an Advanced Oxidation Process (AOP). These AOPs involve the *in situ* generation of highly reactive hydroxyl radicals (·OH). Ozonation consists of both direct molecular attack of ozone and indirect reaction via ·OH radicals and, therefore, very often it is classified as an AOP. In practice, both of the direct and indirect oxidation reactions will occur. However, one type of reaction will dominate depending on various factors, such as temperature, pH and chemical composition of the water. The ·OH radical is the second most powerful oxidizing species (second only to fluorine) with an oxidation potential of 2.80 V [8]. The most important characteristic of this radical is its non-selectivity, which allows it to attack a large group of organic chemicals and convert them into less complex and harmful intermediate products [9]. On the other hand, molecular ozone is a very selective oxidant and presents also an important oxidation potential (2.07 V) [10].

To date, most of the investigations that are related to the use of AOPs in water reclamation focus on the variation of overall parameters, such as Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) or turbidity. Related works that use the UV/H_2O_2 and ozonation processes can be found in the literature [2,4,11,12]. To characterize the fate of Natural Organic Matter (NOM) content, High-Performance Size Exclusion Chromatography (HPSEC) has been used more widely than Liquid Chromatography – Organic Carbon Detection (LC-OCD) in studies that use ozone and ·OH radicals for the treatment of synthetic solutions containing humic substances [13], natural waters [14–16] and secondary effluents [17]. The use of LC-OCD seems to be more aimed at studies dealing with the largest NOM fractions which are responsible for membrane fouling. Only few literature related to the use of LC-OCD to detect or monitor NOM-constituents can be found (i.e., studies dealing with drinking water treatments [18,19], tertiary treatment for aquifer recharge [20] and photocatalytic oxidation of surface waters [21,22]. Although some authors have already studied the oxidation of NOM present in biotreated effluents by AOP [7,17,23,24], there is still a lack of information about the fate of LC-OCD organic matter fractions during advanced oxidation of municipal biotreated wastewaters.

Similar to the situation that occurs in drinking water treatment due to the presence of NOM, the Effluent Organic Matter (EfOM) present in secondary effluents can cause many technical and environmental problems when the effluents are treated for reclamation purposes. This EfOM is responsible for the majority of the coagulant and disinfectant demand (which results in increased sludge and the formation of potentially harmful disinfection by-product). Moreover, EfOM can interfere with the removal of other contaminants (e.g., competition of adsorption sites in activated carbon). Namely, EfOM is responsible for membrane fouling, contributes to corrosion, acts as a substrate for bacterial growth in distribution systems and can cause eutrophication in receiving water bodies. In addition, EfOM contributes to undesirable color and odor problems and the formation of disinfection by-products and acts as a carrier for metals and hydrophobic organic chemicals [18,25].

The AOPs appear appropriate for the treatment of secondary effluents because of its biorecalcitrant nature. In addition, the efficient removal of EfOM by methods such as AOPs will result in secondary benefits (i.e., water disinfection). It is still commonly perceived that the sustainability of AOPs or other waste water treatment methods should be determined eventually by economics factors. However, given the growing shortage of high quality water, which is expected to worsen with global changes in climate, the water industry and policy makers may have to reconsider the importance of economic issues.

For the above reasons, this work aims to contribute to water reuse knowledge by studying the fate (using LC-OCD) of the different chromatographable Dissolved Organic Matter (DOM) fractions present in secondary effluents during oxidation by UV/H_2O_2 and ozonation. The correlations between DOM degradation and some typical parameters (i.e. biodegradability and Specific UV Absorbance (SUVA)) are also studied.

2. Materials and methods

Two different effluent waters were used in these experiments, including (i) secondary effluent from a Conventional Activated Sludge (CAS) process (WWTP of Gavá, Barcelona), and (ii) effluent from a Membrane Biological Reactor (MBR) (ultrafiltration) located in the Vallvidrera WWTP (Barcelona). The raw CAS effluent was filtered through a conventional filter paper to remove the largest particles. Both effluents were refrigerated at 4 °C before use.

Table 1
Characteristics of DOM fractions in water.

DOM fraction	Molecular weight	Characteristics
Polysaccharides (biopolymers)	>50,000–2 Mio. g/mol	Hydrophilic and not UV-absorbing; may be associated with amino acids and proteins
Humics (HS)	100–100,000 g/mol	Consists of humics (non-soluble), humic acids (insoluble in acids) and fulvic acids (soluble in acids) in varying amounts
Building blocks (HS – hydrolysates)	350–500 g/mol	Intermediates in the degradation process fulvic acids – building blocks – low molecular weight organic acids
LMM organic acids	<350 g/mol	Final degradation product of organics, but also released by algae and bacteria
LMM neutrals and amphiphilics[a]	<350 g/mol	Slightly hydrophobic (amphiphilic), such as alcohols, aldehydes, ketones and amino acids

[a] Note: mentioned as LMM neutrals in further sections.

2.1. Experimental devices

The UV/H_2O_2 experiments were carried out in a jacketed 2-L reactor at 25 °C. Three mercury low pressure lamps were placed inside the reactor (*Philips TUV 8W*, *G8T5*). The photon flow was measured with uranyl-oxalate actinometry and was 1.5×10^{-5} - Einstein s^{-1} at 254 nm. The amount of H_2O_2 that was chosen to run the experiments (2.94 mM) was added at the beginning of the reactions. The H_2O_2 (supplied by Panreac 30% w/v) concentration profile was determined through the metavanadate spectrophotometric procedure at 450 nm [26] throughout all experiments. Reactions were quenched by removing the excess of H_2O_2 with catalase or $NaHSO_3$ according to the analysis to be carried out. The longest reaction took 150 min to nearly consume the initial 2.94 mM of H_2O_2. The ozone experiments were also performed in a 2-L reactor at 25 °C. The gas stream was injected through diffusers at a flow rate of 60 L h^{-1} and an ozone concentration of 40 gO_3 N m^{-3}. The ozone consumption was calculated as the accumulated ozone transferred to the water sample per unit of sample volume as described elsewhere [27]. The highest ozone consumptions were 5.96 mM and 4.47 mM for the CAS and MBR effluents respectively after 150 min of ozonation.

2.2. Analytical methods

2.2.1. LC-OCD technique

The typical water quality parameters that are used to characterize DOM in water, such as TOC, DOC, COD, BOD, and Ultraviolet–visible spectroscopy, are not always sufficient or satisfactory. Therefore, samples were also analyzed with the LC-OCD technique to obtain specific quantitative and qualitative information about DOM fractions during the oxidation process. The quantitative information is based on a continuous carbon mass determination that is similar to TOC analysis. This measurement is performed with an Organic Carbon Detector (OCD). The qualitative analysis consists of a gel chromatographic separation of DOM prior to analysis. This separation takes place with Size Exclusion Chromatography (SEC), in which substances with smaller molecular sizes can access more of the internal pore spaces than those with larger sizes. LC-OCD uses UV detection, a Spectral Absorption Coefficient (SAC) determination at 254 nm and Organic Nitrogen Detection (OND). Combined, these analyses are used to identify the organic fractions that are summarized in Table 1 [28].

The recommended sample handling and storage directions were followed for each analysis.

2.2.2. Typical physicochemical parameters

Samples were withdrawn during the different experiments to monitor the following parameters: TOC, DOC, COD, BOD_5, SUVA (used as an indicator of unsaturated carbon bond levels and aromatic groups), pH, NH_4^+, different anion concentrations and turbidity. In addition, alkalinity and suspended solids were analyzed to characterize the two initial secondary effluents.

TOC and DOC (previously filtered through 0.45 μm Polyethersulfone (PES)) were measured by means of a Simadzu TOC-VCSN analyzer. To analyze the COD, the Standard Methods 5220D procedures were followed. For the evaluation of the BOD_5, the WTW Oxi-Top® measuring system (Weilheim, Germany) thermostated at 20 °C was used following the Standard Methods 5210D procedures. Anion concentrations were determined in an Advanced Compact IC Metrohm ionic chromatographer using a Metrosep A Supp 4–250 columns. Ammonium concentration was measured by an ammonium electrode *SympHony* from VWR®. The turbidity was determined in a Hach 2100P turbidimeter. Alkalinity was quantified by titration with HCl. For solids content determination, Standard Methods 2540 procedures were followed.

3. Results and discussion

3.1. Effluent characteristics

The average physicochemical parameters of the secondary effluents used in this study are summarized in Table 2. On the other hand, Fig. 1 shows the normalized average chromatograms that were obtained through LC-OCD for the CAS and the MBR treated effluents. Despite the complexity of the integration methodology, a visually significant difference in DOM composition was observed. Different biopolymer percentages (13.3% and 1.3% as C for the CAS and MBR waters, respectively) accounted for the main divergence between the effluents. The lower percentage of this

Table 2
Average physicochemical parameters of the two effluents assayed.

	CAS	MBR
TOC (mg C L^{-1})	17.4 ± 3.4	6.8 ± 1.5
DOC (mg C L^{-1})	15.2 ± 3.8	6.3 ± 0.9
COD (mg O_2 L^{-1})	49.7 ± 3.3	10.9 ± 0.01
pH	7.8 ± 0.1	7.4 ± 0.1
UV_{254} (m^{-1})	30.3 ± 0.9	16.3 ± 1.3
Turbidity (NTU)	2.08 ± 0.56	0.31 ± 0.20
VSS (mg L^{-1})	2.6 ± 0.6	0.8 ± 1.1
TSS (mg L^{-1})	4.5 ± 0.7	1.9 ± 0.8
Alkalinity (mg CO_3^{2-} L^{-1})	599 ± 24	432 ± 15
NH_4^+–N (mg N L^{-1})	47.0 ± 4.26	0.28 ± 0.26
NO_2^-–N (mg N L^{-1})	1.13 ± 1.60	0.00 ± 0.00
NO_3^-–N (mg N L^{-1})	1.80 ± 1.91	7.62 ± 2.32
F^- (mg F^- L^{-1})	1.13 ± 0.06	0.76 ± 0.37
Cl^- (mg Cl^- L^{-1})	488 ± 30	275 ± 24
Br^-/BrO_3^- (mg Br^- L^{-1})	0.99 ± 1.40	0.00 ± 0.00
PO_4^{3-} (mg PO_4^{3-} L^{-1})	11.20 ± 6.35	11.90 ± 6.05
SO_4^{2-} (mg SO_4^{2-} L^{-1})	211 ± 24	163 ± 8

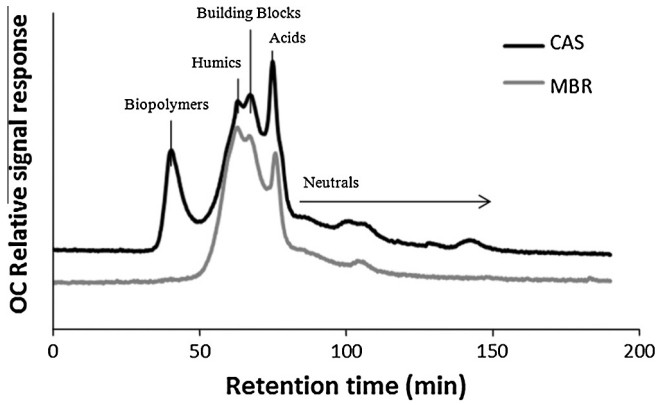

Fig. 1. Average normalized LC-OCD chromatograms for both effluents.

fraction in the MBR effluent was expected because the ultrafiltration membranes block the passage of Extracellular Polymeric Substances (EPSs), which are mainly composed of polysaccharides. Results suggested that humic substances were also retained by the membrane. In accordance with that, organic substances that are composed of proteins, polysaccharides and humics have been found in the sludge cake layer (membrane fouling) of MBRs [29,30].

3.2. CAS effluent

Figs. 2 and 3 show the evolution of the different DOM fractions and their contributions to total DOM during the UV/H_2O_2 and ozone CAS effluent treatments. In both processes, a large reduction of biopolymers occurred. However, the fate of the different DOM fractions and their contribution to the final effluent composition differ between treatments.

At the beginning of the UV/H_2O_2 treatment the reaction rate of biopolymers was higher in comparison to the humics and the Low Molar Mass (LMM) fractions, possibly due to their bigger size. When half of the initial H_2O_2 was consumed (1.50 mM; 30 min of irradiation), the biopolymer concentration was the only that had decreased significantly. Their contribution to the total DOM dropped from 22.4% to 10.7%. Meanwhile, the LMM neutrals, LMM acids and building blocks concentration increased (the building blocks came exclusively from the break-down of humic substances). Humics was the subsequent fraction that decreased in concentration after this first oxidation stage. The decrease in concentration of the smallest fractions did not begin until the biopolymers and humics in the effluent were reduced. The LMM acid fraction was the last to decrease. At the end of the treatment (H_2O_2 consumption: 2.90 mM; 150 min of irradiation), the overall TOC removal was approximately 47.9%. The final DOM consisted of the following: 74.1% LMM compounds, distributed as 31.0% LMM neutrals, 27.1% building blocks and 16.0% acids. Within the high molar mass compounds (25.9%), humic substances were the main fraction that was present in the UV/H_2O_2 effluent (19.6%) (see Fig. 2).

Regarding the ozonation process (Fig. 3), removal of biopolymers but also humics and LMM neutrals occurred from the first oxidation steps. LMM acids accumulation also took place from the beginning of the ozonation. The only contribution to the total DOM that decreased for an ozone dose of 1.7 mM was the biopolymer one (from 14.2% to 2.2%). As the ozonation extended, the accumulation of LMM acids increased to become, after an ozone dose of 3.5 mM, the main organic fraction in the DOM (29.6%). At the end of the treatment (ozone consumption: 6.0 mM), the overall TOC removal was 59.9% and the majority of DOM (90.3%) was composed of LMM compounds as follows: 35.4% LMM acids, 31.3% building blocks and 23.6% LMM neutrals. Only 8.6% of humics and 1.2% of biopolymers were present in the ozonated effluent.

Certain observations were drawn from the fate of the DOM fractions during the advanced oxidation treatments. The UV/H_2O_2

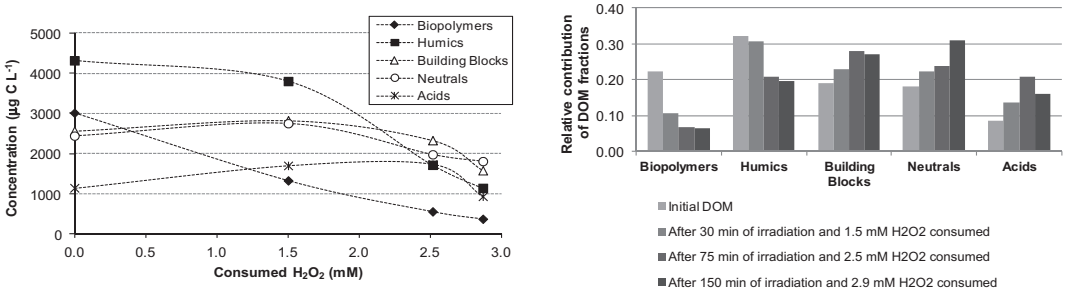

Fig. 2. Evolution of DOM fractions and contribution to the total DOM for the CAS effluent treated with UV/H_2O_2.

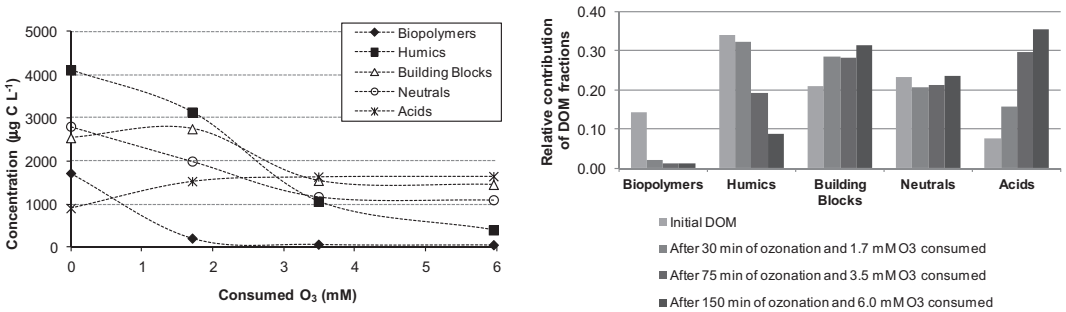

Fig. 3. Evolution of DOM fractions and contribution to the total DOM for the CAS effluent treated with ozone.

oxidation process occurred through hydroxyl radicals, which are non-selective. Thus, the largest and most abundant organic fractions were attacked to a greater extend. Following the non-selective oxidation mechanism, the final DOM contained all type of LMM compounds and humic substances (Fig. 2).

In contrast, oxidation was concentrated on the largest fractions (biopolymers and humics) but also on the LMM neutrals during ozonation from the beginning. However, the treatment efficiency and the removal rate of LMM acids were clearly lower, which lead to their accumulation. These results correspond with the findings of Gong et al. [31]. Although the CAS effluent pH (between 7.7 and 8.0) implies that two ozone attack mechanisms (molecular ozone and hydroxyl radicals) occur [32], the high alkalinity and the high chloride concentration in the medium would minimize the indirect via due to scavenging effects. Therefore, the molecular attack of ozone would govern the reaction and increase the selectivity of the oxidation process [10]. Consequently, the early removal of biopolymers, humic substances and neutrals is explained. Ozone reacts with proteins through polypeptide backbone oxidation, peptide bond cleavage, protein–protein cross-linking and a range of amino acid side chain modifications [33]. Wang et al. [34] showed that O_3 depolymerizes polysaccharides by reacting with the glycosidic linkages in those molecules. O_3 selectively oxidates the β-D-glycosidic linkages of polysaccharides to aldonic esters. The oxidation of the α-D-glycosidic linkages during O_3 oxidation is also possible. Moreover, the high aromaticity of humic substances and the likely slight hydrophobicity and aromaticity of the LMM neutrals [27] justifies the significant initial removal of these two fractions. At this point, the removal of building blocks appears to be masked by their continuous formation from the break-down of humics. On the contrary, the accumulation of the acidic fraction is supported by the low oxidation rate of LMM acids by molecular ozone [35]. In addition, other studies have reported that the amounts of carboxylic acids generated during ozonation are usually much higher than those of aldehydes and ketones. Even after long oxidation periods, the saturated reaction products accumulate in the solution and are not mineralized [36,37]. In conclusion, very severe oxidation of biopolymers, humics and neutrals is necessary to begin the degradation of the less aromatic and unsaturated fractions in the ozonation process, while simultaneous oxidation of all fractions takes place during the non-selective hydroxyl radical oxidation.

These results are in accordance with the information obtained from the biodegradability ratio (BOD_5/COD) determination. Although biodegradability increased during both treatments, it reached its highest value at the end of the ozone treatment (0.46 for O_3 and 0.26 for UV/H_2O_2). This behavior was expected because AOPs are able to improve biodegradability by oxidizing recalcitrant compounds into biodegradable compounds [11,38,39]. The higher biodegradability of the ozonated effluent occurred because 90.3% of the total DOM was LMM compounds and about 40% of them were LMM acids, which are readily biodegradable compounds. The final composition of DOM in the UV/H_2O_2 treatment presented a lower percentage of LMM compounds. In addition, 19.6% of the final DOM composition was humic substances, which are known as hardly biodegradable compounds [20]. Therefore, the parameter biodegradability ratio is consistent with the LC-OCD results.

Some interesting conclusions can be extracted from the joint analysis of another typical water quality parameter, such as the SUVA, and the LC-OCD. SUVA results from dividing the UV Absorbance at 254 nm (UV_{254}), which represents organic compounds that contain unsaturated carbon bonds and aromatic groups and have a strong absorbance at 254 nm, by the DOC of the water sample. This parameter normalizes the aromatic measurement to the total organic content of the water. Moreover, the SUVA is strongly correlated with the ^{13}C NMR aromaticity. Therefore, the SUVA can be used to indicate the effluent aromaticity [40]. SUVA has been used as an indicator of the fraction of humic substances present in different water matrices [40–42].

The obtained SUVA trends were the same for both treatments. In particular, the SUVA decreased during the first 30 min, resulting in poorly aromatic effluents, and then remained nearly constant until the end of the oxidation process. After 30 min of UV/H_2O_2 reaction, most biopolymers were removed from the effluent and 9.6% of the mineralization had taken place. Percentages of the other DOM fractions increased. In addition, the humic fraction in the effluent was the most abundant (30.6%) (see Fig. 2). During these 30 min of irradiation, the SUVA decreased from 1.59 to 0.70 L m^{-1} mg C^{-1}. Similar behavior was observed during the ozonation treatment. After 30 min of ozonation and 10.3% mineralization, the reduction of SUVA was 68% (from 2.35 to 0.75 L m^{-1} - mg C^{-1}). At that point, nearly all of the biopolymers were removed (see Fig. 3). However, the humic contents were not significantly reduced and accounted for the predominant organic fraction in the effluent (32.3% of total DOM). Thus, the observed SUVA decrease did not correspond to a reduction in the percentage of humics in DOM. The treatments provided enough oxidation of the humic substances to achieve high aromaticity depletion, but were not able to induce sufficient cleavage to attain characteristic structure loss. The decreased SUVA could be explained by the presence of electron-rich sites (double bonds, aromatic rings) in the humics molecules, which react rapidly with hydroxyl radicals and ozone to produce various hydroxylation products, mainly of the aromatic rings. The aromaticity of the functional groups would be destroyed but simpler substances would not be generated immediately. Jansen et al. [43] also noted that steric obstruction, which is related to the coiled structure of the humic substances, could prevent O_3 and ·OH radicals from cleaving molecular core bonds and only allow the split off from the periphery of larger humic molecules. Thus, the main structure would become smaller but remain intact. The oxidized group may eventually cleave the molecule into smaller ones (building blocks). However, the decrease in

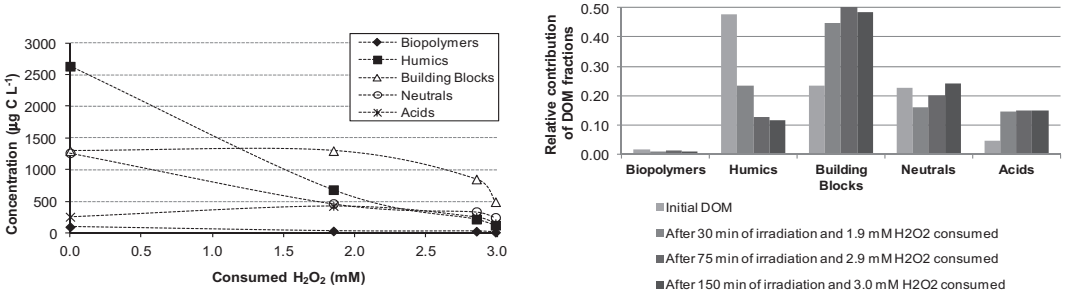

Fig. 4. Evolution of DOM fractions and contribution to the total DOM for the MBR effluent treated with UV/H_2O_2.

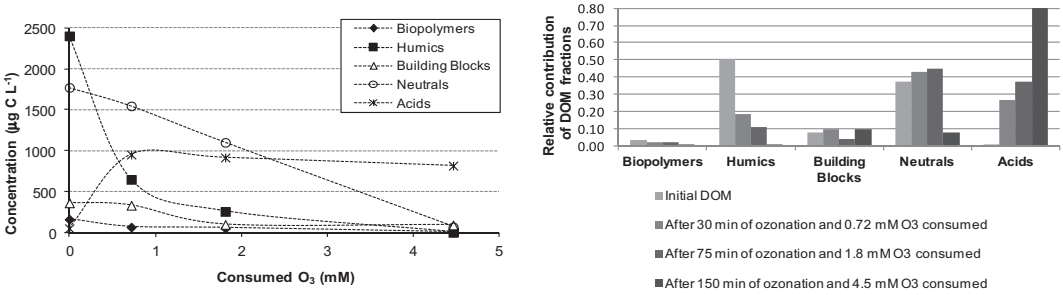

Fig. 5. Evolution of DOM fractions and contribution to the total DOM for the MBR effluent treated with ozone.

UV Absorbance is immediate as soon as the aromaticity is lost. Therefore, the complete depletion of humic substances and low SUVA should not be directly correlated.

Similar results were obtained by Kim and Yu [44] and Korshin et al. [45], where the chlorination of NOM provoked a significant change in UV_{254} absorbance, but only small disinfection byproduct amounts were produced during the first oxidation steps. In photocatalytic (TiO_2) and ozone-based oxidation studies of cresols (commonly used to simulate NOM and humic components), various hydroxylation products of the aromatic carbons (methylcatechols, methylhydroquinone and methylbenzoquinone) were identified [46,47]. Ozone and o-, m-, and p-cresol methyl group reactions that yield the corresponding benzaldehydes and ozone and double bond reactions have also been reported [48]. Moreover, the presence of xylene, toluene and phenol (also used to simulate some NOM components) degradation products prior to ring cleavage has been proven extensively.

3.3. Membrane Biological Reactor (MBR) effluent

The evolution of the different DOM fractions in the MBR effluent during UV/H_2O_2 and ozone treatment is shown in Figs. 4 and 5, respectively.

In the absence of biopolymers, the attack at the beginning of the reaction was mainly directed at humics, which was the most abundant and aromatic fraction in the MBR effluent. However, some differences were observed between both oxidation treatments.

In the UV/H_2O_2 treatment, the LMM neutral concentration decreased continuously throughout the reaction. In this case, the initial accumulation of this fraction during the CAS effluent UV/H_2O_2 oxidation was not observed because the hydroxyl radical availability was larger and the concentration of the other larger organic fractions was smaller. After 30 min of irradiation (H_2O_2 consumption: 1.85 mM) and when 47.8% of TOC and 74.2% of humics were eliminated, the accumulation of building blocks, which come from the break-down of humics, was already significant. Further oxidation led to the progressive degradation of small neutral compounds and, to a greater extent, building blocks. In addition, the LMM acid concentration noticeably decreased in the last oxidation stage. At the end of the treatment, the building blocks contribution stood out within the DOM composition: biopolymers 0.8%, 11.7% humics, 48.6% building blocks, 24.0% LMM neutrals and 14.9% LMM acids.

Regarding ozonation, high alkalinity and chloride concentration were expected to favor ozone molecular attack over the indirect mechanism during MBR effluent oxidation. After 30 min of reaction (ozone consumption: 0.72 mM), TOC and humic concentrations decreased by 37.8% and 72.8%, respectively. Moreover, no building block fraction increments were observed and a significant increase in LMM acid concentration occurred during the removal of the humics fraction. At this point, neutrals (43.3%) and LMM acids (26.8%) were the main contributors to total DOM. The higher oxidant to carbon concentration ratio and the reaction selectivity for unsaturated compounds could explain the lack of building block accumulation in this effluent. Further ozonation led to the progressive and exclusive degradation of the LMM neutrals fraction. This degradation resulted in a final DOM that was composed solely of LMM acids, which proves their recalcitrance and accumulation during the ozonation of secondary effluents.

In summary, the UV/H_2O_2 process, which operates through unselective hydroxyl radical mechanism, degrades all type of organic compounds. After the first oxidation stage, DOM is composed of humics and all the small fractions (building blocks, LMM neutrals and LMM acids). This distribution remains the same despite further oxidation and mineralization. In contrast, the selectivity of the molecular ozone prioritizes the high molar mass compounds and building block degradation. The degradation byproducts are almost exclusively LMM acids, which accumulate from the early ozonation process stages.

The difference between the biodegradability ratios in the final MBR effluents that were obtained by ozonation and UV/H_2O_2 (0.74 and 0.45 respectively) was even higher than in the CAS case due to the greater accumulation of LMM acids during the ozonation of the MBR effluent. Thus, the ozonation process appears to result in more biodegradable effluents independently of the treated matrix.

Again, the SUVA parameter, which reflects the normalized aromaticity of waters, decreased for both processes during the first 30 min (from 2.38 to 0.8 L m^{-1} mg^{-1} and from 2.59 to 0.98 L m^{-1} - mg^{-1} during the UV/H_2O_2 and ozone processes, respectively). The decrease in SUVA corresponded to humic content removal, but not to the complete elimination of this fraction (see Figs. 4 and 5), which confirms the findings from the CAS case.

4. Conclusions

In general, the ozonation and UV/H_2O_2 technologies successfully eliminated the different DOM fractions that were present in the two secondary effluents. During the first 30 min of oxidation, both processes obtained a large reduction of biopolymers, which are known to play an important role in membranes fouling during the treatment of biologically processed wastewater.

The oxidation mechanisms of the two treatments and the alkalinity of the effluent played important roles in the degradation sequence of the different DOM fractions and in the final composition of the oxidized effluent. The main mechanism of ozonation was direct ozone oxidation. The selectivity of the molecular ozone induced a large reduction of the biopolymers and aromatic humic substances and to the accumulation of LMM acids from the early stages of the ozonation process. This accumulation was more significant when ozonation was extended in MBR effluents, which demonstrated the recalcitrance of short chain acids to direct reaction with ozone. The higher biodegradability of ozonated effluents agreed with this fact. Biodegradability of tertiary treatment efflu-

ents must be taken into account since readily biodegradable organic matter acts as a substrate for bacterial growth in distribution systems and can cause eutrophication in receiving water bodies.

In contrast, the UV/H_2O_2 process operated through a non-selective hydroxyl radical oxidation, which progressively led to an effluent with lower biopolymer content and to an important increase of the LMM compounds percentage (building blocks, neutrals and LMM acids). Significant amounts of humic substances still remained after extended oxidation treatments.

Monitoring the organic matter fractions with LC-OCD demonstrated that the reduction of effluent aromaticity (decreasing SUVA) was not strictly correlated with the complete depletion of humic substances in the effluents for both advanced treatments.

In summary, although ozonation and UV/H_2O_2 efficiently removed the different DOM fractions, the final composition of the treated effluents was significantly different between the involved oxidation processes.

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APPENDIX II

Regeneración de aguas de rechazo de Osmosis Inversa (OI) mediante la combinación del Proceso de Oxidación Avanzada (POA) UV/H_2O_2 con tratamiento biológico

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Published in *VII Congreso "La investigación ante la sociedad del conocimiento". Sostenibilidad y medioambiente – Libro de resúmenes* (Alcoy, 2011)

Edited by "Escuela Politécnica Superior de Alcoy" (ISBN: 978-84-694-9814-9)

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RESUMEN

Los procesos de tratamiento de aguas residuales y reutilización con membranas se están expandiendo rápidamente. Mediante el proceso de Osmosis Inversa (OI) se eliminan eficientemente compuestos orgánicos, inorgánicos y biológicos, obteniendo un agua de alta calidad. Sin embargo, uno de los mayores inconvenientes es el destino final de las aguas de rechazo. Con la finalidad de tratar estos efluentes, se propone la combinación de un Proceso de Oxidación Avanzada (POA) como es el proceso UV/H_2O_2 con un posterior tratamiento biológico mediante un sistema de Reactor Secuencial Discontinuo (SBR). Se emplearon 40 mg L^{-1} de H_2O_2 y tres ciclos de 24 horas en el SBR. Después de realizar el tratamiento a un efluente real de rechazo de un proceso de OI de reutilización, se obtuvo una eliminación final del 63,7 y 68,6 % de Carbono Orgánico Total (COT) y Demanda Química de Oxígeno (DQO), respectivamente. La combinación propuesta, parece ser adecuada para reducir la carga contaminante vertida al medioambiente de este tipo de efluentes.

INTRODUCCIÓN

Durante los últimos años, la escasez y la calidad del agua se han convertido en una de las mayores preocupaciones a nivel mundial. Cada día se contaminan grandes cantidades de agua, especialmente en los países desarrollados. Restablecer la calidad de las aguas residuales es una tarea esencial para evitar continuar contribuyendo a la contaminación del Medio Ambiente (MA) así como para posibilitar su reutilización, disminuyendo, de esta manera, el consumo de agua potable [1, 2].

Los procesos de separación con membranas son cada vez más utilizados en el tratamiento de aguas residuales y reúso, ya que combinan la estabilidad del proceso con la producción de agua de excelente calidad. El proceso de Osmosis Inversa (OI) es uno de los más efectivos para la eliminación de un gran rango de microcontaminantes, iones, y materia orgánica (MO) durante los tratamientos de reutilización de agua [3]. Uno de los mayores obstáculos para la implementación de estos procesos es la necesidad de tratar el efluente de rechazo. Aunque actualmente su vertido no está regulado, unas buenas prácticas medioambientales sugerirían el tratamiento de este tipo de efluentes concentrados antes de verterlos y que se diluyan en el MA. Mientras que el contenido de sal de estos efluentes supone un riesgo medioambiental, los compuestos disruptores endocrinos, fármacos, pesticidas, etc., que son altamente rechazados por las membranas, son una preocupación creciente [4].

El rechazo de OI suele caracterizarse por su alto contenido en MO poco biodegradable y compuestos inorgánicos, limitando el uso de tratamientos convencionales de aguas residuales [5]. Los Procesos de Oxidación Avanzada (POA) son capaces de degradar MO recalcitrante y aumentar la biodegradabilidad del efluente, siendo, por tanto, adecuados para tratar el rechazo de OI. Por otro lado, la combinación de un POA con un posterior tratamiento biológico, menos costoso, permite eliminar mayores cantidades de MO utilizando menos energía y menores dosis de oxidante.

Así pues, el objetivo principal de este trabajo es la evaluación de la combinación del POA UV/H_2O_2 con un tratamiento biológico del tipo Reactor Secuencial Discontinuo (SBR) para tratar el rechazo de un proceso de OI de la línea de reutilización de la Estación Depuradora de Aguas Residuales (EDAR) de El Prat de Llobregat (Barcelona, España).

EXPERIMENTAL

Etapa química: UV/H_2O_2

El proceso UV/H_2O_2 consiste en la generación de radicales ·OH de alto poder oxidante (2,80 V) mediante la fotólisis del H_2O_2 con radiación ultravioleta. Los experimentos se han llevado a cabo en un reactor encamisado (25 ºC) de 2 L provisto de tres lámparas de mercurio de baja presión de 254 nm y caudal fotónico $1{,}5 \cdot 10^{-5}$ Einstein s^{-1}.

Etapa biológica: Reactor Secuencial Discontinuo (SBR)

Los SBR son reactores de lodos activos con la peculiaridad de que la forma de operación es en discontinuo (llenado-vaciado). En los procesos biológicos, la actividad metabólica de los microorganismos (entre los que destacan las bacterias) es la responsable de la degradación de MO. Los SBR continuamente aireados de 1 L se inocularon con lodos aerobios del tratamiento secundario de la EDAR de El Prat de Llobregat (Solidos Suspendidos Volátiles Totales (SSVT) = 100 mg L^{-1}).

Análisis realizados

El análisis de la Demanda Química de Oxígeno (DQO) se realizó siguiendo el procedimiento del Standard Methods 5220D. El Carbono Orgánico Total (COT) se cuantificó con el analizador Shimadzu TOC-VCSN. El método utilizado para la medición de la Demanda Biológica de Oxígeno a los 5 días (DBO_5) fue el 5210D del Standard Methods a 20ºC mediante el sistema OxiTop® de WTW. La turbidez se midió mediante el turbidímetro HACH 2100P. La aromaticidad, expresada como absorbancia a 254 nm (UV_{254}), se evaluó en el espectrofotómetro Perkin Elmer UV/VIS. Por último, la concentración de H_2O_2 (Panrreac 30 % w/v) se cuantificó con el método espectrofotométrico basado en la reacción con metavanadato de amonio [6].

RESULTADOS Y DISCUSIÓN

En la Tabla 1 se presentan los parámetros fisicoquímicos más representativos del efluente a tratar. Cabría destacar el valor realmente bajo del ratio de biodegradabilidad (DBO_5/DQO = 0,06), de acuerdo con un alto contenido en MO poco biodegradable. Además, el efluente posee una elevada alcalinidad y contenido en sales. También se determinó que el rechazo no era tóxico según el análisis de Microtox® con bacterias *Vibrio fischeri*.

Tabla 1. Composición del rechazo de OI de El Prat de Llobregat.

COT (mgC L^{-1})	18,5	**Turbidez** (NTU)	0,75
DQO (mgO_2 L^{-1})	46,5	**pH**	7,87
DBO_5 (mgO_2 L^{-1})	2,75	**Alcalinidad** (mg$CaCO_3$ L^{-1})	686
UV_{254} (cm^{-1})	0,372	**Conductividad** (mS/cm)	5,94

Una vez caracterizado el efluente, se compararon dos estrategias de adición del H_2O_2 (inicial y secuencial) con la finalidad de evaluar la importancia del efecto secuestrante del radical ·OH debido al exceso de H_2O_2 en el medio. Para ello, se utilizaron 20 mgH_2O_2 L^{-1} y se evaluó la contribución de radiación UV. En la Figura 1 se muestran las eliminaciones finales alcanzadas a través de las dos estrategias.

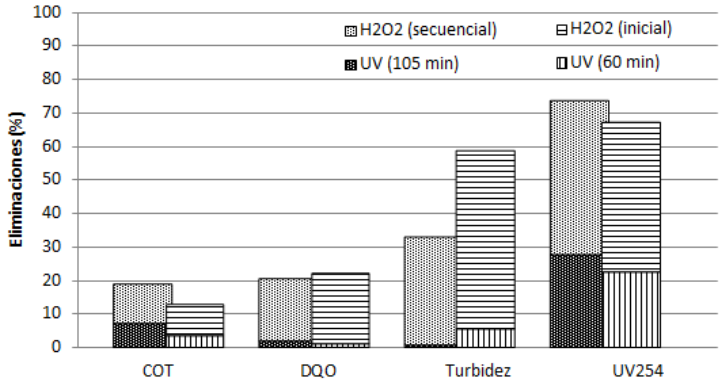

Figura 1. Eliminaciones finales de las diferentes estrategias de adición del H_2O_2.

Tal y como puede observarse, el hecho de añadir todo el H_2O_2 al inicio de la reacción resulta más apropiado, ya que, aunque el grado de mineralización obtenido es inferior, el grado de oxidación y el ratio de biodegradabilidad son mayores, implicando mejores condiciones para la combinación con un tratamiento biológico. Además, los tiempos de radiación menores, favorecerían también la opción de dosificación única de H_2O_2.

Por otro lado, se compararon las eliminaciones finales de los principales parámetros analizados de tres dosis de oxidante añadidas al inicio de la reacción (5, 20 y 40 mgH_2O_2 L^{-1}). Se obtuvieron mayores porcentajes de eliminación cuanto mayor era la dosis de H_2O_2 aplicada. Para la combinación del POA con el SBR, se descartó el experimento con menor dosis de H_2O_2 por qué no alcanzaba el ratio de biodegradabilidad recomendado en la bibliografía para la aplicación de un tratamiento biológico.

Durante el tratamiento químico se extrajeron muestras para hacer un seguimiento de: COT, DQO, Turbidez, UV_{254} y [H_2O_2], así como también se analizó la DBO_5 de las muestras finales. Paralelamente, se analizó la concentración de algunos microcontaminantes pertenecientes al grupo de fármacos a lo largo del proceso UV/H_2O_2. Las eliminaciones finales alcanzadas para la mayor dosis de H_2O_2 aplicada fueron correspondientemente: 26,0, 41,0, 54,8 y 77,4 %.

En la segunda etapa de tratamiento del rechazo de OI, etapa biológica, se compararon tres sistemas SBR alimentados con: el efluente sin tratar (R1), el efluente tratado con UV/20mgH_2O_2 L^{-1} (R2) y otro tratado con UV/40mgH_2O_2 L^{-1} (R3). Se realizaron tres ciclos consecutivos de 24 horas para cada alimento, aunque el seguimiento de Carbono Orgánico Disuelto (COD) y DQO solo se llevó a cabo durante

las 8 primeras horas. A modo de ejemplo, se presenta la Figura 2 correspondiente al seguimiento de COD y DQO del SBR R3.

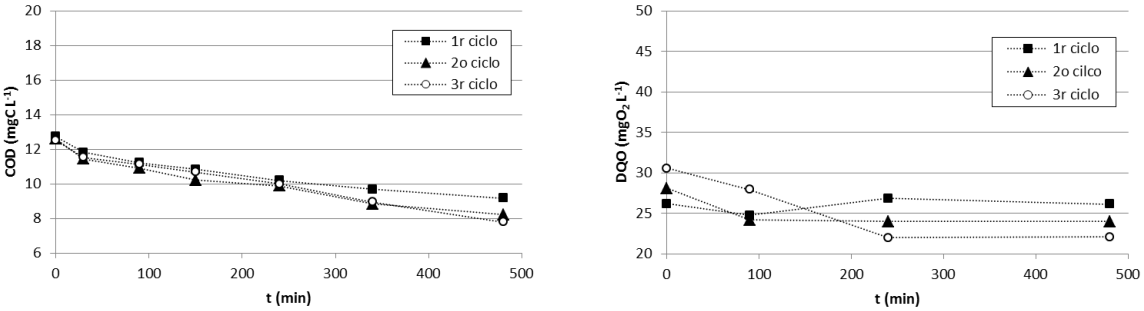

Figura 2. Seguimiento de COD (izquierda) y DQO (derecha) del SBR pretratado con UV/40mgH_2O_2 L^{-1}.

La MO del R1 no experimentó ningún cambio tal y como se esperaba, debido a su baja biodegradabilidad. Por el contrario, la biomasa fue capaz de degradar el sustrato de los otros SBR. Además, se observó un aumento en las velocidades de degradación del sustrato entre ciclos debido al acondicionamiento de las bacterias. Las eliminaciones de COD y DQO alcanzadas en el tercer ciclo de los microorganismos del SBR alimentado con el sustrato más oxidado (R3) fueron 37,7 y 27,6 % respectivamente.

CONCLUSIONES

El tratamiento propuesto para regenerar efluentes de rechazo de OI, consistente en la combinación del proceso UV/H_2O_2 con un tratamiento biológico SBR, parece apropiado para reducir la carga contaminante de dichos efluentes. Las eliminaciones de COT y DQO globales fueron 63,7 y 68,6 %, respectivamente.

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APPENDIX III

Pharmaceuticals and organic pollution mitigation in reclamation osmosis brines by UV/H_2O_2 and ozone

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Published in *Journal of Hazardous Materials* 263 (2013) 268-274

Journal of Hazardous Materials 263 (2013) 268–274

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Pharmaceuticals and organic pollution mitigation in reclamation osmosis brines by UV/H_2O_2 and ozone

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HIGHLIGHTS

- High concentrations of drugs were identified in a municipal water reclamation RO brine.
- UV/H_2O_2 exhibited higher performance than ozone in the removal of the analyzed pharmaceuticals.
- Atenolol and Carbamazepine appeared to be the most ozone-resistant pharmaceuticals.
- Acceptable levels of elimination of Paroxetine and Trimethoprim required high H_2O_2 doses.
- Matrix complexity may be responsible for deviations from expected removal kinetics.

ARTICLE INFO

Article history:
Received 15 December 2012
Received in revised form 25 April 2013
Accepted 15 May 2013
Available online 22 May 2013

Keywords:
AOP
Micropollutants
Retentates
RO concentrate

ABSTRACT

One significant disadvantage of using reverse osmosis (RO) for reclamation purposes is the need to dispose of the RO retentates. These retentates contain a high concentration of micropollutants, effluent organic matter (EfOM) and other inorganic constituents, which are recalcitrant to biological treatment and may impact the environment. The occurrence of 11 pharmaceuticals (concentrations ranging from 0.2 to 1.6 $\mu g\,L^{-1}$) and their mitigation in RO retentates by a UV/H_2O_2 process and ozonation was studied using a wide range of oxidant dosages. Eleven pharmaceuticals were identified at. Initial observed kinetic constants (k_{obs}) were calculated for the different pharmaceuticals. Other typical wastewater parameters were also monitored during the UV/H_2O_2 and ozonation reactions.

The range for k_{obs} was found to be 0.8–12.8 L mmol $O_3{}^{-1}$ and 9.7–29.9 L mmol $H_2O_2{}^{-1}$ for the ozonation and UV/H_2O_2 process, respectively. For ozonation, Atenolol, Carbamazepine, Codeine, Trimethoprim and Diclofenac showed the lowest initial k_{obs} (in the order mentioned). Atenolol and Carbamazepine appeared as the most ozone resistant pharmaceuticals, exhibiting the lowest percentage of elimination at low ozone doses. On the other hand, despite the non-selectivity of HO•, differences in the initial k_{obs} were also observed during the UV/H_2O_2 process. Trimethoprim, Paroxetine and Sulfamethoxazole exhibited the lowest initial k_{obs} values (in the order mentioned). Trimethoprim and Paroxetine also exhibited the lowest percentage removal when low H_2O_2 doses were assayed. The compounds that were identified as problematic during ozonation were more efficiently removed by the UV/H_2O_2 process. UV/H_2O_2 generally appeared to be a more efficient technology for removing pharmaceuticals from RO brines compared to ozonation.

1. Introduction

Over the last few decades, water scarcity and water quality have become issues of major concern. Large amounts of water have been continuously contaminated, especially in developed countries. The restoration of water quality is essential for avoiding higher levels of contamination and for enabling water reuse, which can decrease potable water consumption [1]. The presence of pharmaceuticals among other microcontaminants in the aquatic environment is now being well-established. Although some pharmaceuticals (e.g. ibuprofen, paracetamol) are effectively removed by conventional biological treatments, others (e.g. carbamazepine, diclofenac, naproxen and atenolol) are barely affected [2,3]. The presence of pharmaceuticals in the aquatic environment is of concern because the effects of low-level but long-term exposure

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http://dx.doi.org/10.1016/j.jhazmat.2013.05.030

of aquatic life to pharmaceuticals due to the bioactive nature of pharmaceuticals are still largely unknown. Moreover, although there is no evidence of the impact of pharmaceuticals on human health, the precautionary principle should be applied in the case of indirect potable water reuse. Therefore, additional advanced treatment steps should be considered to reduce the discharge load of pharmaceuticals and other micropollutants into sensitive receiving waters.

Membrane processes are good candidates for wastewater reuse due to the combination of process stability and excellent water quality production in these processes. Reverse osmosis (RO) is one of the most effective membrane treatments for removing a wide range of organic pollutants, bacteria and viruses, dissolved organic matter and inorganic salts [4–6]. However, one of the major drawbacks of RO is the generation of huge volumes of concentrates that are commonly discharged to water bodies. The organic materials in membrane retentates include organic matter in the carrier drinking water, refractory chemicals wastewater from public use (e.g. pesticides, personal care products, pharmaceuticals and endocrine disruptors) and residuals from wastewater treatment processes (e.g. soluble microbial products, partially biodegraded organics and anti-scaling chemicals). In addition, membrane retentates contain biological materials (i.e., bacteria, viruses, oocysts and cell fragments) that also represent a potential environmental hazard. Although the discharge of membrane retentates is not currently regulated, safe environmental practices would suggest that such a concentrated waste stream to be treated before its release and dilution into the environment [7,8]. Treating these concentrates would minimize the environmental impacts associated with their discharge or management.

Because there is an urgent need for environmentally friendly management options for RO brines, diverse technologies for the treatment of RO brines have been investigated, including coagulation/flocculation, activated carbon adsorption, ozonation, UV/H_2O_2, Fenton, photocatalysis, sonolysis, electrochemical oxidation and river bank filtration, among others [3,5,8–10]. However, these studies focused on very diverse aspects of the technologies and there is still a lack of information about the efficiency of advanced oxidation processes (AOP) in removing micropollutants from these complex matrices and to improve the quality of the effluents. AOP appear to be appropriate for the treatment of waste streams that are highly concentrated in recalcitrant micropollutants (i.e., streams that correspond to rejections higher than 98%). Specific information on the efficacy of AOP for the removal of pharmaceuticals from RO concentrates is needed. On the other hand, it is still commonly perceived that the sustainability of AOP or other wastewater treatment methods should be eventually determined by economic factors. However, given the growing shortage of high quality water, which is expected to worsen with global changes in climate, the water industry and policy makers may have to reconsider the priority given to economic issues. Moreover, the energy inputs that are expended in RO treatment and the fact that only around 25% of the RO inlet flowrate is treated should also be taken into account. Therefore, the UV/H_2O_2 process and ozonation, which are investigated in this study, appear to be good treatment options for minimizing the potential negative effect of this problematic effluent upon the environment and public health.

In this paper, reclamation RO concentrates from a Municipal Waste Water Treatment Plant (MWWTP) were subjected to ozonation and UV/H_2O_2 treatments. Different pharmaceuticals were identified in the RO retentate. The decrease in the concentration of the pharmaceuticals was determined for different oxidant doses. The initial observed kinetic constants (k_{obs}) were calculated for the different pharmaceuticals to assess the initial efficiency of each AOP to remove the pharmaceuticals. The degree of treatment required to totally eliminate the micropollutants was also determined. Additional objectives in the study were the measurement of mineralization (total organic carbon (TOC) removal), the degree of oxidation (chemical oxygen demand (COD)), changes in aromaticity (measured as UV_{254} absorbance), acute toxicity and biodegradability (measured as the 5-day biochemical oxygen demand to COD (BOD_5/COD)). These typical water quality parameters enable the post-treatment effluent quality to be assessed to evaluate the suitability of implementing a biological step after chemical oxidation and to develop correlations between micropollutant removal and typical organic parameters.

2. Materials and methods

2.1. Lab-scale reactors

The oxidation experiments were carried out in 2-L jacketed reactors at 25 °C. The UV/H_2O_2 reactor was equipped with three low pressure mercury lamps which emit at a wavelength of 254 nm (Philips TUV 8 W, G8T5). The photon flow rate was measured to be 1.5×10^{-5} Einstein s^{-1} using uranyl oxalate actinometry [11]. The H_2O_2 doses in the experiments ranged from 0.04 to 0.72 mg H_2O_2 mg TOC^{-1}. Reaction time for the highest dose was 96.9 min. In the ozonation experiments, the ozone-containing stream was injected into the effluent through diffusers at a flow rate of 133.5 L h^{-1} and an ozone concentration of 10 g O_3 Nm^{-3} to attain various transferred ozone doses (from 0.14 up to 6.93 mg O_3 mg TOC^{-1}). Ozonation time to achieve the highest ozone dose was 68.9 min. The ozonation set-up is described elsewhere [12].

2.2. Analysis

Various methods and devices were used to both characterize the effluent and monitor the oxidation processes. Some of these methods and devices are briefly described here. The concentration of H_2O_2 (supplied by Panreac, 30% w/v) was determined using the metavanadate spectrophotometric procedure at 450 nm [13]. TOC was measured using a Simadzu TOC-VCSN analyzer. To measure the COD and filtrated COD, procedure 5220D from Standard Methods [14] was followed. The BOD_5 was evaluated using a WTW OxiTop® measuring system (Weilheim, Germany) by following procedure 5210D from Standard Methods [14]. The anion concentrations were determined using an Advanced Compact IC Metrohm ionic chromatograph with a Metrosep A Supp 4-250 column. Bromide and bromate ions were analyzed by ionic chromatography (881 Compact IC pro Metrohm) with conductivity detection following the Spanish standard UNE-EN ISO 10304-2:1995. The ammonium concentration was measured by an ammonium electrode *SympHony* from VWR® and turbidity was determined using a Hach 2100P turbidimeter. Alkalinity was quantified by titration with HCl as described in 2320B Standard Methods procedure [14]. The Microtox® test, which uses luminescent *Vibrio fischeri* bacteria, was performed to assess the acute toxicity of the samples. The Microtox® analysis covers the potential toxicity of a wide range of substances.

Samples were prepared for pharmaceuticals analysis by vacuum filtering all the water samples through 0.7-μm glass fiber filters, followed by filtration through 0.45-μm nylon membrane filters. An aqueous solution of 5% Na_2EDTA was added to the filtered water samples to achieve a final concentration of 0.1%. Aliquots of water were then spiked with surrogates and pre-concentrated onto lipophilic-hydrophilic balanced Oasis HLB (200 mg, 6 mL) cartridges from Waters Corporation (Milford, MA, USA) using an automatized solid phase extraction (SPE) system (ASPEC GX-271,

Gilson). The cartridges were rinsed with 5 mL of HPLC grade water and dried under vacuum for 20 min. Elution was subsequently carried out with 8 mL of methanol, followed by 6 mL of ethyl acetate. The extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 1 mL of methanol/water (1/9). For the internal standard calibration, 10 μL of a 1 mg L^{-1} standard mixture of isotopically labeled compounds were added to the prepared samples that were analyzed using high performance liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). LC analysis was performed using an Agilent HP 1100 HPLC (Palo Alto, CA, USA), which was equipped with an autosampler and connected in series to a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer, equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved using a Purospher Star RP-18 endcapped column (125 mm × 2.0 mm, particle size 5 μm), preceded by a C_{18} guard column (4 × 4, 5 μm), both supplied by Merck (Darmstadt, Germany). The method detection limits (MDLs) and method quantification limits (MQLs) were determined for the wastewater samples as the minimum amount of analyte detected at a signal-to-noise ratio of 10. Therefore, the MDLs ranged from 0.1 to 17 ng L^{-1} and the MQLs ranged from 0.3 to 55 ng L^{-1} for effluent wastewaters [15]. Recoveries achieved for all target compounds ranged from 65 to 119%. The overall method precision, calculated as the relative standard deviation (RSD), was satisfactory, with RSD values ranging from 1 and 10% (see Table 3).

2.3. RO brines

The RO brines studied here originate from a tertiary treatment in El Prat del Llobregat MWWTP, Barcelona (Spain). The secondary effluent from the conventional activated sludge treatment is subjected to coagulation/flocculation, lamellar settling, disinfection and ultrafiltration, before finally passing through an RO unit to produce indirect potable water quality. This water is reused to mitigate saline intrusion and to supplement groundwater supplies. The RO retentate is currently being discharged into coastal surface waters.

Tables 1 and 2 show the physicochemical characteristics of the MWWTP RO retentate. The low biodegradability ratio of the retentate was due to a high recalcitrant organic matter content (BOD_5/COD = 0.03); the high alkalinity and the high chloride concentration of the retentate should also be highlighted.

The initial concentrations of the eleven identified pharmaceuticals were of the same order of magnitude as found by other authors that used the same type of matrices [16]. The initial concentrations of the pharmaceuticals ranged from 0.2 to 1.6 μg L^{-1} and are detailed in Table 3.

Table 1
Average physicochemical parameters of RO brine.

TOC (mg C L^{-1})	27.6	UV_{254} (cm^{-1})	0.595
TN (mg N L^{-1})	22.9	Turbidity (NTU)	1.07
COD (mg O_2 L^{-1})	77.0	pH	8.3
BOD_5 (mg O_2 L^{-1})	2.2	Alkalinity (mg $CaCO_3$ L^{-1})	914
Microtox®	Non-toxic	Conductivity (mS cm^{-1})	5.96

Table 2
Average anion and cation concentrations in the RO brine.

Cl^- (mg Cl^- L^{-1})	1540	NH_4^+ (mg NH_4^+ L^{-1})	3.23
Br^- (mg Br^- L^{-1})	9.64	Na^+ (mg Na^+ L^{-1})	1065
NO_3^- (mg NO_3^- L^{-1})	83.7	K^+ (mg K^+ L^{-1})	135
PO_4^{3-} (mg PO_4^{3-} L^{-1})	3.96	Ca^{2+} (mg Ca^{2+} L^{-1})	477
SO_4^{2-} (mg SO_4^{2-} L^{-1})	569	Mg^{2+} (mg Mg^{2+} L^{-1})	145

Table 3
Average pharmaceutical concentrations in the RO brine. Pharmaceutical % recoveries and Relative Standard Deviations (RSD) of the analytical method.

Pharmaceutical	% recoveries (RSD)	Conc. (μg L^{-1})	Type
Indometacin	108 (±4)	0.895	Non-steroidal anti-inflammatory drug
Diclofenac	104 (±1)	0.605	Non-steroidal anti-inflammatory drug
Naproxen	109 (±3)	1.080	Non-steroidal anti-inflammatory drug
Propyphenazone	116 (±4)	0.258	Analgesic and antipyretic
Paroxetine	99 (±3)	0.508	Antidepressant
Sulfamethazine	87 (±8)	0.635	Sulphonamide antibacterial
Sulfamethoxazole	114 (±3)	1.638	Sulphonamide bacteriostatic antibiotic
Atenolol	65 (±10)	1.028	Beta blocker
Codeine	109 (±4)	0.673	Opiate
Trimethoprim	119 (±7)	0.235	Chemotherapeutic
Carbamazepine	73 (±8)	1.038	Anticonvulsant and mood-stabilizing

3. Results and discussion

The characterized RO brine was treated by ozone and UV/H_2O_2 processes. The target pharmaceuticals were monitored by LC–MS/MS throughout the oxidation processes. Because it is still commonly perceived that the sustainability of AOP or other wastewater treatment methods should be eventually determined by economic factors, extended ozonation or UV/H_2O_2 treatments of these RO concentrates could be excessively expensive. For this reason, the initial k_{obs} values, which provide information on pharmaceutical removal at the lowest initial oxidant doses, were calculated. The k_{obs} were calculated using the initial experimental degradation rate (the variation in the amount of pharmaceutical per oxidant consumed) divided by the corresponding initial concentration (using mole units). The k_{obs} values for ozonation and the UV/H_2O_2 process are detailed in Table 4.

The percentage removal of the pharmaceuticals at the different oxidant doses (mg of oxidant per mg of initial TOC of the effluent) are plotted for both processes in Figs. 1 and 2. In ozonation, organic compounds can be oxidized via two mechanisms: reaction with molecular ozone (direct pathway) and reaction with hydroxyl radicals generated by ozone decomposition (indirect pathway). Molecular ozone reacts selectively with organic compounds and the reaction rates can vary over several orders of magnitude. In contrast, hydroxyl radicals are not selective and the corresponding reaction rates are typically much higher $>10^9$ M^{-1} s^{-1}. Due to $[HO^\bullet]/[O_3]$ ratios typically in the 10^{-9} to 10^{-7} range [17] and the particularly high alkalinity and chloride concentration, which act as scavengers of $HO^\bullet$, the indirect pathway is not the dominant mechanism in the ozonation of these RO brines. Moreover, the

Table 4
Ozone and UV/H_2O_2 initial experimental k's for the analyzed pharmaceutical.

	k's ozone (L mmol O_3^{-1})		k's UV/H_2O_2 (L mmol $H_2O_2^{-1}$)
Paroxetine	12.8	Diclofenac	29.9
Sulfamethazine	12.2	Propyphenazone	28.1
Sulfamethoxazol	9.6	Indometacin	26.7
Naproxen	9.5	Naproxen	26.4
Propyphenazone	9.1	Sulfamethazine	26.0
Indometacin	8.4	Codeine	20.1
Diclofenac	7.5	Carbamazepine	18.8
Trimethoprim	6.7	Atenolol	17.8
Codeine	6.5	Sulfamethoxazol	15.0
Carbamazepine	4.3	Paroxetine	11.2
Atenolol	0.8	Trimethoprim	9.7

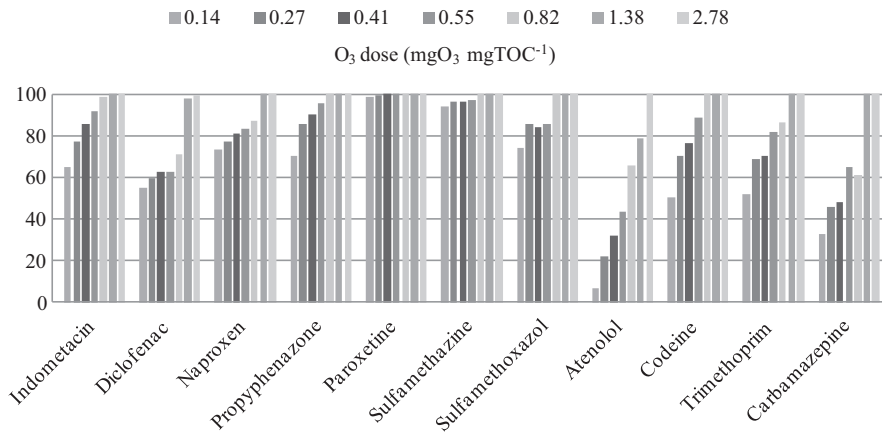

Fig. 1. Pharmaceutical removal at different ozone doses.

elimination of all of the pharmaceuticals took place before the initial ozone demand (IOD; the transferred ozone dose required to obtain a measurable dissolved ozone level) (3.27 mg O_3 mg TOC^{-1}) was satisfied and it is from this point on that the removal of trace organic contaminants via $HO^{\bullet}$ becomes more important [18]. Therefore, oxidation of compounds, especially if the compounds have high direct reaction rates with molecular ozone, will occur almost exclusively via the direct reaction. Sulfamethoxazole, Sulfamethazine and Naproxen, among others, showed high k_{obs} values and good percentage removals even at low ozone doses (see Fig. 1) according to Reungoat et al. [19] because these pharmaceuticals have electron-rich functional groups, such as aniline, pyrimidine and naphthalene, that are highly reactive with molecular ozone. Atenolol, Carbamazepine, Codeine, Trimethoprim and Diclofenac showed the lowest initial k_{obs} values (in the order mentioned). Atenolol and Carbamazepine showed the lowest percentage of removal at low ozone dosages. In addition, Carbamazepine, Atenolol and Diclofenac were the only drugs that did not show higher than 80% removal when an ozone dose of 0.82 mg O_3 mg TOC^{-1} was used. The poor performance of ozone in eliminating Atenolol can be explained by the lower direct reaction rate of Atenolol with ozone $<10^3$ M^{-1} s^{-1}. Other pharmaceuticals, such as Diclofenac, Trimethoprim and Carbamazepine, were expected to show higher k_{obs} values and percentage removals because they also have electron-rich functional groups and have already shown to be easily removed from effluents even at low ozone doses [19]. These differing results evidence the considerable complexity inherent in this type of treatment when applied to practical wastewaters due to the high number of factors involved, such as the reactivity of effluent organic matter (EfOM) and variable water quality, that can affect the observed removals [20].

Hydroxyl radicals degrade compounds in UV/H_2O_2 treatment. Trimethoprim, Paroxetine and Sulfamethoxazole exhibited the lowest k_{obs} values (in the order mentioned). In addition, Trimethoprim and Paroxetine exhibited the lowest percentage removals when low H_2O_2 doses were assayed. A removal higher than 80% was obtained for all of the drugs, with the exception of Trimethoprim, Paroxetine and Codeine, when a dosage of 0.11 mg H_2O_2 mg TOC^{-1} was used. The UV/H_2O_2 process showed higher performance for removing compounds that were problematic in ozonation, such as Atenolol, Diclofenac and Carbamazepine. In contrast, Paroxetine and Trimethoprim required high H_2O_2 doses to achieve acceptable percentage removals, as seen in Fig. 2. The poor removal exhibited by the pharmaceutical Trimethoprim was unexpected because most of pharmaceuticals show similar $HO^{\bullet}$ reaction rate constants [3] and similar removals have been previously reported for Trimethoprim and other target pharmaceuticals (i.e., atenolol and carbamazepine) [20]. As discussed above, the reactivity of EfOM and variable water characteristics can affect micropollutants removal, thus explaining the controversial results. Moreover, the pharmaceuticals identified possess different chemical functionalities that would make them reactive to direct photolysis. The

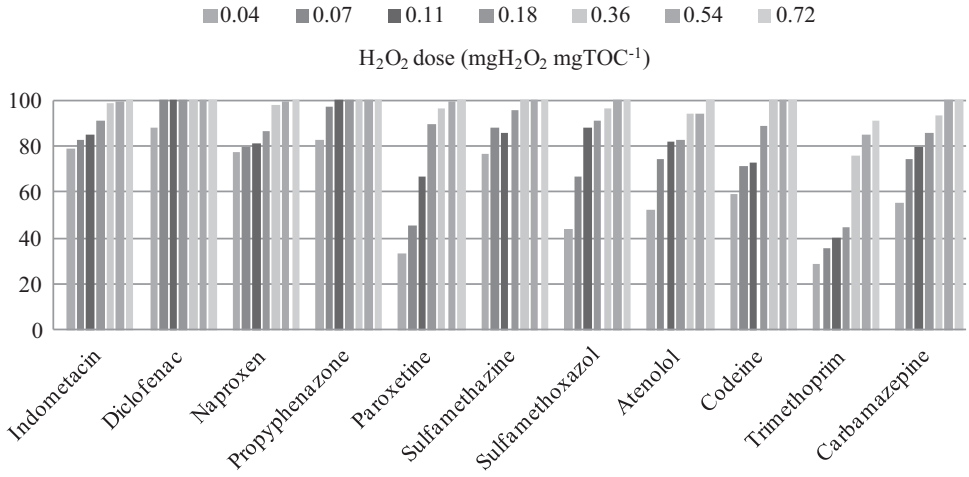

Fig. 2. Pharmaceutical removal at different H_2O_2 doses in the UV/H_2O_2 process.

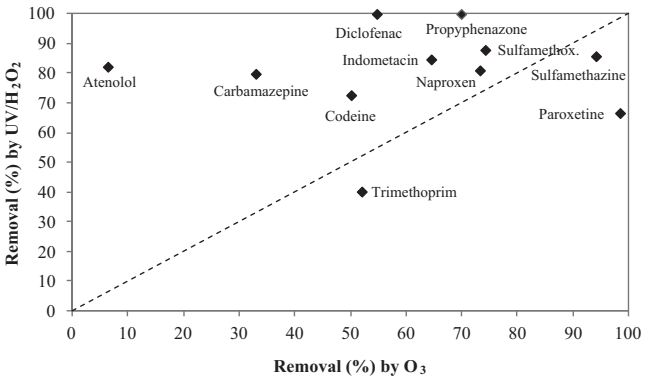

Fig. 3. Comparison of removal by ozonation and the UV/H_2O_2 process for doses of 0.08 mmol O_3 L^{-1} and 0.09 mmol H_2O_2 L^{-1}, respectively.

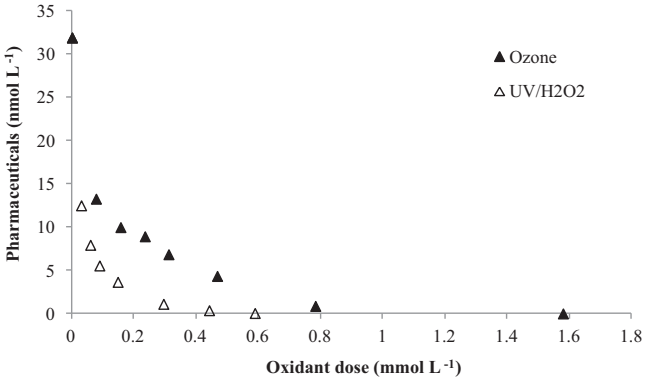

Fig. 4. Removal of the sum of pharmaceuticals as function of oxidant dosage.

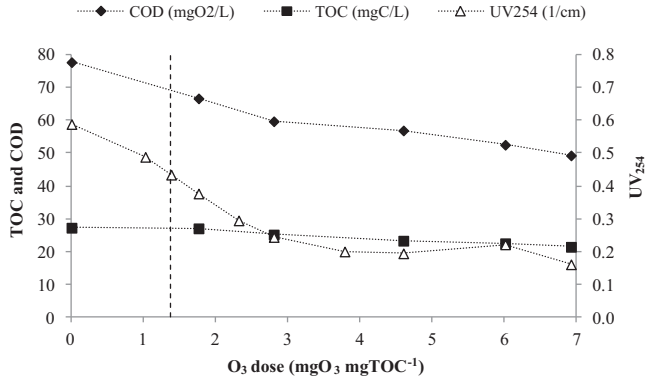

Fig. 5. Monitoring of typical water quality parameters during ozonation.

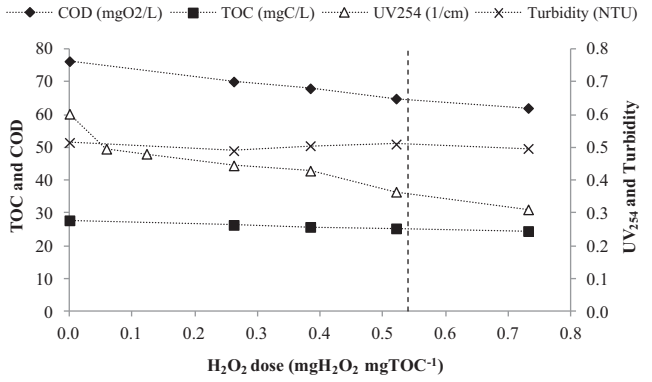

Fig. 6. Monitoring of the typical water quality parameters during the UV/H_2O_2 process.

contribution of direct UV photolysis could be partially responsible for the better removal of some pharmaceuticals.

To illustrate the performance of both processes in pharmaceutical removal, Fig. 3 compares the removal by ozone and UV/H_2O_2 processes when moderate and equal doses of oxidants (in mol) were applied (0.034 mmol O_3 mmol TOC^{-1} and 0.038 mmol H_2O_2 mmol TOC^{-1}, which correspond to 0.14 mg O_3 mg TOC^{-1} and 0.11 mg H_2O_2 mg TOC^{-1}, respectively). The results showed that UV/H_2O_2 was more efficient than ozone at removing the pharmaceuticals from the RO brines in most cases, with the exception of Trimethoprim, Paroxetine and Sulfamethazine. The same conclusion can be drawn from Fig. 4, where the amount of H_2O_2 needed to remove the sum of the analyzed pharmaceuticals can be observed to be significantly lower compared to the amount of ozone required.

The evolution of typical parameters such as COD, TOC, aromaticity (UV_{254} absorbance) and turbidity for ozonation and the UV/H_2O_2 process was also determined. These parameters are plotted in Figs. 5 and 6 for ozonation and the UV/H_2O_2 process, respectively. Although the final degradation achieved with both AOPs was different due to the different reaction conditions assayed, the processes were compared by choosing as a reference the oxidant dose that provided complete elimination of most of the pharmaceuticals (1.38 mg O_3 mg TOC^{-1} and 0.54 mg H_2O_2 mg TOC^{-1}). The moles of oxidant required in the ozonation process was almost double that required in the UV/H_2O_2 process (0.34 mol O_3 mol C^{-1} vs. 0.19 mol H_2O_2 mol C^{-1}, respectively). Table 5 shows the TOC, COD and UV_{254} removals and the biodegradability ratios obtained for these dosages. Reaction times needed to achieve these dosages of oxidants are also shown in the table. The results show that ozonation led to negligible mineralization and lower removal of COD and aromaticity than the UV/H_2O_2 treatment. However, the biodegradability ratio (BOD_5/COD) of the resulting effluents reached satisfactory and similar values for both processes. Thus, implementing a biological step after chemical oxidation could reduce the organic load of the effluents in a cost-effective way, improving the quality of the effluent for a more environmentally friendly discharge. For the Microtox® assay, no inhibition of bacteria was observed for the RO retentate or for any of the effluents resulting from the oxidation processes. Therefore, the samples did not show any non-specific toxicity before and after treatment. The results show that UV/H_2O_2 treatment appears to be the best candidate process for treating RO concentrates.

On the other hand, the percentage removal of TOC, COD or UV_{254} was not similar for the two processes at the specific oxidant doses for which complete elimination of pharmaceuticals was obtained. However, parallel trends were observed for both processes when

Table 5
Removals and biodegradability ratios achieved at the "total elimination" doses for the AOPs studied.

Ozone	Oxidant dose	Time (min)	ΔTOC (%)	ΔCOD (%)	ΔUV_{254} (%)	BOD_5/COD
Ozone	1.38 mg O_3 mg TOC^{-1}	5.4	0.6	11	26	0.34
UV/H_2O_2	0.54 mg H_2O_2 mg TOC^{-1}	61.7	9.6	16	42	0.30

the specific UV absorbance (SUVA) parameter (calculated as UV_{254} over COD) was represented as function of the oxidant consumed around the pharmaceutical total elimination dosages. The effluent exhibited very similar SUVA values (0.62 and 0.56 L mg O_2^{-1} m^{-1} for ozone and UV/H_2O_2, respectively) independent of the oxidation process that was used at dosages of 1.38 mg O_3 mg TOC^{-1} and 0.54 mg H_2O_2 mg TOC^{-1}. Thus, pharmaceutical removal appeared to be correlated with the SUVA parameter. Further research in this direction is required to corroborate whether this behavior can be extended to other oxidation treatments, such that SUVA can be considered as a surrogate for assessing contaminant removal efficiency.

The oxidation of the contaminants present in these waste effluents can potentially produce transformation products even more toxic than the mother compounds. Moreover, the large number of toxicity tests that can provide information on effluent quality often results in highly variable responses which are difficult to interpret. For these reasons, various authors [7,21] agree that complete oxidation of all organics to carbon dioxide is the best level of organics control. In addition, because advanced processes are necessary to remove the micropollutants from RO retentates, an extension of treatment could be considered in order to improve the effluent quality for a more environmentally friendly disposal. For these reasons, further ozonation of the RO retentate was performed to obtain higher mineralization, removal of UV_{254} absorbance and to rule out any non-specific toxicity due to the formation of residual oxidants [22] or toxic secondary oxidation by-products that has been reported by Petala et al. [23]. Fig. 5 shows that high ozone doses are needed to obtain a certain degree of TOC removal. The highest ozone dose assayed (6.93 mg O_3 mg TOC^{-1}) produced 21% of mineralization, 37% of COD removal and 73% of UV_{254} absorbance removal. At this dose, the biodegradability ratio did not increase significantly (up to 0.37) and Microtox® analysis, once more, did not show any inhibition of bacteria. The bromate concentration in the effluent increased from a negligible initial value to 1.1 mg BrO_3^- L^{-1}, due to the presence of high bromide levels during ozonation. This bromate value did not exceed the threshold (3 mg BrO_3^- L^{-1}) proposed by Hutchinson et al. [24] for the protection of aquatic organisms, which is based on long-term adverse effects. However, the bromate level must be evaluated, depending on the final use of the ozonated effluent because the World Health Organization has recommended a provisional guideline value of 25 μg L^{-1} for drinking water [25]. In addition, the IOD of the brine was 3.27 mg O_3 mg TOC^{-1}. As previously discussed, all the analyzed pharmaceuticals were completely removed at this ozone dose. Moreover, the IOD led to the completion of the first oxidation stage of UV_{254} and COD (see Fig. 5). Therefore, such an ozone dose ensures good improvement in the effluent quality and the complete removal of the micropollutants studied ensuring fast kinetics and ozone transfer [26].

While the pH and turbidity levels remained constant during the UV/H_2O_2 process, these levels increased during ozonation up to maximum values of 8.76 and 332 NTU, respectively. Ozonation stripped the CO_2 and volatile fatty acids [27] from the wastewater, slowly increasing the pH during the experiments and leading to the formation of a white precipitate. The maximum registered pH corresponded to an ozone dose of approximately 5.7 mg O_3 mg TOC^{-1}. The wastewater pH subsequently started to decrease, while the turbidity increased. Both the decrease in the alkalinity and the Ca^{2+} concentration (27 and 21%, respectively, at the highest ozone dose) corroborated calcite precipitation at these ozone doses, as previously reported by Westerhoff et al. [7]. In addition, significant differences between the COD and filtrated COD values were observed at this ozone dose, which could also indicate the precipitation of a calcium-organic acid material, as indicated by the same authors.

4. Conclusions

Both ozonation and UV/H_2O_2 technologies performed well in the removal of various analyzed pharmaceuticals from the RO brine of a reclamation plant. However, the UV/H_2O_2 process appeared to be a more promising and efficient tool for treating these concentrates. The UV/H_2O_2 process removed the pharmaceuticals and improved the effluent quality, while using significantly less oxidant compared to ozonation.

The main mechanism in ozonation is the attack of contaminants by molecular ozone. However, the predominant mechanism in the UV/H_2O_2 process involves the reaction of hydroxyl radicals. The different mechanisms produced significant differences in the order of priority with which pharmaceuticals were removed. The UV/H_2O_2 process exhibited a higher performance than ozone, in being able to remove compounds that were problematic for ozone, such as Atenolol, Diclofenac and Carbamazepine. In contrast, Paroxetine and Trimethoprim required high H_2O_2 doses for acceptable levels of pharmaceutical elimination, whereas ozonation produced a good level of removal from the first stages of oxidation. Further research should be made in order to deeply assess the responsibility of these complex matrices on deviations from expected kinetics of pharmaceuticals during their removal by AOPs.

Due to the strongly recalcitrant nature of some of the pharmaceuticals studied, high oxidant doses were necessary to ensure the complete removal of all the monitored microcontaminants from the brines studied in this work (0.54 mg H_2O_2 mg TOC^{-1} and 1.38 mg O_3 mg TOC^{-1}). Effluents with a biodegradability ratio (BOD_5/COD) higher that 0.3 were obtained when complete removal was achieved. This result suggests the combination of an AOP for pharmaceuticals removal with a subsequent biological treatment step. This combination could lead to high organic matter removal and sufficiently high effluent quality for an environmentally friendly disposal of the effluent.

Acknowledgements

This work was partly supported by the Generalitat de Catalunya (Consolidated Research Groups: Enginyeria de Processos d'Oxidació Avançada 2009-SGR-1466 and Water and Soil Quality Unit 2009-SGR-965).

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APPENDIX IV

Application of bioassay panel for assessing the impact of advanced oxidation processes on the treatment of reverse osmosis brine

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Published in *Journal of Chemical Technology and Biotechnology* 89 (2014) 1168-1174

Research Article

Received: 15 January 2014 Revised: 21 March 2014 Accepted article published: 22 April 2014 Published online in Wiley Online Library: 30 April 2014

(wileyonlinelibrary.com) DOI 10.1002/jctb.4389

Application of bioassay panel for assessing the impact of advanced oxidation processes on the treatment of reverse osmosis brine†

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Abstract

BACKGROUND: The persistence of microcontaminants through conventional wastewater treatments is a matter of concern and it suggests the implementation of advanced treatment steps. Although there is evidence that reverse osmosis (RO) is the most efficient treatment for the the removal of these compounds, it has the drawback of producing significant amounts of highly polluted brine. In this work, chemical analyses and toxicity bioassays were combined to evaluate the removal of different pharmaceuticals and of dioxin-like compounds from RO brine through oxidative processes such as ozone, UV and UV/H_2O_2.

RESULTS: The removal of the selected pharmaceuticals required a relatively high oxidative capacity, either by ozonation or by the combination of UV radiation and H_2O_2. Bioassays showed a significant dioxin-like activity in brine samples, whereas antibacterial or estrogenic activities were negligible. UV by itself was the least efficient in removing this dioxin-like activity. Ozonation appeared as the most competent treatment.

CONCLUSIONS: The results of this work indicate the usefulness of advanced oxidation methods, especially ozonation, to remove biologically active micropollutants from brine samples. They also show that only the combination of chemical analyses and bioassays allows complete characterization of the efficiency of advanced water treatment processes to remove recalcitrant pollutants.

Supporting information may be found in the online version of this article.

Keywords: RO brine; RYA; AOP; micropollutants; bioassay

INTRODUCTION

Municipal and industrial wastes are treated in wastewater treatment plants (WWTP) and the effluents generated are discharged to receiving streams. These effluents contain pollutants of concern since secondary or even advanced treatments are unable to remove all pollutants and chemicals.[1] Therefore they require additional treatment in order to be reused for groundwater recharge or irrigation. Membrane processes are promising candidates for the treatment of wastewater effluents for reuse due to the combination of process stability and the production of high quality water. Reverse osmosis (RO) is one of the most effective membrane treatments. However, one of the major drawbacks of RO is the generation of huge volumes of concentrates (brine) that are commonly discharged to water bodies. Although their discharge is not currently regulated, safe environmental practices would suggest treatment before its release into the aquatic environment.[2,3]

Advanced oxidation processes (AOPs) appear to be appropriate for treatment of the RO brines, which are highly concentrated in recalcitrant micropollutants.[3–6] The literature on this kind of effluent is focused on very diverse aspects and there is still a lack of information about the toxicity assessment of the treated RO brines. In a previous work, the removal of 11 pharmaceuticals from RO brine by ozone and UV/H_2O_2 treatments was evaluated. The

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† *This work was given at a scientific meeting (EAAOP3 – October 2013 – Almeria)*

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evaluation of the toxicity of the RO retentate using the Microtox assay was also included.[7] However, no inhibition of *Vibrio fischeri* was observed for the RO retentates or for any of the effluents resulting from the oxidation processes.

Bioassays provide the unique possibility to monitor water (and other matrices) quality based on the biological activity of the putatively present pollutants, rather than depending on their specific structural nature. These assays can be tailored to cover from rather crude general toxicity tests to very specific biological activities, presumed to be relevant in a particular case. For example, the presence of antibiotic activity in wastewaters can be quantified by measuring their effects on sensitive bacterial strains.[8] While antibiotics themselves are not particularly damaging for the environment, it has been demonstrated that their presence induces resistance forms in soil and water microbes, a phenotype that can be transmitted to pathological microbial strains.[9,10] Specifically addressed to study ligand–receptor interactions, recombinant yeast assays (RYA) use yeast to evaluate the presence of specific biological activities by partially reproducing mammalian/vertebrate signalization pathways. Two RYA assays were used in this work, one harboring the human estrogen receptor (ER–RYA)[11] and a second one expressing the human aryl hydrocarbon receptor and the ah receptor nuclear translocator complex (AhR-RYA).[12,13] These systems are designed to allow the precise quantitation of the ligand concentration (or, better said, of its biological activity) by measuring the enzymatic activity of the reporter. The ER-RYA is able to detect and quantify estrogenic compounds,[11] whereas the presence of AhR ligands is linked to the ectopic activation of detoxification of phase I and II metabolic enzymes.[14] This effect, termed dioxin-like activity, is central on the subletal toxicity of a variety of organic pollutants, including pharmaceuticals, polycyclic aromatic hydrocarbons (PAHs), organochlorine compounds (OCs) and dioxins.[15,16] It is important to stress that RYA (and other single-cell based similar assays) do not provide direct information on the concentration of any particular molecule in the solution, but rather integrate the total biological activity (estrogenicity, dioxin-like, etc.) present in a given sample, irrespectively of the chemical nature of the active compounds or of the complexity of sample composition. These or similar bioassays have been used to monitor the efficiency of water treatment processes to remove specific toxic activities.[17–19]

Our objective in the present work is to combine chemical analyses (addressed to defined sets of compounds, pharmaceuticals in this work) and bioassays (targeting biological activities rather than specific compounds) to characterize the removal of contaminants from tertiary RO brine by UV radiation and two different AOPs, i.e. ozonation and UV/ H_2O_2 process.

MATERIAL AND METHODS

Sampling of RO brines

The RO brines studied originate from a tertiary treatment of a WWTP in a coastal area of Catalonia (Spain). The secondary effluent from the conventional activated sludge treatment is subjected to sand filtration, then microfiltration and finally part of this water is fed to a two-stage RO system. In order to be sure that no impurity can damage the RO membrane, two bag filters (5–20 μm) were placed before the RO system. A mixture of sand filtration effluent and RO permeate plus chlorination was suitable for golf court irrigation.

Lab-scale reactors

The oxidation experiments were carried out in 2 L jacketed reactors at 25°C. The UV/H_2O_2 reactor was equipped with three low pressure mercury lamps which emit at a wavelength of 254 nm (Philips TUV 8 W, G8T5). The photon flow rate was measured to be 1.7×10^{-5} Einstein s^{-1}. The initial concentration of hydrogen peroxide was 30 mgH_2O_2 L^{-1} (H_2O_2 consumed in the experiments ranged from 0.01 to 0.90 mgH_2O_2 mgTOC^{-1}). Reactions were quenched by removing the excess H_2O_2 with catalase or $NaHSO_3$ according to the analysis to be carried out. Reaction time for the highest dose was 109.1 min. In the ozonation experiments, the ozone-containing stream was injected into the effluent through diffusers at a flow rate of 133.5 L h^{-1} and an ozone concentration of 10 gO_3 Nm^{-3} to attain various transferred ozone doses (from 0.05 up to 3.00 mgO_3 mgTOC^{-1}). Ozonation time to achieve the highest ozone dose was 31.2 min.

Chemical analysis

Different methods and devices were used to both characterize the effluent and monitor the oxidation processes. The concentration of H_2O_2 (supplied by Panreac, 30% w/v) was determined using the metavanadate spectrophotometric procedure at 450 nm.[20] Total organic carbon (TOC) was measured using a Simadzu TOC-VCSN analyzer. To measure the chemical oxygen demand (COD), procedure 5220D from Standard Methods was followed. The biological oxygen demand at 5 days (BOD_5) was evaluated using a WTW OxiTop® measuring system (Weilheim, Germany) by following procedure 5210D from Standard Methods (16). The anion concentrations were determined using an Advanced Compact IC Metrohm ionic chromatograph with a Metrosep A Supp 4–250 column. Turbidity was determined using a Hach 2100P turbidimeter. Alkalinity was quantified by titration with HCl as described in 2320B Standard Methods procedure.[21]

The analytical method for pharmaceutical determination was adapted from the protocols described in Gros *et al.*[22] Samples were vacuum filtered through 0.7 μm glass fiber filter by 0.45 μm nylon membrane filters. An aqueous solution of 5% Na_2EDTA was added to achieve a final concentration of 0.1%. Then, aliquots of water were spiked with surrogates, and pre-concentrated onto lipophilic-hydrophilic balanced Oasis HLB (200 mg, 6 mL) cartridges from Waters Corporation (MA, USA). The sample volume was 250 mL for the different points of the tertiary treatment analyzed and 500 mL for the permeate and samples of each dosage applied. Pre-concentration was carried out by an automatized solid phase extraction system (ASPEC GX-271, Gilson). The cartridges were rinsed with 5 mL of HPLC grade water and dried under vacuum for 20 min. The elution was carried out with 8 mL of methanol. Extracts were evaporated to dryness and reconstituted with 1 mL of methanol/water, 1/9. For the internal standard calibration, 10 μL of a 1 mg L^{-1} standard mixture of isotopically labeled compounds was added to the prepared samples, and analyzed using high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). LC-MS/MS analysis was performed using a Transcend LC system coupled to triple quadrupole TSQ Vantage, both from Thermo Fisher Scientific (CA, USA). Analytes were separated on a Halo C-18 endcapped column (50 mm × 2.1 mm, particle size 2.7 μm) preceded by a Halo C-18 guard column (5 mm × 2.1 mm, 2.7 μm). A simple binary gradient consisting of (A) 0.1% HCOOH or 20 mmol L^{-1} of NH_4OAc for acid and neutral conditions, respectively, and (B) acetonitrile was employed for chromatographic separation.

Tandem mass spectrometry detection was performed in multiple reactions monitoring mode using heated electrospray ionization. The validation of the method showed satisfactory parameters like sensitivity (limits of detection below 5 ng L^{-1} for most of the compounds), accuracy (absolute recoveries above 60%) and repeatability (relative standard deviations below 8%) (see Table 2).

Biological assays

Microtox

Acute toxicity of the raw RO brine and samples at different oxidant doses from both AOPs was measured by Microtox® toxicity test, using luminescent *Vibrio fischeri* bacteria. Analysis was conducted according to the standard Microtox® test procedures recommended by the manufacturer (Azur Environmental, Delaware, USA). Toxicity is expressed as Effective Concentration that reduces the bioluminescence to 50% (EC_{50}) value, the concentration of sample that causes a 50% reduction in light emission after 15 min of contact.

Antimicrobial activity test

Quantitative analyses of bacterial growth inhibition[8] were performed using *Escherichia coli* (*E. coli*, ATCC25922 strain). The bacterial cell culture (OD_{600}=0.1) was distributed in a 96 well plate (final volume, 100 µL). 2 µL of sample or a reference antibiotic solution (Ampicillin from Sigma, both diluted in water) was added and plates were incubated at 37°C. Bacterial growth was recorded at regular intervals as Optical Density at a wavelength of 600 nm (OD_{600}) (Synergy 2 spectrofluorometer, BioTec) until negative controls (Milli-Q water) reached OD_{600}=2. Growth inhibition for each sample was calculated by interpolation of the final OD_{600} values to those from a serial dilution of ampicillin, from 0.1 to 3 mg L^{-1}.

RYA assays

The ER-RYA was performed using the yeast strain BY4741 (MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0) from EUROSCARF (Frankfurt, Germany) transformed with plasmids pH5HE0 (hER) and pVitBX2 (ERE-LacZ).[11] For the AhR yeast assay, we used the YCM4 yeast strain,[12] harboring a chromosomally integrated construct that co-expresses the hAHR and ARNT genes under the Gal1-10 promoter and the pDRE23-Z (XRE5-CYC1-LacZ) plasmid.[13,16] These two RYA assays were used to quantify the presence of estrogens and dioxin-like activity in samples, using already described protocols.[13] Briefly, a grown yeast culture was adjusted to OD of 0.1 and split into 100 µL aliquots in silylated 96-well polypropylene microtiter plates (NUNC™, Roskilde, Denmark).[11] Sample extracts solved in methanol were applied to the wells in a serial dilution scheme based on 1:3 dilution steps. Plates were incubated for 4 h at 30°C under mild shaking. After incubation, 100 µL YPER™(PIERCE™, Rockford, IL, USA) was added to each well and further incubated at 30°C for 30 min. Afterwards, 100 µL of assay buffer supplemented with 0.1% 2-mercaptoethanol and 0.5% of 4-methylumbelliferone ß-D-galactopyranoside solution (Bio-Rad Laboratories, Hercules, CA, USA) was added to the lysed cells. Plates were read at 355 nm excitation and 460 nm emission wavelengths. Fluorescence was recorded for 11 min (one measurement per 42 s); ß-galactosidase activity values were calculated as rates of the increment of arbitrary fluorescence units with time, using standard linear regression methods. To test possible inhibitory activity, yeast cultures were incubated for 6 h with 10 nmol L^{-1} estradiol (ER-RYA) or 1 µmol L^{-1} ß-naphthoflavone (AhR-RYA), added to a 1:60 dilution of each sample, and processed as before. Samples were tested in duplicate. Estrogenic or dioxin-like activities were calculated as estradiol equivalents (E2Eq, ER-RYA) or ß-naphthoflavone equivalents (BNFEq., AhR-RYA), by adjusting the data to a first-order Hill equation, as described.[16]

Table 1. Physicochemical parameters of RO brine

TOC (mgC L^{-1})	24.4	UV_{254} (cm^{-1})	0.464
DOC (mgC L^{-1})	24.2	Turbidity (NTU)	0.53
IC (mg C L^{-1})	149.8	pH	7.4
TN (mgN L^{-1})	73.3	Alkalinity ($mgCaCO_3$ L^{-1})	583
COD (mgO_2 L^{-1})	76.9	Conductivity (mS cm^{-1})	8.22
BOD_5 (mgO_2 L^{-1})	2.3	Residual oxidants (mgBr L^{-1})	0.42

Table 2. Average pharmaceutical concentrations in the RO brine. Pharmaceutical % recoveries and relative standard deviations (RSD) of the analytical method

Pharmaceutical	Group	Conc. (ng L^{-1})	% Recoveries ± RSD
Naproxen	Analgesic and anti-inflammatory	169	112 ± 1
Ketoprofen		259	128 ± 7
Diclofenac		935	93 ± 1
Gemfibrozil	Lipid regulator	1275	85 ± 8
Diazepam	Antidepressant and antiepileptic	102	111 ± 1
Lorazepam		45	125 ± 3
Carbamazepine		17	62 ± 6
Clarithromycin	Antibiotic	77	129 ± 1
Sulfamethoxazole		87	109 ± 4
Trimethoprim		81	61 ± 8
Atenolol	Betablocker	28	108 ± 4

RESULTS AND DISCUSSION

Characterization of the RO brine

Previous to their treatment with UV/H_2O_2 or ozone, the RO brines were physicochemically characterized (Table 1). As can be observed in Table 1, the low biodegradability ratio due to a high recalcitrant organic matter content (BOD_5/COD = 0.03) and the high alkalinity and chloride concentration of the retentate (1511 $mgCl^-$ L^{-1}) were remarkable within the average physicochemical parameters.

Eleven pharmaceuticals were identified by LC-MS/MS in the RO brine and monitored during the two oxidation processes performed. The initial concentrations of the pharmaceuticals ranged from 17 to 1275 ng L^{-1} (Table 2). These concentrations were relatively lower than those obtained in other work[7,23] probably due to the different placement and influential area of the WWTPs. More than 95% of the pharmaceuticals present in the input wastewater were rejected by the membranes and accumulated in the residual brine (Table 3). The removal efficiencies of the wastewater treatment technologies of the WWTP were in agreement with the reported removal ratios by Oulton *et al.*[24] On average, RO brine showed concentrations from 2–3-fold higher than those observed in the input wastewater (Table 3). Although the brine only represents around 30% of the processed water volume, the high presence of pharmaceuticals and other pollutants (see below)

Table 3. Dioxin-like activity and total pharmaceutical (Ph) contents in waste water samples after the different processing steps of tertiary treatment

Processing step	BNF equivalents (nM)	95% Confidence Interval	% Total flux	% input (dioxin-like activity)	Total Ph (ng L^{-1})	% input (Ph)
Sand Filter	4600	(3900 - 5600)	100	100	1500	100
Microfilter	4600	(2200 - 2600)	100	51	990	67
Brine	5600	(5000 - 6200)	30	36	3100	63
RO output	69	(65 - 73)	70	1	10	0.5

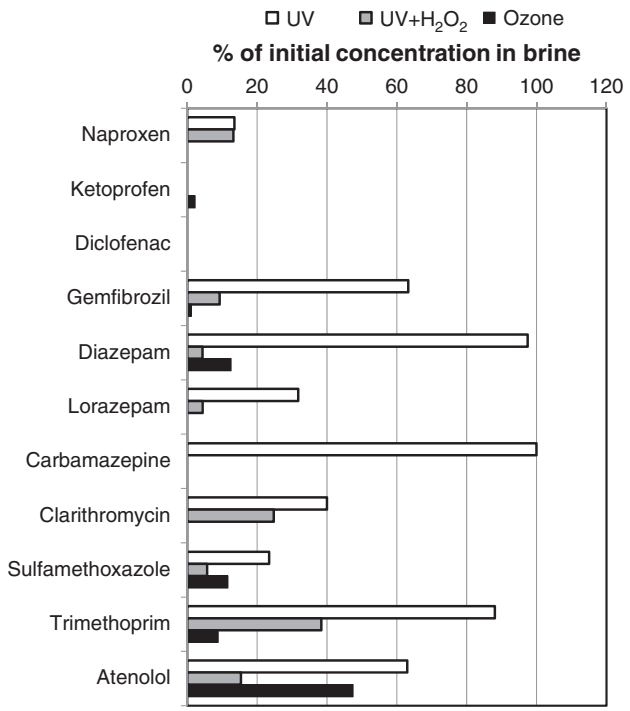

Figure 1. Maximal removal of different pharmaceuticals from RO brine after UV irradiation, single (UV, empty bars) or combined with peroxide treatment (UV/H_2O_2, grey bars), or ozonation (O_3, solid bars). Data are represented as percentages of the initial concentration in the brine.

represents a potential hazard for the environment and justifies the need for an extra treatment step.

Effect of AOP in water quality

Removal of pharmaceuticals from RO brine by AOPs

The susceptibility of the different pharmaceuticals to UV, UV/H_2O_2, and ozone treatments is shown in Fig. 1. Only Ketoprofen and Diclofenac were readily decomposed by UV (below 5% of the input value after the first minutes of treatment), suggesting that additional/alternative oxidative treatments are required for the elimination of a significant fraction of pharmaceuticals. In contrast, ozonation and UV/H_2O_2 process showed much higher removal rates for most of the pharmaceuticals, although some of them, such as Atenolol, Clarithromycin and Trimethoprim, were relatively recalcitrant to one or both treatments (Fig. 1). For most cases, high oxidant doses (2 mgO_3 mgTOC^{-1} in the ozonation case and 0.5 mgH_2O_2 mgTOC^{-1} in the UV/H_2O_2 process) were necessary to ensure significant removal (higher than 80%) for most of the contaminants present in the matrix. This fact corroborated the strongly recalcitrant nature of some of the pharmaceuticals studied.

Table 4. Pharmaceutical removal pseudo-first-order kinetic constants for both AOPs

	ks ozone (min^{-1})		*ks* UV/H_2O_2 (min^{-1})
Gemfibrozil	0.37	Ketoprofen	2.57
Clarithromycin	0.34	Lorazepam	0.76
Diclofenac	0.33	Diclofenac	0.63
Naproxen	0.33	Diazepam	0.63
Carbamazepine	0.31	Atenolol	0.16
Trimethoprim	0.30	Carbamazepine	0.16
Sulfamethoxazole	0.18	Sulfamethoxazole	0.15
Lorazepam	0.12	Naproxen	0.06
Diazepam	0.11	Gemfibrozil	0.05
Ketoprofen	0.07	Clarithromycin	0.02
Atenolol	0.04	Trimethoprim	0.01

Pharmaceutical removal pseudo-first-order kinetic constants (*ks*) were determined for both AOPs using removal values up to approximately 80%. The calculated values ranged from 0.04 to 0.37 min^{-1} and 0.01 to 2.57 min^{-1} for ozonation and UV/H_2O_2 processes respectively. Results are shown in Table 4.

Three different ranges of values were established (see different shades in Table 4) for *ks* in order to make a comparison not dependent on the particular oxidation conditions used in each oxidation case. It is noteworthy that pharmaceuticals such as Gemfibrozil, Clarithromycin, Naproxen and Trimethoprim, which showed the highest *ks* in ozonation, exhibited poor values during the UV/H_2O_2 process. It is also remarkable the opposite behavior of Ketoprofen. The observed differences could be related to the different mechanism pathways involved in the two treatments, as observed and commented on in previous work.[7,25] However, it is difficult to predict the kinetics of microcontaminants in this kind of matrix due to the high number of factors involved, such as the reactivity of the effluent organic matter (EfOM) and variable water quality, that can affect the observed removals.[7,25]

Near-total removal of pharmaceuticals was accomplished only when the highest oxidant doses were applied, where both technologies exhibited similar performances (Fig. 1).

Water parameters of RO brine after oxidation processes

Table 5 shows the TOC, COD and aromaticity (UV absorbance at 254 nm, UV_{254}) removals obtained for the most extended treatments. The reaction times required to consume the corresponding amounts of oxidants are also shown in the table. Removals achieved through ozonation were the highest. As expected, the addition of H_2O_2 to the photolytic process significantly improved the elimination of organic content. It is also well known that AOPs are able to enhance the biodegradability of treated effluents. As a

Table 5. TOC, COD and UV_{254} removals achieved by the oxidation processes at the highest doses

	Oxidant dose	Time (min)	ΔTOC (%)	ΔCOD (%)	ΔUV_{254} (%)
O_3	3 mg O_3 mg^{-1} TOC	31.2	18.7	39.6	64.2
UV/H_2O_2	0.9 mg H_2O_2 mg^{-1} TOC	109.1	12.0	27.5	64.8
UV	0.11 Einstein mg^{-1} TOC	109.1	0.5	2.1	28.9

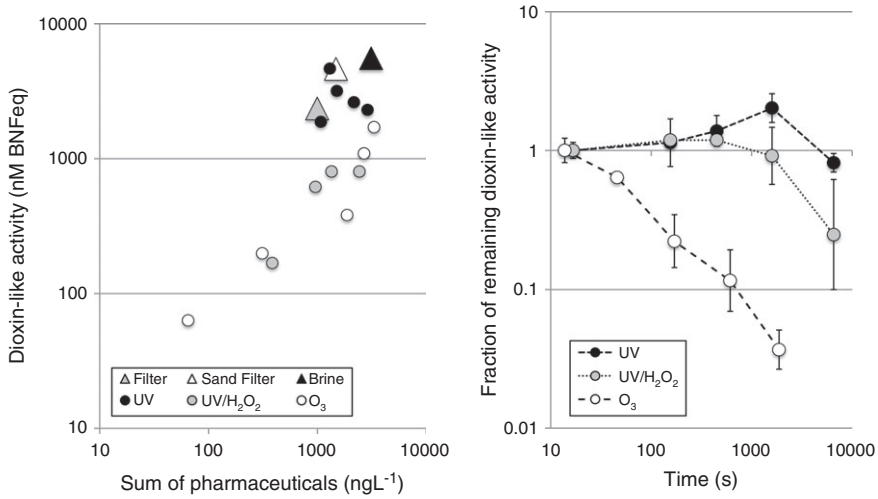

Figure 2. Analysis of biological activity in treated and untreated water samples. (A) Correlation between dioxin-like activity (Y-axis, expressed as nMBNFeq.) and chemical composition (X-axis, expressed as ng L^{-1} of total concentration of pharmaceuticals) for different samples. Triangles correspond to sand filtered (empty), micro filtered (gray) and brine (solid triangle) water samples (see also Table 3). Circles correspond to brine samples treated with UV (solid), UV/H_2O_2 (gray) or O_3 (empty circles); times of treatment are not indicated (see Fig. 2(B)).(B) Changes in dioxin-like activity in brine samples treated with UV (solid), UV/H_2O_2 (grey) or O_3 (empty circles), expressed as fraction of the initial activity of the sample. Time of treatment (X-axis) is expressed in seconds; initial activity was taken during the first 10–15 s of treatment. Whiskers indicate 95% confidence intervals.

result, the biodegradation ratios reached were 0.29, 0.25 and 0.21 for ozonation, UV/H_2O_2 and UV alone, respectively.

Measurement of biological activity of water samples through the reclamation treatment

Evaluation of antimicrobial activity

Samples were suspected to have antimicrobial activity because of the significant antibiotic concentration detected and because of possible antibiotic synergic effects between them. Nevertheless, the *E. coli* antibiotic activity bioassay detected very low antimicrobial activity in brine samples, and only after an important pre-concentration step (100 000 fold, not shown). By comparing growth inhibition of the pre-concentrated brine samples with the standard Ampicillin growth inhibition curve (Supplementary Figure S1), a residual antibiotic concentration equivalent to 15 μg of Ampicillin per L of brine was estimated. Although this low concentration is likely unable to induce any biological effects (including the onset of resistance strains) on receiving waters, the methodology can be used for evaluation of wastewater and brine from other sources (like hospital water discharges) in which the concentration of microbiocides is several orders of magnitude higher than in standard urban wastewaters (unpublished observations). Although no antimicrobial activity was detected, the test was also performed for the different sample points of the tertiary treatment.

Toxicity evaluation

Data from the Microtox® acute toxicity test performed with raw water samples from the main steps of the tertiary treatment process showed no acute bacterial toxicity for any of them. The level of antibacterial activity of the pre-concentrated RO brine was consistent with the negative results obtained with Microtox® (which is, after all, a microbial acute toxicity test), and indicates a relatively low level of this particular type of contamination in the samples. In addition, it illustrates the capability of the antimicrobial bioassay to evaluate very low levels of antibiotics in water samples. Other authors also obtained the same results with the Microtox® test; the RO brine was non-toxic to the microorganism used in the test, *Vibrio fisheri*.[7,26,27]

RYA evaluation

ER-RYA analyses revealed no significant estrogenic activity in any of the water samples, a result consistent with the relatively low levels of biological activity observed in the toxic tests.

In contrast, AhR-RYA revealed a substantial dioxin-like activity in water samples previous to the RO process and also in the RO brine, probably reflecting the presence of organic anthropogenic compounds (Table 3). Filtration reduces the input dioxin-like activity by some 50%, probably due to the removal of particulate matter, but the activity is still significant even after microfiltration. RO brine concentrated the dioxin-like compounds, which were 99% excluded from the RO output (Table 3). The distribution of dioxin-like activity through the different steps of the treatment was very similar to the one observed for the total pharmaceuticals (Table 3). However, and although the presence of dioxin-like activity has already been related to the presence of the pharmaceuticals in natural media,[28] the observed dioxin-like effect was very likely related to the presence of other anthropogenic

compounds in the samples.[29] Whatever their nature may be, the data show that both pharmaceuticals and dioxin-like compounds were excluded from the RO permeate and concentrated in the RO brine. Nevertheless, the observed activity correlated with the presence of pharmaceuticals, and was essentially removed by the RO process (Table 3, see also Fig. 2(A)), consistently with the absence of any quantifiable amount of pharmaceutical in the RO permeate (Table 3).

Removal of dioxin-like activity by oxidation processes

AhR-RYA was used to assess the evolution of dioxin-like activity through the two AOPs performed, and also with UV radiation itself. Although the most drastic removal of dioxin-like activity was observed during the first seconds (<20 s) of each treatment applied (58%, 88% and 69% for UV, UV/H_2O_2 and ozonation, respectively), a particular behavior was observed for all processes from this point on. For this reason, Fig. 2(B) was elaborated using normalized AhR activities referred to this point of treatment. UV radiation did not decrease significantly the total dioxin-like residual activity of the RO brine after more than an hour of treatment (Fig. 2(B), solid circles). Besides, an increase of the activity was observed, probably due to the formation of by-products that exhibited high response to dioxin-like assay. Similarly, UV combined with H_2O_2 resulted in a negligible decrease for the dioxin-like response during several minutes of treatment (more than 15 min), although a final reduction of 97% of the initial activity response was accomplished after 109.1 min of treatment (Fig. 2(B), grey circles). On the contrary, ozonation resulted in a continuous and rapid decrease of dioxin-like activity, achieving almost total response removal in 10 min, with an apparent half-life of only 50 s (Fig. 2(B), empty circles). This value is comparable with the ones calculated for the different pharmaceuticals (2–20 s^{-1}, Table 4), and underscores the efficiency of the ozonation method. There was a pseudo-linear relationship between dioxin-like activity and the total amount of pharmaceuticals for all treated samples (see the circles in Fig. 2(A)). This indicates that the non-characterized dioxin-like components present in brine showed a susceptibility to the different treatments similar to the pharmaceuticals analyzed in this work. Previous results with the same or similar RYA assay showed EC_{50} values around the mg L^{-1} mark for different pesticides and other common pollutants, while some specific compounds, like some PAHs, dioxins, and other organochorine compounds, show EC_{50} values between 2 and 20 μg L^{-1}.[13,30] While these values are clearly higher than the maximum observed concentration of pharmaceuticals in the samples, they are not uncommon in environmental samples. AhR ligands have been detected in both wastewaters and superficial waters.[17,18,29,31,32] Therefore, their removal from brines and wastewaters in general by the oxidation procedure is an interesting result, as they are not eliminated (at least, not completely) by standard secondary treatment procedures.

CONCLUSIONS

The results show the efficiency of AOPs for the removal of micropollutants in RO brines, both measured as the degradation of specific pharmaceuticals or by the combined dioxin-like activity of the samples. The nearly complete removal of pharmaceuticals by UV/H_2O_2 and ozone can be achieved only by relatively long treatments and under severe oxidative conditions (0.5 mgH_2O_2 mgTOC^{-1} and 2 mgO_3 mgTOC^{-1}). UV itself is only able to eliminate a reduced subset of compounds. Although little differences were observed between both AOPs considering only the chemical data, the dioxin-like activity test showed significant response variances among treatments after the first 20 s of treatment. Ozonation resulted in a continuous and rapid decrease of dioxin-like activity, with an apparent half-life of only 50 s whereas, for the UV/H_2O_2 process, dioxin-like activity response remained constant for several minutes of reaction (more than 15 min). Notwithstanding these considerations, our data demonstrates that the AOPs for the inactivation of brine represent an integral solution of wastewater treatment with a potential application even for highly polluted inputs. Furthermore, the reported results, combined with other previous reports, confirmed the utility of combining chemical analysis together with bioassays to assess the fate of micropollutants through advanced procedures of waste water treatment.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the Generalitat de Catalunya (Consolidated Research Groups: Enginyeria de Processos d'Oxidació Avançada 2009-SGR-1466, Water and Soil Quality Unit 2009-SGR-965 and Toxicologia Ambiental unit, 2009-SGR-924). SP acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Economy and Competitiveness. The studies have partly been supported by the Spanish Ministry of Economy and Competitiveness Consolider-Ingenio 2010 Scarce CSD2009-00065.

Supporting Information

Supporting information may be found in the online version of this article.

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APPENDIX V

BAC filtration to mitigate micropollutants and EfOM content in reclamation reverse osmosis brines

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Published in *Chemical Engineering Journal* 279 (2015) 589-596

Chemical Engineering Journal 279 (2015) 589–596

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

BAC filtration to mitigate micropollutants and EfOM content in reclamation reverse osmosis brines

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HIGHLIGHTS

- BAC filtration alone only improved some water quality parameters of the RO brine.
- The highest EfOM removals were achieved by the combination of ozone and BAC.
- To ensure the total removal of pharmaceuticals was necessary the integration of an AOP with BAC.
- ATP analyses and FISH technique allow the assessment of the BAC filters biomass.
- β-Proteobacteria was the main bacteria phylum identified in the three biofilters.

ARTICLE INFO

Article history:
Received 26 January 2015
Received in revised form 30 April 2015
Accepted 5 May 2015
Available online 12 May 2015

Keywords:
Ozone
UV/H_2O_2
Biological activated carbon
Pharmaceuticals
Advanced oxidation processes
Biofilter

ABSTRACT

The effluent organic matter (EfOM) including micropollutants present in the concentrate streams generated from reverse osmosis (RO) based municipal wastewater reclamation processes entails environmental and health risks on its disposal to the receiving environment. The suitability of a biological activated carbon (BAC) process to treat municipal wastewater RO concentrate was evaluated at lab scale during 320 days of operation. BAC alone and combined UV/H_2O_2–BAC and ozone–BAC were performed. The combination of both advanced oxidation processes with the BAC filter improved considerably the water quality parameters. Overall eliminations for dissolved organic carbon, chemical oxygen demand and ultraviolet absorbance at 254 nm ranged between 50–66%, 48–66% and 73–87%, respectively, improving considerably the removals obtained without pretreatment step (28%, 19% and 37%, respectively). Moreover, although some pharmaceuticals were partially removed by the BAC filter, the integration of the UV/H_2O_2 or the ozone step was necessary to achieve the total removal of those micropollutants. Finally, biomass assessment techniques allowed determining the diversity of different BAC filter scenarios.

1. Introduction

It is generally assumed that not all polluting agents are removed through conventional wastewater treatment plants (WWTP). These persistent compounds include the emerging pollutants group, constituted by chemicals of very diverse origin. They are characterized by their high production and consumption volumes, which entails their continuous presence in the environment even at low concentrations [1]. Whereas their occurrence is fairly well-established, their long-term effects and environmental consequences are not clearly identified [2]. Thus, additional advanced treatment steps should be considered to reduce their discharge into receiving waters.

In recent years, reverse osmosis (RO) has been applied to further treatment of the secondary effluents of wastewater treatment plants [3]. The resultant permeate is usually used for irrigation or aquifer recharge. Despite the high quality effluent generated, salts, biological constituents and organics, including micropollutants, coming from secondary effluent, are concentrated in the rejected effluent [4]. Consequently, one of the major drawbacks of RO is the need to dispose this concentrate. These waste effluents are usually discharged to surface waters, oceans or groundwaters. Although their discharge is currently not regulated, safe environmental practices would suggest their treatment before its release and dilution into the environment [3,5].

Advanced oxidation processes (AOPs) have been applied to treat RO retentates in order to reduce their high concentration in

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http://dx.doi.org/10.1016/j.cej.2015.05.018
1385-8947/

recalcitrant micropollutants [4,6–12]. AOPs are those processes which involve *in situ* generation of hydroxyl radicals (HO•) and their reaction with organics converting them into simpler compounds or even leading to their total mineralization. The HO• radical is a powerful oxidant species with a reduction potential of 2.80 V. Its non-selectivity is also remarkable [13]. Furthermore, taking advantage of the biodegradability enhancement achieved by AOPs, the use of a subsequent biological step has also been previously suggested to minimize even further the organic load of the target effluent [14].

Granular activated carbon (GAC) filters have been used for long time to remove by adsorption undesirable organic compounds including biodegradable organic matter, micropollutants, halogenated hydrocarbons and taste and odor compounds [15]. GAC offers an effective mean to remove organic compounds due to its irregular creviced, porous particle shape and affinity for attaching to itself most organics even at low concentrations [16,17]. However, one of the major limitations of GAC is saturation which implies the need to regenerate it, with the economic costs it entails. On the other hand, crevices and macropores of activated carbon are also an excellent support material for the development of microbial biofilms as they provide protection from shear stress to microorganisms colonies [15–18]. Such colonized filters are referred in the literature as biological activated carbon (BAC) filters. When the GAC media particles start becoming exhausted for adsorption, the rough porous surfaces are amenable to indigenous microbial communities establishment. This transition from GAC to BAC filter is a time-dependent process where simultaneous adsorption and biodegradation processes can coexist [15,16,19]. Precisely, biodegradation mechanism consists on a first adsorption of organic matter, removed from water into macropores, where it is detained long enough to promote its slow biodegradation by attached bacteria [16]. Extending GAC service life and decreasing backwash frequency are the main benefits of BAC filters [17]. Pre-oxidation of high recalcitrant effluents prior to BAC filtration is a commonly used combination. It results in an increase of the biodegradability of the inlet effluent, therefore promotes biological activity of the biofilm and consequently extends GAC media life [17,20–22].

Active biomass characterization is important during BAC filter processes in order to stablish connections between the degradation process and the biomass involved. Various methods have been used by different authors to assess the biomass activity. These include heterotrophic plate counts (HPC) [23], phospholipid extraction method [24], adenosine tri-phosphate (ATP) analyses [15,20,25] and respirometric measurements [19,26] among others. Likewise, determination of microbial communities is also essential information for a better understanding of BAC filters performance. Few studies have been conducted using culture-dependent methods on drinking water BAC applications [25,27,28]. However, only a very small fraction of microorganisms in the environment is cultivable on the commonly applied media. Culture-independent molecular methods are therefore preferred above culture-dependent in most sorts of microbiological investigations in wastewater treatments [28,29]. Within them, 16S ribosomal ribonucleic acid (rRNA) gene clone library analysis [25,28,30,31] and fluorescence *in situ* hybridization (FISH) [32,33], with rRNA-targeted, probes are known to be very powerful tools for the identification of microorganisms in microbial biofilms.

This study aims to evaluate the performance of biologically enhanced granular activated carbon filtration in minimizing the environmental impacts associated to the direct discharge of reclaimed RO brine coming from a WWTP located in Catalonia (Spain). The BAC filter performance on the removal of micropollutants and dissolved organic carbon (DOC) was compared with the performance of two integrated systems which consist of UV/H_2O_2 or ozonation coupled with a BAC filtration. This work focused on the biological step of the three proposed treatments since the occurrence of different micropollutants in reclamation RO retentates and their mitigation by both AOPs was already assessed in previous studies [11,12]. ATP analyses and FISH technique were applied to ensure and assess the biological activity in GAC filters.

2. Materials and methods

2.1. Experimental devices

The photo-oxidation pretreatment was carried out in 2 L jacketed reactor at 25 °C. Three low pressure mercury lamps (Philips TUV 8W, G8T5) emitting at a wavelength of 254 nm were placed inside the UV/H_2O_2 reactor. The photon flow measured with uranyl-oxalate actinometry was $1.7 \cdot 10^{-5}$ Einstein s^{-1} [34]. Reaction time depended on the matrix organic load and the average reaction time was 98 min. 30 mgH_2O_2 L^{-1} were added at the beginning of the reactions and they were considered ended when an average dose of 0.82 mgH_2O_2 mgDOC^{-1} was reached. Catalase enzyme was added to quench the excess of H_2O_2 prior feeding the BAC filter.

In the ozonation pretreatment, ozone-containing stream was injected into the effluent (2 L and 25 °C) through diffusers at a flow rate of 133.5 L h^{-1} with an ozone concentration of 10 gO_3 Nm^{-3}. The average transferred ozone dose achieved was 2.2 mgO_3 mgDOC^{-1}. In this case, reaction time was also dependent from the ozone demand of the different matrices (average reaction time: 19 min). The complete ozonation set-up is described elsewhere [35].

Three biological filters were operated. Two of them were fed with the resultant effluent from each assayed AOP and the other one was fed with RO raw brine. Filtrasorb® 400 agglomerated coal based granular activated carbon (Chemviron Carbon, Belgium) was used as a filter media. Its effective size was between 0.6 and 0.7 mm and its mean particle diameter was 1.0 mm. The set-up consisted of 3 cm inner-diameter glass columns packed with approximately 5 cm of GAC. All columns were protected from the light to minimize the potential effects of photodegradation and were run under aerobic conditions by aerating the feeding solution (until saturation) just before its entry into the columns. The columns were fed at an average flow rate of 0.79 mL min^{-1}. Contact time resulted 13.4 min (empty bed contact time (EBCT): 44.7 min). Since this study was focused on biodegradation as a removal mechanism, GAC was soaked during few days in the WWTP RO brine tank to be close to its saturation state before being packed into the columns. After that, columns were initially inoculated with secondary sewage sludge in order to accelerate the colonization process. The startup strategy is detailed elsewhere [36].

2.2. Analytical methods

Samples were withdrawn during both AOPs and also at the inlet and outlet of each biofilter to monitor the following parameters: DOC (previously filtered through 0.45 μm polyethersulfone (PES)), chemical oxygen demand (COD), ultraviolet absorbance at 254 nm (UV_{254}), turbidity and pH. DOC was measured by means of a Simadzu TOC-VCSN analyzer. To measure the COD, procedure 5220D from Standard Methods [37] was followed. Absorbance was determined by Perkin Elmer UV–Vis spectrophotometer with *Lambda 20* software. The turbidity was determined using a Hach 2100P turbidimeter. Other parameters like alkalinity were also analyzed, which was quantified by titration with HCl as described in 2320B Standard Methods procedure [37]. For the evaluation of the biological oxygen demand at 5 days (BOD_5), the WTW

OxiTop® measuring system (Weilheim, Germany) was used following the Standard Methods 5210D procedures [37]. On the other hand, the biodegradability of the BAC filters outlet effluents was assessed using aerated batch reactors inoculated with WWTP activated sludge (Total Volatile Suspended Solids (TVSS) of 100 mg L^{-1}).

Anion concentrations were determined in an Advanced Compact IC Metrohm ionic chromatographer using a Metrosep A Supp 4–250 column, and ammonium concentration was measured by Orion 9512 ammonia electrode from Thermo Scientific. H_2O_2 concentration (Panreac 30% w/v) was determined through the metavanadate spectrophotometric procedure at 450 nm [38]. The Microtox® test, which uses luminescent *Vibrio fischeri* bacteria, was performed to assess the acute toxicity of the effluent. Total residual oxidant (TRO) concentration was measured using a pocket colorimeter II (Hach, USA) according to the n,n-diethyl-p-phenylenediamine (DPD) colorimetric method for total chlorine, Standard Method 4500G [37].

Eleven pharmaceuticals were measured in order to characterize the raw water. Pharmaceuticals monitoring was also performed along both AOPs [11,12] and also at the outlet of the BAC filter fed with RO brine at the end of the operational period. Pharmaceuticals in the effluents of the two other columns were not analyzed since these were nearly removed by the AOPs applied as pretreatment. Analytical method for pharmaceutical determination was described in Justo et al. [12]. The validation of the method showed satisfactory sensitivity (detection limits below 5 ng L^{-1} for most of the compounds), accuracy (absolute recoveries above 61%) and repeatability (relative standard deviations below 8%) (see Table 5).

2.3. Biofilm assessment

Measurement of ATP was used to validate the presence of biological activity in the GAC filters. ATP is used as primary energy currency by all organisms. Therefore, it can be used as an indicator of biomass content [20]. The procedure followed for liquid phase analysis was BacTiter-Glo™ Microbial Cell Viability Assay (Promega Biotech Iberica, Spain) using a GloMax® 20/20 luminometer (Promega Biotech Iberica, Spain). In short, BacTiter-Glo™ reagent and an equal volume of sample (100 μL: 100 μL) were transferred to an Eppendorf tube. Then, the content was mix on an orbital shaker for 3 min at room temperature, and finally the luminescence was recorded after 30 s and expressed as Relative Light Units (RLU). ATP was measured before and after sample filtration through 0.22 μm filter of polyethersulfone (PES) to quantify total and free ATP respectively; ATP present from living cells was determined by calculation [39]. The followed methodology to estimate the ATP contained on GAC attached cells was based on that developed by Velten et al. [20]: 200 mg of GAC and 100 μL of phosphate buffer were added to an Eppendorf tube. After 3 min orbital shaking at room temperature, 300 μL of BacTiter-Glo™ were added. After 3 more minutes of orbital shaking, 200 μL of the supernatant were transferred into a new Eppendorf tube and luminescence was measured after 30 s. ATP measurements were performed in triplicate.

Bacterial phylum distribution in backwashing waste of the three BAC filters during the last stage of the experimentation was identified by FISH technique [29,40] followed by scanning confocal laser microscopy (SCLM). Prior to hybridization, the biomass was fixed using 4% paraformaldehyde. The specific oligonucleotide probes (Thermo Fisher Scientific, Germany) used are defined in Table 1 and more details are available at probeBase [41]. Hybridizations were performed at 46 °C for 90 min with different percentage of formamide (F) (Table 1). The probes used for *in situ* hybridization were labeled with the dyes FLUOS (green) or Cy3 (red), and also 4′,6-diamidino-2-phenylindole (DAPI) (blue) was added in order to fluorescence Deoxyribonucleic Acid (DNA). Fluorescence signals were recorded with an Axioskop 2 epifluorescence microscope (Zeiss, Germany).

Table 1
Targeted organisms and the corresponding formamide (F) percentages for the oligonucleotide probes used.

Probe	Probe sequence (5′–3′)	F (%)	Targeted organisms
EUB338	GCT GCC TCC CGT AGG AGT	20/35	Bacteria
ALF1B	CGT TCG YTC TGA GCC AG	20	α-proteobacteria
BET42a	GCC TTC CCA CTT CGT TT	35	β-proteobacteria
GAM42a	GCC TTC CCA CAT CGT TT	35	γ-proteobacteria

2.4. Reverse osmosis brine

The RO brines came from a tertiary treatment in a WWTP located in Tarragona (Catalonia, Spain). The RO concentrate is currently being discharged into coastal sea waters. During the experimentation period (1 year), nine different effluents were collected. Their average physicochemical characteristics are summarized in Tables 2 and 3. The low biodegradability ratio of the concentrate ($BOD_5/COD = 0.09$) confirmed a high biorecalcitrant organic matter content. Besides, the high alkalinity and the high chloride concentration of the retentate should also be underlined.

3. Results and discussion

3.1. Pretreatment of RO brine

The characterized effluent was treated by UV/H_2O_2 (0.82 mgH_2O_2 $mgDOC^{-1}$) and ozonation (2.2 mgO_3 $mgDOC^{-1}$) in order to ensure almost total elimination of micropollutants. Then, the two pretreated solutions were used to feed the corresponding biofilter. The resulting COD, DOC and UV_{254} average values from UV/H_2O_2 and ozonation treatments are summarized in Table 4. The mechanisms involved in the studied processes are different [11]. Thus, different final removals of DOC, COD and UV_{254} were achieved, and also different results in the pharmaceuticals eliminations were obtained [11,12]. These results suggested also different performance of the two pretreated biofilters.

The achieved COD removal resulted higher when ozonation was applied (percentage eliminations: 27 ± 3% and 39 ± 8% for UV/H_2O_2 and ozonation, respectively). However, mineralization was very similar for both treatments (DOC elimination percentages: 15 ± 5% and 18 ± 5% for UV/H_2O_2 and ozonation, respectively). Apart from the satisfactory efficiency that the performed AOPs showed in terms of COD and DOC removal, they decreased the aromaticity in higher percentage (51 ± 8% and 63 ± 7% for UV/H_2O_2 and ozonation, respectively) [42]. Turbidity and pH were also

Table 2
Average physicochemical parameters of RO brine.

Parameter	Units	Average ± SD*
DOC	$mgC\ L^{-1}$	23.7 ± 3.8
COD	$mgO_2\ L^{-1}$	61.5 ± 7.9
BOD_5	$mgO_2\ L^{-1}$	5.5 ± 3.1
pH	–	6.9 ± 0.5
Turbidity	NTU	0.37 ± 0.09
UV_{254}	cm^{-1}	40.5 ± 7.1
TN	$mgN\ L^{-1}$	49.0 ± 22.2
Alkalinity	$mgCaCO_3\ L^{-1}$	308 ± 177
Conductivity	$mS\ cm^{-1}$	7.3 ± 3.1
Microtox®	EC_{50}	Non-toxic

* Standard deviation (SD).

Table 3
Average anion and cation concentrations in the RO brine.

Ion	Units	Average ± SD
F^-	$mgF^- L^{-1}$	2.8 ± 1.4
Cl^-	$mgCl^- L^{-1}$	1627 ± 265
Br^-	$mgBr^- L^{-1}$	6.0 ± 3.3
NO_2^-	$mgNO_2^- L^{-1}$	21.2 ± 6.3
NO_3^-	$mgNO_3^- L^{-1}$	15.9 ± 6.8
PO_4^{3-}	$mgPO_4^{3-} L^{-1}$	15.6 ± 10.0
SO_4^{2-}	$mgSO_4^{2-} L^{-1}$	1200 ± 242
Na^+	$mgNa^+ L^{-1}$	1637 ± 675
K^+	$mgK^+ L^{-1}$	90.9 ± 26.3
Ca^{2+}	$mgCa^{2+} L^{-1}$	469.1 ± 67.5
Mg^{2+}	$mgMg^{2+} L^{-1}$	235.7 ± 78.8
NH_4^+	$mgNH_4^+ L^{-1}$	38.4 ± 10.0

measured along the oxidation processes. Both parameters decreased during UV/H_2O_2 process (final pH: 6.8 ± 0.3; average turbidity removal: 12%). In contrast, turbidity increased during ozonation due to the precipitation of calcite or calcium-organic acid material (great variability of the increase depending on the effluent treat) while pH increased until an average final pH of 7.8 ± 0.4. The same results were observed by Westerhoff et al. [5].

Bromide is suspected to be oxidized by hydroxyl radicals during advanced oxidation of real effluents, especially when high concentrations of Br^- are present. Bromide oxidation products (hypobromous acid and hypobromite) can be quantified as TRO and represent a real interest for disinfection as they could limit bacterial regrowth [39,43], and for environmental release, as they are carcinogenic. In this study, TRO were analyzed because of the biofilters placed after the AOPs. Bromide has also shown to be highly reactive with molecular ozone. As expected, after UV/H_2O_2 treatment, TRO were reduced about 87% because the presence of hydrogen peroxide reduces the formation of bromide oxidation products. On the contrary, when ozone was used the TRO increased from 0.74 to 3.21 $mgBr_2 L^{-1}$.

On the other hand, it is well known that AOPs are capable of enhance biodegradability by oxidizing persistent organic compounds into more biodegradable intermediates which would then be treated in a biological oxidation process with a considerably lower cost [14]. Chemical oxidation for complete mineralization is generally expensive because of the energy and chemical reagents consumption. Therefore, the main role of the chemical pretreatment is partially oxidizing the biologically persistent part (including micropollutants group) to produce more biodegradable reaction intermediates [14]. In the case of study, biodegradability was analyzed for the 4th RO effluent treated. Its initial biodegradability ratio resulted 0.11. After UV/H_2O_2 and ozonation, the values reached were 0.36 and 0.38, respectively. The enhancement of biodegradability ratio encouraged the implementation of a subsequent biological step.

3.2. Effect of pretreatment on overall water quality

Figs. 1–3 show the inlet and outlet DOC and COD values for the BAC filters fed with raw RO brine, UV/H_2O_2 pre-oxidized and pre-ozonated brines, respectively, during 318 days operating in a continuous-flow mode.

Table 4
Averaged DOC, COD and UV_{254} values achieved by UV/H_2O_2 and ozonation.

Parameter	Units	UV/H_2O_2 effluent Average ± SD	Ozone effluent Average ± SD
DOC	$mgC L^{-1}$	19.6 ± 3.0	18.9 ± 2.7
COD	$mgO_2 L^{-1}$	43.9 ± 5.9	37.1 ± 5.0
UV_{254}	m^{-1}	20.2 ± 3.3	14.1 ± 4.3

To analyze the results obtained, three different operational periods were established (black continuous lines delimit the different operational periods) mainly based on the profile obtained for the eliminations of DOC and COD achieved with BAC filter fed with raw RO brine [17]. During the first period of operation (until day 130) DOC and COD eliminations were similar for the three BAC columns and the average values were 87% and 58%, respectively. These similarities could indicate that despite the strategies performed before GAC packaging in order to saturate the media, this objective was not achieved to the desired extent. During that period adsorption seemed to be the dominant DOC and COD removal mechanism, the adsorption capacity of the GAC was still high while the colonization process seemed negligible. The fact that highest removals were reached during this adsorption stage as suggested by other authors [16] confirmed these suppositions. In a second stage (until day 240), GAC absorption capacity was decreasing, the biofilm was growing up and biodegradation mechanism started growing in importance. From this point on, the nature of the matrix, in particular the biodegradation degree of the effluent to be treated, gained significance. Although the DOC and COD percentage removal decreased for the three columns due to the GAC saturation, the eliminations reached with the pretreated effluents were higher than the eliminations obtained by the filter fed with raw RO brine (52% and 44% for DOC and COD, respectively for BAC filter without pretreatment). As abovementioned, the ozonated effluent was slightly more biodegradable and therefore the removals achieved by the corresponding BAC filter were higher in comparison to the UV/H_2O_2 case (58% and 46% for UV/H_2O_2 BAC filter and, 70% and 54% for pre-ozonated column, for DOC and COD, respectively). Finally, the dominant degradation mechanism in the last period was biodegradation since GAC was almost saturated after 240 days of operation. Regarding RO brine BAC filter, DOC removal (22%) was almost 70% higher than COD removal (13%) which means that the fraction that was being mineralized was already very oxidized. This behavior was also significant when the RO brine was pretreated with UV/H_2O_2 (60%). The average percentage removals were again higher for the more biodegradable pretreated effluents (44% and 27% for UV/H_2O_2 BAC filter and, 57% and 43% for ozonated column, for DOC and COD, respectively). From the results obtained from the biodegradability test of the BAC filters effluents it could be concluded that the contact time assayed in the three BAC filters was enough because significant further biodegradation was not observed during the test.

Aromaticity removal could also be discussed for the three different stages. Similarly to DOC and COD, the highest removal of UV_{254} took place during the pure adsorption period. During the adsorption step, aromaticity removal across the BAC filter fed with raw RO brine (94%) was slightly higher than the ones reached with the pretreated effluents (90.0% for UV/H_2O_2 and 84% for ozonation). In the second established period, the aromaticity percentage removal decreased in the three columns (55%, 67% and 61% for RO brine, UV/H_2O_2 and ozone BAC filters, respectively). When the main mechanism of degradation was biodegradation, the less biodegradable effluent exhibited the lowest removal (36%, 52% and 48% for RO brine, UV/H_2O_2 and ozone biofilters, respectively).

No significant differences between the established periods were observed regarding pH and turbidity. pH slightly increased throughout the BAC filters fed with RO brine and with UV/H_2O_2 pretreated brine up to an average value of 7.8 ± 0.3 and 7.9 ± 0.2, respectively. Meanwhile, it maintained practically inalterable (7.8 ± 0.3) for the ozonated fed. With respect to turbidity, no significant changes were observed for the RO brine and UV/H_2O_2 biofilters (0.53 ± 0.20 NTU and 0.59 ± 0.14 NTU for RO brine and UV/H_2O_2). Ozone BAC filter was able to remove the turbidity

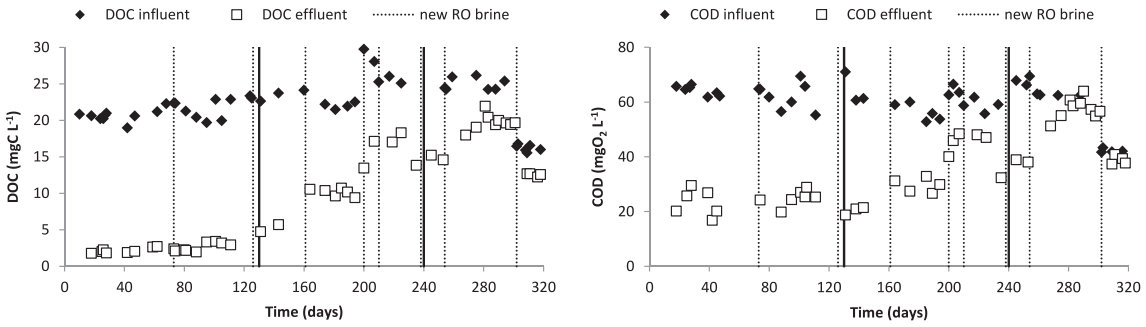

Fig. 1. Monitoring of DOC and COD for the BAC filter fed with RO brine.

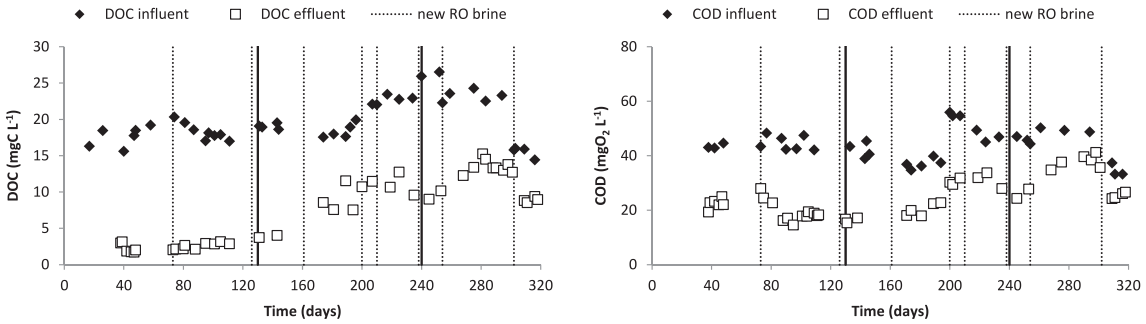

Fig. 2. Monitoring of DOC and COD for the BAC filter fed with UV/H_2O_2 treated effluent.

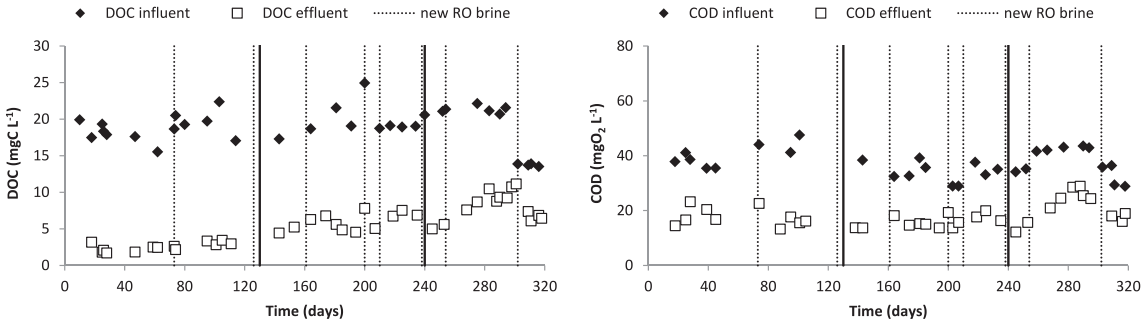

Fig. 3. Monitoring of DOC and COD for the BAC filter fed with ozonated effluent.

generated during ozonation reaching an average value of 0.79 ± 0.56 NTU.

Total nitrogen and ions listed in Table 3 were analyzed at the inlet and outlet of the three columns. In general, anions and cations concentration remained practically inalterable except phosphate and ammonium concentration that slightly decreased in the three effluents. Moreover, total nitrogen also showed a slight reduction across the biological filter. A small part of this reduction could be attributed to the nitrogen and phosphorous requirements for the synthesis of new cells. Another contribution to the ammonium and total nitrogen removal could be the simultaneous nitrification and denitrification that generally occurs in the biofiltration columns [16].

As commented before, the presence of TRO could limit bacterial growth. For this reason, the TRO were also measured at the outlet of the biofilter to guarantee that the biomass present in the biofilters was able to endure the presence of TRO. The TRO values resulted practically negligible after the columns fed with RO pretreated effluents (0.02 and 0.04 $mgBr_2\ L^{-1}$ for UV/H_2O_2 and ozonation, respectively). A slight TRO signal was detected (0.10 $mgBr_2\ L^{-1}$) when RO brine was biofiltered. The TRO reductions achieved in the biofilter fed with ozonated effluent and the biofilter fed with RO brine and also the good performance of both biofilters, ensure that the biomass present was able to assume the presence of these TRO.

BAC filter backwashing is a common practice to control the growth of the biofilm [17]. Several backwashing were conducted during experimentation (days: 140, 210, 260 and 311) to avoid bacteria clogging and the consequent increase in pressure drop. For this purpose, tap water was pumped in countercurrent through the columns to fluidize the GAC column during few minutes [16]. In agreement to other authors [24], results showed that backwashing of the filter did not change general biological activity within the BAC filter.

3.3. Micropollutants removal

As mentioned before, most micropollutants were mitigated during the two AOPs performed with the applied doses [11,12]. In this work, the occurrence of 11 pharmaceuticals measured in the raw RO brine was assessed after BAC biofiltration. Table 5 shows the pharmaceutical concentrations detected in the RO brine

Table 5
Pharmaceutical concentrations in the RO brine, % recoveries and relative standard deviations (RSD) of the analytical method and removal (%) by BAC filter.

Pharmaceutical	Group	Conc. (ng L^{-1})	% Recoveries ± RSD	Removal (%)
Naproxen	Analgesic and anti-inflammatory	254	112 ± 1	91
Ketoprofen		628	128 ± 7	39
Diclofenac		283	93 ± 1	54
Gemfibrozil	Lipid regulator	3443	85 ± 8	92
Diazepam	Antidepressant and antiepileptic	135	111 ± 1	48
Lorazepam		195	125 ± 3	83
Carbamazepine		98	62 ± 6	86
Clarithromycin	Antibiotic	46	129 ± 1	70
Sulfamethoxazole		26	109 ± 4	12
Trimethoprim		20	61 ± 8	35
Atenolol	Betablocker	361	108 ± 4	90

(8th feed) and the percentage removal achieved through the biofilter.

Different removal efficiencies were observed for selected pharmaceuticals. Complexity of the treated matrix, properties of the support used in biofilters, EBCT and the operational time influence the removal of pharmaceuticals [22]. Maeng et al. [44] investigated the role of biodegradation in the removal of selected pharmaceutically active compounds (PhACs) during silica sand columns passage under biotic and abiotic conditions. They indicated that biodegradation represents an important mechanism for the removal of PhACs during soil passage. The average removal of analgesic and anti-inflammatory group in the current study (61%) resulted similar to the removal efficiency obtained by Maeng et al. [44]. In contrast, carbamazepine was more easily eliminated by BAC column than by silica sand package, where they obtained different results compared to the other neutral PhACs selected. Other authors obtained contradictory results for the removal of sulfamethoxazole in laboratory-scale and pilot plant experiments [45]. In this case, they attributed these results to the different initial concentrations. Baumgarten et al. [46] also reported initial concentration of sulfamethoxazole being a driving factor for its biodegradation. Lee et al. [36], who treated an effluent from membrane bioreactor (MBR) with ozone and biofiltration with anthracite for removing pharmaceuticals and personal care products (PPCPs), did not observed significant changes in PPCPs concentrations in the biofilter. Other authors suggest that even though it is hypothesized that adsorption capacity of the activated carbon in the BAC filters is largely exhausted, the removal of specific organic micropollutants may occur by a combination of adsorption and biodegradation [22].

3.4. Integrated pre-oxidation and BAC performance

Pretreatment of RO brine with UV/H_2O_2 or ozonation led to the removal of recalcitrant micropollutants and also enhanced biodegradability [11,12]. The results exposed in this work demonstrated that their integration with BAC filtration was able to improve overall removals of the analyzed parameters compared to RO brine biofiltration without pretreatment. Table 6 shows the average effluent concentrations of the analyzed parameters (DOC, COD and UV_{254}) at the end of the combined treatment. These values correspond to the final operational period (from day 241 to 318) where biological removal was the dominant process in organics removal. While BAC filtration of raw brine reached removals of 28%, 19% and 37% for DOC, COD and UV_{254}, respectively, combined treatments provided at least 2 times higher removals. The combined UV/H_2O_2–BAC process was able to attain removals of 50%, 48% and 73% of DOC, COD and UV_{254}, respectively. The combined ozone–BAC process performed higher removals than the combination of UV/H_2O_2–BAC process (66%, 66% and 87% of DOC, COD and UV_{254} removals, respectively). Other authors also reported similar results in integrated systems. Lee et al. [21] reported slightly higher overall removals when treating RO brine with lower organic load using ozonation as pretreatment. In this case, ozonation with 6.0 $mgO_3\ L^{-1}$ and 20 min of contact time was combined with a lab-scale BAC column operated at an EBCT of 60 min (removal efficiencies for DOC and COD: 70% and 89%, respectively). The combination ozone–BAC, performed by Reungoat et al. [22], to treat conventional secondary effluent led to 50% of DOC removal and to the elimination of a wide range of organic micropollutants by more than 90%; despite the fact that lower ozone doses (0.5 $mgO_3\ mgDOC^{-1}$) were used.

Table 6
DOC, COD and UV_{254} values achieved after biofiltration of raw RO brine and after the integrated systems UV/H_2O_2–BAC and ozone–BAC.

Parameter	Units	BAC effluent	UV/H_2O_2–BAC effluent	Ozone–BAC effluent
DOC	$mgC\ L^{-1}$	17.2 ± 3.4	11.8 ± 2.3	8.1 ± 2.0
COD	$mgO_2\ L^{-1}$	50.0 ± 10.0	31.7 ± 6.7	21.2 ± 5.5
UV_{254}	m^{-1}	25.7 ± 5.9	10.8 ± 2.0	5.1 ± 1.3

3.5. Results from biofilm assessment

ATP from the three influents and effluents was measured after few months of biofilters operation (day 90) to assess the presence of biological activity in the GAC filters. Raw RO brine contained low amounts of cellular ATP (8%) because the secondary WWTP effluent is subjected to sand filtration and microfiltration before RO. The cellular ATP content was very low in both AOPs treated influents (4% and 0.3% of ATP cellular in UV/H_2O_2 and ozonated effluents, respectively). As other authors mentioned, both AOPs performed are able to damage and inactivate suspended bacteria in the RO brine [15,39]. For this reason, measurement of ATP had been used to evaluate disinfection rate of AOPs [39]. It stands to reason that after a GAC filter with biological activity, total and cellular ATP should increase because of the GAC biomass colonization [15]. The greater increase of ATP content after going through the corresponding BAC filters was for raw RO brine effluent, followed by UV/H_2O_2 and ozone effluents in this order. Cellular ATP percentage resulted as follows: 90% for RO brine effluent, 98% for UV/H_2O_2 pretreated effluent and 94% for the ozonated RO brine. On the other hand, a decrease in dissolved oxygen concentration detected after filtration through the BAC confirmed that they were biologically active, as other authors obtained [22].

Regarding ATP contained on cells attached into GAC, it was measured twice (day 90 and day 105). ATP slightly increased for both BAC filters fed with pretreated effluent (5% and 2% for UV/H_2O_2 and ozone treated RO brine) indicating that the average biomass growth rate was very low and the BAC filters already attained steady biological performance. The ATP of the biofilter fed with raw RO brine increased 40% which means that the biomass concentration had not reached yet a steady stage by day 105. These differences between pretreated and non-pretreated RO brine were possibly due to the ease of bacterial colonization of the more biodegradable pre-oxidized effluents. As commented before, simultaneously to the development of biomass in the filter, organic carbon removal decreased as a result of the saturation of adsorption capacity of the GAC which corresponds to the proximity from first period established to the second one (day 130).

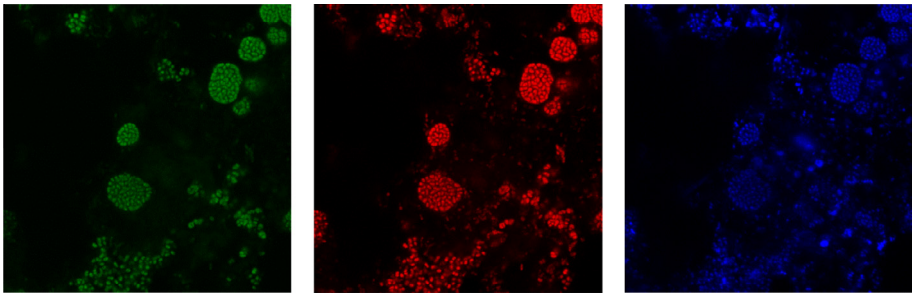

Fig. 4. FISH analysis images of samples collected on day 311 from the BAC filter fed with RO brine. Green: β-proteobacteria; Red: bacteria; Blue: DNA (numbered from left to right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In order to identify the main bacteria phylum present in the three BAC filters, FISH technique was applied to biomass samples from backwashing water (day 311) using the 16S rRNA probes shown in Table 1. The probe EUB338, specific for the domain Bacteria, was used to evaluate the bacterial presence in the microbial community. Additional probes for α- (ALF1B), β- (BET42a) and γ- (GAM42a) proteobacteria were also employed for a more accurate characterization. Fig. 4 is presented as representative proof of the results obtained by FISH technique. Images were obtained through SCLM, where β-proteobacteria (green dye), bacteria (red dye) and DNA (blue dye) were visualized in the backwashing water of the biofilter fed with raw RO brine. Hybridization process showed that bacterial strains of BAC filters fed with RO brine and RO brine pretreated with UV/H_2O_2 mainly consisted of β-proteobacteria. In the case of the BAC filter fed with pre-ozonated RO brine β-proteobacteria, which were also the predominant, coexisted with γ-proteobacteria. No α-proteobacteria could be detected by FISH technique in backwashing waters. All proteobacteria should fluoresce with the bacterial probe (EUB338) and it can be observed that there were a high number of green colored cells that match with the red fluoresced and only a few red cells (BET42a) that did not match, indicating a very low degree of unspecific staining. In the same way, all bacteria should fluoresce with DAPI dye. β-Proteobacteria has been found as the dominant one in other prone environments to microorganisms colonization such as biofilm formed in water distribution systems [32], membrane fouling in membrane bioreactors [47] and BAC filters used in drinking water [25,30].

4. Conclusions

The results presented in this work show that BAC filtration performed some improvements on water quality parameters of RO brines. Average DOC removal was 28%, and approximately 60% of the pharmaceuticals content was depleted in the biological treatment. Nevertheless, the integration of a pre-oxidation stage using UV/H_2O_2 or ozone with a biological filtration was necessary to completely remove the high concentration of micropollutants present in the municipal RO brines and to reduce the DOC, COD and UVA_{254} parameters close to values typically found in a conventional secondary effluent. The integration allows minimizing the environmental impact of their direct discharge. The combination of ozone and BAC filter led to the highest removals of DOC, COD and UV_{254} (66%, 66% and 87% of DOC, COD and UV_{254} removals, respectively).

The presence of biological activity in the three BAC filters studied was confirmed by ATP measurements. Moreover, ATP study showed earlier steady performance in the pre-oxidized fed biofilters. β-Proteobacteria was the main bacteria phylum identified in the three biofilters by FISH technique.

Acknowledgements

The authors gratefully acknowledge financial support from the Spanish Ministry of Economy and Competitiveness Consolider-Ingenio 2010 Scarce CSD2009-00065 and the collaboration of the *Water and Soil Quality Research Group* (IDAEA-CSIC).

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APPENDIX VI

Reverse osmosis concentrate treatment by chemical oxidation and moving bed biofilm processes

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Published in *Water Science and Technology* 68.11 (2013) 2421-2426

Reverse osmosis concentrate treatment by chemical oxidation and moving bed biofilm processes

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ABSTRACT

In the present work, four oxidation techniques were investigated (O_3, O_3/UV, H_2O_2/O_3, O_3/H_2O_2/UV) to pre-treat reverse osmosis (RO) concentrate before treatment in a moving-bed biofilm reactor (MBBR) system. Without previous oxidation, the MBBR was able to remove a small fraction of the chemical oxygen demand (COD) (5–20%) and dissolved organic carbon (DOC) (2–15%). When the concentrate was previously submitted to oxidation, DOC removal efficiencies in the MBBR increased to 40–55%. All the tested oxidation techniques improved concentrate biodegradability. The concentrate treated by the combined process (oxidation and MBBR) presented residual DOC and COD in the ranges of 6–12 and 25–41 mg L^{-1}, respectively. Nitrification of the RO concentrate, pre-treated by oxidation, was observed in the MBBR. Ammonium removal was comprised between 54 and 79%. The results indicate that the MBBR was effective for the treatment of the RO concentrate, previously submitted to oxidation, generating water with an improved quality.

Key words | chemical oxidation, MBBR, reverse osmosis concentrate

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INTRODUCTION

Although reverse osmosis (RO) is an established and diffused technology, it generates a concentrated stream that causes environmental impacts if discharged without any treatment (Westerhoff *et al.* 2009). Treatment of RO concentrate, also named retentate and brine, has been the object of some studies. Dialynas *et al.* (2008) investigated several techniques to treat RO concentrate: coagulation, activated carbon adsorption, electrochemical treatment, photocatalysis and sonolysis. Ng *et al.* (2008) utilized biological activated carbon (BAC) and capacitive deionization (CDI) to remove the organic matter and inorganic ions from RO brine. Lee *et al.* (2009) combined ozonation, BAC and CDI to treat RO concentrate. Advanced oxidation processes were combined with biodegradation to remove the organic matter content of a RO concentrate (Westerhoff *et al.* 2009). Electro-oxidation was employed to treat a concentrate containing emerging pollutants (Pérez *et al.* 2010). Advanced oxidation processes were also applied to remove pharmaceutical and personal care products from the retentate of an industrial RO unit (Abdelmelek *et al.* 2011). Although oxidation processes have been used to remove organic matter from RO concentrate, they can be combined with biological processes to enhance treatment performance. Additional and more sophisticated techniques are necessary when inorganic ions should be removed to acceptable levels.

RO brine may contain recalcitrant organic matter and usually presents low biodegradability, expressed by the low ratio BOD_5/COD (biochemical oxygen demand/chemical oxygen demand). Ozone and hydrogen peroxide can oxidize some recalcitrant compounds originally found in RO brine, improving its biodegradability and rendering the wastewater suitable for biological treatment. Among the biological treatment techniques, the moving-bed biofilm reactor (MBBR) presents some important advantages such as high surface area for biofilm adhesion, high sludge age and robustness.

In the present work, several oxidation techniques (O_3, O_3/UV, H_2O_2/O_3, O_3/H_2O_2/UV) were investigated to treat RO concentrate. Additionally, the performance of a MBBR fed with RO concentrate, previously submitted to chemical oxidation, was investigated in order to obtain water for reuse, especially in a locale where water stress is intense.

doi: 10.2166/wst.2013.510

METHODS

The concentrate or retentate used in the present work came from a sewage treatment plant located in Barcelona, Spain. The wastewater from the secondary clarifier of the treatment plant was filtered (sand filtration and membrane microfiltration) before feeding the RO unit. In the sewage treatment plant, the RO permeate is used as reuse water and the concentrate is discharged into the sea. Several samples of RO retentate were collected and transferred to the laboratory during the experimental work. Samples were stored under refrigeration (<5 °C) until use.

A 2-L oxidation reactor was used in assays operated in a batch mode with the following oxidants: O_3, O_3/UV, O_3/H_2O_2 and O_3/H_2O_2/UV. A low pressure mercury lamp (Philips TUV 8W, G8T5, 254 nm), presenting photon flow of $1.5{\cdot}10^{-5}$ Einstein.s^{-1}, was employed in the radiated assays. Ozone dose was 10 gO_3 Nm^{-3}, leading to transfer ratios of 9 to 11.5 mgO_3 mg^{-1} DOC (dissolved organic carbon). H_2O_2 concentration was 10 mg L^{-1} in the peroxidation assays. The contact time of the oxidation assays always lasted 20 min. The pH of the retentate was not adjusted and was close to 7. Assays were conducted at room temperature (19–23 °C). Figure 1(a) shows the oxidation unit used in the experiments. After oxidation the RO concentrate was stored under refrigeration and then used to feed the moving-bed reactor.

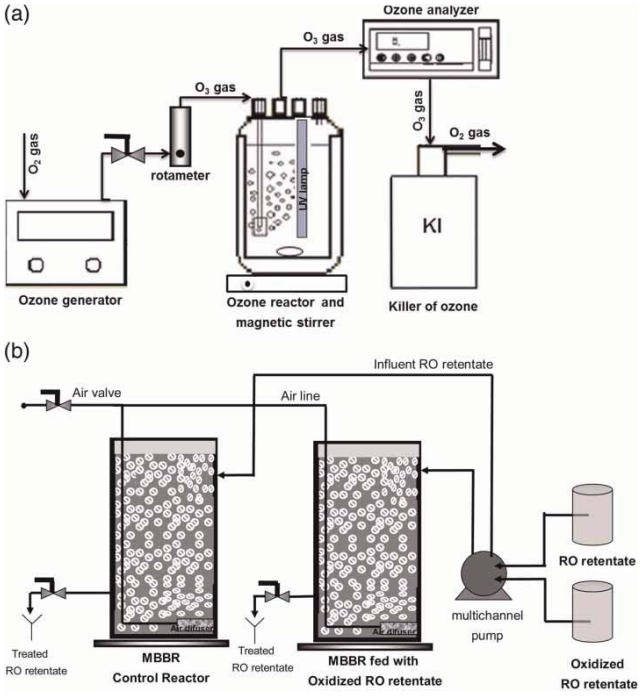

Figure 1 | Experimental set-up for RO retentate treatment: (a) set-up used in oxidation experiments (O_3, O_3/UV, O_3/H_2O_2 and O_3/H_2O_2/UV); (b) MBBR control reactor and MBBR fed with oxidized RO retentate.

Two 0.5-L capacity MBBRs were employed, one of them (control) fed with the RO concentrate and the other one fed with the concentrate previously treated by chemical oxidation. Figure 1(b) illustrates the MBBR set-up. The glass reactors contained 40% of their useful volume occupied by the supports (biomedia). Plastic biomedia from Dynamic Aqua Science Inc, type AMD Bio Media™, model MR-01, presenting a specific superficial area of 650 m^2 m^{-3}, were employed in the experiments. Air was supplied at the reactor bottom through a porous diffuser to promote oxygen transfer and biomedia circulation inside the reactor. Reactors were operated at room temperature (19–23 °C) in parallel for 6 months with a hydraulic retention time of 24 h.

COD and volatile suspended solids (VSS) determinations were made according to established procedures (APHA 2005). BOD_5 was determined by using the WTW OxiTop® measuring system (Weilheim, Germany) following the Standard Method 5210D procedure. DOC was determined, after sample filtration through a 0.45 μm membrane, in a Shimadzu TOC-VCSN analyzer. Absorbance at 254 nm (UV_{254}) was measured in a spectrophotometer UV-vis, Lambda 20 (Perkin-Elmer) in order to evaluate its variation during the oxidation process and also calculate SUVA (specific UV absorbance) according to Method 415.3 (EPA 2005). Ammonium, nitrate, nitrite and other ions were determined by ionic chromatography using the equipment Advanced Compact IC Metrohm with Metrosep A Supp 4–250 columns.

Microscopic observations of the biofilm accumulated on the biomedia surface were made using an optical microscope (Optika) equipped with digital camera (Moticam 2300) and software (Motic Images Plus 2.0).

RESULTS AND DISCUSSION

RO concentrate characteristics

The RO concentrate used during the experiments presented the composition shown in Table 1.

Some samples presented peaks of COD, DOC and, mainly, ammonium. The standard deviation values shown in Table 1 indicate that data scattering was high. Coefficients of variation of 25% were observed for DOC and COD and 36% for NH_4^+.

Table 1 | RO retentate characteristics

DOC (mgC L^{-1})	24 ± 6.0	NH_4^+ (mgNH_4 L^{-1})	127 ± 46	Cl^- (mgCl L^{-1})	1,394 ± 54
COD (mgO_2 L^{-1})	83 ± 20	Na^+ (mgNa L^{-1})	1,318 ± 103	NO_3^- (mgNO_3 L^{-1})	38 ± 3.4
Surfactants (mg L^{-1})	4.4 ± 3.8	K^+ (mgK L^{-1})	93 ± 2.0	NO_2^- (mgNO_2 L^{-1})	17 ± 0.4
UV_{254} (cm^{-1})	0.507 ± 0.016	Ca^{2+} (mgCa L^{-1})	485 ± 21	PO_4^{3-} (mgPO_3 L^{-1})	39 ± 1.0
pH	7.0	Mg^{2+} (mgMg L^{-1})	199 ± 13	SO_4^{2-} (mgSO_4 L^{-1})	1,144 ± 67

Organic matter removal in the oxidation experiments

All the tested oxidation processes were able to remove COD and DOC of the RO retentate, as shown in Table 2. In general, it is difficult to drop COD and DOC values when their initial values are already low (about 80 and 24 mg L^{-1}, respectively). Although the tested oxidation processes showed a satisfactory efficiency in terms of COD and DOC removal, they were more effective to drop the wastewater absorbance at 254 nm (63–74%), which indicates that the presence of double bound carbon compounds and aromatic substances was reduced, increasing the wastewater biodegradability. BOD determinations made before and after each oxidation process allowed calculation of the ratio BOD_5/COD. This ratio increased after oxidation. The BOD_5/COD was low (0.06) for the concentrate and increased to 0.15–0.26 after oxidation. The residual DOC of the treated concentrate was between 13 and 19 mgC L^{-1}. Although BOD and COD determinations can be influenced by salinity, in the present work the inorganic matrix was not changed after oxidation. Thus, if a possible effect of salinity occurred on both determinations, it was probably the same for the two wastewaters (retentate and oxidized retentate).

Lee *et al.* (2009) observed an increase of 1.8 to 3.5 times on RO retentate biodegradability after ozonation, using ozone doses of 3 to 10 mg L^{-1} and reaction times of 10 and 20 min. These authors also verified that ozonation generated low molecular weight compounds (<1 kDa) from high molecular weight compounds. In the RO retentate, compounds with molecular weight higher than 100 kDa corresponded to 80% of the organic matter. The SUVA, which corresponds to the ratio UV absorbance (254 nm)/DOC, decreased significantly from 2.1 L mg^{-1} m^{-1} for the retentate to values in the range of 0.8 to 1.1 L mg^{-1} m^{-1} for the oxidized retentate. As observed by Weishaar *et al.* (2003), SUVA is correlated with the organic matrix aromaticity. Thus, the pre-treatment with oxidants was able to oxidize a significant fraction of the aromatic compounds originally found in the retentate.

MBBR performance

Organic matter removal

The MBBR was fed with a stream presenting low organic matter content and, thus, was operated with a very low organic load (about 0.05 kgCOD m^{-3} d^{-1}). This was necessary due to the difficulty of treating biologically such diluted and complex wastewater. Operating under such conditions, with a dissolved oxygen level always above 7.0 mg L^{-1}, the MBBR was able to remove the residual organic matter to a larger extent, as shown in Table 3.

The results shown in Table 3 reveal that oxidation contributed significantly for organic matter removal. In particular, the combination of ozone, peroxide and UV (O_3/H_2O_2/UV) led to the highest removals of COD (36%) and DOC (53%), in contrast with the result attained in the control experiment, which presented lower removals (18%, COD and 14%, DOC).

Table 2 | COD, DOC, UV_{254} removals and BOD_5/COD ratios attained after oxidation

Oxidation processes	COD removal (%)	DOC removal (%)	UV_{254} removal (%)	BOD_5 / COD
O_3	33 ± 6	15 ± 0.2	63 ± 1.8	0.15
O_3/UV	42 ± 5	29 ± 1.2	73 ± 1.2	0.18
O_3/H_2O_2	48 ± 7	37 ± 1.9	67 ± 1.0	0.20
O_3/H_2O_2/UV	54 ± 5	44 ± 0.8	74 ± 0.7	0.26

Table 3 | COD and DOC removals attained in the MBBR

Processes	COD removal (%)	DOC removal (%)
Control	18 ± 8	14 ± 9
O_3 + MBBR	27 ± 3	39 ± 0.2
O_3/UV + MBBR	29 ± 8	49 ± 1.2
O_3/H_2O_2 + MBBR	25 ± 10	45 ± 1.9
O_3/H_2O_2/UV + MBBR	36 ± 10	53 ± 0.8

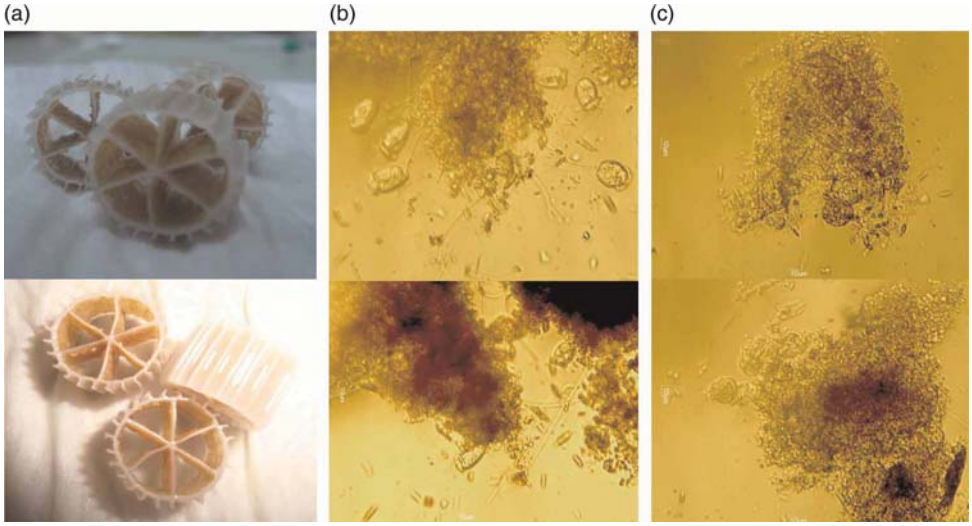

Figure 2 | Attached biomass characteristics: (a) thin biofilms on the biomedia surface; (b) protozoan and filaments observed in the control reactor; (c) dense agglomerates of biomass in the reactor fed with retentate previously oxidized.

Attached biomass characteristics

Attached biomass was carefully scraped from the carriers and used for volatile solids (VS) determination and also for microscopic observation. In general, carriers were recovered by thin biofilms (Figure 2(a)) presenting an important microbial diversity. A higher diversity was observed in the MBBR fed with the RO retentate (control reactor). In this case, protozoan and filaments were observed, as illustrated in Figure 2(b). Examination of the attached biomass collected from the MBBR operating with pre-oxidized wastewater revealed a lower diversity, in terms of protozoan population and the absence of filaments, as illustrated in Figure 2(c).

The attached biomass content, expressed as VS, was determined as 1,500 mg L^{-1} (average) in the control reactor and 960 mg L^{-1} (average) in the MBBR operated with pre-oxidized wastewater. In terms of average specific COD removal the following results were obtained: 0.013–0.017 $mgCOD_{removed}\ mg^{-1}VS$ (MBBR fed with oxidized retentate) and 0.010 $mgCOD_{removed}\ mg^{-1}VS$ (MBBR control). The low values of specific COD removal are a consequence of the low organic load applied to the reactors and the relative recalcitrance of the pollutants found in the retentate.

The knowledge of microbiota involved in biofilms is important for operation and control of MBBR systems. The literature about biofilms grown on MBBRs is relatively scarce, especially, information about community composition, as pointed out by McQuarrie & Boltz (2011).

Ammonium removal

Removal of nitrogen was very high in the control reactor and moderate in the reactor fed with pre-oxidized wastewater (Table 4). It should be remarked that the control reactor was always fed with the same influent (RO retentate), whereas the MBBR was fed with different influents (produced by different oxidation processes). Thus, the control reactor had enough time to adapt and develop an effective nitrifying microbial community. In addition, the biodegradability increase promoted by retentate oxidation may have favored the implantation of heterotrophs in the reactor. As a consequence, these organisms competed for space and oxygen with nitrifiers and hindered nitrification. Another point to take into account is the influent ammonium concentration in the MBBR fed with pre-oxidized retentate. In general, it was lower than that

Table 4 | Amonium removal results

Processes	Removal NH_4^+ (%)	Inlet NH_4^+ (mg L^{-1})	Residual NH_4^+ (mg L^{-1})	Produced NO_3^- (mg L^{-1})
Control	91 ± 9	109 ± 50	12 ± 13	299 ± 11
O_3 + MBBR	64 ± 9	83 ± 10	29 ± 5	156 ± 3
O_3/UV + MBBR	66 ± 4	83 ± 8	26 ± 5	157 ± 29
O_3/H_2O_2 + MBBR	69 ± 7	72 ± 3	24 ± 3	134 ± 13
O_3/H_2O_2/UV + MBBR	73 ± 4	69 ± 2	19 ± 3	145 ± 11

observed in the control reactor influent (109 mg L^{-1}, average). Even so, ammonium concentration in the reactor fed with pre-treated retentate was below 30 mg L^{-1} (average). This result suggests that hydraulic retention time should be increased to improve nitrification and assure a lower level of ammonium in the treated effluent.

Global performance

Analysing the global results obtained by the combined treatment process (oxidation + biological treatment in a MBBR), it can be verified that oxidation effectively contributed to reach higher levels of organic matter removal (COD and DOC), as shown in Figure 3, where the results of the control reactor were also presented just for comparison.

When the concentrate was pre-treated by oxidation, the final effluent DOC was comprised between 6 and 12 mg L^{-1}, whereas for the control reactor the effluent DOC was always in the range of 21 to 68 mg L^{-1}.

CONCLUSIONS

The RO retentate investigated in the present work had a relatively low and variable organic matter content (24 ± 6 mg L^{-1}, DOC and 83 ± 20 mg L^{-1}, COD) and a relatively high and variable ammonium concentration (127 ± 46 mg L^{-1}). In addition, it presented moderate levels of chloride (1,394 ± 54 mg L^{-1}), Na^{+} (1,318 ± 103 mg L^{-1}) and Ca^{2+} (485 ± 21 mg L^{-1}). Additionally, the retentate was considered not suitable for biological treatment, since its BOD_5/COD ratio was very low (0.06).

The retentate treatment by oxidation techniques was able to remove COD, DOC, and UV absorbance at 254 nm. As expected, the results obtained with ozonation were improved when this technique was combined with UV radiation (O_3/UV) and peroxidation (O_3/H_2O_2). An additional improvement was observed when ozonation was combined with peroxidation and UV radiation (O_3/H_2O_2/UV). Ozonation, mainly when combined with radiation and/or peroxidation, promoted oxidation of aromatic compounds and other substances originally found in the retentate. As a consequence, SUVA decreased significantly from 2.1 to 0.8–1.1 L mg^{-1} m^{-1}, indicating a drop on the organic matrix aromaticity and biodegradability was increased, as expressed by the augmentation of the ratio BOD_5/COD from 0.06 (retentate) to 0.15–0.26 (oxidized retentate).

Biological treatment of the retentate in a MBBR (control reactor) led to low removals of COD and DOC, respectively, 18 and 14%. When the MBBR was fed with pre-oxidized retentate, the removal efficiencies increased, attaining 36 and 53%, COD and DOC, respectively, for the retentate treated by O_3/H_2O_2/UV. However, ammonium removal was more effective in the control reactor (91%) than in the reactor fed with retentate treated by O_3/H_2O_2/UV (73%). Less time for nitrifiers adaptation and more favorable conditions for heterotroph growth, probably, contributed to hinder nitrification in that reactor.

Oxidation of the retentate contributed to obtain good results in terms of organic matter removal and reduction of organic matter aromaticity. The effluent of the combined process (oxidation + MBBR) presented low DOC levels, in the range of 6 to 12 mgC L^{-1}.

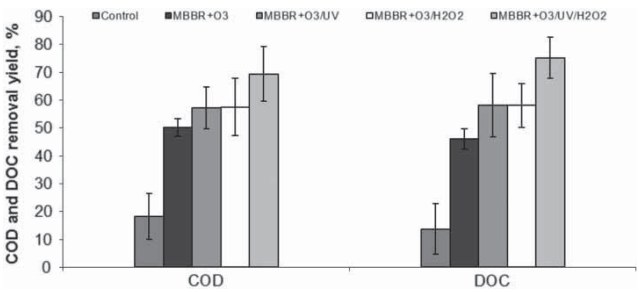

Figure 3 | COD and DOC removals attained in the MBBR control reactor and in the combined experiments (oxidation processes + MBBR).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of the Brazilian agency CAPES (S.M.R Vendramel fellowship) and the Spanish agency Fundación Carolina.

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First received 12 June 2013; accepted in revised form 12 August 2013. Available online 25 October 2013

CONGRESS COMMUNICATIONS

During the development of this thesis, the author took part in several conferences and meetings presenting different part of her work. There is a list with the contributions to those events:

ORAL presentations:

- Alicante – Spain (June 2014), **XI Reunión de la Mesa Española de Tratamiento de Aguas**: *Utilización de Procesos de Oxidación avanzada para el reúso de aguas*. Marce, M., Justo, A., De Luca, A., Cruz, A., Ramos, D.D., González, O., Dantas, R. F., Sans, C., Esplugas, S. (ISBN: 978-84-616-9173-9)

- Almería – Spain (October 2013), **3rd European Conference on environmental Applications of Advanced Oxidation Processes**: *Pharmaceuticals and toxicity evaluation of tertiary reverse osmosis concentrate treated by UV/H_2O_2 and ozone*. Justo, A., González, O., Aceña, J., Mita, L., Cruz, A., Casado, M., Pérez, S., Piña, B., Sans, C., Barceló, D., Esplugas, S. (ISBN: 978-84-15487-99-9)

- Las Vegas – Nevada (September 2013), **World Congress and Exhibition** (3rd joint IOA and IUVA World Congress and Exhibition that combine the 20th IOA World Congress and 6th IUVA World Congress): *Assessment of UV/H_2O_2 and ozone treatment for removing pharmaceuticals and toxicity from reverse osmosis retentates*. González, O., Justo, A., Aceña, J., Mita, L., Cruz, A., Casado, M., Pérez, S., Piña, B., Sans, C., Barceló, D., Esplugas, S.

- Paris – France (May 2013), **9th International Conference on Biofilm Reactors**: *Reverse Osmosis Concentrate Treatment by Chemical Oxidation and Moving Bed Biofilm Processes*. Vendramel, S., Justo, A., González, O., Sans, C., Esplugas, S.

- Santander – Spain (May 2013), **NOVEDAR young water researchers Workshop**: **Innovative technologies for the XXI Century WWTP and future perspectives**: *Pharmaceuticals mitigation in reclamation reverse osmosis brines by advanced oxidation processes*. Justo, A., González, O., Aceña, J., Pérez, S., Barceló, D., Sans, C., Esplugas, S.

- Almería – Spain (October 2012), **X Reunión de la Mesa Española de Tratamiento de Aguas**: *Aplicación de Procesos de Oxidación Avanzada para el reúso de aguas*. Micó, M. M., Justo, A., Cruz, A., De Luca, A., González, O., Dantas, R. F., Sans, C., Esplugas, S. (ISBN: 978-84-15487-33-3)

- Istanbul – Turkey (June 2012), **International Conference on Recycling and Reuse**: *Pharmaceuticals and Organic Pollution Mitigation in Reclamation Osmosis Brines through UV/H_2O_2 and Ozone*. Justo, A., González, O., Aceña, J., Pérez, S., Barceló, D., Sans, C., Esplugas, S. (ISBN: 978-975-404-919-0)

- Alcoy – Spain (November 2011), **VII Congreso, La investigación ante la sociedad del conocimiento. Sostenibilidad y Medioambiente**: *Regeneración de aguas de rechazo de Osmosis Inversa (OI) mediante la combinación del Proceso de Oxidación Avanzada (POA) UV/H_2O_2 con tratamiento biológico*. Justo, A., González, O., Sans, C., Esplugas, S. (ISBN: 978-84-694-9814-9)

- Los Angeles – California (July 2011), **Fourth IWA Specialty Conference on Natural Organic Matter: From Source to Tap and Beyond**: *Characterization and monitoring of EfOM through UV/H_2O_2 and ozonation processes.* González, O., Justo, A., Bacardit, J., Ferrero, E., Malfeito, J.J., Sans, C.

- Paris – France (May 2011), **Ozone and UV: Leading-edge science and technologies** (20th Ozone World Congress and 6th Ultraviolet World Congress): *Monitoring of EfOM fractions along ozonation of CAS and MBR secondary effluents by LC-OCD.* González, O., Justo, A., Bacardit, J., Ferrero, E., Malfeito, J.J., Sans, C. (ISBN: 978-2-9528298-8-5)

POSTER presentations:

- Barcelona – Spain (October 2014), **13th Mediterranean Congress of Chemical Engineering**: *Combination of advanced oxidation processes and biofiltration for reclamation reverse osmosis brine*. Justo, A., González, O., Sans, C.

- Barcelona – Spain (November 2011), **12th Mediterranean Congress of Chemical Engineering**: *Treatment of reclamation reverse osmosis brines through the Advanced Oxidation Process (AOP) UV/H_2O_2 combined with a biological reactor*. Justo, A., González, O., Sans, C. (ISBN: 978-84-615-4777-7)

- Barcelona – Spain (September 2011), **8th IWA International Conference on Water Reclamation & Reuse**: *Characterization and fate of dissolved organic matter (DOM) in secondary effluents treated by UV/H_2O_2 oxidation.* González, O., Justo, A., Bacardit, J., Ferrero, E., Malfeito, J.J., Sans, C.

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RESUMEN EN CASTELLANO

Actualmente, la situación de escasez de agua y la calidad de la misma son cuestiones de gran preocupación a nivel mundial. Es por ello que, restablecer la calidad de las aguas que han sido previamente utilizadas, es esencial para evitar seguir contribuyendo a la contaminación del medio ambiente y caminando hacia el ideal de "vertido cero". Esta tesis, presentada como compendio de artículos, se ha centrado en la aplicación de tecnologías avanzadas para el tratamiento de efluentes procedentes de Estaciones Depuradoras de Aguas Residuales (WWTPs) que normalmente son vertidos al medio acuático sin tratamiento extra, y sin embargo contienen aún materia recalcitrante como por ejemplo, microcontaminantes. Los efluentes tratados han sido: efluentes secundarios procedentes de WWTPs municipales y procedente del efluente de rechazo producido en el tratamiento terciario con Ósmosis Inversa (RO).

Los tratamientos empleados han sido los Procesos de Oxidación Avanzada (AOPs) UV/H_2O_2 y ozonización, los cuales se caracterizan por la generación in situ de radicales hidroxilo de alto poder oxidante. Por otro lado, aprovechando que éstos son capaces de mejorar la biodegradabilidad del efluente tratado, también se ha estudiado la integración con tratamientos biológicos. como son los filtros Biológicos de Carbón Activo (BAC). Esta tecnología aprovecha la saturación del Carbón Activo Granular (GAC) para la colonización de la biomasa en su superficie.

Por lo que respeta a efluentes secundarios, se ha caracterizado la Materia Orgánica del Efluente (EfOM) durante su oxidación con UV/H_2O_2 y ozono mediante la técnica de Cromatografía Líquida con Detección de Carbono Orgánico (LC-OCD). Se ha concluido que ambas técnicas parecen apropiadas para la oxidación de las diferentes fracciones de EfOM. No obstante, se han observado algunas diferencias en las características de las aguas resultantes debido a los diferentes mecanismos de oxidación implicados en los AOP utilizados.

Por otro lado, en los estudios realizados con efluentes de rechazo de RO, se ha evaluado la degradación de diferentes fármacos a diferentes dosis de oxidante aplicadas para ambos AOPs, así como la combinación de análisis químicos con bioensayos para caracterizar la eliminación de estos microcontaminantes. Por último, se ha evaluado la combinación de los tratamientos UV/H_2O_2 y ozonización con unos filtros Biológicos de Carbón Activo (BAC). Esta tecnología aprovecha la saturación del Carbón Activo Granular (GAC) para la colonización de la biomasa en su superficie. Los resultados han sido satisfactorios para todos los tratamientos propuestos, obteniendo cinéticas de degradación de fármacos diferentes en función del tratamiento aplicado y también de las características del efluente de rechazo. Los bioensayos aplicados han proporcionado información útil para una mejor caracterización de los efluentes resultantes. Por último, la integración de los AOPs como paso previo a un tratamiento biológico ha permitido reducir los parámetros típicos de calidad del agua significativamente.

1. Introducción

El desarrollo tecnológico e industrial ha procurado a la humanidad prosperidad y bienestar, pero ha traído consigo un alarmante deterioro del medio ambiente. Actualmente,

especialmente en los países desarrollados, se generan cantidades importantes de aguas residuales, cuyo vertido a los cauces naturales provoca un impacto considerable sobre el medio ambiente. La contaminación de las aguas es, por tanto, uno de los principales motivos de preocupación en la actualidad [5,9,11].

La mayoría de los métodos de depuración de aguas residuales se limitan a reproducir el proceso de autodepuración natural que durante siglos ha sufrido el agua tras su vertido a los cauces aunque, lógicamente, dados los enormes volúmenes y agentes contaminantes a tratar, esta depuración debe ser acelerada por medios artificiales. La incorporación de nuevas tecnologías de depuración en las Estaciones Depuradoras de Aguas Residuales (WWTPs) es muy importante para la mejora de la calidad de las aguas de nuestros ríos y acuíferos, ya que estas estaciones son una de las principales vías de entrada de contaminación al medio acuático.

La reutilización, entendida como uso de unas aguas que ya han sido previamente utilizadas, ha estado siempre presente en nuestro país. En este contexto, siempre se ha visto la reutilización como una fuente alternativa o complementaria de recurso para usos que no sean de boca. Algunos de los beneficios de la reutilización y regeneración de aguas residuales se citan a continuación: (i) permite recuperar efluentes que serían vertidos al medio acuático, aumentando los recursos disponibles; (ii) puede substituir a caudales destinados a usos no potables, y (iii) se reduce la cantidad de contaminantes vertidos al medio receptor (“vertido cero”).

Los procesos de depuración convencionales no son capaces de eliminar todos los contaminantes presentes en las aguas. Entre ellos se encuentran los llamados microcontaminantes o contaminantes emergentes [16,17]. Éstos incluyen productos de uso diario tales como detergentes, fármacos, productos para el cuidado y la higiene personal, aditivos de gasolinas, plastificantes, etc. Corresponden a contaminantes previamente desconocidos o no reconocidos como tales, cuya presencia en el medio ambiente no es necesariamente nueva, pero sí lo es la preocupación por las posibles consecuencias de la misma.

Los efluentes tratados en esta tesis, son efluentes que habitualmente son vertidos al medio acuático sin recibir ningún tratamiento extraordinario. El primer grupo lo constituyen dos efluentes secundarios (uno procedente de un tratamiento secundario Convencional de Fangos Activados (CAS) y otro de un Bioreactor de Membrana (MBR)), mientras que el segundo tipo de efluente tratado corresponde a rechazos que se producen durante el tratamiento terciario con la tecnología de Ósmosis Inversa (RO). Los efluentes secundarios contienen una matriz compleja de materia orgánica llamada Materia Orgánica del Efluente (EfOM) que consiste en: Materia Orgánica Natural recalcitrante (NOM), trazas de compuestos orgánicos sintéticos (microcontaminantes) y productos solubles microbianos [21–23]. Respecto al efluente de rechazo de RO, en él se concentran sales y la EfOM procedente de los tratamientos secundarios. Sobre todo, por lo que respecta a los rechazos de RO, aunque actualmente su vertido no está regulado, unas buenas prácticas medioambientales sugerirían el tratamiento de este tipo de efluentes concentrados antes de verterlos y que se diluyan en el medio ambiente [26–28]. Ambos efluentes suelen caracterizarse por su significativo contenido en

materia orgánica poco biodegradable haciendo apropiado el tratamiento mediante los Procesos de Oxidación Avanzada (AOPs).

Los AOPs implican la generación *in situ* de una especie sumamente reactiva, como es el radical hidroxilo ($HO^{\cdot}$), que es la especie más oxidante después del flúor con un potencial de oxidación de 2.80 V [33]. El radical hidroxilo no es selectivo ni estable, siendo estas dos características una gran ventaja. Esto hace que pueda atacar fácilmente a un gran grupo de sustancias químicas orgánicas para convertirlas en productos intermedios menos complejos.

Las tecnologías de oxidación que se han utilizado en el presente trabajo son: UV/H_2O_2 y ozonización. El proceso UV/H_2O_2 se basa en generación de radicales $HO^{\cdot}$ debido a la fotólisis del H_2O_2 [29]. En cambio, en la ozonización, el ozono puede actuar degradando la materia orgánica a través de dos vías: (i) la propia molécula de ozono es la encargada de producir la degradación de contaminantes gracias a su gran poder oxidativo en condiciones neutras o ácidas, siendo un ataque selectivo preferentemente a compuestos insaturados y compuestos alifáticos con grupos funcionales específicos [39]; (ii) la otra vía de ataque, se produce por medio de radicales libres hidroxilo, todavía más oxidantes y no selectivos, formados al introducir el ozono en el seno de una disolución básica, donde los grupos OH^-, actúan como catalizadores.

Por otro lado, aprovechando el aumento de la biodegradabilidad del efluente que se consigue al aplicar un AOP, la combinación de éstos con un posterior tratamiento biológico, menos costoso, permite eliminar mayores cantidades de materia orgánica utilizando menos energía y menores dosis de oxidante. Aunque se han estudiado otras integraciones, en la que se ha profundizado más en este trabajo ha sido la integración de los AOPs con unos filtros Biológicos de Carbón Activo (BAC) para el tratamiento de efluentes de rechazo de RO. Esta tecnología aprovecha la saturación del Carbón Activo Granular (GAC) para la colonización de la biomasa en su superficie alargando la utilización del GAC [67]. El mecanismo de biodegradación consiste en primero adsorber en los macroporos la materia orgánica eliminada del agua, donde se detiene el tiempo suficiente para promover la biodegradación mediante las bacterias adheridas [66]. Es importante la caracterización de esta biomassa para conocer más sobre el proceso biológico que tiene lugar.

2. Objetivos

El objetivo principal de esta tesis es la evaluación de la aplicación de algunos procesos avanzados a diferentes efluentes procedentes de WWTPs que habitualmente se vierten al medio acuático. El objetivo final es la regeneración de estos efluentes y/o la minimización de la contaminación del medio receptor. El objetivo principal se puede dividir en cuatro trabajos de investigación específicos:

a) Estudiar la evolución de la EfOM presente en dos efluentes secundarios (CAS y MBR) durante la oxidación con UV/H_2O_2 y ozonización utilizando la técnica de LC-OCD (Apéndice I).

b) Evaluar los AOPs UV/H_2O_2 y ozonización durante el tratamiento del rechazo de RO y monitorizar la oxidación de algunos fármacos presentes mediante la técnica LC-MS/MS para diferentes dosis de oxidante (Apéndice II y III).

c) Combinar los análisis químicos con diferentes bioensayos para caracterizar la eliminación de los fármacos en el efluente de rechazo de RO durante la aplicación de procesos de fotólisis, UV/H_2O_2 y ozonización (Apéndice IV).

d) Valorar la integración de filtros BAC con pretratamiento mediante UV/H_2O_2 o ozonización para tratar el rechazo de RO, así como evaluar la actividad biológica de los biofiltros (Apéndice V y VI).

3. Materiales y métodos

Ambos tratamientos de oxidación se realizaron en reactores encamisados de 2 L a 25 ^oC. El reactor UV/H_2O_2 está provisto de tres lámparas tres lámparas de mercurio de baja presión de 254 nm. En los experimentos de ozonización, el ozono se genera *in situ* y se inyecta en el reactor en fase gaseosa mediante unos difusores.

A continuación se detallan los principales métodos analíticos y técnicas empleados:

- Determinación del Carbono Orgánico Disuelto (DOC): mediante el analizador *Shimadzu 5055 TOC-VCSN*.
- Determinación de la Demanda Química de Oxígeno (COD): método 5220 D del Standard Methods [88].
- Espectrofotometría UV (UV_{254}): mediante el espectrofotómetro *Perkin Elmer UV/Vis*.
- Determinación de la Demanda Bioquímica de Oxígeno a los 5 días (BOD_5): método 5210 D del Standard Methods [88].
- Cromatografía Líquida con Detección de Carbono Orgánico (LC-OCD): técnica capaz de facilitar información tanto cualitativa como cuantitativa de la NOM.
- Análisis de los fármacos: estos han sido analizados por Cromatografía Líquida acoplada a un Espectrómetro de Masas (LC-MS/MS).
- Bioensayos: Microtox®, test de actividad antimicrobiana y Ensayos de Levadura Recombinante (RYA).
- Evaluación del biofilm: mediante la técnica de Hibridación Fluorescente *in situ* (FISH) y los análisis de Adenosín trifosfato (ATP).

4. Resultados y discusión

a) *Caracterización y destino de la EfOM tratada con UV/H_2O_2 y ozonización (Apéndice I):*

En este trabajo se han analizado las siguientes fracciones de EfOM disuelta (Materia Orgánica Disuelta (DOM)): biopolímeros (BP), Sustancias Húmicas (HS), compuestos fragmentados de las HS (BB), compuestos Ácidos de Bajo Peso Molecular (LMMA) y

compuestos Neutros de Bajo Peso Molecular (LMMN), para dos efluentes secundarios, CAS y MBR.

Aunque ambas tecnologías se mostraron eficientes para eliminar prácticamente toda la DOM en condiciones de oxidación severas, se pudieron observar algunas diferencias entre ellas. Desde la primera etapa de oxidación los dos procesos fueron eficaces en la degradación de BP. Además, la ozonización, por dominar el ataque directo de la molécula de ozono, se mostró más eficaz en la eliminación de HS y otros subproductos de oxidación, con la excepción de los LMMA, que se acumularon desde el inicio de la reacción. La presencia exclusiva de LMMA en el efluente final de MBR, con mayores dosis de oxidante, confirmó su carácter recalcitrante a la ozonización. Por el contrario, el mecanismo radicalario no selectivo del proceso UV/H_2O_2 condujo a efluentes finales de CAS y MBR compuestos por HS y el resto de compuestos de bajo peso molecular.

Además, mediante la monitorización de las fracciones de la materia orgánica por la técnica LC-OCD se ha demostrado que la reducción de la aromaticidad del efluente (medida como Absorbancia UV Específica (SUVA)) no se corresponde rigurosamente con el descenso de las HS de los efluentes para ninguno de los AOPs aplicados.

b) *Mitigación de los fármacos y contaminación orgánica en los efluentes terciarios de rechazo de RO mediante UV/H_2O_2 y ozonización (Apéndice III):*

Se estudió la presencia de once fármacos (sus concentraciones variaron desde 0.2 a 1.6 g L^{-1}), así como su eliminación en el rechazo de RO mediante los procesos UV/H_2O_2 y ozonización utilizando un amplio rango de dosis de oxidante. También se monitorearon los parámetros típicos de calidad de las aguas durante ambos procesos.

El rango de las constantes cinéticas iniciales (K_{obs}) calculadas para los diferentes fármacos fueron: 0.8-12.8 L mmol O_3^{-1} y 9.7-29.9 Lmmol $H_2O_2^{-1}$ para la ozonización y el proceso UV/H_2O_2, respectivamente. Los fármacos que experimentaron las K_{obs} iniciales más bajas en la ozonización fueron Atenolol, Carbamazepin, Codeina, Trimetoprim y Diclofenac (en el orden mencionado). Además, tanto Atenolol como Carbamazepine manifestaron ser los más resistentes al ozono, mostrando bajos porcentajes de eliminación a bajas dosis de ozono. Por otro lado, a pesar de no ser el radical hidroxilo selectivo, durante el proceso UV/H_2O_2 también se observaron diferencias en las K_{obs} iniciales. Trimetoprim, Paroxetin y Sulfametoxazol (en el orden mencionado) mostraron las K_{obs} iniciales más bajas, siendo Trimetoprim y Paroxetin los que mostraron menores porcentajes de eliminación a bajas dosis de H_2O_2 aplicadas.

c) *Aplicación de bioensayos para la evaluación del impacto de los AOPs en el tratamiento del rechazo de RO (Apéndice IV):*

En este estudio se combinaron los análisis químicos y algunos bioensayos de toxicidad para evaluar la eliminación de diferentes fármacos y los compuestos similares a las dioxinas

(dioxin-like compounds) del rechazo de RO mediante los procesos de oxidación UV, UV/H_2O_2 y ozonización.

Para la eliminación de los fármacos analizados se requirieron dosis relativamente altas de oxidante, tanto para la ozonización como para el proceso UV/H_2O_2. Mediante el bioensayo de RYA se determinó una actividad significativa de compuestos similares a las dioxinas en el rechazo de RO, mientras que las actividades antibacterianas y estrogénicas fueron prácticamente nulas. La radiación UV por si sola fue la menos eficiente en la eliminación de la actividad de los compuestos similares a las dioxinas. En cambio, el proceso de ozonización demostró ser el más eficiente para la eliminación de este tipo de actividad.

d) *Aplicación de filtros BAC para la eliminación de los microcontaminantes y EfOM en los efluentes terciarios de rechazo de RO (Apéndice V):*

En este trabajo se evaluó a escala laboratorio la idoneidad de los filtros BAC para tratar los efluentes de rechazo de RO procedentes de una WWTP municipal. Para ello se compararon tres tratamientos: un filtro BAC, y otros dos filtros BAC combinados con los pretratamientos UV/H_2O_2 (UV/H_2O_2-BAC) y ozonización (ozono-BAC), durante un periodo de operación de 320 días.

La combinación de ambos AOPs con un biofiltro mejoraron considerablemente los parámetros de calidad de aguas analizados. Las eliminaciones totales de DOC, COD y UV_{254} en las integraciones de UV/H_2O_2 y ozonización con BAC variaron entre: 50-66%, 48-66% y 73-87%, respectivamente, mejorando considerablemente las obtenidas mediante el biofiltro sin pretratamiento (28%, 19% y 37%, respectivamente). Además, aunque algunos de los fármacos analizados fueron parcialmente eliminados en el biofiltro sin pretratamiento, la integración con los AOPs fue necesaria para la eliminación completa de estos microcontaminantes. La presencia de biomasa se demostró mediante los análisis de ATP y la diversidad de la biomasa se identificó mediante la técnica FISH, obteniendo que las β-proteobacteria eran las más abundantes en los tres biofiltros.

5. Conclusiones

Los tratamientos aplicados mostraron resultados satisfactorios en todos los casos. El análisis global de todos los resultados permite concluir:

a) Los diferentes mecanismos de oxidación implicados en el proceso UV/H_2O_2 y ozonización, debido a la significativa alcalinidad de los efluentes tratados, condujeron a efluentes finales con composiciones diferentes de las fracciones de DOM. Ambos AOPs permitieron eliminar la fracción de BP durante los primeros 30 minutos de tratamiento, los cuales son conocidos por jugar un papel importante en el *fouling* de las membranas durante el tratamiento de efluentes biológicamente procesados.

b) En general el proceso UV/H_2O_2 se mostró como la tecnología más eficiente para eliminar completamente los fármacos del rechazo de RO en comparación con la ozonización. Los compuestos identificados como "problemáticos" durante la ozonización fueron eliminados con mayor eficiencia mediante el proceso UV/H_2O_2, aplicando menores dosis de oxidante comparado con la ozonización.

c) Los resultados del trabajo demostraron la eficiencia de los AOPs, en especial de la ozonización, para eliminar los microcontaminantes biológicamente activos del efluente de rechazo de RO. Los diferentes resultados obtenidos, evidencian la complejidad de la aplicación de estos tratamientos a efluentes reales debido al gran número de factores implicados, como pueden ser la reactividad de la EfOM y la variable calidad del agua. Por otro lado, se demostró que la combinación de los análisis químicos y los bioensayos permite la caracterización completa de la eficiencia de los procesos de tratamiento de agua avanzados para eliminar los contaminantes recalcitrantes.

d) La integración de unos filtros BAC con un pretratamiento con UV/H_2O_2 u ozonización para tratar el rechazo de RO condujo a obtener mejoras en los parámetros de calidad del efluente final. Mediante la combinación de la ozonización con los filtros BAC se obtuvieron las mayores eliminaciones de los parámetros estudiados. Po su parte, las técnicas de evaluación de la biomasa permitieron determinar la diversidad de la biomasa de los diferentes biofiltros.

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