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ENVIRONMENTALLY FRIENDLY REMOVAL OF WATER EMERGING CONTAMINANTS BY PHOTOCATALYTIC OZONATION AND BIOFILTRATION

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Tese no âmbito do Doutoramento em Engenharia Química orientada pelo Professor Doutor Rui Carlos Cardoso Martins e Professora Doutora Rosa Maria Quinta-Ferreira apresentada ao Departamento de Engenharia Química da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

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Environmentally friendly removal of water emerging contaminants by photocatalytic ozonation and biofiltration

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RESUMO

No futuro a gestão da água deverá necessariamente passar pela recuperação dos efluentes municipais. O crescimento exponencial da população implica um aumento na utilização de produtos farmacêuticos e cuidado pessoal (PFCPs), causando um avolumar de contaminação dos efluentes municipais e de outros recursos aquáticos por contaminantes emergentes, tais como os parabenos. Os parabenos são amplamente usados como conservantes. Por outro lado, a presença de bactérias tem igualmente sido detetada na água tratada o que levanta sérias preocupações para a saúde humana. Os processos avançados de oxidação como a ozonólise e processos foto-assistidos apresentam-se como soluções adequadas no tratamento de efluentes. O desempenho destas metodologias deve ser avaliado em termos do seu impacto na toxicidade e na capacidade de desinfecção da água.

Neste sentido, foram testadas a oxidação fotocatalítica com radiação UVA e a ozonólise catalítica e fotocatalítica para degradação de uma mistura de parabenos (metil, etil, propil, butil e benzilparabeno). A eficiência de catalisadores à base de metais nobres (Ag, Au, Pd e Pt) dopados no TiO_2 foi testada, assim como a de materiais de baixo custo. No que respeita à oxidação fotocatalítica, a taxa de degradação dos parabenos seguiu uma cinética de pseudo primeira ordem e a constante cinética aumentou com o número de carbonos ligados ao anel aromático. Para os melhores catalisadores, Ag e Pd - TiO_2 a concentração inicial de parabenos desceu para menos de metade em 180 min de reação. A análise da toxicidade para os melhores catalisadores demostra que as soluções tratadas são menos perigosas ambientalmente que a mistura inicial. A ozonólise é capaz de oxidar a mistura inicial dos parabenos com uma elevada dose de ozono transferido (DOT) (cerca de 170 mg/L). No entanto, a redução da carência química de oxigénio (CQO) foi baixa (25%). Para os melhores catalisadores, 0.5% Pt e Pd–TiO_2 a degradação total dos parabenos foi alcançada com uma DOT de 70 mg/L. Mais ainda, com estes catalisadores foi possível obter uma remoção de CQO de 36%. Estudos feitos com fatias cerebrais de ratos Wistar indicam que a mistura de parabenos tratada por ozonólise simples e catalitíca não produz qualquer efeito nos sinais neuronais ao contrário da mistura inicial. De forma a melhorar a degração da mistura de parabenos estas duas metodologias foram aplicadas em conjunto com os mesmos catalisadores. 0.5% Ag-TiO_2 foi o melhor catalisador obtendo a remoção total dos parabenos com 46 mgO_3/L. A presença de radiação UVA levou a um aumento do consumo de energia comparativamente à ozonólise catalítica com os mesmos catalisadores mas diminuiu o tempo de reação. Da análise dos subprodutos conclui-se que a hidroxilação aparenta ser o mecanismo de reação mais significativo na degradação dos parabenos. Na ozonólise fotocatalítica foi analisado o efeito da carga de metal (0.1, 0.5 e 1 wt.% para Ag, Pd e Pt-TiO_2) na degradação dos parabenos e na remoção de CQO e verificou-se que ambos são dependentes do tipo de metal nobre usado. A ozonólise fotocatalítica usando um baixa quantidade de 0.1 wt% Ag-TiO_2 provou ser eficiente na degradação dos parabenos em água ultrapura. Por isso, foi importante verificar o efeito de diversos parâmetros na eficiência dos processos. Foi analisado o efeito do pH, da presença de bloqueadores de radicais, da matriz efluente municipal e de iões como HCO_3^-, Cl^- e SO_4^{2-}.

A ozonólise catalítica com rochas vulcânicas permitiu com um DOT de 55 mg/L com 0.5 g/L de catalisador obter a remoção total dos parabenos. Os radicais hidroxilo são os principais responsáveis pela degradação dos parabenos. A toxicidade em espécies não alvo: cladóceros, microalgas, amêijoas, macrófitas e agrião, das amostras tratadas por ozonólise simples e catalítica foi comparada com a de diluições da mistura inicial de parabenos. A ozonólise catalítica permite uma redução da quantidade de ozono para a remoção total de parabenos (3 vezes), e a solução tratada foi mais tóxica de que a amostra obtida do tratamento com a ozonólise simples o que está relacionado com os subprodutos formados.

A *Corbicula fluminea* é uma espécie de água doce, um bivalve invasor amplamente disperso, causando significativos impactos ecológicos e industriais. Então devem ser implementadas estratégias adequadas para a gestão desta praga. Estes bivalves em 6 h conseguem remover *E. coli* da água até concentrações abaixo do limite de detecção. Os resultados obitdos por esta metodologia foram comparados com a ozonólise simples e oxidação fotocatalítica com TiO_2, Ag, Au, Pd-TiO_2. Este estudo sugere que apesar da boa performance do ozono a biofiltração com *C. fluminea* pode ser um solução adequada para minimizar a presença de bactéria no efluente reduzindo impactos ambientais e económicos.

Palavras-chave: Parabenos; *Escherichia coli*; Biofiltração; Foto-degradação; Ozonólise; Contaminantes emergentes; Catálise heterogénea

ABSTRACT

Water scarcity is one of the major drawbacks of this century. Future water management must encompass municipal wastewater reclamation. The exponential population growth implies an increase of pharmaceutical and personal care products (PPCPs) utilization which means a higher contamination of municipal wastewaters and water sources by emerging contaminants such as parabens. Parabens are widely used as a preservative and antimicrobial. On the other hand, the presence of bacteria has also been detected in treated water which may cause important concerns to human health. Thus, when dealing with municipal wastewater reuse, special attention must be given to the impact of reclaimed water. The advanced oxidation processes such as ozonation and photo-assisted processes appear as suitable solution for parabens degradation. The performance of such methodologies was evaluated in terms of their impact over toxicity and water disinfection capacity.

Therefore, photocatalytic oxidation under UVA radiation and catalytic ozonation, photocatalytic ozonation were tested to promote a parabens mixture (Methyl, Ethyl, Propyl, Butyl and Benzylparaben) degradation. The efficiency of noble metals (Ag, Au, Pd and Pt) doped on TiO_2 was verified as catalyst as well as a low-cost solution (volcanic rock). In what regards photocatalytic oxidation, the degradation rate of single parabens followed a pseudo-first order kinetics and the kinetic constant increased with the number of carbons on the chain linked to the aromatic ring. For the best catalysts, Ag and Pd on TiO_2, a reduction higher than 50% of the initial parabens concentration was achieved after 180 min of reaction. The toxicity analysis points out that, for the best catalysts, the treated solutions are less environmentally harmful than the initial mixture. Single ozonation was able to totally oxidize the initial parabens mixture using a high transferred ozone dose(TOD) (around 170 mg/L). However, the chemical oxygen demand (COD) reduction was very low (25%). For best catalysts, 0.5% Pt and Pd–TiO_2, total degradation of parabens was achieved for a TOD of 70 mg/L. Moreover, these catalytic systems led to a COD removal of 36%. The studies made with Wistar rat brain slices, indicate that the parabens mixture treated using single or catalytic ozonation had no effect on the measured neuronal ROS signals, unlike the raw mixture. Then these two methodologies were coupled to enhance the parabens mixture degradation with the same catalysts. The best catalyst was 0.5% Ag-TiO_2 leading to total parabens removal using 46 mgO_3/L. The presence of UVA irradiation increased the energy consumption compared with catalytic ozonation with these catalysts but it can decrease the time of reaction. From the by-products analysis, it was concluded that hydroxylation appears to be the most significant reaction pathway and the main responsible for parabens degradation. For photocatalytic ozonation the effect of metal loading (0.1, 0.5 and 1 wt.% for Ag, Pd and Pt-TiO_2) on the parabens degradation and COD removal is very dependent on the type of noble metal used. Photocatalytic ozonation process using a low amount of 0.1 wt% Ag-TiO_2 proved to be efficient on the parabens degradation under ultrapure water conditions. Therefore, it was important to verify the effect of multi parameters on the process efficiency. The effect of pH, presence of radical scavengers, municipal wastewater matrix and several ions (HCO_3^-, Cl^-, SO_4^{2-}) was analyzed.

The catalytic ozonation with volcanic rock allowed total parabens degradation for a TOD of 55 mg/L using 0.5 g/L of catalyst and natural pH values allowed a reduction on the TOD value. Moreover, hydroxyl radicals are the main responsible by parabens degradation by this process. The toxicity of treated samples by single and catalytic ozonation was compared with the several dilutions of the initial parabens mixture over several non-target species: cladocerans, microalgae, clams, macrophytes and cress. Catalytic ozonation allows reducing the amount of ozone (about 3-fold) required for total removal of parabens, the resulting treated solution was more toxic than the sample taken at the endpoint of the single ozonation treatment which was related with the by-products formed.

Corbicula fluminea, is a widely spread freshwater invasive bivalve with significant ecological and industrial impacts. In this context, suitable pest management strategies must be implemented. The bivalves can remove *E. coli* until concentrations below the detection limit in about 6 h and clearance rate as function of the number of clams was analyzed. Results obtained by this methodology were compared with ozonation and photocatalytic oxidation using TiO_2, Ag, Au, Pd-TiO_2. Despite of ozone good performance this study suggests that application of biofiltration using *C. fluminea* can be a suitable solution to minimize the presence of bacteria in wastewater, reducing environmental and economic impacts.

Keywords: Parabens; *Escherichia coli*; Biofiltration; photo-degradation; Ozonation; Emerging contaminants; Heterogeneous catalysis

List of Contents

List of Figures

I. Introduction

II. State of the art

III. Detoxification of parabens using UV-A enhanced by noble metals – TiO_2 supported catalysts

IV. Noble metal – TiO_2 supported catalysts for the catalytic ozonation of parabens mixtures

V. Photocatalytic Ozonation using doped TiO_2 Catalysts for the Removal of Parabens in Water

VI. Effect of Noble Metals (Ag, Pd, Pt) Loading over the Efficiency of TiO_2 during Photocatalytic Ozonation on the Toxicity of Parabens

VII. Study of the Influence of the Matrix Characteristics over the Photocatalytic Ozonation of Parabens Using Ag-TiO_2

VIII. Parabens degradation using catalytic ozonation over volcanic rocks

IX. Ecotoxicity variation through parabens degradation by single and catalytic ozonation using volcanic rock

X. Biofiltration using *C. fluminea* for *E. coli* removal from water: Comparison with ozonation and photocatalytic oxidation

List of Tables

II. State of the art

III. Detoxification of parabens using UV-A enhanced by noble metals – TiO₂ supported catalysts

IV. Noble metal – TiO₂ supported catalysts for the catalytic ozonation of parabens mixtures

V. Photocatalytic Ozonation using doped TiO₂ Catalysts for the Removal of Parabens in Water

VI. Effect of Noble Metals (Ag, Pd, Pt) Loading over the Efficiency of TiO₂ during Photocatalytic Ozonation on the Toxicity of Parabens

VII. Study of the Influence of the Matrix Characteristics over the Photocatalytic Ozonation of Parabens Using Ag-TiO_2

VIII. Parabens degradation using catalytic ozonation over volcanic rocks

IX. Ecotoxicity variation through parabens degradation by single and catalytic ozonation using volcanic rock

I. Introduction

Modern society requirements are leading to an increasingly stress over the natural resources. The fresh water bodies are continuously affected by contamination from municipal and industrial polluted streams. This involves a great threat to ecosystems and human health (Ganiyu et al., 2015). The shortening on the amount of water supply sources and their quality is pushing towards the spreading of the implementation of municipal wastewater (MWW) reclamation and reuse strategies (Bixio et al., 2006). In this ambit, in the last decade attention has been driven to the occurrence of persistent organic compounds in municipal wastewaters, natural waters and even drinking water, such as pharmaceuticals and personal care products - PPCPs (Rivera-Utrilla et al., 2013). These contaminants are found in secondary MWW (Petrovic et al., 2005; Hollender et al., 2009; Molins-Delgado et al., 2016a; Ahmed et al., 2017) and their presence is related with the incorrect disposal of unused drugs and personal care products in the sewage and is also due to human excretion (Heberer, 2002). In this regard, some specific compounds such as pesticides, dioxins and dioxins-like, aromatic hydrocarbons, flame retardants and industrial compounds were classified by European Union as priority substances (Directive, 2013). Besides, a group of 10 substances are recommended to be monitored (Decision, 2015), which means about 16 contaminants.

The traditional methodologies applied in municipal wastewater treatment plants (MWWTP) are unable to satisfactorily remove emerging contaminants (Martínez-Bueno et al., 2007). Therefore, continuous discharge from MWWTP into the environment is a source of persistent compounds. Thus, they are reaching and accumulating in the natural water courses/rivers which is an increasing concern for these streams that are used as water sources for human applications as, for example, drinking water source through indirect reuse (Lee and von Gunten, 2010). Moreover, MWW treated water is starting to be considered an important component of water resources supply (Li et al., 2015). Thus, the precautionary principle imposes that those compounds must be removed from human use water (Huber et al., 2003) and particularattention must be given to the possible synergetic or additive toxic effect of mixtures of pollutants (Schwarzenbach et al., 2006).

Some of these compounds are considered as endocrine disrupting chemicals (EDCs) which are substances able to interfere with the endocrine system. The exposure to such substances is a matter of public concern since they are alleged to be related with some tumours (Molins-Delgado et al., 2016b) as well as with reproductive problems (Esplugas et al., 2007). On the other hand, the spread of antibiotics throughout the ecosystems leads to the proliferation of antibiotic-resistant bacteria which easily disseminate posing serious human health danger (Chen et al., 2016). Indeed, antibiotics are considered as pseudo-persistent in the environment due to their continuous discharge (Watkinson et al., 2009). It should also be stressed that although MWWTP are able to remove up to 2 logarithmic cycles of bacteria they do not prevent antibiotic resistance prevalence and it is estimated that these treatments lead to the disposal of more than 10^9 antibiotic resistant coliforms per minute to the environment (Michael et al., 2013; Vaz-Moreira et al., 2014) which also constitutes an important health issue.

The World Health Organization (WHO) and International Water Association (IWA) recommend that a preventive risk management must be implemented so that safe drinking water may be provided to all populations (Omar et al., 2017). Thus, in a way of protecting the main water sources for human applications (surface and groundwater) as well as to envisage reclaimed municipal wastewater as a safe water supply, it is essential to improve MWWTP performance. This involves the application of tertiary treatment approaches able to remove chemical and biological microcontamination (bacteria and viruses). Ozonation can be highlighted due to its unique feature that combines specific molecular ozone reactions and unselective radical pathways involving hydroxyl radicals (von Gunten, 2003a) which enables the removal of a wide range of contaminants as well as water disinfection (von Gunten, 2003b). The potential of ozonation makes it an interesting process for full-scale applications in water reclamation plants. The main drawback of this method is the low mineralization, and further research is necessary to optimize and understand this process. The use of solid catalysts and/or light appear as suitable options to enhance ozone action. In this context, studies must be performed to find active and stable catalytic materials. Of special interest is the use of solar light that is highly available in countries where water shortage is an important problem such as those in the Mediterranean area. On the other hand, ozonation can be integrated with biological depuration.

Invasive species are also an important ecological and industrial problem and a new management axis for invasive bivalves is arising. This new approach aims finding environmental applications for these species. For example, the bivalves' high filtration rate and relative tolerance to contaminants make them interesting for controlling eutrophication in natural and man-made water reservoirs (McLaughlan and Aldrige, 2013). Moreover, some studies have been made to find an application of this species to wastewater treatment (Elliot et al., 2008; Binelli et al., 2014; Pipolo et al., 2017; Ferreira et al., 2018). The resistance to chemicals used on the wastewater treatment such as coagulant and flocculants reveals that these species can be suitable for alternative water management strategies. Furthermore, their high capacity of filtration can be helpful to remove particles and other contaminants from water. The use of these species as biofilters on the decontamination of wastewater can innovatively combine pest management approaches with low cost solutions for water and wastewater decontamination. In fact, an application for the mollusks removed from the invaded sites can be given by using them on the assembly of biofilters for water and wastewater decontamination. Biofiltration using invasive bivalves can compete or be integrated with other wastewater treatment technologies. The success of that approach would contribute to the solution of two environmental problems: wastewater and water decontamination at lower cost giving an environmental application to nuisance species reducing their impact due to the competition for food and space against native species as well as an enhanced economic impact in water dependent facilities. However, some care must be taken when dealing with these species to avoid their further spreading. This could lead to unwanted effects over the surrounding ecosystems.

I.1. Objectives

Wastewater reclamation continues to be an objective difficult to achieve due to the inefficiency of conventional wastewater treatments. Ozonation coupled with heterogeneous catalysis or even in the presence of UVA or sunlight radiation seems to be a suitable option for the abatement of emerging chemical and biological contaminants. Thus, one of the aims of the present thesis is to analyse this process with eyes guided towards water reuse. On the other hand, an innovative approach will be addressed studying biofiltration systems for water disifection, giving an environmental application to nuisance species. The usage of *Corbicula fluminea* can be considered as pest management due to their invasive character. Bearing in mind the environmental impact of parabens, this group of PPCPs was selected as model compounds. In what regards biological contamination, *E. coli* was chosen as the target species.

The photocatalytic ozonation is a good candidate option for emerging contaminants removal but the challenge is to find a suitable catalyst that can be photoactive under UVA or even sunlight radiation. Therefore, the first objective of the present thesis was to screen the efficiency of titania based catalysts for the photocatalytic oxidation and ozonation of emerging contaminants. Although simulated waters were used, the best materials were tested under real water treatment conditions.

One of the main drawbacks of the catalysts is related with the production costs. Therefore, a low-cost solution, a volcanic rock, was also tested on the abatement of emerging contaminants. Moreover, the catalyst efficiency addressed also the impact of the treatment in terms of treated water toxicity over a wide range of species. This is essential when water reclamation (for example for irrigation purposes) is the target.

The same processes were tested on the abatement of biological contamination using *Escherichia coli* as target species. Meanwhile, *Corbicula fluminea E. coli* removal capacity was also evaluated and compared with the chemical disinfection processes.

Overall, the objectives to be achieved in this thesis are the following:

- Selection of active and stable photo-catalysts;
- Optimization of UVA photo-catalytic ozonation for the abatement of the pollutant character of PPCPs;
- Use of low cost material for the catalytic ozonation process and analysis of its impact in terms of water toxicity over a wide range of living species;
- Ozone and photocatalytic oxidation studies towards the inactivation of bacteria;
- Water dissinfection using *C. fluminea*.

I.2. Thesis structure

The thesis is divided into eleven chapters, as can be seen in the Figure I. 1. At the initial one, a brief introduction is given on the water problematic that will be addressed. Moreover, the objectives and scope of this work on wastewater treatment encompassing water disinfection and chemical decontamination are described. On the second chapter an overview about the application of ozonation for PPCPs removal from water is performed. This allows to understand what has been done in this field and what are the future challenges. On the other hand, the use of invasive bivalves is also analyzed for water and wastewater decontamination through nutrients, enteric pathogens and emerging contaminants removal. The aim is to find an environmental application for these pests, the *C. fluminea* invasive bivalves.

The core of this thesis is centered on results obtained during the treatment processes optimization for emerging contaminants abatement included in chapters III, IV, V and VI. The chapters III, IV and V refer to the analysis of several methodologies for PPCPs (the selected molecules were parabens) decontamination. It starts with the use of UVA radiation, then ozonation and finally the combination of the previous two approaches. Noble metal doped TiO_2 were tested as catalysts for these three processes. The selection of the catalytic material had into account COD and TOC removal as well as the treated water toxicity assessment over different species. After selecting the most promising technology and catalyst, the effect of different noble metal loads on the parabens mixture degradation was analysed. The effect of real wastewater matrix as well as their constituents' ions regarding photocatalytic ozonation efficiency on the removal of parabens was followed in chapter VII. Moreover, the mechanism degradation was also assessed focused on the pH and hydroxyl radical scavenger effect.

Chapters VIII and IX deal with the application of low-cost materials as a solution for parabens descontamination through catalytic and photocatalytic ozonation. On chapter VIII the characterization of two volcanic rocks was performed and their catalytic and photocatalytic activity was assessed. Afterwards, in chapter IX, the impact of the catalytic treatment process over the treated water toxicity wihin a wide range of species was evaluated considering the possibility of water recovery.

Biofiltration for the removal of *E. coli* bacteria using *C. fluminea* was presented on the chapter X. Ozonation and photocatalytic oxidation under UVA radiation were also applied for comparative purposes with this environmental approach.

The final remarks with the most important conclusions of each chapter and the recommendations of future work were presented at the final chapter (XI).

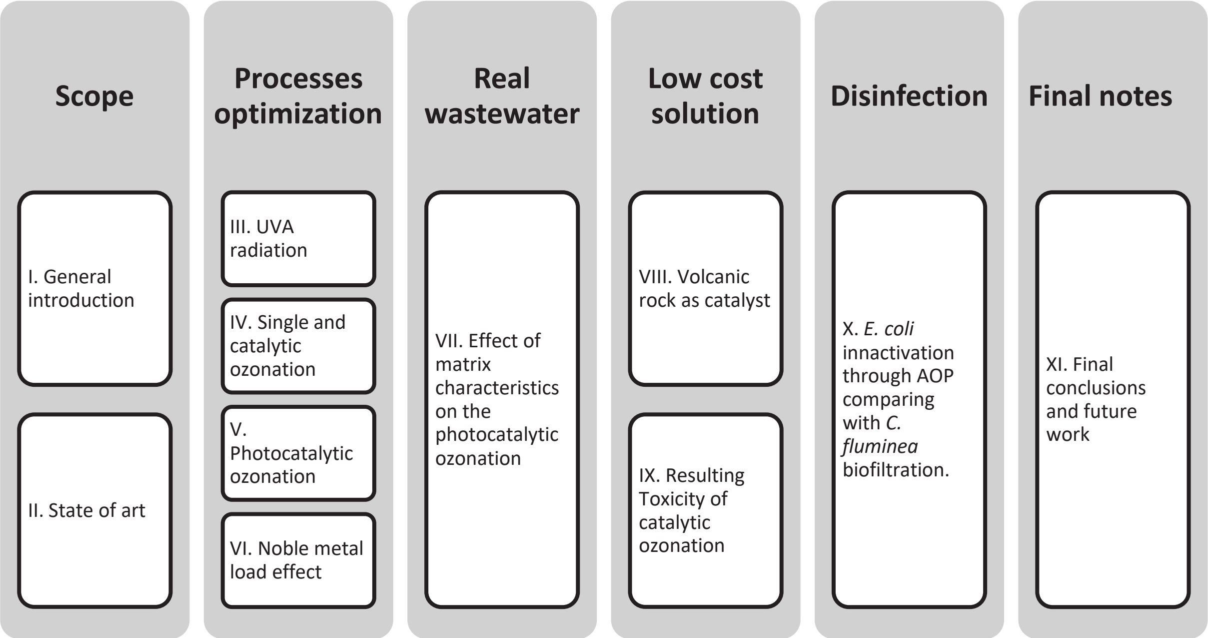

Figure I.1. Summary guidance of the thesis structure.

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II. State of the art

II.1. Aplication of ozonation for pharmaceuticals and personal care products removal from water

This section gives a literature overview on the application of ozone-based processes for the removal of emerging contaminants from water and wastewater. It is based on the publication: Gomes, J., Costa, R., Quinta-Ferreira, R.M., Martins, R.C. (2017) Application of ozonation for pharmaceuticals and personal care products removal from water (Review), Science of the Total Environment 586, 265-283.

II.1.1. Ozonation for microcontaminants removal

Conventional drinking water treatment processes (coagulation-flocculation, filtration and chlorination) are not suitable for removing emerging contaminants as, for example, atrazine a widely applied pesticide (Saquib et al., 2010). Ozone is a widely spread oxidant for drinking water treatment due to its oxidation and disinfection potential (von Gunten, 2003a, b). Moreover, it can remove taste and odour (Hua et al., 2006). Under water treatment conditions, ozone can lead to hydroxyl radicals (Deborde et al., 2005) which are less specific and react fast with a wide range of molecules. Ozone is an electrophilic molecule which specifically reacts with high electronic density sites of the molecules such as unsaturated bonds and aromatics, while HO• radical is less specific oxidizing all kind of organic functional groups. However, in water these radicals can be scavenged due to the substances present in the matrix such as HCO_3^-, CO_3^{2-}, NO_3^-, NO_2^-, Cl^-, Br^- and natural organic matter.

Westerhoff et al. (2005) compared several conventional drinking water treatments with ozonation on the removal of several EDCs and PPCPs. Aluminium sulphate, iron chloride coagulation and chemical softening removed less than 25% of the EDCs and PPCPs. Hydrocodone, erythromycin as well as hydrophobic polycyclic aromatic hydrocarbons and pesticides achieved the highest depletion. It was concluded that hydrophobicity may be a reliable parameter to predict pollutants removal by this treatment approach. The use of activated carbon (AC) by adjusting the dose led to up to 98% of compounds removal independently of the contaminant concentration. However, it should be considered that coagulation is usually designed to remove suspended solids. Thus, only EDCs and PPCPs capable to be adsorbed on the flocs formed can be removed which may explain the low efficiency of this method, whereas

AC removes target dissolved compounds able to adsorb on its surface. In fact, the authors verified that the octanol-water partition coefficient (K_{OW}) was able to generally predict the compounds removal by AC. Comparing the oxidation performance of chlorine and ozone, it was concluded that compounds easily oxidized by chlorine are also very reactive with ozone. Among these compounds are those encompassing activated aromatic rings; 6 (of 60) of the tested substances for example, aliphatic compounds with chlorine groups were poorly oxidized by both oxidants while 24 were better removed by ozone. This group of compounds includes those encompassing nucleophilic sites that are more reactive with ozone than with hypochlorous acid. This may be related with the production of hydroxyl radicals during ozonation which are powerful oxidants (Westerhoff et al., 2005).

From a literature survey, Ahmed et al., (2017) concluded that ozonation is highly efficient for the degradation of EDCs and pesticides. This behaviour was related with their high K_{ow} and susceptibility for reacting with ozone. Even if this process is able to remove many pharmaceutical compounds, perindopril, phenytoin, sertraline and ketoprofen are among those that are removed by ozone oxidation in less extent. Ozone tends to react preferably with hydrophobic fractions of organic compounds such as hydrophobic acid and neutral species. After treatment, the aromatic character decreases while aliphatic and ketones load increases with special relevance when low ozone doses are applied (Jin et al., 2016)

In this context, ozone is prone to remove emerging contaminants from water. Table II.1.1. shows the kinetic constants for direct oxidation by ozone (k_{O_3}) or towards radical degradation through hydroxyl radicals ($k_{HO^\bullet}$) for several PPCPs that can be found in municipal wastewater, natural and drinking water.

Tay et al., (2010) verified that for parabens degradation (methyl, ethyl, propyl and butylparaben) the kinetic constants increase with the chain length (Table II.1.1.) since the number of available sites for reaction increases.

Huber et al., (2003) showed that from 9 pharmaceutical compounds tested (bezafibrate, carbamazepine, diazepam, diclofenac, 17α-ethinylestradiol, ibuprofen, iopromide, sulfamethoxazole and roxithromycin), 5 (carbamazepine, diclofenac, 17α-ethinylestradiol, sulfamethoxazole and roxithromycin) were completely degraded by ozonation with kinetic constants higher than 10^4 $M^{-1}.s^{-1}$. The authors also concluded that the kinetic constants determined during oxidation using ultrapure water can be used to predict the behaviour of the pharmaceuticals while degraded in other matrix such as natural waters which are used as source of drinking water. However, some authors refer a decay on pharmaceutical compounds decomposition rate when real matrices are used (for example: Ternes et al., 2002; Lee and von Gunten, 2010) due to the presence of compounds that can compete with the oxidants. Thus, even if the kinetic constants obtained using ultrapure water can be used as a first approach for designing full scale treatment units, tests involving actual effluents must be performed at the lab and pilot scale to verify the accuracy of the prediction before the scale-up.

The diversity of structurally different micropollutants found and the diverse water matrixes make difficult the prediction of ozonation behavior on the removal of a broad range of contaminants (Lee et al., 2013). However, the study from Lee et al., (2013) showed that the knowledge of the chemical kinetics by inferring the contribution of molecular ozone and hydroxyl radicals as well as the wastewater characteristics (ozone dose per DOC - Dissolved Organic Carbon) allows to predict the elimination of specific micropollutants in the effluent. Thus, the combination of reaction kinetics and hydraulic of the reactor allows to satisfactorily predict the behavior of the full-scale reactor based on bench scale studies (Hollender et al., 2009).

Therefore, the data gathered in Table II.1.1. allow a first approach on designing ozonation reactors for the removal of specific contaminants (S) following the kinetic model proposed by von Gunten (2003a) describing a second order reaction between the pollutant and ozone or hydroxyl radicals (Equation II.1.1).

$$-\frac{d[S]}{dt} = k_{O_3}[O_3][S] + k_{HO^\bullet}[HO^\bullet][S] \qquad \text{(Equation II.1.1.)}$$

The hydroxyl radicals concentration ($[HO^\bullet]$), that is not easily measured, can be related with ozone concentration ($[O_3]$) through R_{ct} proposed by Elovitz and von Gunten (1999). This parameter is constant for a wide range of reactions and can be defined as the ratio between $\frac{[HO^\bullet]}{[O_3]}$. R_{ct} is usually in the range $10^{-9} – 10^{-7}$, but, if advanced oxidation conditions are applied (for example by combining ozone with hydrogen peroxide) it can be over 10^{-7} (von Gunten, 2003a). R_{ct} depends also on water characteristics such as temperature, pH, alkalinity and dissolved organic matter (Elovitz et al., 2000) that must be bear in mind while modelling the ozonation system.

Table II.1.1. Second-order kinetic constants for some emerging contaminants reaction with ozone (k_{O_3}) and hydroxyl radicals ($k_{HO\bullet}$). Otherwise stated, the data were gathered at pH 7 and T = 20 – 25ºC)

	Compound	k_{O_3} **(M^{-1}.s^{-1})**	**Ref.**	$k_{HO\bullet}$ **(M^{-1}.s^{-1})**	**Ref.**
Lipid Lowering	Bezafibrate	590	Huber et al. (2003)	7.4×10^{9}	Huber et al. (2003)
	Fenofibric acid	3.43	Rosal et al. (2010b)	6.55×10^{9}	Rosal et al. (2010b)
	Gemfibrozil	5×10^{4}	Lee and von Gunten (2012)	1.0×10^{10}	Razavi et al. (2009)
Ansiolitic/Antidepressant	Oxazepam	~1	Lee et al. (2014)	9.1×10^{9}	Lee et al. (2014)
	Diazepam	0.75	Huber et al. (2003)	7.2×10^{9}	Huber et al. (2003)
	Venlafaxine	-	-	8.46×10^{9}	Abdelmelek et al. (2011)
	Meprobamate	< 1	Lee and von Gunten, (2012)	4×10^{9}	Lee and von Gunten (2012)
Anti-Epileptic	Gabapentin	2.2×10^{4}	Lee et al. (2014)	9.1×10^{9}	Lee et al. (2014)
	Carbamazepine	3×10^{5}	Huber et al. (2003)	8.8×10^{9}	Huber et al. (2003)
	Phenytoin	< 10	Lee and von Gunten (2012)	5×10^{9}	Yuan et al. (2009)
	Primidone	1	Real et al. (2009)	7×10^{9}	Lee and von Gunten, 2012
	Levetiracetam	< 1	Lee et al. (2014)	3.8×10^{9}	Lee et al. (2014)
Anti-Inflammatory	Diclofenac	~ 1×10^{6}	Huber et al. (2003)	7.5×10^{9}	Huber et al. (2003)
	Ibuprofen	9.1	Huber et al. (2003)	7.4×10^{9}	Huber et al. (2003)
	Naproxen	2×10^{5}	Huber et al. (2005)	9.6×10^{9}	Packer et al. (2003)
	Mefenamic acid	6.4×10^{6}	Lee et al. (2014)	10^{10}	Lee et al. (2014)
	Ketorolac	4.4×10^{5} (pH 2)	Rivas et al. (2011)	-	-
Pesticides	Clorofibric acid	< 20	Huber et al. (2005)	4.7×10^{9}	Packer et al. (2003)
	Atrazine	6	Acero et al. (2000)	3×10^{9}	Acero et al. (2000)
	Alachlor	3.8	Yao and Haag (1991)	7×10^{9}	Haag and Yao (1992)
	Carbofuran	620	Yao and Haag (1991)	7×10^{9}	Haag and Yao (1992)
	Endrin	< 0.02	Yao and Haag (1991)	1×10^{9}	Haag and Yao (1992)
	Methoxychlor	270	Yao and Haag (1991)	2×10^{10}	Haag and Yao (1992)
Steroidal estrogen	17α-ethinylestradiol	3×10^{6}	Huber et al. (2003)	9.8×10^{9}	Huber et al. (2003)
Contrast agent	Iopromide	< 0.8	Huber et al. (2003)	3.3×10^{9}	Huber et al.(2003)
	Iohexol	-	-	3.21×10^{9}	Jeong et al. (2010)
Antibiotics	Sulfamethoxazole	2.6×10^{6}	Huber et al. (2003)	5.5×10^{9}	Mezyk et al. (2007)
	Roxithromycin	7×10^{4}	Huber et al. (2003)	-	-
	Erytromycin	-	-	3.00×10^{9}	Abdelmelek et al. (2011)
	Clarithromycin	4×10^{4}	Huber et al. (2003)	7.5×10^{9}	Huber et al. (2003)
	Sulfapyridine	2×10^{5}	Lee et al. (2014)	8.2×10^{9}	Lee et al. (2014)

Table II.1.1. Second-order kinetic constants for some emerging contaminants reaction with ozone (k_{O_3}) and hydroxyl radicals ($k_{HO\bullet}$). Otherwise stated, the data were gathered at pH 7 and T = 20 – 25ºC) (Cont.)

	Compound	k_{O_3} (M^{-1}.s^{-1})	**Ref.**	$k_{HO\bullet}$ (M^{-1}.s^{-1})	**Ref.**
	Nalidixic acid	-	-	6.74×10^{9}	Abdelmelek et al. (2011)
	Ofloxacin	-	-	7.66×10^{9}	Santoke et al. (2009)
	Trimethoprim	3×10^{5}	Lee and von Gunten (2012)	8.92×10^{9}	Abdelmelek et al. (2011)
	Flumequine	6.4 (pH 2)	Rivas et al. (2011)		
Beta Blockers	Atenolol	1.7×10^{3}	Benner et al. (2008)	7.05×10^{9}	Song et al. (2008)
	Metoprolol	2.0×10^{3}	Song et al. (2008)	8.39×10^{9}	Song et al. (2008)
	Acebutolol	1.9×10^{3}	Benner et al. (2008)	4.6×10^{9}	Benner et al. (2008)
	Propranolol	1×10^{5}	Benner et al. (2008)	1.0×10^{10}	Benner et al. (2008)
	1-Phenoxy-2-Propanol	320	Benner et al. (2008)	-	-
	4-Methoxy-1-Naphtalene Sulfonic acid	3600	Benner et al. (2008)	-	-
	Sotalol	1.4×10^{4}	Lee et al. (2014)	10^{10}	Lee et al. (2014)
	Valsantan	38	Lee et al. (2014)	6.7×10^{9}	Lee et al. (2014)
Others	Caffeine (stimulant)	2.5×10^{3} (pH 2)	Rivas et al. (2011)	8.5×10^{9}	Vinchurkar et al. (1999)
	DEET (insecticide)	< 10	Gerrity et al. (2012)	4.95×10^{9}	Song et al. (2009)
	Bisphenol A	7×10^{5}	Deborde et al. (2005)	1×10^{10}	Deborde et al. (2005)
	Triclosan (Anti-Septic)	4×10^{7}	Suarez et al. (2007)	1×10^{10}	Lee and von Gunten (2012)
	Musk ketone (Fragrance)	< 1	Lee and von Gunten (2012)	2×10^{8}	Lee and von Gunten (2012)
	TCEP (Flame retardant)	< 1	Lee and von Gunten (2012)	6×10^{8}	Watts and Linden (2009)
	Furosemide (Diuretic)	6.8×10^{4}	Lee et al. (2014)	10^{10}	Lee et al. (2014)
	Hydrochlorothiazide (diuretic)	1.64×10^{4} (pH 9)	Real et al. (2010)	5.7×10^{9}	Real et al. (2010)
	Acesulfame (Sweetener)	88	Kaiser et al. (2013)	4.55×10^{9}	Kaiser et al. (2013)
	Lidocaine (Anesthetic agent)	7.3×10^{4}	Lee et al. (2014)	10^{10}	Lee et al. (2014)
	Tramadol (Analgesic)	4×10^{3}	Zimmermann et al. (2012)	6.3×10^{9}	Zimmermann et al. (2012)
	Fluconazole (Antimicotic)	2	Lee et al. (2014)	4.4×10^{9}	Lee et al. (2014)
	Benzotriazole (antifreezer)	20	Leitner and Roshani (2010)	6.2×10^{9} (pH 10.2)	Leitner and Roshani (2010)
	Antypirine (Analgesic)	6.15×10^{5} (pH 2)	Rivas et al. (2011)	-	-
	Acetominophen (Pain reliever)	2.5×10^{5} (pH 2)	Rivas et al. (2011)	-	-
	Methylparaben	2.5×10^{5} (pH 6)	Tay et al. (2010)	6.8×10^{9}	Tay et al. (2010)
	Ethylparaben	3.4×10^{5} (pH 6)	Tay et al. (2010)	7.7×10^{9}	Tay et al. (2010)
	Propylparaben	4.1×10^{5} (pH 6)	Tay et al. (2010)	8.6×10^{9}	Tay et al. (2010)
	Butylparaben	4.4×10^{5} (pH 6)	Tay et al. (2010)	9.2×10^{9}	Tay et al. (2010)

The contamination of natural water streams due to the discharge of MWWTP is of concern as these streams feed water supply systems. Hua et al., (2006) detected the presence of carbamazepine (antiepileptic), caffeine (stimulant), cotinine (metabolite of nicotine) and atrazine (herbicide) in water coming from a drinking water treatment plant which is fed by Detroit River (Canada). The traditional treatment system encompassing flocculation/coagulation and dual media sand filtration was unable to reduce those compounds concentration. The introduction of an ozonation step before filtration removed them with efficiencies ranging from 66 – 100%. Moreover, this process was able to maintain efficiency whichever the seasonal fluctuations of the inlet water composition.

Also, adsorption using AC and reverse osmosis (RO) are usually reported as suitable technologies for pharmaceutical compounds removal from water due to the high removal efficiencies obtained (for example: Heberer, 2002; Ternes et al., 2002) and are considered competitors with ozonation in this field. However, other considerations must be taken into account while selecting a treatment technology such as operating costs, complexity (and cost) of the equipment required, as well as the wastes generated during the process. Bearing in mind this, although efficient, filtration technologies can be extremely costly when compared with the alternatives (Joss et al., 2008). A deeper comparison between these technologies is given in section II.1.4. The dose of ozone required depends on the target pollutants as well as on the water characteristics.

Ternes et al., (2002) verified that 0.5 mgO_3/L reduced up to 90% of diclofenac and carbamazepine while 1.5 mgO_3/L were required for 50% of degradation of bezafibrate. On the other hand, clofibric acid showed to be stable even when applying 3.0 mgO_3/L. However, ozonation is generally able to remove micropollutants while also inactivate bacteria and viruses at low ozone doses such as 0.5 gO_3/g DOC, which corresponds to 2.6 mgO_3/L (Lee et al., 2014). The degradation of the pollutants during this process is due to direct molecular O_3 attack but also by hydroxyl radicals ($HO^\bullet$). While ozone reactions can be described by kinetic constants from 10^3 – 10^7 $M^{-1}.s^{-1}$ at pH 7 (Table II.1.1.), $HO^\bullet$ is less selective and thus higher kinetic constants (10^9 – 10^{10} $M^{-1}.s^{-1}$) are possible (Buxton et al., 1988). The effluent characteristics and operating conditions dictate the relevance of each pathway (Elovitz et al., 2000). However, the knowledge of the specific ozone dose (gO_3/g DOC) and the kinetic constants for ozone and hydroxyl radicals reactions allow to predict the efficiency of ozonation during municipal wastewater treatment (Lee et al., 2014).

The difficulty of the biological MWWTP systems on removing PPCPs is clearly patent on the work from Rosal et al., (2010a). These authors detected over seventy PPCPs in wastewater samples taken in a MWWTP near Madrid. Among them, paraxanthine, caffeine and acetaminophen were found at a higher concentration. While the conventional biological treatment of the MWWTP was able to remove only below 20% of most of the PPCPs, ozonation with ozone doses lower than 3.6 mgO_3/L permitted the removal of most of the compounds including those refractories to biological degradation.

Nevertheless, it is understood that there are certain chemical species that can affect the efficiency of micropollutants reaction with ozone. Among them is nitrite that acts as a radical scavenger (Lee and von Gunten, 2010) inhibiting ozonation efficiency. On the other hand, there are species that become more dangerous when in contact with ozone. The formation of bromate is worrying in those waters in which Br^- is present (Zhang et al., 2008). In fact, ozone may lead to the formation of brominated by-products such as BrO_3^- and organic brominated compounds with well-known carcinogenic characteristics. For example, for BrO_3^-, the world health organization (WHO) recommends values below 10 µg/L in drinking water (Zhang et al., 2008). Thus, an appropriate control of the operating parameters such as the amount of dissolved ozone is required to minimize the production of such compounds during water decontamination. Zhang et al., (2008) verified that the appearance of 0.1 mg/L dissolved ozone (corresponding to 0.4 mg of ozone consumed per mg of initial DOC) was able to satisfactorily treat the primary effluent as well as controlling bromine by-products formation.

Although ozonation is usually very reactive with high electronic density sites of high molecular weight organic contaminants, their decomposition leads to the formation of low molecular by-products with stronger refractory behaviour towards further ozone oxidation or towards radical degradation. Thus, even if the initial pollutants can be totally depleted by single ozonation, mineralization is usually low. For example, Dantas et al., (2007) analysed the degradation of bezafibrate using an ozone inlet gas concentration of 40,000 mg/L. Even if the initial compound was totally removed only 20% of mineralization was reached. Similar conclusions were attained when sulfamethoxazole was used as target molecule (Dantas et al., 2008). This may be a drawback of the application of ozonation for water reclamation since the obtained products may present stronger toxic features than the initial pollutants.

II.1.2. Strategies to improve ozone action for water reclamation

Even if most of the micro-contaminants are still not regulated and their impact over human health and ecosystems is not clarified, the precautionary principle imposes that they should be removed from water. Thus, the toxicity of the treated water should be monitored which implies the development of automatic tools for toxicity assessment involving several species for a broaden analysis. In middle to long term perspective, forecasting stronger water shortening problems allied with increasing social concern regarding the effect of such pollutants which will surely lead to tighter water related legislation, it makes sense to search for more efficient treatment alternatives. In this context, research efforts can be focused on improving ozone action over pollutants aiming higher mineralization. This may involve, for example, promoting hydroxyl radicals production through the use of hydrogen peroxide or light. Moreover, the application of catalysts can be envisaged as an alternative to improve mineralization. The integration of ozone-based systems with other treatments is also subject of intense study.

The use of catalysts can be an interesting approach to enhance ozone action over pollutants (Kasprzyk-Hordern et al., 2003) by improving mineralization. Especial interest is given to the heterogeneous materials that allow an easy separation from the liquid and reuse. Heterogeneous catalytic ozonation can follow 3 different pathways (Nawrocki and Kasprzyk-Hordern, 2010) ozone adsorption on the catalytic surface leading to the production of hydroxyl radicals that will decompose organic compounds in the liquid bulk; adsorption of organic compounds on the catalyst followed by oxidation by dissolved molecular ozone or adsorption of both ozone and organic pollutants with subsequent surface reaction.

Table II.1.2. overviews the different materials tested for catalytic ozonation of emerging contaminants and the efficiencies obtained. Among them, AC is clearly widely used in these studies. AC doped with ceria revealed to enhance the catalytic activity of this material for the ozonation of sulfamethoxazole (Gonçalves et al., 2013a), erythromycin (Gonçalves et al., 2014) and bezafibrate (Gonçalves et al., 2015). The presence of Fe_2O_3/CeO_2 adsorbents/catalysts supported in AC allowed attaining 80% of DOC removal of a solution of sulfamethoxazole (Akthar et al., 2011).

Table II.1.2. List of catalysts applied for the catalytic ozonation of water emerging contaminants and main results achieved.

	Catalyst	Contaminants	Removal efficiency	Reference
Carbon Based (AC)	Carbon Nanofibers (CNF)	Metolachlor	82% of metolachlor removal and 20% of TOC removal in 2min	Restivo et al. (2012)
		Bezafibrate	Total degradation of bezafibrate in 15min and TOC removal of 73% in 300min	Gonçalves et al. (2013b)
		Atrazine Bezafibrate Metolachlor Nonylphenol	Atrazine 75% of degradation and TOC removal of 12%; Metolachlor 78% of degradation and TOC removal of 35%; Nonylphenol 100% of degradation and TOC removal of 21%(contact time of 7.6min)	Restivo et al. (2013)
	Ce-doped AC	Sulfamethoxazole	SMX total degradation in 30min and 73% of TOC removal in 180min	Gonçalves et al. (2013a)
		Erythromycin	TOC removal of 38% in 300min	Gonçalves et al. (2014)
		Bezafibrate	Total degradation in 10 min and 77.1% of TOC removal in 300min	Gonçalves et al. (2015)
	N-CNF	Atrazine Metolachlor Nonylphenol	TOC removal: Atrazine(3%), Metolachlor(8%), Nonylphenol (7%).	Restivo et al. (2016)
	AC activated with NH_4Cl	Amoxicilin	Total degradation and 54.1% of mineralization in 50 min	Moussavi et al. (2015)
	P110 Hydraffin AC	17-α-Ethynylestradiol	90% of TOC removal in 120 min	Beltrán et al. (2009)
Mn based	MnOx/SBA - 15	Clofibric acid	Total degradation and 43.8% of TOC removal in 60 min	Sun et al. (2015)
		Atrazine Linuron	________________	Rosal et al. (2010c)
	MnO_2	Bisphenol A	90% of Bisphenol A degradation in 30 min	Tan et al. (2017)
Mg based	MgO	Acetaminophen	Total degradation in 15min and 94% of TOC removal in 30 min	Mashayekh-Salehi et al. (2017)
Ce based	Fe_2O_3/CeO_2 - AC	Sulfamethoxazole	Total degradation and 86% of TOC removal in 3 min	Akthar et al. (2011)
	Mn-Ce-O	Diclofenac Sulfamethoxazole	Total diclofenac and 95% SMX degradation in 30 min 63% of COD abatement in 120 min	Martins et al. (2015)
	Ce – Red Mud	Bezafibrate	Removal of 96% of Bezafibrate in 5 min (with best catalyst)	Xu et al. (2016)
Alumina Based	Cu/Al_2O_3	Benzotriazole	90% of mineralization in 15 min	Roshani et al. (2014)
	Mn/Al_2O_3	Benzotriazole	60% of mineralization in 15 min	Roshani et al. (2014)
	$Mn\text{-}Cu/Al_2O_3$	Benzotriazole	75% of mineralization in 15 min	Roshani et al. (2014)
	$MnOx/Al_2O_3$	Fenofibric acid	________________	Rosal et al. (2010b)
		Atrazine Linuron	________________	Rosal et al. (2010c)
	$^{\gamma}$-Al_2O_3	Diclofenac Sulfamethoxazole 17-α-Ethynylestradiol	Total degradation of compounds in 8 min and TOC removal of 40% in 60 min	Pocostales et al. (2011)
	Co_3O_4/Al_2O_3	Diclofenac Sulfamethoxazole 17-α-Ethynylestradiol	Total degradation of compounds in 8 min and TOC removal of 50% in 60 min	Pocostales et al. (2011)
	β-$FeOOH/Al_2O_3$	Ibuprofen Ciprofloxacin	Total removal of Ibuprofen in 9 min, and 90% of TOC removal in 40 min; Total removal of Ciprofloxacin in 6 min and 88% of TOC removal in 40 min.	Yang et al. (2010)

Table II.1.2. List of catalysts applied for the catalytic ozonation of water emerging contaminants and main results achieved. (Cont.)

	Catalyst	Contaminants	Removal efficiency	Reference
Other materials	Pd – FeOOH	Clofibric acid	81% of mineralization in 6h and total conversion of clofibric acid in < 15 min	Sable et al. (2015)
	ZSM – 5	Cumene 1,2 – dichlorobenzene 1,2,4 – trichlorobenzene Ibuprofen	100% removal of Cumene, 1,2 – dichlorobenzene, 1,2,4 – trichlorobenzene and 50% removal of Ibuprofen in 30 min (at pH 3)	Ikhlaq et al. (2014)
	Co-Fe_3O_4	2,4 – dichlorophenol 2,4,6 – trichlorophenol Phenazone	79% of Phenazone, 98% of 2,4 – dichlorophenol, 98% of 2,4,6 – trichlorophenol mineralized in 40 min	Lv et al. (2012)
	TiO_2	Naproxen Carbamazepine	TOC removal of 62% for naproxen and 73% for carbamazepine	Rosal et al. (2008a)

Restivo et al., (2012) tested carbon nanofibers (CNF) and multiwalled carbon nanotubes (MWCNT) for the catalytic ozonation of the herbicide metolachlor in semi-batch and continuous operation. While both single and catalytic ozonation were able to efficiently remove the initial contaminant (> 73%), mineralization was greatly improved when the catalyst was tested. In fact, even after 8h of single ozonation a large part of DOC was found in the sample. Meanwhile, catalytic ozonation was able to reach 20 and 35% of DOC degradation at the steady-state at contact times of 1.9 and 7.6 min, respectively. MWCNT also showed higher catalytic activity than AC during the catalytic ozonation of bezafibrate (Gonçalves et al., 2013b). Restivo et al., (2013) verified that carbon nanofibers supported on monoliths are able to improve mineralization of mixtures of atrazine, bezafibrate, erythromycin, metolachlor and nonylphenol even if no significant differences were found for parent compounds degradation when compared with single ozonation. Moreover, toxicity towards *A. fischeri* is also lower when catalytic ozonation treated water is tested. Although the catalyst showed good stability during long runs, an initial deactivation step was recorded and related with the generation of oxygen groups of the surface. The use of natural water as matrix reduced the catalytic activity in some experiments. This was related with the presence of some inorganic compounds with scavenging properties HCO_3^-, SO_4^{2-} and Ca^{2+}. Carbon nanofibers doped with N (N-CNF) promoted ozone production of radicals that enhanced atrazine, metolachlor and nonyphenol degradation (Restivo et al., 2016) leading to higher DOC removals when compared with single ozonation. The higher reactivity when N-CNF are used was attributed to the higher electronic density promoted by N groups. Moussavi et al. (2015) compared chemically activated carbon with NH_4Cl (NAC) with standard activated carbon (SAC) for the degradation of the antibiotic amoxicillin by ozone. NAC is characterized by a larger electronic density surface related with hydroxyl groups. Among the AC materials tested by Beltrán et al., (2009), P110 Hydraffin AC led to higher mineralization (90%) of a solution of 17-α-Ethynylestradiol by catalytic ozonation. Besides lowering ozone consume, this catalytic system also leads to lower mortality among *D. magna*.

Alumina and alumina supported materials show good catalytic activity on ozonation of both gas and aqueous contaminants (Einaga and Futamura, 2004; Yang et al., 2009). Cu, Mn and the mixed Mn-Cu supported in Al_2O_3 catalysts enhanced the mineralization of benzotriazole (Roshani et al., 2014). The highest mineralization attained was 90% in 15 min when Cu/Al_2O_3 was applied at neutral conditions when compared to the 46% of DOC removed by single ozonation. Mn-Cu/Al_2O_3 led to 77% of DOC removal in 30 min at alkaline medium. Mn/Al_2O_3 promoted the fastest ozone decomposition at pH 2 leading to 75% of mineralization in 10 min. Moreover, all the catalysts showed stability regarding metal leaching besides reusability capacity. It should be referred though that the type of buffer applied has a strong influence in catalytic activity. Phosphate can act as radical scavenger and may also coordinate with the catalyst active sites. A decrease on the catalytic activity when a phosphate buffer was applied was also detected by Martins et al. (2015) while testing a Mn-Ce-O catalyst. This can be an important drawback for catalytic ozonation when applied to real secondary effluents. However, Rosal et al., (2010b) verified a positive effect of MnO_x/Al_2O_3 over the hydroxyl radical/ ozone ratio during the ozonation of fenofibric acid either when using ultrapure water or spiked secondary municipal wastewater at the presence of inorganic compounds and radical scavenger species. Although γ-Al_2O_3 or Co_3O_4/Al_2O_3 had no significant impact over the degradation of diclofenac, sulfamethoxazole and 17α-ethylnylstradiol (that were removed either in pure water or secondary effluent in 10 min), DOC and COD degradation were much higher in catalytic ozonation than with single ozone specially when Co_3O_4/Al_2O_3 was applied (Pocostales et al., 2011). When a spiked secondary municipal wastewater was tested, a first quick COD depletion step was observed related with the degradation of the highly reactive pharmaceuticals with ozone followed by a slower stage which is due to the oxidation of refractory compounds dissolved in the effluent or by-products of the pharmaceuticals degradation. The higher mineralization degree achieved during the ozonation of ibuprofen and ciprofloxacin using β-FeOOH/Al_2O_3 was related with the strong Lewis acid sites that enable water chemisorption improving ozone interaction with the catalyst surface to produce reactive oxygen species (Yang et al., 2010).

The impregnation of 0.5% (w/w) of Pd in FeOOH enhanced the catalyst stability during the catalytic ozonation of clofibric acid (Sable et al., 2015). The material calcinated at 200 ºC (maghemite and hematite predominant phases) revealed the highest catalytic activity with up to 81% of mineralization in 6 h of reaction.

Cerium is known to be an important catalyst in catalytic ozonation due to its capacity of giving or withdrawing electrons (Martins and Quinta-Ferreira, 2009; Li et al., 2011). Xu et al., (2016) recently tested the use of cerium doped red mud as a low-cost catalyst for bezafibrate degradation. Red mud is a residue produced during the Bayer process. Several Ce sources and catalyst preparation methods were tested and it was concluded that Ce(IV) and the precipitation method led to the best catalytic activity. Mn-Ce-O showed interesting features on improving ozone action on the degradation of a mixture of diclofenac and sulfamethoxazole (Martins et al., 2015).

MnO_x/Al_2O_3 and MnO_x/SBA-15 improve the efficiency of hydroxyl radicals production in a fixed bed reactor for the decomposition of the herbicides atrazine and linuron (Rosal et al., 2010c). Moreover, these catalysts optimized ozone consume by lowering the amount of ozone per converted organic compound. The better catalytic activity observed for MnO_x/SBA-15 was attributed to its larger surface area and better MnO_x distribution. The application of MnOx/SBA-15 catalyst enhanced DOC removal during ozone decomposition of clofibric acid which was related with the formation of lower amounts of small molecular carboxylic acids due to the generation of hydroxyl radicals (Sun et al., 2015).

3D MnO_2 porous hollow microspheres enhanced bisphenol A ozonation due to their well-defined voids that promoted the contaminant adsorption and ozone retention (Tan et al., 2017). α-MnO_2 showed best results which was attributed to its richness in lattice oxygen which promotes the production of highly reactive oxygen species such as $O_2^{\bullet-}$ and $HO^{\bullet}$.

ZSM-5 zeolites showed good catalytic activity towards the ozone decomposition of cumene; 1,2-dichlorobenzene; 1,2,4-trichlorobenzene and ibuprofen (Ikhlaq et al., 2014). This efficiency was related with the high adsorption capacity of these materials specially those involving high SiO_2/Al_2O_3 ratios. Since the presence of radical scavenger species had no significant effect over the process, it was concluded that catalysis occurs due to the enhancement of molecular ozone over adsorbed organic species.

Recently, Mashayekh-Salehi et al., (2017) identified MgO nanoparticles as active and stable for the removal of acetaminophen from water by ozonation.

One of the drawbacks of using slurry catalytic ozonation systems is the recovery of the catalyst after treatment. The development of magnetic particles can sort out this shortcoming (Lv et al.,

2012) since a magnet can easily separate them from the fluid. Lv et al., (2012) prepared magnetic Co doped Fe_3O_4 for the catalytic ozonation of herbicides 2,4,6-trichlorophenoxyacetic acid, 2,4-dichlorophenol and 2,4,6 – trichlorophenol and the anti-inflammatory phenazone. The high DOC removal was related with the conversion of ozone into hydroxyl radicals at the catalyst surface which quickly reacted with the organic compounds in the liquid bulk. Contrarily, Rosal et al., (2008a) determined that the catalytic mechanism of TiO_2 on the decomposition of naproxen and carbamazepine is related with the adsorption of the reaction intermediates on Lewis acid sites and not with the production of hydroxyl radicals.

The use of catalytic materials shows interesting results on the detoxification of water. The mechanism behind the catalytic behaviour and its stability depends largely upon the type of catalyst and the water characteristics. The full-scale application though faces challenges such as the design and operation of multiphase reactors as well as catalysts stability and commercial availability (most of the test materials are lab produced) that is inhibiting the wide industrial spread of this technology.

When high mineralization levels are required and if stable catalysts are selected, catalytic ozonation can be a competitive methodology. In fact, the only drawback (when compared with single ozonation) is related with the cost of initial catalyst acquisition. Whereas, for example when adsorption on AC followed by disinfection is used, wasted AC must be regularly disposed or regenerated. To the best of our knowledge there is no data regarding the operating costs of catalytic ozonation systems for water reclamation. However, some results involving a commercially available catalyst (involving the TOCCATA ® process) reveal that the presence of catalyst enhances ozone mass transfer leading also to a lower dissolved ozone concentration and improving DOC removal (Crousier et al., 2016). Thus, depending on the effluent type, lower ozone doses can be necessary for the treatment (Fontanier et al., 2006) which may reduce the energy requirements of the ozone generator. Still further studies involving pilot scale units encompassing economic analysis are required.

The use of hydrogen peroxide as a co-oxidant in ozonation is usually pointed out as an interesting strategy to improve the process efficiency. In fact, H_2O_2 through reaction with ozone promotes the production of hydroxyl radicals. While three O_3 molecules are required for one $HO^{\bullet}$, aided by hydrogen peroxide, one molecule of ozone is able to produce one hydroxyl radical (Acero and von Gunten, 2001). This process is considered as both efficient and low cost (Beltrán et al., 1998).

Rosal et al., (2008b) analysed alkaline ozonation (pH ~ 8) and ozone aided by hydrogen peroxide processes on the treatment of a secondary municipal effluent. Up to 33 organic compounds (pharmaceuticals and metabolites) were found in a concentration range of 3 – 2100 ng/L. Whichever the process used, removal efficiency of the individual compounds was always higher than 99% after 5 min. Nevertheless, organic carbon depletion kinetic constants were much lower for alkaline ozonation than for O_3/H_2O_2. In fact, DOC decay was 15% for the former process after 1 h of reaction while most part of the depletion was in the first 5 min of reaction. When H_2O_2 was applied (pulses of 0.15 mL/ 5L every 5 min of 30% w/v H_2O_2) mineralization reached 90%. Combining both hydrogen peroxide injection ($H_2O_2:O_3$ molar ratio below 0.5) with pH >8 allowed complete mineralization after 1h (Rosal et al., 2009). This corresponded to ozone doses up to 150 mg/L which is a very high value but, according to the authors, the value is near those found for wastewater treatment. In a first step (first 5 – 15 min of reaction) DOC removal was fast, followed by a slower period (up to 20 times slower) related with the oxidation of more refractory compounds. Thus, this approach is suitable since, even if the initial PPCPs are easily removed whichever the ozone-based process applied, only mineralization can ensure that toxic intermediates are not formed during the oxidation.

However, care must be taken during the ozonation of water containing natural organic matter (NOM) or carbonate ions that, depending on their concentration and solution pH may promote or scavenge hydroxyl radicals reactions (Staehelin and Hoigne, 1985) and thus will directly influence the role of ozone during the treatment. The scavenging effect of the matrix can however be overcome by increasing the oxidant dosage (Zwiener and Frimmel, 2000). Zwiener and Frimmel (2000) achieved degradation values more than 98% of clofibric acid, ibuprofen and diclofenac using 5.0 mgO_3/L and 1.8 mgH_2O_2/L. Nevertheless, some ozone can be consumed by the presence of dissolved organic carbon in the matrix. Thus, for drinking water higher oxidants loads are recommended so that quantitative removal of the pharmaceutical compounds is really accomplished. But the increased operating costs with the reactants must be considered.

Saquib et al., (2010) verified that the O_3/H_2O_2 system is efficient for removing atrazine from water and thus with higher potential for the degradation of persistent pollutants from drinking water than single ozonation. Duguet et al. (1985) concluded that hydrogen peroxide improves both organics removal and ozone mass transfer yield while treating drinking water. Nevertheless, H_2O_2 concentration and injection point must be carefully selected. In fact, the costs of H_2O_2 must be considered. Besides, when in excess, these reactants act as radical scavengers reducing the process efficiency.

Atrazine is a widely used herbicide and its concentration in natural waters can be sometimes found above the legal limit (0.1 µg/L) (Beltrán et al., 2000). Beltrán et al., (2000) tested ozonation, O_3/H_2O_2 and O_3/UV for the degradation of this compound in surface waters. Among the tested processes O_3/UV (ozone dose 2.72 mg/L and 1.9×10^{-6} Einstein/(L.s) was the most efficient and the kinetic models that were established determined that atrazine was mainly degraded through hydroxyl radicals. It was concluded that the water matrix applied can have a key role on the degradation efficiency since some compounds may present initiating, promoting or inhibitory character over free radicals.

UVA photolysis is not satisfactory for the removal of emerging contaminants (Encinas et al., 2013). However, its combination with ozone and TiO_2 supported in AC is able to improve DOC removal. Even if some efficiency decrease was observed when the process was applied to a spiked secondary effluent, the presence of inorganic compounds in the water matrix only affected caffeine depletion. There is a synergetic effect between ozone and the irradiated photocatalyst. In fact, ozone presents a high electron trapping effect which avoids the electron-hole recombination. In this process, ozonide radicals ($O_3^{\bullet -}$) are produced which led then to the highly reactive radicals (Mena et al., 2012).

Beltrán et al., (2012) verified that photocatalytic ozonation over TiO_2 using UV lamps (maximum emission wavelengths of 254, 313, and 366 nm) promoted the highest DOC removal of a solution of diclofenac, sulfamethoxazole and caffeine both in ultrapure water and secondary wastewater while demanding a lower amount of ozone. Photocatalytic ozonation enhanced by Fe_3O_4 or Fe(III) using black light lamps removed up to 35% of DOC of a mixture of nine pharmaceuticals (acetaminophen, antipyrine, caffeine, carbamazepine, diclofenac, hydrochlorothiazide, ketorolac, metoprolol and sulfamethoxazole) spiked in primary municipal wastewater (initial COD and DOC of 228 and 56 mg/L, respectively). This is an interesting increasing level of mineralization when compared with the 13% achieved by single ozonation. Moreover, all the ozone-based processes led to total pharmaceuticals removal within 10 min. It should be still referred that when Fe(III) was used as photocatalyst, toxic effect over *D. magna* was detected for the treated wastewater which was attributed to the production of ferryoxalate. Diclofenac showed high reactivity when exposed to artificial light ($\lambda > 300$ nm) but amoxicillin was stable. The use of ozone was able to remove both compounds but it was necessary to combine ozone + TiO_2 + light to promote complete mineralization of the simulated wastewaters. However, when secondary effluent was applied, DOC removal did not reach 68% in 3h which was attributed to the formation of refractory low molecular weight carboxylic acids such as oxamic acid (Moreira et al., 2015). Moreira et al., (2016) tested TiO_2-coated glass

Raschig rings as photocatalysts in photocatalytic ozonation of urban wastewater and surface water using LEDs (dominant wavelength 382 nm) as light source in continuous mode. The photocatalytic ozonation process was more efficient than single or catalytic ozonation (over TiO_2) on the removal of organic contaminants. Only fluoxetine, clarithromycin and 17-α-ethinylestradiol were detected after treatment. Moreover, this system was also able to degrade different groups of microorganisms and antibiotic resistance related genes. However, according to the authors, the potential for regrowth must be still assessed since part of the treated wastewater microbiota was still viable. No estrogenic effect was detected for the compounds produced during the treatment.

The results so far published for photocatalytic ozonation regarding chemical and biological contaminants removal show that this technology may have potential for water recovery applications. This can be especially important when high quality reclaimed water is necessary. But still deeper economic viability analyses are required so that such technology can be widely used at real scale. Besides, a deep comparison with other strategies such as ozonation integrated with biofiltration must be performed to conclude about the competitiveness of this approach.

Solar photocatalytic oxidation of emerging contaminants is an interesting approach especially in countries with high number of sun light hours. However, the retention times required for an effective contamination removal is an important drawback for this technology. Its combination with ozone may be a solution for this shortcoming (Márquez et al., 2014). TiO_2/O_3/light revealed high oxidation potential for the detoxification of a mixture of atenolol, hydrochlorothiazide, ofloxacin and trimethoprim both in ultrapure water and secondary municipal effluent. This process was able to lead to 85% of mineralization and 90% of toxicity removal.

The most used photocatalyst (TiO_2) only absorbs 5% of solar radiation which corresponds to the UV range of the electromagnetic spectrum. This can be overcome by doping TiO_2 or modifying its surface with metal ions or coupling semiconductors (Malato et al., 2009). Rey et al., (2014) prepared TiO_2 photocatalysts coated with WO_3 and tested their catalytic activity on the photocatalytic ozonation of a mixture of ibuprofen, metropol and caffeine dissolved in real municipal secondary effluent. The presence of WO_3 promoted the catalyst response to visible light by enhancing the transfer of photogenerated electrons from the conduction band of TiO_2 to WO_3 and holes from the valence band of WO_3 to TiO_2 which avoids electron-hole recombination and increases the process efficiency. On the other hand, the catalytic activity was also improved when WO_3 dispersion and catalyst surface area were higher. WO_3-TiO_2 photocatalytic ozonation was able to remove up to 64% of DOC in 2 h.

Quiñones et al., (2015a,b) verified that solar photocatalytic ozonation (Fe(III)/O_3/light, Fe(III)/H_2O_2/O_3/light and TiO_2/O_3/light) slightly enhanced a mixture of six emerging compounds (acetaminophen, antipyrine, bisphenol A, caffeine, metoprolol and testosterone) mineralization (near 40% when compared with 35% for single ozonation) due to the enhancement of hydroxyl radicals production. Moreover, the solar photocatalytic ozonation treated effluent showed lower toxicity towards *D. magna*.

It should be highlighted that the use of solar photocatalytic systems avoids the energetic costs related with lamps. However, the design and scaling up of such reactors is not an easy task since it must be ensured that a good distribution of the sunlight is achieved bearing in mind the absorption and scattering effect of the catalyst particles (Spasiano et al., 2015). Besides, economical evaluation must be carefully assessed to guaranty that costs savings are really achieved when using solar light. While designing a solar photocatalytic system, one of the main capital constraints is the cost of the land since large illuminated areas are required (Spasiano et al., 2015). Also, material for the walls must be carefully selected. Pyrex glass is used for the reactors construction since this is the best cost-effective material due to its good transmittance (Blanco et al., 1999). Moreover, aluminium sheets must be used as reflectors. According to Ajona and Vidal (2000), the collector manufacturing costs constitute up to 60% of the total capital. While using heterogeneous catalysts, which usually are applied in suspended beds, the costs for liquid-solid separation (sedimentation or filtration) and catalyst replacement after deactivation must be considered.

Ultrasonication is able to thermally decompose water and organic compounds due to implosive collapse of bubbles in the liquid (Suslick, 1990). Moreover, it is able to improve mass-transfer and solid catalyst surface properties (Ince et al., 2001). In fact, 42 kHz ultrasound waves were able to enhance the photocatalytic oxidation of norflurazon (Sathishkumar et al., 2016). Thus, ultrasounds can improve catalytic ozonation action over pollutants. Ziylan and Ince (2015) tested sonocatalytic ozonation using Fe based materials for the degradation of ibuprofen. Single ozonation at pH 9 was able to totally remove the parent compound but with low mineralization (15%). The best results were obtained using sonocatalytic ozonation over zero valent iron – graphite at pH 3 with total ibuprofen removal and up to 58% of mineralization in 4h. It was verified that the ibuprofen reaction by-products react very slowly with molecular ozone and hydroxyl radicals are the main responsible for their degradation. It should be remarked that even after 4h of reaction only 58% of mineralization was achieved. This is not an impressive mark when considering the costs of sonication of large volumes of wastewater and the complexity of the reactor. Probably such procedure can be only competitive for the treatment of some specific industrial wastewaters with a composition requiring more sophisticated oxidation procedures and involving smaller flowstreams. The combination between ultrasound and ozonation was also able to reduce the fouling of the following membrane bioreactor due to the reduction of extracellular polymeric substances (Prado et al., 2017). However, the increased energy requirements make this approach not viable especially in developing countries.

Some EDCs and PPCPs are known by their hydrophobicity. Thus, in the traditional MWWTP, based on activated sludge reactors, they tend to accumulate in the bio-sludge (Bolz et al., 2001; Andersen et al., 2003). Some works reveal that up to 45 – 90% of EDCs entering in MWWTP can be adsorbed and accumulate in the sludge and will circulate along the treatment process (Zhang et al., 2008). Moreover, some sludge management strategies involve the application of the sludge in farmland which will promote the spreading of the contaminants through the soils and aquifers. If ozonation is applied as a refining treatment after the biological effluents processing it will be not able to avoid micropollutants deposition in the activated sludge. Thus, this chemical approach can be envisaged as a pre-treatment before activated sludge. This way it may promote persistent pollutants removal avoiding their adsorption in the sludge. The main drawback of such procedure is related with the large volumes of municipal wastewaters that must be processed. Ozone in situ generation is energy consuming and the operating costs can discourage the full-scale application of this methodology. However, the rising of health problems and the growing shortening on water with quality for human application will surely be a driving-force for changing decision making and stakeholders priorities forcing policy to put environmental interests over the economics. Although some authors refer that ozonation improves MWW biodegradability it should be considered that this wastewater is biodegradable and the removal of the PPCPs (that typically occur in these streams at so low concentrations) will not substantially affect wastewater biodegradability. Thus, the main goal of ozonation will surely not be further biodegradability enhancement and rather to remove those persistent and harmful chemicals that are not degraded through the biological processes.

When compared with alternative technologies such as RO and AC adsorption, ozonation presents as advantage the fact that contaminants are destroyed rather than concentrated or transferred to another phase. Moreover, RO and AC adsorption imply higher operating costs when compared with ozonation (Joss et al., 2008; Mousel et al., 2017). However, as it was identified before, ozonation is usually not able to totally mineralize the organic contaminants.

Thus, the toxicity of intermediates generated must be considered which usually is not an easy task. On the other hand, reclaimed water must go through a final disinfection step and there the production of halogenated nitrogenous disinfection by-products (N-DBPs) is a problem difficult to prevent by controlling operating conditions (Chu et al., 2012). The application of a further treatment step after ozonation to eliminate both ozone oxidation by-products and N-DBPs precursors is usually a good option. Biofiltration shows interesting features on removing those by-products compounds and thus it is very used in drinking water treatment (Onstad et al., 2008; Chu et al., 2012). Biofiltration is a safe/affordable post-treatment methodology since it implies low cost implementation and operation. In fact, several low-cost substrates can be used such as sand and some industrial results point out the interest in this integrated treatment approach (as it will be discussed later). For example, our spin-off company (Adventech http://www.adventech.pt/pt/empresa) that is devoted to the development, design and implementation of advanced wastewater treatments usually applies sand biofiltration as a final step of the treatment scheme since this is a very low-cost technology leading to a high-quality final effluent without the need of sophisticated equipment and control. Another possible integrated scheme could incorporate AC adsorption followed by a disinfection step. However, it should be considered that using AC the pollutants are only transferred from the liquid to the solid phase. Thus, further management strategies must me then applied to the wasted AC. This may incorporate AC disposal or regeneration through (for example) oxidation. Fenton's reagent (dark or light aided) and UV/H_2O_2 can also compete with ozonation on PPCPs removal for water reclamation (Prieto-Rodríguez et al., 2013; Yuan et al., 2015). Nevertheless, Fenton's reagent requires wastewater pH adjustments since it is known that the best results are obtained at pH 3. Moreover, homogeneous catalyst (generally iron salts) is needed which normally implies its separation after the treatment. In what concerns UV/H_2O_2 the preliminary energy consumption results detailed by Yuan et al., (2015) show that this process may lead to lower costs than ozonation. However, reactor design, operation and maintenance were not considered, and thus further studies must be performed to conclude about the best option.

Chu et al., (2012) tested BAC filters after ozonation at a pilot scale to improve the removal of the precursors of N-DBP's prior to a disinfection stage. By itself ozonation was not able to substantially reduce the formation of the precursors. In fact, some of them were even enhanced since ozone oxidation was able to improve the biodegradability of N-organic compounds. The subsequent BAC filter was able to reduce those compounds and a synergetic effect between ozonation and biofiltration was concluded on controlling the formation of DBPs.

The presence of a biological aerated biofilter (BAF) was able to reduce the toxicity (towards *A. fischeri* light emission) related with the presence of unknown intermediates produced during the oxidation of micropollutants by ozonation of a secondary effluent (Li et al., 2015). Due to the presence of a relatively high nitrogen concentration in the influent (TN ~25 – 30 mg/L), a denitrification biofilter (DNBF) was applied before ozonation. However, the accumulation of nitrite in this unit reduced ozonation performance as each mg of nitrite consumes 3.43 mgO_3 ($k = 3.7\times10^5\ M^{-1}.s^{-1}$). Thus, DNBF must be controlled to avoid accumulation of nitrite during the process. One way may consist in increasing carbon dosage in the DNBF which will increase the operation costs. Even if the sequence DNBF/ozonation/BAF was able to meet reclaimed water thresholds it is arguable the need of the DNBF first stage since. If not properly controlled, it can even reduce ozonation efficiency on the degradation of microcontaminants. If the final water nitrogen content is a problem, probably the final biofilter could be designed so that a denitrification zone could be considered.

Lee et al., (2012) directly compared the performance of ozonation followed by biofiltration with RO for removing PPCPs and micropollutants from a secondary effluent. The ozone reactor consisted in 3 columns with a retention time of 5 min each. After, air was bubbled to remove dissolved ozone before the wastewater being forwarded to the biofilter. This unit consisted in anthracite that was seed with the mixed liquor of the primary biological reactor used in the MWWTP. This integrated approach was able to remove up to 82% of the PPCPs and micropollutants using an ozone dose of 8 mg/L. This is a value very close to the one obtained by RO (90%). However other factors must be considered while comparing these technologies. Thus, even if a slightly higher efficiency is obtained with RO, if one takes into account energy consumption and waste generation, ozone + biofiltration can be more attractive than RO. In fact, while for the former energy consumption is within the range 0.042 – 0.084 kWh/m^3, the latter requires 0.29 – 0.41 kWh/m^3. Besides, it should be considered the need of frequent membrane backwash and replacement due to fouling in RO. Moreover, it must be considered the management of the retentate generated by RO (that corresponds to 15 – 25% of the feed water) that, due to the high concentration in PPCPs and micropollutants and to the higher salinity, is not easily disposed or treated. Contrarily, in ozonation+ biofiltration all the feed water is virtually recovered, without waste generation. Thus, this seems a better approach for water decontamination.

Water reclamation can be efficiently achieved through membrane processes such as RO and nanofiltration (NF) (Liu et al., 2014). These processes can remove organics, inorganics as well as biological contamination (Dialynas et al., 2008; Wintgens et al., 2008). Several units are now being applied to recover water especially from municipal wastewaters treatment plants for example for recharge water in water catchment sites (Benner et al., 2008). Nevertheless, some drawbacks can be referred such as the need of high pressure which entails high energy costs as referred before. Besides, membranes fouling will require suitable management strategies. Moreover, membrane separation generates a concentrate with high pollutant load with high environmental risk and that will require special attention. These streams are usually diluted in the environment (Westerhoff et al., 2009; Bagastyo et al., 2011) but with unknown impact over the ecosystems. In fact, the concentrate from a MWW will include micropollutants with high environmental risk. The application of a chemical oxidation process such as ozonation may be a logical option to manage this retentate (Ganiyu et al., 2015) as well as to improve the quality of the permeate (Acero et al., 2015). In fact, RO is able to transform 80-85% of the initial wastewater in pure water and only 15-20% in retentate containing the pollutants. Thus, a smaller flowstream must be chemically treated which apparently reduces the ozonation treatment prices (Westerhoff et al., 2009). However, it must be taken into account that the concentration of pollutants in the retentate is higher which may increase the ozone demand for total degradation of the contaminants. Moreover, the increased salinity may be a drawback reducing ozonation efficiency. It should be though referred the study from Miralles-Cuevas et al., (2017) that compared the application of ozonation aiming the reuse of a secondary effluent at a pilot scale in two situations: ozone was directly applied to the effluent and in the case where ozonation was used for the removal of microcontaminants from the retentate produced when the municipal wastewater goes through NF. The authors refer that the ozone dose to remove 90% of the microcontaminants was 50% lower (4.5 mgO_3/L) than the one required for the same goal when ozonation is applied directly to the secondary effluent. Nevertheless, the energetic costs associated with membrane separation systems as well as the problems related with membrane fouling, makes not clear the advantage of their integration with ozonation when compared with ozonation as a single step. Of course, a deeper economic viability analysis should be performed before the final decision.

Justo et al., (2013) evaluated the potential of ozonation on the minimization of the pollutant character of concentrates coming from water reclamation facilities based on RO. These streams were at that time discharged into coastal waters and 11 pharmaceuticals were detected in it. Molecular ozone was the main oxidant in the process and low mineralization levels were achieved with single ozonation. In fact, some micropollutants such as atenolol, diclofenac and carbamazepine show recalcitrant behaviour being necessary a high oxidant dose (1.38 mgO_3/mg DOC, corresponding to 38 mgO_3/L) for total removal of the emerging contaminants.

The combination of ozone with hydrogen peroxide (Westerhoff et al., 2009) was able to remove up to 75% of the dissolved organic carbon from the RO concentrate. However, very high ozone dosage was still required. Moreover, ozonation stripped CO_2 from the solution. This increased pH promoting precipitation of calcite and calcium organic compounds which can be an operating problem at the full-scale. However, this can be avoided by maintaining pH controlled.

Ozonation was also able to significantly reduce the organic content (between 60-80% of COD removal) of the rejects of NF and RO from a unit used for recovering water from a tannery effluent (Pophali et al., 2011). The ozone load varied from 2.4 – 3.4 gO_3/g COD (537 mgO_3/L) and 2.8 – 4.5 gO_3/g COD (348 mgO_3/L) for NF and RO retentates, respectively. These doses (as well as the one determined by Justo et al., (2013)) are much higher than the typical values found in literature when ozonation is applied to remove emerging contaminants from secondary effluents as a single step. These different values from those obtained for example by Miralles-Cuevas et al., (2017) point out the impact of the water characteristics over the process efficiency. That must be considered while designing a proper treatment scheme.

Benner et al., (2008) determined the second-order reaction constants for the reaction of beta blockers (Table II.1.1.) with ozone and hydroxyl radicals during the ozonation of brine from a water recovery facility from MWWTP. While kinetic constants between 10^3 – 10^5 $M^{-1}s^{-1}$were found for ozone, ~10^{10} $M^{-1}s^{-1}$ values were determined for $HO^{\bullet}$ with efficient degradation degrees which means that ozonation can efficiently remove these contaminants from RO brines. Moreover, the application of a chlorination step permitted to increase ozone stability while also improving disinfection without the formation of significant amounts of bromate (< 40 µg/L) pointing out the interest on this methodology as a complement of the existing water reclamation facilities. Contrarily, Saquib et al., (2010) verified that UF as a pre-treatment of water contaminated with atrazine had no important impact over O_3/H_2O_2 removal efficiency. But this must be due to the high efficiency of that specific oxidation process.

The combination between ozonation (ozone dosage of 6.0 mgO_3/L during 20 min) and biological activated carbon (BAC) filter (60 min of empty bed contact time) was able to remove 3 times more DOC from RO brine coming from a water reclamation facility processing secondary municipal effluent when compared with the case where ozonation was not applied (Lee et al., 2009). The treated effluent was then further treated in a capacitive deionization (CDI) process which was able to remove up to 80% of the ions. It was concluded that the application of ozone + BAC reduce the fouling problems in CDI and in the following RO process used for brine treatment and recovery. Although high quality water is for sure obtained using this integrated solution, the economic feasibility must be thoroughly assessed. Probably cheaper technologies, for example involving the direct application of ozonation to the secondary effluent (avoiding the RO step) followed by a biofiltration step to improve final water quality, would be enough for reaching reusable water at lower costs. However, the selection of the management strategy must involve economic considerations bearing in mind the water standards required for the recovered water application.

Thus, ozone is able to reduce the environmental impact of other water recovery technologies. Its integration with membrane processes can in fact lead to zero discharge of pollutants from MWWTP (Liu et al., 2014). Ozone is very efficient on the removal of antibiotics present in MWWTP secondary effluent concentrate coming from NF (Liu et al., 2014). NF was applied as advanced downstream of secondary treatment. Ozone action can be enhanced by $UV_{254\ nm}$ leading to a slight reduction of dissolved organic carbon (40%) but with interesting results regarding reduction of toxicity (58%).

Microfiltration (MF) is also applied for drinking water treatment. However, fouling can also be an important drawback for this system. Ozonation is also tested as a pre-treatment of membrane systems aiming to avoid this issue. Oh et al., (2007) analysed a hybrid approach involving ozonation and MF in order to evaluate the effect of ozone on reducing fouling related to the presence of pharmaceuticals in water. Pre-ozonated streams led to lower reduction of transmembrane pressure even if very low mineralization was achieved by ozone. Thus, it was concluded that pharmaceuticals and not their by-products are responsible for membrane fouling. Also, the direct exposure of fouled membrane to ozone was able to satisfactorily recover the membrane performance even when other cleaning processes were not efficient. The application of MF followed by ozonation was not so efficient on pharmaceuticals removal which was attributed to the rejection of some dissolved compounds that initiate ozone decomposition in hydroxyl radicals. Ozonation, as stand-alone process, was able to remove > 95% of pharmaceuticals but leading to less than 10% of mineralization. However, it is not clear the benefit in terms of DOC removal of the MF step. It should be though highlighted that the aim of the authors was to analyse the role of ozone in preventing/cleaning the MF membrane fouling. This hybrid methodology was also applied as pre-treatment before RO for water recovery from sea water (Oh et al., 2009). The effect of ozonation over hydrophilic (PHI) and hydrophobic (PHO) MF membranes fouling was distinct. While for PHI higher fouling was detected for the pre-ozonated seawater (due to the improvement on calcium complexation due to ozone), the increase on organics hydrophilicity and the decrease on their molecular size due to the ozone oxidation reduced fouling of PHO.

II.1.3. Applications at pilot and full scale

The ozonation industrial set-up is simple since, besides the ozone generator, only a bubble column for gas/liquid contact and an ozone depletion tank would be required (Joss et al., 2008). Thus, bearing in mind the interest on the application of ozonation as a tertiary treatment approach for removing microcontaminants for water reclamation, some works are reported in literature showing the performance of pilot and/or full-scale units. Table II.1.3. summarizes the main industrial treatment schemes involving ozonation found in literature for water treatment. Table II.1.3. shows the operating conditions, efficiencies and, when available, costs.

Table II.1.3. Summary of the main operating conditions and results found for industrial application of ozonation based processes for PPCPs removal from water.

Reference	Treatment Scheme	Operation Conditions	Ozone dose	Efficiencies	Energy and Costs
Gerrity et al. 2011	Secondary effluent – Sand filtration (SF) or Ultrafiltration (UF) - Ozone/ H_2O_2 – BAC.	pH:6.9; Alkalinity: 90 mg $CaCO_3$/L; BAC 1.4m bed depth; Residence time: 5min; 3.5mgH_2O_2./L.	5mgO_3/L	O_3/H_2O_2 + BAC $PPCPs_{Removal}$ = 95% (Except benzophenone). $TOC_{Removal}$ = 33%.	UF required higher costs compared to SF. SF require higher O_3 doses for suitable disinfection levels
Flyborg et al. 2010	1) NF at VRF 5,10, and 20; 2) NF of treated WW at VRF 60 and O_3; 3) O_3 treated WW and NF at VRF 60.	Membranes nominal area 7.9m^2; pH:6.1 to 6.9; T: 20.7-24.6°C.	5mgO_3/L	$PPCPs_{Removal}$: 1) VRF 5, 10, 20 were 39, 18 and 20%, respectively; 2) NF + O_3:99%; 3) O_3 + NF:99%;	____________
Margot et al. 2013	1)Ozone – SF; 2) PAC – UF 3) PAC – SF.	pH: 7.2 (±0.4); T: 17.1(±3.5)°C; DOC:7.3(±1.9)mg/ L; TSS:14.8(±5.3) mg/L;	5.7mgO_3/L (Average)	$PPCPs_{Removal}$: 1)More than 70%; 2)More than 70% (13mgPAC/ L and UF); $Toxicity_{Reduction}$: 1) 75%; 2) 84%(PAC-UF); $Estrogenic_{Reduction}$: 1) 89%; 2)77%(PAC-UF).	Electricity (kWh.m^{-3}): 1) 0.117; 2)0.9(15mgPAC/ L-UF); 3)0.08(15mgPAC/ L-SF); Total Cost (€.m^3): 1) 0.176; 2)0.803(15mgPA C/L-UF); 3)0.161(15mgPA C/L-SF).
Stalter et al. 2010b	Biological activated sludge: 1)Ozone – SF; 2) PAC – SF.	pH: 7.5; DOC:9.4 mg/ L; 20mgPAC/L;	6.58 mgO_3/L	Toxicity reduction was verified for five different species. Ozonation slightly increased the adverse effects on species but sand filtration reduce these effects.	____________
Reungoat et al. 2012	Denitrification-Pre-Ozonation-Dissolved air flotation and Sand filtration-Ozone and Biological activated carbon(BAC)	T: 22°C; pH: 6.7; DOC:6.5-8.1 mg/L; BAC: Residence time 18min	Pre-ozonation: 2mg O_3/L; 3.9-6.5mg mgO_3/L;	$PPCPs_{removal}$: More than 90%(for a wide range); $Estrogenic_{Reduction}$: More than 95%; Non-Specific $Toxicity_{Reduction}$: up to 70%; $DOC_{removal}$: up to 50%.	____________

Table II.1.3. Summary of the main operating conditions and results found for industrial application of ozonation based processes for PPCPs removal from water. (Cont.)

Reference	Treatment Scheme	Operation Conditions	Ozone dose	Efficiencies	Energy and Costs
Slater et al. 2010a	Biological treatment-Ozonation-Sand Filtration	pH:8.3; DOC: 5.4-5.9 mg/L; Median discharge: 6190m^3/day;	2.2-5.9 mgO$_3$/L	Toxicity reduction verified with ozonation linked to sand filtration.	_____________
Zimmermann et al. 2011	Secondary effluent-Ozonation-Sand Filtration	pH: 7.0; T: 20 °C; DOC: 2.4-4.8 mg/L; TCC: 3.3-8.4x10^6 cells/mL.	0.8-5.2 mgO$_3$/L	PPCPs$_{removal}$: 100%(for higher ozone doses); TCC$_{reduction}$: 0.5-1.5 log units;	_____________
Hollender et al. 2009	Secondary effluent-Ozonation-Sand Filtration	pH: 7.0; T: 17 °C; DOC: 5.4 mg/L;	3.2mgO$_3$/L	PPCPs$_{removal}$: 100% (except compounds such as atenolol, benzotriazole(>85%))	Energy consumption: 0.035 kWh/m^3; 0.01-0.015 kWh/m^3 (O$_2$ production)
Nakada et al. 2007	Conventional activated sludge-Sand Filtration-Ozonation	Residence time:27min; DOC:3.7 mg/L; pH: 7	3mgO$_3$/L	PPCPs$_{removal}$: More than 80% (most of the target compounds)	_____________
Escher et al. 2009	Activated sludge-Ozonation-Sand Filtration	DOC:5.8mg/L; Fr:5000-5500 m^3/day; Residence time: 3-10min.	3mgO$_3$/L	Estrogenic$_{reduction}$: 99.5%; Non-Specific Toxicity$_{Reduction}$:up to 76%. Photosyntesis inhibition$_{reduction}$: 89%.	_____________
Huber et al. 2005	Conventional activated sludge and Ozonation	48 m^3/day Residence time:8-8.8min; pH:7 DOC: 7 mg/L; T:16 °C; SS: 15 mg/L.	0.5-5 mgO$_3$/L	PPCPs$_{removal}$:~100%(except iopromide (40%)); *E.coli*$_{inactivation}$: inefficient in the presence of higher SS (even with 5 mgO$_3$/L)	_____________
Zucker et al. 2015	Biofiltration-Ozonation-Soil aquifer treatment (SAT)	Fr: 120 m^3/day Residence time:6-7min (ozonation);5min (biofiltration);22 day (SAT) DOC: 10.2 mg/L.	10 mgO$_3$/L.	DOC$_{removal}$: 88%; PPCPs$_{removal}$:~100%(except iopromide (52%) and primidone(65%))	_____________

Escher et al., (2009) analyzed during three consecutive years the performance of an ozonation tank (36.1 m^3 working as a plug flow with a residence time of 3 – 20 min) on the treatment of secondary effluent coming from activated sludge. The wastewater treatment plant was in Zurich serving a population equivalent to 25000 people. The treated water (5000 to 5500 m^3 per day) is discharged in a creek corresponding to 50% of the total water flux, thus a small dilution is observed which increases the problematic of the presence of emerging contaminants at the outlet of the treatment plant. The authors specially addressed the toxic character of the treated water. In fact, as ozonation is not able to totally mineralize the organic compounds, some of the by-products produced can present even higher toxic features when compared with the initial compounds. It was assessed that the treatment efficiency was improved with the increasing ozone dose until 0.5 gO_3/g DOC (2.5 mgO_3/L), after this it became constant. Ozonation was able to reduce up to 76% of bioluminescence *Aliivibrio fischeri* and also growth inhibition of *Pseudokirchneriella subcapitata*. The reduction was even larger concerning the inhibition of photosystem II in algae as well as estrogenicity. The presence of suspended solids (SS) showed negligible impact on ozonation efficiency for pharmaceuticals removal in a pilot scale ozone treatment plant operating with municipal wastewaters with different SS loads (Huber et al., 2005). Nevertheless, that parameter may be important for disinfection as inactivation of *E. Coli* did not fulfill the standards when high SS concentrations were applied even for a strong ozone dose (5 mg/L) probably due to some shielding effect of the SS.

The main drawback associated with the application of ozonation as a tertiary treatment for water reuse is related with the large volumes of wastewater that are processed in the municipal wastewater treatment plants. For very high wastewater flows, the costs associated with ozone generation can be prohibitive. However, volume reduction factors (VRF) reached by NF may lead to reasonable volumes to handle through ozonation. Flyborg et al., (2010) analyzed a pilot plant involving NF and ozonation for water recovery using as feed secondary effluent from Henriksdals (Sweden) MWWTP. With a VRF of 60, only NF was not very effective for pharmaceutical compounds removal. However, applying ozonation to the NF permeate flow the sum of both processes was able to remove up to 99% of the compounds. In fact, only oxazepam was detected after ozonation but even this substance had a reduction of 94%. Thus, NF is only suitable for water recovery if aided by ozonation.

Ozone action can be enhanced by the presence of hydrogen peroxide (as already referred). Gerrity et al., (2011) analyzed a pilot scale O_3/H_2O_2 unit at the Reno-stead water reclamation facility during 5 months of operation. The aim was to reach a suitable alternative for indirect potable reuse (IPR). The effluent was pre-treated in a conventional activated sludge reactor and O_3/H_2O_2 was applied after UF or sand filtration. Ozone was dosed as 0.8 gO_3/gDOC (5.8 mgO_3/L) while hydrogen peroxide was added at a molar ratio of 1.0 ($molH_2O_2$:$molO_3$) and the reaction took place in 5 min. While O_3/H_2O_2 was very suitable on removing trace organic contaminants, the total organic carbon abatement was very slight. In this context, care must be taken regarding the potential toxicity of the by-products. Besides, the possibility of formation of bromates and N-nitrosodimethylamine (NDMA) during ozonation must not be ruled out. However, the authors verified that the application of a BAC process effectively mitigated these issues. Moreover, this strategy enabled a significant disinfection since a large inactivation on total and fecal coliforms was observed after the oxidation step. However, some regrowth was observed in BAC. Still, the water satisfied the California Department of Public Health Title 22 requirements for recycled water. A detailed cost analysis is still needed to select whether UF or sand filtration is the most suitable pre-treatment before O_3/H_2O_2 + BAC. While UF has more investment and energetic costs, the use of sand filtration would require higher ozone doses to accomplish disinfection levels.

Dan region reclamation project is a big water reclamation project in Israel for water reuse from secondary municipal wastewater. It is able to produce 130 million m^3/year of water for irrigation (Zucker et al., 2015). It is based on soil aquifer treatment (SAT). However, the increasing DOC on the wastewater and the growing demand for reused water is increasing organic and ammonium load. Thus, the infiltration fields are becoming clogged and SAT efficiency is reduced. Moreover, some trace organic compounds (polar and non-biodegradable) are not removed by this method and are able to reach the environment. Ozonation was tested to improve the process using a reaction time of 6 – 7 min, ozone dosage of 1.0 - 1.2 mgO_3/mg DOC (10.2 – 12.2 mgO_3/L) for a water flow of 4.3 – 5 m^3/h. Biofiltration with indigenous population (the media was inoculated with sludge from the primary effluent) was used to remove particles and reduce the ozone demand. Moreover, this previous step was able to remove ammonium, nitrite and DOC. Nevertheless, it was quite inefficient regarding trace organic compounds abatement (only acesulfame and iopromide were removed by 60% and 30%, respectively). On the other hand, the subsequent ozonation step led to low DOC abatement but strongly removed the aromaticity of the effluent (measured by the ratio between absorbance at 254 nm and DOC). Moreover, the trace organic compounds were abated depending upon their characteristics. While diclofenac, sulfamethoxazole, carbamazepine and venlafaxine were efficiently removed, iopromide and primidone (which are known to be less prone to reaction with ozone) were only abated in 52% and 65%, respectively. The application of these pre-treatment steps reduced the SAT hydraulic retention times and the clogging. Besides, ozone removed satisfactorily trace organic compounds and pathogens from reclaimed water.

Hollender et al., (2009) concluded that biological sand filtration is able to remove biodegradable compounds formed during ozonation such as the carcinogenic NDMA. A full-scale ozonation reactor for the elimination of micropollutants (using 0.47 – 0.6 gO_3/g DOC which corresponds to 2.4 – 3.0 mgO_3/L) required about 12% of energy (0.035 kWh/m^3) of a typical WWTP with an average feeding of 5 g DOC/m^3. To this it must be added the energy costs for pure oxygen production from air (0.01 – 0.015 kWh/m^3). Sand filtration by itself is inefficient on removing pharmaceutical compounds due to their low hydrophobicities. However, Nakada et al., (2007) verified that sand filtration followed by ozonation globally led to more than 80% of removal of

24 selected pharmaceutical active compounds in a MWWTP in Tokyo. It was verified that, while compounds encompassing carbon- carbon double bounds or aromatic rings were very susceptible to ozonation, amide groups were refractory to ozone oxidation. Also, Zimmermann et al. (2011) verified that the integration between ozonation and sand filtration led to high disinfection levels based on both total cell counts (TCC) and *E. coli*. However, 2.5 log units of TCC regrowth was still observed while no *E. coli* regrowth was detected at the end of the treatment. The authors verified that the lab-scale results regarding micropollutants and disinfection obtained were overestimated when compared with the full-scale operation which was related with the shielding effect of colloids present in the real system. Care must be taken when scaling up the lab results obtained for full scale application. Thus, pilot unit analysis may be recommended.

Ozonation used as a single process for the removal of micropollutants during water reclamation presents as drawback the partial oxidation usually reached that may lead to toxic by-products. Stalter et al., (2010a) analysed the behaviour of a full-scale MWWTP (operating with a median discharge of 6190 m^3/day and with an ozone dose of 0.4 - 1 mgO_3/mg DOC corresponding 2.3 – 5.7 mgO_3/L) and concluded that some development retardation on *Oncorhynchus mykiss* individuals occurred when exposed to water samples from ozonation reactor even if estrogenic activity was significantly removed. This behavior must be related with the partial degradation of the contaminants during ozone oxidation. The adverse effect was though removed after sand filtration since most of the ozonation intermediates are biodegradable and/or adsorbable. Thus, sand filtration seems to be an easy to handle inexpensive procedure to increase ozonation efficiency. Nevertheless, the authors refer that further studies should be performed to address the efficiency of sand filtration on ozone by-products removal.

Reungoat et al., (2012) compared the performance of ozonation followed by BAC in three Australian wastewater reclamation plants. Before ozonation, the trace organic compounds concentrations were in the same order of magnitude for all plants which means that they are omnipresent in all treated effluents. The treatment plants operated with different ozone doses (0.2 -0.3; 0.4-0.5 and 0.6-0.8 mgO_3/mg DOC corresponding to 2 – 5 mgO_3/L) and empty bed contact times in BAC filter (18; 9 and 45 min). While ozonation was able to satisfactorily remove trace organic compounds and reduce both non-specific toxicity (31-39%) and estrogenicity (up to 87%), mineralization was quite low (<10%). The biodegradation promoted in BAC filtration was able to remove between 20 – 50% of DOC while the trace contaminants concentration was reduced (globally considering ozonation and BAC) to up to 99%. This integrated configuration was able to abate 70% and 95% of non-specific toxicity and estrogenicity, respectively. Thus, ozonation is able to degrade most of the trace compounds present in the wastewater. However, when high mineralization levels are required ozonation by itself is not the best solution due to the low DOC removals that are generally reached. As referred before, biofiltration (such as BAC) seems to be an easy to implement and low-cost refining step to abate potential by-products produced during ozonation leading to a higher quality reclaimed water. Also, this integration shows interesting results in what regards microorganisms elimination (Lüddeke et al., 2015; Reaume et al., 2015). However, the performance of such methodology can be affected by water characteristics such as alkalinity, ionic strength, high organic loads and turbidity (Zanacic et al., 2016). Thus, the ozone dose must be adjusted bearing in mind all these parameters that must be regularly monitored.

II.1.4. Comparison between ozonation, reverse osmosis and powdered activated carbon for water reclamation process

Membrane filtration and powdered activated carbon (PAC) are competitors of ozonation in water reclamation field bearing in mind the revised pilot/industrial scale results shown in section II.1.3. and the high removal efficiencies usually reported. Table II.1.4. compares the costs, advantages and disadvantages of these processes (Joss et al., 2008; Stalter et al., 2010b; Lee et al., 2012).

Table II.1.4. Comparison between ozonation, RO and PAC for water reclamation processes

Process	Costs (kWh/m^3)	Advantages	Disadvantages
Ozonation	0.04 - 0.30	- Up to 99% of water recovery; - No waste production.	- Possibility of toxic intermediates; - Safety procedures for ozone handling.
RO	0.29 – 0.41	- Reduction of wastewater volume.	- Membrane fouling; - Energy costs; - Concentrate difficult to handle
PAC	30% more expensive than ozonation	- No sophisticated equipment necessary.	- Contaminated PAC difficult to handle; - Possible release of contaminated PAC to the environment

Margot et al., (2013) analyzed during more than a year two alternative pilot plants for the removal of more than 70 emerging compounds of a secondary effluent from the MWWTP of Lausanne, Switzerland. A plug-flow ozonation reactor (129 m^3 with a reaction time of 20 min) followed by sand filtration was compared with PAC in a slurry tank (using Norit SAE SUPER^{TM}) followed by either sand filtration or UF. Both systems were able to remove more than 70% of the selected compounds (injected ozone dose 5.65 mgO$_3$/L and 13 mg/LPAC). However, PAC was able to remove a larger range of compounds while ozone is more specific.

Both systems satisfactorily reduce toxicity with stability for long term operation. Also, a comparative study was performed between ozonation (0.7 mgO_3/mg DOC corresponding to 5.65 mgO_3/L) and AC (20 mg/L) for the full-scale elimination of micropollutants after municipal wastewater conventional biological treatment (Stalter et al., 2010b). These two end of pipe techniques are regarded as the most suitable for this purpose. The authors refer interesting micropollutants removal/degradation for both systems. However, it is important also to infer about the potential effect of the exposure of aquatic species to the by-products produced during the treatment. For this chronic toxicity tests *in vivo* using several taxonomic species (higher plants - *Lemna minor*, annelids – *Lumbriculus variegatus*, insects- *Chironomid riparius*, gastrops – *Potamopyrgus* and bivalves – *Dreissena polymorpha*) were applied. *L. minor* and *C. riparius* were not sensitive to detect differences before and after water treatment (either by ozonation or AC). The production of by-products with toxic features during ozonation leads to decrease biomass and reproduction of *L. variegatus*. PAC treatment is more expensive than ozonation considering the cumulative energy demand (Joss et al., 2008; Mossel et al., 2017). Besides, contaminated PAC handling is not easy, and some can be released from the treatment tank which due to its high contamination is an environmental problem. To avoid it reaching the MWWTP outflow, it is likely that sand filtration is not enough and thus membrane filtration would be required increasing drastically the energetic and technical needs. Moreover, wasted PAC must be regularly regenerated (for example by oxidation) or disposed.

Membrane filtration such as RO permits the reduction of the wastewater volume as well as high micropollutants retentions. However, the energetic costs of the operation as well as the problems related with membrane fouling are important drawbacks. Moreover, it should be considered that a concentrate stream with high pollutant load and salinity is produced which is difficult to handle. Moreover, the high energy and equipment requirements make this approach not very interesting when compared with the alternatives (Joss et al., 2008).

Ozonation seems to be a less energy demanding treatment per m^3 of treated water. Nevertheless, these costs are dependent of ozone dose (Mousel et al., 2017). Moreover, it permits up to 99% of water recovery with no waste production. In addition, ozonation is easier to adapt when changes occur on the characteristics of the inlet wastewater. However, the possibility of the production of toxic intermediates due to the low mineralization achieved can be a drawback. But the integration of a biofiltration step involving low operation costs may overcome this shortcoming if necessary. Still, the safety procedures required for ozone handling must be considered due to its toxicity. In fact, specialized personal is required for the operation of an ozonation plant.

It should be stressed, however, that the bench scale results found in literature show that UV/H_2O_2 is also an interesting alternative for PPCPs removal from water (Justo et al., 2013; Yuan et al., 2015; Shu et al., 2016). The results reported by Yuan et al., (2015) show that UV/H_2O_2 demands lower energy costs (0.279 €/m^3) than ozonation (0.344 €/m^3). However, other considerations must be still addressed for the selection of the technology such as the reactor design as well as other operation and maintenance costs. This will imply further pilot/industrial scale tests.

II.1.5. Summary and future perspectives

The relevance of water for life on Earth is pushing both academics and stakeholders towards the development of suitable methodologies able to safeguard the natural water sources as well as to find suitable alternatives for water supply. Wastewater reclamation and reuse covers both objectives. Ozonation, operating at ambient conditions of pressure and temperature appears as a suitable alternative as a tertiary process able to remove emerging contaminants that threat both ecosystems and human health. Besides, since this process gathers both the oxidation potential of molecular ozone with the unselective reactivity of hydroxyl radicals it is an interesting technology for water reclamation.

The low mineralization level usually reached by ozonation may lead to the accumulation of intermediates in the water. Some degradation by-products from chemical oxidation may be more toxic than the initial contaminants (Gao et al., 2016). A keystone for boosting the application of ozone-based technologies for water recovery from wastewater will imply the safeguarding of ecologic and human health impact of the reclaimed water. Thus, the potential toxic (acute and chronic) character of transformation by-products must be accurately assessed preferably using species from the several trophic levels of the food chain. In this sense, suitable bioanalytical tools must be applied aiming also their automation so that they can be used routinely (Macova et al., 2010). The monitoring of full-scale treatment units regarding disinfection and trace contaminants depletion is resources and time consuming. Differential UV_{254} and total fluorescence can be used as surrogate parameters which demand minimal equipment and cost (Gerrity et al., 2012).

The use of solid catalysts seems to enhance ozone action over pollutants and some interesting results are already found in literature. Particularly AC is being widely tested. However, it should be also pointed out the recent interest for low cost materials that will decrease the costs associated with the catalyst acquisition. However, literature still lacks from pilot scale studies involving these catalytic materials able to give reliable data regarding their long-term stability under water treatment conditions. In fact, it is necessary to clarify the effect of inorganic species over catalytic activity before proposing the full-scale application of such procedure.

The use of hydrogen peroxide and/or radiation aided ozonation enhances mineralization and is a good perspective for industrial application. The optimal dose of this oxidant must though be carefully selected due to the scavenging effect when in excess. Moreover, if solar light is applied the costs associated with the treatment may be reduced even if further economic studies are required. In this sense further studies must be developed to select an active and stable catalyst able to interact with a wider range of wavelengths. Additionally, the reactor design can be a challenge.

The integration of ozonation with other processes able to further refine the treated water by removing the potential toxic ozonation by-products seems to be now the best option. In fact, it is forecasted that a higher social concern regarding reused water safety will increase water legislation. Especially integration of ozonation with a biologically active filter such as sand filtration able to remove the biodegradable organic matter produced during the oxidation or using this filtration system to remove possible precursors of toxic intermediates before applying ozonation are interesting approaches. This system involves simple apparatus and low-cost maintenance. The challenge is now to optimize this integration by understanding the biofiltration mechanisms and the effect of such system in disinfection.

Another interesting scientific goal would be to improve ozone application to primary effluents to avoid emerging contaminants migration to the sludge in the following primary biological system which leads to their spreading along the process and constitutes another ecological concern.

Some bench scale results seem to point out that UV/H_2O_2 may be less energy demanding than ozonation for PPCPs removal from water. However, further pilot scale studies encompassing deeper economic analysis are required to select the best technology.

The present work revises the processes listed above showing this way the present status of the scientific research and industrial applications of the ozone based technologies for water reclamation whether in separated systems or coupled with other treatment techniques aiming at presenting a scientific look for the various approaches that literature is offering now to all those interested on implementing sound solutions for sustainable water reuse to overcome the serious threaten of freshwater scarcity free from harmful emerging contaminants.

II.2. Environmentally applications of invasive bivalves for water and wastewater decontamination

The potential of invasive bivalve species on the removal of contaminants from water and wastewater is now overviewed. This section is based in the publication: Gomes, J., Matos, A., Quinta-Ferreira, R., M., Martins, R.C. (2018) Environmentally applications of invasive bivalves for water and wastewater decontamination (Review), Science of the Total Environment, 630, 1016-1027.

II.2.1. Application of invasive bivalves for the removal of nutrients from water

The discharge of effluents into natural freshwater courses leads to an increase in their nutrients content (Antsulevich, 1994; Smith et al., 1999) promoting eutrophication. Water eutrophication leads to seasonal blooms of algae and cyanobacteria that promote the degradation of the ecosystems. Besides, some toxins may be produced during this process that may have adverse impacts to human health if the natural reservoir is used for supplying water for human applications (Cooke and Kennedy, 2001). In this context, methodologies must be developed for the recovery of eutrophic sites. The capacity of bivalves for removing suspended materials from the water column makes them interesting low costs solutions for the restoration of these sites (McLaughlan and Aldridge, 2013). Moreover, the used bivalves harvesting may promote nutrients recycling since the animals may be used as soil fertilizers, poultry feeding or, if presenting adequate quality, human consume (Jönsson and Holm, 2009). However, mollusks cultures also release nutrients especially when their activity is stimulated (such as at the spring and summer). The feces and pseudofeces are a source of N, P and carbon. Thus, an increased oxygen uptake rate may occur with consequent reduction of the nitrification rates (Nizzoli et al., 2011). Nevertheless, Holmer et al., (2015) study reveals that the benefits of using mussels for nutrients mitigation overcome the drawbacks. Moreover, their nutrient removal efficiency is higher in their early stage. Thus, the mussels are recommended to be harvested within their first year of production. Recently, Waajen et al., (2016) identified quagga mussel (*Dreissena rostriformis bugensis*) as a suitable controller for algal blooms in eutrophic ponds. However, further studies must be performed with long term experiments to infer about the possible impacts of this invasive species. In fact, the application of an invasive species must be careful to avoid further spreading. Thus, their use should be restricted to already invaded sites.

Song et al., (2014) proposed a floating-bed device encompassing water spinach (*Ipomoea aquatica*), Asian clam (*Corbicula fluminea*) and biofilm carrier for the recovery of eutrophic water. The direct contribution of the clam on the removal of nitrogen and phosphorus was less than 10%. However, *C. fluminea* was a key component for the efficiency of the process since the N and P fed and excreted by the clam were more bioavailable for water spinach and biofilm. Moreover, chlorophyll A was essentially removed by this mollusk.

Eutrophication due to fish farming can also be controlled through the application of freshwater bivalves. *Diplodon chilensis* with a high filtration rate (1.31 /h. ind) was able to significantly reduce chlorophyll A, phosphorous and nitrogen from salmon culture tanks (Soto and Mena, 1999).

A pilot project involving the application of triangle sail mussel (*Hyriopsis cumingi*), silver carp and submerged macrophytes was implemented to recover near shore zone of Gonghu bay (Xangai) from eutrophication (Gao et al., 2017). The mussels were able to graze phytoplankton and detritus leading to clear water. This stimulated the growth of submerged macrophytes which are essential to improve water quality. However, careful must be taken as the mussels spread fast. Thus, as it will be discussed latter, preventive measures must be taken to avoid their spreading into non-invaded sites. In this study, the mussels were engaged in nets at a biomass of 150 g/m^3. Pearl oyster (*Pinctada imbricata*) was also able to reduce the contamination of the waters of Port Stephens (Australia). The oysters removed up to 703 g metals, 7452 g of nitrogen and 545 g of phosphorus per ton of biomass (Gifford et al., 2005). The main problem of using pearl oyster harvesting for eutrophication removal may be the accumulation of toxic compounds in the edible parts of the oyster. However, it was detected that most of the metals accumulation does not occur in the eaten parts of the animals (muscle and gonad). The exception goes to cadmium which may be a public health problem.

A real scale application of mussel farming for the removal of phosphorus and nitrogen from the Baltic Sea (Gren et al., 2009) refers a cost ranging from 0.1 and 11 billion Euros per year corresponding to 2 to 11% of savings compared with alternative procedures. Nevertheless, other authors (Stadmark and Conley, 2011) do not support the idea of using mussels for the removal of nutrients from Baltic Sea. This is due to the accumulation of nitrogen and phosphorus due to the mussels' excretion which may interfere in the biogeochemical cycles of those nutrients. This is especially relevant during the summer where the nutrients release by the bivalves is the highest rate (Nizzoli et al., 2011). While the effect of these accumulated excreted nutrients may be diminished in areas with high water depth and current speed due to their dispersion, these sites will most likely not need the application of biofiltration measures for controlling nutrients accumulation. Of course, the reduction of nutrients release at the source should be preferable.

Thus, the use of invasive bivalves for the recovery of water in reservoirs is not consensual. In fact, these species may lead to unwanted impacts, for example, over biomass and composition of the site phytoplankton (Kirsch and Dzialowski, 2012). Some authors refer that zebra mussels are able to remove cyanobacteria (Higgins and Zanden, 2010). However, others report that this bacteria growth may be promoted by the presence of zebra mussels (Sarnelle et al., 2005; Knoll et al., 2008). This increase was related with the incapacity of mussels to feed from these bacteria that are rejected through pseudofeces to the water column and can growth (Juhel et al., 2006). Moreover, as zebra mussels strongly reduce the dissolved amounts of nitrogen and phosphorus, the conditions may become more adequate for selective cyanobacteria growth as these bacteria are able to fix nitrogen from the atmosphere (Smith and Bennett, 1999). Dressenid mussels are also known as ecosystem engineers due to their capacity to alter aquatic ecosystems (Schwalb et al., 2015). Besides, their biofiltration capacity may promote large reductions of phytoplankton and consequently fish (Higgins and Zanden, 2010). *Limnoperna fortunei* is also reported to reach high filtration rates from 130 to 725 mL/ind.h (von Rückert et al., 2004; Sylvester et al., 2005; Pestana et al., 2009; Frau et al., 2013). Thus, this bivalve is able to reduce phytoplankton in more than 95% in 24h (Cataldo et al., 2011). Moreover, it was observed that cyanobacteria growth was favored in invaded sites (Cataldo et al., 2012).

Asian clam is considered as one of the 100 worst invasive species in Europe (Crespo et al., 2015). Their action can strongly change the microbial community of the invaded sites. For example, Novais et al. (2016) verified that *C. fluminea* stimulates fungal biomass and bacterial diversity in an invaded estuarine. Gathering up all previous discussion, the use of invasive bivalves for nutrients removal from water is not consensual. In fact, very different results were obtained. In this context, the use of these animals for eutrophic waters recovery must be carefully monitored and the potential long-term impacts must be assessed. Moreover, due to their high invasive character, their application must be limited to water reservoirs where those species are already present. Besides, containing actions must be putted in action to avoid the further spreading of the pest. Still, an interesting point is the potential of these species for the recovery of aquaculture water. This economic activity uses a large amount of nutrients for fish farming leading to a water rich in N and P. The biofiltration capacity of the bivalves may be well applied for water recovery in this sector.

II.2.2. Removal of pathogens from water

There are in literature some works applying invasive bivalves on the removal of enteric pathogens from municipal treated effluents (Mezzanotte et al., 2016), spring water (Ladeiro et al., 2014), urban lake water (Ismail et al., 2015), river (Selegean et al., 2001; Ismail et al., 2016) and synthetic water (Silverman et al., 1995; Silverman et al., 1997; Graczyk et al., 2003; Faust et al., 2009). These studies presented the effect of different bivalves over bacteria, viruses and protozoa removal. Due to diversity of enteric pathogens, the removal was analyzed individually for different bivalve species and compared among them.

Water related infectious diseases, mainly gastroenteritis, represent a serious problem in developing countries, mainly associated with the use of compromised drinking water sources, the reuse of wastewater for agriculture and food production, or even through the contamination of aquatic environment where children use to play. Also, several developed countries have experienced many waterborne outbreaks due to cross contamination of public access waters (Giannakis et al., 2017). Thus, it is clear that microbiological contamination of water sources, either due to bacteria, viruses or protozoaires, represent a serious risk to public health and poor access to safe water and sanitation increases the risk of exposure to parasitic, viral, and bacterial pathogens, which can lead to several human diseases (Ulloa-Stanojlović et al., 2016). Thus, the efficient monitoring of microbiological water quality is crucial to assure the safely use of treated waters.

Some bacteria, mainly from human origin, such as fecal coliforms and *Escherichia coli*, are usually used as bioindicators to evaluate water quality and wastewater treatment efficiency (Orruno et al, 2014). The presence of these bacteria is assumed to indirectly indicate that other pathogens may also be present. However, their absence does not guarantee the absence of all other pathogens, as the inactivation of different microorganisms depends on their nature and the type of used treatment process (Bonadonna et al., 2002).

Recently, special attention has also been given to the potential risk of the induction of viable but nonculturable bacteria (VBNC) during wastewater treatment processes (Orruno et al, 2014). The presence of VBNC in effluent wastewaters, and its possible regrowth after land application (such as irrigation) could result in potential human infection. Also, during normal used enumeration procedures, the presence of VBNC would lead to the underestimation of the total bacteria present (van Frankenhuyzen et al., 2011), inducing a potential false negative result, and therefore erroneous control of treatment efficiency.

Despite different treatment approaches have been used, the presence of enteric pathogens in water sources and wastewater treatment plants remains a reality, leading to the need for looking for suitable solutions able to remove these biological contaminants from water.

The application of invasive bivalves can be faced as a way for removing bacteria from water. In fact, bacteria can act as a food source to bivalves (Zobell and Landon, 1937). McHenery et al., (1979), described the presence of enzyme lysozyme in the digestive system of *Mytilus edulis,* proving the role of bacteria in bivalves nutrition. Still, the removal performance is very dependent upon the species of bivalves applied in the biofilter. Besides, also the type of pathogen to be removed has strong influence on the process efficiency.

The filtering capacity of the bivalves is very dependent upon the particles size. This parameter differs on the type of bivalve. Zebra mussels can filter particles with sizes from 0.7 to 40 µm (Ten Winkel and Davids, 1982; Sprung and Rose, 1988; Lei et al., 1996; Silverman et al., 1996). Lei et al., (1996) proven that microspheres equal to or greater than 1.5 µm in diameter were almost totally removed by zebra mussel. In the case of *C. fluminea*, filtration was efficient for particles sizes as small as 1 µm (McMahon and Bogan, 2001). Previous studies suggest that the upper limit for particle size retention by this bivalve is about 20 µm (Way et al., 1990). Nevertheless, further studies reveal that Asian clam can filter spherical algae particles with diameters above 50 µm (Boltovskoy et al., 1995). These studies show once more the ambiguity of the results. It seems that the effect of particle size on filtering capacity is very dependent on the kind and type of particle to be filtered.

Escherichia coli is a small bacterium with typical size of about 2 µm (Brock and Madigan, 1991). This value is near to the lower limit size for particle filtration of these two bivalves. This can be a factor contributing for a low bacteria removal capacity by biofiltration using these species. However, the filtration capacity of these bivalves can be enhanced if bacteria are adsorbed on particles with the optimum size for bivalves consumption (Selegean et al., 2001). This is probably true for municipal wastewaters and natural waters where bacteria can adsorb at the colloids surface. Moreover, bacteria may also aggregate leading to larger particles easily filtered by the bivalves. This may explain the efficiency detected on the removal of this bacteria by several invasive biofiltering species.

Silverman et al., (1995) compared the *Dreissena polymorpha, Corbicula fluminea* and *Carunculina texasensis* efficiency on the removal of *E. coli* from artificial pond water. This artificial pond water has a small amount of salts. At the laboratory conditions was verified for *D. polymorpha* a consumption of 3×10^7 bacteria/mL in 30 min. One indicator often used for performance evaluation of bivalves is their clearance rate (Cohen et al., 1984; Silverman et al., 1995; Ismail et al., 2016). Therefore, to compare the clearance rate values in terms of *E. coli* removal the results were analyzed in the same basis mL/h. bivalve (Table II.2.1.). The clearance rate per one bivalve was 137, 97 and 7 mL/h for *D. polymorpha, C. fluminea* and *C. texasensis* respectively. *D. polymorpha* clearance rate based on the mussel dry weight was higher than the ones observed for *C. fluminea* and *C. texasensis* by 30 to 100 times, respectively. This difference can be related with the gill surface area. Moreover, the dry weight of *C. fluminea* is relatively higher compared with *D. polymorpha*. That is why when this comparison was made per bivalve the difference between these two species was not so sharp. Later, was verified that the gill surface area represents an important parameter that must be considered on the clearance rate (Silverman et al., 1997). Silverman et al., (1997) studied 6 species of lotic unionids bivalves (*Elliptio dilatata, Lampsilis ovata, Ptychobranchus fasciolaris, Fusconaia flava, Villosa lienosa* and *Cyclonaias tuberculata*) regarding to clearance rates of *E. coli* on the dry weight tissue or gill surface area basis. In terms of dry weight tissue, the values ranged from 5 to 19 mL/g.min, whereas looking for gill surface area basis this wide range was reduced to 2 to 4.6 µL/mm^2.min. In fact, in this study the clearance rates based on the gill surface area was also analyzed for *C. fluminea* and *D. polymorpha*, where the Asian clam presents best performance with about 25.5 µL/mm^2.min for the 7.7 µL/mm^2.min of zebra mussel (Silverman et al., 1997). Therefore, the gill surface area can be very important to enhance the biofiltration of bacteria.

Another parameter influencing clearance rate for the removal of *E. coli* is the initial concentration of bacteria. More recently, Ismail et al. (2015) tried to take advantage of the native freshwater mussel *Anodonta californensis* to improve the water quality from an urban lake contaminated with *E. coli*. The analysis of initial concentration of bacteria effect regarding clearance rate, showed that an increase of initial concentration promoted higher clearance rates. However, this increase is not linear because for initial concentrations of 10^7 and 10^8 CFU/100 mL the clearance rate was similar. The values of clearance rate ranged from 177 to 246 mL/h in batch experiments. These clearance rates were determined without achieving the total removal of *E. coli*. In fact, a maximum of 2.5 log of *E. coli* reduction was achieved. In the flow-through experiments with an inlet flow rate of 6 mL/h, from an initial concentration of 3700 CFU/100 mL, 1log of *E. coli* concentration reduction (120 CFU/100 mL) was recorded for 22 days. For this kind of system, it was verified at the last day of experiment a reduction of the clearance rate in about 54% compared to the maximum clearance rate achieved during the experiment.

On the same way, Ismail et al., (2016) examined the capacity of two different bivalves, one native (*A. californensis*) and another invasive (*C. fluminea)* to remove *E. coli* in natural water from a river. In this study, at laboratory scale was established a relationship between the type and bivalve size and the clearance rates. The lengths of native bivalves are much higher than those from invasive species. *A. californensis* ranged from 13 to 73 mm, while *C. fluminea* ranged from 8 to 24 mm. The clearance rate per one bivalve was determined as 100 and 75 mL/h for *A. californensis* and *C. fluminea*, respectively. However, the mean calculated dry weight was 0.2 g and 0.07 g to *A. californensis* and *C. fluminea*, respectively. This means that to achieve the same clearance rate in the real application it will be needed a higher surface area (higher number of individuals) when *A. californensis* is used. By means of the allometric equations, clearance rate was related to soft tissue dry weight. Standardizing dry tissue mass to a value of about 0.3 g of bivalves leads to mass-specific clearance rates of 415 mL/h.g to *A. californensis* and 770 mL/h.g for *C. fluminea* (Ismail et al., 2016). Thus, per specific mass, the *C. fluminea* clearance rate is almost 2-fold higher than the one determined for *A. californensis*. These results reveal that the type and size of bivalve are important parameters that must be taken into account when considering the real application of this biofiltration system to remove *E. coli*.

Bivalves are also usually used as indicators of water contamination due to their capacity of bioaccumulation. Particularly, the detection of fecal bacteria (such as *E. coli*) in mussels is an indication of fecal contamination. The use of these species as indicators of contamination shows some advantages regarding bacterial detection when compared with traditional monitoring methods (Selegean et al., 2001). In rivers and other streams where the water is in constant moving it is difficult to guarantee that representative samples are taken (Selegean et al., 2001). In that kind of environments without toxicity for bivalves, zebra mussel and Asian clams (for example) can work as contamination biomonitoring since they quickly bioaccumulate bacteria. Selegean et al., (2001) verified in laboratory experiments that zebra mussel accumulated up to 90% of its maximum capacity of *E. coli* in the first 4.5 h of contact. Thus, the zebra mussel's accumulation of these bacteria happens very fast. On the other hand, in this study at the same time was measured the decay of *E. coli* concentration in water. The *E. coli* concentration reduces about 50% compared to the initial one in 24 h and 90% after 48 h (Selegean et al., 2001). Thus, besides the mussels' contamination indication capacity, they have a strong ability to remove fecal pollution from water. This shows that their integration in water/wastewater decontamination systems may be feasible.

Mezannote et al., (2016) evaluated *E. coli* removal from a treated municipal wastewater with zebra mussel. Using an initial concentration of about of 2 x 10^3 CFU/100mL and ten mussels per 300 mL of the effluent sample, an *E. coli* reduction of 83% was achieved after 4 h of contact and almost 100% after 48 h. Moreover, a lower reduction on *E. coli* concentration in the control without mussels (about 1 log) was also verified that could be related with the toxic factors present in the wastewater.

The bivalves referred above are freshwater species and, therefore, were applied on the removal of bacteria in freshwater. However, the problem of fecal contamination is also spread to seawater. In this context, studies of contamination with enteric pathogens over seawater species can be also found. Therefore, it is important to consider the capacity of marine bivalves to uptake *Escherichia coli*. Based on the interesting results regarding the application of filtering bivalves on the depletion of bacteria from water/wastewater, Table II.2.1 overviews the clearance rates, and most significant results on *E. coli* removal using different types of bivalves: clams (*C. fluminea*), mussels (*A. californiensis, C. texasensis, D. polymorpha, E. dilatata, L. ovata, P. fasciolaris, F. flava, V. lienosa, C. tuberculata*).

Table II.2.1. Overview of clearance rates and reduction of *E. coli* for different types of bivalves.

Species	**Conditions**	**Clearance rate and E. coli reduction**	**References**
Dreissena Polymorpha	Artificial pond water spiked with 3x10^7 *E.coli*/mL;	137 mL/h.bivalve	Silverman et al., 1995
Corbicula fluminea	Artificial pond water spiked with 3x10^7 *E.coli*/mL;	97 mL/h.bivalve	Silverman et al., 1995
Carunculina texasensis	Artificial pond water spiked with 3x10^7 *E.coli*/mL;	7 mL/h.bivalve	Silverman et al., 1995
Elliptio dilatate	Artificial pond water spiked with 10^7 *E.coli*/mL;	31 mL/h.bivalve	Silverman et al., 1997
Lampsilis ovata	Artificial pond water spiked with 10^7 *E.coli*/mL;	139 mL/h.bivalve	Silverman et al., 1997
Ptychobranchus fasciolaris	Artificial pond water spiked with 10^7 *E.coli*/mL;	181 mL/h.bivalve	Silverman et al., 1997
Fusconaia flava	Artificial pond water spiked with 10^7 *E.coli*/mL;	120 mL/h.bivalve	Silverman et al., 1997
Villosa lienosa	Artificial pond water spiked with 10^7 *E.coli*/mL;	120 mL/h.bivalve	Silverman et al., 1997
Cyclonaias tuberculata	Artificial pond water spiked with 10^7 *E.coli*/mL;	108 mL/h.bivalve	Silverman et al., 1997
Dreissena polymorpha	150 mussels; 38 L of river water; 1 x 10^3 CFU/mL;	-500 CFU/mL (50%) after 24h; - 900 CFU/mL (90%) after 48h.	Seleagen et al., 2001.
Anodonta californiensis	Mountain lake surface water spiked with 10^7 CFU/mL	246 mL/h.bivalve	Ismail et al., 2015

Table II.2.1. Overview of clearance rates and reduction of *E. coli* for different types of bivalves. (Cont.)

Species	**Conditions**	**Clearance rate and E. coli reduction**	**References**
Anodonta californiensis	Mountain lake surface water spiked with 10^6 CFU/mL	177 mL/h.bivalve	Ismail et al., 2015
Anodonta californiensis	Mountain lake surface water spiked with 10^5 CFU/mL	180 mL/h.bivalve	Ismail et al., 2015
Corbicula fluminea	River water spiked with 10^3-10^4 CFU/mL	75 mL/h.bivalve	Ismail et al., 2016
Anodonta californiensis	River water spiked with 10^3-10^4 CFU/mL	100 mL/h.bivalve	Ismail et al., 2016
Dreissena polymorpha	10 mussels; 300 mL of municipal wastewater; 2 x 10^3 CFU/100 mL;	-1.6 x 103 CFU/100 mL of reduction (83%) in 4h; - 100% after 48h.	Mezannote et al., 2016.

The studies at laboratory scale allows to ascertain the clearance rates of bivalves to each enteric pathogen at optimal conditions. The laboratory studies under suitable conditions to potentiate bivalves behavior turns difficult to compare with natural field data. In the real conditions, (for example natural waters) multifactor exist, such as food, organic matter, flow conditions, water temperature, weather, etc., that can significantly affect, positively or negatively, the filtration capacity of bivalves. Thus, this clearance rate determined under optimal conditions must be corrected for the real conditions.

Human enteric viruses are associated with waterborne gastroenteritis, a serious concern on public health. Such viruses, including enteroviruses, adenoviruses, noroviruses, rotaviruses, and others, are typically transmitted through fecal-oral route (Church, 1986). High concentrations of virus are excreted in stool of infected individuals, which may end in environmental contamination through discharge of inappropriately treated wastewater effluents (Theron et al., 2002). Survival characteristics of these viruses under environmental conditions are well described, and their presence in effluents is reported in several countries from different continents (Arraj et al., 2008; Hewitt et al., 2011; Hewitt et al., 2013).

Nowadays, the effectiveness of microbiological removal during wastewater treatment processes is mainly evaluated through the monitoring of bacterial indicators of fecal contamination, such as fecal coliforms and *E. coli*. However, the efficient removal of these bacteria does not necessarily mean the efficient removal of pathogenic viruses, once they are more resistant to both environmental factors and inactivation processes (El-Senousy et al., 2014; Qiu et al., 2015), persisting for longer periods in the environment and representing important threats to public health. Thus, more effective wastewater treatment processes are urgent to prevent environmental viral contamination.

As for bacteria, bioaccumulation ability of bivalves has also been evaluated towards viral removal from different types of waters. Mezannote et al., (2016) evaluated poliovirus and rotavirus removal from a spiked treated municipal wastewater with zebra mussel. These two viruses are very known due to their human health impact. Poliovirus is the responsible agent for poliomyelitis and rotavirus is a contagious virus that can cause gastroenteritis. In the absence of mussels, after 24 h a decrease on poliovirus titer was detected. Thus, the viral reduction verified in 24 h using zebra mussels was not considered significant and could be related with environmental factors that promoted poliovirus inactivation. Nevertheless, a significant reduction was verified on rotavirus titer after 8 h (about 89%) when in contact with the mussels. Moreover, the results reveal that zebra mussel can inactivate the viruses. In fact, despite of viruses remaining in the soft tissues or in the liquid phase, they were not able to transmit to other species, such as waterbirds which feed from zebra mussels. Thus, possibility of avian-to-human transmission of this kind of viruses was broken using this biofiltration system.

The likelihood of mussels being a source for transmission of avian influenza (AI) viruses was also studied. Low pathogenic AI virus (H3N8) concentration can be significantly reduced (2.5 log of the initial concentration 5 log) after 24 h of contact with *C. fluminea* (Faust et al., 2009). Faust et al., (2009) also examined the effect of the presence of *C. fluminea* on the reduction of infectivity of highly pathogenic AI form of H5N1. For this, the water and tissue coming from a treatment system involving those bivalves were inoculated in wood ducks (*Aix sponsa)* and compared to the original virus (without coming from a biofiltration system). The ducks inoculated with the samples provided from Asian clam treatment exhibited normal behavior without mortality. Contrarily, 100 % of mortality was experienced for the ducks exposed to the initial virus (control). Avian influenza virus H5N1 removal was also analyzed using *D. polymorpha*. Zebra mussel shows capacity of accumulating a low pathogenic form of H5N1. After 48h of contact the mussels were placed into freshwater for 14 days, and the virus could still be detected in mussels (Stumpf et al., 2010).

Normally the depuration methodologies applied for shellfish to reduce *E. coli* acceptable levels according to legislation are enough. However, the depuration methodologies regarding virus removal may be ineffective (Formiga-Cruz et al., 2002). The presence of human adenovirus, Norwalk-like virus and enterovirus in the shellfish (*Mytilus galloprovinciallis, Mytilus edulis, Crassostrea gigas,* and *Ostrea edulis*) was verified in Greece, Spain, Sweden and the United Kingdom (Formiga-Cruz et al., 2002). Later, Lowther et al., (2012) analyzed the norovirus contamination presence in the commercial oyster production areas from United Kingdom. In the total of 39 oysters production areas, Norovirus was detected in 76.2% of the samples.

Moreover, in the winter the detection increases. On this way, the virus removal by bivalves from wastewater must keep in mind that the predominant viruses depend on the season of the year.

Nappier et al., (2008) examined the virus retention onto oysters (*Crassostrea virginica* and *Crassostrea ariakensis*) in terms of number. The oysters were seeded with Hepatitis A, poliovirus, bacteriophage (MS2), murine norovirus 1 and human norovirus (at 10^3 PFU/mL). In this study was verified that all population of oysters was not capable to retain the virus. Only 4 and 7% of the total of oysters presented poliovirus and MS2, respectively. The *C. ariakensies* shows a higher number of animals with a positive test of different viruses comparatively to *C. virginica*. Additionally, the depuration rates can be different between oysters at different salinities (8, 10, and 12 ppt). At the end of 29 days of depuration the murine norovirus 1, norovirus and Hepatitis A, presents positive result for *C. ariakensies* independently of the salinity considered. On this way, the presence of active virus on the edible oysters can work as vehicle of viral infections.

Some of these results showed that bivalves can accumulate enteric pathogens for extended periods and may also be able to reduce the infectivity of virus. Nevertheless, such results are not consensual. Still, further studies are required to ensure that bivalves can really remove viruses or decrease its infectivity.

Serious human health concerns are also posed by protozoal fecal pathogens, which are estimated to be the origin of about 4 billion cases of diarrhoea that occur worldwide each year (Daniels et al., 2014). Many of these diarrhoea cases are acquired through the ingestion of contaminated water, with *Giardia* and *Cryptosporidium* species, representing the most common protozoa spread by this route (Imre et al., 2017).

Individuals infected with these gastrointestinal protozoa excrete into the environment many infectious stages (cysts, or oocysts) resistant to conventional water treatment processes (Ulloa-Stanojlović et al., 2016). Furthermore, these parasites can remain infectious for long periods (months to years) in water and be transported for long distances through water courses (Daniels et al., 2014). According to the World Health Organization, the diseases caused by these protozoa, giardiasis and cryptosporidiosis, respectively, belong to the group of Neglected Diseases, due to their importance in waterborne diseases and challenge for water-producing industries, health officials, and researchers in the environmental field (Ulloa-Stanojlović et al., 2016).

Bivalves can also be used for biomonitoring the presence of protozoa on water resources and remove them. The most commonly studied microorganisms are *Cryptosporidium parvum, Giardia lamblia* and *Toxoplasma gondii* due to their detection in water resources (Graczyk et al., 2003; Ladeiro et al., 2014) and with potential parasitic effect over humans.

The main used bivalves for biomonitoring and removing these intestinal parasites from water are *D. polymorpha* and *C. fluminea.* Graczyk et al., (2003) exposed *D. polymorpha* and *C. fluminea* along six-weeks at laboratory conditions to water contaminated with *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts. Both bivalves revealed great performance over the protozoa removal since no oocyst and cysts were identified in water and sediment after biofiltration.

The clearance rate of *C. parvum* oocyst was analyzed under laboratory conditions for *C. fluminea* (Graczyk et al., 1998). From a 38 L tank filled by 200 clams contaminated with 3.8 x 10^7 oocysts total parasites removal from water was achieved after 24 h. This leads to a clearance rate of 7.9 x10^3 oocysts/clam.h (Graczyk et al., 1998). The clearance rate for *G. lamblia* cysts by *C. fluminea* was 8.7 x10^2 cysts/clam.h (Graczyk et al., 1999). As it can be seen, the clearance rate for *C. fluminea* regarding to protozoa is also very dependent of the kind of protozoa. On the other hand, Ladeiro et al., (2014) studied the potential of zebra mussel to bioaccumulate *C. parvum, G. lamblia* and *T. gondii*. The results showed that the bioaccumulation was proportional to ambient contamination. After 7 days of exposure were found an average number of 1.4, 8.7 and 31 (oo)cysts per mussel tissue for *C. parvum, G. lamblia* and *T. gondii*, respectively. These differences could be related with the size dimension of protozoa (Ladeiro et al. 2013). *C. parvum* has the normal size between 4-5 µm while the typical size of *G. lamblia* and *T. gondii* is about 12-15 µm (Ladeiro et al., 2013). As the zebra mussel has food size preference between 15 to 40 µm (Ten Winkel and Davids, 1982) it is expectable that it can filter or retain higher amounts of *G. lamblia* and *T. gondii* comparatively to *C. parvum.* On the other hand, *C. fluminea* reveals higher performance on the filtration of *C. parvum* comparatively to *G. lamblia* (Graczyk et al., 1998). Looking for the size of these pathogens and the food preference size 5-20 µm of Asian clam (Way et al., 1990) it was expectable other result in terms of clearance rate of these pathogens. Comparing the studies of Graczyk et al. (1998) and Ladeiro et al., (2014) it is possible to say that *C. fluminea* has preference for lower pathogen size relatively to *D. polymorpha.* However, previously for *E. coli* removal (typical size 2 µm) the reverse was concluded (Silverman et al., 1995). Thus, it is impossible predicting the filtration capacity of these two bivalves in terms of pathogens sizes. So, the type of pathogen must also be considered. In fact, it is important to look for the type of pathogen which needs to be removed from water or wastewater. Table II.2.2. shows the differences between *C. fluminea* clearance rates for different protozoa.

Table II.2.2. Clearance rate for protozoa using *C. fluminea*.

Specie	Kind of protozoa	Conditions	Clearance rate	References
Corbicula fluminea	*Cryptosporidium parvum*	-38L of drinking water; -200 clams; - initial concentration 3.8 x 10^7 oocysts.	7.9 x 10^3 oocysts /h. bivalve	Graczyk et al., 1998
Corbicula fluminea	*Giardia lamblia*	-38 L of drinking water; -160 clams; -Initial concentration 1x10^5 cysts.	8.7 x 10^2 cysts /h. bivalve	Graczyk et al., 1999

As referred above for *E. coli*, protozoa pathogens can also be found in the seawater. Therefore, biofiltration of protozoa by marine bivalves must be considered. Willis et al., (2014) exposed *Crassostrea virginica* to *C. parvum* contamination in a static tank to analyze bioaccumulation within 7 days and 1day experiments. In the 1-day exposure for an initial concentration 1000 oocysts/L, the clearance rate was about 9 oocysts/oyster.h. The 10 times initial concentration reduction promotes a proportional decrease on the clearance rate about 1.2 oocysts/oyster.h (Willis et al., 2014). Other important aspect is the decrease on clearance rate along wide periods of exposure. Along the 7 days experiments the mean clearance rate was about 1.4 oocysts/oyster.h for higher initial concentration (1000 oocysts/L) (Willis et al., 2014). Normally, when bivalves are continuously exposed to pathogens along wide periods, they may reach the saturation and consequently it will occur a decrease on clearance rate. So, the exposure time must be carefully designed.

Regarding the disinfection of wastewaters or water sources using these low cost and ecological/industrial problematic species, it is important trying to understand the mechanism behind their efficiency in removing the enteric pathogens. For example, it is important to understand if pathogens are really removed from the water source or if the bivalves only can inactivate temporally the pathogens. This analysis needs to be made specie to specie since the results reported in literature are somehow contradictory.

In order to understand the removal mechanism, Ismail et al., (2015) analyzed the water resulting from biofiltration using *A. californiensis* after 24 h. *E. coli* present in water was analyzed as well as the fecal matter produced by the mussel. Moreover, the bivalve tissue composition after contacting with *E. coli* was compared with the one coming from the control beaker. The analysis revealed that 3% of *E. coli* remains in the mussel tissue, whereas the main portion of *E. coli* was quantified in the fecal matter (about 58 %) and the rest remained in water (39%). The high amount of *E. coli* in the mussel feaces reveals that *E. coli* was removed from water but it is afterwards excreted by the mussel. Still bacteria may be inactive after biofiltration. Nevertheless, the possibility of bacteria being able to reproduce and contaminate again the water sources may not be ruled out in this case.

Selegean et al., (2001) proven that zebra mussel can accumulate with great efficiency *E. coli* present in river water. Moreover, it was verified that along the depuration time the amount of *E. coli* in the tissues reduced. In fact, after 80 h *E. coli* concentration on zebra mussels decreased to the initial levels. Zebra mussel can accumulate *E. coli* over several days in their tissues but, after this, bacteria is eliminated. On the same way, in a previous study, *D. polymorpha* revealed a great capacity to remove bacteria from artificial pond water and only about 26% of initial bacteria concentration remains in their soft tissues after 48 h (Silverman et al., 1995).

In 1981, tests with a seawater mussel (*Mytilus edulis*) were made on bacteria removal. The authors refer that bacteria could disappear from seawater due to joint activity of appropriate enzymes allocated on the digestive system of the mussel and lysozyme (Birkbeck and McHenery, 1982). However, no accordance was achieved regarding the role of bacteria as food source for the mussel.

On global vision of the results, it is concluded that the preferential mechanism for *E. coli* removal by biofiltration is very dependent on the type of bivalve used. Besides the type of bivalve, other factors can influence the enteric pathogens removal such as the experimental conditions and the type of water source applied. Some experimental conditions can be more appropriate for enteric pathogens processing by bivalves.

Love et al., (2010) verified, in a flow-through system during 5-days trials, the influence of temperature, salinity, turbidity, pH and algae conditions on the depuration of some enteric human pathogens (*Escherichia coli, Enterococcus fecalis,* coliphage MS2, poliovirus and hepatitis A virus) using oysters (*Crassostrea virginica*) and hard-shell clams (*Mercinaria mercinaria*). The water temperature of 25 ºC improves the depuration efficiency of oysters for all enteric pathogens tested, while the high salinity (28 ppt) increased the depuration of *E. coli* and *E. fecalis* for both mussels and MS2 for oysters. Regarding the other parameters tested, only turbidity improves the *E. fecalis* depuration for hard shell clams. After 44 h of depuration the percent of microorganism removed was almost total in the case of oysters. Whereas for hard shell clams, the MS2 and Hepatitis A virus were still found in high amounts.

The viruses are more difficult to depurate compared to bacteria due to their presence in intra and extracellular digestive system (Le Guyader et al., 2006; Love et al. 2010).

Graczyk et al., (1998) verified that for *C. fluminea*, during 14 days of exposure *C. parvum* oocysts were found in the clam tissues and feces along the experiment. The oocysts released in the water were always surrounded by feces. In terms of *Giardia* cysts, it was verified that it mainly accumulates in *C. fluminea* tissues. The presence of cysts in the feces along 3-week of exposure was never detected (Graczyk et al., 1999). The main explanation for this absence in feces can be related with the digestion resistance of cysts (Graczyk et al., 1999).

The protozoa pathogens removed by zebra mussel and Asian clams from water were mainly accumulated in the tissue of both bivalves as described by Graczyk et al., (2003). These authors found that 35% and 16% of the protozoan seeded were identified in *D. polymorpha* and *C. fluminea*, respectively. In order to understand the real accumulation of protozoan pathogens on mussel tissues, Ladeiro et al., (2014) measured during a 7 days experiment for zebra mussel *T. gondii* accumulation in muscle, gills and digestive glands. After 7 days of exposure *T. gondii* accumulation was mostly detected in the muscle. At the first and third day of exposure *T. gondii* higher accumulation was also verified in the gills and digestive glands. However, for one week of experiments occurs a significant decrease on the amount of these pathogens on these organs (Ladeiro et al., 2014). This could be related with the possible digestion or rejection of *T. gondii* by the zebra mussel. However, this study is in accordance with the previous one revealing that a high number of protozoa can be accumulated in the mussel tissues. As can be seen for the other pathogens analyzed, zebra mussel presents the highest capacity to accumulate these microorganisms in their tissues. This can be related with the higher gill size (Silverman et al., 1995).

Moreover, for marine species at seawater conditions Willis et al., (2014) for 7 days exposure experiments verified that 49% of the total *C. parvum* placed into 10 L static tank were accumulated in *C. virginica* tissues, whereas 38% was detected in fecal material of the oyster and the remaining was found in tank water. The first day of experiment revealed a decrease of tissue accumulation (about 30% of accumulation in the tissue) which means that in longer exposures occurs the refilter of the oocysts initially rejected by oysters.

The removal of different enteric pathogens was analyzed at four secondary wastewater treatment plants in Sweden (Ottoson et al., 2006). Norovirus, Enterovirus, *Gyardia* cysts, *Cryptosporidium* oocysts, *E. coli* and Enterococci were selected as reference. The process of wastewater treatment at these plants consists in chemical precipitation followed by activated sludge. The results reveal that the most difficult pathogens to remove from wastewater were viruses and *Cryptosporidium* oocysts. The cysts and bacterial removal were about 2 to 2.6 logs, while the viruses and oocysts were 0.9 to 1.3 logs. Other important aspect is that some viruses only appear in the winter (November and February) as is the case of norovirus, while other pathogens, such as *Giardia* cysts and enterovirus, were always detected (Ottoson et al., 2006). This study comes to highlight the need for demand a suitable solution to promote the removal of different pathogens with higher efficiency from treated wastewater looking for wastewater reclamation.

The biofiltration process is highly dependent upon abiotic factors such as temperature, dissolved oxygen and salinity (Sprung and Rose, 1988). However, the type and amount of food may affect the filtration efficiency. For example, the formation of pseudofeaces was mainly detected when an excess of food is found in water. This is a mechanism the bivalves use for removing the excess of particles and keep the gills working (Sprung and Rose, 1988). Moreover, the bivalves also select the particles concerning their size and weight as well as chemical composition. The rejected particles are removed through pseudofeaces. However, that selection fails when the animals are subjected to large periods of starvation (Ten Winkel and Davids, 1982). This must be taken into account while designing a biofiltration system so that conditions are stablished to avoid pseudofeaces formation so that active pathogens are not accumulated in them.

II.2.3. Application of invasive bivalves for wastewater treatment

The presence of recalcitrant chemical contaminants in municipal wastewater is an important point of concern nowadays. In fact, compounds such as pharmaceutical, personal care products, drugs of abuse and their metabolites are continuously reaching municipal wastewater treatment plants (Fatta-Kassinos et al., 2011). The conventional wastewater treatment plants are not designed for the removal of these complex substances. Thus, they are reaching natural water courses with potential adverse impacts over ecosystems and human health. Several approaches were performed to develop tertiary processes for the refinement of secondary municipal wastewaters in order to avoid the discharge of those pollutants to water courses or even to reached treated water safe for reuse (Li et al., 2015; Gomes et al., 2017; Miralles-Cuevas et al., 2017; Sora and Fumagalli, 2017). The lipophilic character of most of these compounds makes them suitable for biofiltration using bivalves. In this context, Binelli et al., (2014) proposed a secondary effluent management strategy involving the invasive *Dreissena polymorpha* as host for biofiltration. This mussel was selected due to its high biofiltration capacity (5 to 400 mL/bivalve.h) (Ackerman et al., 1999) and resistance to variations of water properties.

Moreover, the mussel capacity to attach to surfaces makes it easier the biofilter assembly. The pilot plant consisted on a 1000 L tank with 20 Plexiglas® panels where the mussels were attached (around 40,000 individuals). Chemical oxygen demand (COD) removal is a parameter that allows following up global organic contaminants depletion during the treatment. The biofilter was able to remove up to 42% of the initial COD after 4 h when compared with 19% of the control experiment. Nevertheless, it was concluded that zebra mussels COD abatement efficiency is highly dependent upon the initial COD. In fact, their efficiency is higher when initial COD values are higher than 30 – 40 mg/L. This may be related with the lowering pumping or filtration rates demonstrated by bivalves when low food concentrations are available (Davenport and Woolmigton, 1982). This behavior implies, when the previous wastewater treatment leads to high COD abatements, the mixing between secondary and primary municipal wastewaters for improving biofiltration efficiency. However, the use of an excessive percentage of inflow wastewater led to an increasing mortality of the mussels probably due to the presence of some toxicants to this bivalve in the initial wastewater. Moreover, the presence of excessive particulate matter that clogged the gills may not be ruled out. Also, extreme food loads decrease filtration rates (Davenport and Woolmigton, 1982). The authors tested the capacity of zebra mussels on the removal of several pharmaceutical and drug abuse compounds. Although the presence of the mussels was apparently irrelevant on the removal of diclofenac and naproxen, biofiltration led to a high abatement on paracetamol (4000 μg in 24 h). Also, interesting removal efficiencies were determined for clarithromycin, ofloxacin, ibuprofen and cocaine. The removal mechanism was not determined but some hints are given: the mussel may process the compounds and excrete them through faeces and pseudofeaces that are removed from the water by sedimentation or, on the other hand, some bioaccumulation in the soft tissues may occur.

Zebra mussels are envisaged as biofouling agents leading to serious damages to water treatment facilities. However, their biofiltration potential may also lead to benefits to treated water quality. Elliot et al., (2008) verified that zebra mussels were able to provide an initial stage of water depuration by removing algae. Moreover, low maintenance to the biofilter was required since the mussels survived for more than 3 months. Thus, the potential of zebra mussels to improve water quality may be used in infested water treatment facilities if further spreading of the pest is avoided. In this context, biofiltration may be considered as a tertiary treatment in municipal wastewater treatment plants (Figure II.2.1.).

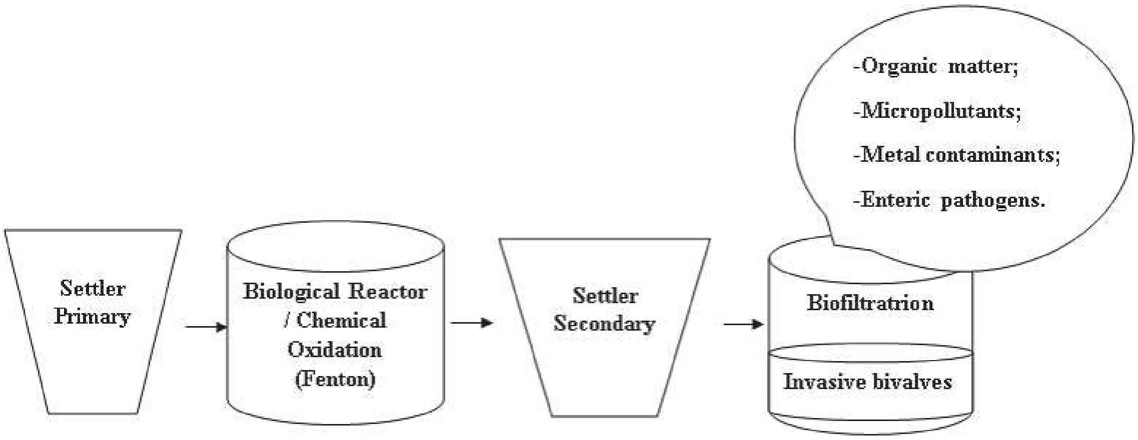

Figure II.2.1. Scheme proposal for integrated wastewater treatment.

The integration on wastewater treatment facilities was also envisaged as a possible axis for *C. fluminea* pest management strategy. Ferreira et al., (2018) tested Asian clams biofiltration capacity for the organic charge removal of winery wastewater. The seasonal character of this effluent leads to streams with different toxic impact over the clams during the year. In fact, the composition and organic load of the winery wastewater is highly dependent of the season of the year (Ioannu et al., 2015). This characteristic may be an important drawback for the application of biofiltration using *C. fluminea* for the initial winery wastewater treatment. In fact, even if interesting COD results were obtained by Ferreira et al. (2018) when the biofilter was directly applied to the raw effluent (100% of COD removal after 7 days), an induction period ~ 2 day was verified. This may be related with the acclimation of the clams to the effluent characteristics. Thus, the continuous change on the effluent composition would imply permanent adaptation which would jeopardize the treatment efficiency. Moreover, high clam mortality rate was observed at those conditions. The use of Fenton's process as a pre-treatment was able to significantly reduce the winery effluents toxicity towards *C. fluminea*. Moreover, Fenton's process may also contribute to some equalization of the effluent feeding the biofilter characteristics. Thus, the clams would be subjected to lower levels of stress due to changes in the feeding composition. In fact, when biofiltration was applied to the pre-treated effluent, 100% of COD removal was obtained in ~ 3 days of treatment and low clams mortality was verified when compared with the case where the raw effluent was used. Another advantage of biofiltration was the capacity of the clams for removing the dissolved iron remaining after Fenton's process treatment. This integrating treatment strategy efficiency was proved at the pilot-scale by Pipolo et al., (2017). This approach was compared with a benchmark (Fenton's process followed by activated sludge) and the results show that the process may be competitive.

The main drawback of the application of *C. fluminea* when compared with *D. polymorpha* is their incapacity to attach to substrates. This increases the complexity on the assembling of the biofilter since suitable supports holding the clams must be prepared.

Metallic contaminants are produced in several human activities. These pollutants tend to accumulate and may be toxic to several species including humans even at low concentrations (Inglezakis et al., 2003). The most suitable techniques able to interesting removal percentages for metals removing from water and wastewater encompass precipitation, ion exchange and membranes separation (Gomes et. al., 2010; Martins et al., 2017). However, these processes tend to be expensive due to the chemicals used or energy required. The high filtration capacity and bioaccumulation of invasive bivalves may be used as a low-cost alternative for removing such pollutants from water.

Rosa et al., (2014) explored the potential of *C. fluminea* for the removal of metallic contaminants from acid mine drainage (AMD). The capacity of metal removal was highly dependent on the metal considered and the effluent dilution level applied. Optimal purification levels were attained when 4% AMD was applied. Among the tested metals (Al, Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn) most of the content removed from the water column was detected in the clams' shells. However, the metals sequestration and elimination through pseudofeces may not be ruled out. The efficiency of biofiltration on AMD depuration was also clear in what regards toxicity removal since a strong decrease on AMD toxicity over selected species (*A. fischeri*, *P. subcapitata* and *D. magna*) was observed after the treatment.

The ability of *D. polymorpha* for removing metallic elements from municipal effluent was tested at the pilot scale (Magni et al., 2015). High removal percentages were observed for most of the tested metals after 24 h of biofiltration. The highest removal (100%) was obtained for Cr while negligible depletion was observed for Ni.

Even though these interesting results, several points must be still analyzed such as the mechanism behind the metals removal, the behavior of the mollusks after long term exposure to the metal rich effluents and the fate of wasted animals. At this point, Magni et al., (2015) propose incineration or storage in landfills. In fact, those procedures are already implemented the sewage sludge with the advantage that probably with the bivalves the material weight would be lower.

Table II.2.3. resumes all the works involving biofiltration integrated and as single process for the removal of organic matter and micropollutants (such as heavy metal and pharmaceutical compounds).

Table II.2.3. Removal of organic matter and micropollutants over invasive bivalves.

Type of experiment	Target compounds	Removal	Reference
***Dreissena polymorpha* as biofilter with recirculation of 50 % inlet / 50% outlet; (1000L pilot-plant)**	-Paracetamol; -Clarithromycin; -Ofloxacin; -Atenolol; -Ibuprofen; -Cocaine	- 4 mg (24h); - 0.38 mg (24h); - 0.28 mg (24h); - 0.25 mg (24h); - 0.22 mg (24h); - 0.14 mg (24h);	Binelli et al., 2014
Fenton (1st) + *Corbicula fluminea* (2nd) (Vessels of 600mL)	Winery wastewater (COD=2000 mg/L)	-52% of COD removal ($[H_2O_2]$ / $[Fe^{2+}]$ =0.206 M/ 0.03 M) (2h); - Remain 48% after 48h of contact with clams.	Ferreira et al., 2018
***Corbicula fluminea* as biofilter (only) (Vessels of 500mL)**	Winery wastewater (COD=2000 mg/L)	-100% of COD removal (7 days),	Ferreira et al., 2018
Fenton (1st) + *Corbicula fluminea* (2nd) (20L reactor)	Winery wastewater (COD=1260 mg/L)	-84% of COD removal ($[H_2O_2]$ / $[Fe^{2+}]$ =0.206 M/ 0.03 M) (2h); - Remain \12% after 72h of contact with clams.	Pipolo et al., 2017
***Corbicula fluminea* as biofilter of 4% Acid mine drainage; (Lab Scale 500mL)**	-Al; -Fe; -Ni -Mn; -Si; -Zn.	-3.9 mg/L (7days); -3.6 mg/L (7days); -0.01 mg/L (7days); -0.82 mg/L (7days); -1.1 mg/L (7days); -2.2 mg/L (7days);	Rosa et al., 2014
***Dreissena polymorpha* as biofilter with recirculation of 50 % inlet / 50% outlet; (1000L pilot-plant)**	-Al; -Cr; -Fe; -Ni -Mn; -Pb;	-74 µg/L (24h); -2.86 µg/L (24h); -343 µg/L (24h); -1.76 µg/L (24h); -14 µg/L (24h); -2.75 µg/L (24h);	Magni et al., 2015

The application of invasive bivalves as biofiltering hosts for wastewater treatment seems an attractive pest management strategy. In fact, this way an environmental application can be given to nuisance organisms. This avoids the use of the traditional control methodologies such as chemical disinfection. In fact, the use of biocides may have side effects over the ecosystems since toxic by-products can be generated. Moreover, it is unavoidable that some disturbance occurs over non-target organisms.

One potential drawback associated with this wastewater treatment approach is related with the costs associated with the transport of the bivalves from the infested sites for the biofilters assembling. However, this can be coordinated with the physical removal of the bivalves from the invaded spots which is a typical method employed for controlling this invasive species (Sousa et al., 2014). Moreover, if necessary, cost-effective cultivation may be regarded as another alternative to complement the bivalves collected from invaded areas (McLaughlan and Aldridge, 2013).

Even though the interesting results observed using biofiltration based on invasive bivalves for wastewater treatment it is likely that some resistance is encountered regarding the wide application of this technology. In fact, the high invasive character of those species and their ecological and economic impacts brings about concern regarding their further spreading. In this context, care must be taken in the biofilters assembly so that the animals are perfectly held and that releases of organisms are completely avoided. This can be done by adding downstream processes such as sand filters or oxidation processes (such as ozonation) able to contain any form of the invasive organisms (even in their planktonic life stages) so that further invasion to the surrounding ecosystems is completely circumvented.

Another important operating issue is the faith of the contaminated animals. In fact, after use it is likely that the bivalves accumulate contaminants. For example, as it was referred before, pathogens (bacteria and viruses) can still be detected in the bivalves soft tissues even if in some cases inactive. The drawback of contaminated solids disposal is recurrent for most wastewater treatment strategies. One of the most widely spread effluents depuration technologies are based in activated sludges. The excess of sludge (incorporating contaminants) must also be safely disposed. Typically, this sludge is dried and landfilled or incinerated. Another activated sludge management strategy may include anaerobic digestion. Those solid waste management techniques may be also applied to spent bivalves. One advantage is that probably biofiltration will lead to lower weights of waste.

Gathering up all this it seems that biofiltration using invasive bivalves is a feasible technology to be integrated in wastewater treatment plants. Nevertheless, some more pilot scale studies are required to test the efficiency of this process for long term application. In fact, it is necessary to optimize the operating conditions especially in what concerns the food (or volume of effluent) to bivalves number ratio and the organisms renewal rate. Besides the mechanisms behind biofiltration efficiency must be further explained to understand the fate of pollutants during biofiltration in order to establish an adequate spent bivalves disposal methodology.

II.2.4. Concluding remarks

Invasive bivalves constitute a serious environmental and economic problem that must be solved. Their relative tolerance to toxicants joined with their high biofiltration/bioaccumulation features is opening a new management axe for these alien species. This new approach involves their application for eutrophic sites recovery, water disinfection as well as wastewater treatment. Pest management strategies can thus be integrated with water treatment approaches. Although interesting results are reported regarding their efficiency on those tasks, some drawbacks may arise leading to strong resistance to their wide usage that must be taken onto consideration when designing such a biofiltration system:

1. Their high biofiltration capacity may significantly reduce phytoplankton in the water column leading to changes in the biological diversity of the invaded sites;

2. The mechanism behind their efficiency on pathogens removal during water disinfection depends upon the type of bivalve and pathogen to be removed. Thus, careful must be taken so that effective inactivation occurs. This aims to avoid that bivalves became sources of diseases transmission;

3. The further spreading of these alien species to uninvaded sites must be avoided;

4. The fate of the contaminated animals must be taken into account bearing in mind environmental and economic constrains.

In this context, pilot-scale studies must be carried out to analyze the long-term efficiency of such depuration systems. This way the potential negative impacts of invasive bivalves while used in biofiltration may be addressed carefully. Moreover, their long-term exposure behavior may also be addressed. The biofiltration mechanisms must be studied for different bivalves and pathogens to avoid further contamination. Also, the development of downstream measures to avoid the release of animals from the biofilters is necessary so that further spreading of the pest occurs. In what regards the use of invasive species for eutrophic reservoirs recovery, this must be limited to already invaded sites rather. Spent bivalves' management probably may involve the procedures used for activated sludge disposal.

It seems thus that the environmental application of invasive bivalves may be an interesting approach for pest management. These procedures integrate water/wastewater treatment with pest control avoiding the use of unnecessary chemical products.

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III. Detoxification of parabens using UV-A enhanced by noble metals – TiO_2 supported catalysts

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III.1. Introduction

Advanced oxidation processes (AOPs) are considered suitable alternatives or complements to traditional wastewater treatment methodologies. The increasing demands of the population are leading to effluents with highly refractory contaminants such as pharmaceutical and personal care products. Alternatives such as ozonation, Fenton, catalytic oxidation, and photochemical technologies can be considered interesting solutions (Tay et al., 2010; Hansen and Andersen, 2012, Gmurek and Miller, 2012, Gmurek et al., 2012; Domínguez et al., 2014; Gmurek et al., 2015; Martins et al. 2015; Martins et al. 2016) for the degradation of wastewaters containing this kind of pollutants. In particular, the heterogeneous photocatalysis is a technology that deserves to be considered since the correct combination of catalysts with UV irradiation can truly improve the wastewater treatment efficiency. The semiconductor titanium dioxide (TiO_2) is one of the most used photocatalysts, because it has good optical and electronic properties, besides low cost, chemical stability, easy handling and non-toxic characteristics (Chong et al. 2010; Pelaez et al. 2012). This semiconductor, with enough light energy, can improve the photocatalytic degradation of pollutants. Two pathways can be considered, the photogenerated holes (h^+ (equation III.1)) can oxidize adsorbed water and the photoexcited electrons (e^- (equation III.1)) may promote the reduction of an electron acceptor (such as O_2). These two reactions can produce hydroxyl (•OH) and superoxide ($\bullet O_2^-$) radicals, respectively (Pelaez et al., 2012).

$$TiO_2 + h\upsilon \longrightarrow h^+ + e^- \qquad \text{Equation III.1}$$

The main problem of TiO_2 is that it requires UV-A light ($\lambda < 400$ nm) to start the reductive and oxidative reactions on its surface, corresponding to a photon energy higher than 3 eV (Chong et al. 2010). Many studies have been made for the development of visible light active TiO_2 photocatalysts (Emeline et al. 2007; Wang et al., 2012; Pelaez et al., 2012). In our work, nanoparticles of noble metals (Pt, Pd, Au, Ag) supported on TiO_2 were used. The noble metals used can help TiO_2 on photoreactions because they can absorb visible light due to surface plasmon ressonance effect (Zheng et al., 2011; Wang et al., 2012). Additionally, the deposition of these metallic nanoparticles on titania surface promotes the interfacial charge transfer on the conduction band because nanoparticles scavenge the photogenerated electrons. Therefore, the recombination phenomenon of electron-hole pairs is much lower when compared with TiO_2 alone (Anpo et al. 2003; Jakob et al. 2003; Bahruji et al. 2010). During the irradiation of the photocatalysts many reactions occur but the most important for mineralization of organic compounds is the generation of reactive oxygen species (ROS). These include hydroxyl radicals that can hydroxylate aromatic compounds and the resulting intermediates to produce carbon dioxide and water (Chong et al. 2010). So, with noble metals deposited on a semiconductor it is possible to improve this reaction because electron-hole recombination is more difficult to occur. Thus, an improvement on the degradation of organic compounds with consequent higher mineralization will normally be expected. Oros-Ruiz et al. (2013) compared the effect of metallic nanoparticles (Au, Ag, Cu and Ni) supported on titanium dioxide with that of single TiO_2. The authors applied 6 h of UV-C light for degrading an antibiotic (trimethoprim) and concluded that the presence of metallic particles increases the mineralization level achieved. Complete mineralization of ciprofloxacin was obtained after 180 min of UV-C irradiation using 0.5 g/L of metallic (1.5%Au, 1.5%Ag, 1%Cu) nanoparticles deposited on TiO_2. However, with simulated sunlight irradiation, applied for 360 min, total mineralization was not achieved (Durán-Álvarez et al. 2016). So, for the same amount of catalyst, the results are very affected by the kind of irradiation applied. Moreover, the increase of noble metal nanoparticles concentration on TiO_2 surface can block the UV light thus affecting the photocatalytic activity. Mohamed and Khairou (2011) verified that for Ag loading of 1.6 wt% the photogenerated hole trapping effect was negligible. However, for increased loading values this effect prevails and photocatalytic activity declines. Peng and Wang (2007) studied the effect of Pt loadings (0.01, 0.1, 0.6, 1 wt.%) supported on titanium dioxide for oxidation of formaldehyde and concluded that 0.6 wt.% was the most active material.

Nowadays, a group of emerging pollutants is being detected in the aqueous media due to municipal wastewater discharges or even due to the incorrect disposal of industrial effluents containing these mixtures. In fact, these substances are difficult to remove with conventional wastewater treatments (Kasprzyk-Hordern et al., 2008, Bolz et al., 2001; Gibs, et al., 2007). Some of these emerging pollutants are called endocrine disrupting chemicals (EDC's) because they can interfere with the hormonal system causing, possibly, adverse effects on the physiology of the organisms (Pojana et al. 2007). Parabens, which are present in most pharmaceutical and personal care products, are within these pollutants (Meeker et al., 2013, Calafat et al., 2010). Gmurek et al. (2015) studied the photolysis of a mixture of parabens (10 mg/L each, pH 7) with UV-C light. In these conditions the total degradation of parabens was achieved after 2 h, but in terms of mineralization irradiation caused 20% of total organic content (TOC) decrease. The results were improved by the addition of hydrogen peroxide to UV-C irradiation. The photocatalytic degradation of methylparaben (MP) was studied using TiO_2 as catalyst and simulated solar irradiation by Velegraki et al. (2015). With 0.5 g/L of TiO_2, at pH 5.2, complete elimination of 1 mg/L of MP was achieved after 35 min, whereas for the same conditions, with 10 mg/L of MP, 240 min of reaction were required (Velegraki et al. 2015). Using UV-A irradiation it was possible to achieve 90% of MP degradation in 120 min (Lin et al. 2009).

To the best of our knowledge, there are no studies about the degradation of parabens mixtures with heterogeneous catalysts in the presence of UV-A irradiation. In this ambit, the aim of our work focus on the evaluation of the photolytic and photocatalytic degradation of a mixture of five parabens, methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BuP) and benzylparaben (BeP) that can be found in industrial and municipal wastewaters. The catalytic activity of noble metals (Ag, Au, Pd and Pt) at loadings of 0.5 wt.% deposited on TiO_2 was compared with that of pure TiO_2. Moreover, time and fluence based pseudo-first order kinetic constants were determined for this system for the first time.

Normally, when mineralization is not totally achieved on the degradation of those compounds, it means that even though parabens can be removed, organic compounds more difficult to oxidize were produced along the degradation process. So, it is necessary to know if the toxicity after the treatment is lower than that of the initial mixture of parabens, to ensure safe disposal or reuse of the water. This toxicity assessment was evaluated using three species: *Aliivibrio fischeri*, *Corbicula fluminea,* and *Lepidium sativum*. This allowed a more accurate inference of the impact of such streams over the ecosystems. No studies involving toxicity assessment over three different species with the resulting samples of photocatalytic parabens degradation has been made. Thus, in this work aiming to give a broader idea about the potential impact of these waters over the ecosystems, we analyze the effect of treated samples over three species, which allows covering a wide range of trophic levels. *Aliivibrio fischeri* is very sensitive marine bacteria usually used as standard for toxicity assessment. *Corbicula fluminea* is a freshwater clam which allows inferring about the potential impact over a more complex organism. The germination index determined for *Lepidium sativum* allows understanding the potential effect of the treated water if reused for agriculture purposes.

III.2. Material and methods

III.2.1. Chemicals and Catalysts

Methylparaben (MP), ethylparaben (EP), propylparaben (PP), benzylparaben (BeP) were obtained from Sigma-Aldrich. Fluka was the provider of butylparaben (BuP). The purity of all parabens is higher than 99%. Titanium (IV) isopropoxide (97%) was purchased from Aldrich Chem. $KAuCl_4$ (98%), $PdCl_2$ (5 wt.% solution in 10 wt.% HCl), H_2PtCl_6 (99%) and $AgNO_3$ (99%) from Sigma Aldrich were used as metal source in the preparation procedure. Ethanol (99.8%) was purchased from POLCHEM (Poland).

The sol-gel method was used for preparing Au – modified TiO_2 from titanium (IV) isopropoxide, known to be the titanium source origin for the anatase-type TiO_2. Titanium (IV) isopropoxide (35 mg) were added to 35 ml of ethanol with an appropriate amount of the precursor – $KAuCl_4$ (0.5 wt.%). The mixture was stirred for 5 min at 500 rpm. After stirring, 1.5 ml of distilled water and 0.105 ml of methanol were added to the mixture. The solution was stirred at room temperature for 2 h, followed by a 24 h thermal treatment (45 ^oC) and calcination at 400 ^oC for 2 h. The other catalysts were prepared by photodeposition of noble metals at the surface of titanium dioxide. All the photocatalysts were obtained by UV-reduction of the precursor (Pt^{4+}, Pd^{2+}, Ag^+ ions) in the TiO_2 suspension. An aqueous solution of isopropanol containing TiO_2 (2 g) and H_2PtCl_6 (0.5 wt.%) or $PdCl_2$ (0.5 wt.%) was degassed with nitrogen and irradiated by UV-Vis light (1000 W Xe lamp) for 6 h. Whereas 70 cm^3 of ethanolic solution-containing TiO_2 (2 g) containing $AgNO_3$ (0.5 wt.%) was degassed with nitrogen and irradiated by UV-Vis light (1000 W Xe lamp) for 100 min. The modified TiO_2 photocatalysts were separated by centrifugation and dried at 65 - 120 ^oC for 12 h.

III.2.2. Experimental procedure

The solution used in these tests was prepared from a mixture of the five parabens (10 mg/L each) in ultrapure water (Gmurek et al., 2015). The experiments were carried out in a 2 L glass reactor with light source axially placed inside the reactor. The light source consisted of 3 lamps Philips TL 6W BLB (tube diameter of 16 mm), made of blacklight blue glass, which transmits UV-A radiation, with the main emission wavelength around 365 nm. The photon flux inside the reactor E_o= 5.75 x 10^{-7} Einstein/Ls (8.9 J/s.m^2) was obtained using ferrioxalate actinometer (Kuhn et al. 2004), normally used for this wavelength. The degradation experiments occurred at controlled temperature (25 ± 1 ºC) by a thermostatic bath during 180 min. The mixture of parabens was placed inside the reactor with magnetic agitation at 700 rpm. This stirring speed was optimized in preliminary studies, to ensure homogeneity of media and chemical regime.

Samples were taken along the reaction ensuring that no more than 10% of the reactor initial volume was exceeded. The amount of catalyst introduced in the reactor was 0.07 g/L and was placed in contact with the solution 5 min before turning on the light, to test the catalyst adsorption capacity without irradiation. Besides this, the reactor was fed with 0.2 L/min of pure oxygen stream (99.9%, Praxair) during the degradation experiments.

Kinetic studies were made for the degradation rate of single parabens assuming pseudo-first order kinetics (Lin et al., 2009; Gmurek et al, 2015; Velegraki et al., 2015; Bouarioua and Zerdaoui, 2017).

$$-\frac{dC}{dt} = k'.C \qquad \text{(Equation III.2)}$$

k' is the pseudo-first order degradation rate constant for each paraben (min^{-1}). The pseudo-first order degradation rate constant based in the fluence (UV-A dose), was also determined using a photon flux of 8.9 J/s.m^2.

III.2.3. Analytical Methods

The concentrations of the five parabens along the reaction time were analytically determined by high-performance liquid chromatography HPLC (UFLC, Shimadzu). The injection volume was 20 µL and the mobile phase consisted of a mixture of 50:50 methanol: acidic water (0.1% orthophosphoric acid) isocratically fed at a flow rate of 0.5 mL/min. A chromatography column, C18 from SiliaChrom, at 40 ºC was used and parabens were detected at 255 nm.

For determining total organic carbon (TOC) a TOC analyser (TOC-V CPN model, Shimadzu, Japan) coupled to an autosampler (model V-ASI, Shimadzu, Japan) was used. This TOC analyser uses nondispersive infrared analysis after oxidative combustion for measuring total organic carbon of the samples. Chemical oxygen demand (COD) was measured according to the standard method 5220D (Greenberg et al., 1985). Potassium hydrogen phthalate, obtained from Panreac, was used to prepare a calibration curve with COD values within the range 0-100 mg O_2/L. Absorbance after 2 h of digestion at 150 ºC (ECO25 – Velp Scientifica) were obtained at 445 nm in a WTW photolab S6 photometer.

pH was determined using a Crison micropH 2002 apparatus. UV-Vis absorbance spectra for the mixture of parabens were obtained with a T60 spectrophotometer from PG instruments. The surface areas of catalysts, *Brunauer–Emmet–Teller* (S_{BET}), and average pore diameters were determined using nitrogen (-196 °C) with an accelerated surface area and porosimetryanalyzer (ASAP 2000, Micrometrics).

The crystalline structure of the catalysts powders was evaluated by X-ray diffraction (XRD) analysis, through a diffractometer (Bruker D8 Advance). The diffractometer works with Cu Kα radiation (2.2kW ceramic tube). Using a 1D LynxEye detector (Silicon Drift Detector) covering an angle of ~3º and with ~25% energy resolution. Simultaneous differential scanning calorimetry (DSC)/Thermogravimetric analysis (TGA), normally called SDT, was made to evaluate thermal resistance of catalysts at high temperatures (maximum 1200 ºC). For this analysis a SDT Q600 (TA Instruments) was used with a heating rate of 10 ºC/min and a scan temperature from 25 to 1200 ºC.

III.2.4. Toxicity assessment

The toxicity of the initial parabens mixture and of by-products resulting from the photocatalytic degradation was assessed using three species: *A. fischeri, C. fluminea* and *L. sativum*. The aim of this part of the work was to verify if the treatments applied allow achieving a less harmful effluent for the mentioned species.

The inhibition of luminescence was measured to evaluate the toxicity of samples for bacteria *A. fischeri* using a LUMIStox 300 apparatus (Dr. Lange). The untreated and treated samples, for the different reaction conditions, were inoculated with the bacteria solution at 15 °C. The produced light was measured after 15 min and 30 min of incubation and compared with a blank, wherein bacteria was placed in a culture media solution (2% NaCl Solution).

Corbicula fluminea individuals were collected from a canal in Mira, Portugal (N40°25'06.90''/W8°44'13.18''), where a well-established population is found. Individuals with shell length in the range 20-30 mm were selected and immediately transported in field water to the laboratory, where they were gradually acclimated. Two replicates were made for each effluent concentration (50, 75 and 100% v/v) with a blank control (dechlorinated water).

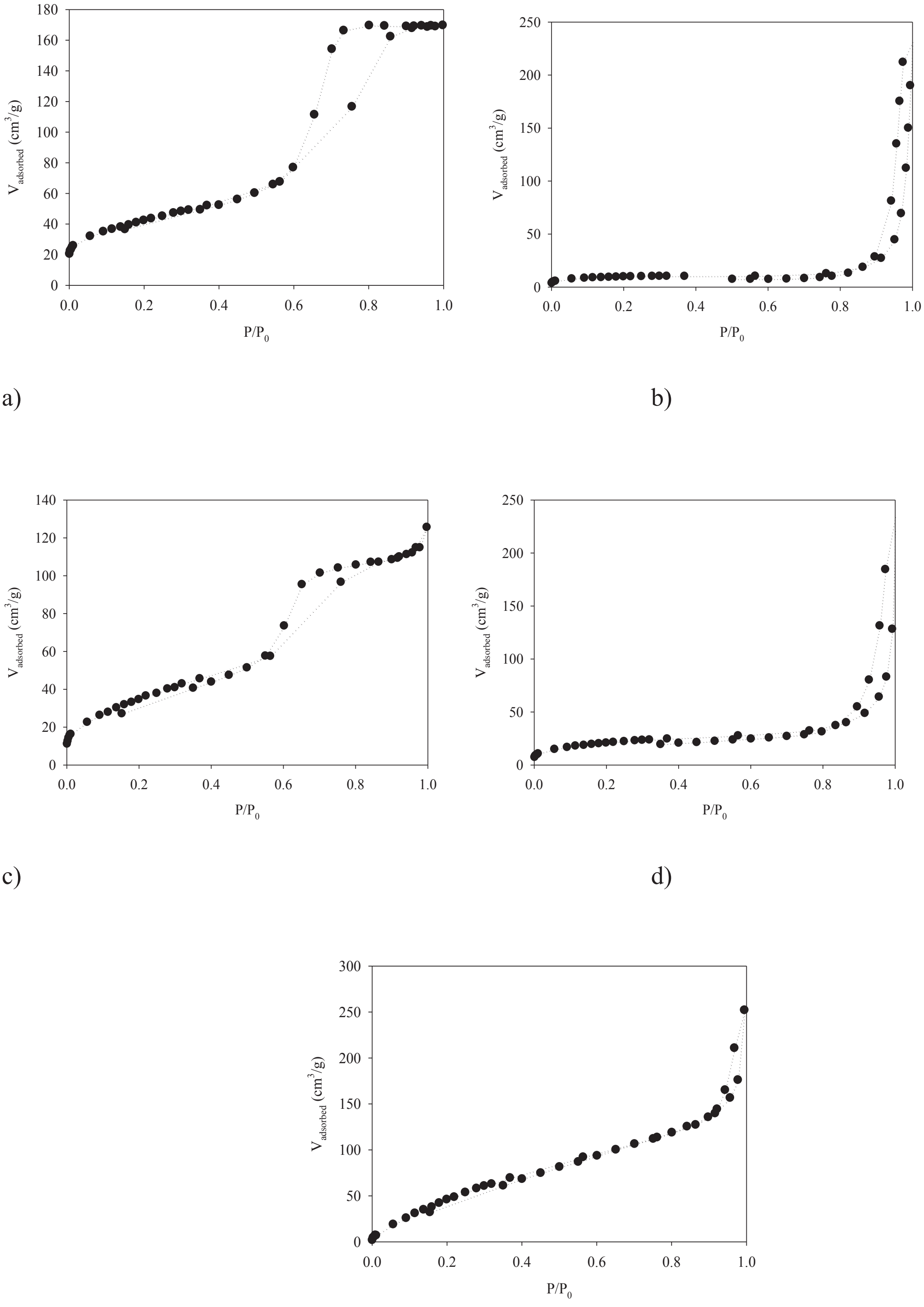
After 24 h acclimation period the clams were added to the effluents samples (20 clams per liter). The vessels were kept at constant temperature 20 ± 2 °C, under a 16 h^{Light} : 8 h^{Dark} photoperiod cycle and continuous aeration. The mortality of clams was assessed every 24 h along the test time based on siphoning activity evidence or resistance to valve opening when forced with a blunt dissection needle (Gomes et al., 2014).

For evaluating the phytotoxicity of initial and treated effluents, the number of *L. sativum* germinated seeds and the radicle growth were determined, while those germinated in contact with different concentrations of the raw or treated effluent were used to calculate the germination index (GI) (Trautmann and Krasny, 1997). In this test, 10 seeds of *L. sativum* were placed over a filter paper on a petri dish evenly spread. The volume of sample used was 5 mL for various concentrations of the effluent (50, 75, 100 % V/V). The petri dishes filled with the samples were placed in an oven for 48 h at constant temperature, 27 ^{o}C, in the dark. After this period, the number of seeds germinated and radicle growth in each sample was recorded. GI is the product of relative seed germination (RSG) and relative radicle growth (RRG). The phytotoxicity was evaluated based on the germination index according to the Trautmann and Krasny (1997) criteria. All the experiments were run at least in duplicate.

III.3. Results and Discussion

III.3.1. Catalysts Characterization

The BET analysis shows that the specific surface areas increase in the following order (values between brackets): Ag-TiO_2 (34 m^2/g) < Pd-TiO_2 (73 m^2/g) < Au-TiO_2 (135 m^2/g) < TiO_2 (149 m^2/g) << Pt-TiO_2 (238 m^2/g). Figure III.1 shows the N_2 isotherms for TiO_2 (a); Ag-TiO_2 (b); Au-TiO_2 (c); Pt-TiO_2 (d) and Pd-TiO_2 (e). Isotherms of type IV(a) were obtained for TiO_2 and Au-TiO_2 (Thommes et al., 2015) while type II isotherms were identified for Ag-TiO_2, Pd-TiO_2 and Pt-TiO_2. The BET surface area increase observed for Pt-TiO_2 when compared with pure TiO_2 may be related with the formation of large metal nanoparticles in TiO_2 surface and low dispersion of the metals. In fact, previous characterization studies for these catalysts involving SEM-EDS confirmed a low metal dispersion for Pt-TiO_2 whilst a good dispersion was achieved for the remaining materials.



a) b)

c) d)

e)

Figure III.1. N_2 isotherms obtained for a- TiO_2, b- Ag- TiO_2, c- Au- TiO_2, d- Pt- TiO_2 and e- Pd- TiO_2.

The XRD analysis of the catalysts used in these tests reveals that in all of them the anatase phase is clearly predominant (Figure III.2). The presence of noble metals onto TiO_2 surface did not change the XRD patterns of pure TiO_2 (anatase phase). This is in accordance with results found in literature for similar catalysts. For example, Huang et al. (2008) did not found the diffractive peak of platinum for 1.5 wt.% Pt-TiO_2 on XRD analysis, due to the low amount of noble metal used for doping TiO_2.

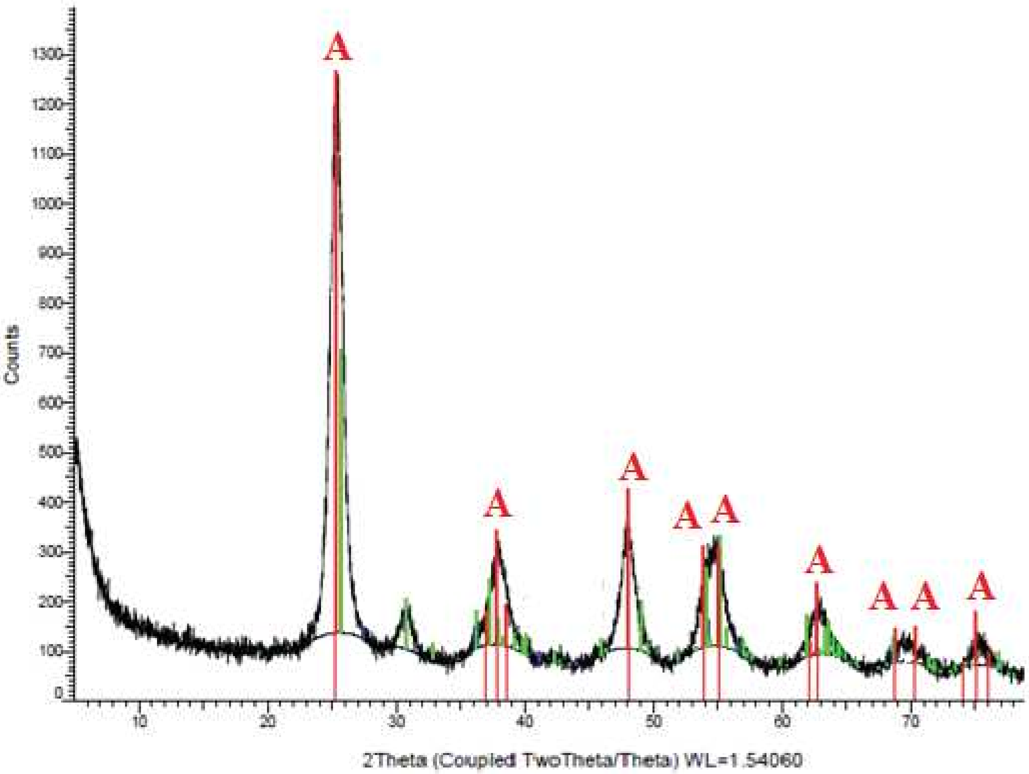


Figure III.2. X-ray diffraction patterns obtained for pure titanium dioxide, the "A" corresponds to reflections from anatase phase.

The SDT analysis of the catalysts powders allows evaluating the thermal stability at high temperatures (1200 ºC). The maximum weight loss at the maximum temperature was 10% for 0.5% Pt-TiO_2, and the minimum was 3% for the 0.5% Pd-TiO_2. For 0.5% Pd-TiO_2, 0.5% Au-TiO_2, 0.5% Ag-TiO_2 and pure TiO_2 the major weight loss was detected between room temperature and 200 ºC and was due to water evaporation from the surface of the catalyst (Figure III.3). For these catalysts, at higher temperatures no significant weight loss was glimpsed, which means that they are very thermally resistant and stable. For 0.5% Pt-TiO_2 in the initial temperature range (up to 200 ºC) 3% of weight loss was observed, and after this temperature 7% of weight loss was verified which can be attributed to the decomposition of organic solvents. However, a weight loss below 10% at this range of temperatures is not enough to consider this catalyst thermally unstable.

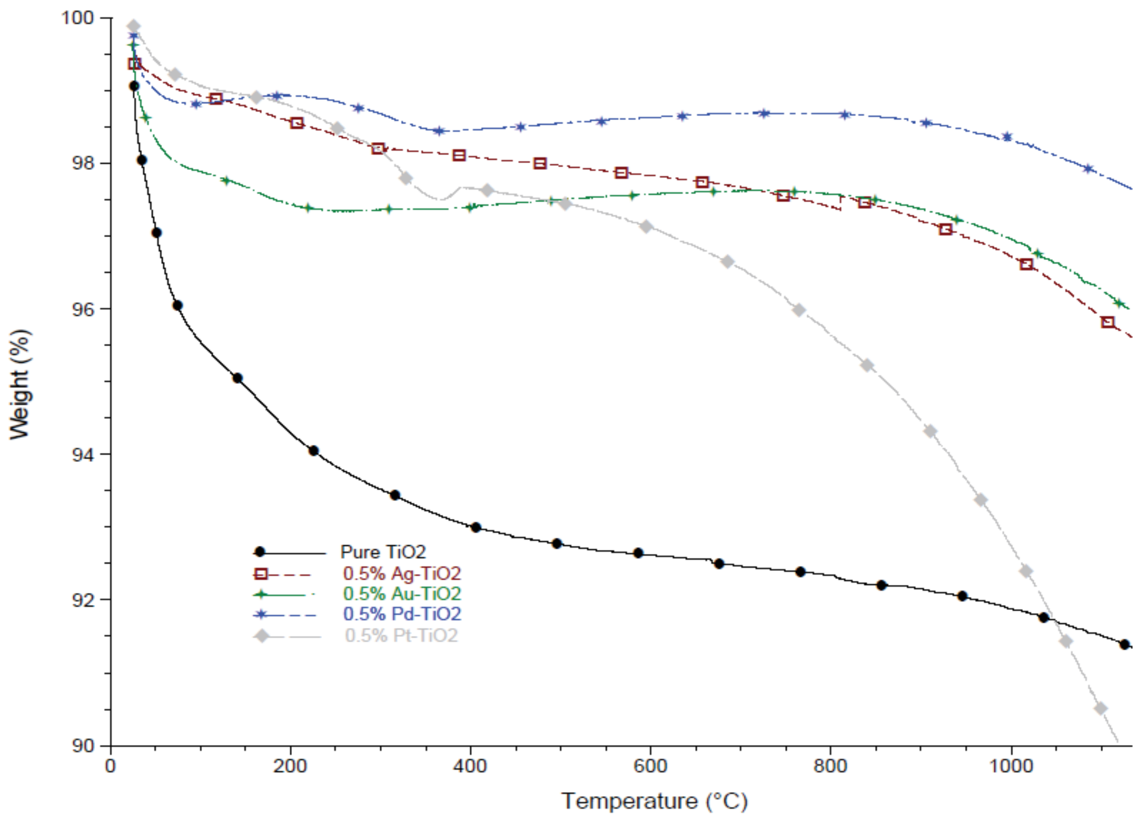


Figure III.3. SDT analysis of doped noble metals on titanium dioxide photocatalysts

III.3.2. Parabens photocatalytic degradation

The concentrations of the five parabens along the reaction time were analytically determined by high-performance liquid chromatography (HPLC). The direct photolysis of the parabens mixture was inefficient for their degradation (Figure III.4), achieving only 4% of benzylparaben degradation as the best result. For the other parabens, removal values were negligible in 180 min of reaction. In fact, the mixture absorbance spectrum shows that the parabens preferably absorb at a wavelength of 254 nm while the lamps used as light source have their maximum emission at 365 nm. Thus, under these conditions, a strong degradation of the contaminants should not occur.

The addition of catalysts can improve the efficiency of degradation (Oros-Ruiz et al. 2013; Cuerda-Correa et al. 2016; Durán-Álvarez et al. 2016; Ofiarska et al. 2016). The load of the tested catalysts (TiO_2, Au-TiO_2, Ag-TiO_2, Pt-TiO_2 and Pd-TiO_2) was 0.07 g/L, for minimizing the scattering and screening effects, which results in non-uniform light intensity distribution (Mendéz-Arriaga et al. 2008). Figure III. 4 shows the single parabens degradation versus time, for different catalysts, tested with UV-A irradiation. The results clearly show that palladium and silver supported on titanium dioxide were the most efficient catalysts for all experiments. However, total parabens degradation was not achieved for these catalysts at these experimental conditions in 180 min. The most difficult paraben to degrade at the conditions used is the methylparaben (MP) possibly because it presents the lowest ester chain attached to the benzenic ring less reaction sites are available for hydroxyl radicals. On the other hand, the heaviest paraben, benzylparaben (BeP), showed the highest degradation efficiency. This may occur because the higher molecular weight as well as two benzene rings that lead to more reaction sites are available in the molecule making it more prone for degradation. In fact, Gmukek et al. (2015) verified that the first order kinetic constant for the degradation of these 5 parabens through UVC-H_2O_2 followed a linear increase with the number of carbon atoms in the ester chain.

The results also show that the presence of noble metal nanoparticles on the titanium dioxide surface improves the parabens degradation (Figure III. 4). For all parabens, the degradation was higher for catalysts with metals deposited when compared with pure TiO_2. Previous studies reveal that the presence of loading metals on the semiconductor surface decreases the band gap (Wang et al., 2012; Zheng et al., 2011). Normally for TiO_2 alone the values of the band gap were above 3 eV requiring wavelengths lower than 400 nm (Pelaez et al. 2012; Wang et al. 2012). In our work, UV-A irradiation was used with the main wavelength at 365 nm which is near the wavelength necessary for exciting the electrons from the valence band to the conduction band of TiO_2. For anatase-type TiO_2, like pure TiO_2 used in our degradation experiments, the band gap is normally 3.2 eV, which corresponds to UV-light ($\lambda \leq 387$ nm) (Pelaez et al., 2012). This can be an explanation for the inefficiency of the low efficiency of pure TiO_2 for the parabens degradation, since the number of photogenerated electrons and consequent holes in the valence band of the semiconductor would be small. In fact, the methylparaben degradation under solar irradiation was tested for three different kinds of TiO_2 nanoparticles (Velegraki et al., 2015), namely Degussa P25 (70% anatase, 30% rutile), Kronos VIp 7000 (> 85% anatase) and 7001 (> 85% anatase), with a specific surface area of 55 > 225 and > 225 m^2/g, respectively. Normally, Degussa P25 is mainly activated under UV light irradiation ($\lambda < 400$ nm). On the other hand, Kronos VIp 7000 and 7001 are considered highly active under visible irradiation. In this study it was concluded that for 1 mg/L of MP and catalyst loading of 0.1 mg/L at pH 5.2, Degussa P25 reached a complete elimination of MP within 35-45 min. For the same conditions with the other TiO_2 powders (Kronos VIp and 7001) with higher anatase percentages only 9 and 7% of degradation MP were obtained, respectively (Velegraki et al., 2015). This supports the idea mentioned above related to the activity of TiO_2

nanoparticles (anatase-type) on photocatalytic degradation. The presence of the rutile phase on TiO_2 nanoparticles possibly reduces the band gap allowing TiO_2 to be excited with solar radiation ($\lambda > 400$ nm). Indeed, Zheng et al. (2011) proved that for radiation with a wavelength around 400 nm using naked-TiO_2 (anatase phase) no oxidation of benzene was observed. However, degradation can be improved by the addition of electron traps, such as deposited noble metals, to retard the electron-hole recombination in TiO_2.

The noble metals used in this work improved the photocatalytic activity of titanium dioxide (Figure III. 4), since their presence on the titanium dioxide surface improves electron transfer from the conduction band to metallic nanoparticles. This allows the occurrence of reduction reactions with these electrons present on the surface of the metal nanoparticles, while the photogenerated holes remain on the surface of the photocatalyst, where oxidation reactions can take place. The presence of metals delays the electron-hole recombination increasing the photocatalytic efficiency of TiO_2 (Liao et al. 2012). Subramanian et al. (2004) showed that in the presence of Au nanoparticles, when using UV- laser pulse, fewer electrons remain on the TiO_2 particles and proved, in the subnanosecond time scale, that charge recombination is significantly decreased in the presence of gold nanoparticles. Anpo and Takeuchi (2003) used electron spin resonance (ESR) signals to measure the intensity of Ti^{3+} on Pt-loaded TiO_2 and unloaded TiO_2 under UV light irradiation. The latter intensity increased while few changes were observed for that of Pt-TiO_2. These results indicate that in the presence of Pt the electrons coming from the valence to conduction band are quickly transferred from TiO_2 to platinum particles. These works explain the difference between the catalytic behavior of pure TiO_2 nanoparticles and of noble metals loaded TiO_2. However, it is important to understand why different noble metals can achieve different levels of parabens degradation. Figure III. 4 shows that parabens degradation increases according to the following order: TiO_2 < Au-TiO_2 < Pt-TiO_2 < Ag-TiO_2 ~ Pd-TiO_2.

Globally, our results depict that palladium, silver and platinum supported on TiO_2 have a higher efficiency on parabens degradation. Possibly Pd, Ag and Pt modified TiO_2 can produce more •OH radicals and superoxide radical ($•O_2^-$). Ofiarska et al. (2016) studied the effect of the presence of Pt on TiO_2 for two cytostatic drugs degradation and concluded that Pt significantly increased the production of •OH radicals, probably due to the better electron/hole separation. Moreover, the presence of superoxide radical ($•O_2^-$) was revealed. For hydrogen production from water-ethanol solutions, the activity of Au catalysts is about 30% lower than that of Pt on TiO_2, prepared by photodeposition (Bamwenda et al. 1995). Oros-Ruiz et al. (2013) show that

Ag and Au supported on TiO_2 achieved the highest photoactivities for degradation of trimethoprim, because these metals have the highest electron affinity, when compared with Cu and Ni. However, the difference on the efficiency of photoactivity between Ag and Au was not so large.

Among the noble metals tested in our work, the Au and Pt have the highest electron affinity, 223 and 205 kJ/mol, respectively. On the contrary, the lowest values were 125 and 54 kJ/mol, for Ag and Pd, respectively. In terms of electronegativity (Pauling scale), the metals can be ordered as Ag (1.93) < Pd (2.20) < Pt (2.28) < Au (2.54). With these electronegativities, it is possible to conclude that photogenerated electrons from the conduction band of TiO_2 are easily trapped by noble metal nanoparticles, producing more holes (h^+) on the valence band of TiO_2. Therefore, the Au, Pt, Pd and Ag allow higher production of •OH radicals. Meanwhile, the highest electron affinity occurs for gold (223 kJ/mol), so few superoxide radicals ($•O_2^-$) were produced because photogenerated electrons are probably highly retained in gold nanoparticles. Moreover, since gold also has the highest electronegativity (2.54, Pauling scale) it can attract more electrons, so lower amounts of O_2 can be reduced, leading, consequently, to lower parabens degradation.

The size, dispersion and interface contact of the noble metal Au and Pt with the semiconductor may be another reason for explaining the decrease on photocatalytic degradation (Zheng et al 2011). In fact, the previous results obtained for SEM-EDS show that a good dispersion of Pt was not achieved which may explain the lower efficiency of Pt-TiO_2 catalyst. Moreover, palladium and silver have the lowest electron affinity (54 and 125 kJ/mol, respectively), therefore possibly a higher amount of superoxide radicals ($•O_2^-$) can be produced with a consequent higher degradation of parabens (Figure III. 4). In fact, Velegraki et al. (2015) verified the effect of different radical scavengers on the methylparaben degradation (1 mg/L) with TiO_2 (0.5 g/L P25), under solar irradiation, for pH 5.2. They concluded that the presence of 5 mM 1,4-benzoquinone suppresses the MeP degradation, suggesting the predominance of superoxide radical ($•O_2^-$), a reactive species, on the degradation of this compound. Which can explain the highest catalytic activity of Ag and Pd-TiO_2.

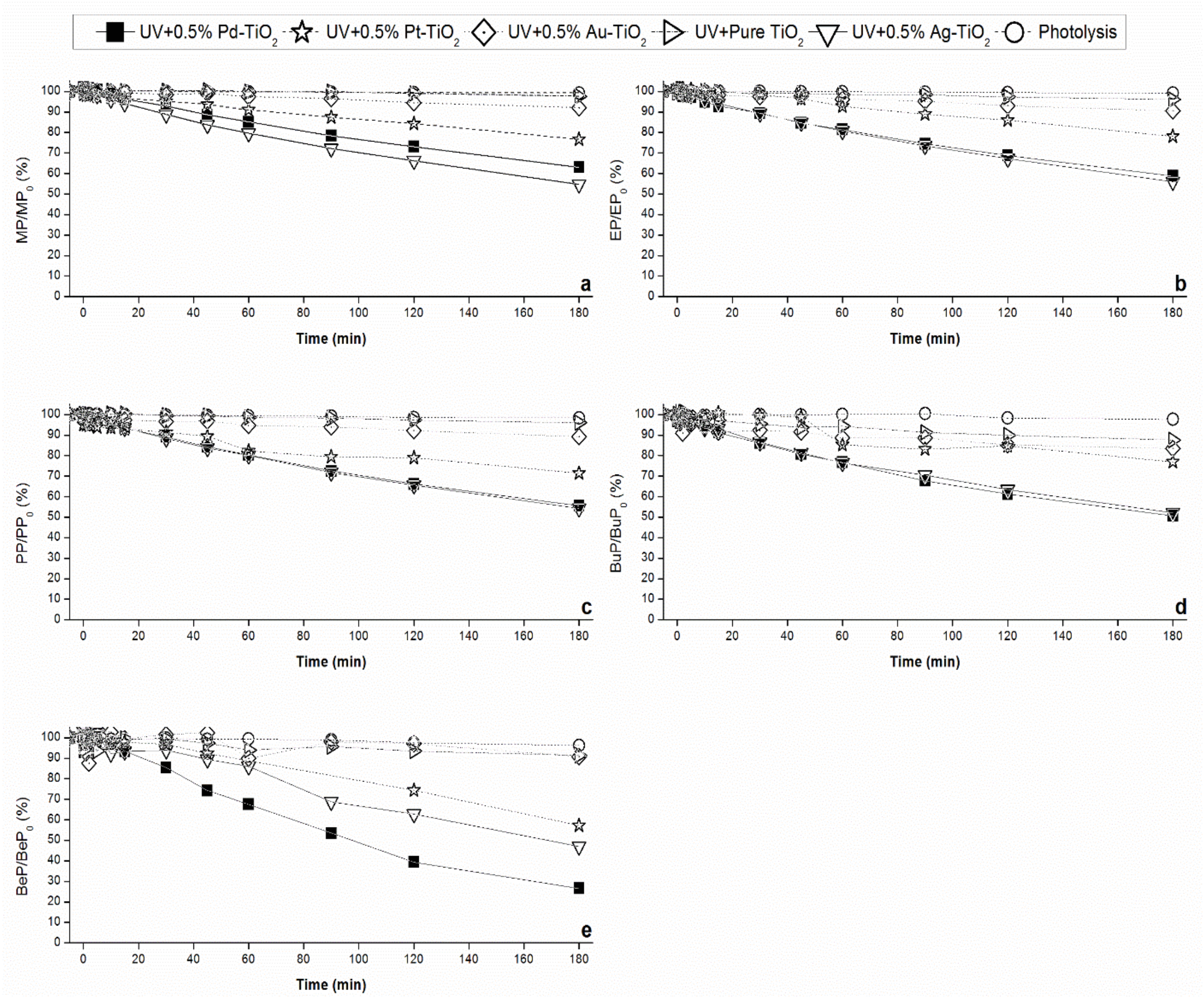


Figure III. 4. Normalized concentration of individual parabens (a – MP; b – EP; c – PP; d – BuP and e – BeP) during single and photocatalytic degradation of the mixture as function of time.

III.3.3. Single parabens degradation kinetic rate

Methyl-, ethyl-, propyl-, butyl- and benzylparabens photocatalytic degradation was studied using different noble metals nanoparticles deposited on titanium dioxide surface as photocatalysts. The photocatalytic degradation of parabens can be described by a pseudo-first order kinetic (Bourarioua and Zerdaoui, 2017) following eq. (III.2). When photolysis and photocatalytic oxidation using pure TiO_2 were applied, it was impossible to determine the pseudo-first order kinetic constants because the degradation of parabens in these conditions was very low after 180 min, as can be seen in Figure III. 4. For the other conditions tested, the pseudo-first order kinetic model fitted well the experimental results with coefficients of determination (R^2) ranging from 0.810 to 0.997. The different pseudo-first order kinetic constants for the catalysts used are summarized in Table III. 1. The values of these constants are between 0.00047 min^{-1} for 0.5% Au-TiO_2 on methylparaben degradation, and 0.00751 min^{-}

^{1} for 0.5% Pd-TiO_2 on benzylparaben degradation. These values generally increase with the molecular chain length linked to aromatic rings. For the methylparaben degradation, in general, the constant values were the lowest ones while the highest values were obtained for benzylparaben degradation (Table III. 1).

Table III. 1. Pseudo-first order kinetic rate constants (k') for MP, EP, PP, BuP and BeP degradation during photocatalytic oxidation for different catalysts and respective determination coefficients (R^2)

Catalyst	**k'$_{MP}$ (min^{-1}) (R^2)**	**k'$_{EP}$(min^{-1}) (R^2)**	**k'$_{PP}$(min^{-1}) (R^2)**	**k'$_{BuP}$(min^{-1}) (R^2)**	**k'$_{BeP}$(min^{-1}) (R^2)**
Pd-TiO_2	0.00275 (0.996)	0.00295 (0.996)	0.00336 (0.997)	0.00377 (0.995)	0.00751 (0.995)
Ag-TiO_2	0.00347 (0.996)	0.00330 (0.997)	0.00353 (0.996)	0.00371 (0.990)	0.00414 (0.960)
Pt-TiO_2	0.00135 (0.996)	0.00148 (0.995)	0.00167 (0.963)	0.00150 (0.830)	0.00345 (0.870)
Au-TiO_2	0.00047 (0.970)	0.00053 (0.975)	0.00057 (0.930)	0.00077 (0.810)	0.00071 (0.840)

As described above in the section Material and Methods, the determination of pseudo-first order rate constant based on the photon flux was also performed using E_o 8.9 J/s.m^2. Table III. 2 shows these photocatalytic reaction rate constants for all parabens, using noble metals supported in TiO_2 as catalysts. The highest photocatalytic constant, about 14.10 x 10^{-6} m^2/ J, was achieved for benzylparaben degradation with 0.5% Pd-TiO_2 (Table III. 2).

Table III. 2. Photocatalytic reaction rate based on the photon flux (k), for the various parabens and catalysts

Catalyst	**k_{MP} x 10^6 (m^2 / J)**	**k_{EP} x 10^6 (m^2 / J)**	**k_{PP} x 10^6 (m^2 / J)**	**k_{BuP} x 10^6 (m^2 / J)**	**k_{BeP} x 10^6 (m^2 / J)**
Pd-TiO_2	5.15 (0.996)	5.52 (0.996)	6.30 (0.997)	7.07 (0.995)	14.10 (0.995)
Ag-TiO_2	6.49 (0.996)	6.19 (0.997)	6.60 (0.996)	6.96 (0.990)	7.75 (0.960)
Pt-TiO_2	2.57 (0.996)	2.71 (0.988)	3.28 (0.933)	2.88 (0.833)	6.45 (0.870)
Au-TiO_2	0.88 (0.970)	0.99 (0.975)	1.08 (0.930)	1.45 (0.810)	1.32 (0.840)

III.3.4. Mineralization and COD removal

The efficiency of photolysis and photocatalytic degradation was compared in terms of mineralization, expressed in total organic content (TOC) and degradation of organic matter expressed in chemical oxygen demand (COD). TOC and COD of the initial mixture of parabens was 35 mg/L (±2%) and 95 mgO_2/L (±8%), respectively. TOC and COD removal by single photolysis was insignificant, about 4 and 5%, respectively. In terms of mineralization, the efficiency of the catalysts followed the order (efficiency between brackets): pure TiO_2 (11%) ~

Au-TiO_2 (11%) < Pt-TiO_2 (19%) < Ag-TiO_2 (22%) < Pd-TiO_2 (25%). The catalysts show the same efficiency order than the one discussed previously regarding parabens degradation. The best results in terms of mineralization were achieved with 0.5% Pd-TiO_2, with a final TOC value, after 3 h, of 26 mg/L, corresponding to a mineralization of about 25%. The pure TiO_2 and Au-TiO_2 just reached around 10 to 15% of each paraben degradation (Figure III. 4), and this low removal was also noticed in what regards COD and TOC abatements. In fact, for Au-TiO_2 only 8 and 11% of COD and TOC depletion were achieved, respectively. The corresponding values for TiO_2 were 10 and 11%. Pd-TiO_2 led to the highest abatement on TOC and COD, around 25% and 19%, respectively, which, taking into account that total degradation of parabens was not achieved, corresponds to a good mineralization level. Velegraki et al. (2015) measured the TOC removal for different stages of methylparaben (initial concentration 10 mg/L) degradation with solar irradiation, at pH 5.2 and for a catalyst loading of 0.5 g/L (Degussa P-25). The authors verified that for 39 % of MP degradation around 20 % of TOC removal was achieved. In our study, for palladium and silver (the best catalysts on parabens degradation and organic matter removal) with 0.07 g/L of catalyst loading and a mixture of five parabens (10 mg/L of each one), a better TOC removal was observed, for the same degradation of MP; nevertheless, it should be remarked that Velegraki et al. (2015) used solar light while in our case UV-A irradiation was applied.

Thus, on a global analysis, regarding mineralization (values between brackets) the best results were obtained for 0.5% Ag-TiO_2 (22%) and 0.5% Pd-TiO_2 (25%). However, for COD removal 0.5% Pt-TiO_2 can also be considered as a promising catalyst since 18% of COD were removed using this catalyst during 3 h.

The results showed that photolysis alone has a low efficiency on COD and TOC removal and that it can be improved by photocatalytic degradation. However, the resulting refractory compounds of photocatalytic degradation are difficult to remove. Moreover, with single photolysis and photocatalytic oxidation using pure TiO_2 and 0.5% Au-TiO_2, a great amount of parabens from the initial mixture persists after the treatments. Therefore, it is important to evaluate the toxicity of the effluent obtained after performing the treatments.

III.3.5. Toxicity assessment

The toxicity studies were made for inferring if the treated solutions are still toxic to different species when compared with the initial parabens mixture.

Acute toxicity of the samples was evaluated by different methods including the luminescence inhibition of the marine bacteria *A. fischeri*, after 15 and 30 min of exposure. The luminescence inhibition of *A. fischeri* was above 80% for all the samples obtained from the photocatalytic experiments, indicating that the parabens concentration is still very high and thus the level of degradation was low. According to Miralles-Cuevas et al. (2017), the luminescence inhibition test is not sensitive to changes when the values are higher than 80%. Thus, our results, only allow the conclusion that the treated effluents are still toxic towards *A. fischeri*.

L. sativum and *C. fluminea* are more resistant species than *A. fischeri*. For single photolysis, pure TiO_2 and 0.5% Au-TiO_2 toxicity responses were like those of the initial mixture of parabens, because the concentration of parabens after treatment at these conditions are near the initial ones. In the case of Asian clams, for the same conditions, the presence of parabens in high dosage was clearly detected because the response of *C. fluminea*, which included abnormal foot extension and paralysis, was again similar before and after the treatments.

The toxicity assessment of *L. sativum* was classified in terms of germination index for different concentrations (100, 75 and 50 % V/V). For the initial mixture of parabens this value was around 40%, which according to the Trautmann and Krasny (1997) criteria, means that the sample is very phytotoxic. After the treatments with the three best catalysts (Pt-TiO_2, Ag-TiO_2 and Pd-TiO_2) the phytotoxicity of the initial mixture of parabens was reduced (Figure III. 5). It should be reminded that for the other operating conditions (photolysis and photocatalytic oxidation using pure TiO_2 and Au-TiO_2) the toxicity of the treated solutions was similar to that of the parent mixture of parabens. In terms of catalytic oxidation of parabens, the worst catalyst among those three selected before (Ag-TiO_2, Pd-TiO_2 and Pt-TiO_2), Pt-TiO_2, shows the higher phytotoxicity towards *L. sativum*, with a germination index of around 57%. This represents a moderate phytotoxic behavior that was also observed with 0.5% Ag-TiO_2, that led to a similar germination index (61%). These values can be related with the low degradation of benzylparaben, 40% and 50%, respectively, achieved by these two photocatalysts. In fact, for 0.5% Pd-TiO_2 the germination index achieved 70% and, in this case, the degradation of that compound was higher (75%) in comparison with that of the other two materials.

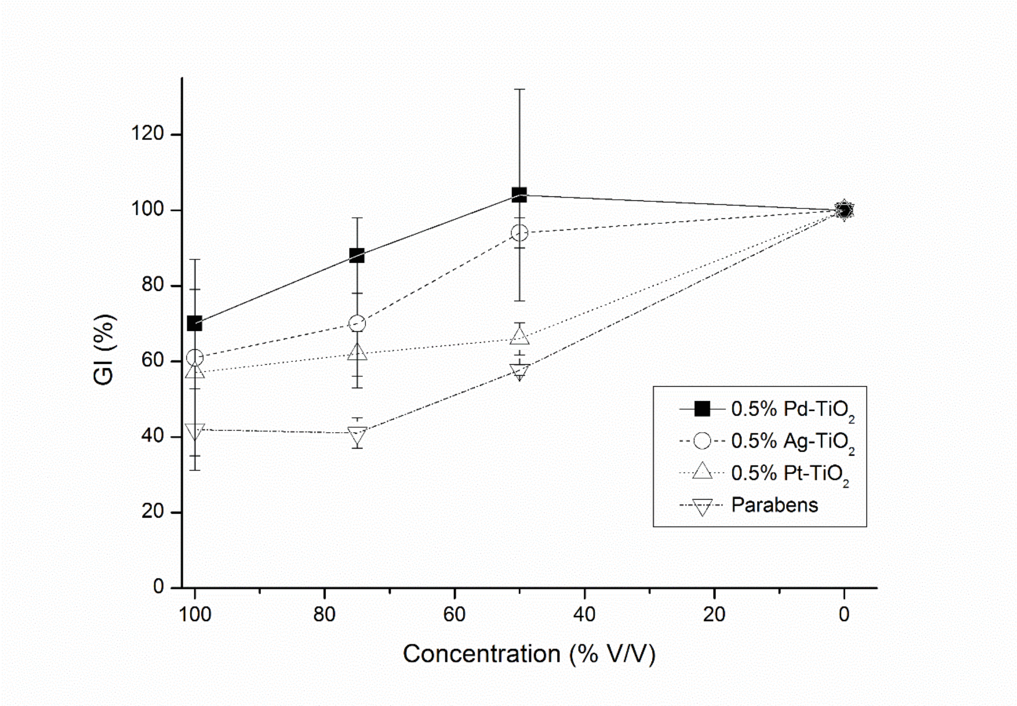


Figure III. 5. GI for the initial parabens mixture and after 180 min of photocatalytic oxidation using 0.5% Pd-TiO_2, 0.5% Ag-TiO_2 and 0.5% Pd-TiO_2, as a function of the sample concentration.

Figure III. 5 also shows that for undiluted sample solutions (100% of sample) moderate phytotoxic values were obtained for all the treated solutions. However, this phytotoxicity level changes to non-phytotoxic when a concentration of 75% (V/V) of the sample obtained by photocatalysis with Pd-TiO_2 was used. This indicates that a small dilution of that sample leads to an effluent with low phytotoxic features. The solution with a concentration of 50% (V/V) of the sample, obtained after photocatalytic oxidation using Pt-TiO_2, is also associated with a moderate phytotoxic level.

Asian clams are intolerant to parabens, when submitted to a mixture of five parabens at concentrations higher than 7.5 mg/L each, as can be seen in the normalized response described above in the acute toxicity tests (Figure III. 6). In our preliminary studies, for Au-TiO_2, pure TiO_2 and single photolysis the parabens concentration after treatment was higher than 8.5 mg/L, so the clam's response with these samples showed also abnormal foot extension and paralysis. This points out the high impact that the same samples still have on *C. fluminea*. For the treatment involving 0.5% Pt-TiO_2 the parabens concentration after 180 min decays to values lower or around 7.5 mg/L, but when in contact with that sample, *C. fluminea* do not show the response described before (abnormal foot extension and paralysis). In fact, a reduction of toxicity was verified comparing it with that of the initial parabens, since for the maximum concentration (100%) of that treated sample the mortality was around 55% when compared with the 100% of mortality obtained for the raw mixture at the same conditions (Figure III. 6).

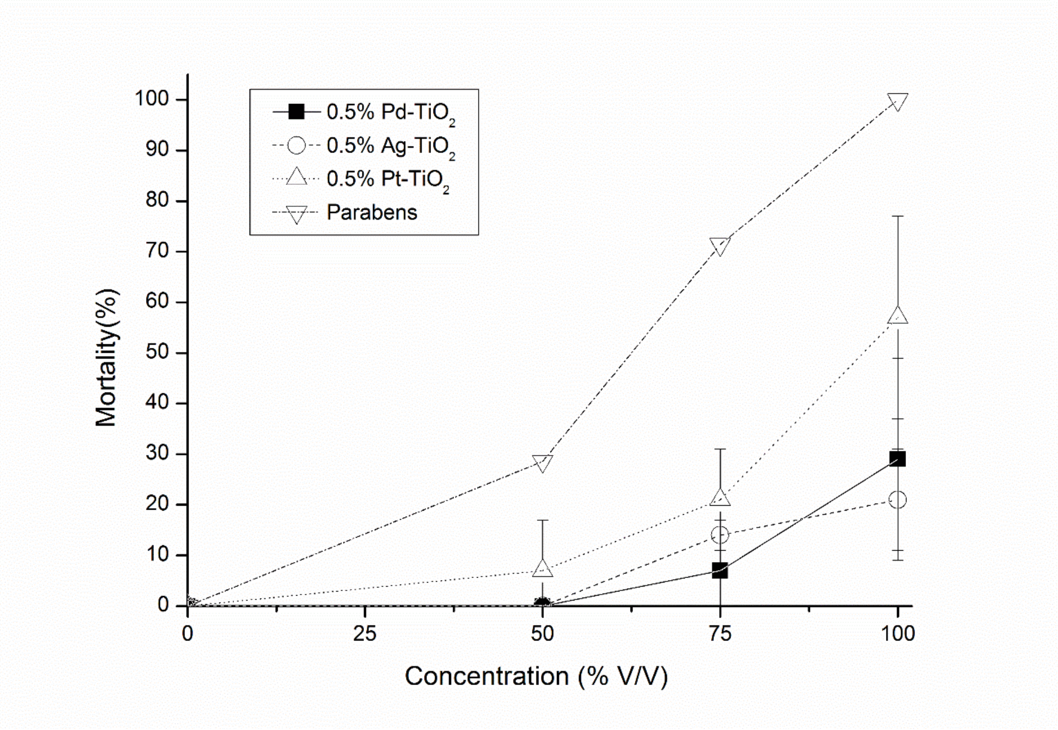


Figure III. 6. Concentration-response data for *C. fluminea* exposed during 72 h to different concentrations of the resulting solutions, obtained after 180 min of photocatalytic oxidation using 0.5% Pd-TiO_2, 0.5% Ag-TiO_2 and 0.5% Pd-TiO_2, and to the initial effluent.

For the other treatments applied (photocatalytic oxidation using Ag-TiO_2 or Pd-TiO_2), the treated solutions were observed not to be able to kill more than 50% of the exposed clams at the maximum concentration (100% V/V) (Figure III.6). The 0.5% Ag-TiO_2 and 0.5% Pd-TiO_2 clearly reduce toxicity leading to 21% and 29% mortality at the maximum concentration, respectively (Figure III. 6).

These results show that 0.5% Pt-TiO_2, 0.5% Ag-TiO_2 and 0.5% Pd-TiO_2 reduce the toxicity of parabens towards *C. fluminea*. Thus, gathering up all these results, it seems that these materials are promising catalysts for parabens detoxification through photocatalytic oxidation.

III.4. Conclusions

The photocatalytic oxidation of a parabens mixture (MP, EP, PP, BuP and BeP) using noble metals (Ag, Au, Pd, Pt), supported in TiO_2 and pure TiO_2 under UV-A irradiation, was performed. The results indicate that 0.5% Ag-TiO_2 and 0.5% Pd-TiO_2 were the best catalysts in terms of parabens degradation. However, all the tested materials improved the parabens degradation compared with single photolysis. The parabens degradation rate was generally higher for the compounds with the longer molecular chain length. The COD and TOC removals were improved by the presence of catalysts in photo experiments. For TOC removal the best results were obtained for the same catalysts that lead to higher degradation of parabens. On the other hand, higher COD removal was achieved with Pd-TiO_2 and Pt-TiO_2, but the difference with Ag-TiO_2 was not so sharp. The values for the degradation kinetic constants generally increase with molecular chain length linked to aromatic rings. The materials catalytic activity was related with the metals dispersion over TiO_2 as well as with the noble metals electron affinity.

The low COD and TOC removals obtained make it necessary to verify if the treated solutions were less toxic than the initial mixture of parabens. For this reason, toxicity analysis was evaluated for all catalysts tested. The *A. fischeri* luminescence inhibition was impossible to determine because the toxicity after treatment remains high for these sensitive bacteria. The GI for *L. sativum* increased from 40% (before treatment) to 70% (after 180 min of treatment) when 0.5% Pd-TiO_2 was used as photocatalyst. Moreover, the Asian clam mortality decreased from 100% to 21% for the treated solution obtained applying photocatalytic oxidation using 0.5% Ag-TiO_2.

Globally, 0.5% Ag-TiO_2 and 0.5% Pd-TiO_2 showed to be the most active catalysts for the detoxification of industrial wastewater containing parabens mixtures.

III.5. References

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IV. Noble metal – TiO_2 supported catalysts for the catalytic ozonation of parabens mixtures

It is based on the publication: Gomes, J., Bednarczyk, K., Gmurek, M., Stelmachowski, M., Zaleska-Medynska, A., Bastos, F. C., Quinta-Ferreira, M. E., Costa, R., Quinta-Ferreira, R. M., Martins, R. C. (2017a) Noble metal – TiO_2 Supported Catalysts for the Catalytic Ozonation of Parabens Mixtures. Process Safety and Environment Protection. 111, 148-159.

IV.1. Introduction

Parabens are commonly used as antimicrobial and preservatives in food, textiles, drinks, pharmaceutical and personal care products (Canosa et al., 2007a; Calafat et al., 2010; Meeker et al., 2013).

As a result, these compounds are widely present in urban and industrial effluents and have been detected in living organisms (Peng et al., 2006), soil (Núñez et al., 2008), air, dust (Canosa et al., 2007a, b), and aquatic resources such as rivers (Bolz et al., 2001; Kasprzyk-Hodern, et al., 2008) and even drinking water (Gibs et al., 2007). The presence of parabens in water is alarming due to the ecological and human health risks they pose and has prompted an increasing number of studies in this area. Darbre et al., (2004) showed that parabens are linked to human breast tumors. Parabens can be easily assimilated by the human body and have been found in urine (Janjua et al., 2008; Calafat et al., 2010), milk (Schlumpf et al., 2010) and serum (Janjua et al., 2007).

The main sources of parabens in waterbodies are wastewater treatment plant discharges (Bledzka et al., 2014), which means that conventional wastewater treatment is not able to deal with these compounds (Bledzka et al., 2014). Parabens biodegradation occurs by natural means to some extent, but it is a slow process. The process is also retarded by the length of the alkyl chain and the chlorination degree (Bledzka et al., 2014).

Advanced oxidation processes (AOP), either as an alternative or a complement to traditional wastewater treatment, have been attracted increasing attention in the context of parabens removal from effluents. Ozonation (Tay et al., 2010), photo-chemical technologies (Hansen and Andersen, 2012; Gmurek and Miller, 2012; Gmurek et al., 2012; Gmurek et al., 2015), and photosonochemical degradation (Daghrir et al., 2014) have been successfully used to degrade these compounds. Fenton's process, using the optimized combination of H_2O_2/Fe^{2+}, was tested for the degradation of methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BuP), with generally good results despite the long reaction time (Domínguez et al., 2014). Cost-efficiency makes AOP's attractive as a solution for treating effluents contaminated parabens (Martins et al., 2016).

Among the AOP above, ozonation is a promising technology for the treatment of recalcitrant organic compounds. Ozone is a powerful oxidant, which can act in a direct pathway and/or produce hydroxyl radicals that can degrade refractory compounds (Martins and Quinta-Ferreira, 2009). Tay et al., (2010) published data on ozone degradation for a mixture of five parabens Methylparaben (MP), Ethylparaben (EP), Propylparaben (PP), Butylparaben (BuP) Benzylparaben (BeP), with initial concentrations 15.2, 16.6, 18.0, 19.4 and 22.8 mg/L, respectively. For pH 6.9 and 0.67 g/h ozone, the authors reported parabens removal efficiency of 99% in 12 min. However, mineralization was low, 32% of total organic carbon (TOC) abatement for 3 hours of ozonation. Ozonation alone can be a source of refractory by-products once total mineralization levels are difficult to achieve. The use of heterogeneous catalysts with ozonation may improve the process, allowing higher levels of mineralization and increased time-effectiveness (Martins and Quinta-Ferreira, 2009; Martins and Quinta-Ferreira, 2011; Cuerda-Correa et al., 2016).

Lin et al., (2009), studied the photocatalytic degradation of methylparaben (MP). Only 5% of the initial MP was removed after 120 min of photolysis; the removal rate increased to 90% when 2 g/L of TiO_2 was added to the process in the same conditions. The TiO_2 catalyst is widely used because it is inexpensive and non-toxic, being a good choice for degradation of parabens (Lin et al., 2011; Chuang et al., 2012; Atheba et al., 2013; Petala et al., 2015).

In the literature several mechanisms are proposed for catalytic ozonation. Kasprzyk-Hordern et al., (2003), proposed three possible pathways that explain how the heterogeneous catalysts enhance ozone action over contaminants: chemisorption of organic compounds followed by oxidation due to the ozone dissolved in the bulk; adsorption of ozone on the catalyst leading to the production of hydroxyl radicals that will interact with the pollutants in the liquid or chemisorption of both ozone and organic molecule followed by surface reaction between chemisorbed species. The adsorption of ozone molecules on catalyst surface can occur by different ways, such as, physical adsorption, formation of hydrogen bonds with surface hydroxyl groups, molecular adsorption into weak Lewis sites and dissociative adsorption with strong Lewis sites (Bullanin et al., 1995). Béltran et al., (2002) described the adsorption of ozone on TiO_2 free active sites, followed by decomposition of ozone. Meanwhile, Rosal et al., (2008), described that also the adsorption of organic molecules on catalyst surface was possible in TiO_2, followed by reaction between adsorbed ozone and contaminants. However, the authors also refer the possibility of oxidation due to molecular ozone or hydroxyl radicals (produced due to the decomposition of ozone in the catalyst surface) in the bulk.

The catalysts selected (Au, Ag, Pt, Pd supported onto TiO_2 and pure TiO_2) have been used in the photocatalytic oxidation of benzene (Zheng et al., 2011). Recently, Durán-Alvarez et al., (2016), investigated the photocatalytic degradation of ciprofloxacin using mono Ag and Au (1.5% wt) metallic nanoparticles supported on TiO_2. For a concentration of catalyst of 0.5 g/L with UV-C complete degradation of the compound was achieved after 90 min of reaction, with total mineralization occurring in around 180 min, for both catalysts.

The work presented here studied the increase of ozonation efficiency promoted by the noble metals supported on titanium dioxide. To the best of our knowledge this was the first time that ozonation was used with those catalysts. Some authors used only ozonation with unloaded TiO_2 (Beltrán et al., 2002; Yang et al., 2007; Benitez et al., 2011). Literature reports the use of these catalysts for other oxidation processes (Lin et al., 2000; Peng and Wang, 2007; Zhang and He, 2007; Saqer et al., 2009). Noble metals are more expensive compared to transition metals. However, they have the capacity to regenerate, resistance to deactivation, and high specific activity that may be interesting for industrial applications. In fact, these metals may enhance TiO_2 redox capacities increasing its efficiency as catalyst.

One of the main concerns about parabens is their potential impact on ecosystems and human health. In this context, the toxicity of the parabens mixture was considered as a key dimension to evaluate the performance of the treatment processes under study. The toxicity of the mixture before and after treatment was assessed using the *Aliivibrio fischeri*, *Corbicula fluminea* and *Lepidium sativum* as model species. The impact of treated mixtures on neuronal activity (Wistar rats) was also investigated. This combination of tests covers a broad spectrum of organism complexity levels, and therefore provides a broad view of the potential impact the initial contaminants and the compounds resulting from their decomposition may have on environment and human health. The main aim of the toxicological analysis run here was to infer the treatments potential for toxicity removal rather than formal risk assessment.

Nowadays, the quality of drinking water is one of the major issues concerning human health, being the evaluation of the treated effluents in mammalian preparations highly desirable. It is now well established the involvement of reactive oxygen species (ROS) in a large range of health problems including common central nervous system diseases. For this reason, this work addressed the effect of untreated and treated effluents on a neuronal preparation. In the last decades brain plasticity has been largely studied at neuronal synapses from the hippocampus. In particular, the mossy fiber synapses from area CA3, undergo a form of plasticity (LTP-Long term potentiation) that is considered to represent a model for learning and memory formation at the cellular level (Fekete et al., 2008). These synapses are involved in various forms of neurological disorders and have unique features including one of the highest synaptic zinc concentrations in the brain (Frederickson et al., 2000). Upon release, zinc interacts with a variety of cellular proteins (Quinta-Ferreira et al., 2004), leading, when in excess, to the formation of reactive oxygen species (ROS) and eventually cell death (Oteiza et al., 2004; Nicoll and Schmitz, 2005; Sensi et al., 2011). In this work the toxicity of treated effluents was also evaluated on mammalian neuronal cells by measuring ROS signals (Setsukinai et al., 2003) using the fluorescent indicator H_2DCFDA.

The main aim of this work was to evaluate the degradation of a mixture of five parabens, methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BuP) and benzylparaben (BeP) using single and heterogeneous catalytic ozonation. The processes that were assessed were O_3, O_3 / 0.5% Ag-TiO_2, O_3 / 0.5% Au-TiO_2, O_3 / 0.5% Pd-TiO_2, O_3 / 0.5% Pt-TiO_2 and O_3 / TiO_2. This will allow to infer about the potential of using noble metals to enhance TiO_2 catalytic activity. Moreover, special interest was given to the environmental impact of raw and treated waters by covering a wide range of species.

IV.2. Materials and methods

IV.2.1. Chemicals and Catalysts

Methylparaben (MP), ethylparaben (EP), propylparaben (PP), benzylparaben (BeP), were obtained from Sigma-Aldrich, while butylparaben (BuP) was purchased from Fluka. The solution used in this work consisted of a mixture of these five parabens (10 mg/L each) in ultrapure water. This is a commonly used parabens concentration found in literature dealing with the degradation of these pollutants (Lin et al., 2009; Lin et al., 2011; Gmurek et al., 2015; Martins et al., 2016).

Titanium (IV) isopropoxide (TIP) (97%) was purchased from Aldrich Chem. and was used as titanium source for the preparation of TiO_2 nanoparticles. TiO_2 – based catalysts were obtained by two methods as described below:

a) sol-gel method (titania doped with transition metals):

Au – modified TiO_2 was prepared from titanium (IV) isopropoxide, known to be the titanium source origin for the anatase-type TiO_2. Titanium (IV) isopropoxide was mixed with an appropriate amount of the dopant precursor – $AuCl_4K$ (0.5 wt.%), methanol and distilled water. The solution was stirred at room temperature for 2 h, followed by a 24 – hour thermal treatment (45ºC) and calcination at 400ºC for 2 h.

b) photodeposition of noble metals at the surface of titanium dioxide:
The catalysts were obtained by UV-reduction of Pt^{4+}, Pd^{2+}, Ag^{+} ions in the TiO_2 suspension. An aqueous solution of isopropanol containing H_2PtCl_6 (0.5 wt.%) or $PdCl_2$ (0.5 wt.%) was degassed with nitrogen and irradiated by UV-Vis light (1000 W Xe lamp) for 6 h. An aqueous solution of ethanol containing $AgNO_3$ (0.5 wt.%) was degassed with nitrogen and irradiated by UV-Vis light (1000 W Xe lamp) for 100 min. The modified TiO_2 catalysts were separated by centrifugation and dried at 65 - 120 ºC for 12 h.

IV.2.2. Experimental procedure

The experiments were carried out in a 2L glass reactor with controlled temperature (25 ± 1 º C) by a thermostatic bath. The reactor was covered with aluminum foil to avoid the interference of light. The previously prepared parabens solution was placed inside the reactor with magnetic agitation at 700 rpm. This stirring speed was optimized in preliminary studies, to ensure homogeneity of media and chemical regime. Ozonation time in all the tests was the same (120 min), being samples withdrawn along the reaction ensuring that no more than 10% of the reactor initial volume was taken. Samples were immediately centrifuged at 3500 rpm (Nahita 2655). Moreover, the samples used in toxicity assessment were filtrated using cellulose acetate filters with pores of 0.45 μm. In catalytic experiments, the catalyst was placed in contact with the solution 5 min before applying ozone, to test the catalyst adsorption capacity without ozone contact. In this work the amount of catalyst used was 0.07 g/L. The aim was to use a minimal catalyst dosage. In fact, supported catalysts based on noble metals allow a good degradation efficiency of parabens using a very low catalyst loading. Moreover, the use of high catalyst amounts may lead to unrealistic catalytic activity connected with unnoticeable impurities present in the catalyst or some adsorption effects of ozonation by-products (Nawrocki, 2013).

Ozone was produced by an ozone generator (802N, BMT) from pure oxygen stream (99.9%). The ozone concentration incoming and leaving the reactor was measured by gas ozone analysers (BMT 963 vent, BMT) and (BMT 964 vent, BMT) respectively. With these values the ozone consumption could be calculated and also the amount of ozone necessary to degrade the parabens mixture. Therefore, the results were expressed as a function of the transferred ozone dose (TOD). On the other hand, to know the ozone mass transfer, the injected ozone dose (IOD) was determined. These two variables were calculated as follows:

$$\mathrm{IOD}=\int_0^t \frac{Q_{Gas}}{V_{Liquid}}\times[O_3]^{in}\times dt \qquad \text{(Equation IV.1)}$$

$$\mathrm{TOD}=\int_0^t \frac{Q_{Gas}}{V_{Liquid}}\times\left([O_3]^{in}-[O_3]^{out}\right)\times dt \qquad \text{(Equation IV.2)}$$

Where Q_{Gas} represents the gas flow rate (0.2 L/min), V_{Liquid} is the volume of the effluent used in the reactor, $[O_3]^{in}$ and $[O_3]^{out}$ are the ozone concentrations incoming and leaving the reactor, respectively. These two parameters (IOD and TOD) were expressed in mg O_3/L. The remaining ozone that leaves the reactor was destroyed by a solution of 2% (w/v) potassium iodide (Panreac).

IV.2.3. Analytical Methods

The concentrations of the five parabens along the reaction time were analytically determined by high-performance liquid chromatography HPLC (UFLC, Shimadzu). The injection volume of samples was 20 μL and the mobile phase (0.5 mL/min) consisted in a mixture of 50:50 methanol: acidic water (0.1% orthophosphoric acid). A chromatography column, C18 from SiliaChrom, at 40ºC was used and parabens were detected at 255 nm. Chemical oxygen demand (COD) was determined according to the standard method 5220D (Greenberg et al., 1985). Potassium hydrogen phthalate, obtained from Panreac, was used to prepare a calibration curve with COD values within the range 0-100 mg O_2/L. This calibration curve was selected because the COD values for the initial mixture of parabens in ultrapure water was around 95 mgO_2/L. Absorbance values after 2h of digestion at 150 ºC (ECO25 – Velp Scientifica) were measured at 445 nm in a WTW photolab S6. A TOC analyser (TOC-V CPN model, Shimadzu, Japan) coupled to an autosampler (model V-ASI, Shimadzu, Japan) was used for determining total organic carbon (TOC) using nondispersive infrared analysis after oxidative combustion. pH was determined using a Crison micropH 2002 apparatus. The surface area of catalysts, *Brunauer–Emmet–Teller* (S_{BET}) were analysed using nitrogen (-196°C) with an accelerated surface area and porosimetry analyzer (ASAP 2000, Micrometrics). The microstructural analysis of the surfaces was performed using a TESCAN VEGA 3 SBH - Easy Probe Scanning Electron Microscopy (SEM) with a tungsten heated cathode. The SEM images were acquired with a working tension of 5 kV and using the secondary electrons detector. The Energy Dispersive X-Ray Analysis (EDS) of the electrodes was conducted with the Bruker QUANTAX system that includes the Bruker Nano XFlash® detector. The Bruker Nano XFlash® detector is an energy dispersive X-ray detector that works according to the principle of the silicon drift detector (SDD). For the data analysis, the ESPRIT 1.9 Software was used. The EDS analysis was performed with an accelerating potential of 20 kV and the time for the spectrum accumulation was 60 s (real time) with a dwell time of 16 ms. The Energy-Channel calibration was performed with a copper standard using the ka line (8.0463 keV). Several different points in the samples were tested.

IV.2.4. Toxicity evaluation

Before toxicity evaluation, it was necessary to correct the pH of the samples to values within the range 6.5 - 7.5. The required pH was obtained by adding sodium hydroxide or sulfuric acid solutions to the samples.

The inhibition of luminescence was measured as an indicator of the toxicity of the samples towards *A. fischeri* using a LUMIStox 300 apparatus (Dr. Lange).The organisms were exposed to untreated and treated samples for the different reaction conditions, at 15 °C. Luminescence was measured after 15 min and 30 min and compared with a blank, where bacteria were placed in culture media solution (2% NaCl solution).

C. fluminea individuals were collected from a canal in Mira, Portugal (N40°25'06.90''/W8°44'13.18''), where a well-established population (density above 2000 clams m^{-2}) exists. Clams were collected by sieving sediment into a 5-mm mesh bag. Individuals with shell length in the range 20-30 mm were selected and immediately transported in field water to the laboratory, where they were gradually acclimated to the test conditions. The toxicity tests were run in vessels containing 7 clams in 350 mL of liquid. Two replicates were prepared for each effluent concentration (25, 50, 75 and 100% V/V) along with a blank control. The effluents were dosed after 24 h of acclimation. The vessels were kept at constant temperature 20 ± 2 °C, under a 16 h^{Light}: $8h^{Dark}$ photoperiod cycle and continuous aeration. The mortality of clams was assessed every 24 h for 72 h based on the animal's siphoning activity and their resistance to valve opening when forced with a blunt dissection needle (Gomes et al., 2014).

To evaluate the phytotoxicity of the initial and treated effluents, the number of germinated seeds and the radicle growth of *L. sativum* were determined to calculate the germination index (GI) (Trautmann and Krasny, 1997). In this test, 10 seeds of *L. sativum* were evenly spread on a filter paper in a petri dish. The volume of effluent sample used was 5 mL for the same concentration levels described above. The experiments were run in duplicate. The petri dishes filled with the samples were placed in the oven for 48h at constant temperature of 27 ^{o}C. Then the number of seeds germinated and the radicle growth of each one was recorded. Germination index (GI) is the product of relative seed germination (RSG) by relative radicle growth (RRG) as in equations IV.3-5.

$$\mathrm{GI}\ (\%) = \frac{\mathrm{RSG}\ (\%) \times \mathrm{RRG}\ (\%)}{100} \qquad \text{(Equation IV.3)}$$

$$\mathrm{RSG}\ (\%) = \frac{N_{SG,T}}{N_{SG,B}} \times 100 \qquad \text{(Equation IV.4)}$$

$$\mathrm{RRG}\ (\%) = \frac{L_{R,T}}{L_{R,B}} \times 100 \qquad \text{(Equation IV.5)}$$

Where $N_{SG,T}$ and $N_{SG,B}$, are the arithmetic means of the number of seeds germinated for each sample and blank with ultrapure water, respectively; $L_{R,T}$ and $L_{R,B}$ correspond to the average radicle length for each sample and blank, respectively. The phytotoxicity was classified according to the germination index using Trautmann and Krasny (1997) criteria.

IV.2.5. Neuronal ROS studies

The neuronal work was carried out in hippocampal slices (400 µm thick) from Wistar rats (10-13 weeks old, 16-18 days of pregnancy). The slices were obtained from the hippocampus of the brain of animals sacrificed by cervical dislocation, placed in artificial cerebrospinal fluid (ACSF) with the following composition (in mM): NaCl 124; KCl 3.5; $NaHCO_3$ 24; NaH_2PO_4 1.25; $MgCl_2$ 2; $CaCl_2$ 2 and D-glucose 10 (reagents from Sigma, Sintra, Portugal), pH 7.4. For these experiments the treated media were prepared by adding the constituents of ACSF to the untreated and treated parabens mixture, at pH 7.4. The slices were loaded, for 1 h, with 20 µM of the permeant ROS indicator H_2DCFDA (Life technologies, Carslband, CA), that was added to the ACSF oxygenated (95 % O_2, 5 % CO_2) medium. Afterwards the slices were transferred to the experimental chamber and kept in the oxigenated ACSF solution. In the experimental chamber they were perfused by this medium or by a treated solution, at 1.5–2 mL min^{-1} and 30–32 °C.

The optical data were recorded from the hippocampal CA3 area, at the mossy fiber synapses, and consisted of transfluorescence signals from a microscope (Zeiss Axioskop) with a tungsten/halogen lamp (12 V, 100 W). Light was selected using excitation (480 nm, 10 nm bandwidth) and emission (> 500 nm, high-pass) filters. The emitted light, after passing through a water immersion lens (40x, N.A. 0.75), reached a photodiode (Hammamatsu, 1 mm^2) that was followed by a current/voltage (I/V) converter with a very high (1 GΩ) feedback resistance. The processing, conversion and analysis of the data occurred at 1.67 Hz, using a 16-bit analog/digital converter (National Instruments), and the Signal Express^{TM} software, respectively. Each point in the graphs represents the average of 100 consecutive data points. All average points were corrected for autofluorescence by subtracting a value calculated from dye-free slices. After correction the data (represented as mean ± s.e.m) were normalized by the average of the initial 10 points recorded in ACSF. All experiments were carried out in agreement with the European Communities Council Directive, minimizing animal suffering and using only the number of animals required to produce reliable scientific data.

IV.3. Results and Discussion

IV.3.1. Catalysts characterization

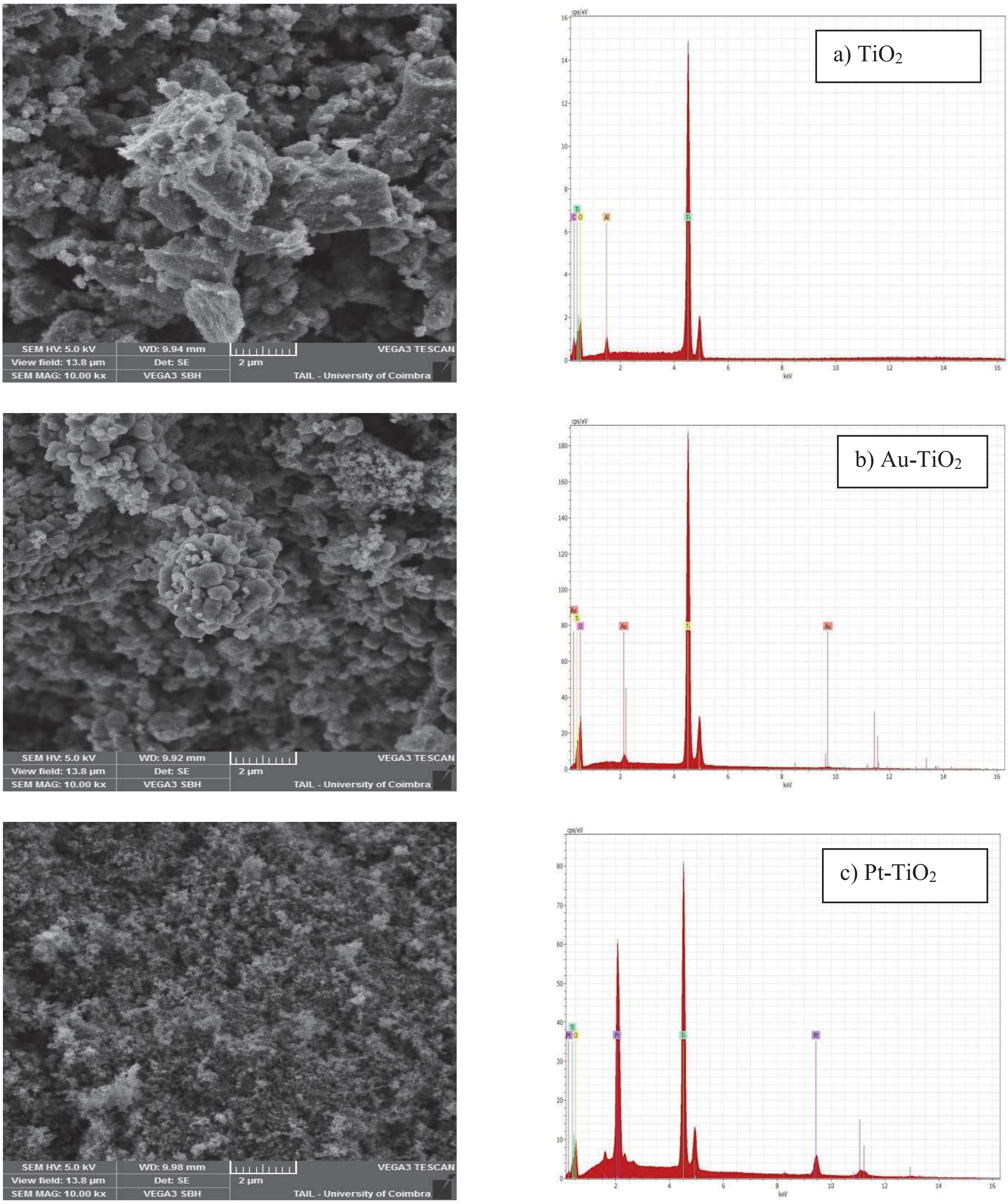
N_2 isotherm analysis of the prepared catalysts allowed the determination of their BET surface (Table IV.1). It is possible to see that the specific surface area of pure TiO_2 is higher than when supporting noble metals. The exception goes to Pt-TiO_2. This catalyst presents the highest surface area and pore volume, which may mean a higher active surface for catalytic reactions.

Ag-TiO_2 presents the lowest specific surface area which may reduce its adsorption capacity and thus its catalytic activity. On the other hand, Pd-TiO_2 has a good surface area (73 m^2/g), so that good catalytic activity may be expected. Gold supported on TiO_2 shows an interesting surface area (135 m^2/g). If a direct relationship between surface area and catalytic activity is expected, it can be concluded that Pt-TiO_2, Au-TiO_2, TiO_2 and Pd-TiO_2 can be promising catalysts.

Table IV.1. Specific surface area of the tested catalysts

Catalyst	**Surface area (m^2/g)**
Ag-TiO_2	34
Au-TiO_2	135
Pd-TiO_2	73
Pt-TiO_2	238
TiO_2	149

The fresh catalysts before catalytic ozonation were analysed by Scanning Electron Microscopy (SEM) for surface microstructure characterization. Moreover, the existing elements on their surface were identified by Energy-Dispersive X-Ray Spectroscopy (EDS). The results are gathered in Figure IV.1. From SEM images it can be observed that the catalysts particles present a diameter below 0.500 µm. According to a previous work from our research group (Martins and Quinta-Ferreira, 2009), in catalytic ozonation the use of particles with diameters below 125 µm already ensured that internal mass transfer resistances were negligible. Thus, chemical regime was guaranteed in all experiments which allow the comparison of the performance of the catalysts.


SEM HV: 5.0 kV
WD: 9.94 mm
VEGA3 TESCAN
View field: 13.8 µm
Det: SE
2 µm
SEM MAG: 10.00 kx
VEGA3 SBH
TAIL - University of Coimbra
cps/eV
a) TiO$_2$
Ti
C
O
Al
Ti
keV
SEM HV: 5.0 kV
WD: 9.92 mm
VEGA3 TESCAN
View field: 13.8 µm
Det: SE
2 µm
SEM MAG: 10.00 kx
VEGA3 SBH
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cps/eV
b) Au-TiO$_2$
Au
Ti
O
Au
Ti
Au
keV
SEM HV: 5.0 kV
WD: 9.98 mm
VEGA3 TESCAN
View field: 13.8 µm
Det: SE
2 µm
SEM MAG: 10.00 kx
VEGA3 SBH
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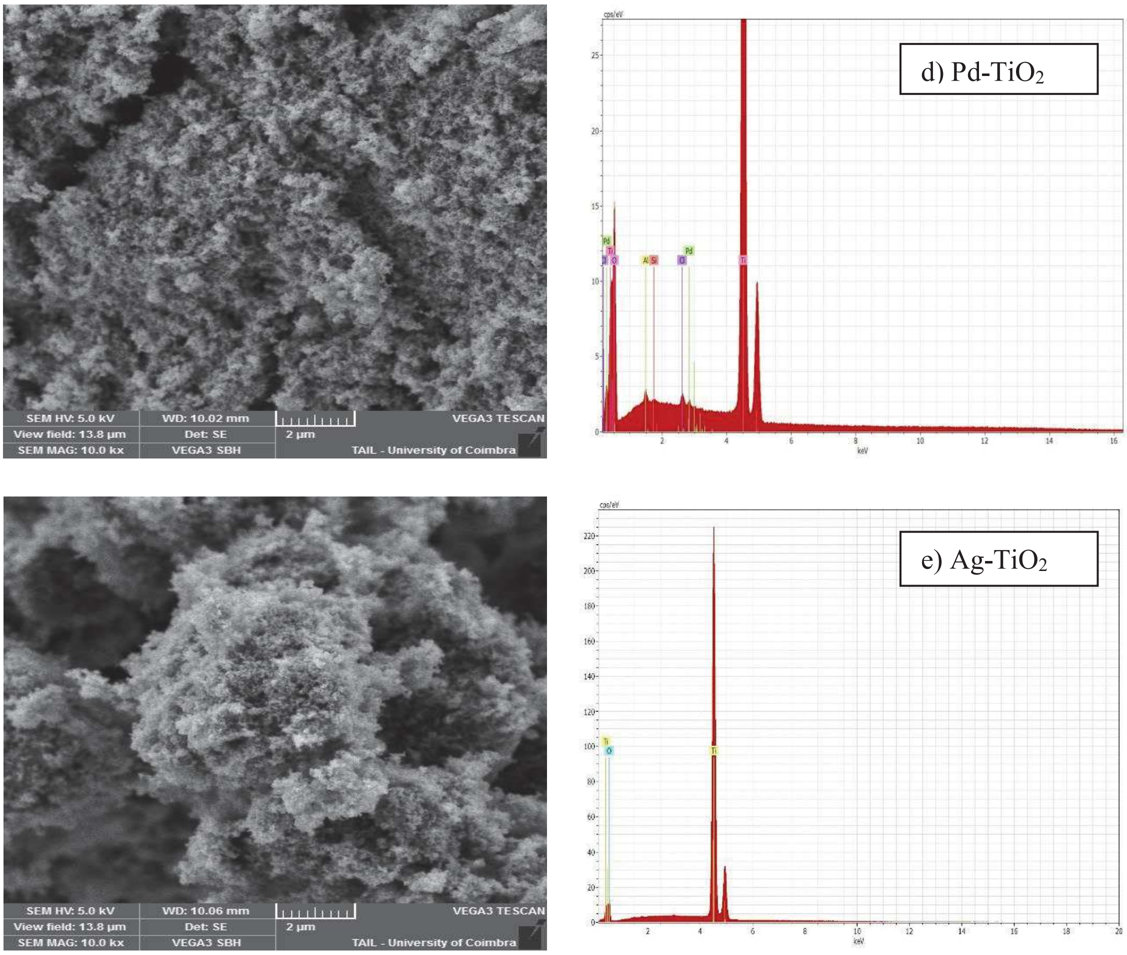

Figure IV.1. SEM images for different materials and EDS analysis for the classification of elements (a- TiO_2; b- Au-TiO_2; c- Pt-TiO_2; d- Pd-TiO_2; e- Ag-TiO_2)

SEM images show that the particles of TiO_2 (Fig.IV.1a) and Au-TiO_2 (Fig. IV.1b) present a linear morphology with well-designed particles and good shape (magnification of 10000x). In higher magnification (50000x, data not shown), it is possible to observe that the nanoparticles of gold are well dispersed on the surface of titanium dioxide. The images for Pt-TiO_2 (Fig. IV.1c) Pd- TiO_2 (Fig. IV.1d) and Ag-TiO_2 (Fig. IV.1e) show particles with a certain roughness on their surfaces. The titanium and oxygen are present in all spectra of the EDS analysis. For Pt-TiO_2, EDS analysis on a point randomly selected from the SEM image, shows a great amount of platinum (40% wt) well above the 0.5 % wt, expected theoretically, which means that this metal is not well dispersed on the surface of titanium dioxide. On the other hand, for Pd on Pd-TiO_2 and Ag on Ag-TiO_2 catalysts, where the amount of these elements is nearly zero and below the detection threshold, respectively. This means that these metals are well dispersed. It should be reminded that several points were analyzed for these catalysts and spectra were very similar which confirms that the metal was well dispersed on the catalyst surface. On this analysis other elements appear in residual concentrations such as aluminum, chlorine and silicon which are related with some contamination with the precursors used for the catalysts preparation. Importantly, as can be seen in the Figure IV.1b and in higher magnification (50000x, data not shown), the nanoparticles of gold are well dispersed, with a well-defined morphology as describe above. It should be reminded that Au-TiO_2 was prepared using the sol-gel method while the other tested catalysts were prepared by photo-deposition. Therefore, it seems that the sol-gel methodology for preparation of catalysts is more efficient than photo-deposition, in what regards metal dispersion.

IV.3.2. Degradation of parabens using single and catalytic ozonation

For analyzing the influence of different catalysts with ozonation on the degradation of parabens, five different catalysts 0.5% Ag-TiO_2, 0.5% Au-TiO_2, 0.5% Pd-TiO_2, 0.5% Pt-TiO_2 and pure TiO_2 were used. All experiments were performed under the same conditions referred above. The pH of the ultrapure water with 10 mg/L of parabens at the beginning of ozonation was 5.7 and at the end of reaction, pH value falls to 3.5 probably because of the formation of low molecular weight carboxylic acids due to parabens partial oxidation.

Figure IV.2 shows the normalized concentration of single parabens (a – MP, b-EP, c – PP, d-BuP and e – BeP) for ozonation and catalytic ozonation as a function of transferred ozone dose (TOD). The analysis of Figure IV.2 when TOD is 0, i e, when the effluent is only in contact with the catalysts without ozone, suggests that parabens are poorly adsorbed into catalysts. The exception is BuP and BeP that reach up to 35 % and 45 % of removal on TiO_2. BuP and BeP are larger molecules presenting higher electronic density which may improve electrostatic interactions between TiO_2 surface and these molecules.

From Figure IV.2, it is easily seen that for all the conditions applied, parabens were totally degraded. Single ozonation is the less efficient treatment. However, ozone alone can reach total parabens degradation with higher quantity of transferred ozone. In fact, ozone is very reactive with aromatic compounds (Martins and Quinta-Ferreira, 2009). This effectiveness was improved by the addition of solid catalysts (Fig. IV.2). For achieving total degradation of all parabens by single ozonation it was necessary around 170 mg/L of TOD which, in comparison with the worst result of catalytic ozonation, the catalyzed system required less 80 mg/L of TOD (Fig. IV. 2). This difference on degradation of parabens with single and catalytic ozonation makes it clear the interest on exploring the catalytic process. In fact, the amount of ozone applied is an important parameter in this treatment as O_3 production is a costly step since it must be generated *in situ*.

Benzylparaben, having the highest molar mass, corresponds to the lowest molar concentration. Moreover, presenting two aromatic rings in its structure, this molecule is more reactive as more high electronic density sites are available for oxidation. In fact, as described on introduction section the ozone is very reactive with aromatic compounds (Martins and Quinta-Ferreira, 2009). Therefore, during ozonation or catalytic ozonation it was necessary less quantity of ozone for degrading benzylparaben molecules. For BeP degradation the best results were observed for 0.5% Pd-TiO_2 and pure TiO_2, which obtained total degradation using around 55 mg/L of TOD, whereas with single ozonation, it was necessary more than the double of ozone, around 124 mg/L. Comparing the performance of catalysts, 0.5% Au-TiO_2 required the highest TOD obtaining total degradation of BeP for 68 mg/L of TOD.

The best results for degradation of the five parabens were detected for 0.5% Pt-TiO_2, 0.5% Pd-TiO_2 and TiO_2. Results can be influenced by the type of paraben, but in general these three catalysts have the best results. For MP, EP, PP and BuP the TOD required for total degradation was around 70 mg/L for 0.5% Pt-TiO_2, pure TiO_2, and 80 mg/L for 0.5% Pd-TiO_2. Globally, for all the conditions applied, parabens removal followed the order BeP > BuP > PP > EP > MP. Thus, it seems that the number of carbons in the ester chain linked to the aromatic ring is related with the paraben reactivity.

Noble metals (Pd, Pt, Ag, Au) are good redox catalysts, that can decompose O_3 with formation of oxygen-metal bound strong enough for effective ozone decomposition. For aqueous ozone decomposition, with the 1.5 g of catalyst and 0.175 L/min of water flow, the order of catalytic activity of different noble metals (loading of 5%) supported on TiO_2, was Pt > Pd > Ag (Lin et al., 2002). This can explain the higher activity of Pt and Pd catalysts for the removal of parabens when compared with the other materials tested in our work. Zhang and He (2007), analyzed noble metals (Pt, Pd, Ag) supported on TiO_2 for the catalytic oxidation of formaldehyde and found that the order of activity was 1% Pt-TiO_2 > 1% Pd-TiO_2 > 1% Ag-TiO_2 > pure TiO_2. In our case this difference of activity order can be explained by the nature of the compounds and the use of ozone instead of oxygen as oxidant

The low parabens adsorption onto the catalysts (Figure IV.2) seems to point out that parabens chemisorption followed by oxidation by ozone does not seem to be the main reaction pathway. Béltran et al., (2002) described the adsorption of ozone on TiO_2 free active sites, and at acidic conditions the hydroxyl radicals are generated from ozone decomposition. Yang et al., (2007) measured the signals of hydroxyl radicals by spin trapping-EPR technique. The results revealed that for catalytic ozonation the signals were stronger compared with ozonation alone, so TiO_2 enhances the production of hydroxyl radicals. In this context, the most plausible mechanism of reaction may be the adsorption of ozone on the catalysts leading to the production of hydroxyl radicals that will interact with the pollutants in the liquid bulk. The lower efficiency of 0.5% Ag-TiO_2 on parabens degradation must be related with lower surface area of this catalyst. Therefore, less active sites are available for decomposition of ozone, consequently lower amount of hydroxyl radicals can be produced.

The effect of Pt loadings (0.01, 0.1, 0.6, 1 wt%) supported on titanium dioxide for oxidation of formaldehyde was verified by Peng and Wang (2007). These authors concluded that the catalyst performance is associated with the dispersion of platinum on TiO_2 and the 0.6 wt% Pt loading was the most active. That is why in this work it was decided to use 0.5% wt of noble metals in TiO_2 for the catalytic ozonation studies.

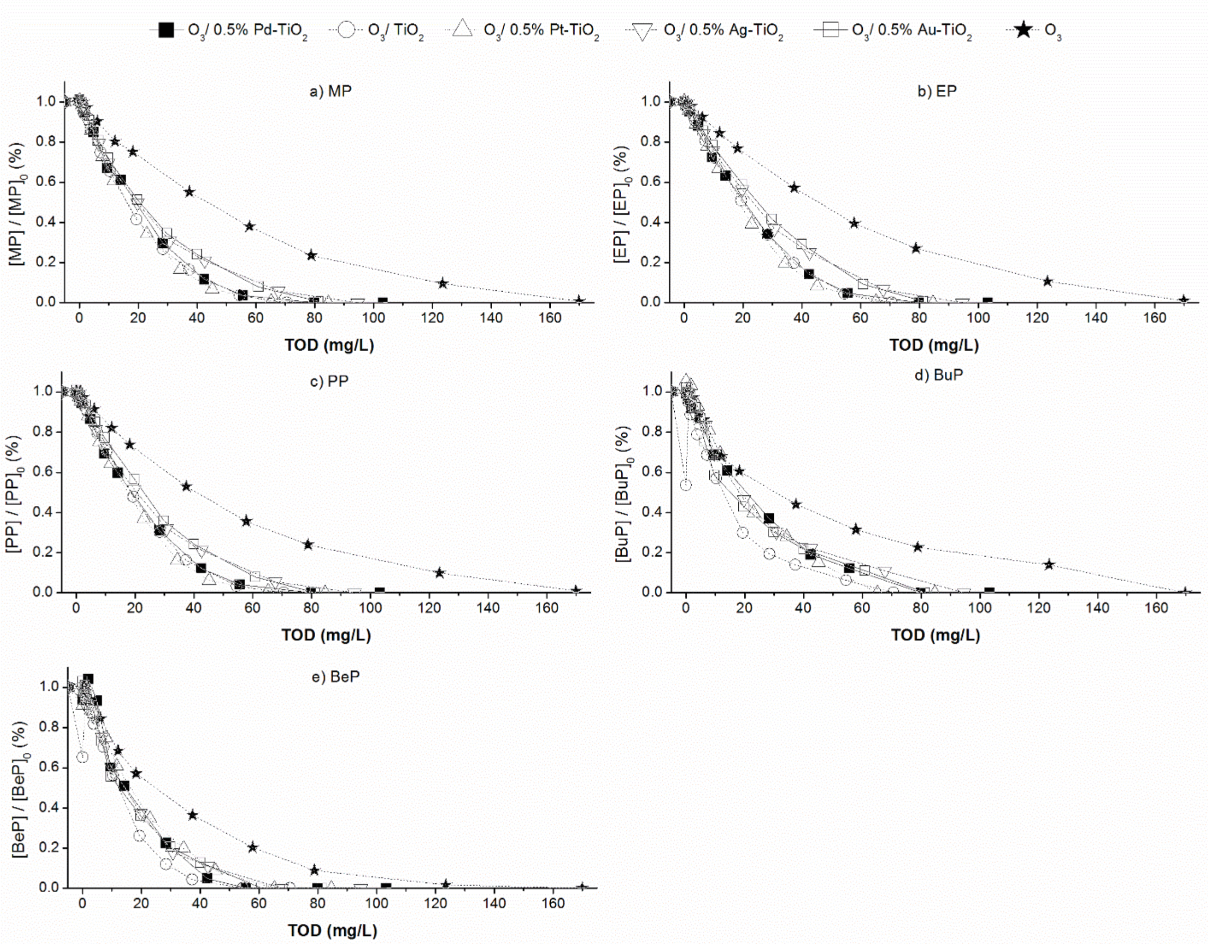

Figure IV.2. Normalized concentration of individual parabens (a – MP; b – EP; c – PP; d – BuP and e – BeP) during single and catalytic ozonation of the mixture as a function of TOD.

IV.3.3. COD and TOC removal

In this section the aim was to evaluate the efficiency of catalytic ozonation and single ozonation in terms of organic content removal from the mixture of five parabens. The initial concentration of COD was 95 mgO_2/L. Combination of catalysts with ozonation increases the efficiency on COD removal. Figure IV.3 shows this information, revealing that catalytic ozonation led to higher COD removal than single ozonation. However, all the processes present similar COD abatement at the beginning of the process until a TOD of ~30 mg/L. From Figure IV.2, it can be observed that the parabens removal rate is very high until that point. Thus, most likely, until that stage COD abatement is mostly due to parabens decomposition which are very reactive even when only ozone is applied. After this, differences are more noticeable for COD abatement.

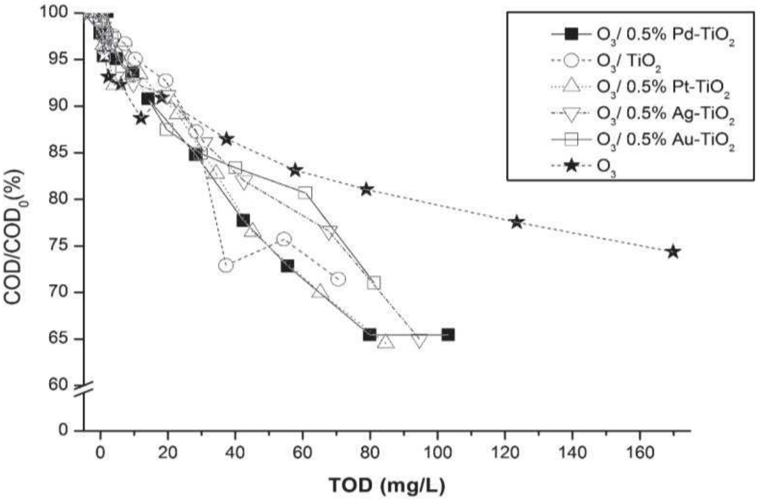

Figure IV.3. COD abatement as a function of TOD.

Similar conclusions are taken from the analysis of Figure IV. 4 that compares TOD and IOD for all the processes. While no significant differences are found between single and catalytic systems in what regards TOD until TOD ~30 mg/L, after this a higher TOD is found for single ozonation. Until the referred stage, the COD remaining in solution (Figure IV.3) is not significantly different for all the conditions applied. But, after that COD is much higher for single ozonation than for the catalytic systems. Thus, more ozone is required for oxidation which leads to a higher TOD (Figure IV.4).

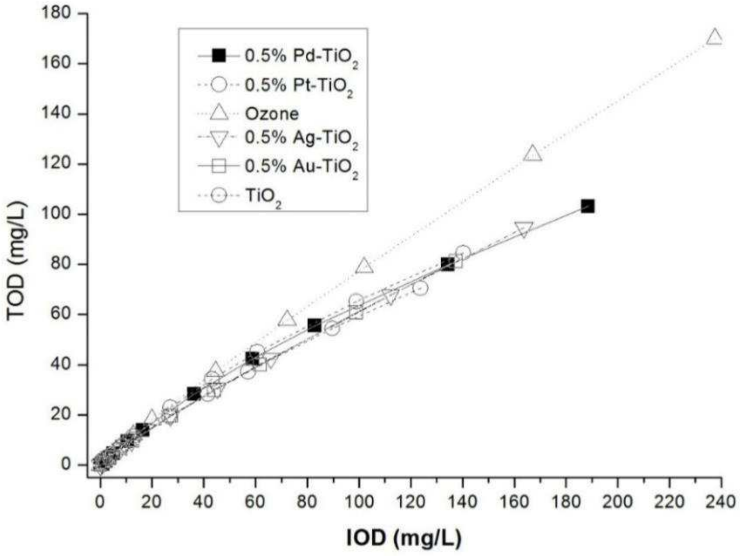

Figure IV.4. TOD as a function of IOD during single and catalytic ozonation.

Single ozonation for 72 mg/L of TOD led to removal only 17% of COD. For catalytic ozonation the worst results were obtained with the catalysts 0.5%Ag-TiO_2 and 0.5%Au-TiO_2. For these two materials it was possible to remove 23% of COD. For this TOD, COD depletion was 28% for pure titanium dioxide, while for platinum and palladium it was possible to obtain up to 32% of COD removal. Tay et al. (2010) studied the effect of single ozonation on COD removal and achieved 61% of removal relative to the initial amount. In this study an ozone dose of 0.67 g/h was used, being the initial concentration of each paraben 100 μM and the time for ozonation 3 h.

The best final results for COD removal were detected for platinum and palladium supported on titanium dioxide, which led to a COD removal of around 36% for 80 mg/L of TOD. For silver it was possible to achieve the same COD removal, but the value of TOD required was slightly higher, around 95 mg/L. So, it was necessary more 15 mg/L of ozone for removing the same amount of organic matter. Contrarily to parabens degradation, at the tested conditions even with catalytic ozonation it was not possible to achieve total degradation of the effluent organic content.

Bearing in mind COD abatement values, it seems that a straightforward relationship between surface area and catalytic activity does not exist. Considering BET surface area, COD removal should increase according to the following order Ag-TiO_2 < Pd-TiO_2 < Au-TiO_2 < TiO_2 << Pt-TiO_2, but COD abatement results show a different sequence (Au-TiO_2 < TiO_2 < Ag-TiO_2 < Pd-TiO_2 ≈Pt-TiO_2). Only Pt-TiO_2 follows the activity order predicted by the BET surface area. The differences found for the other materials are related with the fact that catalytic activity is also very influenced by the active sites of the catalyst and not only by the specific surface area.

The efficiency of single and catalytic ozonation was also compared in terms of mineralization expressed in total organic carbon (TOC). In fact, even if ozone is very reactive with the high electronic density sites of high molecular weight organic compounds, from their decomposition low molecular weight by-products are produced. Among them are low chain carboxylic acids which might be responsible for pH lowering along the reaction (from 5.7 to 3.5). These molecules are usually less prone to further oxidation and thus the mineralization level achieved by ozonation is low. However, the presence of catalyst may improve ozone action over pollutants due to the production of hydroxyl radicals or by promoting surface reactions between ozone and organic contaminants leading to higher mineralization levels. The TOC of the initial parabens mixture was 35 mg/L. For single ozonation TOC removal was insignificant (3%). In terms of mineralization (measured as TOC removal), the efficiency of the catalysts followed this order (efficiency between brackets): Ag-TiO_2 (6%) < Pt-TiO_2 (11%) < Au-TiO_2 (12%) < Pd-TiO_2 (18%). The best results were achieved with Pd-TiO_2, where the TOC value after 2h was 29 mg/L, corresponding to a mineralization of around 18%. It should be highlighted, that although Pt-TiO_2 was quite efficient regarding COD removal, it only achieved 11% of mineralization, therefore this catalyst pushes the reaction towards partial oxidation rather than promoting the production of carbon dioxide and water as end products. Concerning the degradation of the five parabens used the results from HPLC show that the best catalysts were 0.5% Pt-TiO_2, 0.5% Pd-TiO_2 and pure TiO_2. However, on COD removal pure TiO_2 was not so good. Thus, on a global analysis, for the degradation of the parabens mixture and COD and TOC removal, the best results were obtained with 0.5% Pt-TiO_2 and 0.5% Pd-TiO_2.

The results show that ozonation alone has a low efficiency on COD and TOC removal, and this can be improved by catalytic ozonation. The treated and untreated effluents have COD values within the legal limits for discharge into the natural water courses. However, it is important to evaluate if the solutions remaining after the treatment are more or less toxic compared to the initial mixture of five parabens. Moreover, to try to understand their effect over the ecosystems, it is essential to analyze the toxic impact over species from several trophic levels.

IV.3.4. Toxicity assessment

All the conditions tested for the degradation of the parabens proved to be efficient, because for all of them the final concentration of each paraben was below the detection limit of HPLC. However, the COD values indicate the presence of refractory compounds which were difficult to remove with single or catalytic ozonation for the catalysts tested. These compounds may still cause significant toxicity. Hence, the toxicity of resulting solutions after 120 min of single and catalytic ozonation treatment was evaluated using *A. fischeri, C. fluminea, L. sativum* and *Wistar* rat brain slices and compared with the toxicity of the initial mixture of parabens.

Table IV.2 shows the results obtained in the tests with *A. fischeri.* The initial parabens solution led to a luminescence inhibition of around 95% after 15 and 30 min of exposure, a value that indicates a high toxic effect (Miralles-Cuevas, et al., 2017). Compared to this, all the treatment conditions tested lowered the toxicity of the parabens mixture. The worst result was for single ozonation where the value of luminescence inhibition after 30 min of exposure was 53.6%, corresponding to a toxicity reduction of 40 % relatively to the initial solution. Table IV.2 also shows that the compounds resulting from catalytic ozonation are less toxic than those obtained by applying single ozone, although the difference is not so large. For 30 min of exposure the luminescence inhibition for pure TiO_2 and 0.5% Ag-TiO_2 was 41.4% and 48.2%, respectively, corresponding to the best and worst results obtained with the catalysts. *A. fischeri* is very sensitive marine bacteria which may explain the toxicity still detected for the treated samples. Nevertheless, samples are considered nontoxic for *A. fischeri* when luminescence inhibition is below 30 % (Miralles-Cuevas, et al., 2017). For all the samples coming from catalytic ozonation, the value reached after 15 min of exposure was not very far from that threshold. Thus, all the catalysts show potential on acute toxicity effective abatement.

Table IV.2. Results obtained for *A. fischeri* luminescence inhibition after 15 and 30 min of exposure.

	Luminescence inhibition after sample exposure for 15 min (%) (95 % CI in brackets)	**Luminescence inhibition after sample exposure for 30 min (%) (95 % CI in brackets)**
Initial Parabens Solution	95.6 *(95.5 – 95.6)*	95.1 *(95.0 – 95.1)*
Single Ozonation	38.6 *(35.0 – 42.1)*	53.6 *(48.9 – 58.3)*
0.5% Pd-TiO_2	33.7 *(32.7 – 34.8)*	47.5 *(46.9 – 48.1)*
0.5% Pt-TiO_2	32.9 *(31.9 – 33.9)*	46.6 *(44.6 – 48.6)*
0.5% Au-TiO_2	31.4 *(30.0 – 32.8)*	45.2 *(44.4 – 46.0)*
0.5% Ag-TiO_2	35.2 *(31.4 – 39.1)*	48.2 *(45.5 – 50.8)*
Pure TiO_2	26.5 *(26.0 – 27.0)*	41.4 *(41.3 – 41.6)*

Figure IV.5 (a, c, e, g, i, k) shows the mortality of Asian clams exposed to different concentrations of solutions resulting from catalytic and single ozonation treatment. For these organisms the median lethal concentration (LC_{50}) of the initial parabens mixture, after 72 h of exposure, was estimated as 56 % (v/v) (probit method). The probit method is commonly used in toxicity tests with living organisms, where the answer is binomial (death/no death) and normally the relationship between the response and various concentrations is sigmoidal. The regression can be used to determine many endpoints to compare the toxicity of different compounds on living organisms, such as LC50 (the median lethal concentration). In our case that concentration was determined in volumetric percentage (V/V). In other words, to obtain 50% of mortality of *C. fluminea* after 72h it was necessary to put them in contact with an aqueous with a V/V concentration of 56% of the initial mixture of parabens. For all the treatments applied, the resulting solutions were not able to kill more than 50 % of the exposed clams at the maximum concentration (100 %V/V) (Figure IV.5 a, c, e, g, i, k).

Figure IV.5 (a, c, e, g, i, k) also shows that 100% mortality was only achieved for the initial parabens mixture at the maximum concentration (100% V/V). For the treated mixtures, the maximum mortality was around 29 % for the sample treated with single ozonation. Ozonation coupled with solid catalysts always allowed better results in terms of toxicity and in most cases no toxicity has been registered for all the dilutions performed. The worst catalyst was pure TiO_2, leading to 21 % mortality at maximum concentration. These results show clearly that single and catalytic ozonation reduce the toxicity of parabens towards *C. fluminea*.

In a perspective of water reclamation for agricultural purposes, it is important to infer about the phytotoxicity of both the initial parabens and the treated samples. For that seeds of *L. sativum* were used.

These tests with *L. sativum* give similar results to those previously described for *A. fischeri* and *C. fluminea*. Thus, the toxicity was reduced after treatment. The germination index for maximum concentration (100% V/V) of the initial parabens mixture was very low, 40 %, meaning that the phytotoxicity of the sample was very high (Figure IV.5 b, d, f, h, j, l).

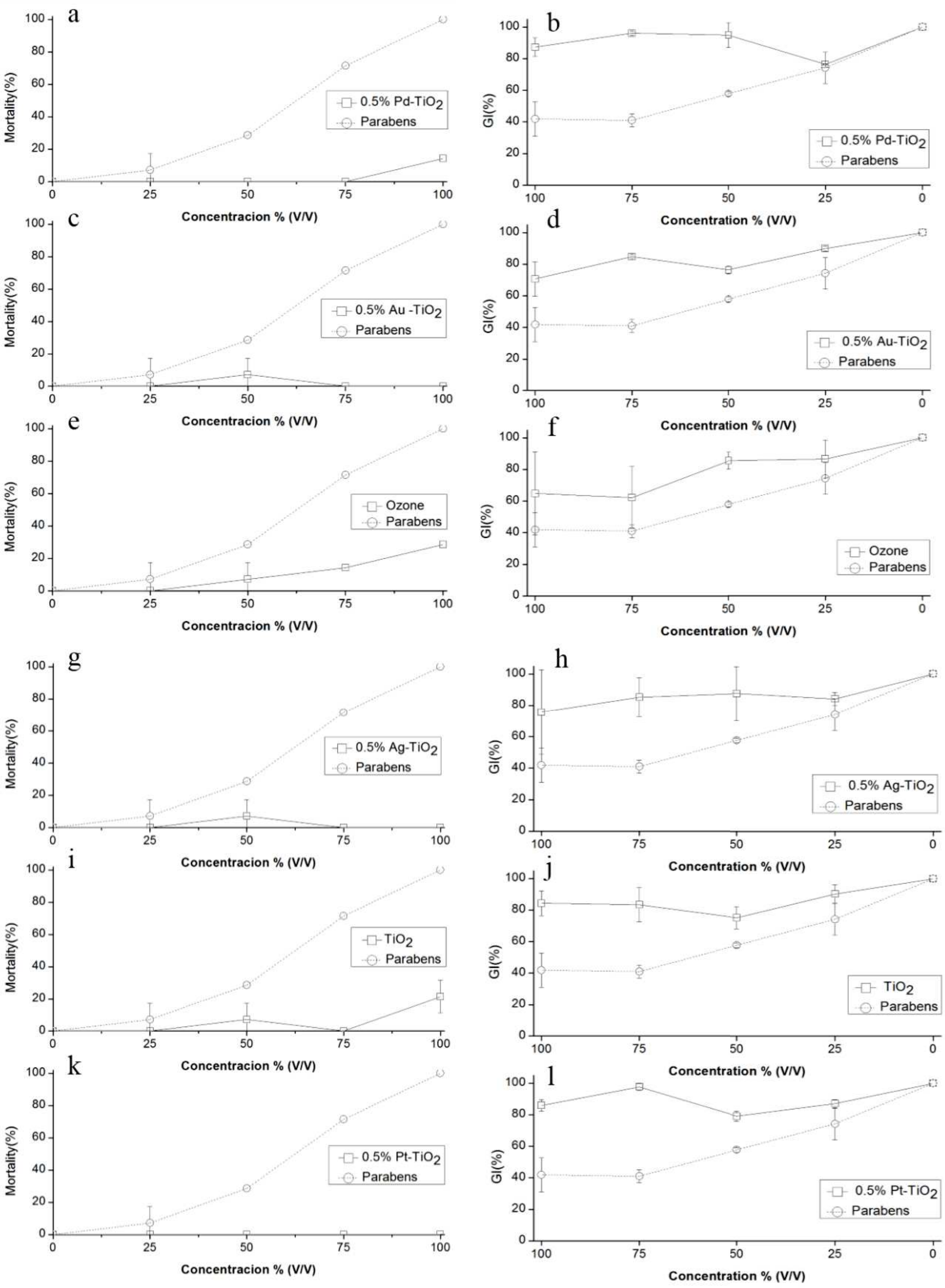

Figure IV.5. Concentration-response data for *C. fluminea* and GI respectively, representing the effect of treated effluents compared with the initial parabens mixture a, b - 0.5% Pd-TiO_2, c, d -0.5% Au-TiO_2, e, f – Ozone, g, h - 0.5% Ag-TiO_2, i, j - TiO_2, k, l - 0.5% Pt-TiO_2, compared with the initial effluent.

Figure IV.5 (b, d, f, h, j, l) also shows that the treated solutions coming from catalytic ozonation were less phytotoxic compared with those from single ozonation. The only exception is the sample coming from catalytic ozonation using Au-TiO_2 where the differences for single ozonation are not so evident. In fact, using 100% of sample GI of 65% and 71% were obtained for the samples coming from single and catalytic ozonation, respectively. For all catalysts at maximum sample concentration the germination index was around 80%, which according to Trautmann and Krasny (1997) criteria, represent that these solutions are considered non-phytotoxic. Only single ozonation, corresponding to the maximum concentration, led to a moderately phytotoxic behavior. The best results were detected for palladium and platinum catalysts because the germination index was around 90%. It seems thus that 0.5% Pt-TiO_2 and 0.5% Pd-TiO_2 can constitute interesting catalytic materials for the degradation of parabens mixtures using ozone leading to interesting toxicity removal results.

The impact of treated effluents on synaptic activity can be seen in Fig. IV.6 that shows the results of the application of one effluent treated by catalytic ozonation (Pd-TiO_2), on neuronal ROS activity. In this case only the solutions obtained after catalytic ozonation using the best catalysts (Pd-TiO_2 and Pt-TiO_2) were tested. In all cases, after the treatment that consisted in single or catalytic ozonation, using the catalysts (Pd-TiO_2 and Pt-TiO_2), the parabens media do not cause significant neuronal ROS changes. The graph for effluent treated with Pd-TiO_2 as an example of the behavior, indicate that all normalized values remain close to those of the baseline, formed by the first 10 min in ACSF. Thus, both in the presence (30 min), and upon replacement of the treated mixture by ACSF (30 min), the amount of detected ROS remains stable. The values measured at the end of application of the treated and reperfused ACSF solutions, namely in the periods 35 - 40 min / 65 - 70 min are 1.00 (± 0.02) / 1.00 (± 0.01) for O_3, 0.96 (± 0.03) / 0.98 (± 0.05) for Pt-TiO_2 and 1.03 (± 0.01) / 1.00 (± 0.03) for Pt-TiO_2 (mean ± s.e.m, n = 2). These facts suggest that chemical elements present in the untreated effluent, that caused an enhancement of the normalized ROS signal, with respect to baseline (data not shown), were efficiently degraded during the treatments, giving rise to a safe water from the neuronal activity point of view.

This wide variety of toxicity tests allows achieving a better and more complete answer for different trophic levels regarding the potential impact of initial and treated samples.

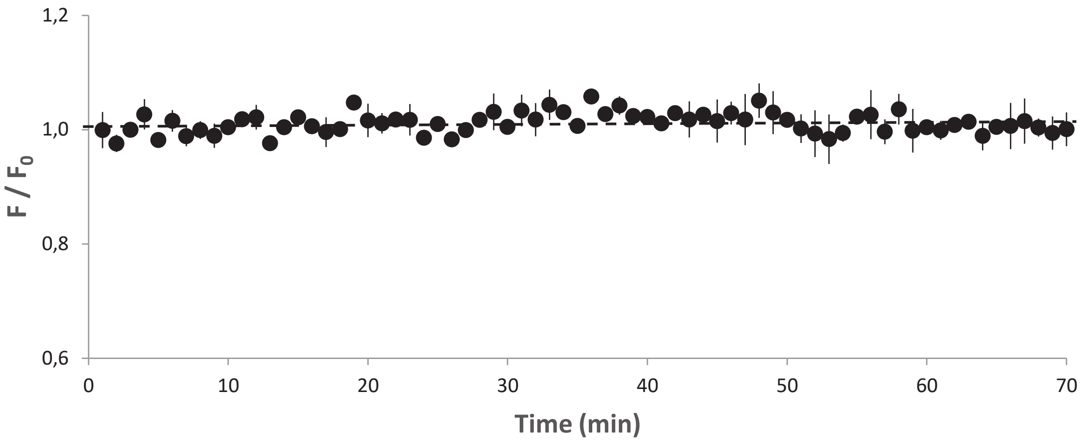

Figure IV.6. Effect of treated effluent on neuronal ROS signals from brain slices detected with the fluorescent probe H_2DCFDA. Effluent treated by Pd-TiO_2 ozonation (n = 2) does not alter the ROS signals

IV.4. Conclusions

The efficiency of parabens degradation using several catalysts with ozonation was compared with single ozonation. Single ozonation was able to totally remove parabens but the addition of catalyst allowed to reduce the amount of ozone required for that result. In terms of parabens degradation, the best catalysts were Pt-TiO_2, Pd-TiO_2 and TiO_2.

The use of catalytic ozonation also enhanced COD and TOC removal. In terms of TOC, 0.5% Pd-TiO_2 was the best catalyst and achieved 18% of TOC abatement when compared with the 3% attained for single ozonation. For 0.5% Pt-TiO_2 and 0.5% Pd-TiO_2 36 % of COD depletion was reached around at the end of reaction.

Due to the relatively low mineralization achieved for all the processes, it is necessary to verify if the resulting solutions are less toxic than the initial parabens. In this context, toxicity assessment was evaluated before and after single and catalytic ozonation and revealed that these systems can be highly efficient on impact reduction of the initial parabens over *A. fischeri*, *C. fluminea*, *L. sativum* and *Wistar* rats.

The bacteria luminescence inhibition after exposure to samples for 30 min decayed from around 95% to 41%, when TiO_2 was used as catalyst in ozonation. In the same direction, for initial parabens the GI was around 40% and increased to 90% after catalytic ozonation (0.5% Pt-TiO_2 and 0.5% Pd-TiO_2). The clams are very susceptible to initial parabens concentration because after 72 h all the clams exposed died, while after all catalytic ozonation experiments (except for

TiO_2) no mortality was observed for the same conditions. The neuronal studies indicate that the treated effluents considered do not affect neuronal ROS signals, unlike what happens with the raw one.

Gathering all the results, it can be concluded that, globally, 0.5% Pt-TiO_2 and 0.5% Pd-TiO_2 show to be the most active catalysts for parabens mixture detoxification.

IV.5. References

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V. Photocatalytic Ozonation using doped TiO_2 Catalysts for the Removal of Parabens in Water

It is based on the publication: Gomes, J., Leal, I., Bednarczyk, K., Gmurek, M., Stelmachowski, M.; Diak, M., Quinta-Ferreira, M.E., Costa, R., Quinta-Ferreira, R.M., Martins, R.C. (2017b) Photocatalytic Ozonation using doped TiO_2 Catalysts for the Removal of Parabens in Water. Science Total Environment, 609, 329–340.

V.1. Introduction

The demand for potable water will be one of the main challenges of this century, since the water resources are being continuously polluted by different types of emerging contaminants. The application of conventional wastewater treatment technologies for its degradation reveals a lack of efficiency (Bolz et al., 2001; Gibs et al., 2007; Kasprzyk-Hordern et al., 2008; Yu et al., 2011; Bledzka et al., 2014). A particular case of emerging contaminants are the widely used parabens. These compounds are used as antimicrobial and preservatives in drinks, pharmaceutical and personal care products (Canosa et al.,2007; Calafat et al., 2010; Meeker et al., 2013) and appear as a group of emerging compounds reaching the water resources since they are hardly degraded. Some parabens (methyl, ethyl and propyl) were found in rivers and in wastewater treatment plants (Yu et al., 2011). The presence of these contaminants on water and the scarcity of water resources, make as priority the implementation of municipal wastewater (MWW) reclamation and reuse strategies (Bixio et al., 2006). On this way, several new technologies, such advanced oxidation processes, are required to improve the degradation of parabens.

Ozone is a powerful oxidant and under water treatment conditions can act in a direct pathway or produce hydroxyl radicals, which can oxidize a large spectrum of organic compounds (Martins and Quinta-Ferreira, 2009a,b; Gomes et al., 2017a). However, predominantly single ozonation cannot mineralize the organic compounds. Moreover, refractory by-products are produced that can be more or less toxic compared to the initial organic pollutants (Martins and Quinta-Ferreira, 2009a). The UVA irradiation with photocatalysts possibly improves the degradation of organic compounds. In this system, the photogenerated holes and electrons are the main responsible for this improvement of degradation. For this process, molecules such as oxygen and water are required to generate superoxide ($O_2^{\bullet-}$) and hydroxyl radicals ($^{\bullet}OH$), capable of mineralizing the contaminants into water and carbon dioxide (Chong et al., 2010). In fact, photocatalysis in the absence of oxygen or even single photolysis normally are not efficient on the degradation of some organic compounds (Lin et al., 2011; Oros-Ruiz et al., 2013; Velegraki et al., 2015; Ofiarska et al., 2016; Gomes et al., 2017a). Therefore, for improving the efficiency of the process, the addition of a compound that will act as electron scavenger is necessary. Thus, the application of a more reactive molecule than oxygen, such as ozone, would be interesting. Following that, for the elimination of hardly degraded organic contaminants, the combination of photocatalysis and ozone, will probably push the reaction towards mineralization. In fact, ozone is a stronger oxidant than oxygen which will speed up the degradation rate leading to a more effective process (Agustina et al., 2005). Moreover, Hernández-Alonso et al. (2002) confirmed by Electron Spin Resonance (ESR) signals analysis that oxygen adsorbed on TiO_2 surface has lower electroaffinity compared to ozone. Thus, ozone will allow to trap a higher amount of photogenerated electrons reducing the electron-hole recombination phenomenon. This will enhance the contaminants degradation efficiency.

In this context, the combination of ozonation, heterogeneous catalysis and UVA irradiation allows an increase in photogenerated electron-holes pairs at the photocatalyst surface, that can reduce electron acceptor or oxidize water and contaminants. This will improve the production of reactive oxygen species, such as hydroxyl radicals ($^{\bullet}OH$), superoxide radicals ($O_2^{\bullet-}$) and ozonide radicals ($O_3^{\bullet-}$) (Mehrjouei et al., 2015). Bearing this in mind, photocatalytic ozonation appears as a promising technology that will improve contaminants mineralization for water reclamation. Nevertheless, it is necessary to select a photocatalyst with a band gap lower or equal to the photon energy resulting from UVA irradiation. Titanium dioxide (TiO_2) is the most frequently used photocatalyst, because this semiconductor presents good optical and electronic properties, low cost and non-toxic characteristics. This photocatalyst normally presents a band gap higher than 3eV ($\lambda < 400$nm) (Chong et al., 2010; Pelaez et al., 2012). Doping TiO_2 with nanoparticles of noble metals (such as Pt, Pd, Au, Ag) decreases the band gap energy allowing TiO_2 absorption in UVA and visible region (Zheng et al., 2011; Wang et al., 2012).

Titanium dioxide with enough photon energy enhances the photogeneration hole-electron pair (eq. V.1). The adsorption of ozone molecules on photocatalyst surface can occur by different ways, such as, physical adsorption, formation of hydrogen bonds with surface of hydroxyl groups, molecular adsorption into weak Lewis sites and dissociative adsorption with strong

Lewis sites (Bulanin et al., 1995). This way, the photogenerated electrons can react with ozone molecules adsorbed on the catalyst surface, that works as electron acceptor producing ozonide radicals (eq. V.2) (Rivas et al., 2006; Rodríguez et al., 2013; Mehrjouei et al., 2015). Besides, ozone works as electrons scavenger decreasing the recombination rate of hole-electron pairs. This recombination phenomenon also decreases in the presence of metallic nanoparticles on the semiconductor catalyst surface that scavenge the photogenerated electrons (Anpo and Takeuchi, 2003; Bahruji et al., 2010). It is expected that the mechanism of photocatalytic ozonation is similar to photocatalysis, therefore the photogenerated holes can react with water molecules adsorbed on the catalyst surface to produce hydroxyl radicals (eq.V. 3), this reaction preferably occurs at acidic conditions (Mehrjouei et al., 2015).

$$TiO_2 + h\upsilon \longrightarrow h^+ + e^- \qquad \text{Equation V.1}$$

$$O_{3(ads)} + e^- \longrightarrow O_3^{\bullet -} \qquad \text{Equation V.2}$$

$$H_2O_{(ads)} + h^+ \longrightarrow {}^{\bullet}OH \qquad \text{Equation V.3}$$

The ozonide radicals generated during ozone adsorption can react directly with organic contaminants or, at acidic conditions, the hydroxyl radicals formation can be favored, as shown by eq. V.4 (Hernández-Alonso et al., 2002).

$$O_3^{\bullet -} + H^+ \longrightarrow HO^{\bullet}_3 \longrightarrow {}^{\bullet}OH + O_2 \qquad \text{Equation V.4}$$

Hydroxyl radicals can also be formed from the subsequent rapid equilibrium of ozonide, as can be seen in the eq. V.5 (Merenyi et al., 2010).

$$O_3^{\bullet -} \longleftrightarrow O^{\bullet -} + O_2 \longrightarrow O^{\bullet -} + H_2O \longleftrightarrow {}^{\bullet}OH + OH^- \qquad \text{Equation V.5}$$

In photocatalytic ozonation, it is predictable that other reactive oxygen species can appear, such as superoxide radicals ($O_2^{\bullet -}$), because as it can be seen in equation V.4, oxygen molecules, are resultant products of hydroxyl radicals formation from hydrogenation of ozonide, and can be reduced by the remaining photogenerated electrons. These superoxide radicals can oxidize organic compounds or react with other intermediates to produce hydroxyl radicals (Mehrjouei et al., 2015).

The photocatalytic ozonation has been recently studied on the degradation of emerging contaminants. The photocatalytic ozonation (O_3/UV/TiO_2) led to complete mineralization of amoxicilin and diclofenac after 30 and 120 min respectively (Moreira et al., 2015). Rodríguez et al. (2013) showed that at acid conditions (pH 4) the total polyphenolic content and total organic carbon (TOC) removal of atenolol, hydrochlorothiazide, ofloxacin and trimethoprim mixture, were improved under TiO_2/O_3/UVA irradiation. This was related to the formation of hydroxyl through ozonide resulting at these conditions (Rodríguez et al., 2013). For these compounds with O_3/TiO_2/solar radiation 70% of TOC and 90% of toxicity removal were achieved in 2 h (Márquez et al., 2014).

Bearing in mind the novelty and interest of this technology for water reclamation, the aim of this work was to evaluate the degradation of a mixture five water contaminants namely methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BuP) and benzylparaben (BeP) under photocatalytic ozonation. For this purpose, the noble metals Ag, Au, Pd, Pt, at loadings of 0.5 wt.%, doped onto TiO_2 were tested. The next goal was to check if metals can improve TiO_2 photocatalytic activity since they are capable to reduce the energy band gap and avoid electron-hole recombination. Moreover, photocatalytic ozonation (O_3/catalyst/UV-A irradiation) was compared with photolytic ozonation (O_3/UV-A irradiation). Furthermore, the toxicity assessment was performed. To cover a wide range of trophic levels, three different species such as *Aliivibrio fischeri* (bacteria), *Corbicula fluminea* (clam) and *Lepidium sativum* (plant) were used. To the best of our knowledge, this is the first time noble-metal doped TiO_2 catalysts are applied in photocatalytic ozonation for parabens degradation.

V.2. Materials and Methods

V.2.1. Chemicals and catalysts preparation

Titanium (IV) isopropoxide (97%) was used as titanium source for the preparation of catalysts and was purchased from Aldrich Chem. Butylparaben (BuP) was purchased from Fluka and methylparaben (MP), ethylparaben (EP), propylparaben (PP), benzylparaben (BeP), were obtained from Sigma-Aldrich. The solution used in these tests was prepared from a mixture of these five parabens (10 mg/L each) in ultrapure water. This concentration is commonly used in studies involving these contaminants [15, 33, 34, 35]. The standards of 4-Hydroxybenzoic acid (4-HBA), 3,4-Dihydroxybenzoic acid (3,4-diHBA), 2,4-Dihydroxybenzoic acid, Hydroquinone, 1,4-Benzoquinone and 3,4,5-Trihydroxybenzoic acid were acquired from Sigma-Aldrich.

Titanium (IV) isopropoxide was used to prepare all catalysts. It is known to be a source for the anatase-type TiO_2. An adequate amount of the $KAuCl_4$ (0.5 wt.%), methanol and distilled water were mixed with titanium (IV) isopropoxide for preparing Au – modified TiO_2. This mixture was stirred at room temperature during 2 h, followed by a 24 h thermal treatment at 45 °C and calcination at 400 °C for 2 h.

The other catalysts (Pt-TiO_2, Pd-TiO_2 and Ag-TiO_2) were obtained through photo-deposition by UV-reduction of Pt^{4+}, Pd^{2+}, Ag^{+} ions in the titanium dioxide suspension. An aqueous solution of isopropanol containing H_2PtCl_6 (0.5 wt.%) or $PdCl_2$ (0.5 wt.%) was degassed with nitrogen and irradiated by UV-Vis light (1000 W Xe lamp) for 6 h. An aqueous solution of ethanol containing $AgNO_3$ (0.5 wt.%) was degassed with nitrogen and irradiated by UV-Vis light (1000 W Xe lamp) for 100 min. The modified TiO_2 catalysts were separated by centrifugation and dried at 65 - 120 °C for 12 h.

V.2.2. Experimental apparatus

The experiments were carried out in a 2 L glass photoreactor equipped with 3 lamps (Philips TL 6W BLB, tube diameter of 16 mm) which transmit UV-A irradiation (main emission 365 nm), at controlled temperature (25±1 °C) by a thermostatic bath during 120 min (Figure V.1). The photon flux was obtained using ferrioxalate actinometer according to the method proposed by Kuhn et al. (2004) and was calculated at 5.75×10^{-7} einstein/ (Ls). In photocatalytic ozonation experiments, 5 min before ozone fed and the light irradiation started, the reaction solution containing parabens (10 mg/L each) as well as catalysts (70 mg/L) was placed inside the reactor to test the catalyst adsorption capacity (Gomes et al., 2007b). The stirring speed was previously optimized at 700 rpm to ensure chemical regime, which means that external and internal diffusion limitations can be neglected. Moreover, the catalyst particles had an average diameter of 500 nm (determined through SEM). According to previous studies (Martins and Quinta-Ferreira, 2009a) at those conditions chemical regime is ensured. During the reaction, the mixture of parabens in ultra-pure water samples were withdrawn at 1, 2, 5, 10, 15, 30, 45, 60, 90 and 120 min and immediately centrifuged at 3500 rpm (Nahita 2655 Centrifuge) to remove the catalyst and posterior analysis of degradation by HPLC. The volume taken never exceeded 10% of total volume. The inlet ($[O_3]^{in}$) and outlet ($[O_3]^{out}$) ozone concentration were measured using two ozone analysers (BMT 963 and 964 vent, BMT), allowing the determination of transferred ozone dose (TOD) according to eq. V.6

$$\mathrm{TOD}=\int_0^t \frac{Q_{Gas}}{V_{Liquid}}\times\left([O_3]^{in}-[O_3]^{out}\right)\times dt \qquad \text{(Equation V.6)}$$

Where Q_{Gas} represents the gas flow rate (0.2 L/min), V_{Liquid} is the volume of the effluent used in the reactor. The TOD were expressed in mg O_3/L.

Ozone was produced by ozone generator (802N, BMT) from pure oxygen stream (99.9%). The remaining ozone that leaves reactor was trapped by a solution of Potassium iodide (Panreac).

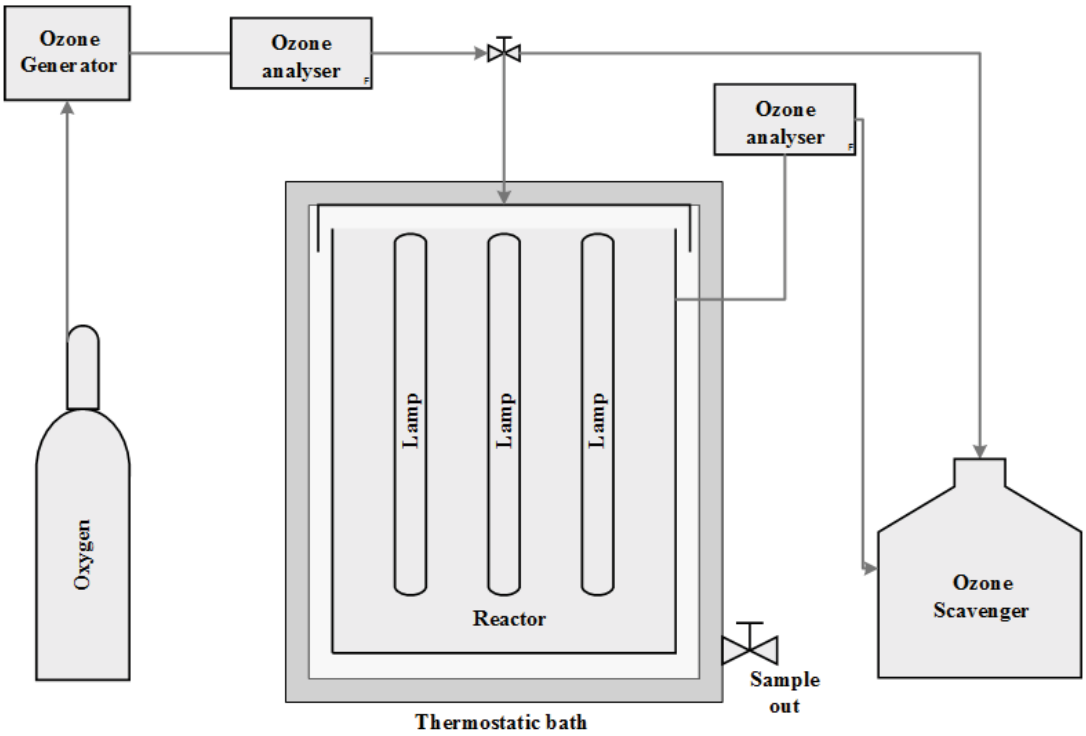

Figure V.1. Scheme of experimental photoreactor

V.2.3. Analytical methods

The microstructural analysis of the catalyst surface and particles average diameter were performed using a TESCAN VEGA 3 SBH - Easy Probe Scanning Electron Microscopy (SEM) with a tungsten heated cathode. The dispersion of metals on catalyst surface was analyzed by Energy Dispersive X-Ray Analysis (EDS) using a Bruker QUANTAX system that includes the Bruker Nano XFlash® detector, which works according to the principle of the silicon drift detector (SDD). EDS analysis was carried out using an accelerating potential of 20 kV and real time for the spectrum accumulation of 60 s at a dwell time of 16 ms. Energy-Channel calibration was used with a copper standard (K_a 8.0463 keV). EDS was performed to several random selected points of the samples. The crystalline structure of the catalysts powders was evaluated by X-ray diffraction (XRD) analysis, through a diffractometer (Bruker D8 Advance). The diffractometer works with Cu Kα radiation (2.2 kW ceramic tube). Using a 1D LynxEye detector (Silicon Drift Detector) covering an angle of ~3º and with ~25% energy resolution. SDT Q600 (TA Instruments) was used for thermogravimetric analysis (TGA), for evaluating the thermal resistance of catalysts at higher temperatures (maximum 1200 ºC). The BET (*Brunauer–Emmet–Teller* (S_{BET})) specific surface area of the catalysts were analyzed using nitrogen (-196 °C) with an accelerated surface area and porosimetry analyzer (ASAP 2000, Micrometrics). According to De Lange et al. (2014), adsorptive characterization using N_2 can be employed if the solids present a specific surface area higher than 5 m^2/g.

XPS (X-ray photoelectron spectroscopy) analyses were carried out with a Kratos AXIS Ultra spectrometer using monochromatic Al Kα X-Rays source of an excitation energy equal to 1486.6 eV. The spectra were obtained using an analysis area of 300 μm x 700 μm. The power of anode was set at 150 W and the hemispherical electron energy analyzer was operated at a pass energy 20 eV for all high-resolution measurements. All measurements were performed with the use of a charge neutralizer due to semiconducting character of the samples. The background subtraction of XPS spectra was performed with Shirley algorithm and the adventitious carbon main peak (C 1s, 284.8 eV) was used for a final calibration of each spectrum.

High-performance liquid chromatography HPLC (UFLC, Shimadzu, equipped with a diode array detector), was used for the determination of each paraben concentration along the reaction time. The injection volume of samples was 20 μL and the mobile phase (0.5 mL/min) consisted in a mixture of 50:50 methanol: acidic water (0.1% orthophosphoric acid). A C18 (SiliaChrom) column at 40 ºC was used and the parabens detection occurred at 255 nm. The identification of by-products followed the same procedure. Reaction samples were directly injected, and the compounds identification was performed by comparing peaks retention times with those obtained when each compound standard solutions were injected. Calibration curves were obtained for each substance. The LOD was calculated using the coefficient of 3.3 with quotient between standard deviation and slope of calibration curve. On otherwise, the LOQ was calculating using the coefficient of 10 with the same quotient.

The total organic carbon (TOC) for initial and treated solutions was determined using a TOC analyser (TOC-V CPN model, Shimadzu, Japan) coupled to an autosampler (model V-ASI, Shimadzu, Japan). Chemical oxygen demand (COD) was measured according to the standard method 5220D (Greenberg et al., 1985). Potassium hydrogen phthalate, obtained from Panreac, was used to prepare a calibration curve with COD values within the range 0-100 mg O_2/L. After

2 h of digestion at 150 ºC (ECO25 – Velp Scientifica), the absorbance was measured at 445 nm in a WTW photolab S6 photometer.

pH was determined using a Crison micropH 2002 apparatus. The pH of the ultrapure water with 10 mg/L of parabens at the beginning of photocatalytic ozonation was 5.7 and at the final pH value after 120 min of reaction falls to 3.2 whichever the operating conditions tested.

V.2.4. Toxicity assessment

The inhibition of luminescence was measured to evaluate the toxicity of samples against *A. fischeri* bacteria using a LUMIStox 300 apparatus from Dr. Lange GmbH (HACH). The untreated and treated samples, for the different reaction conditions, were inoculated with the bacteria solution at 15 °C in a LUMISTherm (HACH). Luminescence was measured directly in the LUMIStox 300 after 15 min of incubation and compared with a blank, where bacteria were placed in a NaCl solution (2%). *C. fluminea* (clams) individuals were collected from a canal in Mira, Portugal (N40°25'06.90''/W8°44'13.18''). Individuals with shell length in the range 20-30 mm were selected and transported in field water to the laboratory, where they were gradually acclimated for one week before experiments. Two replicates were used for each effluent concentration (50, 75 and 100% V/V) with a blank control (dechlorinated water). The effluents were dosed after 24 h acclimation to the test pots in a ratio of 1 L of test medium to 20 clams. The vessels were kept at constant temperature (20 ± 2 °C), under a 16 h^{Light}: 8 h^{Dark} photoperiod cycle and continuous aeration. The mortality of clams was assessed every 24h along the test period based on siphoning activity evidence or resistance to valve opening when forced with a blunt dissection needle (Gomes et al., 2014). To evaluate the phytotoxicity of the samples, the number of *L. sativum* (plant) germinated seeds and the radicle growth were determined to calculate the germination index (GI) (Trautmann and Krasny, 1997). In this test, 10 seeds of *L. sativum* were evenly spread over a filter paper on a petri dish. The volume of sample used was 5 mL. The tests were performed using the treated effluents (120 min of photocalytic ozonation) (100% V/V). The petri dishes filled with the samples were placed in an oven for 48 h at constant temperature of 27 ºC in the dark. After this, the number of seeds germinated and radicle growth of each one is recorded. GI is the product of relative seed germination (RSG) and relative radicle growth (RRG). The phytotoxicity was classified using the germination index according to Trautmann and Krasny (1997) criteria. The pH of all the samples was corrected to 7 using a NaOH solution prior to the toxicity tests.

V.3. Results and Discussion

V.3.1. Catalyst characterization

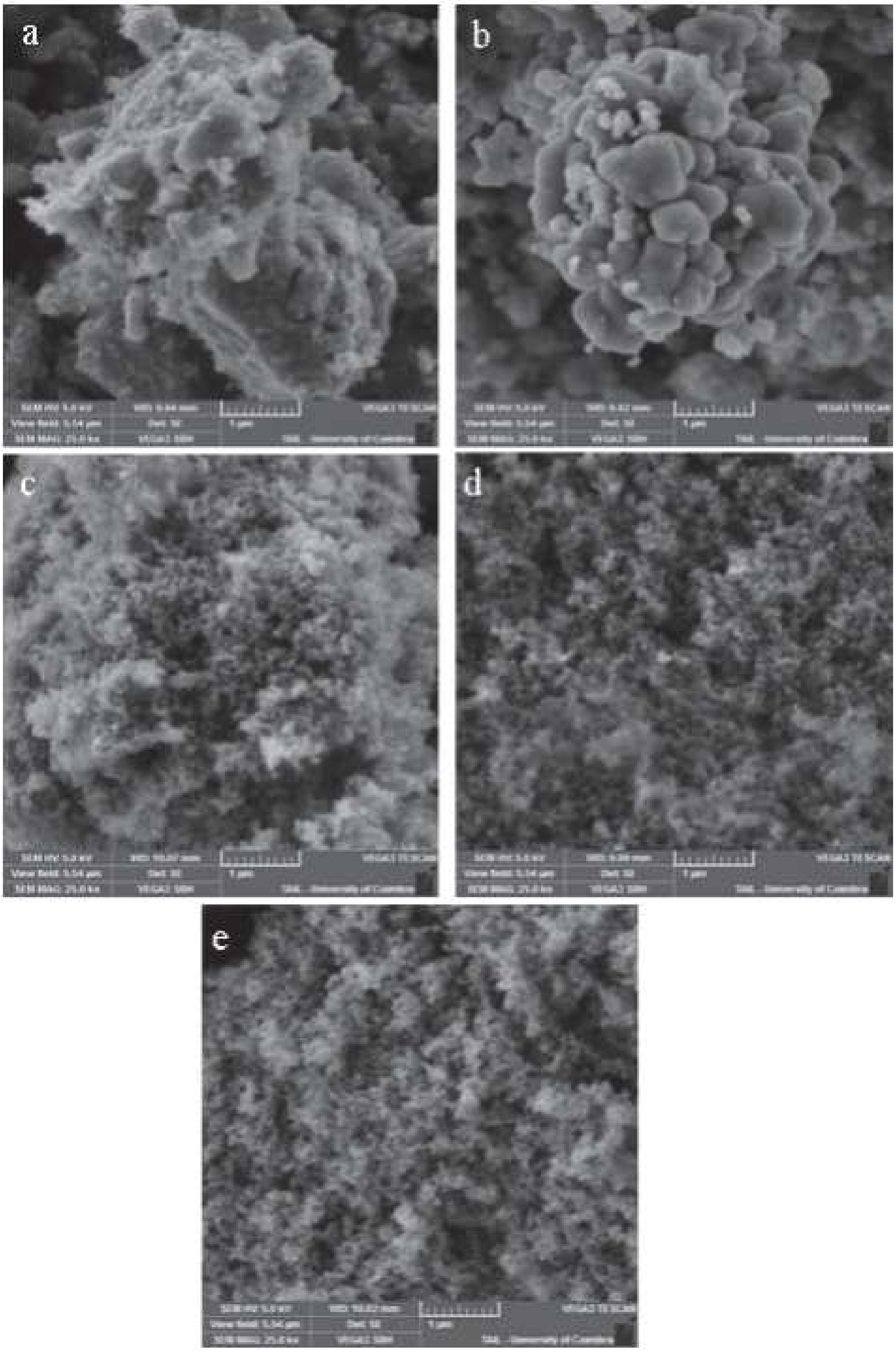

Figure V.2. SEM images (25000x) of different materials (a- TiO_2; b- Au-TiO_2; c- Ag-TiO_2; d- Pt-TiO_2; e- Pd-TiO_2)

The SEM analysis (Fig. V.2) shows that sol-gel preparation method used for Au-TiO_2, did not change significantly the shape and morphology of original TiO_2 particles (Fig. 2a). Besides, the well-defined shape of Au-TiO_2 particles (Fig. V.2b), with higher agglomeration of particles can be observed. The size of particles is higher comparatively to other catalysts (Fig. V.2 c, d, e). On the other hand, the shape and morphology of TiO_2 particles suffer some alterations after photo-deposition of noble metals (Ag, Pt and Pd) on surface. For these catalysts the size of the particles is lower at the nanoscale and the materials show a certain roughness on their surfaces (Fig. V.2 c, d, e). It can be concluded that the sol-gel methodology for preparation of catalysts is more efficient than photo-deposition in what regards metal dispersion. On the other hand, EDS analysis revealed a good dispersion of metals on TiO_2 surfaces except for Pt-TiO_2 which confirmed a low metal dispersion (Gomes et al., 2017a).

The surface composition of the samples was determined from the intensities of the characteristic photoelectron lines taken in high resolution, and atomic sensitivity factors provided by the manufacturer of the XPS system. The relative concentrations of elements in Pt-TiO_2 as well as in Pd-TiO_2 and Ag-TiO_2, are provided in Table V.1. Based previous research where Au-TiO_2 catalysts exhibits the lowest photochemical activity the XPS analysis was not performed. Pt shows the lowest incorporation percentage, while for Ag the percentage found was near the theoretical one.
Morphological and surface characterization of the samples (Pt, Pd, Ag and Au nanoparticles photodeposited on TiO_2) were studied and published elsewhere (Diak et al., 2015; Grabowska et al., 2016; Gomes et al., 2017a,b).

Table V. 1. Relative elemental concentrations obtained from the XPS composition analysis

Catalysts	O (%)	Ti (%)	C (%)	Ag content (at%)	Pt content (at%)	Pd content (at%)
0.5 Pt	48.70± 0.55	20.73± 0.01	30.46± 0.54	-	0.125± 0.01	-
0.5 Pd	55.88±0.05	23.66±0.62	20.24±0.66	-	-	0.23±0.01
0.5 Ag	58.63±0.66	23.84±0.25	17.06±0.88	0.47±0.04	-	-

The XRD analysis of the catalysts showed that anatase phase is predominant which means that band gap of pure titanium dioxide is probably close to 3.2 eV. Therefore, the UVA irradiation used in this work is enough to cause the production of photogenerated electron-hole pairs. However, it is likely that application of noble metals for TiO_2 doping results in the reduction of band gap. For the other catalysts, doped with noble metals, no significant variations occurred on XRD patterns compared to the one observed for pure TiO_2. It is probably due to the low load of metals used for their preparation (0.5 % wt.) (Gomes et al., 2017b).

For evaluating the thermal stability of the catalysts powders, the thermogravimetric analysis was performed for a wide range of temperature from 25 ºC to a maximum of 1200 ºC, with a heat rate of 10 ºC/min. The maximum weight loss, to Au - TiO_2, Pd - TiO_2 and pure TiO_2, occurred between room temperature and 300 ºC, which represents the evaporation of water from the surface of the catalysts. For Pt and Au-TiO_2, the thermogravimetric analysis reveals high weight loss between 400 and 1100 ºC which can be attributed to the decomposition of organic solvents. But weight losses lower than 10% in this range of temperatures confirms the thermo stability of the catalysts (Gomes et al., 2017b).

The BET analysis of catalysts powders reveals that specific surface areas of catalysts increase accordingly (values between brackets): Ag-TiO_2 (34 m^2/g) < Pd-TiO_2 (73 m^2/g) < Au-TiO_2 (135 m^2/g) < TiO_2 (149 m^2/g) << Pt-TiO_2 (238 m^2/g) (Gomes et al., 2017a). The higher specific surface area of Pt-TiO_2 can be related to the low metal dispersion confirmed previously by EDS analysis.

V.3.2. Photocatalytic ozonation

Total parabens degradation for all tested conditions in the photocatalytic ozonation experiments was achieved (Figure V.3). The presence of catalysts improves the parabens degradation, as can be seen in Figure V.3. Using the catalysts, the transferred ozone doses required for total depletion are lower compared to the single photoozonation. In fact, for single photoozonation total parabens degradation was achieved using about 118 mg/L of TOD, while for the worst catalyst in terms of efficiency of parabens degradation (0.5% Au-TiO_2) only about 84 mg/L of TOD was necessary. This reduction on the amount of ozone makes the usage of catalyst coupled with UVA irradiation an interesting option on parabens degradation. Futhermore, in a previous work, the single ozonation without UVA irradiation required around 170 mg/L of TOD for achieving total parabens degradation (Gomes et al., 2017a). Therefore, the presence of UV-A irradiation allows a reduction around 30% in the amount of ozone. This reduction was further enhanced in the presence of catalysts (Figure V.3).

From all tested compounds, the benzylparaben has highest molecular weight and comprises two aromatic rings. So, these two characteristics can explain the lower amount of ozone necessary for achieving its complete degradation. Martins and Quinta-Ferreira (2009b) studied the ozonation for phenolic content removal and verified that ozone is very reactive with the nucleophilic sites of phenolic ring. 0.5% Ag-TiO_2 was the most efficient catalyst on parabens degradation leading to total degradation of BeP using 30 mg/L of TOD. On other hand the worst catalyst, 0.5% Au-TiO_2, needed about the double amount of TOD (60 mg/L) for achieving the same result. The BET analysis reveals that specific surface area of Au-TiO_2 (135 m^2/g) is higher than the one of Ag-TiO_2 (34 m^2/g). It seems that the type of active sites rather than the surface area available for reactions determines the process efficiency. As described above, for the remaining parabens the 0.5% Au-TiO_2 was the most inefficient catalyst. As can be seen in Figure V.3, pure TiO_2 was more efficient than Au-TiO_2. This result can be probably explained by the electronegativity of gold. According to Pauling scale, the electronegativity of the noble metals used in these catalysts decreases following this order (values between brackets): Au (2.54) > Pt (2.28) > Pd (2.20) > Ag (1.93). Gold is the noble metal with the highest capacity to retain photogenerated electrons (eq. V.1), therefore lower amount of ozone can be reduced. Besides this, higher electron affinity of gold (223 kJ/mol) when compared with the other metals difficult the release of electrons, so few ozonides radicals ($O_3^{\bullet-}$) can be produced (eq. V.2), and consequently lower amount of hydroxyl radicals ($^{\bullet}OH$) may be produced (eq. V.4) (Oros-Ruiz et al., 2013). Therefore, with pure TiO_2 probably the photogenerated electrons (eq. V.1) were not retained and were available to reduce O_3. The difference between pure TiO_2 and the remaining catalysts maybe the result of the noble metals present on the catalyst surface (Zheng et al., 2011; Wang et al., 2012). On one hand, more electrons are generated since the energy gap is lower, but the presence of metals reduces electron-hole recombination. Besides the metals with low electron affinity makes the photogenerated electrons available to react with ozone hence producing higher amount of ozonide and hydroxyl radicals.

The catalytic ozonation with these catalysts was investigated previously (Gomes et al., 2017a). The best results for parabens degradation (MP, EP, PP, BuP and BeP) were achieved for platinum and palladium supported on TiO_2 surface. Total parabens degradation was complete for a TOD of about 70 and 80 mg/L with these two catalysts, respectively (Gomes et al., 2017a). In the present study, the best results on parabens degradation were obtained using Ag-TiO_2 and Pt-TiO_2. For Pd-TiO_2 the total parabens (MP, EP and PP) degradation was achieved using about 64 mg/L of TOD, which is a small difference for the TOD required by ozonation using this catalyst (80 mg/L) without UVA. In the case of pure TiO_2, the total degradation of parabens was achieved using a TOD of 70 mg/L, which is a similar result to the one obtained with ozone and catalyst (Gomes et al., 2017a). The same behavior occurred with 0.5% Au-TiO_2. Therefore, the presence of light had no significant improvement on the catalytic activity of these catalysts. This may be related to the high electronegativity of Au and the low photoactivity of pure TiO_2

for UVA. Thus, the amount of hydroxyl radicals produced by these two catalysts will not be largely improved by the presence of light. Contrarily Ag-TiO_2 was the most photoreactive catalyst because total parabens degradation was achieved using a TOD of 40 mg/L which is much lower (94 mg/L) compared to catalytic ozonation without UVA irradiation (Gomes et al., 2017a) for the same catalyst. This good result for 0.5% Ag-TiO_2 can be explained by the higher amount of photogenerated electrons available for reducing ozone. In fact, silver has the lowest value for electronegativity and the electron affinity of 125 kJ/mol which means that not too much energy is necessary for releasing an electron compared with platinum (205 kJ/mol) and gold (223 kJ/mol).

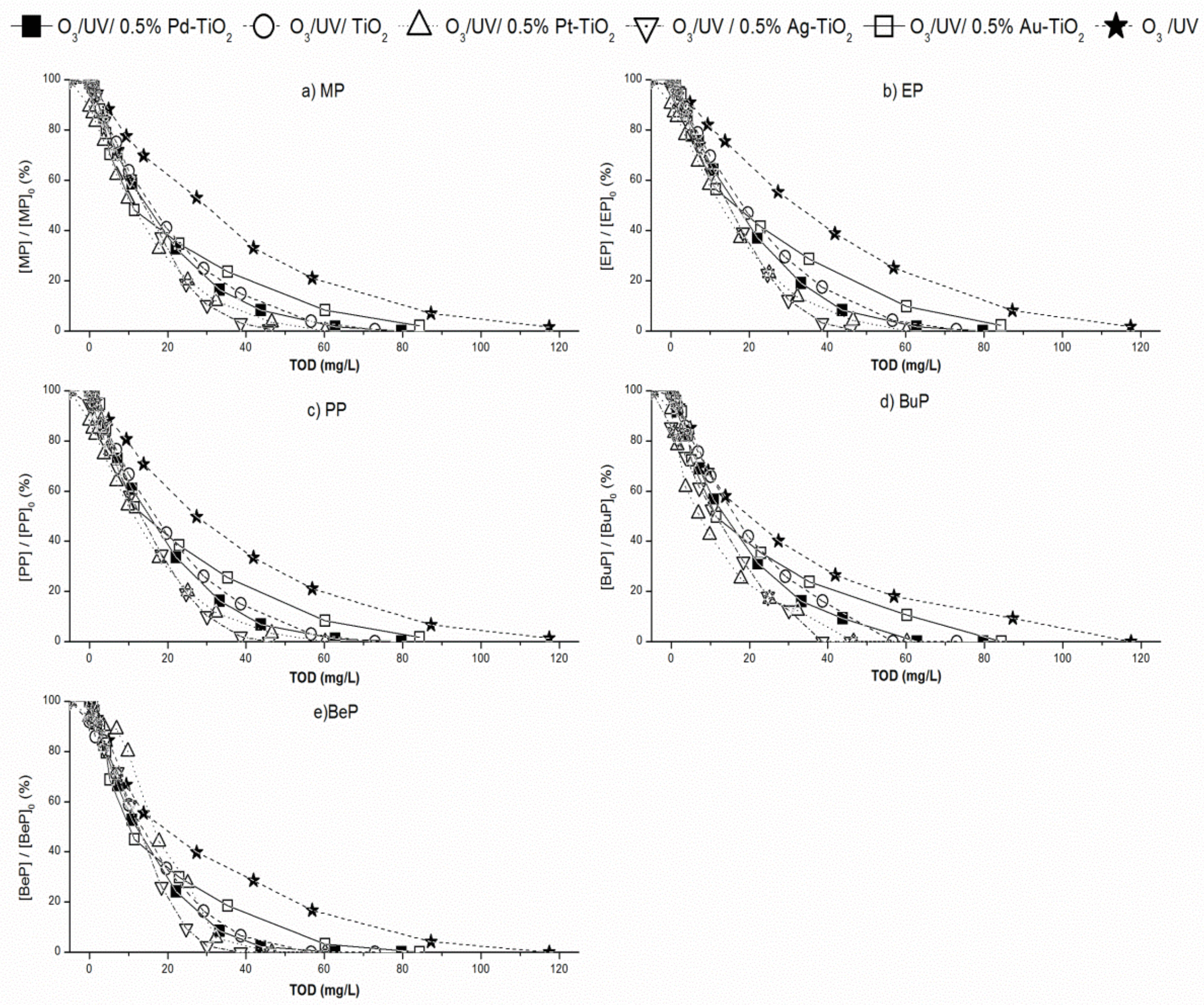

Figure V. 3: Normalized concentration of individual parabens (a – methylparaben (MP); b – ethylparaben (EP); c – propylparaben (PP); d –buthylparaben (BuP) and e – benzylparaben (BeP)) during photolytic and photocatalytic ozonation of the mixture as a function of TOD.

V.3.3. COD removal and mineralization of parabens mixture

The initial parabens mixture has a COD value of 95 mg/L. Even if total degradation of the parent parabens is achieved for all treatments applied, the COD is never totally removed, which means that the degradation of parabens produces some refractory by-products. The presence of catalyst improves photolytic ozonation on COD removal (Figure V.4). The photolytic ozonation using a TOD of 118 mg/L can remove 27% of the initial COD. In fact, after an ozone dose of 88 mg/L the further increase on oxidant had no significant effect over COD removal. Gomes et al. (2017a) using a TOD of 118 mg/L with single ozonation achieved a COD removal around 22%, therefore the presence of UVA irradiation does not change significantly the amount of organic matter removed when compared with O_3 by itself. Ozone is the main responsible for parabens degradation with this kind of radiation without photocatalysts. On the other hand, the lowest value of COD, corresponding to 50% of the initial COD, was achieved for the Pt-TiO_2 using a TOD of 60 mg/L. However, Ag-TiO_2 reduced the COD to 44% of the initial value using a TOD of 46 mg/L (Figure V.4). In a previous study, for catalytic ozonation with Ag-TiO_2 the COD removal was very low (around 6% with a TOD of 95 mg/L(Gomes et al., 2017a)). So, with this result it may be concluded that the improvement on organic matter degradation was due to the UVA irradiation. Maybe the presence of photogenerated electrons and holes promotes organic matter degradation through an increase of reactive oxygen species. Until a TOD of 20 mg/L for Ag-TiO_2 the behavior of COD removal was like photolytic ozonation, achieving for this TOD 6% of COD removal. After this COD removal suffers a significant abatement, around 20% for an increase of 10 mg/L of TOD. This can be explained by the fact that until a TOD of 20 mg/L total degradation of BeP and about 90% of remaining parabens are removed. Ozone by itself is very reactive with these contaminants due their nucleophilic sites. Thus, until a TOD of 20 mg/L the COD removal is most likely due parabens abatement. Therefore, the main differences on COD removal verified afterwards between photolytic ozonation and photocalytic ozonation using Ag-TiO_2 will be due to intermediates decomposition which are probably less reactive with ozone.

Using a TOD of 25 mg/L, the catalytic system involving Pd-TiO_2 only removed 2% of initial COD. Thus, using a low ozone dose in the presence of UVA irradiation applying Pd-TiO_2 as catalyst inhibits the degradation of organic matter. Possibly for this ozone dose occurs the production of refractory intermediate compounds that are still accounted as COD, since the parent parabens degradation for this TOD is above 75%. After this TOD value, the photocalatyic ozonation with Pd-TiO_2 leads to higher COD removal rates and, using a TOD of

80 mg/L, a COD abatement of 41% is achieved (Figure V. 4). Gomes et al. (2017a) for the catalytic ozonation of the same mixture using Pd-TiO_2 attained 35% of COD removal. So, with this catalyst the presence of light had no significant improvement on COD degradation. However, with catalytic ozonation the TOC removal was 18% (Gomes et al., 2017a), and in the present study this value increased to 38% for the same value of TOD (80 mg/L). So, for this catalyst the presence of light maybe improves mineralization rather than partial oxidation.

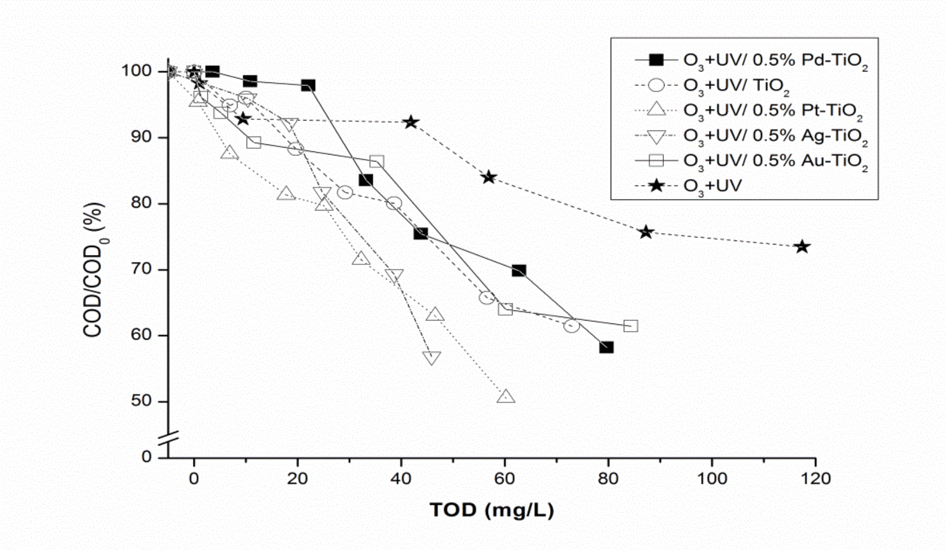

Figure V. 4. COD removal as function of TOD during photolytic and photocatalytic ozonation.

TOC analysis to the treated solution after 120 min was performed as a measure of the mineralization degree. Table V.2 summarizes the TOC and COD removal and the oxidation yield (μCOD_{Partox}). The oxidation yield was calculated to compare the selectivity of different treatments, following eq.V. 7 and V. 8 (Hellenbrand et al., 1997).

$$\mu COD_{Partox} = \frac{COD_{Partox}}{COD_0 - COD} \quad \text{Equation V.7}$$

$$COD_{Partox} = \left(\frac{COD_0}{TOC_0} - \frac{COD}{TOC}\right) . TOC \quad \text{Equation V.8}$$

The μCOD_{Partox} was calculated to understand if COD is removed by partial oxidation or mineralization. This parameter can be within the range of 0 (total mineralization) and 1 (100% of partial oxidation). As can be seen in Table V.2 the best results for μCOD_{Partox} were obtained for 0.5% Pd-TiO_2 which means that about 92% of COD removed was transformed into carbon dioxide and water. TOC removal increases according the following order: 0.5% Au-TiO_2 < photolytic ozonation < Pure TiO_2 < 0.5% Ag-TiO_2 < 0.5% Pt-TiO_2 < 0.5% Pd-TiO_2. This is different from the order obtained for COD removal, where the 0.5% Pt-TiO_2 has the highest value of COD removed (about 50%) and 0.5% Ag-TiO_2 the second highest value (44%). The values of μCOD_{Partox} for these catalytic systems reveal that 84% and 75% of COD removed was transformed in CO_2 and H_2O, when Ag-TiO_2 and Pt-TiO_2 were applied respectively. Meanwhile these values of μCOD_{Partox} were obtained using a lower TOD comparing with Pd-TiO_2 (Table V.1). The photolytic ozonation has the highest μCOD_{Partox} which means that ozone and UVA removed organic matter by partial oxidation way. Ag-TiO_2 was selected as the best catalyst since it required lower TOD to achieve total removal of parabens mixture. However, if the oxidation yield (function of COD and TOC removals) is considered, Pd-TiO_2 led to the best results. Globally in terms of efficiency on organic matter degradation the Ag, Pt and Pd-TiO_2 appear as promising catalysts for UVA aided ozonation.

Table V.2. TOD, COD removal, TOC removal and Oxidation yield for different treatments applied after 120 min of reaction

Condition	TOD (mg/L)	$COD_{removal}$ (%)	$TOC_{removal}$ (%)	μCOD_{Partox}
O_3 / UV	118	27	26	0.68
O_3/UV/0.5% Au-TiO_2	84	38	25	0.40
O_3/UV/TiO_2	73	39	28	0.30
O_3/UV/0.5% Pd-TiO_2	80	41	38	0.08
O_3/UV/0.5% Ag-TiO_2	46	44	37	0.16
O_3/UV/0.5% Pt-TiO_2	60	50	37	0.25

Since TOC is not totally removed in any of the processes tested, it means that organic by-compounds are still present in solution after treatments. Therefore, it is necessary to understand if the resulting solutions are less or more toxic over different species compared to the initial mixture of parabens.

V.3.4. Toxicity assessment

The initial mixture of parabens was highly toxic to the different species tested (Table V.3). The treatments applied reduced the toxicity of the initial mixture. For this toxicity assessment the pH of different effluents was adjusted to a near neutral value, because the species used are sensitive to a non-neutral pH value.

C. fluminea (no data shown) was submitted to acute toxicity tests during 72 h. The mortality of clams was measured at each 24 h and the final results represent to the cumulative mortality after 72 h. In these tests the mortality of the clams at different concentrations of treated effluents (100, 75, 50 % V/V) was examined. For the samples obtained after 120 min of photolytic ozonation and photocatalytic ozonation with different catalysts no mortality was verified for all concentrations tested after 72 h of exposure. However, for the initial mixture of parabens 100%

mortality was achieved after 72 h. Therefore, the treatments applied to the initial mixture of parabens clearly reduced the toxicity towards *C. fluminea* even though the total COD removal was not achieved. This indicates that the refractory by-products produced are less toxic than parabens.

Table V.3 presents the results obtained in the tests with *A. fischeri* and *L. sativum*. For *A. fischeri* the initial mixture of parabens leads to 95% luminescence inhibition after 15 min of exposure. Miralles-Cuevas et al. (2017) suggested that if the luminescence inhibition was higher than 30%, the samples can be considered toxic. So, the initial mixture of parabens is very toxic for these bacteria. The photocatalytic ozonation provided significant reduction of initial toxicity. For the treated samples, the higher luminescence inhibition was achieved for 0.5% Pt-TiO_2 treated effluent, about 61%, which corresponds to a reduction of about 30% compared with the initial mixture of parabens. For 0.5% Pd-TiO_2, 0.5% Ag-TiO_2 and pure TiO_2 the luminescence inhibition was not so far from 30%, so these three catalysts exhibit a potential effect on acute toxicity over *A. fischeri* abatement. Gomes et al. (2017a), for the same parabens mixture achieved about 39% and 26% of luminescence inhibition when bacteria was exposed to the samples coming from single ozonation and catalytic ozonation applying pure TiO_2, respectively. Thus, it can be concluded that, generally, the treatment involving UVA irradiation, ozone and catalysts leads to a higher toxicity removal.

Table V.3 also shows the results for GI of *L. sativum* in contact with the samples obtained after 120 min of treatment with photolytic and photocatalytic ozonation compared with the initial mixture of parabens. According Trautmann and Krasny (1997) criteria, the initial mixture of parabens, with GI around 40%, must be considered very phytotoxic. As can be seen in Table V. 3, all treatments applied increased the GI for values around and above 90% which means that all treated samples are considered non-phytotoxic. For noble metal doped catalysts, the GI values are above of 100%, which means that the transformation by-products can somehow improve the growth of *L. sativum*. The worst result was obtained for pure TiO_2 with GI around 90%, which according to Trautmann and Krasny (1997) criteria can be considered as non-phytotoxic. Gomes et al. (2017a), using ozonation with these catalysts achieved the GIs of about 80%. Moreover, single ozonation led to a moderately phytotoxic behavior (GI≈ 65%).

Table V.3. *A. fischeri* luminescence inhibition after 15 min of exposure, and GI for *L. sativum* (%) after 120 min treatment and for initial mixture of parabens.

	Luminescence inhibition after 15 min (%) (±95 % CI in brackets)	**Germination Index (%) (±SD)**
Initial mixture parabens	95.6 ± 0.0	42 ± 11
Photolytic Ozonation	59.9 ± 1.8	93 ± 11
0.5% Pd-TiO$_2$	44.2 ± 1.4	108 ± 7
0.5% Pt-TiO$_2$	61.4 ± 1.2	107 ± 11
0.5% Au-TiO$_2$	55.4 ± 1.0	107 ± 5
0.5% Ag-TiO$_2$	43.4 ± 1.4	112 ± 1
Pure TiO$_2$	36.3 ± 0.0	90 ± 0

V.3.5. Implications

The cost evaluation is an important step when deciding the industrial implementation of a treatment technology. Hollender et al. (2009) describes that the energy consumption at a treatment plant for ozone production along with oxygen production is 12 kWh/kg O_3. Table V.4 shows the energy consumption for reaching total parabens degradation at different applied conditions (Ozone + catalyst and Ozone + UVA irradiation + catalyst). The data for ozone treatment without UVA irradiation was determined using the data from previous study (Gomes et al., 2017a).

In the experiments using UVA irradiation, light consumption was about 6.75 - 13 kWh/m^3. This range of energy consumption is explained by the fact that for photolytic experiments 1.5 hours of UVA irradiation were necessary to achieve total parabens degradation (highest value) while for photocatalytic ozonation only 45 min of UVA irradiation were needed. As can be seen in the Table V.3 the presence of UVA irradiation makes the treatment more expensive compared with ozonation and catalyst. The presence of light improves the parabens degradation, but the energy consumption is higher compared with the worst result of single ozonation (2.04 kWh/m^3). The energy consumption is higher with the presence of UVA irradiation, but the use of radiation allows a reduction of retention time required for treatment implying consequently smaller reactors which will probably lower the investment on the layout of wastewater treatment plant. If one considers that the retention time required for a continuous system to achieve total parabens degradation is the same that the reaction time obtained for the batch system (Table V.4), it can be concluded that, for the same wastewater flowrate, the reactor would be 60 % smaller when 0.5% Ag-TiO$_2$ was used as catalyst. It should be though considered that the reactor configuration used for the real scale application will have strong implications over the process efficiency. In fact, if a slurry system is designed, a sedimentation/filtration unit must be considered for catalyst removal and recovery. On the other hand, the immobilization of the photocatalysts in a support would avoid the need of a separation step. However, the process would lose some of the efficiency since light penetration and eventual mass transport limitations would probably be not be negligible.

Table V.4. Energy consumption of parabens degradation using Ozone and UVA irradiation for different catalysts

Conditions	**Energy Consumption single or catalytic Ozonation (kWh/m^3)**	**Energy Consumption single or catalytic Ozonation + UVA irradiation (kWh/m^3)**
O_3	2.04	14.4
O_3/ 0.5% Au-TiO_2	1.01	7.7
O_3/ TiO_2	0.84	7.6
O_3/ 0.5% Pd-TiO_2	0.96	7.5
O_3/ 0.5% Ag-TiO_2	1.13	7.2
O_3/ 0.5% Pt-TiO_2	0.84	7.3

V.3.6. By-products analysis

The photocatalytic ozonation of parabens degradation showed to be efficient in degrading compounds, but total mineralization was never achieved. Therefore, it is important to identify the resulting by-products of the reaction. The hydroxylation appears to be the most significant reaction pathway during photocatalytic ozonation, as can be seen in Figure V.5a. Figure V.5a reveals the possible pathways on parabens (A) degradation in the presence of molecular ozone, hydroxyl radicals and UV light (Lin et al., 2009; Tay et al., 2010; Lin et al., 2011; Gmurek et al., 2015). By-products identification was made by HPLC. The standards of 4-Hydroxybenzoic acid (4-HBA), 3,4-Dihydroxybenzoic acid (3,4-diHBA), 2,4-Dihydroxybenzoic acid Hydroquinone and 1,4-Benzoquinone were used. Their peaks were identified on the chromatograms of the samples obtained during the photocatalytic ozonation for the different catalysts tested. These compounds were quantified and represented as function of TOD along photocatalytic ozonation experiments (Figure V.5b). Hydroquinone, 4-HBA, 3,4-diHBA and 2,4-diHBA are the most common identified by-products resulting from ozonation, hydroxyl radical degradation or photocatalytic oxidation of parabens (Lin et al., 2009; Tay et al., 2010; Lin et al., 2011; Gmurek et al., 2015). Some compounds such as mono-, di- and tri-hydroxyderivatives from parabens may also be found because the hydroxyl radicals and molecular ozone can react with the phenolic ring to produce hydroxylated parabens (Hollender et al., 2009). From the C mass balance bearing in mind the detected by-products, it can be concluded that these compounds represent less than 0.5% of the samples TOC. This means that other by-products are being produced. In fact, from Criegee mechanism several aliphatic compounds (such as carboxylic acids) may be produced through the ring opening. Moreover, other hydroxyderivatives of parabens are surely produced. As can be seen in Figure V.5a, the 4-HBA (B) production can occur through hydroxyl radical attack of O-R bond (R can be methyl, ethyl, propyl, butyl or benzyl group). The removal of this group produces a radical that can react with water to form B or this radical can react again with hydroxyl radical that can break the C-C bond releasing CO_2 resulting on hydroquinone (D) (Lin et al., 2009; Velegraki et al., 2015). The dihydroxylated benzoic acid, such as 3,4-diHBA (C) and 2,4-diHBA production can occur via direct molecular ozone attack of the benzenic ring due to the electrophilic character of this molecule (Fig. V.5a) or can be produced by hydroxyl radical reaction with benzenic ring. 3,4,5-Trihydroxybenzoic acid (data not shown) was also identified, but only when two catalysts were applied (0.5% Ag-TiO_2 and 0.5% Pt-TiO_2) and using high values of TOD. Possibly, at those conditions higher amount of molecular ozone doses are available to react with benzenic rings. Also, as can be seen in the Figure V.5a, due to the presence of UV-light and water the hydroquinone can oxide to produce 1,4 benzoquinone (E). All by-products (identified and other not identified) can react with ozone and hydroxyl radicals to produce low molecular weight compounds that can be mineralized into CO_2 and H_2O.

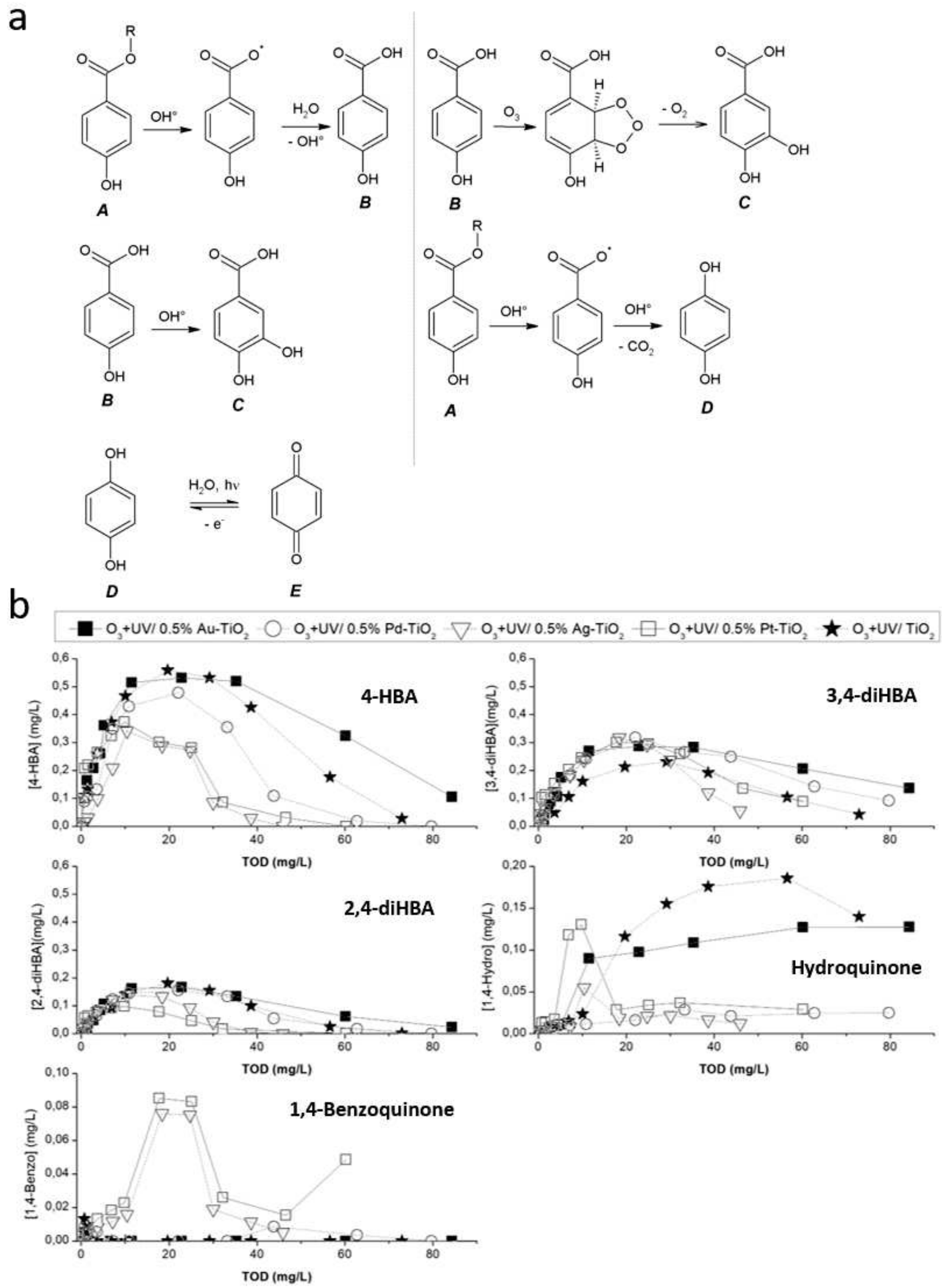

Figure V.5. a) Possible pathways for parabens degradation using photocatalytic ozonation and b) by-products (4-HBA; 3,4 diHBA; 2,4-diHBA; Hydroquinone and 1,4 benzoquinone) formation for differents catalysts tested.

Figure V.5b describes the identified by-products concentrationduring the photocatalytic ozonation experiments. From all identified by-products, the highest concentration was achieved for 4-HBA. As can be seen, the production of this by-product starts to occur on early stages of photocatalytic ozonation. In fact, this compound is referred as an important by-product of parabens oxidation (Lin et al., 2009; Tay et al., 2010; Gmurek et al., 2015). The 4-HBA concentration profile shows a maximum for a TOD of 10 mg/L when 0.5% Ag-TiO_2 and 0.5% Pt-TiO_2 catalysts were applied. A similar behavior was verified for hydroquinone, possibly due to the high concentration of hydroxyl radicals. For these catalysts higher TOD values than 10 mg/L promoted a decrease on 4-HBA concentration. It is probable that 4-HBA decomposition may lead to low molecular weight by-products which were not detected (Lin et al., 2009; Gmurek et al., 2015). As well as 4-HBA decay may lead to an increase of 3,4-diHBA and 2,4-diHBA production, possible due to its hydroxylation with molecular ozone or hydroxyl radicals (Tay et al., 2010; Gmurek et al., 2015). The concentration of 3,4-diHBA is slightly higher compared to 2,4-diHBA, but the concentration profiles are alike along TOD values. The hydroquinone concentration profile for 0.5%Au-TiO_2 and pure TiO_2 can be an explanation for the lower efficiency of these catalysts regarding parabens, TOC and COD removal. The low hydroquinone production observed for these two catalysts may be due to the low concentration of hydroxyl radicals generated in this system. On other hand, as it can be observed, hydroquinone concentration for 0.5% Ag-TiO_2 and 0.5% Pt-TiO_2 decays while 1.4-benzoquinone concentration increases which can be due to higher amount of holes (h^+) on TiO_2 surface in these two catalysts that can promote the oxidation of adsorbed hydroquinone.

The identified by-products remain after photocatalytic ozonation treatment in some of conditions tested, which explains the low TOC and COD removal values obtained for some of the studied processes.

V.4. Conclusions

The present work presents that the application of UVA irradiation with ozonation and noble metal doped on TiO_2 catalysts improves parabens degradation, as well as COD and TOC removal. It was found that the photolytic ozonation can reduce the parabens concentration to below the HPLC detection level. However, this method of oxidation needs a higher amount of ozone, which increases energy consumption around the double compared to the photocatalytic ozonation. Moreover, the presence of UVA irradiation increased substantially the energy consumption, while total degradation of parabens mixture may be achieved using lower amounts of ozone. Therefore, for photocatalytic ozonation, smaller reactors may be used since less time of reaction is required. 0.5% Ag-TiO_2 using the lowest amount of TOD (46 mg/L) was the best catalyst for total parabens degradation. This value of TOD compared with the photolytic ozonation represents a reduction of around three times. Furthermore, the COD and TOC removals were better for 0.5% Pt-TiO_2 (50%) and 0.5% Pd-TiO_2 (38%), respectively. But the differences were not so sharp compared with 0.5% Ag-TiO_2. It should be noticed that for Pt and Pd doping, the XPS analysis showed that both metals are located underneath the TiO_2 surface. This could relate to its lower activity in comparison to Ag, which is almost all located on the TiO_2 surface. However, these values were better when compared with catalytic ozonation. To understand if organic matter was removed by partial oxidation or mineralization, the μCOD_{Partox} was calculated and results reveal that for 0.5% Pd-TiO_2 almost all organic matter was transformed to CO_2 and H_2O. However, with photolytic ozonation, about 70% of organic matter was removed by partial oxidation. Globally, the best results were obtained with 0.5% Ag-TiO_2, 0.5% Pt-TiO_2 and 0.5% Pd-TiO_2 on parabens degradation mixture, COD and TOC removal. The catalytic activity of the noble metals was also related to with their electron affinity and electronegativity.

The toxicity assessment for different species reveals a reduction of toxicity from initial mixture parabens after treatment. For *C. fluminea* no mortality was observed after 72 h of exposure to the treated samples. While for *L. sativum* the level of very phytotoxic for initial mixture of parabens was converted in non-phytotoxic after treatment with all conditions tested. For the most sensitive species, *A. fischeri* the luminescence inhibition was always higher than 30%, which means that by-products resulting from photocatalytic ozonation still have toxic behavior over these bacteria compared to the other tested species. Nevertheless, a reduction in toxicity compared to the initial mixture of parabens was achieved.

The by-products analysis shows that hydroxylated acids and breakdown products can be produced after photocatalytic ozonation of parabens. Among the detected by-products are 4-HBA, hydroquinone, 1,4-benzoquinone, 3,4-diHBA, and 2,4-diHBA.

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VI. Effect of Noble Metals (Ag, Pd, Pt) Loading over the Efficiency of TiO_2 during Photocatalytic Ozonation on the Toxicity of Parabens

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VI.1. Introduction

The conventional municipal wastewater treatments seem to be inefficient regarding the degradation of some specific compounds (Gomes et al., 2017a). The so-called emerging contaminants encompass compounds usually used on pharmaceuticals and personal care products and are progressively appearing on natural aquatic resources and on the wastewater treatment plants (Gomes et al., 2017a). Among these pollutants are the parabens. The parabens are widely used as preservatives and are antimicrobial, mainly in pharmaceutical and personal care products (Bledzka et al., 2014). However, some studies point out that these compounds present carcinogenic potential (Darbre et al., 2004). Thus, their presence in rivers and wastewater treatment plants may constitute a problem of human health (Bledzka et al., 2014). Bearing in mind the low efficiency of the traditional municipal wastewater treatment processes on the degradation of these compounds, scientific efforts have been made to find a suitable solution to their degradation (Tay et al., 2010; Gmurek et al., 2015). In this way, advanced oxidation processes (AOPs) appear as promising technologies.

Ozone is a powerful oxidant, with a high potential oxidation ($E^{\circ} = 2.07$ V), greater than oxygen and hydrogen peroxide, that can oxidize a large amount of organic compounds with high electronic density, such as aromatic compounds (Martins and Quinta-Ferreira, 2009). Heterogeneous catalysis tends to be helpful with improving wastewater treatment by ozonation (Martins and Quinta-Ferreira, 2009). The presence of heterogeneous catalysts on ozonation can enhance the production of hydroxyl radicals due to the decomposition of ozone onto the surface of catalysts (Kasprzyk-Hodern et al., 2003). Besides the hydroxyl radical production, the organic contaminants can adsorb on the catalyst surface, which can suffer an attack by ozone dissolved in bulk, or ozone and organic contaminants can adsorb on the catalyst surface followed by surface chemical reactions (Kasprzyk-Hodern et al., 2003). The higher potential of oxidation of hydroxyl radicals ($E^{\circ} = 2.80$ V) and their low specificity allows reactions with a wider range of organic and inorganic substances. Thus, hydroxyl radicals may improve the mineralization degree achieved by ozonation. In fact, molecular ozone reactions can be a source of refractory by-products no longer reactive with ozone, leading to low mineralization levels (Kasprzyk-Hodern et al., 2003). The choice of a suitable catalyst can be an important factor for the catalytic ozonation performance. This choice can also be influenced by the use of UV-radiation for aiding ozone action.

Titanium dioxide is the most commonly used photocatalyst, due to low cost, easy handling and good optical and electronic properties (Chong et al., 2010). The photocatalytic ozonation using TiO_2 under LED irradiation was used for removal micropollutants from urban wastewater with high efficiency (Moreira et al., 2016). The band gap is 3.2 eV for anatase TiO_2 ($\lambda < 387$ nm), therefore, for exciting an electron from the valence band to the conduction band, UV-light is required (Pelaez et al., 2012). This electron transfer generates a positive hole on the valence band of TiO_2, usually a denominated photogenerated hole. This mechanism can be seen in Equation VI.1:

$$TiO_2 + h\upsilon \rightarrow h^{+}_{VB} + e^{-}_{CB} \qquad \text{(Equation VI.1)}$$

The band gap of TiO_2 can be reduced using metal (such as, Ag, Au, Pd and Pt) or non-metal (such as N and B) dopants, which allows the production of TiO_2 photogenerated electrons and holes using lower energy, such as solar irradiation (Zheng et al., 2011; Pelaez et al., 2012; Petala et al., 2015).

The presence of ozone on photocatalytic experiments with these kinds of catalysts (semicondutors) can enhance the photogeneration of electron–hole pairs because ozone adsorbed on TiO_2 surface works as an electron acceptor producing ozonide radicals ($O_3^{\bullet -}$) Equation VI.2 (Rivas et al., 2006; Rodríguez et al., 2013):

$$O_{3(ads)} + e^{-}_{CB} \rightarrow O_3^{\bullet -} \qquad \text{(Equation VI.2)}$$

Moreover, as can be seen in (Equation VI.3), noble metals on the surface of titanium dioxide may work as scavengers of photogenerated electrons (e^{-}_{SC}) (Gaya and Abdullah, 2008). These two situations can reduce the recombination phenomenon of electron–hole pairs (Anpo and Takeuchi, 2003; Mehrjouei et al., 2015):

$$e^{-}_{CB} \rightarrow e^{-}_{SC} \qquad \text{(Equation VI. 3)}$$

On the other hand, the water adsorbed on the catalyst surface can react with the photogenerated holes to produce hydroxyl radicals ($^{\bullet}OH$) (Equation VI.4) (Chong et al., 2010; Pelaez et al., 2012). The ozonide radical at acidic conditions can also enhance the production of hydroxyl radicals (Hernández-Alonso et al., 2002). In addition, these organic contaminants (such as parabens) adsorbed on catalysts can be oxidized on the photogenerated holes (Equation VI.5) (Mehrjouei et al., 2015):

$$H_2O_{(ads)} + h^{+}_{VB} \rightarrow OH^{\bullet} \qquad \text{(Equation VI.4)}$$

$$\text{Contaminants}_{(ads)} + h^{+}_{VB} \rightarrow \text{Contaminants}^{+\cdot} \qquad \text{(Equation VI.5)}$$

Photocatalytic ozonation has been used for the degradation of emerging contaminants. The degradation of ibuprofen using photocatalytic ozonation under visible light irradiation with the catalyst WO_3 was studied and verified total removal in less than 20 min (Rey et al., 2015). The effect of Pt loading onto TiO_2 surface was studied for the oxidation of formaldehyde and the 0.6 wt.% loading showed the highest dispersion of the dopant over the surface of the catalyst (Peng and Wang, 2007). An increase of Ag loading onto TiO_2 surface can block the UV-light thus affecting the photocatalytic activity and for Ag loading of 1.6 wt.% the photogenerated hole trapping effect was negligible. Moreover, an increase of Ag loading can increase the photogenerated electron scavenging (Mohamed and Khairou, 2011). The effect of doped TiO_2 at 0.5 wt% loading (Ag, Au, Pd and Pt) and pure TiO_2 was compared on the mixture of parabens degradation using UVA radiation coupled with ozone (Gomes et al., 2017b). Thus, the effect of metal loading onto TiO_2 must be carefully analyzed. The photodegradation using Ag_3PO_4 under simulated solar radiation to promote the degradation scof ethyl paraben was studied taking into account pH, initial parabens concentration, catalyst concentration and water matrix (Frontistis et al., 2017a). The photocatalytic degradation of paracetamol promoted by TiO_2 can be influenced by different features of catalysts such as surface acidity (Rimoldi et al., 2017a).

Bearing this in mind, the aim of the present study is to verify the effect of noble metal loading onto TiO_2 surface over the efficiency of photocatalytic ozonation of a mixture of five parabens, methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BuP) and benzylparaben (BeP). For this purpose, the noble metals Ag, Pd and Pt, at loadings of 0.1, 0.5 and 1 wt.%, doped onto TiO_2 were tested using ozone and UV-A irradiation. In order to understand photocatalytic ozonation promoting the mineralization, chemical oxygen demand (COD) and total organic carbon (TOC) were analyzed. Furthermore, the parabens are toxic compounds, so another goal of this work is to verify the ecological impact of raw and treated solutions. For this, the toxicity assessment over three different species *Aliivibrio fischeri, Corbicula fluminea* and *Lepidium sativum* was performed. The results achieved with the loading of 0.5 wt.% onto TiO_2 were obtained from a previous work (Gomes et al., 2017b). The present study allows for understanding that the increase of noble metals load does not necessarily mean an increase in the parabens degradation, TOC and COD removal efficiency. This analysis is important due to the compromise between efficiency and cost of doped catalysts. The increase in noble metal load over titanium dioxide surface can promote a significant impact in terms of investment cost, which is not probably followed by an increase in the efficiency of the process. Moreover, to the best of our knowledge, no studies have been made for analysis of the effect of different loads of noble metals on parabens degradation through the photocatalytic ozonation process. In addition, this work also considers the effect of the different loadings over toxicity reduction. This analysis was performed taking into account the possibility of leading to wastewater reuse. Thus, the impact over different species (plant, clam, bacteria) gives a broader overview about the potential of real application of this methodology to remove emerging contaminants aiming water reuse.

VI.2. Material and Methods

VI.2.1. Chemical and Catalyst Preparation

Methylparaben (MP), ethylparaben (EP), propylparaben (PP), and benzylparaben (BeP) were obtained from Sigma-Aldrich (Poland). Butylparaben (BuP) was purchased from Fluka (Poland). The initial amount of each paraben used was 10 mg/L. Titanium (IV) isopropoxide (TIP) was purchased from Aldrich Chem (Poland) and was used as TiO_2 precursor. Anatase titanium dioxide nanoparticles were prepared by hydrolysis reaction of TIP with water. The photo-deposition method using UV-reduction of Pt^{4+}, Pd^{2+} and Ag^{+} ions in the titanium dioxide suspension was applied to prepare Pt-TiO_2, Pd-TiO_2 and Ag-TiO_2. A solution of isopropanol (70 mL) containing H_2PtCl_6 or $PdCl_2$ (0.1, 0.5 and 1 wt.%) and a solution of ethanol (70 mL) containing $AgNO_3$ (0.1, 0.5 and 1 wt.%) were sonicated with 2 g of titanium dioxide nanoparticles during 10 min. Then, these solutions were stirred in the dark for 2 h and were degassed with nitrogen and irradiated by UV-Vis light (1000 W Xe lamp) for 6 h and for 100 min, respectively. Afterwards, the solids were separated by centrifugation and dried at 65–120 °C for 12 h. The temperatures used to dry the catalysts do not modify their properties. This procedure was only to remove the humidity from the porous (Stelmachowski et al., 2016).

VI.2.2. Experimental Procedure

The photocatalytic ozonation experiments were carried out in a 2 L glass photoreactor with 3 lamps (Philips TL 6 W BLB) with the main emissions at 365 nm (UV-A irradiation), at controlled temperature (25 ± 1 °C) by a thermostatic bath. The photon flux was obtained using ferrioxalate actinometer (Gomes et al., 2017b) and was calculated at 5.75×10^{-7} einstein/Ls. In order to reduce the effect of external sunlight exposure, the photoreactor was covered with aluminum foil, since these photocatalysts are very sunlight sensitive. To test the adsorption capacity of catalyst, this was placed in the reactor with the solution of parabens 5 min before ozone being fed and turning on the light irradiation. The stirring speed was previously optimized at 700 rpm to ensure chemical regime (Martins and Quinta-Ferreira, 2009). Along the reaction, samples were withdrawn and immediately centrifuged at 3500 rpm (Nahita 2655 Centrifuge) to remove the catalyst for analysis. It was guaranteed that the volume removed did not exceed 10% of total reaction volume.

The ozone was produced (802 N, BMT) from pure oxygen stream (99.9%) and the inlet ($[O_3]^{in}$) and outlet ($[O_3]^{out}$) ozone concentration was measured by ozone analyzers (BMT 963 and 964 vent, BMT), for determination of transferred ozone dose (TOD) according to Equation VI. 6:

$$\mathrm{TOD}=\int_0^t \frac{Q_{Gas}}{V_{Liquid}} \times \left([O_3]^{in}-[O_3]^{out}\right) \times dt \qquad \text{(Equation VI. 6)}$$

Where Q_{Gas} represents the gas flow rate (0.2 L/min), V_{Liquid} the volume of the effluent (2 L). The TOD was expressed in mg O_3/L. The remaining ozone that left the reactor was trapped by KI solution.

VI.2.3. Analytical Methods

The analysis of structure, elemental characterization and metal dispersion on catalyst surface were performed using Scanning Electron Microscopy (SEM) with a TESCAN VEGA 3 SBH Easy Probe equipped with Energy dispersive X-Ray analysis (EDS) with a Bruker QUANTAX system that includes the Bruker Nano XFlash® detector. The EDS analysis was performed for randomly points selected from SEM analysis and was carried out using an accelerating potential of 20 kV. Energy-Channel calibration was used with a copper standard (Kα 8.0463 keV). The crystalline structure of the catalysts powders was evaluated by X-Ray diffraction (XRD) analysis, through a diffractometer (Bruker D8 Advance). The diffractometer works with Cu Kα radiation (2.2 kW ceramic tube). XPS (X-ray photoelectron spectroscopy) analysis of 0.1 wt.% of Pt–TiO_2 was carried out with a Kratos AXIS Ultra spectrometer (Manchester, United Kingdom) using monochromatic Al Kα XRays (1486.6 eV). The 150 W was set for anode power and the hemispherical electron energy analyzer was operated at a pass energy of 20 eV. The measurements were performed with a charge neutralizer. The identification of XPS spectra was performed with Shirley algorithm and the adventitious carbon main peak (C 1 s, 284.8 eV) for a final calibration of each spectrum was used. The determination of each paraben concentration was made through High-performance liquid chromatography HPLC (UFLC, Shimadzu, Tokyo, Japan). The detection occurred at 255 nm, with a C18 (SiliaChrom) column at 40 °C. The injection volume of samples was 20 µL and the mobile phase (0.5 mL/min) consisted in a mixture of 50:50 methanol: acidic water (0.1% orthophosphoric acid). The TOC for initial and after treatments solutions was determined using a TOC analyser (TOC-V CPN model, Shimadzu, Tokyo, Japan) coupled to an autosampler (model V-ASI, Shimadzu, Tokyo, Japan). COD was measured according to the standard method 5220D (Greenberg et al., 1985) using a calibration curve obtained for potassium hydrogen phthalate (Panreac). The samples absorbance was measured at 445 nm (WTW photolab S6 photometer, WTW, Weilheim, Germany) after 2 h of digestion at 150 °C (ECO25—Velp Scientifica). The evaluation of thermal resistance of different catalyst was made by thermogravimetric analysis (TGA). The SDT Q600 (TA Instruments, New Castle, DE, USA) was used with a heating rate of 10 °C/min from a temperature of 25 to 600 °C. pH was determined using a Crison micropH 2002 apparatus (Barcelona, Spain).

VI.2.4. Toxicity Assessment

The luminescence inhibition for *A. fischeri* marine bacteria was determined for initial mixture of parabens and for treated solutions using a LUMIStox 300 apparatus from Dr. Lange. The bacteria luminescence after 15 min and 30 min of incubation with blank (NaCl solution of 2%) was compared with the one obtained when *A. fischeri* were in contact with the samples.

C. fluminea individuals with shell length in the range 20–30 mm were collected from a canal in Mira, Portugal (N 40°25′06.90″/W 8°44′13.18″) where the population is well established. Mortality tests were performed in duplicate for treated samples after 120 min of contact with photocatalytic ozonation and compared with a blank control (dechlorinated water). The effluents were dosed after 24 h acclimation period in a ratio of effluent volume to clams number of 1:20. The test occurred at constant temperature (20 ± 2 °C), under a cycle of 16 h Light and 8 h Dark with continuous aeration. The mortality of clams was assessed every 24 h based on the resistance to valve opening when forced with a blunt dissection needle or by siphoning activity evidence (Gomes et al., 2014). These tests had the duration of 72 h. The number of *L. sativum* germinated seeds and the radicle growth were measured to calculate the Germination index (GI) (Trautmann and Krasny, 1997). As can be seen in Equation VI. 7, the GI results from the product between relative seed germinated (RSG) and relative radicle growth (RRG). Seeds were placed on Petri dishes in contact with sample volume of 5 mL. The number of seeds germinated and radicle growth in contact with treated samples and blank were recorded, and the GI was calculated for each one. The Trautmann and Krasny criteria based on GI was used for sample phytotoxic classification (Trautmann and Krasny, 1997):

$$GI\,(\%) = \frac{RSG\,(\%) \times RRG\,(\%)}{100}. \qquad \text{(Equation VI. 7)}$$

pH of the samples was corrected to 7.0 ± 0.5 before toxicity assessment tests.

VI.3. Results and Discussion

VI.3.1. Catalysts Characterization

XRD analysis reveals that the predominant phase is anatase (results not shown). However, rutile and brookite were also identified in a smaller amount. The diffractogram of pure TiO_2 was presented in a previous study (Gomes et al., 2017c). The presence of the doping metals on TiO_2 does not change the XRD pattern, independently of the loading of noble metals considered. The explanation for the absence of diffractive peak of noble metals is due to the low doping dose. For 1.5 wt% of Pt–TiO_2, the diffractive peak of Pt on XRD analysis was not found due to the low amount doping (Huang et al., 2008). The presence of gold, platinum and silver at 1 wt.% load does not change the diffractive peaks of anatase phase from TiO_2 (Zheng et al., 2011). These studies reveal that the lack of detection in the XRD analysis of diffractive peaks of noble metals for different loads used in our study could also be explained by the low doped dose and/or small size of noble metals' nanoparticles.

The SEM-EDS analysis was performed for all catalysts used in these experiments. As an example, in Figure VI.1, the results for different loads of Pd onto TiO_2 were presented. SEM analysis (Figure VI.1) shows that catalysts prepared by photodeposition method reveal a certain roughness on their surfaces, the morphology is not very clean, or the shape is not well designed (magnification of 5000×). For all catalysts with 0.1 and 0.5 wt.% loading of noble metal, the SEM analysis was similar, the shape does not change with the increase of noble metal loading (Figure VI.1). On the other hand, for 1 wt.% of Pd-TiO_2, the shape of catalysts changed comparatively to 0.1 and 0.5 wt.% (Figure VI.1), leading to agglomerates. Another aspect that seems to be relevant is related with the morphology when increasing the catalyst load. Regarding 0.1 wt.% of Pd-TiO_2, it is possible to see a lower amount of white particles that can be related with palladium. When Pd load increases from 0.1 wt.% to 0.5 wt.%, the number of white particles also increases significantly (Figure VI.1a,b). From 0.5 to 1 wt.%, the same behavior occurs (Figure VI.1b,c) but at lower extension comparatively to 0.1–0.5 wt.%. For the first range, the noble metal load increases five times, while, from 0.5 wt.% to 1 wt.%, it just increases two times. The increase of one noble metal loading promotes microsphere formation. The EDS analysis made to randomly selected points of SEM image presents, for the 0.1% of Pd-TiO_2 titanium, oxygen and chlorine as the main elements, whereas the noble metal is not detected. This can be explained by the low amount of doping metal used and its good dispersion over the support. The presence of chlorine in the spectrum of 0.1% Pd-TiO_2 comes from the precursor used to prepare this catalyst. For 0.5 wt%, the presence of aluminium and silicon was also identified, which means that the sample could be contaminated from the precursor of Pd ($PdCl_2$) and/or TiO_2 (Titanium (IV) isopropoxide). At this loading, it is possible that the selected points from SEM to perform the EDS analysis present a low amount of Pd. This can be related with poor dispersion of noble metal onto TiO_2 surface for the 0.5 wt% loading. The weight percentage values determined with EDS analysis are very small due to the low loading tested. Only for 1 wt% Pd loading was possible to quantify about 0.5 wt% in the randomly selected point of EDS analysis. This reinforces the possible poor dispersion of noble metal onto TiO_2 for higher loadings. The XPS analysis was conducted for the lowest load of Pt doped TiO_2 to prove the presence of noble metal at this load. In this analysis, 0.08 wt.% of Pt onto TiO_2 surface was identified. This value is near the expected value.

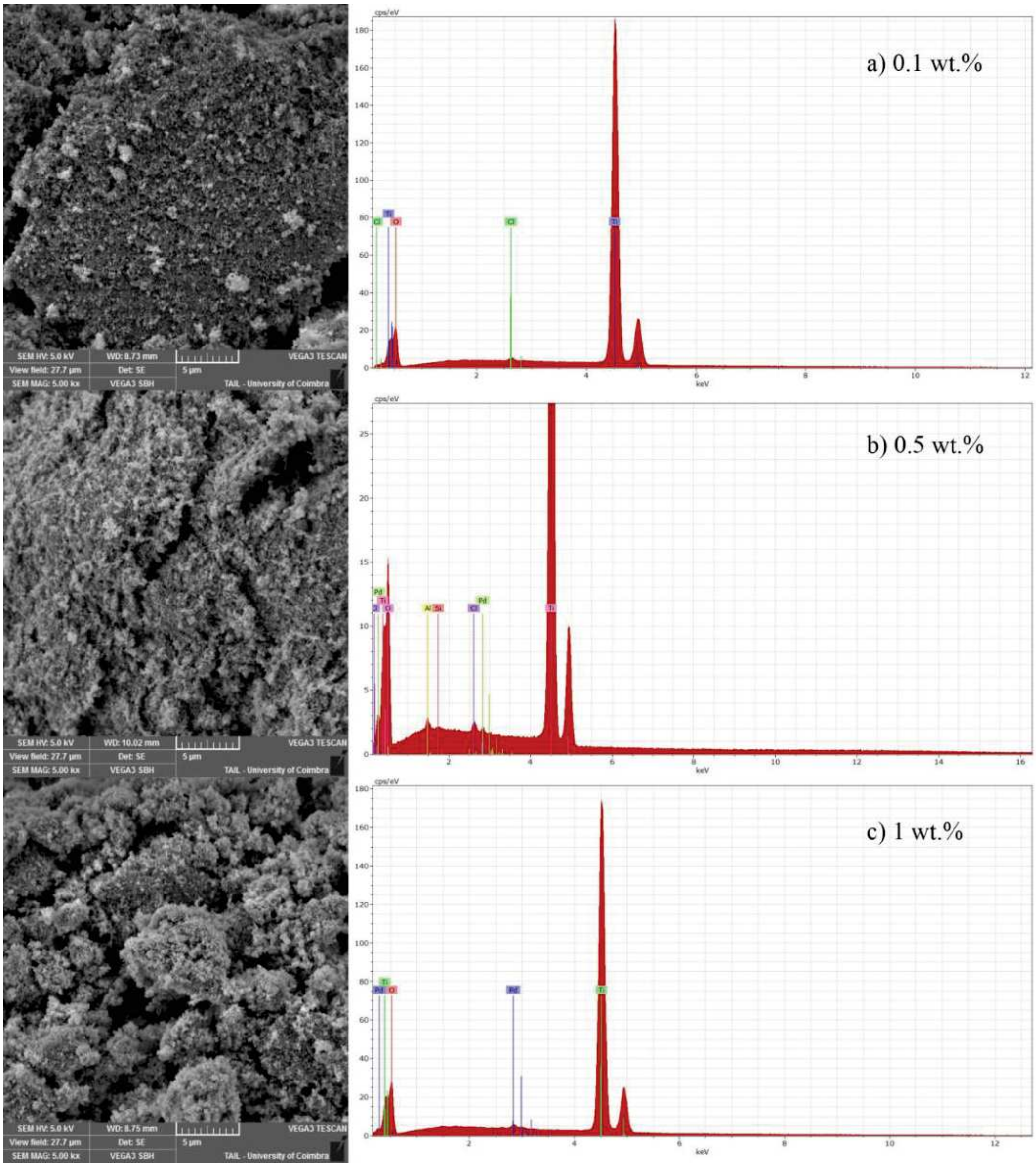

Figure VI. 1. SEM-EDS analysis for catalysts (a) 0.1% Pd-TiO_2; (b) 0.5% Pd-TiO_2; and (c) 1% Pd-TiO_2.

Thermogravimetric analysis was conducted to infer the thermal resistance of catalysts when exposed to high temperatures. The weight loss of catalysts for different noble metal loads as functions of temperature was analyzed. Thermal analysis reveals that catalysts are very stable thermally, since the highest weight loss was just 3%. The presence of different loads of noble metals does not affect significantly the weight loss. For example, in the case of Pd, the maximum weight loss was verified with 0.5 wt% (about 1.5%). This catalyst presents some water on its composition because the greatest weight loss occurs from 25 °C to 100 °C. At 350 °C, another negative variation was verified, which means that the organic contaminants partially identified by SEM-EDS analysis were eliminated at those conditions. The difference in the noble metal load does not change the preparation method. This means that the organic compounds and water present at the catalysts are the same for the different loads. Therefore, the weight loss profile during the TGA is similar for all catalysts.

VI.3.2. Effect of Noble Metal Loading on the Parabens Degradation

Photocatalytic ozonation with Ag, Pd and Pt doped onto TiO_2 catalysts was used to promote the degradation of the mixture of parabens. The effect of different noble metals loads on parabens degradation was analysed as a function of TOD. The UVA radiation is not able to effectively degrade parabens, and just 3% of removal was achieved (Gomes et al., 2017c). On the other hand, for ozone alone, total parabens degradation needed an increase on TOD of 3-fold compared with photocatalytic ozonation. Therefore, the coupling of these two technologies substantially increases the efficiency of parabens degradation comparatively to the contribution of each one alone. As it can be seen, the parabens were all almost totally removed independently of the conditions tested (Figure VI.2). Except for 1 wt% of Ag using a TOD of 78 mg/L, the total degradation for MP, EP and PP was not achieved, contrarily, to what happens for BuP and BeP. In the same way, for 0.1 wt% Pt, total degradation of MP and EP was also not observed. The results for BeP and BuP are not shown. The higher molecular weight compounds (BuP and BeP) were the first ones to be totally degraded using a low amount of TOD (below 30 mg/L for BeP), whichever the catalyst. This may be explained by ozone reactive preference for high electronic density groups such as benzenic rings. The high molecular weight of these compounds leads to a larger number of sites available for reaction. On the other hand, for the parabens with lower molecular weight, the degradation rate was slower, so a higher amount of ozone was necessary for total removal.

Tay et al. (2010) proved that the ozonation degradation constant rates increased with the molecular chain length for the different parabens (MP, EP, PP, BuP). The degradation of our mixture follows this behaviour, namely degradation happens first for BeP/BuP and finally MP (Tay et al., 2010). The kinetics of degradation reactions may be highly dependent upon the matrix (Rioja et al., 2016; Rimoldi et al., 2017b). A study with an emerging pollutant mixture of Tetracycline, Caffeine, Paracetamol and Atenolol verified that the effect of using a mixture was negligible on the decrease of mineralization efficiency (Rimoldi et al., 2017b). Therefore, comparing with our results, the synergistic effect of mixture could be minimal on the parabens degradation. When real water is used, the inorganic salts present in the matrix, especially carbonates, could decrease the removal efficiency (Rioja et al., 2016). On the other hand, some inorganic compounds such as sulfate and chlorine can improve the reverse effect, increasing the efficiency of emerging contaminants removal (Frontistis et al., 2017b).

The results clearly show that, for photocatalytic ozonation, the worst catalyst was 1% Ag-TiO_2. For example, for achieving 95% of MP degradation with this catalyst, a TOD of 78 mg/L was necessary, while, for the best catalyst (0.1 and 0.5% Ag-TiO_2), total MP degradation just needed a TOD of 44 and 39 mg/L, respectively. Thus, for Ag-TiO_2, the increase in the metal load above 0.5 wt% leads to lower efficiency towards parabens removal. However, for Pt-TiO_2 on MP and PP degradation, the decrease in metal loading to 0.1 wt% leads to a process needing a higher amount of ozone compared to 0.5 and 1 wt% of Pt that present similar behavior. Regarding to Pd-TiO_2, in a general way, its efficiency seems to be independent of Pd loading (Figure VI. 2).

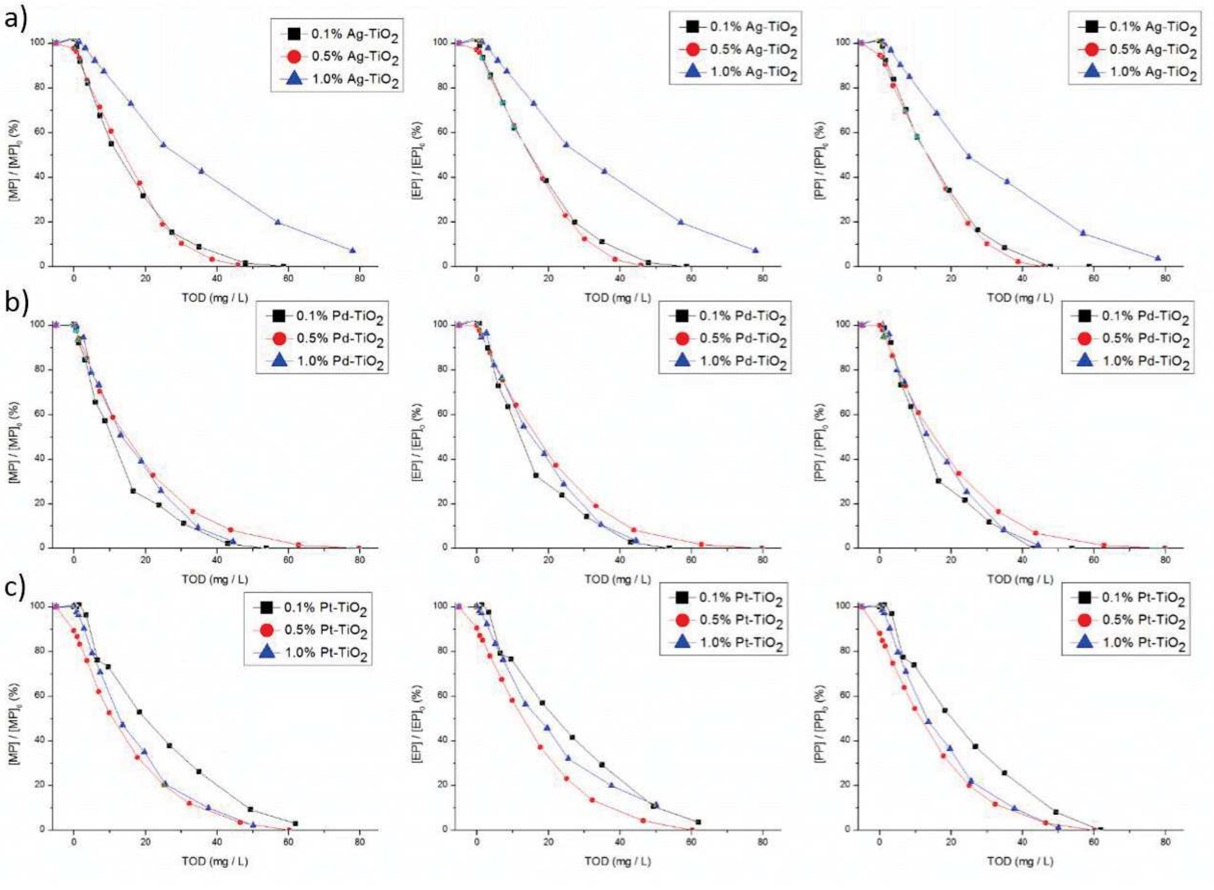

Figure VI.2. Normalized concentration of individual parabens MP, EP, PP as a function of TOD during photocatalytic ozonation for different noble metal loads of (a) Ag–TiO_2; (b) Pd–TiO_2; (c) Pt–TiO_2.

The effect of Pt loading (0.01 wt% to 1 wt.%) onto TiO_2 surface was studied for the oxidation of formaldehyde and concluded that 0.6 wt% was the most active material due to the highest dispersion of the dopant over the surface of the catalyst (Peng and Wang, 2007). This is in accordance with the results obtained in this study, where 0.5 wt% is the most active load for Pt and Ag. The lowest presence of metal loading on TiO_2 surface may reduce the scavenging effect of photogenerated electrons, so the recombination phenomenon of electron–hole pairs can be more recurrent, which can explain the low efficiency of 0.1 wt% in Pt–TiO_2. Therefore, less electrons are available for ozone reduction and few holes may be available to react with adsorbed water to produce hydroxyl radicals, thus reducing the pollutants' oxidation efficiency. Regarding Ag based catalysts, the effect of metal loading until 0.5 wt% was not so sharp in what concerns parabens degradation. The increase of Ag loading from 0.1 wt% to 0.5 wt% allows reducing about 10 mg/L of TOD needed for total parabens degradation. On the other hand, an increase in the metal load to 1 wt% promotes a decrease of the efficiency regarding parabens degradation, resulting in the increase of 30 mg/L of TOD required for parabens depletion. Ag presents a lower electronegativity compared with Pt and Pd (1.93 to 2.28 and 2.20 according to the Pauling scale, respectively). In this way, lower amounts of electrons can be scavenged by Ag. However, when the metal load increases to 1 wt%, the trap of electrons could be higher, and the electrons trapped by Ag were not available for reaction. This can be an explanation for the reduction of efficiency on parabens degradation with this load of silver. For 0.1 and 0.5 wt% of Ag load, the photogenerated electrons are still available to reduce ozone to produce ozonide and hydroxyl radicals. This larger amount of hydroxyl radicals may be helpful for parabens degradation, reducing the amount of TOD needed. Moreover, another explanation for this efficiency reduction can be related to the photohole trapping promoted by a higher load of Ag onto TiO_2 (Hirakawa and Kamat,2005). However, Mohamed and Khairou (2011) say that, up to 1.6 wt% of Ag, this trapping effect was negligible. On the other hand, the excess of Ag may have lead to lower noble metal dispersion over titanium dioxide or even to a shielding effect, not allowing TiO_2 to efficiently absorb radiation so that less photo-electrons and holes were generated.

The recombination phenomenon can be an explanation for the reduction in the efficiency for a lower load of Pt. The noble metal doped onto TiO_2 can reduce the energy band gap (Zheng et al., 2011; Wang et al., 2012), which is the energy needed to move an electron from the valence band to the conduction band of the catalyst, producing photogenerated holes on the surface of the catalyst (Chong et al., 2010; Pelaez et al., 2012). In addition, the presence of noble metals can reduce the recombination phenomenon between electron–hole pairs due to the scavenging of photogenerated electrons (Wang et al., 2012). In this way, the lower amount of Pt minimizes the effect of scavenging electrons, which means an increase of the recombination phenomenon.

Therefore, the ozone reduction and the water oxidation are lower and consequently less production of ozonide and hydroxyl radicals is possible.

The global analysis of the parabens decay results points out that the most efficient catalysts regarding parabens degradation were 0.1, 0.5% Ag-TiO_2.

VI.3.3. COD and TOC Removal

Table VI.1 summarizes COD and TOC removal after 120 min of photocatalytic ozonation for different catalysts. The catalysts lead to slightly different COD and TOC removals. The poorest catalyst on the COD removal, without considering that amount of TOD required, was 0.1% Pd–TiO_2 with only 32% of COD abatement. In the same way, TOC removal presents a similar result. Even if this catalyst presents a good performance regarding parabens degradation, the reaction follows the partial oxidation pathway leading to the formation of organic intermediates instead of carbon dioxide and water.

Taking TOD into consideration, the worst catalyst on the COD removal was 1% Ag-TiO_2. The higher COD removal was attained with 1% Pd-TiO_2 using lower TOD (44 mg/L). In addition, for the 1% Pt–TiO_2, a similar COD removal occurs, but a higher TOD was necessary. Therefore, generally high COD removal can be achieved for all catalysts if TOD increases. Thus, the effect of the amount of ozone is not negligible. Nevertheless, TOC removal does not necessarily follow the same trend since, for some cases where TOD is high (for example for 1% Ag–TiO_2, TOD = 78 mg O_3/L), the mineralization of treated solution is reduced (only 31% of TOC removal). According to this, it is important to analyze the TOC removal besides COD. In terms of TOC removal as a function of TOD, the best result was achieved with 0.5% Ag-TiO_2 (46 mg O_3/L and 37% of mineralization).

In the case of silver, the analysis of the effect of metal load on TOC and COD removal reflects the fact that the increasing amount of Ag decreases TOC and COD removal even using higher TOD (Table VI.1). For palladium, the increase in metal load promotes a higher TOC and COD removal, which differs from what happens regarding parabens degradation. For platinum in terms of TOC removal, the differences could be explained by the different TODs. On the other hand, an increase of metal load promotes a slight improvement in the COD removal, even using lower TOD, which is in accordance with the results achieved for parabens degradation. For the global analysis, the best results in terms of COD and TOC removal were 0.5% Ag-TiO_2 and 1% Pd-TiO_2.

Table VI. 1. Summary results of TOC and COD removal in function of TOD.

Catalyst	TOD (mg/L)	$TOC_{removal}$ (%)	$COD_{removal}$ (%)
1% Pd-TiO_2	44	34	54
1% Pt-TiO_2	50	34	51
1% Ag-TiO_2	78	31	43
0.5% Pd-TiO_2	80	38	41
0.5% Pt-TiO_2	60	37	50
0.5% Ag-TiO_2	46	37	44
0.1% Pd-TiO_2	51	21	32
0.1% Pt-TiO_2	63	43	48
0.1% Ag-TiO_2	59	37	45

These two parameters (COD and TOC removal) are very dependent upon TOD. The analysis for COD and TOC removal was made for the final time of reaction that necessarily corresponds to different TOD values for the different catalysts. In order to understand if the mixture of parabens degradation follows mainly partial oxidation or mineralization pathway, the ratio between COD or TOC and TOD was analyzed. Table VI.2 presents the COD and TOC removal per TOD for all photocatalytic systems tested. From Table VI.2, it was possible to see that higher COD removal in the function of TOD was achieved for 1 wt% of Pd doped onto TiO_2. This result indicates that the parabens degradation using this catalyst progressed towards a partial oxidation pathway. On the other hand, for TOC removal, the best result was achieved for 0.5 wt% Ag-TiO2. Thus, for that catalyst, mineralization occurs to a larger extent.

Table VI.2. COD and TOC removal per TOD for all photocatalytic systems tested.

Catalyst	COD/TOD	TOC/TOD
1% Pd-TiO_2	1.23	0.77
1% Pt-TiO_2	1.02	0.68
1% Ag-TiO_2	0.55	0.39
0.5% Pd-TiO_2	0.51	0.47
0.5% Pt-TiO_2	0.83	0.62
0.5% Ag-TiO_2	0.96	0.80
0.1% Pd-TiO_2	0.63	0.41
0.1% Pt-TiO_2	0.76	0.68
0.1% Ag-TiO_2	0.76	0.63

The best results in terms of COD removal were achieved for higher loads of Pd and Pt. In terms of price, these catalysts present higher costs compared with the silver-based materials. According to the current market values, 25–27 € is the cost per gram of palladium and platinum, while silver is obtained at 0.5 €/g of silver. This represents a high cost reduction. Therefore, accounting for the application costs, the most suitable solution for parabens degradation and COD and TOC removal would be the 0.1 and 0.5% of Ag-TiO_2 catalyst.

VI.3.4. Toxicity Assessment

Since total mineralization was not achieved during photocatalytic ozonation, it is important to analyze the toxic character of the treated samples in order to infer their potential impact over the ecosystems. Thus, the toxicity of the treated samples after 120 min of photocatalytic ozonation with the different catalysts was compared with the initial parabens mixture. Three species were tested: the luminescent bacteria *Aliivibrio fischeri*, the mollusk *Corbicula fluminea* and the plant *Lepidium sativum.*

The luminescence inhibition of *A. fischeri* after 15 min in contact with treated samples decreased compared with the initial mixture of parabens (Table VI.3). Miralles-Cuevas et al., (2017) verified that, for *A. fischeri* light inhibition above 30%, the samples must be considered toxic. Therefore, in the present study, all of the treated samples present toxicity towards these very sensitive bacteria. The sample for 1% Ag-TiO_2, which led to the worst results in terms of parabens degradation, presents the highest light inhibition and lowest germination index, pointing out its highest toxicity. For silver and palladium, the intermediate metal load used (0.5%) leads to the lowest light inhibition. For both catalysts' highest (1%) and lowest (0.1%) metal loads, toxicity increases compared to the intermediate metal load. In the case of platinum, the noble metal load does not affect significantly the luminescence inhibition (Table VI.3).

The mortality of *C. fluminea* strongly decreases when the individuals are in contact for 72 h with the treated solutions (120 min of photocatalytic ozonation) when compared with the initial mixture of parabens. In addition, 1% Pd-TiO_2 reveals the effectiveness on the parabens degradation, COD and TOC removal, but the sample presents higher toxicity towards *A. fischeri* and *C. fluminea*. This could be related with the by-products resulting from the photocatalytic ozonation process. The different metal loads used for Pt do not affect significantly the toxicity towards Asian clams. Contrarily, it seems that the decrease of Ag loading in the catalyst leads to a slightly more toxic treated mixture towards *C. fluminea* (Table VI.3).

After 48 h of contact with treated samples, the GI of *L. sativum* increases, which means that the initial toxicity related with parabens was reduced. According to Trautmann and Krasny criteria, the initial parabens mixture is very phytotoxic, while the treated samples do not present phytotoxicity (Trautmann and Krasny, 1997). The increase of metal load for Pt decreases the GI. As occurs with *A. fischeri,* the intermediate load used for Ag and Pd leads to less toxic samples compared with the remaining tested loads. Moreover, the values of GI for the 0.5% of

Ag and Pt are above 100%, which means that this treated solution can even improve *L. sativum* germination.

In a global way, the best result in terms of toxicity removal over the different organisms tested was the one obtained by photocatalytic ozonation using 0.5% Ag–TiO_2 (Table VI.3).

Table VI.3. Summary results of toxicity tests with different species tested.

Catalyst	*A. fischeri* Light Inhibition (%)	Mortality *C. fluminea* (%)	GI (%)
Initial mixture parabens	96 ± 0.1	100	42 ± 11
1% Pd-TiO_2	63 ± 0.1	29 ± 14	96 ± 22
1% Pt-TiO_2	ND	0	86 ± 11
1% Ag-TiO_2	72 ± 0.3	0	80 ± 26
0.5% Pd-TiO_2	44 ± 1.4	0	108 ± 7
0.5% Pt-TiO_2	61 ± 1.2	0	107 ± 11
0.5% Ag-TiO_2	43 ± 1.5	0	112 ± 1
0.1% Pd-TiO_2	66 ± 1.5	7 ± 7	87 ± 11
0.1% Pt-TiO_2	58 ± 4.6	7 ± 7	112 ± 19
0.1% Ag-TiO_2	69 ± 8.5	21 ± 7	99 ± 0

VI.4. Conclusions

All catalysts and different loads tested in this work were proven to be efficient on the total parabens degradation, COD and TOC removal, as well as toxicity reduction compared with the initial mixture. For lower loads of Pd, TOC and COD removals are poor, which are not in accordance with the results achieved for parabens degradation. Therefore, it can be concluded that the parabens degradation follows a partial oxidation pathway instead of mineralization. Moreover, this provides an increase of toxicity over bacteria and plants when compared with the other treatments. For Pt, the decrease on metal loading doped onto TiO_2 reveals a decrease on the parabens degradation efficiency. However, for the lowest load of Pt, an increase of TOC removal occurs, which means that degradation was directed towards the mineralization way. Herewith, the noble metal loads also affect the reaction pathway. The higher load for Ag reveals the poorest performance in terms of parabens degradation. On the other hand, the higher loads for Pd and Pt present interesting results in terms of degradation and COD removal. However, the usage of these two noble metals doped onto TiO_2 at this load for parabens degradation represents a high cost, so the most suitable solution for parabens degradation would be 0.1 and 0.5% of Ag–TiO_2. The 0.5% Ag–TiO_2 presents the best performance for the photocatalytic ozonation of the parabens mixture, as well as toxicity reduction over different species.

VI.5. References

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VII. Study of the Influence of the Matrix Characteristics over the Photocatalytic Ozonation of Parabens Using Ag-TiO_2

It is based on the publication: Gomes, J., Lopes, A., Gmurek, M., Quinta-Ferreira, R. M., Martins, R. C. (2019). Study of the influence of the matrix characteristics over the photocatalytic ozonation of parabens using Ag-TiO_2. Science of the Total Environment, 646, 1468–1477.

VII.1. Introduction

The exponential growth of population estimates a significant reduction of natural resources. Among them water may be referred. This resource is fundamental to human life and ecosystems. Therefore, water scarcity could be one of the major drawbacks of this century. The reduction of natural water availability will increase the concentration of emerging contaminants on the water sources due to the inefficiency of conventional wastewater treatments (Benijts et al., 2004; Bledzka et al., 2014). The emerging contaminants present in wastewater and water sources are compounds used in a wide spectrum of pharmaceutical and personal care products. The increasing consumption of this kind of products results on their increasing presence in water sources. These compounds must be removed from wastewaters treatment plants before wastewater discharge to natural sources since present high toxicity for humans and other species. Parabens, widely used as preservative and antimicrobial in pharmaceutical and personal care products, can be considered in this group of contaminants (Bledzka et al., 2014). These compounds are endocrine disruptors and have been suspected that, due to their high estrogenicity, can be related with the appearance of some cases of breast cancer (Routledge et al., 1998; Darbre et al., 2004). Routledge et al. (1998) verified that methyl, ethyl, propyl and butyl paraben present estrogenic activity even if lower than 17β-estradiol. Moreover, from this group of parabens the most relevant in terms of estrogenicity was butylparaben (Routledge et al., 1998).

Many approaches regarding parabens degradation can be found in the literature (Hernández-Leal et al., 2011; Tay et al., 2011; Domínguez et al., 2014; Gmurek et al., 2015; Martins et al., 2016; Gomes et al., 2017a, b, c). Most of these approaches reveal great performances in terms of parabens degradation. The main differences between methodologies applied are related, for example, with the energy consumption, the by-products formation, the leaching of catalyst metals into the water medium, etc.

Photocatalytic ozonation appears as promising technology that can take advantages from the oxidative power of ozone coupled with solar or UVA radiation in the presence of suitable semiconductor catalyst (Gomes et al., 2017d). This combination results in the production of radical species (hydroxyl, ozonide, superoxide radicals) which can be very helpful for the organic contaminants degradation, promoting the mineralization pathway. Titanium dioxide appears as the most common semiconductor used on catalytic processes due to the low cost, easy handling and chemical stability. The high band gap of this catalyst can be reduced through noble metal doping which allows its activity at the presence of low energy sources (Pelaez et al., 2012). In fact, according to Chong et al. (2010) it is possible to observe a reduction on the band gap of TiO_2 with the incorporation of noble metals. The incorporation of noble metals on TiO_2 crystal lattice introduces impurity in the band gap reducing the photonic energy requirements. Moreover, the presence of noble metals also enhances the efficiency of the separation between photogenerated electrons and holes avoiding their recombination which will improve the degradation process. The loadings of noble metals normally range from 0.1 to 3 wt. % (Huang et al., 2008; Zheng et al., 2011). According to the noble metal used, high loadings can significantly increase cost of catalysts preparation. On this way and based on previous results (Gomes et al., 2018a) in this study 0.1 wt.% Ag –TiO_2 was selected as photocatalyst. Silver is the noble metal with lower cost in accordance with the market values. Gomes et al. (2018a) verified that this catalyst was efficient on the removal of a mixture of five parabens (Methyl, Ethyl, Propyl, Butyl and Benzylparaben) using a photocatalytic ozonation process.

The photocatalytic ozonation process has been tested on the emerging contaminants degradation and proved to be efficient on their removal (Rodríguez, et al., 2013; Márquez et al., 2014; Fathinia and Khataee., 2015; Moreira et al., 2016; Gomes et al., 2017a;). Several factors can influence photocatalytic ozonation such as the pH of the medium, the presence of radical's scavenger and the water matrix such as secondary municipal wastewater. On this way, the influence of these factors must be analyzed to understand the process at real conditions of wastewater treatment. The medium pH can affect hydroxyl radical production during the photocatalytic ozonation processes (Hernández-Alonso et al., 2002; Kasprzyk-Hodern et al., 2003; Mehrjouei et al., 2015). In fact, at acidic pH hydroxyl radical production can be enhanced (Hernández-Alonso et al., 2002; Mehrjouei et al., 2015). Some species on municipal wastewaters can improve or inhibit emerging contaminants degradation. The presence of carbonates (CO_3^{2-}) and bicarbonates (HCO_3^{-}), that can work as consumer of hydroxyl radical, will affect seriously the degradation contaminants rate in ozonation processes (Kasprzyk-Hodern et al., 2003; Rioja et al., 2016; Rimoldi et al., 2017). Otherwise, the bicarbonate with hydroxyl radicals can produce carbonate radicals ($CO_3^{\bullet-}$), which even if presenting lower potential of oxidation than hydroxyl radicals can be more selective towards electrophilic compounds such as parabens (Augusto et al., 2002; Petala et al., 2015; Kanigaridou et al., 2017). Moreover, species such as Cl^-, SO_4^{2-} can also inhibit the degradation of contaminants when present in the wastewater matrix, since can act as hydroxyl radicals scavengers (Rimoldi et al., 2017; Kotzamanidi et al., 2018). On the other hand, chloride can be oxidized in photocatalytic processes with semiconductors due to the photogenerated holes producing chloride radicals (Equation VII.1).

$$Cl^- + h^+ \longrightarrow Cl^\bullet \qquad \text{(Equation VII.1)}$$

This radical production can be extended if the recombination phenomenon between hole and electron is minimized. Under the tested conditions, ozone and Ag nanoparticles onto the catalyst work as electron traps, and holes can be occupied by chloride, sulfates and water for radical species production (Gomes et al., 2017a; Kotzamanidi et al., 2018). In these complex systems such as real wastewaters the increase or decrease on contaminants degradation rate is very dependent on the type of matrix, the concentration of radical species (HCO_3^-, Cl^-, SO_4^{2-}), the organic matter and the photocatalyst support as well. All above mentioned process parameters should be considered to select the most efficient and suitable AOP methodology.

Bearing this in mind the aim of the present study was exploring the mechanism effect on the mixture of five parabens degradation (Methyl, Ethyl, Propyl, Butyl and Benzylparaben) using a municipal wastewater matrix. The work involved then the analysis of pH, hydroxyl radical scavengers and matrix effect over the process efficiency. To the best of our knowledge this is the first time that the effect of such ions (HCO_3^-, Cl^-, SO_4^{2-}) in the real wastewater was studied. Moreover, the toxicity of the initial and treated wastewaters was assessed after using the *Aliivibrio fischeri* bacteria.

VII.2. Materials and Methods

VII.2.1. Chemicals and water matrix

The methyl, ethyl, propyl and benzylparaben were acquired from Sigma-Aldrich while butylparaben was purchased from Fluka. The mixture of five parabens was used at 10 mg/L of each with ultrapure water (UPW) to analyze the byproducts formation with and without radical scavenger's addition. Moreover, this concentration was already used in others works which will facilitate the comparison of the different technologies efficiency. Iso-propranol at 5 mM was used as hydroxyl radical scavenger (Velegraki et al., 2015). To verify the effect of ozone trapping, a test using potassium iodide at 5 mM as scavenger was performed (Velegraki et al., 2015). On the analysis of the effect of pH, pH 7 was maintained constant with the addition of a buffer of disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4). The standards of 4-Hydroxybenzoic acid (4-HBA), 2,4 -Dihydroxybenzoic acid (2,4-diHBA), 3,4 - Dihydroxybenzoic acid (3,4-diHBA) and hydroquinone were obtained from Sigma-Aldrich. The humic acid (Fluka) was added to the ultrapure water at the concentration of 10 mg/L to analyse the effect of the organic matter presence on the parabens degradation. As sources of HCO_3^-, Cl^- and SO_4^{2-}, sodium bicarbonate, sodium chloride and sodium sulfate were used respectively. The secondary municipal wastewater (MWW) was used as matrix for the parabens mixture degradation. MWW was collected from a Portuguese wastewater treatment plant conceived to treat the wastewater from 130 000 habitants. The wastewater is firstly treated by conventional physical processes and then a biological process is applied. The process starts with a pre-treatment and primary settling. Then the wastewater goes to trickling bed biological reactors to remove most of the organic matter. A secondary settler is then used before the treated wastewater being discharged to a nearby river. The secondary wastewater was then collected after the secondary settler. Those samples were filtered and refrigerated at 4 ºC until use. The main characteristics of the secondary municipal wastewater are presented in the Table VII.1.

Table VII.1. Characterization of secondary municipal wastewater.

Parameters	Values
pH	7.7
COD (mg/L)	50
Cl^- (mg/L)	110
NO_3^- (mg/L)	20
SO_4^{2-} (mg/L)	28

VII.2.2. Catalyst preparation and characterization

The 0.1 wt.% Ag doped on the TiO_2 was used as photocatalyst for the parabens degradation. This catalyst was prepared by a photoreduction method as defined in a previous work (Gomes et al., 2018a). The catalyst was characterized regarding specific surface area using the Brunauer-Emmett-Teler (BET) method through liquid N_2 (−196 °C) with an accelerated surface area and porosimetry analyzer (ASAP 2000, Micrometrics). The catalyst surface was analyzed by Scanning Electron Microscopy (SEM) using a TESCAN VEGA 3 SBH – Easy Probe equipped with tungsten heated cathode. The crystalline analysis of the catalyst powder was evaluated by X-Ray diffraction (XRD), using a diffractometer (Bruker D8 Advance).

VII.2.3. Experimental procedure

The ozone generator (802N, BMT) produced ozone from a pure oxygen stream (99.9%, Praxair) and the inlet ($[O_3]^{in}$) and outlet ($[O_3]^{out}$) ozone gas concentrations were measured by ozone analyzers (BMT 963 and 964 vent, BMT), which allows to determine the transferred ozone dose (TOD) using Equation VII.3.

$$\mathrm{TOD}=\int_0^t \frac{Q_{Gas}}{V_{Liquid}}\times\left([O_3]^{in}-[O_3]^{out}\right)\times dt \qquad \text{(Equation VII.3)}$$

Where Q_{Gas} represents the gas flow rate (0.2 L/min), V_{Liquid} the volume of the effluent (2 L). TOD was expressed in mg O_3/L. Plotting the results against TOD allows to compare the processes efficiency regarding ozone consumption (Domenjoud et al., 2011). Ozone gas inlet and outlet concentrations were recorded along time and Equation VII.3 was numerically integrated to obtain the cumulative amount of ozone absorbed by the liquid phase per unit of volume. The remaining ozone that left the reactor was trapped by 2% of potassium iodide (Panreac) solution.

The reaction occurred in a semi-batch 2 L glass reactor (effluent initially loaded, and gas continuously bubbled in the liquid bulk) at controlled temperature (25 ± 1 °C) by a thermostatic bath was equipped with 3 lamps emitting UVA radiation (main emission peak at 365 nm). The photon flux was calculated as 5.75×10^{-7} Einstein /L.s (Gomes et al., 2017a) based on the ferrioxalate actinometer (Kuhn et al., 2004). The amount of catalyst used in the experiments was 70 mg/L. This load of catalysts was already applied in other works and will allow a comparison between the processes. The aim was to sue the minimal amount of catalyst possible so that unrealistic catalytic effects are avoided (Nawrocki, 2013). The catalyst was placed into the reactor with parabens solution before feeding ozone and turning on the lamps. No adsorption of parabens was verified onto catalyst (Gomes et al., 2018a). During the experiments the stirring speed was about 700 rpm to minimize diffusion limitations (Martins and Quinta-Ferreira, 2009). The samples used for high performance liquid chromatography (HPLC) analysis obtained during the photocatalytic ozonation experiments were centrifuged at 3500 rpm (Nahita 2655, Centrifuge) to remove the catalyst. At the experiments using water matrix the parabens mixture was previously dissolved into secondary municipal wastewater.

VII.2.4. Analytical methods

HPLC (UFLC, Shimadzu, equipped with a diode array detector) was used for following each paraben along the photocatalytic ozonation experiments. A C18 (SiliaChrom) column at 40 ºC was used as stationary phase. 20 μL of sample were injected and a mobile phase flowrate of 0.5 mL/min was used. The mobile phase consisted in a mixture 50:50 of methanol and acidic water. The detection of parabens occurred at 255 nm. The identification and quantification of by-products followed the same procedure. The compounds identification was performed by comparing peaks retention times with those obtained when each compound standard solution was injected. Chemical oxygen demand (COD) was determined following the standard method 5220D (Greenberg et al., 1985). To prepare a calibration curve with COD values within the range 0–100 mg O_2/L the potassium hydrogen phthalate (Panreac) was used. After 2 h of digestion at 150 °C in thermoreactor (ECO25 – Velp Scientifica), the absorbance was measured at 445 nm in a WTW photolab S6 photometer.

The identification and quantification of Cl^-, NO_3^- and SO_4^{2-} was made through ionic chromatography by Millipore, Actions Water Analyzer equipped with a conductivity detector Waters 431. pH was determined using a Crison micropH 2002 apparatus. pH of secondary municipal wastewater after spiked with parabens mixture did not change. However, the pH of spiked ultra pure water (UPW) with the parabens mixture decreased to about 5.

VII.2.5. Toxicity assessment

The toxicity of the samples before and after treatment with photocatalytic ozonation using secondary municipal wastewater as matrix was assessed using the bacteria *A. fischeri*. The samples were inoculated with the bacteria solution at 15 °C in a LUMISTherm. Luminescence was measured directly in the LUMIStox 300 after 15 min and 30 min of incubation and compared with a solution where bacteria were placed in a 2% of NaCl solution (blank). The percentage of luminescence inhibition (D) was calculated according to equation VII.4.

$$D\ (\%) = \left(\frac{I_o - I_t}{I_o}\right) \times 100 \qquad \text{(Equation VII.4)}$$

Where I_0 is the light intensity of bacteria in the blank and I_t the light intensity of bacteria after being in contact with the samples at the measured time (t) 15 or 30 min.

VII.3. Results and Discussion

VII.3.1. Catalyst characterization

The BET surface analysis of catalyst reveals that the specific surface area of 0.1 wt.% Ag-TiO_2 was about 83 m^2/g. This analysis also reveals the pore volume and estimates the average diameter of pores, which were 0.45 cm^3/g and 21 nm, respectively. Although the relatively high specific surface area, no parabens adsorption was verified at the different conditions tested. The XRD analysis reveals that anatase is the predominant phase (Gomes et al., 2018a). In fact, the presence of low amount of noble metal does not change the crystallinity of TiO_2. Other studies show that even higher amounts of noble metal load (until 1.5 wt.%) was not enough to change the XRD pattern of anatase phase of TiO_2 (Huang et al., 2008; Zheng et al., 2011).

Figure VII.1 presents the SEM images depicting the 0.1% Ag-TiO_2 (magnification x5000). Particles with different sizes (within the micro and nano scale) with irregular shape were possible to identify. The presence of noble metal was difficult to conclude due to the low amount. In fact, the presence of silver did not alter the morphology and texture of titanium dioxide pattern (Gomes et al., 2017b).

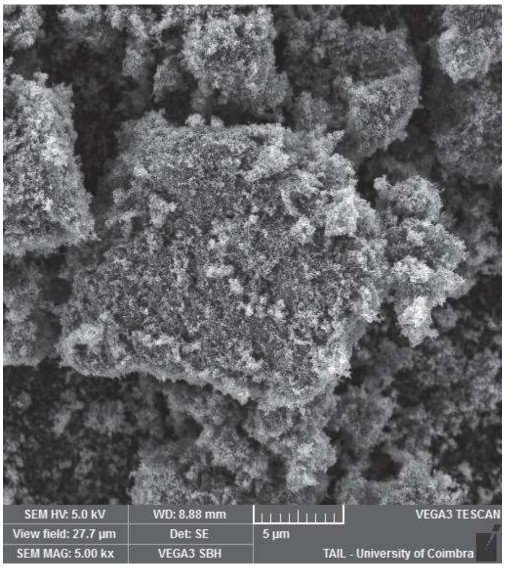

Figure VII.1. SEM image of 0.1% Ag-TiO_2 (5000x)

VII.3.2. Effect of pH

pH should be considered as a very important parameter in ozonation processes (Kasprzyk-Hodern et al., 2003; Martins and Quinta-Ferreira, 2009; Gomes et al., 2018b). The high values of pH promote the self decomposition of ozone into hydroxyl radicals which improves the contaminants degradation (Kasprzyk-Hodern et al., 2003). However, in the photocatalytic ozonation other radical species can appear, such as ozonide and superoxide radical that can favor hydroxyl radicals production at acidic pH's (Hernández-Alonso et al., 2002; Rodríguez et al., 2013; Mehrjouei et al., 2015). Otherwise, besides the production of hydroxyl radicals this kind of species can also work as oxidants or, at acidic/basic conditions, promoting the formation of other radicals (Rodríguez et al., 2013; Fathinia and Khataee., 2015; Mehrjouei et al., 2015; Velegraki et al., 2015). Figure VII.2 presents the effect of pH (3 and 7) on the photocatalytic ozonation of the mixture of parabens in ultrapure water.

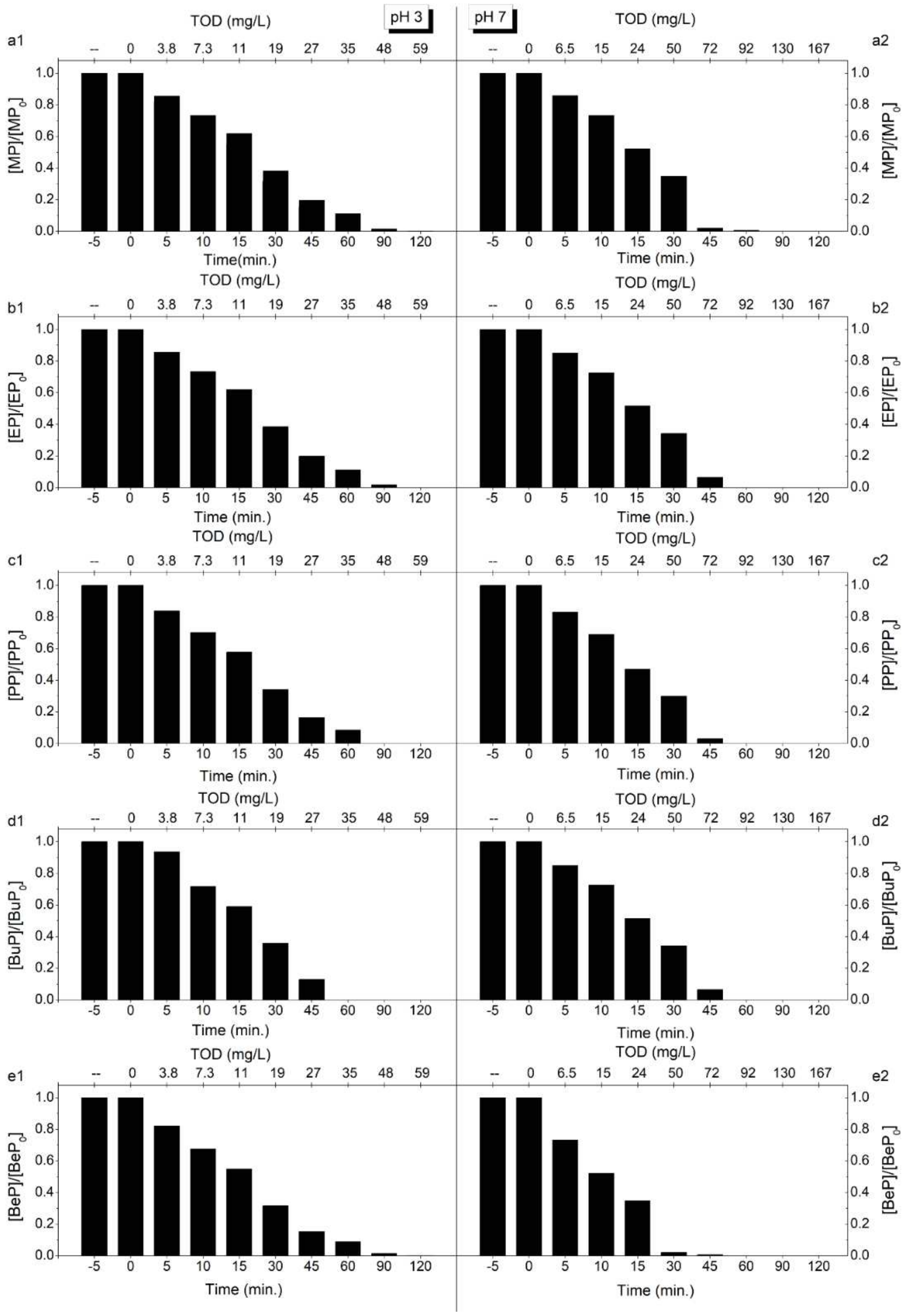

Figure VII.2. Effect of pH on the parabens degradation a) MP; b) EP; c) PP; d) BuP and e) BeP as function of time degradation and TOD during photocatalytic ozonation (Initial parabens concentration 10 mg/L each, 70 mg/L of 0.1%Ag-TiO$_2$ catalyst)

Parabens degradation was investigated from time degradation as well as TOD point of view. When time is considered the fastest degradation was observed in pH 7; however, for that pH, degradation required higher ozone consumption. As it can be seen in Figure VII.2 the parabens degradation does not suffer significant differences when using different pH values, from TOD point of view. TOD approach is industrially important since the energetic cost of ozone generation is an important drawback of this process.

As it can be seen in Figure VII.2 the parabens degradation does not suffer significant differences when using different pH values. The only exception goes for the higher ester chain lengths parabens, such as butyl and benzylparaben, that were more difficult to remove when pH increased. For example, regarding butylparaben total degradation required about 25 mg/L less TOD for acidic conditions. The low pH can be responsible for enhancing the hydroxyl radicals production through ozonide radical reaction (Equation VII.5) (Hernández-Alonso et al., 2002; Rivas et al., 2012; Márquez et al., 2014).

$$O_3^{\bullet-} + H^+ \longrightarrow HO_3^{\bullet} \longrightarrow {}^{\bullet}OH + O_2 \qquad \text{(Equation VII.5)}$$

This can be an explanation for the slightly reduction on amount of TOD necessary for parabens removal using pH 3. At acidic pH, hydroxyl radicals can be quickly produced and at higher conditions according equation 5, whereas at water natural pH (7) molecular ozone may be more available to react with the contaminants.

VII.3.3. Effect of radical scavenger

The hydroxyl radical scavenger used in this work was iso-propanol at a concentration of 5 mM (Velegraki et al., 2015). The effect of the presence of this radical scavenger on parabens mixture degradation at acidic pH was analyzed. The mechanism of parabens degradation will be affected by the presence or absence of hydroxyl radicals. In the presence of hydroxyl radicals the hydroxylation is the main reaction pathway (Tay et al., 2010; Petala et al., 2015; Velegraki et al., 2015). The hydroxyl radical scavenger will favor other reactive oxidative species (such as direct ozone attack, ozonide and superoxide radicals) changing the reaction pathway and the by-products formed as well as the rate of parabens decomposition. The hydroxyl radical can attack benzenic ring of parabens and different moieties (Tay et al., 2010; Petala et al., 2015). This can result in hydroxylated and dealkylated derivatives of parabens (such as 4-HBA, 2,4-diHBA, 3,4-diHBA and hydroquinone). However, due to its electrophilic character, ozone can also attack directly the benzenic ring of parabens producing hydroxylated by-products (Tay et al., 2010; Gomes et al., 2017a). As it can be seen in the Figure VII.3 for benzylparaben removal (e) for low TOD values no significant differences were found when the reaction occurs with or without radical scavenger. This is probably due to the presence of two benzenic rings on benzylparaben molecular structure which are very much reactive with molecular ozone and therefore not very much affected by the radical scavenger. Nevertheless, it should be referred that for all parabens at the presence of the radical scavenger it was not possible to achieve complete removal for the range of TOD values tested. This clearly reveals that hydroxylation is an important reaction pathway for the mixture of parabens degradation.

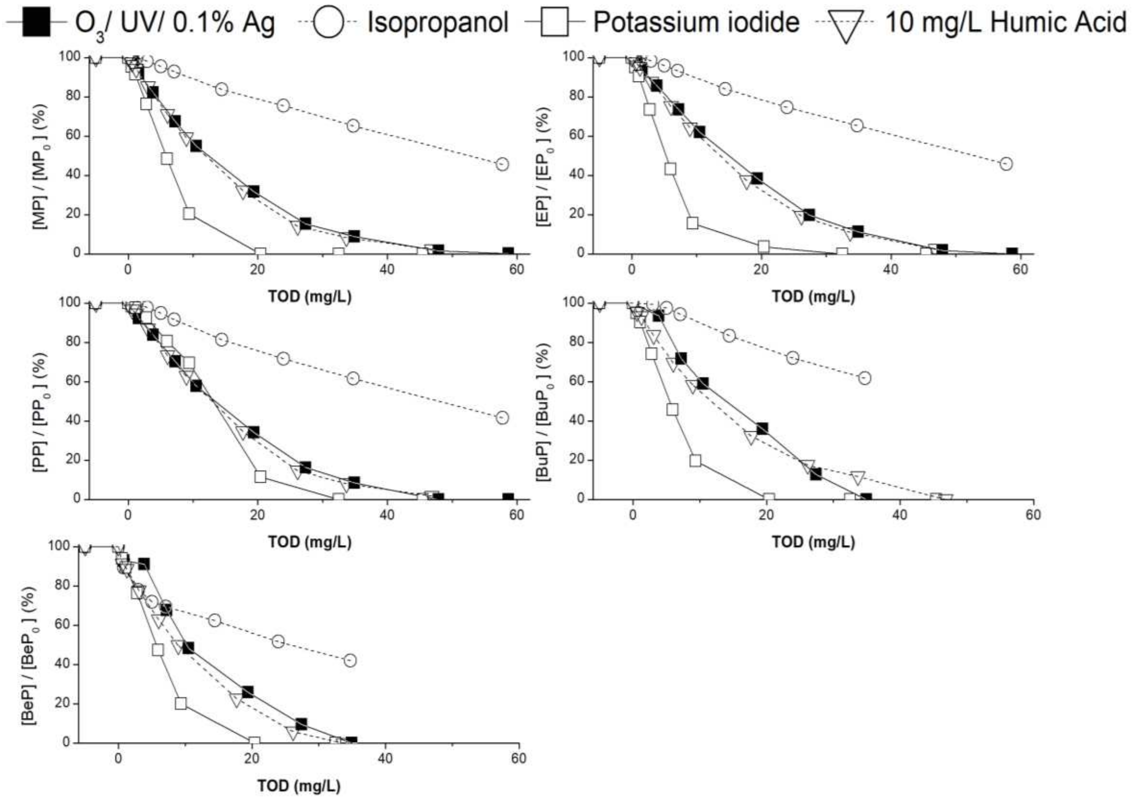

Figure VII.3. Effect of radical scavengers and humic acids on the parabens degradation a) MP; b) EP; c) PP; d) BuP and e) BeP as function of TOD during photocatalytic ozonation (Initial parabens concentration 10 mg/L each, 70 mg/L of 0.1%Ag-TiO_2 catalyst, 5 mM of isopropanol and potassium iodide).

As can be seen in the Figure VII.3 the presence of potassium iodide enhances parabens degradation when compared with the case where KI was not applied. The presence of iodide scavenges molecular ozone. However, I^- can be trapped by hydroxyl radicals and produce $HOI^{-\bullet}$ which at the acidic conditions can generate iodide radical ($I^\bullet$) that can attack very quickly the benzenic rings, converting the parabens compounds in the other by-products. On the other hand, the scavenging of molecular ozone may enhance hydroxyl radical activity for parabens mixture degradation, since ozone can compete with hydroxyl radical for those target pollutants.

Otherwise, when KI was used the photogenerated electron was not trapped by ozone. The hydroxyl radicals and electrons produced can work as adjuvants of organic polutants degradation on wastewater treatment (Deng and Ezyske, 2011). On this way, the free electron can help the parabens mixture degradation through the parabens and oxygen reduction, where this last can result on the superoxide radical production which can also enhance the parabens degradation (Velegraki, et al., 2015; Gomes et al., 2017c).

The organic matter effect on the parabens degradation by photocatalytic ozonation was presented on the Figure VII.3 by testing 10 mg/L of humic acid. The profile of degradation was similar to photocatalytic ozonation with ultrapure water for all parabens. This means that the organic matter has no significant effect over parabens degradation. This behaviour reinforces the idea that hydroxyl radicals had an important role on the parabens mixture degradation, since molecular ozone can be scavenged by the natural organic matter (Kasprzyk-Hodern et al., 2003).

Moreover, the impact of the presence of radical scavenger over by-products formation (4-HBA, 2,4-diHBA, 3,4-diHBA and hydroquinone) was also verified. In fact, to understand the real effect of the hydroxyl radical over the photocatalytic ozonation process the concentration of these four by-products was determined along the TOD values considered. Other by-products can be found such as hydroxylated parabens and dealkylated derivatives, since the reactive oxidative species can attack the moieties or benzenic ring of parabens. Moreover, also the photogenerated holes at the surface of catalyst can oxidize the parabens (Tay et al., 2010; Petala et al., 2015; Velegraki et al., 2015; Frontistis et al., 2017).

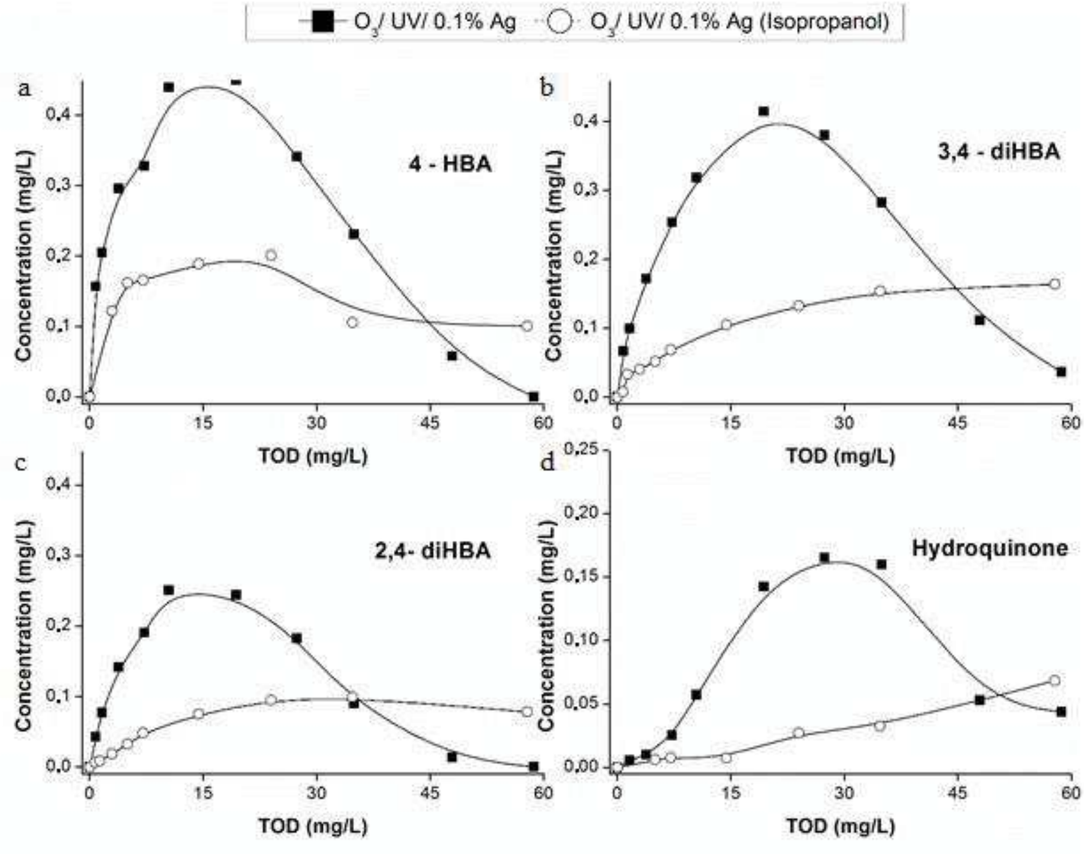

Figure VII.4. Effect of radical scavenger on the by-products formation a) 4-HBA; b) 3,4-diHBA; c) 2,4-diHBA and d) hydroquinone as function of TOD during photocatalytic ozonation (Initial parabens concentration 10 mg/L each, 70 mg/L of 0.1%Ag-TiO_2 catalyst, 5 mM of isopropanol)

As can be seen in the Figure VII.4 the hydroxyl radical presence has a significant effect on by-products formation at initial TOD values and their consequent concentration reduction for the higher values of TOD.

Frontistis et al. (2017) for the photodegradation of ethylparaben (10 mg/L) using simulated solar sunlight and Ag_3PO_4 as catalyst verified that dealkylation and decarboxylation were the main reactions for 4-HBA, benzoic acid and phenol production as by-products. Phenol production was not detected at the conditions tested in our work. Possibly it can be due to the low TOD values considered in our experiments or could be due to the low concentrations of phenol produced which were not detected at the conditions applied in the HPLC. Petala et al., (2015) on the ethylparaben degradation using N-doped TiO_2 under simulated solar radiation studied the phenol production along 8h, and after that time just quantified 10 µg/L. In our study, the early stages of photocatalytic ozonation reveals that 4-HBA, 3,4- diHBA, and 2,4-diHBA production occurs through to the dealkylation by hydroxyl radical attack. Moreover, the decarboxylation of 4-HBA results in the production of hydroquinone, when the 4-HBA starts to decay (Fig.VII. 4a) results in an exponential growth of hydroquinone production (Fig. VII.4d). Another feature that should be analyzed is the higher production of 3,4-diHBA (Fig. VII.4b) relatively to 2,4-diHBA (Fig. VII.4c) when the radical scavenger is absent. This can be related with the hydroxylation of benzenic rings that occur preferably near carbons with the H abstraction and the OH addition. When no scavenger is used all the by-products reveal a decline behavior for high values of TOD. In fact, 4-HBA (Fig. VII.4a), 3,4-diHBA (Fig. VII.4b) and 2,4-diHBA (Fig. VII.4c) were almost totally removed, while hydroquinone (Fig. VII.4d) remains with a residual concentration. However, total mineralization was not achieved even at those conditions. This means that other by-products such as low chain carboxylic acids (Lin et al., 2011; Petala et al., 2015; Velegraki et al., 2015) and other hydroxylated parabens were produced as Tay et al., (2010) verified in the ozonation of methyl, ethyl, propyl and butylparaben. In the presence of the radical scavenger, the quantified by-products can be found at lower concentrations. Moreover, even for high values of TOD their total degradation was not achieved. Meanwhile, 3,4-diHBA and hydroquinone concentrations always present during the reaction. This behavior clearly reveals that hydroxyl radicals are the main responsible by the production of these by-products and consecutive degradation. The other reactive species present in the photocatalytic ozonation process (such as ozone, ozonide and superoxide radicals) have a lower effect on parabens degradation and by-products formation and further decay. Still, ozonide and superoxide radicals may play an important role for hydroxyl radical generation (Mehrjouei et al., 2015).

VII.3.4. Effect of water matrix composition

Many studies can be found with the analysis of different water matrix on parabens degradation (Petala et al., 2015; Velegraki et al., 2015; Frontistis et al., 2017; Kotzamanidi et al., 2018). The effect of different ions presence in the water medium does not generate a consolidated opinion. Some studies can describe these ions as scavenger of hydroxyl radicals and consequently decrease the efficiency of degradation (Kasprzyk-Hordern et al., 2003; Rimoldi et al., 2017). On the other hand, some information can be found revealing that such ions can work as improvers of contaminants degradation (Petala et al., 2015; Kanigaridou et al., 2017; Kotzamanidi et al., 2018). These two antagonic conclusions are valid, since the first conclusion results on the scavenger effect of, for example, bicarbonates and chlorides regarding hydroxyl radical's production. The second idea is related with hydroxyl radicals scavenging effect of some ions that result on the production of other radical species that can be more reactive with specific contaminants than hydroxyl radicals. This way degradation is enhanced. For example, the bicarbonate ion in the presence of simulated solar photocatalytic oxidation using N-TiO_2 reveals an important role of carbonate radical on ethylparaben degradation since this radical has high affinity for electrophilic species (Petala et al., 2015). In our study the usage of secondary municipal wastewater as matrix (MWW) clearly enhanced the efficiency of the mixture of parabens degradation by the photocatalytic ozonation process (Figure VII. 5). Comparing the results with UPW it is possible to observe that with MWW the TOD values required for total parabens removal decreased, which means an increase of efficiency. For highest chain length compounds such as butyl and benzylparaben the behavior was similar to what happens in UPW tests, since the global TOD reduction was verified when compared with other parabens presents at the mixture solution. The concentrations of Cl^- and SO_4^{2-} were followed during the photocatalytic ozonation process with the municipal wastewater matrix. After 120 min of reaction the concentrations decrease from 110 and 28 mg/L to 99 and 24 mg/L for Cl^- and SO_4^{2-}, respectively. This can be an explanation to the improvement of parabens degradation with the municipal wastewater. In fact, this decrease on concentration could result from radical oxidative species production from these ions. Moreover, the presence of Ag and ozone with semiconductors as catalysts and an energy source enough to promote the generation of electron-hole pairs can be working as electron traps, resulting in more available holes to chloride and sulfate radicals production from the oxidation of their ions (Gomes et al., 2017a; Kotzamanidi et al, 2018).

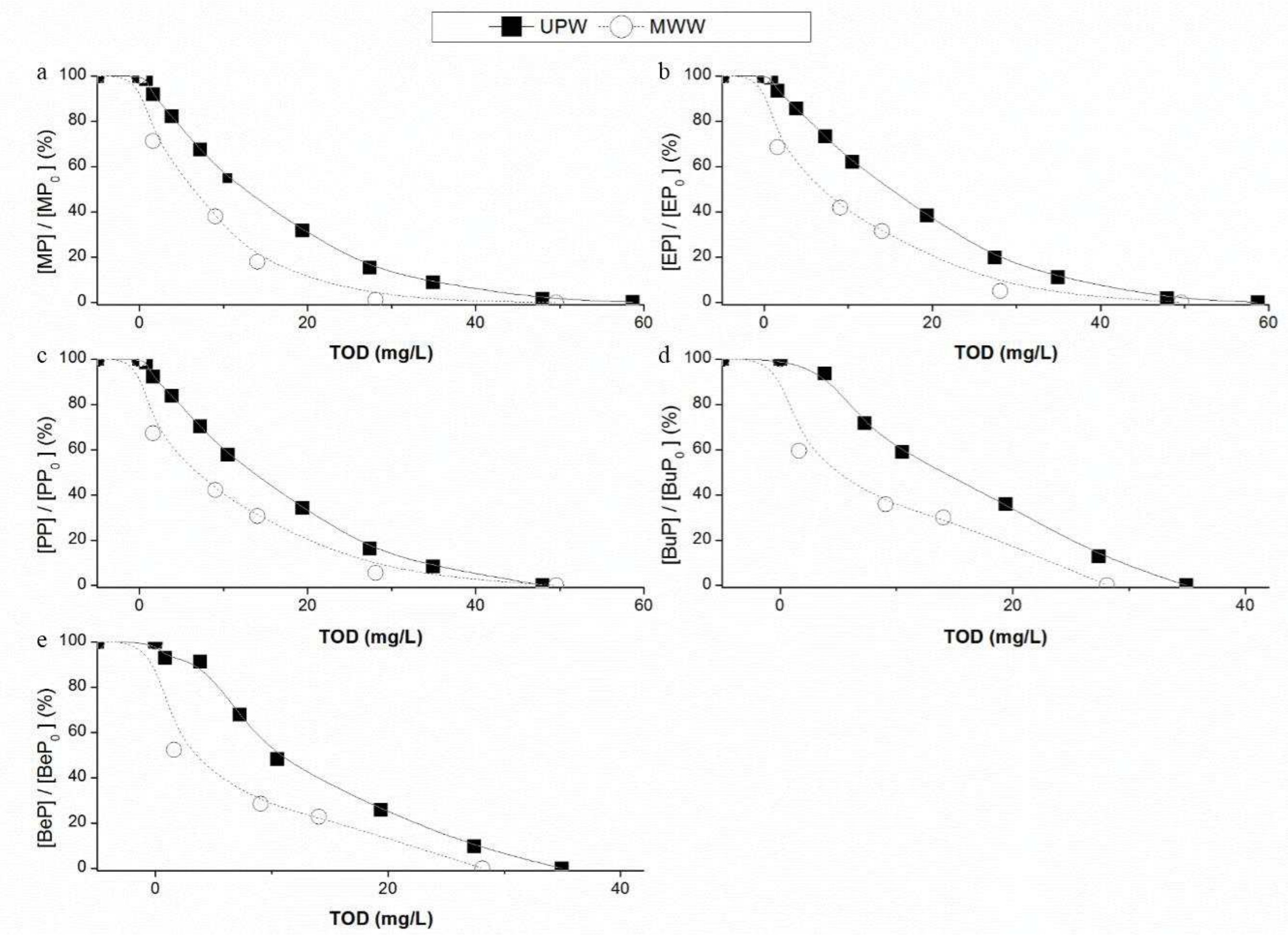

Figure VII.5. Effect of MWW matrix on the parabens degradation a) MP; b) EP; c) PP; d) BuP and e) BeP as function of TOD during photocatalytic ozonation (Initial parabens concentration 10 mg/L each, 70 mg/L of 0.1%Ag-TiO$_2$ catalyst)

To verify if theses ions were responsible for the enhancing on parabens degradation when municipal wastewater was used as matrix, the effect of the concentration of these ions (HCO_3^-, Cl^- and SO_4^{2-}) was analysed. For that, MWW was spiked with 125 mg/L of HCO_3^-, Cl^- and SO_4^{2-}. The experiments with each ion were compared with the case where all of them were used (Figure VII.6). On a general way, for methyl (Fig.VII.6a), ethyl (Fig. VII.6b) and propyl (Fig. VII.6c) parabens for the initial values of TOD the ions presence decreased the efficiency of degradation. Thus, it seems that the initial ozone was scavenged by these ions, instead of promoting the degradation of parabens. Degradation occured at pH 7.7. In this context, chloride radicals production was reduced, since it occurs preferably at acidic conditions, as can be seen in equation VII.6 and VII.7 (Boutiti et al., 2017; Kotzamanidi et al., 2018). Thus, Cl^- ions scavenged by hydroxyl radicals lead preferably to $HOCl^{-\bullet}$ with lower oxidant power.

$$Cl^- + OH^\bullet \longrightarrow HOCl^{-\bullet} \qquad \text{(Equation VII.6)}$$

$$HOCl^{-\bullet} + H^{+} \longrightarrow Cl^{\bullet} + H_2O \qquad \text{(Equation VII.7)}$$

For butyl (Fig. VII.6d) and benzylparaben (Fig. VII.6e) the presence of sulfate ions enhanced their degradation for low TOD values. This can be related with the production of sulfate radical due to the oxidation of sulfate ions on the photogenerated holes (equation VII.2). In fact, sulfate radical presents a higher oxidative potential compared with hydroxyl radicals (Devi et al., 2016; Kotzamanidi et al., 2018). On the other hand, when all ions were tested together the efficacy regarding parabens degradation decreased compared with the case where sulfate ion was used alone. This may possibly be due to sulfate radical scavenging due to the remain species such as HCO_3^- and Cl^- (Ashgar et al., 2015; Monteagudo et al., 2016). According to the Figure VII.3, organic matter does not inhibit the parabens degradation. Otherwise, the municipal wastewater improves the degradation, where this improvement can be truly related with the radical production from ions presence. The efficiency decrease observed when 125 mg/L of these ions were spiked in the water reveals that above a certain concentration, these species inhibit parabens degradation probably due to some radical scavenger effect.

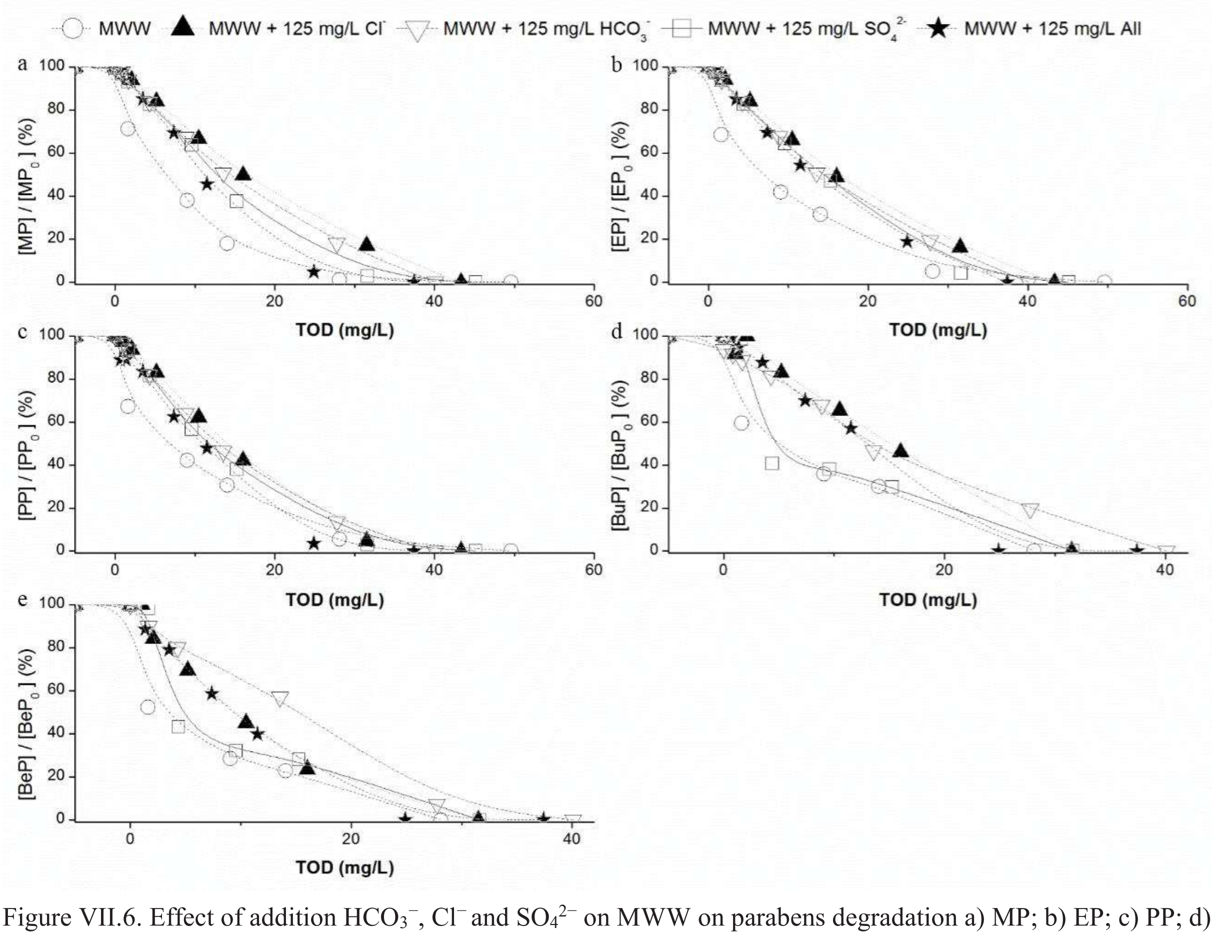

Figure VII.6. Effect of addition HCO_3^-, Cl^- and SO_4^{2-} on MWW on parabens degradation a) MP; b) EP; c) PP; d) BuP and e) BeP as function of TOD during photocatalytic ozonation (Initial parabens concentration 10 mg/L each, 70 mg/L of 0.1%Ag-TiO_2 catalyst, 5 mM of isopropanol)

The initial COD value of spiked municipal wastewater was about 80 mg/L, whereas the final value after treatment was 75 mg/L. This means that several by-products were formed during the photocatalytic ozonation process that still contribute to COD. Therefore, it was important to compare the potential toxic effect of the treated and the initial municipal wastewater.

VII.3.5. Toxicity analysis

The analysis of toxicity was made only for the samples using MWW as matrix. The results related with the UPW were presented in a previous work (Gomes et al., 2018a). The municipal wastewater led to 51% (±1%) of *A. fischeri* luminescence inhibition (contact time of 15 min). This value means that this sample must be considered as toxic. According to Miralles-Cuevas et al. (2017) samples leading to luminescence inhibition for these bacteria above 30% must be considered as toxic. The initial toxicity of municipal wastewater spiked with parabens led to 100% of luminescence inhibition. The photocatalytic ozonation treatment of municipal wastewater spiked with 10 mg/L of each paraben decreased significantly the toxicity. After treatment, the luminescence inhibition decreased to 48% (± 3%), which was even lower than the initial municipal wastewater (without parabens mixture). The presence of ions such as chloride on this water matrix can produce chloride radicals that can react with parabens resulting on halogenated by-products (Devi et al., 2016). This can be an explanation for the relatively high toxicity remaining after treatment. The luminescence inhibiton was also analyzed after bacteria contact during 30 min with the samples. These results can give a preliminary view about the samples chronic toxic effect. For municipal wastewater alone, the luminescence inhibition increased to 68% (± 3%), whereas for the spiked matrix after treatment increased to 61% (± 4%). The increase on toxicity was more meaningful for municipal wastewater alone. This clearly indicates that municipal wastewater alone was worst for bacteria than the samples resulting from the treatment of parabens spiked municipal wastewater through photocatalytic ozonation. This means that the by-products resulting from photocatalytic ozonation treatment are less toxic for bacteria than secondary municipal wastewater alone.

VII.4. Conclusions

The present work shows the effect of different parameters on the parabens degradations during the photocatalytic ozonation using 0.1 wt.% Ag-TiO_2 as catalyst. The parameters analyzed were pH, presence of hydroxyl radical scavenger, impact of using municipal wastewater as matrix. Moreover, the effect of the presence of HCO_3^-, Cl^- and SO_4^{2-} ions on municipal wastewater was also tested. The analysis of these parameters was helpful to understand the mechanism responsible for parabens degradation under this photocatalytic ozonation treatment. In ultrapure water the hydroxyl radicals seem to be the most important specie to improve parabens degradation. However, when a real matrix was used there are other oxidative species that can influence parabens degradation. In fact, the presence of municipal wastewater spiked with parabens allows a reduction on the amount of TOD needed to achieve total parabens degradation. According, to the results achieved for wastewater spiked with different ions, sulfate may have positive effect over parabens degradation. This can be related to the formation of sulfate radicals that present higher oxidative potential compared with hydroxyl radicals. Moreover, the carbonate and chloride radicals can have an important role on water matrix performance regarding parabens degradation, due to their electrophilic character. The reason for parabens removal showing greater performance for municipal wastewater can be related with the fact that these radicals are in enough amount to help on parabens degradation, instead of promoting hydroxyl radicals scavenging. The toxicity assessment reveals a luminescence inhibition reduction when the photocatalytic ozonation was applied. In fact, the treated samples showed a toxicity even lower than unspiked municipal wastewater.

VII.5. References

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VIII. Parabens degradation using catalytic ozonation over volcanic rocks

It is based on the publication: Gomes, J., Quinta-Ferreira, M. E., Costa, R., Quinta-Ferreira, R. M., Martins, R.. C., (2018) Parabens degradation using catalytic ozonation over volcanic rocks Environmental Science and Pollution Research. 25: 7346-7357.

VIII.1. Introduction

Advanced oxidation processes (AOPs), such as ozonation, have been extensively used for enhancing cost-effective treatments for the degradation of contaminants from wastewaters (Gomes et al., 2017a). Ozone is a powerful oxidant capable of degrading a broad spectrum of compounds. However, ozone alone normally cannot totally mineralize organic compounds (Martins and Quinta-Ferreira, 2009). Therefore, for improving efficiency, ozonation can be applied coupled with heterogeneous catalysts. The presence of a catalyst in ozonation process can enhance hydroxyl radicals production presenting higher oxidant power than molecular ozone. However, the catalytic mechanism can also include the adsorption of organic compounds and/or ozone promoting surface reactions (Kasprzyk-Hodern et al., 2003; Nawrocki and Kasprzyk-Hodern, 2010). Besides, catalytic ozonation generally leads to higher mineralization of organic compounds using a lower ozone dose compared to single ozonation. Other technologies can be coupled to ozonation with the aim of enhancing the ozone activity on the degradation of target contaminants, such as UV radiation. However, for taking advantage of this technology on the degradation of emerging contaminants normally it will be necessary the presence of a semiconductor as catalyst. The most widely used semiconductor catalyst is TiO_2 due to its chemical stability, low cost, easy handling, good optical and electronic properties (Chong et al., 2010; Pelaez et al., 2012). The main drawback of pure TiO_2 is the large band gap ($\lambda < 387$ nm for anatase type) which implies high energy for photoelectrons generation. For decreasing the band gap to extend the TiO_2 absorption on the visible region, many approaches were followed. This implied the doping of TiO_2 with non-metal and metal compounds (Pelaez et al., 2012). As an example, N-doped TiO_2 proved to be more efficient on ethylparaben degradation under solar radiation when compared with undoped titania catalysts (Petala et al., 2015). The costs associated with the catalytic materials can be an important drawback on the treatment process. On this way, the use of low-cost materials such as volcanic rocks appear as good solution to improve catalytic or photocatalytic ozonation, because this kind of rocks have metal ions that can be useful on the presence of UVA irradiation and ozone.

Volcanic sand was used in heterogeneous catalytic ozonation for degradation of benzothiazole (Valdés et al., 2008). Martins et al. (2014) studied the utilization of low-cost catalysts such as volcanic rock, sepiolite and iron shavings to enhance the catalytic ozonation on the depuration of olive mill wastewater. Besides, low cost materials, such as pumice, were used as adsorbent of one of the most used reactive dyes in the textile dying industries, reactive black 5 (Heibati et al., 2014).

The pumice and other volcanic rocks are natural materials that can be abundantly found in volcanic regions, such as Azores islands (Portugal). This kind of rocks results from magma erupted from a volcano. Pumice is a volcanic rock characterized by high porosity and low weight. The other volcanic rocks also present high porosity but higher weight when compared with pumice. These rocks are rich in metals that can be useful for catalytic or photocatalytic ozonation and are easily available with low or no costs. Nowadays, special concern is given to emerging contaminants such as parabens, which can be found in wastewater treatment plants and water sources (Yu et al., 2011; Bledzka et al., 2014). These compounds are widely used as antimicrobial and preservatives, mainly in pharmaceutical and personal care products (PPCP's) (Calafat et al., 2010; Meeker et al., 2013). Several studies for degradation of these contaminants have been made using AOPs, such as ozonation (Tay et al., 2010), catalytic ozonation (Gomes et al., 2017b), photocatalytic ozonation (Gomes et al., 2017c), Fenton process (Domínguez et al., 2014; Martins et al., 2016) photocatalytic degradation (Gmurek et al., 2015; Petala et al., 2015; Velegraki et al., 2015) and photosonochemical degradation (Daghrir et al., 2014). All these techniques present good results on parabens degradation.

The goal now is checking if low cost volcanic materials may be efficient to enhance the parabens ozonation. Thus, the aim of this study was evaluating the degradation of a mixture of five parabens, methylparaben (MP), ethylparaben (EP), propylparaben (PP), butylparaben (BuP) and benzylparaben (BeP) using two volcanic rocks coming from Azores islands as catalysts combined with ozonation and photoozonation.

VIII.2. Material and Methods

VIII.2.1. Reagents

The solution was prepared using 10 mg/L in ultrapure water of each one of the parabens: MP, EP, PP, BeP (obtained from Sigma-Aldrich) and BuP (purchased from Fluka).

VIII.2.2. Characterization of Volcanic rocks

The volcanic rocks used in this study were pumice and another material morphologically similar to pumice but with higher density. These rocks were collected in São Miguel (Azores). The rocks will be designated as P1 for volcanic rock and as P2 for pumice. Before experiments, the rocks were washed with high flow of tap water to remove the larger debris present on their surface. Then rocks were placed in a closed flask submersed in distilled water during 24 h. After this, the rocks were dried at 105 ^{o}C in oven for 24 h.

The samples used in the tests were separated by size using multi sieves to obtain particle sizes between 250 to 500 μm and lower than 105 μm. The materials were characterized regarding pHpzc, elemental composition, specific surface area (S_{BET}), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Scanning Electron Microscopy - Energy Dispersive X-Ray Analysis (SEM-EDS).

The point of zero charge (pHpzc) was determined following the method described by Rivera-Utrilla et al. (2001). pH values were determined using a Crison micropH 2002 apparatus. The elemental composition of volcanic rocks regarding N, C, H, S and O was determined using a Fisons Instruments EA 1108 CHNSO. The rocks composition in Si, Al, Fe, Cu, Zn, K, Na, Cr, Mn, Ca and Mg was measured through atomic absorption in a PerkinElmer 3300 spectrometer after acid digestion. The specific surface area of volcanic rocks, *Brunauer–Emmet–Teller* (S_{BET}), was determined using nitrogen (-196 °C) with an accelerated surface area and porosimetry analyzer (ASAP 2000, Micrometrics). The X-ray diffraction (XRD) analysis, was made through a diffractometer (Bruker D8 Advance), that works with Cu Kα radiation (2.2 kW ceramic tube) and using a 1D LynxEye detector (Silicon Drift Detector) covering an angle of ~3^{o} and with ~25% energy resolution. The FTIR analysis of volcanic rocks was carried out using KBr pellets in a Perkin Elmer Spectrum BX-2 and the spectra used was 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. The surface of volcanic rocks was analyzed by a TESCAN VEGA 3 SBH - Easy Probe Scanning Electron Microscopy (SEM) and the qualification of elements present on their surface was performed by Energy Dispersive X-Ray Analysis (EDS) using a Bruker

QUANTAX system that includes the Bruker Nano XFlash® detector. EDS analysis was made on several points randomly selected from SEM analysis and carried out using an accelerating potential of 20 kV and real time for the spectrum accumulation of 60 s at a dwell time of 16 ms. Energy-Channel calibration was used with a Cu standard (K_a 8.0463 keV).

VIII.2.3. Photocatalytic ozonation experiments and analytical techniques

A 2 L glass magnetically stirred photoreactor was used in the degradation experiments. The photoreactor is equipped with 3 lamps (Philips TL 6W BLB, tube diameter of 16 mm) which main emission is 365 nm and a photon flux of 5.75×10^{-7} einstein/Ls (Gomes et al., 2017c). The lamps were only turn on for the photocatalytic experiments. Aluminum foil was used to cover the reactor and avoid the interference of outside light. The experiments were carried out during 120 min at controlled temperature (25±1 ºC) by a thermostatic bath.

In order to evaluate the adsorption of parabens mixture over the volcanic rocks surface, the reaction media was putted in contact with the rocks before ozone being fed and turning on (for the photocatalytic experiments) the UVA irradiation. The total volume taken for analysis during the experiments never exceeded 10% of total volume.

Ozone was generated in an 802N BMT apparatus (~8 mgO_3/Nm^3) from pure oxygen stream (99.9%). The gas outflow leaving the reactor was trapped by a solution of Potassium iodide (Panreac) for the remaining ozone removal before discharge to the atmosphere. The inlet ($[O_3]^{in}$) and outlet ($[O_3]^{out}$) ozone concentration was measured by ozone gas analysers (BMT 963 and 964 vent, BMT). The data collected was used for transferred ozone dose (TOD) determination according to eq. VIII.1.

$$\mathrm{TOD}=\int_0^t \frac{Q_{Gas}}{V_{Liquid}}\times\left([O_3]^{in}-[O_3]^{out}\right)\times dt \qquad \text{(Equation VIII.1)}$$

Where Q_{Gas} represents the gas flow rate (0.2 L/min), V_{Liquid} is the volume of the effluent used in the reactor (2 L). The TOD was expressed in mg O_3/L.

The determination of each paraben and reaction by-products concentration was performed by High-performance liquid chromatography HPLC (UFLC, Shimadzu) with the method previously described (Gomes et al. 2017b).

VIII.3. Results and Discussion

VIII.3.1. Volcanic rocks characterization

The pHpzc of both volcanic rocks is similar, 5.7 (P1) and 5.5 (P2). Below these values of pH the acidic water donates more protons than hydroxide groups to catalyst surface which will be positively charged and able to attract anions or weak acids. Conversely, above these values the surface of the catalyst will be negatively charged attracting cations and metal ions (Stumm and Morgan, 2012). This parameter may have a key role over the impact of pH on the treatment process efficiency.

The FTIR analysis spectra of two volcanic rocks samples reveal a strong absorption peak at 950-1200 cm^{-1} (Fig. VIII.1). This may result from stretching vibrations of Si-O-Si. Huang et al. (2008) reported the FTIR analysis of natural zeolite (similar to pumice) and verified an absorption peak at 1047 cm^{-1} matching with stretching vibration band of Si(Al)-O. As can be seen in Fig.VIII.1a the strongest peak was near to this wavenumber so it seems to be the same vibration. The peak detected from 3400 to 3700 cm^{-1} represents surface OH groups stretching vibrations. The OH bend occurs at 1630 cm^{-1} (Huang et al., 2008). The absorption peaks at 2350 cm^{-1} presented in Fig. VIII.1 corresponds to C-O stretching vibrations, due to the presence of carbon dioxide on atmosphere.

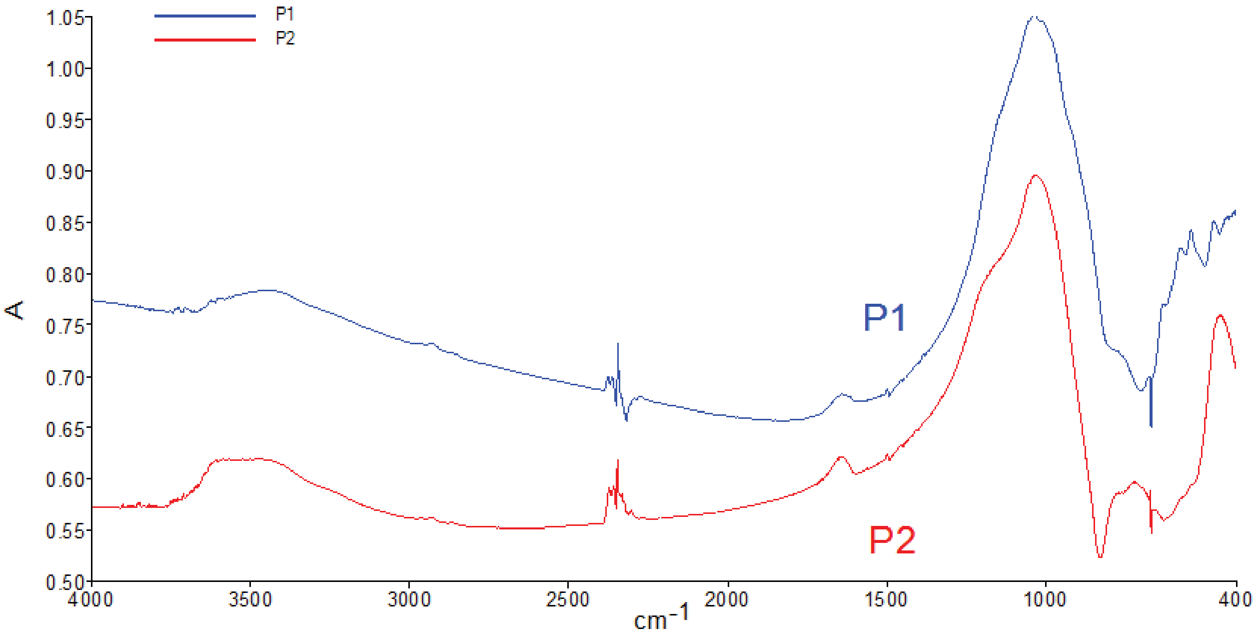

Figure VIII.1. FTIR spectrum of the two volcanic rocks (P1 and P2).

The XRD analysis identified main peaks for P1 at 2θ = 27º, 29.8º, 30.8º, 35º, 35.6º, 42.3º and 56.6º (Fig. VIII. 2). These peaks seem to belong to two different minerals augite [$(Ca,Na)(Mg,Fe,Al,Ti)(Si,Al)_2O_6$] and diopside [$CaMgSi_2O_6$]. In fact, the patterns peaks for augite are at 27º, 29.8º, 35.6º and 42.3º. In what regards diopside these are at 27º, 30.8º, 35º and 56.6º. The elemental analysis confirms the presence of Al, Fe, Ca, Mg and O (Table VIII.1). According to the results for XRD analysis, elemental analysis and EDS analysis of the volcanic rock (P1) it is safe to say that these minerals are present on this sample. These two minerals are characterized by a high percentage of silica, above 50% (Anthony et al., 2017). Rock P2, is a kind of pumice due to the lower density and high porosity. The XRD analysis reveals peaks typical of this material (Fig. VIII.2). Ersoy et al. (2010) performed the characterization of pumice and identified three main peaks at 2θ = 23º, 26.65º and 28º. These peaks seem coincident to those obtained in the Fig. VIII.2. Except for the main peak at 2θ = 21.6º instead of 2θ = 23º described by Ersoy et al. (2010). These two peaks at 2θ = 21.6º and 28º present on Fig. VIII.2 seems to match with the mineral anorthoclase $(Na,K)AlSi_3O_8$. From elemental analysis for P2 (Table VIII.1), as well as based on EDS analysis (Fig. VIII.3), the K, Al, Na and O are found, which reinforces the idea that this mineral is present on pumice. This mineral is characterized by a high percentage of SiO_2 (63%) (Anthony et al. 2017). The peak at 2θ = 26.65º refers the presence of quartz, which is also characteristic of pumice stone.

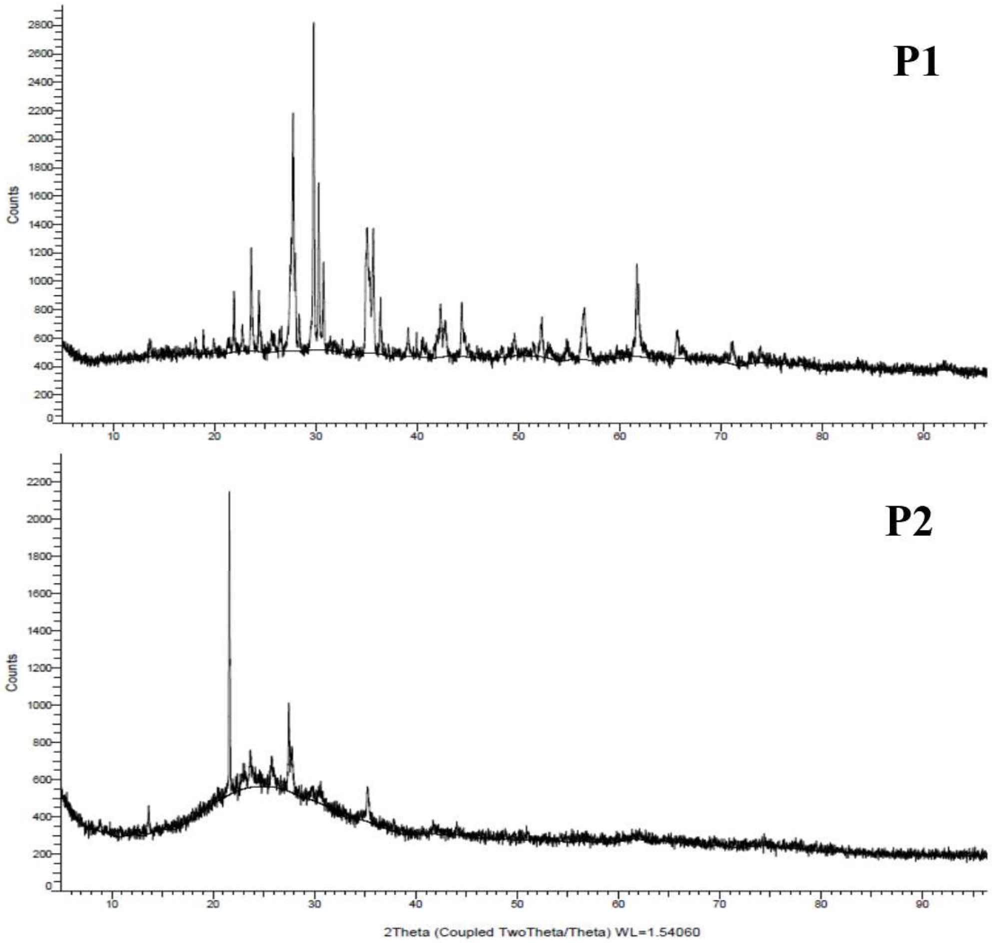

Figure VIII.2. XRD analysis of the two volcanic rocks (P1 and P2).

The SEM-EDS analysis detailing the micro-structure and elemental composition of volcanic rocks are shown in Fig. VIII.3. The SEM images reveal that P1 presents irregular shape. On the other hand, the rock P2 shows a well-defined morphology. The EDS analysis was made on a point randomly selected from SEM images and no differences were verified on identified peaks on both volcanic rocks. The main identified peaks were Ca, K, Ti, O, Fe, Na, Mg, Al, Si. The amount on elemental composition is the only difference between the two volcanic rocks.

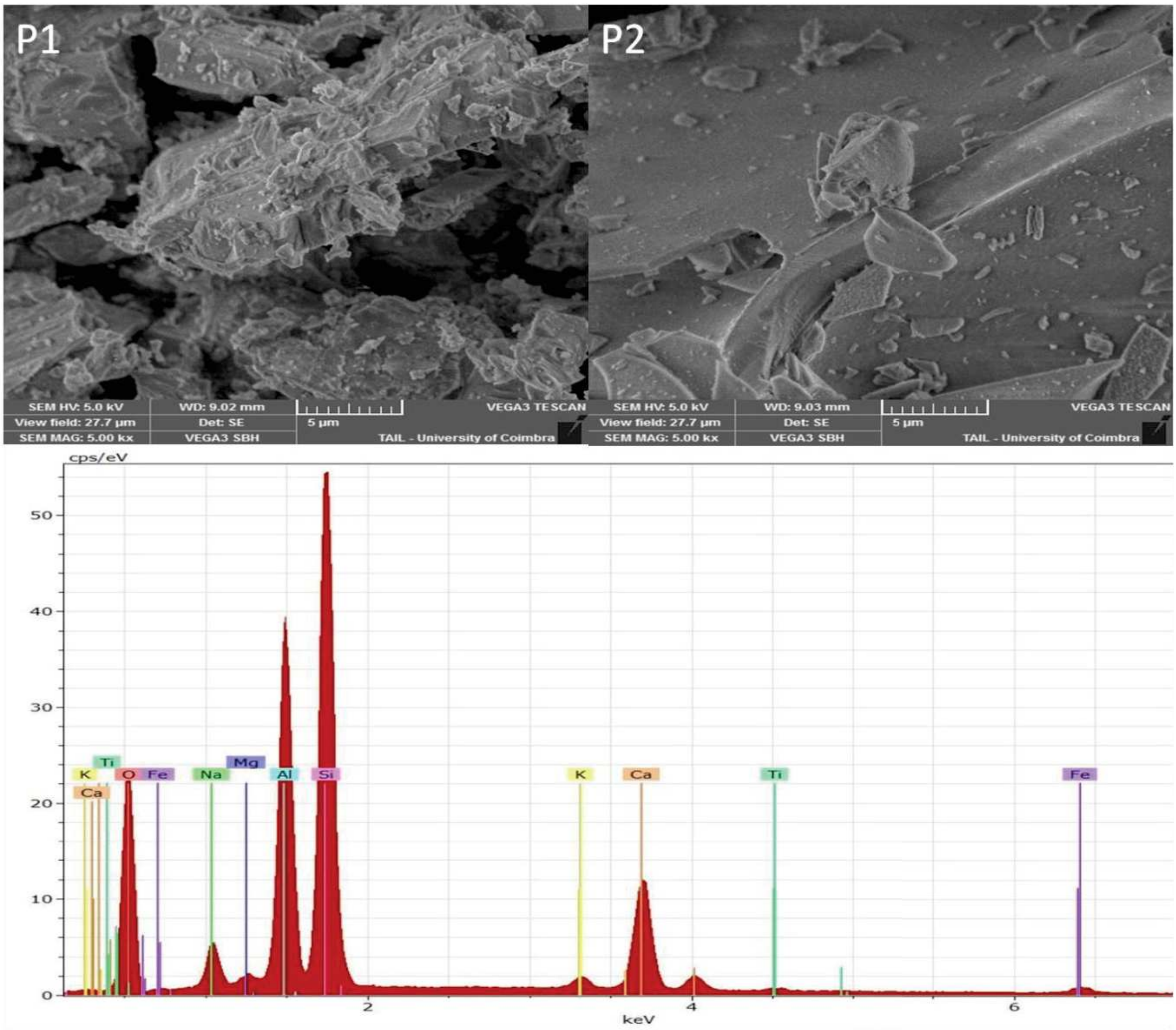

Figure VIII.3. SEM-EDS analysis of two volcanic rocks (P1 and P2)

Table VIII.1 shows the elemental composition for P1 and P2. N, C, H, S, and O contents were obtained through elemental analysis, whereas Al, Fe, Cu, Zn, K, Na, Cr, Mn, Ca, and Mg amounts were attained by atomic absorption. While FTIR and XRD reveal the presence of Si in the samples, Si was not detected through atomic absorption. This may be due to the difficulty of silica digestion. Thus, possibly, also the concentration of Al may be higher than the one reported in Table VIII.1. For P1 the highest elements load was achieved for Al, Fe, Mg, Ca, C and O. Meanwhile for P2 the highest elements load was for Al, K, Fe, Ca, Na, C and O.

Table VIII.1. Elemental composition of the two volcanic rocks (wt of element/wt total %)

	P1	P2
Al (%)	0.302	0.222
Fe (%)	5.576	0.117
Cu (%)	0.010	0.001
Zn (%)	0.005	0.006
K (%)	0.057	0.117
Na (%)	0.055	0.095
Cr (%)	0.002	0.002
Mn (%)	0.022	0.004
Ca (%)	0.199	0.105
Mg (%)	2.004	0.048
N (%)	0.527	0.590
C (%)	7.720	8.499
H (%)	0.421	0.690
S (%)	0.824	*a*
O (%)	1.226	2.184

[a] *Value below the analytical apparatus detection limit*

The BET analysis reveals that surface area obtained for volcanic rock P1 and pumice P2 was 28.3 and 2.98 m^2/g, respectively.

VIII.3.2. Effect of particle size and catalyst load on parabens degradation through catalytic ozonation

In order to establish the optimum particle size and catalyst load for parabens degradation, preliminary studies were made, with two different particle sizes of catalyst (250-500 µm and < 105µm) and four different catalyst loads (0.1, 0.25, 0.5 and 1 g/L) using volcanic rock P1. No significant differences are verified for the different particle diameters on catalytic ozonation efficiency regarding parabens degradation. Thus, for the range of particle sizes used, chemical regime is ensured. In fact, Martins and Quinta-Ferreira (2009) verified that chemical regime was guaranteed for 125-250 µm particle size diameter with a 750 rpm and gas flow rate of 0.2 L/min.

The catalyst load is an important economic and efficiency parameter. The increase on catalyst concentration not necessarily represents an increase of oxidation efficiency, whereas it increases the costs of treatment. Therefore, it is important finding the optimal catalyst load for each process. High concentration of catalyst can increase the number of active sites for oxidation of parabens but does not mean a direct increase of treatment efficiency due to excess of active sites available (Martins and Quinta-Ferreira, 2009). Beltrán et al. (2002) proven that above of 3 g/L of catalyst load no improvement was observed on oxalic acid removal. In the present work no significant differences were verified with the increase of catalyst load on parabens degradation. Since the differences of TOD to total parabens degradation were very small. Thus 0.5 g/L was selected from the range tested to pursue the experiments. This amount of catalyst allows a good compromise between performance on parabens degradation and the amount of leached metals from volcanic rocks.

VIII.3.3. Comparison of the catalytic efficiency of P1 and P2 and effect of UVA irradiation presence on parabens degradation

After chosen the best particle size diameter and catalyst amount (<105 μm and 0.5 g/L), the performance of P1 and P2 on parabens degradation was evaluated with catalytic and photocatalytic ozonation. For comparative purposes, single ozonation results are also reported. Experiments were randomly performed in duplicate and the maximum deviation between means was never higher than 2%.

The best performance on catalytic ozonation regarding parabens degradation was achieved with volcanic rock P1 (Fig. VIII.4). The difference of efficiency on parabens degradation compared to P2 was not so sharp. This can be due to higher surface area of P1 comparatively to P2. Nevertheless, it is clear that the presence of volcanic rock improves process efficiency compared to single ozonation. In fact, while total parabens degradation was achieved using a maximum TOD of 55 mg/L in the former, for single ozonation was necessary about 170 mg/L (Gomes et al., 2017b). The volcanic rocks as catalyst enables a great reduction of ozone amount required at lower cost. Valdés et al. (2008), verified that catalytic ozonation with 10 g/L of volcanic sand can enhance, at lower dose of ozone, the benzothiazole degradation compared to single ozonation. On the other hand, Martins et al. (2014) concluded that using red volcanic rock does not enhance ozone action over pollutants, possibly due to different catalyst composition characteristics. In this study, the two volcanic rocks can enhance the ozonation with low catalyst load. This improvement of ozonation can be due to the higher decomposition of ozone and consequently generation of hydroxyl radical (Kasprzyk-Hodern et al., 2003), this will be further explored latter. According to literature and data from EDS and elemental analysis, these volcanic rocks present in their composition some metal oxides, such as SiO_2, Al_2O_3 and Fe_2O_3 (Ersoy et al., 2010; Anthony et al., 2017). These oxides can work as Lewis centers and promote ozone decomposition (Kasprzyk-Hodern et al., 2003). Tay et al. (2010), worked with a mixture of five parabens (MP, EP, PP, BuP and BeP) and an ozone dose of 0.67 g/h at pH 6.9, and verified a removal of 99% of these parabens in 12 min. These results can be comparable with our work. It seems that increasing pH to 6.9 improves the self-decomposition of ozone into hydroxyl radical. This leads to a similar effect to the one we observed in the presence of catalyst at pH 3.5.

The augite mineral present on P1 has TiO_2 and Fe_2O_3 on its composition, in turn anorthoclasse of P2 only has Fe_2O_3 (Anthony et al., 2017). According to EDS, the TiO_2 must be present on the P2, but at lower amount comparatively with P1 (Fig. VIII.2). These two oxides can work as semiconductors, which can be helpful to improve the parabens degradation on light presence. The application of simulated solar radiation with 0.5 g/L of TiO_2 (Degussa P-25) allows the total degradation of MP after 240 min of reaction (Velegraki et al., 2015). Therefore, the presence of UVA irradiation with ozone and volcanic rocks was tested on parabens degradation. The presence of UVA enhanced only very slightly parabens degradation compared to catalytic ozonation (Fig. VIII.4). Regarding P2 the difference was almost insignificant, for example using TOD of 23 mg/L MP degradation was 70% with and 69% without UVA irradiation. This can be due to the lower amount of Fe_2O_3 available on P2 (about 0.2% of Fe according to Table VIII.1). Normally, from literature data the amount of Fe_2O_3 presents on pumice can be within 1.7 and 2 % (Ersoy et al., 2010; Heibati et al., 2014). On the other hand, the presence of UVA on efficiency of mixture parabens degradation when P1 was applied was more noticeable compared to P2, but not yet significant. For example, using TOD of 25 mg/L PP degradation was 82% with, and 75% without UVA irradiation This increase can be explained by the presence of TiO_2 and higher amount of Fe_2O_3 (5.6% of Fe from Table VIII.1). The difference between efficiencies with or without UVA irradiation indicate that catalytic ozonation seems to be the best choice for parabens degradation, due to the high cost that UVA irradiation represents for a not very significant improvement.

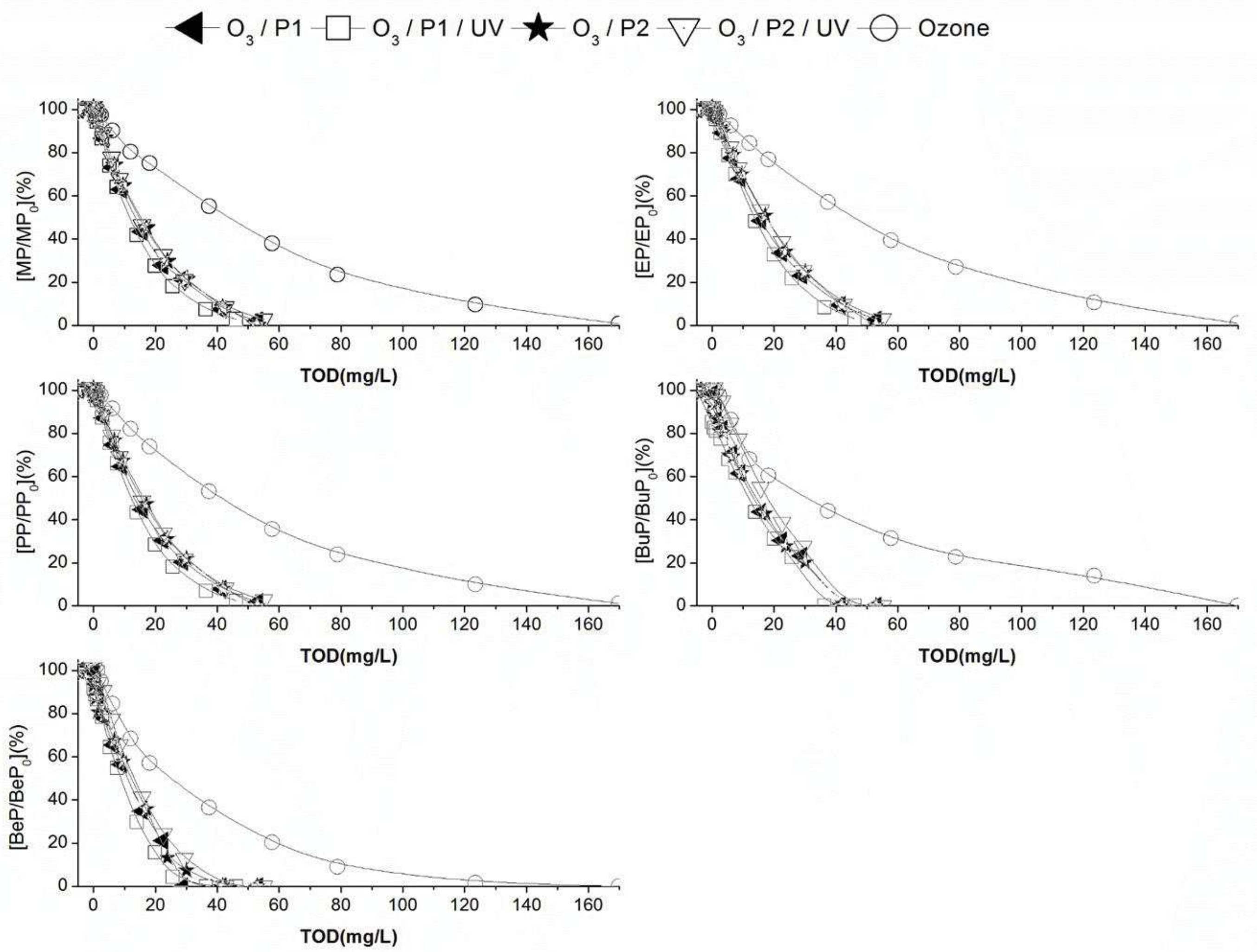

Figure VIII.4. Comparison between catalytic and photocatalytic ozonation over volcanic rocks with single ozonation regarding parabens degradation. (pH 3.5, 0.5 g/L of catalyst)

To compare these results with other processes it is necessary to look for the energy consumption resulting from this process. According to Hollender et al., (2009) the ozone production on wastewater facilities represents an energy consumption of about 12 kWh/kg O_3. In a previous work it was found that the energy consumption for single ozonation process to parabens degradation was 2.04 kWh/m^3 (Gomes et al. 2017c). In the present study using catalytic ozonation the energy consumption for the best volcanic rock (P1) was 0.66 kWh/m^3 (corresponding to 55 mg/L of TOD). Therefore, the catalytic ozonation with volcanic rock presents a higher reduction of energy consumption.

An important feature of a heterogeneous catalyst is its stability regarding metals leaching. These metals are a secondo source of pollution and can contribute as homogeneous catalysts. As can be seen from the Table VIII.2 the amount of iron leached compared with the initial composition of catalyst (corresponding to 27.9 mg/L) was 1.6%. This value reveals that amount of iron available at the end of the catalytic ozonation is small, so the possibility of iron working as homogeneous catalyst may be minimal. Moreover, the catalyst can be considered as stable concerning metals dissolution to the liquid bulk.

Table VIII.2. Amount leached of some metals after catalytic ozonation

	Amount leached (mg/L)
Al	0.35
Fe	0.45
Na	0.15
Mg	0.69

VIII.3.4. Effect of pH

The effect of pH on parabens catalytic ozonation was only analyzed for volcanic rock P1, due to its slightly higher performance compared to P2 and higher specific surface area.

pH is an important parameter to control the surface charge of the catalysts and decomposition of ozone (Kasprzyk-Hodern et al., 2003). According to the results attained, the catalysts were not able to adsorb the initial parabens for different values of pH. Therefore, the most plausible mechanism of reaction may be the adsorption of ozone on the catalyst surface leading to the production of hydroxyl radicals that will interact with the parabens in the liquid bulk. This mechanism will be discussed on next section. The catalyst may contain at the surface hydroxyl groups (Kasprzyk-Hodern et al., 2003). The ability of surface hydroxyl groups to be protonated or deprotonated its dependent upon the solution pH. At pH < pHpzc the surface hydroxyl groups on metal oxides (M-OH) will be protonated ($M\text{-}OH_2^+$). On the other hand, for pH > pHpzc the M-OH will be deprotonated ($M\text{-}O^-$) (Nawrocki et al., 1993). To values below pHpzc the protonated form of M-OH will act as Lewis acid and adsorb anions, while for pH values above pHpzc the deprotonated form will act as Lewis bases and adsorb cations (Kasprzyk-Hodern et al., 2003). Parabens adsorption on the catalyst surface is very dependent on the pH used. In the analysis of pH effect, the impact of adsorption was measured by contacting the catalyst with the effluent during 5 min without feeding the reactor with ozone. For this period, adsorption was negligible for the different pH values that were tested. Methyl, ethyl, propyl and butyl paraben p*Ka* is above of 8.90 (Angelov et al., 2007). Thus, only for higher pH values parabens will be deprotonated and negatively charged. The tested pH values are below this value. Moreover, pHpzc of volcanic rocks are below the pKa of parabens so it was expected that the adsorption would be low.

The increase of pH (Fig. VIII.5) causes an increase in the efficiency of parabens degradation. For example, PP degradation using a TOD of 27 mg/L at pH 3 achieved 80%, while at pH 8 reached 93%. However, no significant differences were verified between the two higher pH's values (7 and 8). The highest efficiency detected for pH 8 may be related with the high decomposition of ozone at basic conditions. Valdés et al. (2009) analyzed the decay of ozone rate constants in the presence of volcanic sand as function of pH. The results reveal that the increase of pH from 2 to 8 doubles the rate constant. This means an increase of hydroxyl radical production for pH above pHpzc. At high pH the presence of hydroxide ions promotes ozone decomposition reactions into hydroxyl radicals and the overall rate is faster compared to lower pH values (Sotelo et al., 1987). Ma and Graham (1999) verified that hydrous manganese dioxide at pH 7 is negatively charged and bound with OH^- ions. These will react with ozone on the surface of the manganese dioxide generating hydroxyl radicals. Therefore, the improvement on parabens degradation promoted by increase of pH may be directly related with higher generation of hydroxyl radicals.

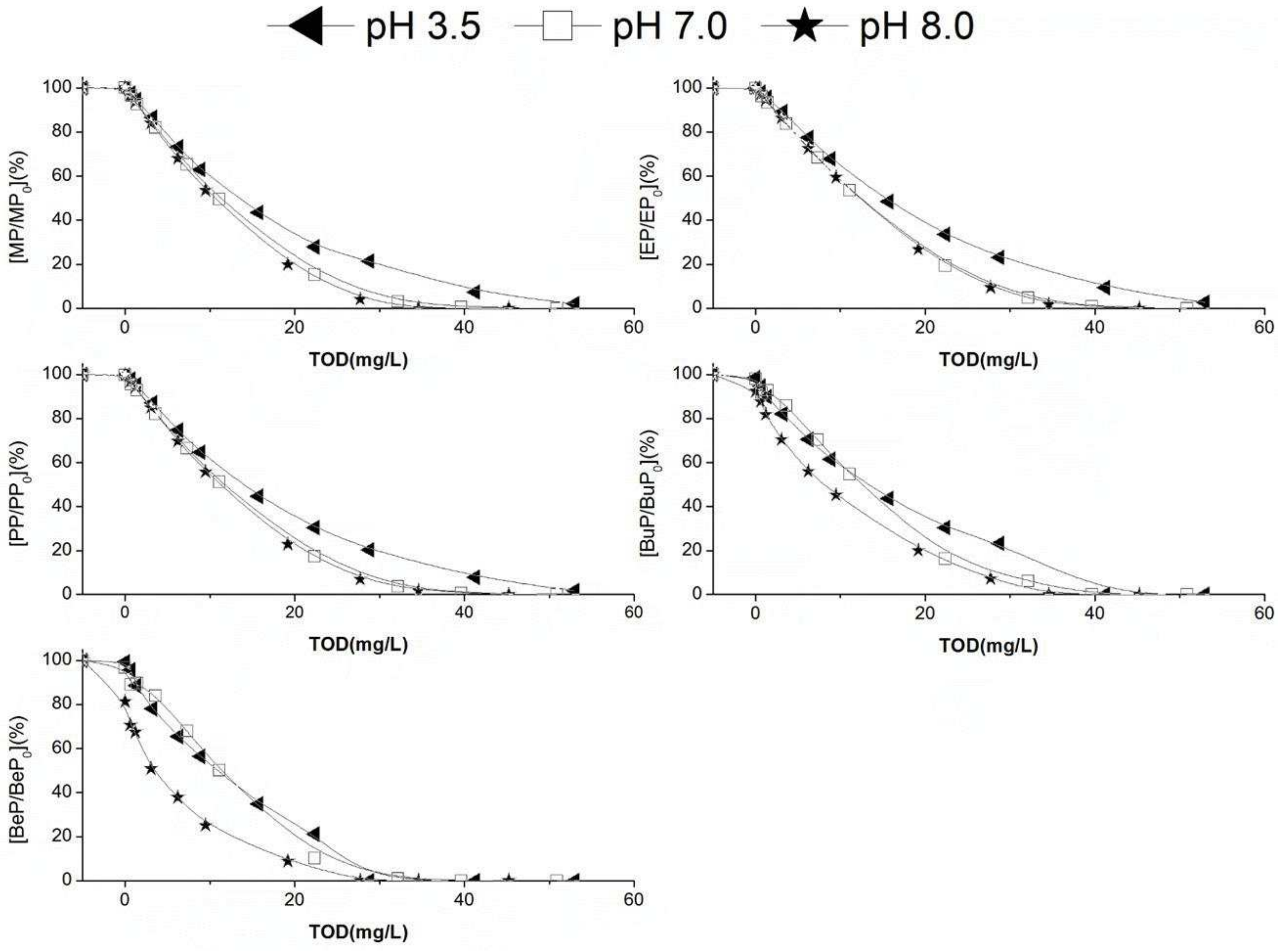

Figure VIII.5. Effect of pH on parabens degradation during catalytic ozonation (0.5 g/L of catalyst)

VIII.3.5. Mechanistic studies: Effect of radical scavenger on parabens degradation and by-products analysis

Catalytic ozonation mechanisms are based on oxidation of compounds through molecular ozone action or based on ozone decomposition to generate highly reactive oxidative species, such as hydroxyl radicals. Besides, another possible pathway may involve both ozone and pollutants adsorption over the catalyst surface followed by surface reactions (Kasprzyk-Hodern et al., 2003).

As described above, the higher pH values enhanced the catalytic ozonation, maybe due to the higher hydroxyl radical production, which increases the overall rate of degradation. On this way, a radical scavenger has been used to understand the contribution of these reactive oxidative species over parabens degradation. Iso-propanol is one of the most common used compounds as hydroxyl radical scavenger (Velegraki et al., 2015). Fig. VIII.6 shows the effect of the addition of 5 mM of iso-propanol on parabens degradation during catalytic ozonation using P1. As can be seen, the presence of the scavenger inhibits the total degradation of parabens comparatively to catalytic ozonation without radical scavenger. To benzylparaben degradation until a TOD of 9 mg/L the degradation curves are similar for the two conditions tested. This can be due to the presence of two benzenic rings which are very reactive with molecular ozone (Kasprzyk-Hodern et al., 2003; Martins and Quinta-Ferreira, 2009). The presence of iso-propanol inhibited the degradation of the other parabens degradation from about 100% to 60%, using a TOD of 42 mg/L. This behavior is similar to single ozonation (Fig. VIII.6). Which means that for single ozonation that occurs at pH 3.5, the main responsible for parabens degradation is molecular ozone, since for this pH hydroxyl radicals production is inhibited. Therefore, it can be concluded that hydroxyl radicals are the main responsible for parabens degradation using a low-cost material (volcanic rock) as catalyst on catalytic ozonation.

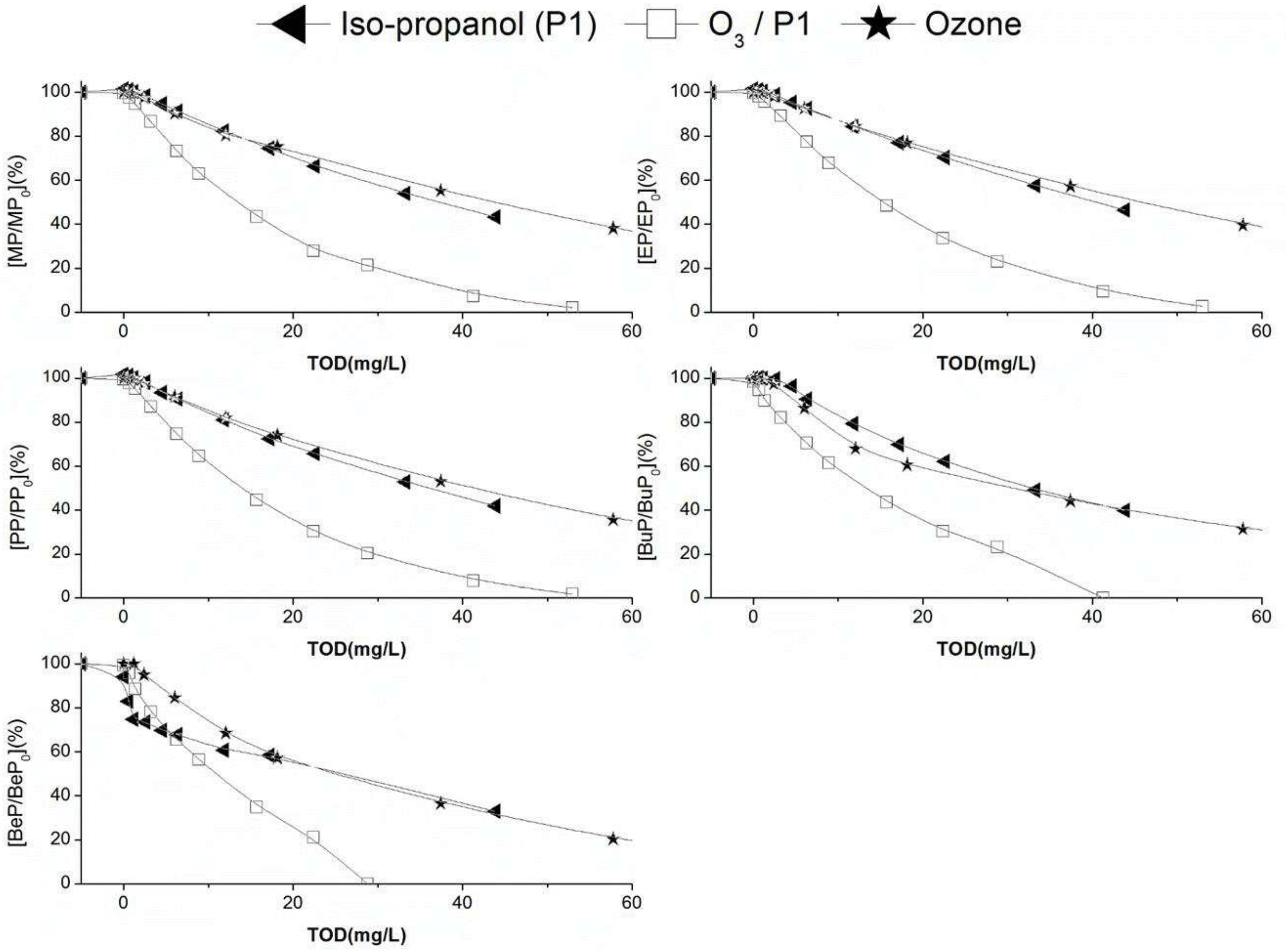

Figure VIII.6. Effect of radical scavenger on parabens degradation during catalytic ozonation

To confirm this mechanism, intermediate compounds from parabens degradation were followed through the reaction. The standards of 4-Hydroxybenzoic acid (4-HBA), 3,4-Dihydroxybenzoic acid (3,4-diHBA), 2,4-Dihydroxybenzoic acid and Hydroquinone were used and compared with the samples chromatograms. These compounds were quantified and represented as function of TOD for catalytic ozonation with and without radical scavenger iso-propanol (Fig. VIII.7). The 4-HBA as well as hydroquinone result from the parabens ester chain breakdown. This reaction is favored by the presence of hydroxyl radicals (Fig. VIII.7). In the presence of radical scavenger, the 4-HBA and hydroquinone production was clearly reduced compared with catalytic ozonation. In the case of hydroquinone, this compound only appears using a higher TOD when iso-propanol was applied. This means that a high amount of molecular ozone was necessary to promote the parabens ester chain breakdown. The remaining by-products identified, 3,4-diHBA and 2,4-diHBA can result via direct molecular ozone attack of the benzenic ring or can be produced by hydroxyl radicals reaction with benzenic ring (Tay et al., 2010). As it can be seen, in the presence of radical scavenger the 3,4-diHBA and 2,4-diHBA follow the same behavior of 4-HBA production, due to hydroxyl radicals absence. Otherwise, the concentration of 3,4-diHBA increase with TOD, possible due to ozone molecular action to improve the hydroxylation of benzenic ring.

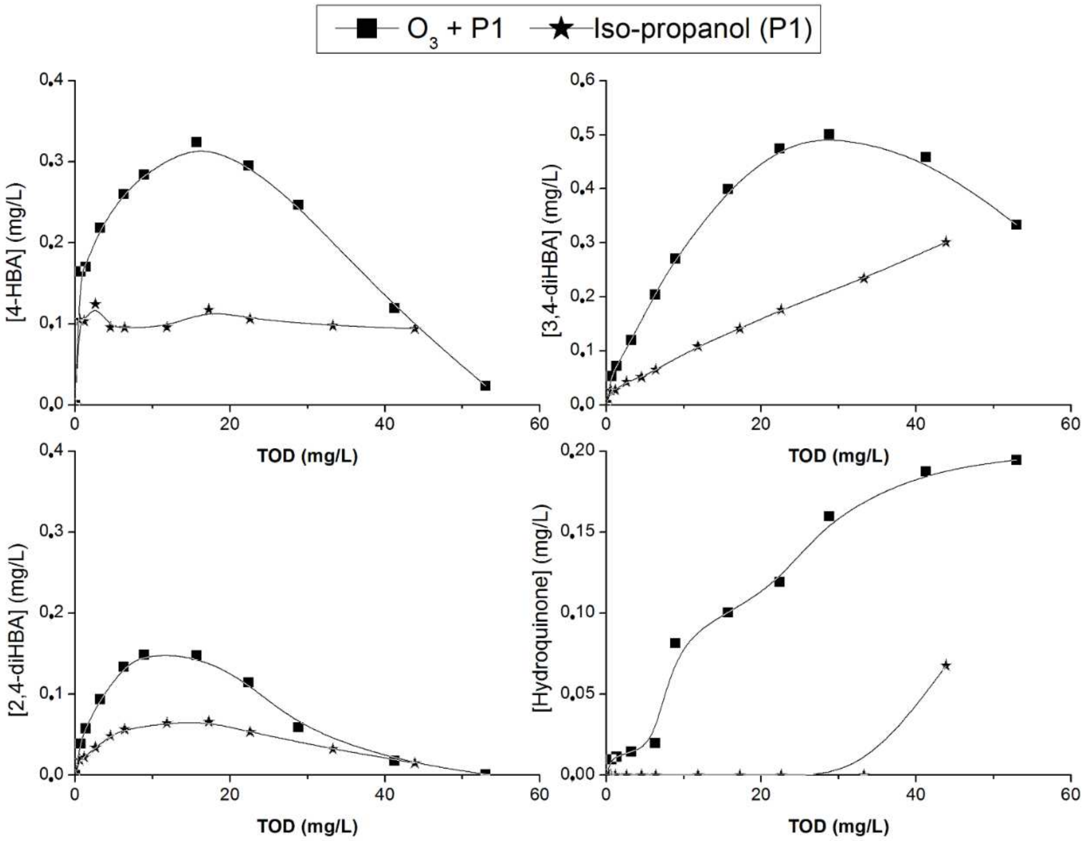

Figure VIII. 7: by-products (4-HBA; 3,4 diHBA; 2,4-diHBA and Hydroquinone) formation for radical scavenger and catalytic ozonation.

VIII.4. Conclusions

The aim of this work was the utilization of a low-cost material as catalyst to enhance ozone activity on the degradation of a parabens mixture. The selected low-cost materials are two abundant volcanic rocks present on Azores (Portugal). The presence of these two volcanic rocks truly enhanced ozone action over pollutants. The total parabens degradation on catalytic ozonation with the P2 (worst performance as catalytic ozonation) was achieved using a TOD of 58 mg/L, while for the same TOD single ozonation only reached 60% of initial concentration reduction. The effect of particle size and catalyst load had no relevant effect on catalytic ozonation for parabens degradation, as well as the presence of UVA irradiation, possible due to the lower amount of semiconductors available on composition. Besides it was verified that for higher values of pH the parabens degradation suffers an improvement. This can be explained by the high ozone decomposition into hydroxyl radicals at basic conditions. The presence of hydroxyl radicals as main responsible of improvement of catalytic ozonation on parabens degradation comparatively to single ozonation was proven through the use of a radical scavenger. The main role of hydroxyl radicals was also proven through by-products analysis. 4-HBA, 3,4-diHBA, 2,4-diHBA and hydroquinone production was clearly influenced by hydroxyl radical absence since significant differences were found on their profile during catalytic ozonation in the absence and presence of a radical scavenger. Our results reveal that two different volcanic rocks, largely presents on volcanic sites can be a low-cost solution for high efficiency catalytic ozonation.

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IX. Ecotoxicity variation through parabens degradation by single and catalytic ozonation using volcanic rock

It is based on the publication: Gomes, J., Frasson, D., Pereira, J. L., Gonçalves, F.J.M., Castro, L.M., Quinta-Ferreira, R.M., Martins, R.C. (2019) Ecotoxicity variation through parabens degradation by single and catalytic ozonation using volcanic rock. Chemical Engineering Journal. 360, 30-37.

IX.1. Introduction

Parabens are widely used as preservative and antimicrobial agents in food and cosmetics (Zhang et al., 2005; Melo and Queiroz, 2010), but mainly in pharmaceutical and personal care products (Huang et al., 2003; Calafat, et al.,2010). Some studies relate these compounds with the onset of human breast cancer (Darbre et al., 2004) and their estrogenic activity, although weak, was already shown (Routledge et al., 1998). In fact, parabens were already detected in human urine, milk and serum (Janjua et al., 2007; Calafat, et al.,2010; Schlumpf et al., 2010). In this context, these substances have also been quantified at the micro- and nanogram per liter range in wastewater treatment plant and surface waters worldwide (Benjts et al., 2004; Hernández-Leal et al., 2011; Haman et al., 2015; Molins-Delgado et al., 2016). As such, concern has been raised regarding the potential impact of parabens in aquatic organisms. On the one hand, the traditional municipal wastewater treatments are not totally effective on the removal of these biorefractory compounds, which makes their discharge into natural waterbodies more likely to occur, threatening aquatic ecosystems. On the other hand, drinking water treatment facilities using contaminated waters may not totally deplete these substances (Benjts et al., 2004; Molins-Delgado et al., 2016). Bearing in mind that freshwater scarcity is nowadays more present than ever, the seek for alternative water sources such as recycled water increases. Even though the uses for recycled water can be restricted, it has the potential to supply e.g. agriculture. This highlights the significance of improving treatment systems to ensure a non-toxic profile of this resource.

Awareness on the environmental hazardous potential of parabens has been leading to an increasing research attention on the aquatic ecotoxicity of these compounds. Dobbins et al. (2009) highlighted the link between the increase in lipophilicity (generally translating alkyl chain length) and the increase of toxicity to the cladoceran *Daphnia magna* (median lethal concentrations following 48-h exposure, 48 h-LC_{50} between 4 and 25 mg/L) and the fish

Pimephales promelas (48 h-LC_{50} between 3 and more than 160 mg/L). The positive relationship between alkyl chain length and the aquatic toxicity of parabens has been generally confirmed in further studies. For example, methyl, ethyl and propyl parabens present moderate toxicity while butyl and benzylparabens revealed high toxicity for the bacteria *Aliivibrio fischeri*, with 15-min luminescence inhibition concentration of 50% (IC_{50}) values ranging within 0.11 to 9.6 mg/L (Bazin et al., 2010). Yamamoto et al. (2011) verified the same trend by recording median toxicity values ranging within 0.7 and 80 mg/L following short-term exposure of freshwater microalgae, cladocerans and fish. These studies addressed the single toxicity of parabens, but it is worth stressing out that these compounds are usually used as mixtures to increase their preservative spectrum, thus analysis of the toxicity of parabens blends is necessary.

Accounting to the increasing presence of these contaminants in natural waterbodies and their hazardous potential, research has been focusing on the development of methodologies for their removal from wastewater matrices before discharge. Among those technologies, the Fenton process (Domínguez et al., 2014; Martins et al., 2016), electrochemical oxidation (Frontistis et al., 2017), single and catalytic ozonation (Tay et al., 2010; Gomes et al., 2017a), photolytic and phototocatalytic oxidation (Hansen and Andersen, 2012; Gmurek et al., 2015; Gomes et al., 2017b), photosonochemical degradation (Daghrir et al., 2014), photocatalytic ozonation (Gomes et al., 2017c) can be mentioned. These methodologies were already proven effective on the degradation of single parabens and mixtures of parabens with higher or lower energy cost. Also, ozone is a powerful oxidant often used to promote the degradation of nucleophilic and high electronic density species such as parabens (Martins and Quinta-Ferreira, 2009; Tay et al., 2010; Gomes et al., 2017a). However, single ozonation can be a source of refractory compounds due to its low mineralization efficiency (Kasprzyk-Hodern et al., 2003). Normally, heterogeneous catalysts can be used to enhance ozonation and improve the mineralization degree (Kasprzyk-Hodern et al., 2003; Martins and Quinta-Ferreira, 2009; Gomes et al., 2017a). The selection of the catalyst must be carried out by looking at the cost production, the stability and performance (Kasprzyk-Hodern et al., 2003; Martins and Quinta-Ferreira, 2009). Low-cost materials such as volcanic rock were already proven efficient for parabens degradation through catalytic ozonation (Gomes et al., 2018).

Still, most of the studies addressing the development of treatment processes targeting parabens degradation do not analyze the ecotoxicity mitigation of the water throughout the degradation process. Nevertheless, some studies show the effect of the treatment over the toxicity but only for the final treated samples and does not consider the toxicity evolution along the treatment time (see e.g. Daghrir et al., 2014; Martins et al., 2016; Gomes et al., 2017a). An appraisal on the ecotoxicity of the treated samples through the treatment time would make it possible to more accurately select the earliest point where the degradation was long enough to significantly reduce toxicity to negligible levels, thus reducing treatment length and costs. In this way, Velegraki et al., (2015) followed the effect of the decrease of methyl paraben concentration along solar photocatalytic degradation in *Artemia* nauplii.

The aim of the present study was to compare single and catalytic (using volcanic rock) ozonation as to their effectiveness in reducing the ecotoxicity of a mixture of five parabens (Methyl, Ethyl, Propyl, Butyl and Benzylparaben). While an ecotoxicity study considering a mixture of parabens is novel *per se*, it is intended to add further by assessing the levels of toxicity throughout the degradation process, providing information about the toxicity of the parent compounds and byproducts formed along the reaction. The applied ecotoxicological battery was designed to properly reflect potential impacts over aquatic ecosystems, thus included species representing different functional levels: the green microalgae *Raphidocelis subcapitata*, the macrophyte *Lemna minor*, the cress *Lepidium sativum* (plant), the cladoceran zooplankter *Daphnia magna*, the benthic bivalve *Corbicula fluminea*.

IX.2. Material and Methods

IX.2.1. Chemicals and dilutions

Solutions were prepared using ultrapure water and five parabens: Methyl (MP), Ethyl (EP), Propyl (PP), Benzyl (BeP) (obtained from Sigma-Aldrich) and ButylParaben (BuP) (purchased from Fluka). A mixture of these parabens (10 mg/L each) was treated by single and catalytic ozonation using different transferred ozone doses (see the methodology details below). Treated solutions, comprising different concentrations of parabens and by-products were chemically analysed and underwent ecotoxicity assessment (see below for details). The standards of 4-Hydroxybenzoic acid (4-HBA), 3,4-Dihydroxybenzoic acid (3,4- diHBA), 2,4-Dihydroxybenzoic acid, Hydroquinone, 1,4-Benzoquinone and 3,4- Dimethoxibenzoic acid (3,4- diMeBA) were acquired from Sigma-Aldrich. As an attempt to isolate the effects of treatment by-products, the ecotoxicity of these treated solutions was compared with that of several direct geometric dilutions from the initial mixture of parabens (10, 7.5, 5, 2.5, 1.25, 0.625 mg/L each paraben).

IX.2.2. Parabens mixture treatment - Experimental procedure

A 2-L reactor magnetically stirred at 700 rpm was used in the single and catalytic ozonation experiments, under controlled temperature conditions (25 ± 1 ºC) achieved with a thermostatic bath. The volcanic rock used as catalyst was collected in São Miguel (Azores, Portugal). A comprehensive characterization of this catalyst can be found in a previous study (Gomes et al., 2018), with main features being the presence of silica and aluminium, augite and diopside as main minerals, and a specific surface area of 28.3 m^2/g. The adsorption of parabens to the volcanic rocks surface was previously found irrelevant (Gomes et al., 2018). The amount of catalyst used for different experiments was fixed at 0.5 g/L.

Ozone was generated in 802N, BMT apparatus from a pure oxygen stream (99.9%). The gas outflow leaving the reactor was trapped by a solution of potassium iodide (Panreac) for the remaining ozone removal before discharge to the atmosphere. The inlet ($[O_3]^{in}$) and outlet ($[O_3]^{out}$) ozone concentrations were measured by ozone gas analysers (BMT 963 and 964 vent, BMT). The data collected was used for transferred ozone dose (TOD) determination according to Equation IX.1.

$$\mathrm{TOD}=\int_0^t \frac{Q_{\mathrm{Gas}}}{V_{\mathrm{Liquid}}}\times\left([O_3]^{in}-[O_3]^{out}\right)\times dt \qquad \text{(Equation IX.1)}$$

Where Q_{Gas} represents the gas flow rate (0.2 L/min), V_{Liquid} is the volume of the effluent used in the reactor (2 L). The TOD was expressed in mg O_3/L.

IX.2.3. Analytical techniques

The concentrations of the five parabens and degradation intermediates (4-HBA, 2,4 and 3,4-di HBA, 3,4-diMeBA, hydroquinone and 1,4-benzoquinone) were analytically determined for different untreated and treated solutions by high-performance liquid chromatography equipped with a diode array detector, HPLC-DAD (UFLC, Shimadzu). The standards of each one were firstly analyzed and a calibration curve was obtained. The mobile phase (0.5 mL/min) consisted in a mixture of 50:50 methanol: acidic water (0.1% orthophosphoric acid). A C18 column from SiliaChrom, at 40 ºC was used and compounds were detected at 255 nm.

Chemical oxygen demand (COD) was determined according to the standard method 5220D (Greenberg et al., 1985). Potassium hydrogen phthalate, obtained from Panreac, was used to prepare a calibration curve with COD values within the range 0-100 mg O_2/L. Absorbance values after 2 hours of digestion at 150 ºC (ECO25 – VelpScientifica) were measured at 445 nm in a WTW photolab S6 photometer. A TOC analyser (TOC-V CPN model, Shimadzu, Japan) coupled to an autosampler (model V-ASI, Shimadzu, Japan) was used for determining total organic carbon (TOC) using nondispersive infrared analysis after oxidative combustion.

After catalytic ozonation, the leaching of metals from the volcanic rocks due to the acidic pH was analysed. pH was determined using a Crison micropH 2002 apparatus. The solution composition in Al, Fe, Cu, Zn, K, Na, Ca and Mg was measured through atomic absorption in a PerkinElmer 3300 spectrometer.

IX.2.4. Ecotoxicity analysis

An ecotoxicological test battery was applied to 16 samples produced as detailed above: 6 samples corresponded to geometric dilutions of the bulk parabens mixture; 5 samples corresponded to sequential stages of the single ozonation treatment; 5 samples corresponded to sequential stages of the catalyzed ozonation treatment. The samples treated with single ozonation were obtained for TOD values of 6, 18, 78, 123, 170 mg/L. On the other hand, the samples used on ecotoxicity tests from catalytic ozonation were obtained for TOD values of 3, 9, 29, 42, 55 mg/L. These TOD values correspond respectively to 5, 15, 60, 90, 120 minutes of reaction time. The pH of all samples was adjusted to values within the range 6.5 - 7.5 to mitigate the interference of different pH levels in the tests outcome. This pH range was achieved by adding sodium hydroxide or sulfuric acid solutions to the samples. The initial pH of treated and diluted samples ranged from 3.5 to 5.2. The range was 3.5-5.2, where 5.2 was for the initial parabens mixture and 3.5 was obtained for highest TOD value of single and catalytic ozonation.

To evaluate the phytotoxicity of the initial mixture of parabens with different dilutions and treated samples for each TOD value, the number of germinated seeds and the radicle growth of *L. sativum* were determined to calculate the germination index (GI) (Equation IX.2). The phytotoxicity was classified according to the germination index using Trautmann and Krasny (1997) criteria. In this test, 10 seeds of *L. sativum* were evenly spread on a filter paper in a petri dish. The volume of sample used to spike the filter paper was 5 mL. The experiments were run in duplicate. The control was set up with ultrapure water. Test treatments were incubated in an oven at constant temperature of 27 ^{o}C for 48 h. Then the number of seeds germinated and the radicle growth in each replicate was recorded. Germination index (GI) is the product of relative seed germination (RSG) by relative radicle growth (RRG) as in equations IX. 2-4.

$$GI\ (\%) = \frac{RSG\ (\%) \times RRG\ (\%)}{100} \qquad \text{(Equation IX.2)}$$

$$RSG\ (\%) = \frac{N_{SG,T}}{N_{SG,B}} \times 100 \qquad \text{(Equation IX.3)}$$

$$RRG\ (\%) = \frac{L_{R,T}}{L_{R,B}} \times 100 \qquad \text{(Equation IX.4)}$$

Where $N_{SG,T}$ and $N_{SG,B}$, were the arithmetic means of the number of seeds germinated for each sample and the control, respectively; $L_{R,T}$ and $L_{R,B}$ correspond to the average radicle length for each sample and the control, respectively.

The inhibition in the growth of the green microalgae *R. subcapitata* as induced by the prepared samples was assessed following guidelines by OECD (2011) adapted to the use of 24-well microplates (Geis et al., 2000). The microalgae have been cyclically maintained in the laboratory as a non-axenic bulk culture in Woods Hole MBL medium (MBL; Stein, 1979), this culture appropriately sourcing the toxicity test through a previously isolated intermediate inoculum. The microalgae (initial cell density of 10^4 cells/mL) were exposed to each (nearly) full-strength sample. To prevent the interference of nutrient scarcity in the growth of the microalgae during the test, the samples were spiked with nutrients so that they reach the same nutrient levels as supplied in MBL. Accounting to both the volume of the nutrient spike and the volume used for microalgae inoculation in each microplate well, samples were not actually tested full-strength but rather slightly diluted (all established at 98.2% strength). Each sample was tested in triplicate, along with the blank control. The microplates were incubated for 72 h under continuous light at 23 ± 1 °C. The algal suspension in each well was thoroughly mixed by repetitive pipetting twice a day. At the end of the test, cell density was quantified spectrophotometrically at 440 nm based on a previously established calibration curve. The biomass yield (cells/mL) was calculated as the difference between cell density at the end and the beginning of the test. The results were made relative to the control yield (% inhibition in yield).

All samples were tested for their ability to impair the growth of the macrophyte *L. minor*, following OECD (2006) guidelines adapted to the use of 6-well plates (Kaza et al., 2007). A laboratory culture of the macrophyte has been cyclically maintained (renewal once a week) in Steinberg culture medium (OECD, 2006). Growth inhibition tests were performed under continuous light at 23 ± 1 °C in triplicate, and six plain-Steinberg control replicates were run in parallel. Each well used as a test replicate contained 10 mL of test solution plus 3 macrophyte colonies of 3 fronds each. To prevent the interference of nutrient scarcity through the test, the samples were spiked with nutrients so that they reach the same nutrient levels as supplied in Steinberg, this meaning that the samples were not actually tested full-strength but rather slightly diluted (93.5% strength). At the beginning of each test, 6 biomass replicates (3 colonies of 3 fronds) were oven-dried for 24 h at 60 °C to record initial dry weight. At the end of the test, the fronds present in each well were collected and oven-dried (at least 24 h at 60 °C) for dry weight records. Yield based on dry weight records was calculated. The results were made relative to the control yield (% inhibition in yield).

Acute toxicity tests with *D. magna* were conducted following the OECD guideline 202 (2004) to address the ability of the samples to promote organism's immobilization. Monoclonal cultures of *D. magna* have been reared in the laboratory in synthetic ASTM hardwater medium (ASTM, 1980) supplied with an organic additive extracted from the algae *Ascophyllum nodosum*. Cultures were renewed three times per week, and the organisms were fed after renewal with 3.0×10^5 cells/mL *R. subcapitata*. Cultures and then the tests were kept under a constant $16h^L$:$8h^D$ photoperiod and temperature of 20 ± 2 °C. The tests were carried out using neonates from the 3rd to the 5th broods, ageing less than 24 h, in test tubes containing 10 mL test sample or blank ASTM control. Nutrient spiking was also ensured at the levels set for ASTM in cultures, meaning that samples were tested slightly diluted (92% strength). Twenty animals were used per treatment, randomly assigned into 4 replicates with 5 animals each, and the test lasted for 48 h. The number of immobilized animals was recorded at the end of the exposure period.

The mortality of *C. fluminea* was analysed with acute toxicity tests. *C. fluminea* was collected in Mira, Portugal (N40°25'06.90''/W8°44'13.18''), where a well-established population (density above 2000 clams /m^2) stands (Rosa et al., 2014). Clams were collected from the canal by sieving sediment into a 5-mm mesh bag. Individuals with shell length in the range 20-30 mm were selected and immediately moved in field water to the laboratory, where they were gradually acclimated to the laboratory conditions changing the water once a week along three weeks before testing. The toxicity tests were run in vessels containing 10 clams with 500 mL of samples or dechlorinated tap water. Two replicates were prepared for each sample along with a blank control. The vessels were kept at constant temperature (20 ± 2 °C), under a 16 h^L:8 h^D photoperiod cycle and continuous aeration. The mortality of clams was assessed every 24 h during 72 h (the maximum period of test) based on the animal's siphoning activity and their resistance to valve opening when forced with a blunt dissection needle (Gomes et al., 2014).

IX.3. Results and Discussion

IX.3.1. Single vs catalytic ozonation regarding organic matter removal

Parabens degradation along single and catalytic ozonation was studied in a previous work (Gomes et al., 2018) and is now briefly described. Total parabens degradation was achieved in both treatments for the highest TOD value (55 and 170 mg/L for catalytic and single ozonation, respectively). From this mixture, benzylparaben is the fastest removed compound due to the presence of two benzenic rings which allows the removal using a TOD value of 29 mg/L in catalytic ozonation and 123 mg/L in single ozonation. For the remaining parabens the behavior was almost the same with small differences for butylparaben. As an example, is described the degradation of methylparaben. In the catalytic ozonation process, from TOD values of 3 to 9 mg/L, MP concentration reduced from 9 to 7 mg/L, whereas the most significant removal was found from TOD value 29 mg/L where the MP concentration reached about 3.5 mg/L. With a TOD value of 42 mg/L the MP concentration was about 0.625 mg/L. In catalytic ozonation, for TOD values of 9, 29, 42 and 55 mg/L the by-products 1,4-benzoquinone and hydroquinone were found with concentrations of 80, 160, 187 and 194 μg/L (hydroquinone) and 4, 28, 38 and 71 μg/L (1,4-benzoquinone). In the single ozonation process for TOD values from 6 to 18 mg/L, the MP concentration reduced from 9 to 7.5 mg/L. When TOD reached 78 mg/L, MP concentration was about 4 mg/L. With a TOD value of 123 mg/L the MP concentration reached about 1.25 mg/L. In what concerns the by-products hydroquinone and 1,4-benzoquinone for the TOD values 18, 78, 123 and 170 mg/L the concentrations were 38, 100, 31 and ~0 μg/L to hydroquinone and for 15, 22, 70 and ~0 μg/L to 1,4-benzoquinone.

Normally, regardless the advanced oxidation process used for contaminants degradation, some refractory compounds can be produced during the reaction. The production of refractory compounds does not allow total mineralization, which means that COD and TOC can be still significantly high after treatment (Kasprzyk-Hodern et al., 2003; Martins and Quinta-Ferreira, 2009). In this way, COD and TOC decrease was verified for different TOD values resulting from ozonation and catalytic ozonation processes. For the initial mixture of five parabens at 10 mg/L each, the initial COD and TOC values were of about 90 mg O_2/L and 35 mg C/L, respectively. As expected, a consistent decrease in COD and TOC values was observed as the dilution of initial parabens mixture increased.

For the highest simple dilution of parabens mixture (0.625 mg/L of each paraben), TOC and COD were not measured since the quantification of such a low quantity of carbons and chemical oxygen demand is difficult with the methodologies used. Moreover, the TOC and COD range obtained with this baseline analysis covered the ranges obtained at the degree of mineralization achieved with catalytic and single ozonation processes, thus feasibly supporting comparative analysis. Parabens were totally removed at the highest value of TOD (170 mg/L) with single ozonation (Gomes et al., 2017a), but COD and TOC values were still high (about 66 mg O_2/L and 28 mg C/L, respectively) after treatment, as shown in Figure IX.1. Catalytic ozonation leads to total removal of parabens using a TOD of 55 mg/L (Gomes et al., 2018). For a TOD value of 55 mg/L, TOC was slightly lower (about 26 mg C/L) than the one reached by single ozonation. An important feature when comparing single and catalytic ozonation is the significant reduction of TOD of about 3 folds required for degradation in the presence of volcanic rock.

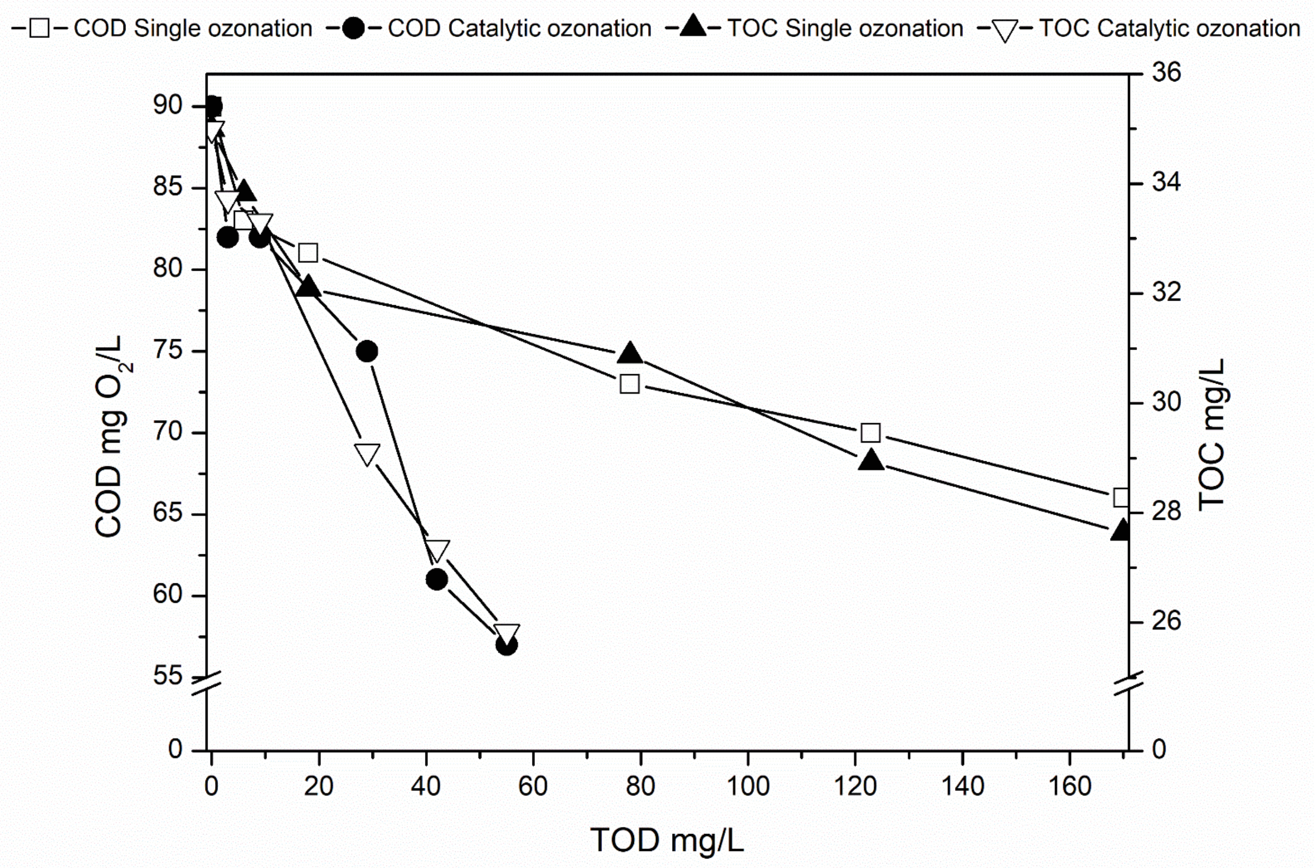

Figure IX.1. COD and TOC reduction through treatment of the initial mixture of parabens with catalytic and single ozonation.

The need of higher TOD values on the single ozonation can denote the production of more refractory compounds since more ozone is being required for further degradation. However, these compounds can be further oxidized using catalytic ozonation (Martins and Quinta-

Ferreira, 2009). In the presence of volcanic rocks, the main responsible for parabens degradation are hydroxyl radicals instead of molecular ozone, which would allow a higher COD removal due to the non-selective process degradation (Gomes et al., 2018). According to the COD and TOC values obtained while analyzing several simple dilutions of the initial parabens mixture, the final COD and TOC obtained for single and catalytic ozonation would correspond to a solution containing 7 mg/L of each paraben. However, HPLC results (Gomes et al., 2018) show that total parabens removal was achieved with both treatment processes. Provided that full parabens removal can be assumed for both ozonation treatment processes applied to the samples, the proper conditions were ensured to address the role of treatments byproducts in defining further the characteristics of the treated samples, namely their toxicity.

Table IX.1 presents the metal concentrations in water after catalytic ozonation for the highest TOD value (55 mg/L). It should be highlighted through Table IX.1 that the volcanic rock is stable regarding active metals leaching to the liquid bulk provided the low metals concentration found in the treated water. However, some toxic effect resulting from these ions cannot be discarded as it will be discussed below.

Table IX.1. Amount leached of selected (based on previous characterization of the volcanic rock used as catalyst Gomes et al., 2018) metals for the highest TOD value in catalytic ozonation.

Elements	**Al**	**Fe**	**Na**	**Ca**	**Mg**
Amount leached (mg/L)	0.13	0.21	1.12	3.18	0.26

IX.3.2. Ecotoxicity evaluation of parabens mixture through simple dilution series

Several studies have been carried out regarding the potential ecotoxicity of parabens (e.g. Terasaki et al., 2009; Yamamoto et al., 2011; Molins-Delgado et al., 2016), and these personal care products have been given attention regarding the need for a structured environmental risk assessment to properly conclude on their hazardous potential to the aquatic biota (e.g. García et al., 2014). However, these studies addressed only a low number of indicator species or consider single exposures of parabens while their mixture (normally used to increase paraben’s preservative spectrum) was not yet considered as far as we known as a variable to assess treatment efficacy or efficiency.

Table IX.2 shows the ecotoxic outcome of simple sequential dilutions of the initial mixture of five parabens. Regarding the animal species, *C. fluminea* was found more tolerant than *D.*

magna, as denoted by the high immobilization rates by the latter observed at lower concentrations (Table IX.2), and confirmed by estimated EC_{50} values via probit analysis – immobilization EC_{50} of 15.6 (95% confidence interval: 14.6-16.7) mg/L compared to a significantly higher LC_{50} of 25.2 (confidence interval: 23.7-26.8) mg/L. The producers tested in the present study were less sensitive to the mixture of parabens than the animals. This is in general agreement with the literature. For example, Yamamoto et al. (2011) found the same trend as they compared *D. magna* and *Pseudokirchneriella subcapitata* (former name for *R. subcapitata*) regarding their sensitivity to single exposures of the five parabens also used here, except for BeP, which was found more toxic to *D. magna*. According to the Trautmann and Krasny (1997) criteria, concentrations of parabens mixture of and lower than 2.5 mg/L each paraben were non-phytotoxic, while for solutions above of 7.5 mg/L each paraben, the *L. sativum* GI values reveal that the tested samples can trigger phytotoxic effects. These results show that *L. sativum* is the most tolerant to parabens mixture of the species tested. Similarly, to *L. sativum, L. minor* at the maximum concentration of parabens mixture tested achieved around 50% of yield inhibition on a dry weight basis. Still, according to our results (Table IX.2) low amounts of parabens (0.625 mg/L of each one, meaning 3.125 mg/L in total) do not show short-term toxicity to any of the five-species tested.

Table IX.2. Mean values (% ± Standard Deviation) obtained for selected parameters following exposure of *D. magna, C. fluminea, L. sativum, R. subcapitata* and *L. minor* to sequential simple dilutions of a parabens mixture (10 mg/L each paraben; total 50 mg/L parabens in solution)

Parabens concentration (mg/L)		***D. magna***	***C. fluminea***	***R. subcapitata***	***L. sativum***	***L. minor***
Total	Each	Immobilization (%)	Mortality (%)	Yield inhibition (%)	GI (%)	Yield inhibition (%)*
50.00	10.0	100 (± 0)	100 (± 0)	84 (± 3)	49 (± 12)	52 (± 4)
37.50	7.50	100 (± 0)	90 (± 10)	78 (± 5)	56 (± 12)	32 (± 2)
25.00	5.00	95 (± 10)	47 (± 15)	83 (± 2)	68 (± 4)	30 (± 5)
12.50	2.50	35 (± 30)	7 (± 11)	65 (± 1)	98 (± 17)	0 (± 8)
6.250	1.25	0 (± 0)	7 (± 6)	3 (± 4)	90 (± 0)	-19 (± 2)
3.125	0.62	0 (± 0)	0 (± 0)	13 (± 2)	100 (± 2)	-50 (± 18)

* based on dry weight.

IX.3.3. Comparison between single and catalytic ozonation on the toxicity evolution

Only few studies can be found about the effect of the degradation treatment applied to remove these contaminants from water over toxicity, relating it with the resulting refractory compounds. This can be a relevant factor for the choice of the most appropriated treatment when looking for water reclamation (Velegraki et al., 2015) and adds significant relevance to this study. Here, to promote the degradation of parabens mixture, single and catalytic ozonation were used. These two processes present different reaction mechanisms. In the first one, the main responsible for degradation is the molecular ozone since the reaction was promote at low pH (~3.5). At acidic conditions it is expected a low production of hydroxyl radical due to the low self-ozone decomposition rate at this pH values (Kasprzyk-Hodern et al., 2003). On the other hand, at the presence of a catalyst several mechanisms may explain the increase on the process efficiency and one of them is the decomposition of ozone into hydroxyl radical (Kasprzyk-Hodern et al., 2003). This was the main pathway responsible by parabens mixture degradation in catalytic ozonation using volcanic rock as concluded in a previous work (Gomes et al., 2018). This allows total parabens mixture degradation using low amount of ozone. Toxicity effect along the treatment processes was analyzed as function of TOD, since with ozone processes, ozone generation is an important point in terms of the process energetic costs. Moreover, plotting the results against TOD allows to compare the processes efficiency regarding ozone consumption (Domenjoud et al., 2011).

At the end of reaction, as previously described, TOC removal was higher for catalytic ozonation and total parabens degradation was achieved. Nevertheless, it cannot be generalized that this means also a less toxic solution for all species. Different species present different sensitivities to the by-products formed so toxicity needs to be analyzed considering a battery comprising diverse species, ideally representing different functional levels in aquatic ecosystems.

D. magna reveals the most sensitive response against the parabens mixture and resulting by-products from both treatments, i.e. single and catalytic ozonation (Table IX.3). Until the final experimental stage (i.e. as the reaction was extended for 5, 15, 60 and 90 min of in treatment processes), 100% immobilization was achieved in both single and catalytic ozonation. This adverse response is certainly related to the by-products formed. For example, for a TOD value of 123 mg/L through single ozonation, the parabens mixture concentration was about 1.25 mg/L each paraben, except for BeP, which is totally removed (Gomes et al., 2018). According to Table IX.2 referring to the tests using simple dilutions of initial parabens mixture, no *D. magna* immobilization was found for this parabens concentration. Thus, this clearly illustrates the noxious potential of the by-products formed to the cladocerans.

We quantified 4-HBA, 2,4 and 3,4- diHBA, 3,4-diMeBA, Hydroquinone and 1,4-Benzoquinone as by-products of single and catalytic ozonation in the present study, and their concentrations were followed for different TOD's values. Kamaya et al., (2005) verified for a wide group of benzoic acids derivates that the EC_{50} of parabens mixtures is lower comparing to the benzoic acids. This means that the parabens mixture was more toxic than the benzoic acids resulting from their degradation. For TOD values of 123 mg/L in single ozonation, small concentrations of 2,4- diHBA (3 µg/L), Hydroquinone (31 µg/L) and 1,4- Benzoquinone (70 µg/L) were detected, but other non-identified by-products should be present and can promote toxic effects observed in *D. magna* (Tay et al., 2010). The toxicity of both these by-products to *D. magna* was already reported in the literature. García et al. (2014) reported an EC_{50} value of 450 µg/L for 1,4-benzoquinone, and Guerra (2001) reported an EC_{50} value of 150 µg/L for hydroquinone. In the present study, it is notorious that the low 1,4- benzoquinone and hydroquinone concentrations at the reference TOD of 123 mg/L cannot explain the highly toxic effect observed (100% *D. magna* immobilization). Nevertheless, their interaction as well as the presence of other unknown by-products, as well as residues of the parabens mixture are likely to explain the observed toxicity. On the other hand, for catalytic ozonation, 85% of immobilization was achieved for the highest TOD's (55 mg/L) (Table IX.3). At this point, the parabens mixture was totally removed but 1,4-benzoquinone and hydroquinone concentrations were high (71 and 194 µg/L, respectively). These concentrations were like those achieved for single ozonation with a TOD value of 123 mg/L, where total *D. magna* immobilization was rather observed. Once again, the residual presence of parabens and other non-identified by-products in treated solutions, as well as their interaction, concur to explain the discrepancy. For the highest TOD in single ozonation (170 mg/L), no *D. magna* immobilization was detected (Table IX.3). We hypothesize that the huge amount of ozone applied allowed the degradation of all quantified by-products, which reinforces the idea that 1,4- benzoquinone and hydroquinone may have great toxicity to the cladocerans. On the other hand, for catalytic ozonation, Al and Fe leached from the catalyst (Table IX.1) can also affect the immobilization of cladocerans, somehow biasing further considerations.

Table IX.3. Mean *D. magna* immobilization (% ± Standard Deviation) observed following single and catalytic ozonation over a parabens mixture, as a function of reaction time (min) and corresponding TOD values (mg/L)

	Single ozonation		Catalytic ozonation	
Reaction time	TOD	*D. magna* immobilization	TOD	*D. magna* immobilization
5	6	100 (± 0)	3	100 (± 0)
15	18	100 (± 0)	9	100 (± 0)
60	78	100 (± 0)	29	100 (± 0)
90	123	100 (± 0)	42	100 (± 0)
120	170	0 (± 0)	55	85 (± 7.5)

To compare ozonation processes over the remaining species regarding to toxicity, data were analysed as function of TOD values (Figure IX.2). The most extensively treated sample of each process (highest TOD value) did not exert toxicity except for microalgae. In microalgae, the highest TOD value of each process induced 36 and 42% yield inhibition compared to the control, for single and catalytic ozonation, respectively, suggesting some susceptibility to by-products. According to Figure IX.2a, within TOD values of 10-50 mg/L, microalgae yield was higher for samples treated with catalytic ozonation. The explanation to this fact can be related with the concentration of hydroquinone and 1,4- benzoquinone. For 29 and 42 mg/L of TOD in catalytic ozonation, hydroquinone was found at 160 and 187 µg/L and 1,4- benzoquinone was quantified at 28-38 µg/L, respectively. The 72h-EC_{50} value of hydroquinone for *Selenastrum capricornutum* (former name of *R. subcapitata*) was reported as 335 µg/L (Devillers et al., 1990), hence not so far from hydroquinone concentration quantified here. This supports the role of hydroquinone in contributing to the observed toxic effect over microalgae. Regarding TOD values of 9 and 29 mg/L in catalytic ozonation, the parabens mixture concentration was reduced to about half compared to the starting point, while hydroquinone concentration increased about two times, and other detected by-products were found at higher concentrations (e.g. 4-HBA and 3,4 – diHBA can be found at a concentration of 247 and 500 µg/L, respectively for a TOD value of 29 mg/L). Altogether, by-products can explain the increased toxicity of samples provided by catalytic ozonation compared to the single ozonation equivalents in microalgae. Still, the role of 4-HBA in the toxicity of the samples must be carefully considered since, although this is the main by-product resulting from parabens oxidation (Tay et al., 2010; Gmurek et al., 2015; Gomes et al., 2017c), it shows a very low toxicity over *P. subcapitata* (72 h-EC50 value of 1367 mg/L; (Kamaya et al., 2006)) when dosed alone. At the defined end of the treatments (170 mg/L and 55 mg/L TOD for single and catalytic ozonation, respectively), toxicity to microalgae remains remarkable (above 35% in both cases) although no parabens were quantified. Catalytic ozonation produces appreciable amount of hydroquinone and 1,4-benzoquinone as discussed earlier, but this is not the case of single ozonation. In this case, other by-products such as low-length carboxylic acids, aldehydes and alcohols may be potentially inducing the noticed toxic effects; their presence is confirmed by TOC values, which remained high.

C. fluminea was tolerant to the treated samples from both treatments. The most significant reduction was observed as TOD increased from 29 to 42 mg/L, corresponding to a 6-times reduction in concentration of MP, EP, PP, BuP and about 27 times for BeP (the most toxic paraben). Although these TOD values also translate in the increase of hydroquinone concentrations, *C. fluminea* was largely insensitive to these and other by-products, meaning that the clams can be affected by parabens mixture rather than by the degradation intermediates of ozonation treatments. This renders the picture on the toxicity of samples undergoing ozonation treatment (Figure IX.2b) essentially similar to that elicited by simple dilutions of the parabens mixture (Table IX.2).

L. minor and *L. sativum* were the most tolerant species to parabens, as indicated by the relatively low inhibition noticed in growth parameters for the raw mixture before treatment (52% yield inhibition for *L. minor* and a GI of 49% for *L. sativum*; Figure IX.2c and IX.2d). Catalytic ozonation reduces more quickly yield inhibition of *L. minor* than single ozonation. However, at 29 mg/L TOD, *L. minor* yield unexpectedly (as parabens concentration decreases) decreases compared to 9 mg/L TOD, standing above the expected inhibition records of 30% as denoted in Table IX.2). This is likely to reflect the toxic potential of hydroquinone and 1,4-benzoquinone (which increase at this point in the treatment reaction), that can react with the molecular constituents of proteins, DNA or lipids. This will affect the cellular metabolism, including enzyme inhibition through covalent binding and oxidative stress (Pandey et al., 2005). The Fe leached could also promote yield inhibition, but this possibility seems unlikely since at 9 and 29 mg/L TOD, Fe leached was similar (about 69 µg/L) while the responses were distinct. The most extensively treated samples by catalytic and single ozonation are non-phytotoxic over *L. sativum* according to Trautman and Krasny (1997) criteria. Still, the toxicological outcome was distinct from that provided by the test with simple dilutions of initial parabens mixture. For example, at 42 mg/L TOD in catalytic ozonation, parabens concentration is around 0.625 mg/L of each paraben, which would translate in 100% of GI (Table IX.2); it rather translated into a 80% GI (Figure IX.2a), the difference being possibly due to the presence of hydroquinone and 1,4- benzoquinone. The putative effect of by-products seems even more evident in samples deriving from single ozonation. Referring to single ozonation using a low TOD value (6 mg/L), decrease on GI until 26% was observed compared to initial parabens mixture, changing the classification of the sample to very phytotoxic. This can be related with the production of hydroquinone (6 µg/L) and 1,4- benzoquinone (8 µg/L) as by-products Also, for catalytic ozonation a slight decrease on GI was verified for low TOD values. Meanwhile at those conditions, the concentrations of hydroquinone and 1,4- benzoquinone were 12 µg/L and 4 µg/L, respectively. Hereupon, as hydroquinone concentration was higher in this case when compared to single ozonation while 1,4- benzoquinone was lower, it can be considered that 1,4-benzoquinone can present a more negative impact on GI of *L. sativum.* This fact can be confirmed by the behavior of GI for single ozonation within 78 and 123 mg/L of TOD values, where this parameter was equal for both TOD's values. For a TOD of 78 mg/L the concentration of parabens was 4 mg/L (MP, EP, PP) 3 mg/L (BuP, BeP), whereas the concentration was about 1.25 mg/L (MP, EP, PP, BuP) while BeP was already not detected using a TOD of 123 mg/L.

Given this decrease in parabens concentration, a consequent increase on GI would be expected. However, the concentration of 1,4- benzoquinone increased almost three times for this range of TOD values, and should have compensated the decrease on parabens mixture concentration. Nevertheless, in this range of TOD values the concentration of hydroquinone also decreases three times, which turns the 1,4- benzoquinone on the main responsible for the toxicity over *L. sativum.* Another support for this theory can be found on the last point of both treatments. Here, no parabens could be found but 1,4- benzoquinone can be detected for catalytic ozonation, so it could explain the higher GI for single ozonation. Still, the possible toxic effect of non-identified by-products cannot be ruled out.

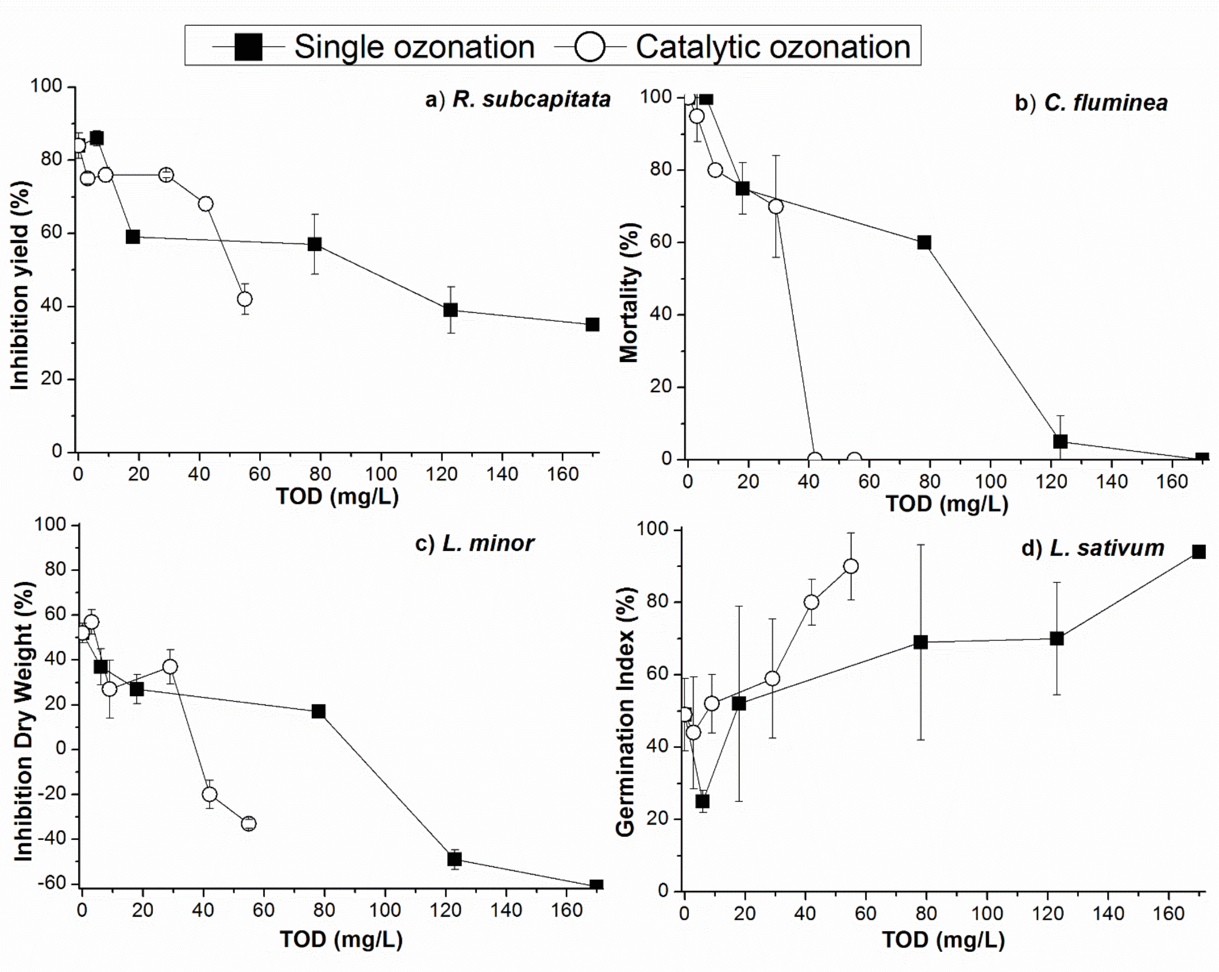

Figure IX.2. Average a) R. subcapitata inhibition yield; b) C. fluminea mortality; c) L. minor inhibition dry weight; d) L. sativum germination index, as function of TOD for single and catalytic ozonation. Error bars represent the standard error and the lines were drawn to facilitate visualization and do not intend to represent any adjusted model.

IX.4. Conclusions

Toxicity assessment should be a key variable to analyze the efficiency of a water treatment process. Moreover, this evaluation should consider the toxic effect over several species rather than use a single indicator deemed adequate, since the toxicity of treated samples may be dramatically different depending on the species tested. The results achieved show that both parabens and degradation by-products through single ozonation and catalytic ozonation treatment produce different effects over the range of indicator species tested. It is noticeable that both treatment processes allowed full degradation of the five parabens mixed to synthesize the test effluent. However, it is also remarkable that toxic intermediates were produced. According to the results obtained and the data from literature hydroquinone and 1,4-benzoquinone may be the most hazardous ones. In fact, fully treated samples following the highest TOD value for both processes (after total parabens removal) present higher toxicity over *D. magna* (just for catalytic ozonation), *R. subcapitata, L. sativum* than a solution containing a parabens mixture with a concentration of 625 µg/L per paraben. This means that the by-products generated through treatment, even at very low concentrations, should be more harmful for the tested species than the parabens mixture. Meanwhile, no toxicity was verified for *C. fluminea* and *L. minor* following exposure to the fully treated samples for both oxidation processes, elucidating on the differential outcomes that can be expected depending on the selected indicator species and thus evidencing the need to consider a wide ecotoxicological test battery while assessing water treatment efficiency.

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X. Biofiltration using *C. fluminea* for *E. coli* removal from water: Comparison with ozonation and photocatalytic oxidation

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X.1. Introduction

Corbicula fluminea is one of the most widely spread bivalves around the world (Araujo et al. 1993). Native from Southeast Asia, this freshwater clam can move between ecosystems due to its high dispersion capacity. *C. fluminea* is able to install in the new ecosystem and compete with the native species for food and space, reducing the available amount of phytoplankton (Lucas et al. 2002; Lopez et al. 2006). Additionally, this species can resist and survive under adverse conditions due to its high physiological tolerance (Morton 1982). Besides the ecologic impacts, this invasive clam can cause industrial problems with economic consequences for water dependent facilities (Karatayev et al. 2003; Pimentel et al. 2005; Rosa et al. 2011). The impacts caused by this species make it necessary to find pest management strategies. Most of the methodologies applied involve the application of chemical products able to kill them. However, this procedure may be a threat for other species as well. Thus, due to the characteristics of *C. fluminea*, an interesting management strategy that can be applied to reduce the economic impacts may be the use of this invasive species as biofilter for wastewater treatment. Such strategy could be integrated with pest management approaches involving physical removal of the clams. However, care must be taken while assembling the biofilter to avoid the spreading of the alien species.

The Asian clam has a high filtration capacity, with filtration rates up to 33 mL/g.h (Cohen et al. 1984). This species can bioaccumulate heavy metals in the tissues and shells and has proven to be an effective bioindicator for these contaminants (Andrès et al. 1999; Marie et al. 2006; Rosa et al. 2015). This bivalve can also bioprocess organic matter, and thus can be useful to remove organic pollutants difficult to treat by the conventional wastewater processes (Ferreira et al. 2018). An integrated solution based on Fenton's process and biofiltration with Asian clam was tested to treat winery wastewater treatment. Ferreira et al. (2018) shown that *C. fluminea* biofiltration host is a promising avenue for winery wastewater treatment coupled with Fenton's process (Ferreira et al. 2018). This filter feeder species promises to be a suitable solution for the wastewater treatment.

Conventional municipal wastewater treatments show some difficulty to remove some pathogens and enteric organisms from the effluents (Koivunen et al. 2003). This can be due to some microorganism's resistance and/or the low disinfection efficiency of these processes. The presence of such pathogens can represent a problem for the human health since they can reach natural water courses in large amounts. One of the most common enteric microorganisms is *Escherichia coli*. This species was used as an indicator of the microbial quality of water and wastewaters, and its presence was a sign of fecal contamination (Wu et al. 2011; Bonetta et al. 2017; Li et al. 2017a). Several methods have been tested with the aim of improving disinfection of *E. coli* from water or wastewaters (Koivunen et al. 2005; Hijnen et al. 2006; Gianakis et al. 2015; Tondera et al. 2015). In those works, the main mechanism to remove bacteria was based on physic-chemical processes, such as photoxidation and ozonation. Moreover, chlorination is one of the most common methods used for water disinfection and recent studies have found that the dosing mode of chlorine (e.g., two-step chlorination or three-step chlorination versus one-step chlorination) could also improve the *E. coli* removal (Li et al. 2017a,b). These processes were shown to be effective but tend to have high costs associated. The use of Asian clam to remove *E. coli* from wastewater allows a solution potentially at lower cost. Moreover, this would contribute to pest management with an environmental application being given to this species.

The capacity of Asian clam to bioprocess or bioaccumulate bacteria has been widely studied (Silverman et al. 1995; Graczyk et al. 2003; Ismail et al. 2016), but fewer studies exist regarding the removal of *E. coli* by *C. fluminea*. Ismail et al. (2016) analyzed the ability of Asian clam and native freshwater mussel *Anodonta californienses* to remove *E. coli* from natural water. *C. fluminea* also proven to be effective on the infectivity reduction of avian influenza virus as well as on the removal of that pathogen from water (Faust et al. 2009). *C. fluminea* and *Dreissena polymorpha* (zebra mussel) have also been presented as potential bioindicators of contamination for two enteric parasites, *Cryptosporidium parvum* and *Giardia lamblia* (Graczyk et al. 2003). In addition to *Cryptosporidium parvum* and *Giardia lamblia,* zebra mussel could also be used for biomonitoring the presence of *Toxoplasma gondii* in watercourses (Ladeiro et al. 2014). Zebra mussel (*D. polymorpha*) has been used on the removal of *E. coli* from municipal wastewater (Mezzanotte et al. 2016). The study of Mezzanotte et al. (2016), at laboratory scale, proved the effectiveness of bivalves (zebra mussel) on *E. coli* removal.

In the literature a few studies have been made using *C. fluminea* on the disinfection of contaminated water spiked with *E. coli*. However, our study further aimed to analyze the rate of bacteria removal as a function of the number of clams used, as well as the mechanism for *E. coli* removal from wastewater. Moreover, the results obtained by this methodology will be compared with ozonation and photocatalytic oxidation. These two advanced oxidation processes were selected for comparative purposes since literature points them out as alternatives for disinfection. Moreover, in previous works their action on the removal of emerging chemical contaminants was optimized (Gomes et al. 2017a,b,c). In fact, ozone shows strong oxidizing capacity as well as antimicrobial characteristics (Wani et al. 2015). This way, ozone is able to inactivate microorganisms including both gram-positive and gram-negative bacteria, bacterial spores, fungi, fungal spores, viruses, and protozoa, among others. Moreover, since the ozone that remains in solution tends to decompose into oxygen, this process is clean of toxic residues which makes it environmentally friendly (Achen and Yousef 2001). Photocatalytic oxidation is also an interesting technology, based on the generation of electron-hole pairs on the surface of semi-conductors through the absorption of radiation. These pairs are able to produce strong oxidant species or even to the oxidation of the pollutants. The most common semiconductor is TiO_2; however, its band gap energy generally requires the use of the UV radiation for the electron-hole pairs production occurs. Its surface doping with noble metals (such as Au, Pd and Ag) reduces the band gap, and thus the energy required for activation is lower, also decreasing the electron-hole recombination. The main drawback of these processes is, usually, the costs associated with the technology. Thus, this paper aims to compare the efficiency and costs of ozonation and photocatalytic oxidation with a low-cost biofiltration system using *C. fluminea* as host. The existing studies with Asian clam only focus on the comparison between bivalves *E. coli* filtration rate. To the best of our knowledge, comparison of biofiltration using invasive species and chemical disinfection technologies is not available in the literature.

X.2. Materials and methods

X.2.1. *Corbicula fluminea* sampling

Asian clams were collected at the beginning of 2017 from a canal in Mira, Portugal (N40°25'06.90’’/W8°44'13.18’’), where a well-established population (density above 2000 clams/m^2) can be found. *C. fluminea* individuals were removed by sieving sediment in a 5-mm mesh bag. The clams with shell length between 20-30 mm were selected to be brought to the laboratory in field water. During a week, clams were gradually acclimated at constant temperature of 20 ± 2 °C under a photoperiod cycle (16 h light: 8 h dark) and continuous aeration. At the end of this acclimation week the clam water is completely dechlorinated tap water.

X.2.2. Photocatalysts preparation and characterization

In order to infer about the best catalyst for photocatalytic oxidation, a screening of noble metals (Au, Pd, and Ag with a wt./wt. of 0.5%) supported in TiO_2 catalysts was performed. Their efficiency was compared with pure TiO_2. The catalysts preparation was described elsewhere (Gomes et al. 2017a). Briefly, titanium (IV) isoproxide was used as precursor for the preparation of TiO_2 particles. 0.5% Ag-TiO_2 and Pd-TiO_2 were prepared by photodeposition of the Ag^+ and Pd^{2+} ions over TiO_2, while 0.5% Au-TiO_2 was produced through the sol-gel method. Gomes et al. (2017d) reports the main characteristics of the tested photocatalysts. SEM-EDS analysis revealed that a good metal dispersion was obtained from all catalysts. Moreover, XPS revealed also that Pd showed the lowest metal incorporation percentage. XRD analysis showed that anatase is the predominant phase in pure TiO_2 particles and no significant variations are found for the XRD patterns of the doped materials probably due to the low amount of dopants used. The materials Brunnauer-Emmet-Teller surface area are as follows (Gomes et al. 2017a): TiO_2 (149 m^2/g), Pd-TiO_2 (73 m^2/g), Au-TiO_2 (135 m^2/g) and Ag-TiO_2 (34m^2/g).

X.2.3. Biofiltration experimental setup

E. coli removal tests were made in 500 mL biofilters with a plastic support for Asian clam, as can be seen in Figure X.1. These tests were carried out at 20 ± 2 °C under a photoperiod cycle (16 h light: 8 h dark) and continuous aeration. The clams used in the removal tests were kept in the dechlorinated water for about one week, where the water was changed every day or every two days. Due to the high bioaccumulation of bacteria from the field water in the clams it was necessary an initial biodepuration of enteric pathogens to avoid disturbing the performance on *E. coli* removal results. The tests were performed in duplicate with a different number of clams, 5, 10, 20 and 30 for 500 mL of distilled water spiked with *E. coli* (10^3 – 10^4 CFU/ mL). Individuals with shell length in the range 20-30 mm were selected to perform these tests. To control *E. coli* adsorption on the plastic support, two biofilters without clams were used as blanks. Samples were taken at 0, 3, 6, 9 and 24 h, for quantification of *E. coli* colony forming units (CFU). Asian clam mortality was also measured and compared with a control blank using two beakers with distilled water and bivalves. Mortality of clams was verified at each time by checking if there was evidence of siphoning activity or, if clams were closed, by proving resistance when forcing valve opening (Gomes et al. 2014). At the end of the tests, the soft tissue of the clams was recovered to analyze *E. coli* presence.

To be sure that *E. coli* is removed by biofiltration and not by adsorption, tests involving only clam shells were also performed following the same procedure as above.

Figure X. 1. Example of biofilter without clams used in the experiments.

X.2.4. Ozonation and photocatalytic oxidation experimental set-up

Ozonation and photocatalytic oxidation experiments were carried out in a glass 2 L magnetically stirred reactor. The reactor is equipped with 3 lamps (Philips TL 6W BLB) emitting UVA radiation (main emission 365 nm). The photon flux entering the reactor was obtained using ferrioxalate actinometer and was calculated as 5.75×10^{-7} einstein. L/s (Gomes et al. 2017a). The tests were carried out at controlled temperature (25 ± 1 ºC) by a thermostatic bath.

To test *E. coli* removal by ozonation, spiked water (2 L) was placed in the reactor before ozone being fed. Ozone was produced (802N, BMT) from a pure oxygen stream (99.9%) and the inlet and outlet ozone gas concentrations were measured by ozone analyzers (BMT 963 and 964 vent, BMT) and these values were used to determine transferred ozone dose (TOD). The gas was continuously fed during the experiment with a flow rate of 0.2 L/min.

In what regards photocatalytic oxidation experiments, the spiked water was stirred with the catalyst (70 mg/L) during 20 min before feeding oxygen and turning on the lamps. It should be referred that the conditions applied for ozonation and photocatalytic oxidation were previously optimized for the removal of parabens from water (Gomes et al. 2017a,b,c). Based on the good results achieved for that processes, the possibility of its application for removing *E. coli* was investigated.

X.2.5. *Escherichia coli* sample preparation and counting

Bacterial cells used for spiking were at the stationary growth phase due to the highest number of membrane intact cells at this phase. *E. coli* (ATCC 25922) was spiked into the sample to achieve a final concentration between ~ $10^3 – 10^4$ CFU/mL. The method used for quantification of bacteria in water samples was based on the membrane filtration according to ISO 9308-1:2014. Briefly, the samples collected were serially diluted in order to achieve plate counts in the 30-300 CFU range. Those dilutions were filtered through 0.45 µm cellulose nitrate membrane, plated in lauryl sulphate agar and incubated at 37 ± 0.2 °C during 24 ± 2 h. The membranes identified with yellow colonies, corresponding to *E. coli*, were counted and the result reported as CFU per mL (Qiu et al. 2015). All the tests were performed in quadruplicate. Thus, the results given are the average of four replicates. The variability of replicates was always below 0.5 log.

E. coli presence in the clams soft tissues was evaluated according to ISO-16649-2, by incorporation in a chromogenic agar medium (TBX - Tryptone Bile X-glucuronide medium®, Oxoid, Thermo-Scientific, Portugal). Inoculated plates were incubated at 44 ± 1 ºC (in order to inhibit the development of other gram-negative bacteria that could be present in soft tissues but enabling *E. coli* grow) during 18-24 h, and blue colonies, corresponding to E. coli, were counted and expressed as CFU per gram of soft tissue.

X.2.6. Regrowth

The disappearance of *E. coli* from samples may not guarantee that the bacteria was totally removed or eliminated. Thus, regrowth tests are required to be sure that no viable cells were still in the solution. The samples without *E. coli* counting were incubated at 37 ± 0.2 °C for 24 ± 2 h and then submitted to the same filtration procedure as described before, in order to verify the presence of viable but not culturable bacteria (Pinto et al. 2011; Lin et al. 2016).

X.3. Results and Discussion

X.3.1. *E. coli* removal from water by *Corbicula fluminea*

The first stage of the study aimed to understand if Asian clams were able to remove *E. coli* from the contaminated water. Figure X.2 shows *E. coli* concentration throughout 24 hours of biofiltration. The initial contaminated water was spiked with about 10^3 CFU/mL.

According to the results, 10 Asian clams can totally remove *E. coli* and/or promote the total disinfection of 500 mL of contaminated water after 9 h of contact. Moreover, no mortality of clams was detected along the experiments. Thus, *C. fluminea* when subjected to this pathogen can resist and remove it from the water. Even without clams a slightly decrease in *E. coli* colonies number was also verified (Fig. X.2). This can be due to the absence of the nutrients to feed bacteria. Surbeck et al. (2010) concluded that survival rate of *E. coli* was strongly related with the concentration of dissolved organic carbon and phosphorous. However, it can be stated that *E. coli* removal observed during biofiltration is really due to clams' activity, since without clams the concentration remains in the same log.

The disappearance of the total countable *E. coli* does not mean necessarily inactivation and disinfection (Bohrerova et al. 2014). Figure X.2 shows that after 9 h of contact with Asian clams *E. coli* was almost totally removed. Nevertheless, to check if some viable but not culturable bacteria was still present in the sample, regrowth tests were performed. For that purpose, the water sample was placed at conditions amenable for *E. coli* growth (37 ºC during 24 h), and re-evaluated. In fact, some colonies were detected after this. These results reveal that even if bacteria are not countable after 24 h of contact with the clams (where no *E. coli* was measured or detected for all carried out dilutions) also some regrowth was detected, some viable cells must still be present and able to growth. However, in this case the concentration present of bacteria after regrowth was residual (about 2 CFU/mL).

According to Ismail et al. (2016), the combination of two bivalves, *C. fluminea* and *A. californiensis*, proved to be effective on *E. coli* removal, reducing the initial concentration (10^4 CFU/100 mL) to 1-1.5 log after 24 h. However, in that study the density of Asian clam compared with *A. californienses* was significantly lower. In the present work involving only *C. fluminea* it was possible to achieve about 3 log reduction in *E. coli* concentration after 24h. On the same way, Mezzannotte et al. (2016) verified that zebra mussel could reduce the initial *E. coli* concentration of 10^3 CFU/100 mL to 10^0 CFU/100 mL from the municipal treated wastewater.

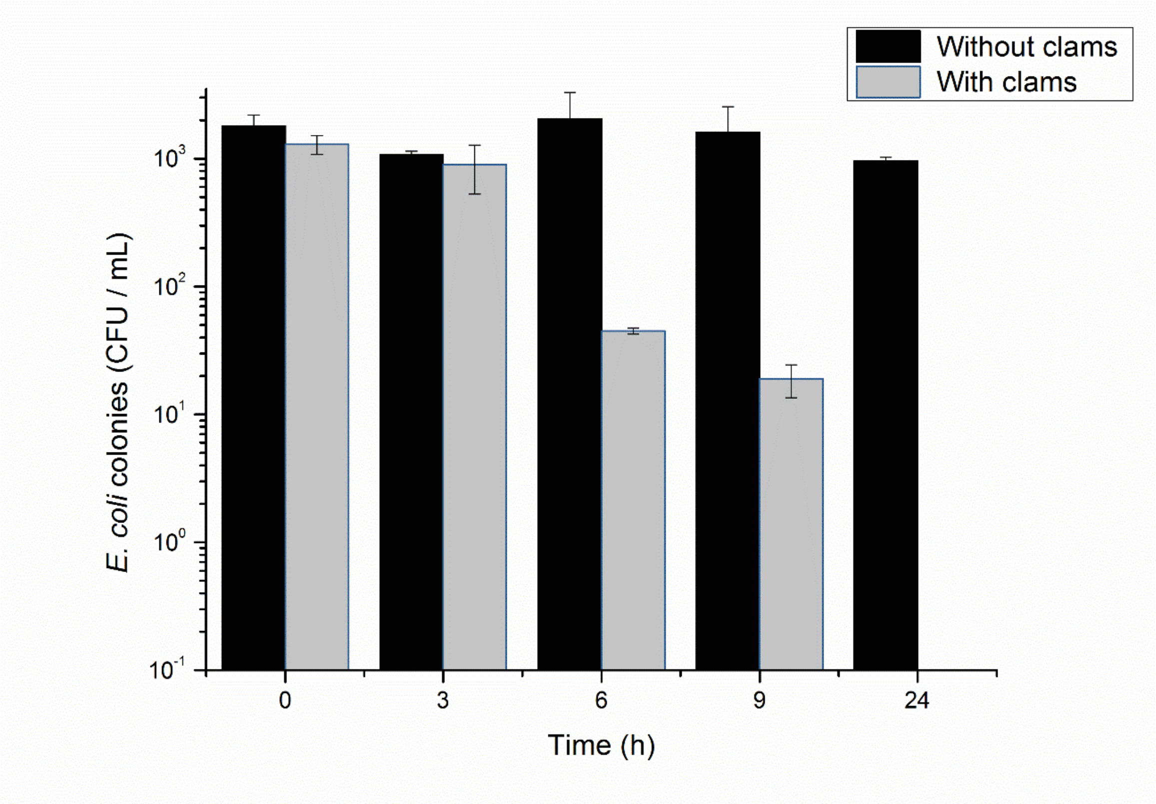

Figure X.2. Average concentration of *E. coli* during biofiltration process using $\log_{10}$ scale (when 10 Asian clams are in contact with 500 mL of *E. coli* spiked water, N = 4)

X.3.2. Effect of the number of *C. fluminea* on *E. coli* removal

It is likely that the efficiency and rate of the *E. coli* removal process by biofiltration is dependent upon the number of clams used.

In this context, the effect of the number of clams on *E. coli* removal was analyzed by carrying out the biofiltration experiment during 24 h using different loads of *C. fluminea*.

Figure X.3 shows the kinetic profile of *E. coli* concentration during the treatment process along 24 h. As it can be seen the increase on the number of clams promotes an improvement on *E. coli* removal by increasing its rate. This is more evident when the number of clams per 500 mL of effluent increases from 5 to 10. In fact, when 5 clams are used the number of bacteria does not significantly change during 9 h of contact. However, with 10 clams, for the same treatment time, a decrease of 1 log was observed. Moreover, by applying 5 clams total *E. coli* removal is not achieved during the 24 h of the experiment. Contrarily, when 10 clams are used, after 24 h of contact there are no countable bacteria in the treated solution (Fig. X.3). However, when the number of clams increased from 20 to 30 removal rate stabilizes and the removal profiles were alike, which means that the number of 20 clams per 500 mL of wastewater seems to lead to the maximum removal yield of the initial *E. coli* concentration applied in these experiments (about 10^3 CFU/mL). Using this number of clams, it was possible to reduce almost 3 log of *E. coli* in 3 h of biofiltration. Possibly for this initial *E. coli* concentration the clearance rate reaches the saturation point with 20 clams (ratio 1:40). This ratio means 1 L of contaminated water for 40 clams. As tests with 0.5 L were performed the number of clams used was 20. Therefore, the addition of more clams will not further improve the rate since an excess of clams is available for that initial concentration of *E. coli*. Ismail et al. (2015) studies the influence of initial *E. coli* concentration on the clearance rate for the same number of bivalves, and for 10^7 and 10^8 CFU/100mL the clearance rate was similar. This means that the number of clams was in excess for the initial *E. coli* concentration. On the same way, in our study the clams achieved the saturation point for the ratio 1:40 (20 clams).

Even if the removal kinetics is the same when 20 or 30 clams were used, the regrowth tests lead to different results when comparing both experimental conditions. The regrowth experiments were performed for the samples collected after 9 and 24 h. This decision was made because after 6 h there was still *E. coli* detected although in a residual number. After 24 h of incubation at 37 ºC, some *E. coli* regrowth was detected for the sample in contact with 20 clams during 9 h. Contrarily, for the same experimental time obtained from the experiment using 30 clams no regrowth was verified. Nevertheless, no regrowth was identified for both conditions when the sample collected after 24 h of biofiltration was analyzed. Therefore, even if the removal kinetic behavior is similar for the both experimental conditions, the highest number of clams can really promote the disinfection of water in a shorter time, removing the total amount of bacteria with no detectable regrowth.

E. coli removal profiles reveal an exponential decay. However, for the experiment using 5 clams, it is possible to see a tailing after that initial exponential decay (Fig. X.3). In fact, after 6 h of biofiltration, the decay of *E. coli* was really slow, possibly due to the clams saturation which led to difficulties on the bioprocessing or bioaccumulation of bacteria.

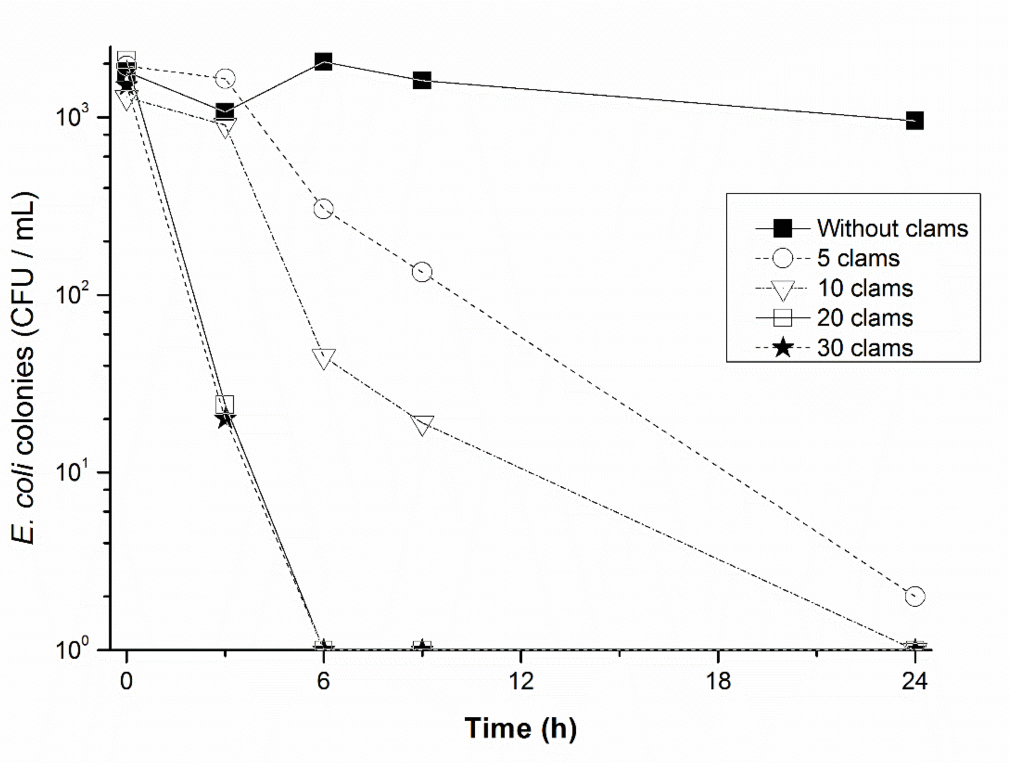

Figure X. 3. Effect of the number of clams on *E. coli* removal using $\log_{10}$ scale (N=4)

The experiments involving different numbers of Asian clams allow determining the clearance rate (CR). The clearance rate is normally used to express the performance of bivalve filtration rate (Cohen et al. 1984; Silverman et al. 1995). It should be pointed out that for the determination of clearance rate for *E. coli* removal*,* the experiment with 30 clams was not considered, since the removal rate for 20 and 30 clams was similar.

The CR of the individual bivalve in each experiment was calculated according to the equation X.1 (Costa et al., 2008):

$$CR= \frac{\mathrm{V}}{\mathrm{n}} \times \frac{\ln \mathrm{Co} - \ln \mathrm{Ct}}{\Delta \mathrm{t}} \qquad \text{(Equation X.1)}$$

where V is the volume of sample and n is the number of bivalves in each biofilter. Co and Ct are the concentrations of *E. coli* at the beginning of the experiments and after 6 h (Δt) of biofiltration. This time was selected due to the significant reduction on *E. coli* verified whichever the conditions. Due to the high agitation promoted by the air diffusion on the biofilter which led to the bacteria suspension homogeneity, the settling rate was not considered in this study. The CR obtained for the different conditions was 31, 28, 32 mL/h. bivalve for ratio 1:10, 1:20 and 1:40 respectively. A good fitting between the model and the data was found (R^2= 0.987). From these values it was possible to obtain the average CR. This corresponds to a clearance rate (mean ±Standard error (SE)) of 30 ± 2 mL/h. bivalve.

The clearance rate for *C. fluminea* was measured in different types of studies. However, the clearance rate can assume a wide range of values even if using the same species (Cohen et al. 1984; Silverman et al. 1995; Ismail et al. 2016). This ambiguity of values can be related with the conditions of the experimental setup, the species size used, the season when the tests were performed. In fact, for some seasons of the year *C. fluminea* appears to be more able for biofiltration than for others. The presence or absence of food, and others parameters can also affect biofiltration rate (Cohen et al. 1984; Rosa et al. 2014; Ismail et al 2016). Ismail et al. (2016) made the analysis of *E. coli* removal with Asian clam in water from river and achieved 75 mL/h.bivalve of clearance rate. In other study with *E. coli* and *C. fluminea* a clearance rate of 97 mL/h.bivalve was verified in an artificial pond water (Silverman et al. 1995). Costa et al. (2008) stated that the filtration rate for zebra mussel was dependent upon the season of the year mainly due to the temperature of water. In this study, a filtration rate of about 35 mL/h.bivalve was detected at the middle of January. Compared with our work (30 mL/h.bivalve), these studies present similar results for the clearance rate of *C. fluminea* with values in the same order of magnitude. The differences observed may be related with the presence of dissolved organic carbon in the river and artificial pond water, whereas in our study no source of food was added to the clams during the biofiltration process.

X.3.3. Mechanisms of removal

E. coli removal tests using Asian clam led to promising results, so it is necessary to understand how the disinfection occurs. The mechanism behind the process was investigated. As described in the introduction section, the Asian clam has the bioaccumulation capacity of chemical contaminants such as heavy metals in their tissues (Marie et al. 2006; Rosa et al. 2015). Moreover, this bivalve can process organic matter, which is helpful in wastewater treatment reducing the chemical oxygen demand to acceptable levels for discharge in the water courses

(Ferreira et al. 2018). Another process that could explain the removal is the adsorption of bacteria to the clams' shells. In fact, the adsorption of metal contaminants onto the shell of bivalves has been documented (Doherty 1990; Rosa et al.2015). Rosa et al. (2015) verified that for Sb, Mn, Se and Sr the most significant removal pathway through *C. fluminea* was the adsorption on the shells.

Contrarily to these studies, in the present work it was identified that the shell adsorption was not the responsible mechanism by the removal of *E. coli* from the contaminated water. Shell adsorption test was done with the highest number of clams (30) since that was the case where the highest surface area of contact between water and shells was available. After 24 h of contact between clam's shells and bacteria no adsorption was verified since the number of bacteria was on the same log of the initial sample (Fig. X.4). This means that *C. fluminea* bioprocess bacteria or bioacumulate it in their soft tissues.

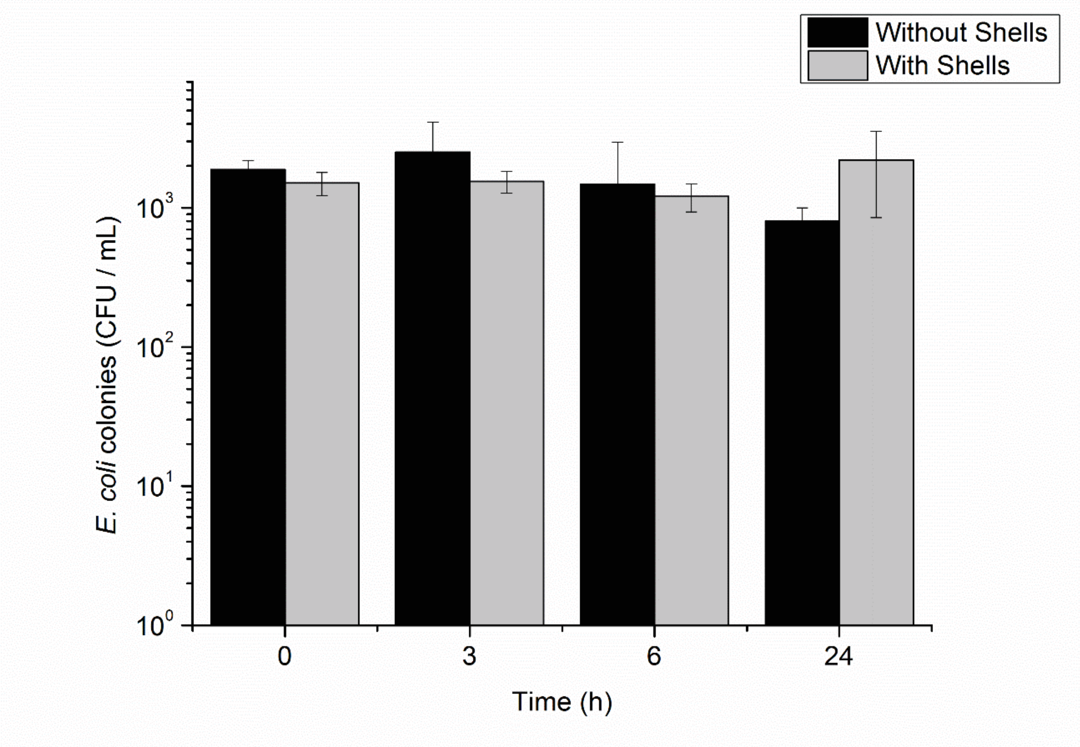

Figure X. 4. Effect of the shell adsorption on *E. coli* removal using $\log_{10}$ scale (N = 4)

In the study performed by Silverman et al. (1995), the removal of 6 x 10^8 CFU of *E. coli* from 20 mL of artificial pond water were achieved with 20 zebra mussels. Furthermore, those authors found that near 26% of the bacteria labeled aminoacids were incorporated into bivalve proteins, and no pure *E. coli* proteins were detected in bivalve samples. These results mean that a strong part of the removed bacteria must have been bioprocessed by the mussel, and not simply trapped in the mussel tissue. In our study, from the analysis of *C. fluminea* soft tissue after *E. coli* biofiltration, only 1-2% of the *E. coli* removed from the contaminated water was found. This seems to mean that up to 98% of the removed bacteria was bioprocessed by the Asian clam. Possibly, the difference between zebra mussel (Silverman et al. 1995) and Asian clam (our study), can be related with higher filtration rate from the zebra mussel (Silverman et al. 1995). To further confirm if some of the bacteria biofiltered by *C. fluminea* can be afterwards released to the water (explaining this way the low number of bacteria found in the clam soft tissues), long duration tests were performed where *C. fluminea* was left aerated in water. Even after 48 h no release of *E. coli* to the water was verified. Thus, this seems to confirm that probably the *E. coli* removed was really processed by *C. fluminea* and not only accumulated. This strengthens the conclusions taken from the fact that only 1-2% of the removed bacteria were still detected in the Asian clam soft tissues.

X.3.4. Biofiltration efficiency comparison with ozonation and photocatalytic oxidation

E. coli concentration (CFU/mL) as function of TOD along ozonation was studied. While a low *E. coli* decrease was observed for a TOD of 0.05 mgO_3/L, no viable bacteria was detected for a TOD of 0.16 mgO_3/L. Thus, it seems that a very low ozone dose is necessary to totally eliminate *E. coli* from this contaminated water. In fact, ozone is a highly reactive molecule with capacity of reacting with several cellular constituents such as the cell wall and DNA structures (Sousa et al. 2017). Regrowth tests were performed to the samples, and no regrowth was detected confirming the high efficacy of ozone for *E. coli* degradation.

Photocatalytic oxidation was also tested for the bacteria removal from the contaminated water. A screening of catalysts was performed involving pure TiO_2, and noble metals (Au, Ag and Pd) doped TiO_2 (0.5% w/w). To check the possibility of *E. coli* removal only due to the contact with the catalytic materials, the solution was stirred with the catalyst (70 mg/L) in the dark during 20 min. After this period, the lamps were turned on. Figure X.5 shows *E. coli* concentration during the process. It should be reminded that the first 20 min correspond only to the contact between the contaminated water and the catalysts. According to these results, Ag-TiO_2 and Pd-TiO_2 can totally remove bacteria without the need of light. For those materials no bacteria were detected in the sample collected after 20 min in contact only with the catalytic materials. Silver, for example, is a well-known bactericide due to the interaction between Ag and the sulphydryl groups of the cell proteins (Kubacka et al. 2008; Suri et al. 2012). Probably similar mechanisms are behind the efficiency of Pd-TiO_2. Since no colonies were detected when regrowth tests involving the catalysts suspension were performed, it was concluded that no viable *E. coli* was adsorbed at the catalytic materials surface. In what regards pure TiO_2 and Au-TiO_2, the contact of the *E. coli* solution with the catalysts without light led to up to 1 log removal. When UVA light was turned on, *E. coli* removal rate was increased for both catalysts with total removal being achieved after 60 min for the two catalytic systems.

Tests were randomly repeated, and no differences were found at the log scale. The use of UVA radiation alone did not provide any change on the initial *E. coli* concentration. So, the effect UVA alone over disinfection efficiency can be neglected.

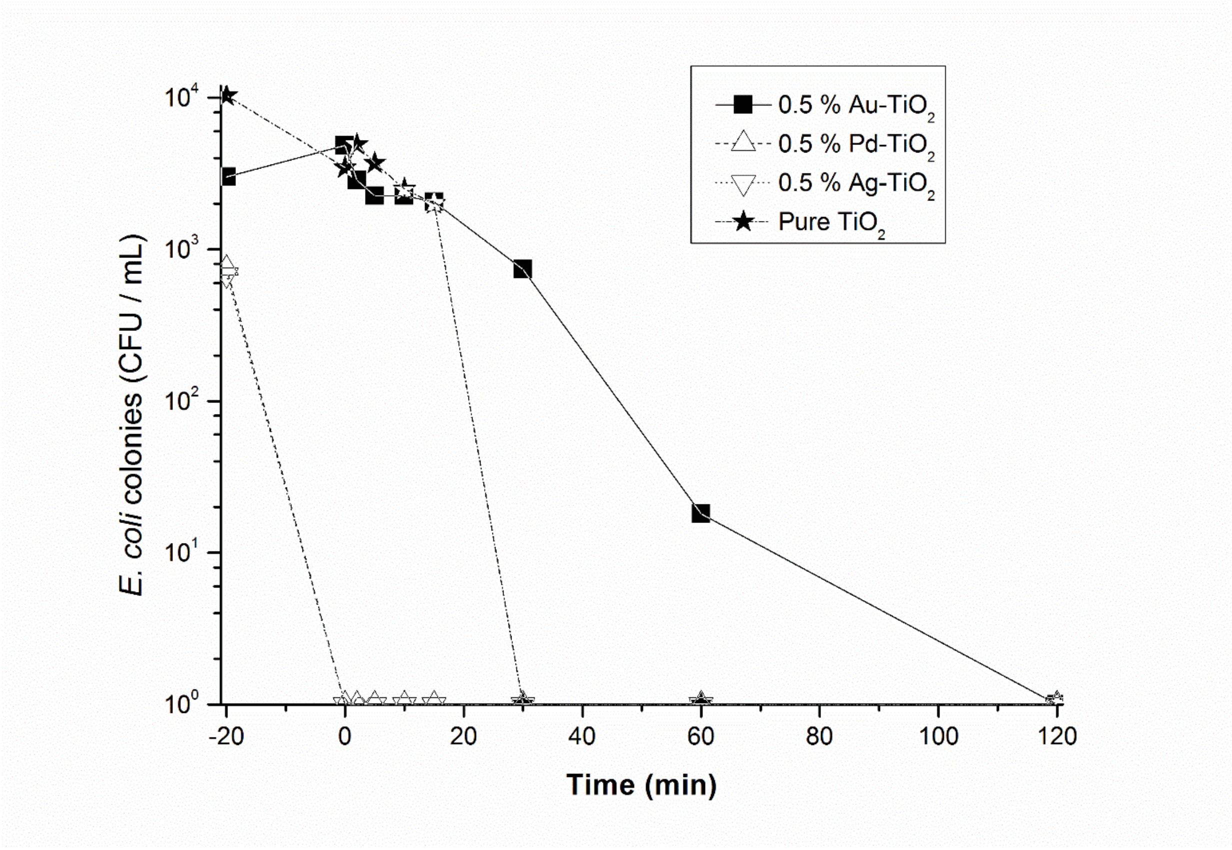

Figure X.5. Effect of different catalysts during photocatalytic oxidation on *E. coli* removal using $\log_{10}$ scale as function of time (N=2)

The results achieved in this work reveal that biofiltration using *C. fluminea*, ozonation and photocatalytic oxidation are efficient systems for the removal of *E. coli*. The selection of a treatment process must also bear in mind the costs associated with it. Considering energy required for ozone production as 12 kWh/kgO_3 (Hollender et al. 2009), and the TOD needed for the total depletion of *E. coli* (0.16 mgO_3/L), energy consumption by ozonation would reach

1.92×10^{-3} kWh/m^3. Here the costs associated with the reactor, ozone injection system and outlet gas ozone burner are not being considered. In what concerns photocatalytic oxidation, the lamp energy costs should only be considered for the cases where Au-TiO_2 and TiO_2 were applied as photocatalysts. For pure TiO_2 60 min of irradiation was required for total *E. coli* removal. This corresponds to an energy consumption of 9 kWh/m^3 which is about 4700 folds higher than the energy demanded by ozonation. It should be reminded that about 15 s of ozonation were enough for the bacteria total abatement. To these energy demands, it must be summed up the costs associated with the catalysts. While TiO_2 can be considered a low-cost material, the use of the noble metals as dopants increases the costs of the catalytic material. In fact, Au can reach 35 €/g (GoldPrice, 2018). As for the Ag-TiO_2 and Pd-TiO_2, the use of light was not required for *E. coli* inactivation, thus only the costs associated with the catalyst must be considered as main drawback. While Ag reaches market prices of about 0.50 €/g (GoldPrice, 2018), Pd is available for about 27 €/g (Infomine, 2018). Moreover, it should be reminded the drawbacks associated with the application of slurry catalytic systems. In fact, at the end of the treatment, separation processes must be applied in order to remove the solids from the effluent and recovering the catalyst for reuse. However, this drawback may be overcome if suitable supports are used.

Regarding biofiltration, besides the efficiency showed on the removal of *E. coli*, it must be considered that this treatment strategy aids on the management of a widely spread pest leading to important ecological benefits (Ferreira et al. 2018). This may avoid the application of conventional pest management approaches involving the biocides with potential side effects over environment and non-target species. Thus, this treatment approach may be integrated with removal strategies of the invasive species from infested natural water courses, which is one of the few efficient methods to control invasive bivalves (Sousa et al. 2014). In this context, the costs associated with bivalves collection for biofilters assembling may be neglected if this procedure is coordinated with pest management strategies in the natural ecosystems. Also, in this line of thought, are the costs associated with the disposal of dead *C. fluminea* drawn from the biofilters, which may involve disposal in the landfills or incineration. It should be reminded that a very low amount of viable *E. coli* was found in clams soft tissues, which means that the bacteria is being processed and not only bioaccumulated. Therefore, the risk of used *C. fluminea* represent a spreading vector of *E. coli* is low. Additionally, the retention times required for biofiltration (9 – 24 h) are of the same order of magnitude of those using by the conventional activated sludge for the municipal wastewater treatment.

Considering all this, ozonation and biofiltration using *C. fluminea* reveal as interesting systems for *E. coli* removal. Comparing these processes, it should be also considered that a higher residence time is required for biofiltration (> 6 h) while only 15 s were enough for the ozonation. This means that the volume of biofilter would be much higher (for the same flowrate) than the one for the ozonation reactor. However, on the other hand, for ozone oxidation more complex equipment is needed, involving ozone generator, ozone injection system and ozone off-gas burner, which will seriously increase the treatment cost. Also, in the biofiltration, it must be ensured that the invasive species are not spread to other ecosystems, requiring safe biolfilters to guarantee that the animals are securely held and that accidental release of the clams (also in their planktonic stages) is totally avoided. All this must be considered when designing a suitable treatment system. As an example, the biofilter could be implemented before a sand filter able to hold the animals. Moreover, to further avoid accidental spreading to non-invaded sites, this proposed treatment technology based on *C. fluminea* biofiltration could be implemented near already infested sites. This would also reduce the clams transportation costs for the biofilters assembly.

X.4. Conclusions

This study reveals that *Corbicula fluminea* can be helpful to consume the bacteria *Escherichia coli* from the contaminated water. The regrowth tests performed when no countable bacteria were detected after contact with the bivalve, revealed that disinfection was effective. The increase on the number of clams enhances the clearance rate of *E. coli* until a certain point. When the number of clams increased, the speed of bacteria removal also increases exponentially, and achieves the optimal value with proportion of 1:40, which means 40 clams per 1 L of contaminated water. When higher proportions were applied, the velocity of *E. coli* removal did not suffer any further increase. The adsorption test with shells revealed that the main mechanism responsible for the bacteria removal was biofiltration and not simple adsorption over the shells. The analysis with the soft tissues of *C. fluminea* revealed that only 1-2% of initial bacteria remains in the bivalve tissues. This result leads us to believe that the main mechanism for *E. coli* removal was bioprocessing and not accumulation. This fact was reinforced by the analysis of bivalve depuration along 48 h, where no bacteria release from *C. fluminea* to the water was detected.

Ozonation is able to totally remove *E. coli* using a low TOD. Among the photocatalyst tested, it was concluded that Ag-TiO_2 and Pd-TiO_2 do not require light to deplete the bacteria. Whereas pure TiO_2 and Au-TiO_2 need 60 and 120 min of UVA irradiation to *E. coli* total removal, respectively. The energy costs associated with the lamps in the photocatalytic oxidation were much higher than those associated with ozone generation. Moreover, the costs associated with the use of noble metals in the catalysts as well as their separation from the liquid for reuse after the treatment must be considered.

Ozonation and biofiltration seems thus interesting solutions for *E. coli* removal. Particularly, the biofiltration results show that the removal of enteric pathogens such as *E. coli* from water can be one of the strategies to make the pest management of *C. fluminea*. Moreover, it is also possible to solve the ecologic and economic problem with this invasive species, coupling pest management strategies with a solution for wastewater decontamination and disinfection.

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XI. Final conclusions and future work

XI.1. Final conclusions

The water scarcity is one of the major challenges for Humanity. Therefore, water reclamation should be considered as a strong possibility to deal with this problematic and scientific community is putting efforts in this way. However, the trail is long and tortuous. The conventional wastewater treatments can lead to the removal of general organic matter but do not remove the recalcitrant compounds that cannot be biologically degraded. Parabens can be considered on the group of these contaminants. In fact, parabens were detected in water courses due to the excretion or release in the wastewaters since they are present in the composition of a wide range of pharmaceutical and personal care products. These contaminants involve serious problems for aquatic toxicity and human health due to endocrine disrupting capacity. Therefore, the necessity to find a suitable technology to deal with this problematic arises as a relevant scientific challenge. Moreover, another important feature is the identification and treatment of bacteria and viruses that can be also found in water courses due to the lack of conventional wastewater treatments to deal with the biological emerging contaminants.

Advanced oxidation processes encompass different types of approaches for wastewater treatment. It was tried to find within this kind of processes a suitable technology to deal with parabens and with bacteria disinfection.

In this context, UVA radiation was tested to promote the decontamination of parabens mixture. The main reason for considering this mixture relates with the fact that several parabens compounds are used together to enhance the antimicrobial and preservative activity. The absorption edge of this mixture is near to the UVC radiation; therefore, UVA radiation alone does not promove any degradation. Therefore, four different TiO_2 noble metals (Ag, Au, Pd, Pt) doped catalysts were considered at the load of 0.5 wt.% to improve the parabens mixture degradation comparing to pure TiO_2. The degradation of parabens followed a pseudo-first order kinetics, and the kinetic constant increased with the number of carbons present in the paraben alkyl chain. In terms of parabens degradation after 180 min the most active photocatalysts were Ag and Pd-TiO_2. The catalytic efficiency promoted by these two catalysts can be strongly related with the generation of reactive oxidative species such as superoxide and hydroxyl radicals. The toxicity decrease over different species was also analysed since the total parabens removal was not achieved. Toxicity suffers a reduction when photocatalytic oxidation is applied except for the most sensitive species, the bacteria *A. fischeri*. Then, the photocatalytic oxidation was compared with the catalytic ozonation. Ozone revealed the capacity to enhance the parabens mixture degradation using a great amount of TOD (170 mg/L) corresponding to 120 min of reaction. Meanwhile, the effect of the same catalysts coupled with ozone was tested aiming to achieve total degradation of the parabens mixture. Indeed, total degradation was achieved for all catalysts with a significant reduction of TOD when compared with single ozonation. In this process, the most active catalysts were Pd and Pt-TiO_2. Here, Ag-TiO_2 does not present significant activity possibly due to the low surface area compared to the other catalysts. On the other hand, the good performance of Pt-TiO_2 could be related with the high surface area. On the same way, pure TiO_2 also presents interesting results in terms of catalytic ozonation since it has the second higher surface area. Moreover, for the all treatments a significant toxicity reduction was observed over all species that were tested (*A. fischeri, C. fluminea, L. sativum*). Thinking in water reclamation the untreated and treated solutions using single and catalytic (Pd-TiO_2) ozonation were tested over the neuronal activity of Wistar rat brain slices. The neuronal reactive oxygen species (ROS) signals of the mammalian preparations reveal that treated solutions do not present toxic effect unlike the initial mixture of parabens.

Ozone and UVA radiation were coupled together to enhance parabens mixture degradation. The reason for this couple be a success is strongly related with the usage of the before mentioned catalysts. In the presence of these doped catalysts the UVA radiation can promote the separation of electron and hole pairs which can reduce ozone and oxidate water, respectively, to promote the ozonide and hydroxyl radical formation. The most active catalyst was 0.5 wt.% Ag-TiO_2 achieving total parabens mixture degradation using a TOD of 46 mg/L. The main by-products analysis was made and their respective profiles of production along the reactions were followed for each catalyst. The toxicity reduction was also analysed for the species used in the previous chapters. For example, the toxicity reduction over *A. fischeri* was more evident in the samples coming from catalytic ozonation than to those coming from photocatalytic ozonation. Nevertheless, for the other tested species the treated water from photocatalytic ozonation was less toxic. This different behaviour can be related with the by-products generated in the photocatalytic ozonation processes. Moreover, the economic analysis in terms of energy consumption was performed. The presence of UVA radiation implies a significant increase on the energy consumption but allows a lower time of reaction.

After the technology selection the interference of different parameters on the parabens mixture degradation was analysed. But first the more suitable noble metal load within 0.1, 0.5 and 1 wt.% for the most promising technology, photocatalytic ozonation, was selected. In this context, the impact of these noble metal loads was analysed regarding parabens depletion, toxicity reduction and the noble metal cost. Gold doped onto TiO_2 was removed from this analysis since led to the worst result in terms of parabens mixture degradation which can be related with the high electronegativity and electron affinity of Au. The parabens mixture, chemical oxygen demand and total organic carbon removal is influenced by the noble metal used. For example, for Ag-TiO_2 the best results regarding to parabens mixture degradation were achieved for the 0.1 and 0.5 wt.% catalysts, while for Pt-TiO_2 the most active loads were 0.5 and 1 wt.% and 0.5 wt.% of Pd-TiO_2 presented the worst result. The COD and TOC removals were analysed as function of TOD. The most active catalyst for COD removal was 1 wt.% Pd-TiO_2 while for TOC removal the best effiency was obtained for 0.5 wt.% of Ag-TiO_2. The effect of the noble metal load on the toxicity reduction was also analysed. An overview of all species reveals that the best results were achieved for 0.5 wt% of Ag and Pd-TiO_2. The general overview discloses multiple answers according to the parameter analysed. Therefore, for the noble metal load selection it was also considered the global market prize. Palladium and platinum present a significantly high global prize comparing to silver.

Thus, globally, 0.1 wt.% Ag-TiO_2 was selected and was tested to evaluate the effect of several parameters over the photocatalytic ozonation performance. The effect of pH, radical scavenger, water matrix and ion species in solution was then investigated. Lower pH leads to the reduction of the TOD values required for the total parabens mixture degradation. This may be related with the formation of the ozonide radical that under acidic conditions can enhance the hydroxyl radicals production. Hydroxyl radical reveals to be the main responsible by the improvement on the parabens mixture degradation through photocatalytic ozonation, since the process performance was reduced when isopropanol was added to the solution. Parabens mixture degradation was improved when secondary municipal wastewater was used as matrix. The main reason can be related with the ion species present in the wastewater such as chloride and sulfate that can lead to additional radicals able to oxidize organic matter. In fact, the increase of the sulfate ions in the real wastewater seems to enhance the photocatalytic ozonation. The spiked real wastewater treated presented lower toxicity than the raw real wastewater without any spike over *A. fischeri*.

The performance of these catalyst was unblamable; however, the costs associated to the catalyst production must not be forgotten. Therefore, the results were compared with low-cost materials, namely two different volcanic rocks from Azores. These materials can be easily found and can be considered as catalysts due to their high amount of metals that can be helpful to enhance ozonation activity. The volcanic rocks were characterized by different technologies and reveal a great amount of silica, iron oxide and alumina. Therefore, the catalytic ozonation was evaluated on the parabens mixture degradation with total removal achieved using 55 mg/L of TOD. These values are similar to to those achieved previously with the noble metal doped catalysts. Photocatalytic ozonation was tested but no significant differences were obtained comparing to catalytic ozonation probably due to the low amount of TiO_2 available on the low-cost materials. The best performance was achieved with the volcanic rock entitled P1. The effect of pH was also analysed for 3.5, 7 and 8. The parabens mixture degradation was improved with the increase of pH which must be related with the high hydroxyl radical production due to ozone decomposition above the catalyst pH_{pzc}. On the other hand, tests with hydroxyl radical scavengers were carried out for the acidic pH to verify that these moieties have a relevant impact on the parabens mixture degradation through catalytic ozonation. Moreover, it was concluded through the evaluation of the by-products generation that the presence of the radical scavengers impacts the reaction mechanism.

The toxic effect of the degradation products provided from the single and catalytic ozonation using the volcanic rocks was analysed over several non-target species (*D. magna, R. subcapitata, C. fluminea, L. minor* and *L. sativum*) comparing to the dilutions of parabens mixture. This comparison allows to infer about the effect of by-products resulting from ozonation processes over different sensitive species. The initial parabens mixture presents a high toxicity. Single ozonation needs a huge amount of TOD (170 mg/L) to achieve the total parabens degradation while catalytic ozonation allows a significant reduction on the TOD amount (55 mg/L). However, this does not mean a toxicity reduction maybe due to the formation of more toxic by-products. In fact, the hydroquinone and 1,4-benzoquinone produced at the end of catalytic ozonation can present a toxicity impact over the tested species, while this does not occur with single ozonation where these by-products were not identified.

Until now parabens decontamination was analysed. However, regarding water reuse, biological emerging contaminants removal must also be assessed. For this *E. coli* abatment was analysed under optimal conditions with different chemical methodologies. Moreover, the possibility of integrating pest management with wastewater treatment was also evaluated. Ozone, photocatalytic oxidation with UVA radiation and biofiltration through *C. fluminea* were compared for *E. coli* abatment. Regarding to biofiltration it was revealed that the ratios of 1:40 and 1:60 (liter of wastewater per clams) present the best results on *E. coli* removal after 6 h. The mechanism of *E. coli* removal was also described, no shell adsorption was verified and the concentration of *E. coli* in mussel tissues reveals that the bioprocessing by clams is the most suitable hypothesis for the removal of bacteria. Ozone reveals a great performance for total *E. coli* elimination using a TOD of 0.16 mg/L. The noble metal doped catalysts tested on the *E. coli* was the same that was previously tested on the parabens decontamination. For Ag and Pd-TiO_2 the dark conditions were enough to totally remove *E. coli* which means that these bacteria were adsorbed on the catalyst and, due to the bactericide activity of this noble metals, were eliminated since no regrowth was observed. The Au-TiO_2 needed about 120 min to achieve total *E. coli* removal while pure TiO_2 just required 30 min under photocatlytic oxidation conditions. These processes reveal great performance under optimal conditions; however, the photocatalytic oxidation was considered the most expensive. Biofiltration with *C. fluminea* can be considered as a pest management strategy since the invasive character of this clam promotes industrial, economical and also environmental problems. Its activity on the removal of fecal contamination points out its potential to be incorporated in wastewater treatment facilities.

XI.2. Future work

The work developed until now should not be seen as an end which finishes with the conclusion of this thesis but rather as a beginning for a further research development. Therefore, despite of the developed work until now a lot of other studies can be made thinking on the industrial application of this kind of processeses to promote the removal of emerging contaminants from wastewater. Taking into consideration the scope of this thesis some possibilities for future work will be analysed here.

Considering the fact that Portugal belongs to the Mediterranean area, the sunlight radiation should be selected for the photocatalysis as energy source. For this, the development of suitable, active and low-cost catalysts should be performed. In this ambit the doping of TiO_2 with non-metals such as N and the co doping with other metals as Ce and Mn must be considered to enhance the TiO_2 perfomance over sunlight radiation. Then the couple with ozone to improve the emerging contaminants degradation should be further analysed. The development of powder catalysts presents advantages in terms of dispersion in batch reactors but problems arise when the continuous reaction would be considered. Therefore, the way to support these active powder catalysts and the use of solid structures previously prepared with active catalyst (such as supported nanotubes) will be analysed.

The optimization of photocatalytic ozonation using municipal wastewater should consider the impact of treated water over human cells, as well as a wide range of non-target species and the impact when used for irrigation of some model food elements. Only this way it can be ensured the safetyness of the recovered water. Moreover, with the real wastewater, photocatalytic ozonation efficiency for bacteria and some viruses abatment should be tested. Then, after optimization kinetic studies should be performed at real water treatment conditions so that suitable models can be used to design pilot scale treatment units. In this ambit, at the pilot plant the operating conditions will be further optimized bearing in mind mass transport resistances at the catalysts and non-ideal behaviour of the reactors. Only gathering these data will be possible to scale-up the process for the industrial level.

Parallel to this, the biofiltration of *E. coli* and other entheric pathogens present in the real wastewater can be performed, since the *C. fluminea* presents potential for *E. coli* removal. Moreover, biofiltration should also be further tested for the abatement of chemical emerging contaminants. Here, some parameters should be checked such as the number of clams, the presence of substrate, food and the shell length. After optimizing these operational conditions at laboratory scale a pilot plant should be designed to verify the performance of *C. fluminea* at real water treatment conditions

The couple of photocatalytic ozonation with the biofiltration can be an interesting target and an economical solution for the water reclamation. A pilot treatment plant with these two methodologies integrated can be tested for simultaneous disinfection and decontamination of real wastewater. However, the sequence of these two methodologies in the process must be studied to reach the best treatment results.