Cristiana Andreia Pinhão Gonçalves

Licenciada em Ciências de Engenharia do Ambiente

Electro-reactor for the removal of PPCPs and microorganisms from effluent: feasibility assessment

Dissertação para obtenção do Grau de Mestre em Engenharia do Ambiente, perfil de Engenharia de Sistemas Ambientais

Orientador: Doutora Maria da Nazaré Parada Figueiredo de Sousa Couto Alves, CENSE, FCT-UNL

Co-Orientador: Professora Doutora Alexandra de Jesus Branco Ribeiro, CENSE, DCEA, FCT-UNL

Júri:

Presidente: Doutora Maria Júlia Fonseca de Seixas

Arguente: Doutor Joaquim Carlos Gomes Esteves da Silva

Vogal: Doutora Maria da Nazaré Parada Figueiredo de Sousa Couto Alves

Março de 2020

Cristiana Andreia Pinhão Gonçalves

Degree in Environmental Engineering Sciences

Electro-reactor for the removal of PPCPs and microorganisms from effluent: feasibility assessment

Dissertation for the master's degree in Environmental Engineering, Environmental Systems Engineering profile

Advisor: Doctor Maria da Nazaré Parada Figueiredo de Sousa Couto Alves, CENSE, FCT-UNL

Co-Advisor: Professor Doctor Alexandra de Jesus Branco Ribeiro, CENSE, DCEA, FCT-UNL

Jury:

President: Doctor Maria Júlia Fonseca de Seixas

Examiner: Doctor Joaquim Carlos Gomes Esteves da Silva

Supervisor: Doctor Maria da Nazaré Parada Figueiredo de Sousa Couto Alves

March 2020

Electro-reactor for the removal of PPCPs and microorganisms from effluent: feasibility assessment

Copyright © Cristiana Andreia Pinhão Gonçalves, FCT – UNL, 2020

A Faculdade de Ciências e Tecnologia e a Universidade Nova de Lisboa tem o direito, perpétuo e sem limites geográficos, de arquivar e publicar esta dissertação através de exemplares impressos reproduzidos em papel ou de forma digital, ou por qualquer outro meio conhecido ou que venha a ser inventado, e de a divulgar através de repositórios científicos e de admitir a sua cópia e distribuição com objetivos educacionais ou de investigação, não comerciais, desde que seja dado crédito ao autor e editor.

Acknowledgements

I would like to express my sincere gratitude to Professor Alexandra Ribeiro for providing me the opportunity to elaborate the present dissertation in collaboration with RESOLUTION LAB and CENSE - Center for Environmental and Sustainability Research. Thank you for trusting in me and for the motivation given.

It has been a challenge, but an unique opportunity for growth and self-improvement that lead me to redefine some aspects of my life.

To Doctor Nazaré Couto a special thank you for all the support, kindness, and patience in the most difficult moments. Having you as my supervisor really inspire me to always want to work harder. Without the attentive advices given and transmitted knowledge, this work could not have been done. Thank you!

Furthermore, I would also like to thank Dr. Cristina Santos and Eng. Olga Paredes for their support in this work, providing the effluent samples. To the Laboratory of Control and Processes for the assistance and to all Quinta do Conde wastewater treatment plant technicians.

To all the inspiring people from the RESOLUTION LAB team that I had the pleasure to meet during the last months and who taught me about laboratory and analytical techniques. To Doctor Paula Guedes for providing me guidance in the different phases of the experimental work and for the scientific knowledge transmitted. To Joana Dionísio for the help given in the laboratory. To Joana Almeida, Cátia Magro and Cátia Santos for transmitting me joyful and curious attitude towards research work and for always being available to helping me.

To Doctor Eduardo Mateus for technical, scientific and logistic support and to Professor Marco Silva for logistic support.

I would also like to acknowledge the project CEMOWAS2 (SOE2/P5/F0505) "Gestão Circular Ecossistémica dos Serviços de Resíduos Orgânicos e de Águas Residuais" for financing this work. This research is anchored by the RESOLUTION LAB, an infrastructure at NOVA School of Science and Technology.

To my family and to my friends for the continuous support and motivation.

Resumo

A visão da água residual tem vindo a mudar ao longo dos anos, à medida que as estações de tratamento de águas residuais (ETAR) começaram a ser consideradas fontes de recursos valiosos que podem ser recuperados. O uso de água residual tratada para fins não-potáveis como irrigação agrícola representa uma opção viável, capaz de dar resposta a situações de escassez hídrica permitindo tirar pressão na exploração de fontes de água doce. No entanto, a água residual tratada pode possuir riscos químicos e microbiológicos, visto que as tecnologias de tratamento secundário e terciário atualmente utilizadas não permitem atingir uma remoção completa de contaminantes emergentes, como fármacos e produtos de cuidado pessoal.

Tecnologias electrocinéticas constituem soluções para o tratamento de água residual para fins de reutilização. A presente dissertação explora a aplicação do processo electrocinético num reator electroquímico para estudar a degradação de cinco compostos orgânicos emergentes: cafeína (CAF), sulfametoxazol (SMX), carbamazepina (CBZ), diclofenaco (DCF) e oxibenzona (OXY). De forma complementar, o efeito do campo elétrico na comunidade microbiológica total do efluente foi avaliado através da contagem de unidades formadoras de colónias que cresceram em meio de agar dextrose de batata, passadas 24 h. Os reatores foram dopados com a mistura de contaminantes com uma concentração de 0.2 mg/L, e foi aplicada uma densidade de corrente fixa de 8 mA/cm² entre elétrodos de óxido de metal misto. No primeiro conjunto de experiências, o processo electrocinético foi aplicado em efluente desinfetado por radiação UV-C à escala laboratorial e três tempos de degradação foram testados: 2 h, 4 h e 6 h. A electrodegradação variou entre 17 ± 6% para CAF após 2 h de tratamento electrocinético, tendo o SMX ficado abaixo do limite de deteção do método, ao fim de 4 h de tratamento. Microrganismos totais cultiváveis do efluente foram reduzidos em cerca de 1.0 unidade logarítmica (Redução em 90%), no primeiro conjunto de experiências. No segundo conjunto de experiências, realizadas em efluente fresco e pelo período de 6 h, verificou-se uma maior inativação dos microrganismos totais cultiváveis, até 3.7 unidades logarítmicas (Redução em 99.98%). Testou-se em paralelo a fotólise por meio de UV-C dos compostos como termo de comparação com o processo eletrocinético. Electrodegradação em efluente fresco permitiu obter para SMX e OXY degradações superiores a 95%.

Tanto os fármacos e os produtos de cuidado pessoal em estudo como os microrganismos presentes no efluente podem ter sofrido oxidação anódica direta e oxidação indireta por espécies intermédias formadas em meio aquoso. Ao permitir remover PPCPs e microrganismos, o processo eletrocinético provou ser viável enquanto tecnologia de remediação e desinfeção para efluente secundário de ETAR.

Palavras-chave: Fármacos e productos de cuidado pessoal, Processo eletrocinético, Microrganismos totais cultiváveis, Desinfeção eletroquímica

Abstract

The vision of wastewater has been changing over the years, as wastewater treatment plants (WWTPs) are now considered supplies of valuable resources that can be recovered. The use of reclaimed water for non-potable uses such as agriculture irrigation, represents a viable option capable of dealing with water scarcity and allowing to alleviate the exploration of freshwater supplies. However, reclaimed water may enclose chemical and microbiological risks, as the currently used secondary and tertiary treatment technologies do not allow a complete removal of emergent contaminants, such as pharmaceuticals and personal care products (PPCPs).

Electrokinetic (EK) technologies are available solutions for wastewater treatment aiming reclamation purposes. This dissertation explores the application of the EK process in one-compartment (1c-cell) electrochemical reactor to study the degradation of five target emergent organic contaminants (EOCs): caffeine (CAF), sulfamethoxazole (SMX), carbamazepine (CBZ), diclofenac (DCF) and oxybenzone (OXY). In addition, the effect of the electric field generated by the low-level direct current (DC) in the effluent total microbial community was also assessed by performing counts of colonies forming units grown in potato dextrose agar media after 24 h of incubation. Reactors were spiked with a mixture of the target PPCPs at a concentration of 0.2 mg/L and a fixed current density of 8 mA/cm^2 was applied between mixed metal oxide electrodes. In Set 1, the EK process was applied in bench scale UV-C treated effluent and three treatment times were tested: 2 h, 4 h and 6 h. Electrodegradation ranged from 17 ± 6% for CAF after 2 h of EK treatment, to removal below the method detection limit for SMX after 4 h of EK treatment. Total culturable microorganisms were reduced by ≈ 1.0 Log_{10} unit (90% removal), in Set 1. In Set 2 experiments, performed in fresh effluent and for a period of 6 h, total culturable microorganisms achieved a higher inactivation, up to 3.7 Log_{10} units (99,98% removal). UV-C photolysis of PPCPs was also tested in Set 2, in comparison to EK treatment. Electrodegradation of PPCPs in fresh effluent was higher than 95% for both SMX and OXY.

Both PPCPs and total microorganisms from secondary effluent can suffer direct anodic oxidation and indirect oxidation by generated intermediate species in aqueous media. By allowing PPCPs and microorganisms removal, the EK process proved to be a viable remediation and disinfection technology for secondary effluent from WWTPs.

Key-words: Pharmaceuticals and Personal Care Products, Electrokinetic process, Total culturable microorganisms, Electrochemical disinfection

Table of Contents

Acknowledgements vii
Resumo ix
Abstract xi
Table of Contents xiii
List of Figures xv
List of Tables xvi
Abbreviations and symbols xviii
1. Introduction 1
1.1 Objectives and questions 3
1.2 Dissertation structure 4
2. State of the art 6
2.1 Water reuse 6
2.2 Pharmaceutical and Personal Care products (PPCPs) 9
2.2.1 Sources and pathways of PPCPs 12
2.3 PPCPs under study 14
2.3.1 Caffeine 14
2.3.2 Sulfamethoxazole 15
2.3.3 Carbamazepine 16
2.3.4 Diclofenac 16
2.3.5 Oxybenzone 17
2.4 Wastewater treatment 17
2.5 Tertiary treatment 19
2.5.1 Removal of microorganisms and PPCPs in tertiary treatment systems 20
2.5.2 UV Disinfection of wastewater 24
2.5.3 Advanced oxidation processes 25
2.6 Electro-based technologies for wastewater treatment 25
2.7 Electrokinetic process 26
2.7.1 Transport mechanisms under an electric field 26
2.7.2 EK remediation of PPCPs 29
2.8 Analytical techniques 29
2.8.1 Solid-phase extraction 30
2.8.2 High-Performance Liquid Chromatograph (HPLC) 31
3. Materials and Methods 34
3.1 Effluent sampling 34
3.2 Experimental design 36
3.3 Effluent disinfection with UV-C radiation – sample treatment 36
3.4 Electro-reactor set up 37
3.5 EK experiments 38

3.6 Chemicals and materials 40
3.7 Analytical methodologies 40
3.7.1 pH and Conductivity 40
3.8 Culturable microorganisms 40
3.9 PPCPs analysis 43
3.9.1 Solid phase extraction 43
3.9.2 High pressure liquid chromatography (HPLC) 44
3.10 Statistical Analysis 45
4. Results and discussion 47
4.1 HPLC calibration 47
4.2 Limits of detection and quantification 48
4.2.1 HPLC 48
4.2.2 Colonies enumeration 49
4.2.3 Method recovery 49
4.3 Initial effluent characterization 50
4.4 Electrokinetic experiments 51
4.4.1 pH, conductivity, and voltage 51
4.5 PPCPS degradation 54
4.5.1 PPCPs degradation in Set 1 – UV treated effluent 55
4.5.2 PPCPs degradation in Set 2 – Fresh effluent 57
4.6 Culturable organisms 61
5. Conclusions 68
6. Future Perspectives 71
7. References 74
8. Appendix 91

List of Figures

Figure 2.1 - Sources and pathways of PPCPs into the environment 13
Figure 2.2 - Tertiary treatment technologies 20
Figure 2.3 - Main transport mechanisms in EK. 26
Figure 2.4 - Schematic SPE procedure 31
Figure 2.5 - Basic components of an HPLC system 32
Figure 3.1 - Location of Quinta do Conde WWTP 34
Figure 3.2 - Simplified flow diagram of Quinta do Conde WWTP unit processes 35
Figure 3.3 - Overview of the experimental design 36
Figure 3.4 - Microcosmos of the electro-reactor 37
Figure 3.5 - Laminar flow chamber used in microbiology experiments and elements used 41
Figure 3.6 - Overview of the microbiology procedure for CFUs enumeration 43
Figure 3.7 - Manifold SPE. Detail of the elution step 43
Figure 4.1 - Chromatogram with stock solution with a mixture of the target PCPPs in a concentration of 7.5 mg/L 47
Figure 4.2 - Chromatograms of recovery assays Set 1 - 0.3 mg/L. a) Water type II spiked at 0.3 mg/L b) EFF UV spiked at 0.3 mg/L 50
Figure 4.3 - Mean initial and final pH in Set 1 and Set 2. 52
Figure 4.4 – Mean and standard deviation of conductivity in Set 1 and in Set 2. 53
Figure 4.5 - Voltage evolution between a pair of electrodes. 53
Figure 4.6 - Chromatograms from EK process in UV-C treated effluent 56
Figure 4.7 – Overall PPCPs mean degradation from UV-C treated effluent and from fresh effluent.... 60
Figure 4.8 - False positive in F1 - EK6 non spiked, after 72 h of incubation at 37 °C 64

List of Tables

Table 2.1 - Risks and benefits of reclaimed water for non-potable uses. 7

Table 2.2 - PPCPs classification and representative compounds........ 10

Table 2.3 - Target PPCPs under study 14

Table 2.4 – Indicative Log_{10} removal of indicator microorganisms and pathogens in wastewater. 22

Table 3.1 - Secondary effluent sampling dates and experimental treatment gave to the effluent prior EK assay 36

Table 3.2 - Main operational parameters used in each assay 38

Table 3.3 - Gradient mode used in HPLC run, at a constant flow of 1.5 mL/min........ 44

Table 4.1 – Comparation of curve equations and calculated linearity parameters for every compound under study, measured in the DAD-B channel (λ = 282 nm). 48

Table 4.2 - Method detection limit of the microbiology method........ 49

Table 4.3 - Method recoveries obtained in spiked effluent........ 50

Table 4.4 - Initial characteristics of the secondary effluent samples collected in Quinta do Conde WWTP 51

Table 4.5 - PPCPs degradation from UV-C treated effluent 55

Table 4.6 - PPCPs degradation from Set 2 58

Table 4.7 - Total culturable microorganisms obtained in Set 1 and Set 2 experiments........ 62

Abbreviations and symbols

H^+	proton
OH^-	hydroxyl ion
•OH	hydroxyl radical
1c-cell	one compartment cell
AC	activated carbon
ACE	acetone
ACN	acetonitrile
AOPs	advanced oxidation processes
ARB	antibiotic resistant bacteria
BDD	boron doped diamond
BOD	biological oxygen demand
CAF	caffeine
CAS	conventional activated sludge
CBZ	carbamazepine
CFUs	colony forming units
CI	current intensity
COD	chemical oxygen demand
CW	constructed wetlands
DC	direct current
DCF	diclofenac
DOM	dissolved organic matter
EC	European Commission
EDCs	endocrine disruptive compounds
EfOM	effluent organic matter
EK	electrokinetic
EOCs	emergent organic contaminants
EU	European Union
F1	fresh secondary effluent sample #1
F2	fresh secondary effluent sample #2
GC	gas chromatography
HA	humic acids
HPLC	high performance liquid chromatography
HRT	hydraulic retention time
Kow	octanol water partition coefficient
LC	liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
LVR	log value reduction

MeOH	methanol
MFCs	microbial fuel cells
MLD	method limit of detection
MLQ	method limit of quantification
MMO	mixed metal oxide
MPs	micropollutants
MS	member states
OXY	oxybenzone
PDA	potato dextrose agar
POPs	persistent organic pollutants
PPCPs	pharmaceuticals and personal care products
RPLC	reverse phase liquid chromatography
RO	reverse osmosis
SMX	sulfamethoxazole
SPE	solid phase extraction
TSS	total suspended solid
UV	ultraviolet
UV-C	ultraviolet radiation in the wavelength of 254 nm
UWWTD	urban wastewater treatment directive
VBNC	viable but nonculturable
WFD	water framework directive
WWTPs	wastewater treatment plants

1. Introduction

Many drivers such as populational growth, urbanization, industrialization and high consumption trends, have been leading to the increase in water demand, with impacts in the quantity and quality of freshwater supplies. Water is indispensable in many economic sectors and currently, around 70% of global freshwater is withdrawn for agriculture irrigation purposes (FAO, 2017). By 2050, it is expected that the water demand will increase by 55%, with agriculture remaining the largest consumer sector (IWA, 2018; WWAP, 2019).

Water shortage periods are becoming more frequent as result of climate change and are a major concern worldwide and the center in political agendas. It is estimated that about 4 billion people in the world experience severe water scarcity during at least one month in the year. Water availability varies temporally and spatially, and local conditions in some countries will be worse than the global situation (Mekonnen & Hoekstra, 2016; Boretti & Rosa, 2019). Water reuse, coupled with water savings and efficiency measures, represents a promising approach with potential to reduce the gap between availability and demand of water, as well as a tool that contributes to a sustainable water management due to the multiple uses in which the effluent can be applied (European Comission, 2016a).

Used in European Union (EU) territory in small extent, particularly by countries in the Mediterranean basin (one of the most affected areas by water scarcity), southern Europe countries such as Greece, Italy, Cyprus, France, Malta, Spain and Portugal have over the years develop their own guidelines for water reuse applications, being the only Member States (MS) with national guidelines in water reuse from all EU. For several years, the EU was lacking a legal instrument on water reuse, capable of harmonizing the different practices already adopted by some MS (Voulvoulis, 2018; EIA, 2018).

The European Commission (EC) presented a proposal for the Regulation on minimum requirements for water reuse in May of 2018 to the European Parliament and the Council of the European Union, having resulted in a provisional agreement in the beginning of December 2019 (European Commission, 2019). Nevertheless, this Regulation is flexible, as the criteria must be adapted according to the specificities of each territory, considering geographical, climatic, social, economic and environmental conditions (EIA, 2018). Wastewater treatment quality standards will continue to follow the Urban Wastewater treatment Directive (UWWTD) (91/271/ECC) requirements, with the addition that the treatment for reuse purposes, will have to be complemented with an advanced treatment process (tertiary treatment) in order to achieve the quality criteria for water according to the use, minimizing human and environmental risks, as stated in the Annex I of the proposal (European Union, 2019).

Effluents from Wastewater Treatment Plants (WWTPs) have been considered point sources of microbial pollution and of a wide variety of micropollutants (MPs) such as Pharmaceuticals and Personal Care Products (PPCPs). PPCPs belong to a wider class of compounds called Emergent Organic Contaminants (EOCs). These MPs arrive to WWTPs as a result of the input into the sewage systems by anthropogenic sources and some compound classes, due to its physicochemical characteristics, make EOCs recalcitrant to the removal along conventional urban WWTPs, that were mostly designed many years ago, not having in mind MPs removal (Bolong et al., 2009; de Oliveira et al., 2020). The

removal efficiency of a MPs in WWTPs varies according to compound intrinsic characteristics, such as hydrophobicity, volatility, solubility, biodegradability, molecular size and external factors, related with the type of treatment applied, the type and mixture of pollutants present in the wastewater, pH, temperature (Luo et al., 2014).

Quality standards for water reuse are based on physicochemical and microbiological parameters. Microbiological monitoring of treated wastewater is crucial to prevent environmental and, ultimately, an health risk related with the use of reclaimed water. Microbiological quality in water is assessed by the identification of indicator microorganisms that are intended to be representative of a certain environment. For fecal contamination, *Escherichia coli* (*E.coli*) is one of the most used bacteria indicator (Naidoo & Olaniran, 1990). As an example, fungal communities are not monitored in final effluents but there are many studies assessing its abundance and diversity in sewage sludge (Gonzalez-Martinez et al., 2018; Assress et al., 2019.), in surfaces and in the air of WWTPs (Viegas et al., 2014) identifying pathogenic fungi in wastewater. In an irrigation scenario, concerns addressing the potential presence of pathogenic microorganisms in effluent, that are not included in routine monitoring, have been made, questioning the long-term impact of reclaimed water in the soil ecosystems due to the input of exogenous microbiota and MPs such as PPCPs at trace levels (Becerra-Castro et al., 2015).

Tertiary treatment (or polishing treatment), is used to disinfect the wastewater, remove nutrients and MPs. The value added in these technologies is increasing year after year, as it also represents a promising research field, boosted by the stricter regulations. According to the quality required for a certain use of water, and the upstream water treatment quality and technology implemented, several types of polishing treatment can be considered according to each case. The are many options of emergent single and/or combined chemical-based, physical, and biological polishing treatments available. Conventional ultraviolet (UV) disinfection has increased its application in WWTPs, as it does not require addiction of chemical reagents (e.g. peracetic acid) or lead to the production of harmful by-products, such as trihalomethanes and haloacetic acids, as it happens in chlorination. UV disinfection rely on the use of mercury lamps, that are fragile, have a relative short life span and in the end of life need to be proper recycled to not constitute a danger (Collivignarelli et al., 2017; Umar et al., 2019).

In the seek for a versatile, cost-effective and sustainable technology, electrochemical reactors have been designed to promote the remediation of several classes of EOCs, into several environmental matrices such as soils (Ferreira et al., 2017a; Guedes et al., 2019; Lopes, 2018; Dionísio, 2019) in effluents (Ferreira et al., 2018; Magro, 2019) and in sewage sludge (Guedes, 2015). In the case of the present dissertation, electrokinetic (EK) process was applied to an effluent matrix. EK remediation, also referred as electrochemical decontamination, performs environmental clean-up based on an application of a low-level direct current (DC), of the order of milliamps, between inert electrodes inserted in a matrix. The induced electric field promotes chemical transformations and transport mechanisms (Acar & Alshawabkeh, 1993).

1.1 Objectives and questions

The present work aims to evaluate the EK process as a wastewater treatment technology able to contribute for a safer and better quality final effluent, by assessing its feasibility as a follow--up/complement of a disinfection step or as a single tertiary treatment technology.

As so, in the present dissertation the following questions are addressed:

1) Does the EK process contribute to an efficient degradation of the PPCPs under study:
   1.1) From effluent after a UV-C disinfection stage?
   1.2) From effluent after the secondary treatment stage?
2) Does the low-level DC applied to the effluent influence the colony forming units grown in agar media?

All assays were conducted using secondary effluent (collected in the secondary settling tank) from Quinta do Conde WWTP (Simarsul, PORTUGAL). The presented electro-reactor was designed at a microcosmos scale, consisting of a one-compartment glass cell (1c-cell) with a pair of rectangular shaped electrodes inserted in the effluent matrix, operated with an effluent working volume of 500 mL, under a constant current intensity of 50 mA. The chosen target contaminants under study used to spike the effluent at a concentration of 0.2 mg/L were: Caffeine (CAF), Sulfamethoxazole (SMX), Carbamazepine (CBZ), Diclofenac (DCF) and Oxybenzone (OXY), due to their widespread consumption and in some cases, recalcitrant characteristics in conventional wastewater systems.

The experimental work can be divided into two main phases according to the type of treatment given to the effluent prior assays:

In the first set of experiments (Set 1) aimed to answer question 1.1), one effluent sample was collected and in a bench-scale UV apparatus, the effluent was exposed to UV-C radiation (wavelength of 254 nm) prior EK assays to simulate a disinfection stage. Different periods of EK treatment were tested as an effluent polishing step. Monitored parameter at the beginning and at the end of experiments were pH, conductivity and PPCPs concentration.

In the second set of experiments (Set 2), all EK assays were performed directly in secondary effluent. In order to answer question 1.2), two fresh effluent samples (F1 and F2), were brought from the WWTP, in different days (14 days apart). In this set, a new typology of experiment was also introduced, and assays with spiked effluent and exposed to UV radiation for 6 h (without DC), were carried out to simulate UV-C photolysis of PPCPs. The experimental procedure during EK experiments was the same, with the difference that the reactors were covered.

To answer question 2), for both 1.1) and 1.2), at the end of each assay, an aliquot of effluent was taken from each reactor to perform enumeration of colony forming units (CFUs) of culturable bacteria or fungi, using the spread plate technique in potato dextrose agar (PDA) media, incubated at 37 °C for 24 h. Log value reductions (LVR) were calculated to evaluate the disinfection efficiency of the EK process.

Organic fraction was extracted by solid phase extraction (SPE) and analyzed by HPLC- DAD-FLD.

1.2 Dissertation structure

The present dissertation is organized according the following chapters:

1. Introduction: Insights related with the scope, main objectives, research questions and dissertation structure.
2. Literature Review: Presentation of theoretical framework that supports the work developed, previous studies and research done related with the scope.
3. Materials and Methods: Description of the materials use and presentation of the experimental plan, namely different stages developed.
4. Results and Discussion: Presentation of the results and respective discussion.
5. Conclusion: Main outcomes.
6. Future Perspectives: Suggestions for future works.
7. References.
8. Appendix: Support material (e.g. relevant chromatograms or tables). A poster elaborated for the *11th Encontro Nacional de Cromatografia – 11ENC,* held in December 2019 in Aldeia dos Capuchos, Congress center, Caparica.

2. State of the art

2.1 Water reuse

The water demand-availability nexus has been driving the seek for alternative sources of water all over the world. A paradigm shift is happening, as the vision of wastewater as a waste is changing, alongside with the strict legislation and technology improvement, the effluent produced in WWTPs is seen as a viable resource and alternative, that can alleviate the exploitation on freshwater supplies and bring many economic, social and environmental benefits to society (Voulvoulis, 2018). From a holistic point of view, wastewater treatment has evolved along the years, to a resource recovery orientated system, aligned with the principles of circular economy.

The use of freshwater is cross-cutting to several sectors. According to the European Environmental Agency (EEA) indicator "Use of freshwater resources in Europe", agriculture, particularly crop irrigation, accounts with 59% of total water use, being the most significant consumer sector exerting the highest pressure in renewable freshwater resources (data from 2017). In southern European countries, crop irrigation tends to be quite intensive, as climate change tends to aggravate the increase of evapotranspiration and precipitation decrease. In addition, water is also used in energy production (18%), manufacturing industries (11%) and household sector (9%) (EEA, 2019). Water reuse (or reclaimed water), can satisfy most water demands, as long it is treated according to the use. According to the ISO 6075 - 1:2014 "Guidelines for treated wastewater use for irrigation projects", water reuse is defined as "the use of treated wastewater for beneficial use". The US Environment Protection Agency (EPA), considers water reuse as the treatment of municipal wastewater in order to achieve a certain quality so it can be beneficially reused. It also distinguishes water reuse according to 5 categories (European Commission, 2016b):

1. ***De facto* reuse** – When the reuse of treated wastewater is practiced but is not officially recognized.

2. **Direct potable uses** – Introduction of reclaimed water directly to a municipal WWTP and/or to an advanced treatment facility. Prior treatment the water is sent to a drinking water treatment plant for further treatment and distribution.

3. **Indirect potable uses** – Wastewater is placed into an environmental buffer (e.g. river, lake, aquifer), to be later used as a source of drinking water.

4. **Non-potable uses** – All uses that does not involve potable uses.

5. **Potable reuse –** Planed augmentation of a drinking water supply, to include reclaimed water.

In fact, the use of reclaimed water for non-potable uses represents a promising approach as the water does not need to achieve drinking water quality. In Table 2.1 an overview of some environmental, social and economic benefits of water reuse for non-potable uses and risks are represented.

Table 2.1 - Risks and benefits of reclaimed water for non-potable uses (Adapted from European Commission, 2016b)

	Risks	Benefits
Environmental	• Potential contamination by emergent organic contaminants (EOCs), their metabolites and degradation products. • Pathogens that can regrowth after a disinfection stage in storage tanks (e.g. bacteria, protozoa, virus) • Spread of antibiotic resistance bacteria and antibiotic resistance genes. • Effect of effluent composition (e.g.mineral salts) in crop productivity when used for irrigation purposes.	• Effluent is an alternative source of water, widely available. • Effluent supplies macro and micro nutrients to plants. • Tool that helps to manage water scarcity (at a regional and river basin scale). • Lower environmental impact than other alternative sources (e.g. desalinization, construction of dams). • Low carbon footprint depending on the used energy source. • Contribute to ecological restauration (e.g. recovery of water channels and creation of ecological corridors in urban areas). • Limited freshwater supplies can be kept for potable uses. • Preservation of the good quality of water bodies.
Social	• Negative public perception. • Lack of engagement of all interested stakeholders: farmers, water sector professionals, researchers, and general society.	• Creation of jobs in the water sector in development, operation and maintenance of advanced water treatment, as well across in all the supply chain. • Promotes quality of life and wellbeing as is a source of water to be used in urban landscapes. • Contribute to the achievement of the UN Sustainable Development Goals (namely goal no 6).
Economic	• Wastewater sector uses the best technology available that has already proven its value in the market. • Defining a water price that is competitive, when compared to drinking water price. • Irrigation machinery and costs associated with maintenance.	• Improvement of water security (in quantity and quality), of water dependent sectors as food industry, agriculture, tourism. • Possibility of industrial symbiosis. • Water reuse technologies represent a new attractive market. • Water reuse schemes can lead to water savings as will avoid the costly extraction, transport and treatment of freshwater supplies. • Financing of reuse projects through EU funds.

In the EU, water reuse has been long ago referred into two different legislative instruments: in the UWWTD (91/271/EEC), article 12, that refers that "treated wastewater shall be reused whenever appropriate", and in the Water Framework Directive (WFD) (2000/60/EC), annex VI, is mentioned as a possible supplementary measure which member states can adopt (Alcande-Sans & Gawlik, 2017). Nevertheless, in this context water reuse lacked a definition and specifications of the water quality required for further reuse.

Many EU MS have shown a proactive attitude and have been implementing their own measures in order to tackle issues related with water shortages, leading water reuse standards to vary significantly, not only in the parameters considered but also in the values associated. Several advances in the water policy in EU have been made during the last years, towards the development of a legal instrument capable of uniform the use of treated wastewater and increase confidence in the use. Reclaimed water has been identified as a priority in the management of water resources in the European Commission communication "A Blueprint to Safeguard Europe's Water Resources". In 2015, the European Circular Economy Plan (COM/2015/0614) referred that water reuse, with the adequate treatment to assure a safe use could constitute a valuable resource capable of taking pressure on over-explored EU water resources. Agriculture and aquifer recharge were identified as being the highest reclaimed water demand sources, with potential to alleviate water scarcity (APA, 2019a; Alcande-Sans & Gawlik, 2017).

On February of 2017, the Joint Research Center of the European Commission made a report proposing minimum requirements for water reuse, on a basis of a risk management approach, targeting agricultural irrigation and aquifer recharge. Nevertheless, major concerns have been pointed out, mainly related with the transnational commercialization of crops irrigated with reclaimed water (Deviller et al., 2020). This report was a key element into the upcoming EU regulation that followed. However, several gaps have been pointed out into the technical report. According to the Scientific Committee on Health, Environment and Emerging Risks (SCHEER), the report lacks information about the following aspects: addressing i) EOCs, (ii) the role of WWTPs effluents in spreading antibiotic resistance genes (and bacteria), (iii) the risk and the possibility of increased toxicity of the disinfection by the use of treated wastewater (Alcande-Sans & Gawlik, 2017; SCHEER, 2017).The pathways and long term effects of exposure of organic MPs in agricultural systems are not included either (Helmecke et al., 2020).

Later, on May 2018, the EC made a "Proposal for a Regulation of The European Parliament and of the Council on minimum requirements on water reuse" (COM/2018/337), based on an impact assessment. In this proposal, the legal instrument proposed was a Regulation, which means that all member states must do the transposition into national law of the EC regulation. The preferred option for agricultural irrigation was a "fit for purpose" - approach, that means the level of treatment of the water for reuse must be set according to the application. The proposal also set in Annex I, minimum quality requirements for water reuse in agriculture and in the Annex II, a request for a Water Reuse Risk Management Plan is made, including public

and environmental hazards (European Commission, 2019). In December 2019, an agreement on a regulation was made. As so, it is expected that MS will issue national regulations based on the EU regulation.

It is estimated that about 1 billion m³ of reclaimed water is reused annually in Europe. This value only represents 2,4% of the total treated urban wastewater effluents in 2019. The potential for reuse is much higher, estimated to be in the order of 6 billion m³ per year by 2025 (European Union, 2020). Across Europe there are some well implemented projects of water reuse, driven by water scarcity and water demand for multiple uses in high populational areas.

Considering the Portuguese case, in 2005 Portugal implemented a regulation on the reuse of reclaimed water for irrigation purposes (urban areas and agriculture), through the NP 4434 by the Portuguese Institute of Quality (Marecos & Albuquerque, 2010). In 2017, the water factory of Tejo Atlântico, that serves the city of Lisbon, produced a total of 3.1 million m³ of recycled water, in which 83% were used for internal uses as equipment washing, reagent preparation, cleaning and irrigation systems, 17% were used for street washing, cars washing, irrigation of green spaces, irrigation of crops, climatization systems (Águas do Tejo Atlântico, 2019). More recently, in 2019 the Portuguese law (D.L 119/2019 21 of August), come to set the use of treated wastewater for non-potable uses, promoting its correct use avoiding harmful effects to the health and to the environment. The level of treatment is supported by a "fit for purpose" concept which means that the resources are directed to where they are most needed and with the guarantee that the water quality is adequate to the use. The production and use of reclaimed water is compatible with the following uses: irrigation (where 5 classes of quality are defined), urban such as ecosystems support, recreational and landscape uses, street washing, firefighting, cooling, flushing, and industrial uses. The microbiological parameters considered for monitoring are *E.coli* and intestinal nematodes (helminth eggs) (APA, 2019a).

2.2 Pharmaceutical and Personal Care products (PPCPs)

The focus of the chemical pollution has passed from the priority pollutants, such as Persistent Organic Pollutants (POPs), to the emergent contaminants that represent a much bigger fraction of the total organic chemicals that are being produced and whose risks are relatively unknown (Daughton, 2004). PPCPs can be described as products used for personal health or cosmetic reasons as well for veterinary purposes, including its metabolites and degradation products (Daughton & Ternes, 1999).

This definition comprises a wide group of organic (carbon based) synthetic compounds, with variable structures and physicochemical properties, that are grouped in sub classes according to their uses. PPCPs are a class of EOCs, that includes human nonprescription

and prescription drugs, veterinary drugs, illegal drugs, nonsteroidal anti-inflammatory drugs (NSAIDS), antibiotics, hormones, lipid regulators, anticonvulsants, antihypertensives as well as sunscreens, synthetic musk's, food additives, insect repellents among others (Dhodapkar & Gandhi, 2019). Table 2.2 shows some representative compounds, as an example, belonging to PPCPs sub classes.

Table 2.2 - PPCPs classification and representative compounds (Adapted from Li & Wong, 2013)

	Sub class	**Representative compounds**
Pharmaceuticals	Antibiotics	Clarithromycin, Erythromycin, Sulfamethoxazole, Sulfadimethoxine Ciprofloxacin, Norfloxacin, Chloramphenicol
	Hormones	Estrone (E1), Estradiol (E2), Ethinylestradiol (EE2)
	Analgesics and anti-inflammatory drugs	Diclofenac, Ibuprofen, Acetaminophen Acetylsalicylic acid
	Antiepileptic drugs	Carbamazepine, Primidone
	Blood lipid regulators	Clofibrate, Gemfibrozil
	β-blockers	Metoprolol, Propanolol
	Contrast media	Diatrizoate, Iopromide
	Cytostatic drugs	Ifosfamide, Cyclophosphamide
Personal Care Products	Antimicrobial agents/Disinfectants	Triclosan, Triclocarban
	Synthetic musks/Fragrances	Galaxolide (HHCB), Toxalide (AHTN)
	Insect repellants	N,N-diethyl-m-toluamide (DEET)
	Preservatives	Parabens (alkyl-p-hydroxybenzoates)
	Sunscreen UV filters	2-ethyl-hexyl-4-trimethoxycinnamate (EHMC) 4-methyl-benzilidine-camphor (4MBC)

PPCPs contribute to an improvement of the overall quality of life and together with increasing medical needs the production of this class of EOCs escalated. As result of the demand, anually, the production of these PPCPs can reach 2 x 10^6 t. Its consumption is therefore expected to continue increasing, following the increasing populational trend (Gibson, 2010; Dhodapkar & Gandhi, 2019).

Some PPCPs are considered Endocrine Disruptors Chemicals (EDCs), meaning that can disrupt the endocrine system of non-target organisms (Cizmas et al., 2015). Only regarding pharmaceuticals, it is estimated that more than 4000 types have been developed to be biologically active, which means these compounds have medical properties that allow them to act in specific metabolic, enzymatic and cellular mechanisms even at low doses. Regarding chronic toxicity, the sub-lethal effects can go from histological changes, biochemical response and gene regulation to behavioral changes (Dhodapkar & Gandhi, 2019; Ebele et al., 2017).

Recently, an increasing awareness about the pseudo-persistence (due to the constant release into the environment), bioaccumulation potential and ecotoxicological effects, lead to major efforts in order to understand the occurrence, distribution and risks of PPCPs in biota. This was made possible due to the advances in sample preparation techniques, analytical tools, and mass detectors with higher sensitivity. The use of liquid chromatography coupled to mass spectrometry (LC-MS) in particular, allowed the detection of polar compounds such as PPCPs, present at trace level concentrations (Dhodapkar & Gandhi, 2019).

Most laboratory experiments are directed to target parent compounds, so information about by-products and metabolites of PPCPs is scarce mostly due to the difficulties in separation and posterior identification (Yin et al., 2017). Nevertheless, most PPCPs are not regulated and there is still lack of correlation about the concentration levels and long-term adverse effects both in humans and aquatic environment and concerns arrive mostly due to possible synergistic interactions of mixtures of PPCPs (Archer et al., 2017).

Sousa et al (2019) conducted the first study providing a seasonal and spatial monitoring of all the 17 EOCs from the EU Watch List (Decision 2015/495). The monitoring was done during a period of a year, in Ave and Sousa River located in the Northern of Portugal. A total of 4 sampling campaigns (one representative of each season) were done in 15 sampling points chosen based on the location of tributaries and urban WWTPs whose discharges could lead to adverse effects on the rivers. 120 surface water samples were collected and analyzed for the 17 EOCs in the Watch list. The analytical methods used were ultra-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) and gas chromatography mass spectrometry (GC-MS). In both rivers, from the 17 EOCs under study, 8 were found in Ave river: an UV filter (2-ethylhexyl-4-methoxycinnamate), 4 pharmaceuticals (azithromycin, clarithromycin, erythromycin and diclofenac) and 3 pesticides (imidacloprid, clothianidin, thiamethoxam). In Sousa river, 13 were found: an estrogen (estrone), an antioxidant (2,6-ditert-butyl-4-methylphenol), 2 herbicides (oxadiazon and triallate) as well as the same EOCs identified in Ave river, except for clothianidin, and with the addiction of 2 pesticide (thiacloprid and methiocarb). The most frequent found EOCs in both rivers were diclofenac, azithromycin and 2-ethylhexyl 4-methoxycinnamate. A risk quotient (RQ) assessment based on the quotient between the measured environmental concentrations and the Predicted No-effect Concentration levels (PNEC), was calculated. Results showed that diclofenac showed a high risk (RQ>1) in both rivers, in most sampling points. The knowledge of this information is of extremely relevant to decision makers, to develop mitigation strategies at source and risks assessments.

Monitoring concentration of PPCPs in urban WWTPs, can help to elucidate possible sources and distributions, as well as assessing underlying factors such as compounds consumption and compound seasonality. The occurrence of PPCPs is considered to be strongly dependent of local diseases and on treatment habits (Salgado et al., 2010).

2.2.1 Sources and pathways of PPCPs

Pharmaceuticals and personal care products are grouped together because they have similar sources and distribution, as both enter the environment as a result of usage, as a by-product or a metabolite. Urban WWTPs receive wastewater from residential households, hospital and industries. The main input of PPCPs is from households, from use and excretion or due to the incorrect disposal of pharmaceuticals that are flushed down the sewer system. Once in urban WWTPs, their removal varies according to their physicochemical properties and the type of technology implemented in the WWTPs (Dhodapkar & Gandhi, 2019).

Urban WWTPs are most likely the principal route of introduction of these compounds into the environment (Daughton & Ternes, 1999), as they fail to remove PPCPs, and act as a point source of release into aquatic systems. By 2050, around 70% of the population will be living in urban areas so WWTPs will suffer an increasing pressure to adapt and to install effective advanced treatment step, capable of removing emerging contaminants in order to produce a safer effluent (IWA, 2018; Dhodapkar & Gandhi, 2019). Among the pharmaceutical group, antibiotics is the class that receives more attention due to its overuse, and systematic release into sewage systems contributing to the spreading of antibiotic genes and bacteria in surface waters (Dhodapkar & Gandhi, 2019; Cuerda-Correa et al, 2019). Around 40% of antibiotics that are produced in the United States are used by the farming industry and for aquaculture (Gibson, 2010).

In urban WWTPs, the production of sewage sludge is one of the more valuable by-products of wastewater treatment due to the high content in nitrogen, phosphorus, and organic matter. Conventional activated sludge fails to remove MPs such as PPCPs and the application of stabilized sewage sludge – biosolids, in agricultural land can also bring many risks, related with the spreading of pathogens or MPs that are not included in legislation, and so are not monitored. Due to strict legislation in EU, landfill disposal of biosolids is banned in many countries (Collivignarelli et al., 2019).

Occurrence of PPCPs has been detected at trace levels (range from ng/L to µg/L) in surface waters due to effluent discharges of treated and untreated wastewater directly into water bodies or due to agricultural run-offs (Wang et al., 2015; Patel et al., 2019). In water bodies, PPCPs may suffer dilution, and be affected by weather conditions (Morone et al., 2019). Figure 2.1 illustrates a simplification and possible entryways of PPCPs.

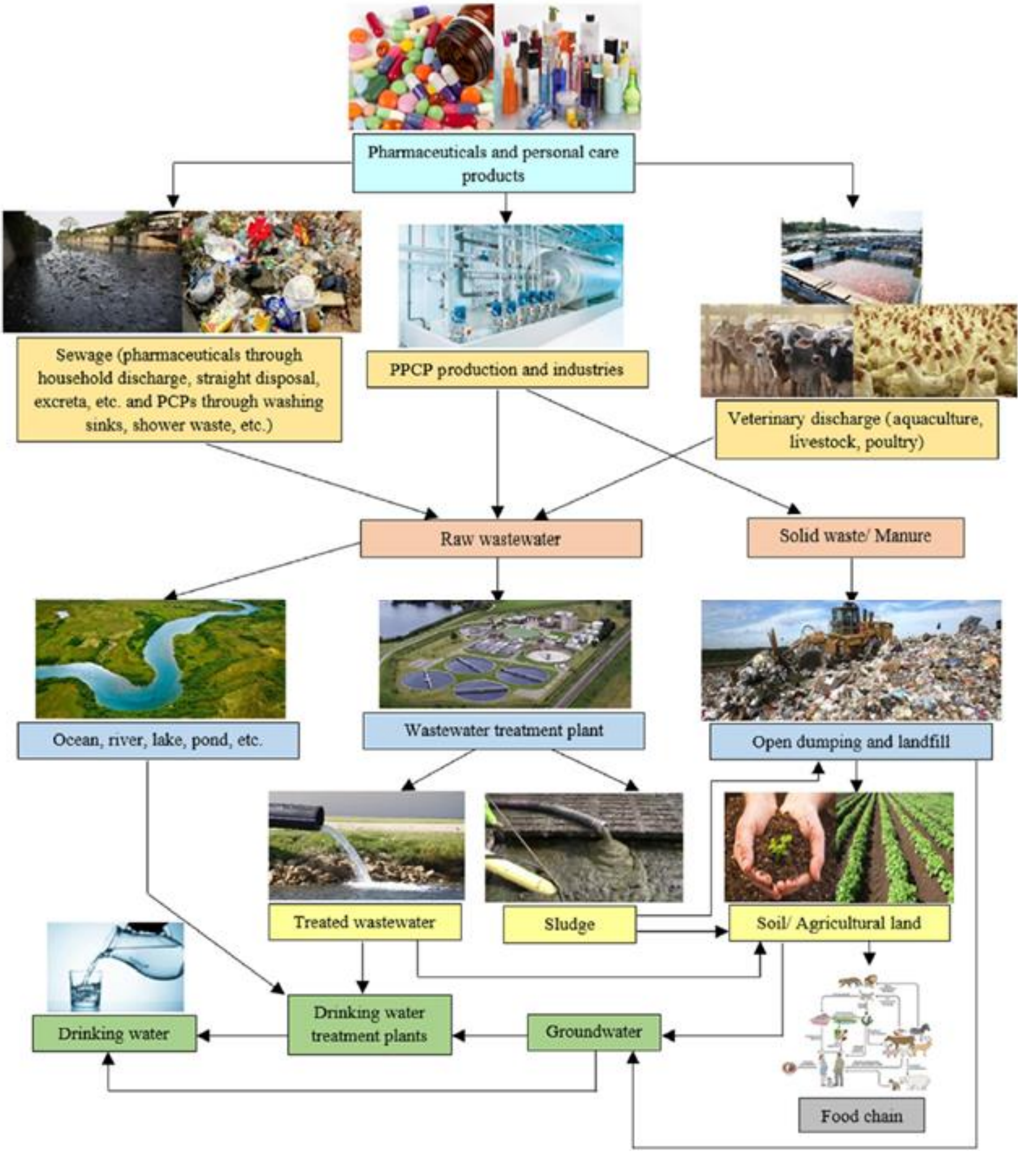


Figure 2.1 Sources and pathways of PPCPs into the environment (Source: Morone et al., 2019)

Therefore, PPCPs have been found in groundwater (Lapworth et al, 2012), in river sediments that act as sinks and accumulate contaminants (Díaz & Peña-Alvarez, 2017), sewage sludge (Ternes et al., 2005), in gardens irrigated with effluent (Biel-Maeso et al., 2019) and even in drinking water (Aristizabal-Ciro et al., 2017).

Water reuse for vegetables crop irrigation brings many concerns, as pharmaceutical active compounds can suffer uptake and translocation to edible parts, and potentially enter the food chain. In arid and semi-arid regions plant transpiration is high, and accumulation of PPCPs in the leaf parts may occur (Dodgen et al., 2015).

2.3 PPCPs under study

In the present dissertation, five model PPCPs (Table 2.3), namely four pharmaceuticals and one personal care product were chosen due to its widespread consumption and frequent occurrence in the environment in result of poor removal from WWTPs. The compounds are: CAF, SMX, CBZ, DCF and OXY.

Table 2.3 - Target PPCPs under study

Compound name	Caffeine (CAF)	Sulfamethoxazole (SMX)	Carbamazepine (CBZ)	Diclofenac (DCF)	Oxybenzone (OXY)
Compound class	CNS stimulant	Bacteriostatic antibiotic	Anticonvulsive	Anti-inflammatory	UV filter
Chemical structure					
Molecular formula[1]	$C_8H_{10}N_4O_2$	$C_{10}H_{11}N_3O_3S$	$C_{15}H_{12}N_2O$	$C_{14}H_{11}Cl_2NO_2$	$C_{14}H_{12}O_3$
Molecular Weight[1] (g/mL)	194.19	253.28	236.27	296.1	228.25
Solubility in water[1] (mg/L)	2.16×10^4 (at 25 °C)	610 (at 37 °C)	17.7 (at 25 °C)	2.37 (at 25 °C)	69 (at 25 °C)
Acid dissociation constant – pKa (at 25 °C)[2]	14.0	pKa1=1.6; pKa2=5.7	13.9	4.15	8.07
Logarithm of the octanol-water partition coefficient - Log Kow[1]	-0.07	0.89	2.45	4.51	3.79
Henry's law constant (atm-m³/mol at 25 °C)[1]	1.1×10^{-11}	6.62×10^{-13}	1.1×10^{-11}	4.73×10^{-12}	1.5×10^{-8}
CAS number[1]	58-08-2	723-46-6	298-46-4	15307-86-5	131-57-7

[1]https://pubchem.ncbi.nlm.nih.gov/, [2]http://www.hmdb.ca/

2.3.1 Caffeine

Caffeine (CAF) is a naturally occurring methylxanthine alkaloid, commonly found in tea and coffee as well in many prescription drugs from analgesics to cold medicines. It is one of the most consumed stimulants globally, acting as a central nervous system stimulant (PubChem, 2019). CAF has been indicated as the most representative pharmaceutically active compound, as well as an anthropogenic marker of surface water pollution, based on its

consumption and constant input into water bodies. It is included in the US EPA List of High Production Volume Chemicals (Buerge et al., 2003; Li et al., 2020).

After human consumption, about 2% of the CAF dose ingested is metabolized in the liver by phase I enzymes (Cytochrome P450) and excreted in the form of metabolites, having suffered demethylation and hydroxylation reactions (PubChem, 2019). CAF metabolites are mainly derivatives of xanthine with methyl functional groups at different positions (He et al., 2018). CAF is susceptible to photolysis, as it absorbs UV light at wavelength >290 nm. The low Henry's law constant ($1.1x10^{-11}$ atm- m^3/mol), indicates that is not expected that CAF suffers volatilization from surface waters, and due to the low Log Kow (-0.07), it is also not expected that CAF suffers accumulation in soil and sediments, being likely to persist in water (PubChem, 2019).

In WWTPs, CAF has shown high removal efficiencies. Buerge et al (2003), analyzed influents and effluent of swiss WWTPs, and the concentration of caffeine detected ranged from 7 – 73 µg/L in influent to 0.03 – 9.5 µg/L in effluent, with an efficiency of elimination between 81% and 99.9% respectively. During the activated sludge treatment, biodegradation has been pointed out as the main process involved in the removal of this compound (He et al., 2018). Lakshmi & Das (2013), studied the removal of CAF from coffee effluent, using an oleaginous yeast, *Trichosporon asahii.* This filamentous fungi, has been previously pointed out as one of the dominant species in activated sludge, contributing also for activated sludge bulking (Zheng et al., 2011). By creating biofilm, the hyphae of *Trichosporon asahii* showed potential for removal of CAF and suspended solids (Lakshmi & Das., 2013).

2.3.2 Sulfamethoxazole

Sulfamethoxazole (SMX), belong to the class of antibiotics and it is a bacteriostatic antibacterial agent from the group of sulfonamides, known for interfering with the folic acid synthesis in susceptible bacteria. It is one of the most used synthetic sulfonamides. It is usually taken in combination with trimethoprim to decrease the risk of bacterial resistance (Pubchem, 2020).

Upon human consumption, SMX is excreted in the urine in the form of two main metabolites: N4-acetyl sulfamethoxazole and sulfamethoxazole-N1-glucuronide (Nguyen et al., 2018). These two metabolites have been reported to be able to transform back to the parent compound, contributing to increase the concentration of SMX into the environment. (Prasannamedha & Kumar, 2020). This was demostrated by Nguyen et al (2018) that studied the biotransformation kinetics of SMX and its two main metabolites (N4-acetyl sulfamethoxazole and sulfamethoxazole-N1-glucuronide) in activated sludge bioaugmented with a strain of *Achromobacter denitrificans*. In this study, the deconjugation of SMX in non-bioaugmented sewage sludge occurred faster than the biotransformation of SMX and the overall results indicate that the bioaugmented sewage sludge with *Achromobacter*

denitrificans contributed to enhance the biodegradation kinetics of SMX in about 100 times more, in comparation with a normal sewage sludge environment.

In WWTPs, due to the low Log kow (0.89), this compound is expected to suffer low sorption to sewage sludge. Besides biodegradation, photodegradation with UV has also been indicated as an effective degradation mechanism from aqueous media (Yang et al., 2017). Nevertheless this compound often resists to WWTPs processes and when present in effluent used for crop irrigation, has been identified to contribute to a decrease the functional diversity of soil microbial communities (Becerra-Castro et al., 2015).

2.3.3 Carbamazepine

Carbamazepine (CBZ) is an anticonvulsant, prescribed for the treatment of epilepsy. Due to its widely use and consumption, occurrence of CBZ pharmaceutical residues is frequent into different environmental compartments. Some authors also consider this compound an anthropogenic marker in surface waters (Zhang et al., 2008).

The principal CBZ metabolites are10,11-dihydro10,11-expoxycarbamazepine (CBZ-epoxide) and trans-10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-diol) (Zhang et al., 2008). CBZ-epoxide represents the primary metabolite, still retaining active properties of the parent compound. A total of 30 metabolites have been identified in human urine (Miao & Metcalfe, 2003), from which 9 have been detected in WWTPs (Ebele et al., 2017). In activated sludge, removal of CBZ is reported to be usually below 10% (Zhang et al., 2008). Photodegradation of CBZ induced by UV radiation can originate acridine, a carcinogenic and mutagenic photoproduct (Patel et al., 2019). Li et al (2013), studied the degradation pathways of CBZ in 3 types of soils (sandy clay loam, loam and silty clay) and after 120 days of incubation under aerobic conditions, mineralization of CBZ was not superior to 2%, for all soils types (Li et al., 2013).

2.3.4 Diclofenac

Diclofenac (DCF) is a non-steroidal anti-inflammatory drug (NSAID). It was one of the first pharmaceuticals being included in the first watch list of European Directive (2013/39/EU), with the aim to gather further data to support prioritization (Kovacic et al., 2016). This compound has been identified having potential risk for the environment and aquatic life.

The primary metabolite is 4-hydroxy-DCF (PubChem, 2020). Removal of DCF in WWTPs is limited and has been reported to occur between 21 and 40% (Zhang et al., 2008). WWTPs sludge adsorption and biodegradation are the main methods for DCF removal, probably due to the high Log kow (4.51), and that the removal efficiency in biological treatment is related with the hydraulic retention time (HRT) (Yan et al., 2019).

Oaks et al (2004) reported a link between the decline of the population of vultures in Pakistan, and renal failure due to DCF residues. The probable cause of exposure was through consumption of livestock treated with DCF. Between 2000 and 2003, it was observed a population decline of 34% to 95%, respectively. Nunes et al (2020) reported toxic effects of DCF on organisms from two different trophic levels, by feeding a benthic fish, *Solea senegalensis,* with a polychaeta *Hediste diversicolor,* previously exposed to DCF at environmental realistic concentrations (ng/L). After 28 days of exposure, oxidative stress was not observed but by assessing the activity of glutathione S-transferase, it was possible to observe that metabolic activity of both microorganisms increased, enabling to demonstrate that DCF causes physiological modifications in the microorganisms.

2.3.5 Oxybenzone

Oxybenzone (OXY) is an organic UV-filter, derived of benzophenone. It has been used as an ingredient in cosmetics and in sunscreens, due to the UV-A and UV-B radiation absorption properties, being considered a broad-spectrum UV filter (PubChem, 2019). OXY is also used in plastics and paints due to its photostable properties (Schneider & Lim, 2019).

UV-filters enter the human organism through percutaneous absorption, and only 4% of the dose is excreted in urine as metabolites and in unaltered form. Nevertheless, the majority is washed-off from the skin and goes to the sewer system entering WWTPs or goes directly to surface water during recreational water activities. OXY has been identified as a contributor for coral reef bleaching, leading to severe ecological impacts into the aquatic media. The presence of OXY in swimming pools can potentially represent a risk, as this UV-filter can react with chlorine present in water and create brominated by-products, known to be hazardous (Schneider & Lim, 2019). Detection of OXY in WWTPs present higher concentrations during the warmer months indicates that this is primarily a seasonal compound (Biel-Maeso et al., 2019). Due to the medium to high lipophilicity, high Log Kow (3.79), OXY may suffer partially sorption to sludge in biological treatment, but further detection in WWTPs effluents has also been confirmed (Schneider & Lim, 2019). In a study done by Matamoros & Salvadó (2012), using a nature based approach to remove EOCs, a surface flow full scale-constructed wetland (CW) and a hybrid pond, both planted with *Phragmites australis* and *Thypa* as a polishing treatment of real WWTPs effluent, showed a mean removal efficiency of OXY superior to 85% after a HRT of 8.5 days and 4 days, in the CW and the hybrid pond, respectively (Matamoros & Salvadó, 2012).

2.4 Wastewater treatment

Wastewater is an inevitable by-product produced by communities. It can be described as the result of the combination of both liquid and solid wastes, or water prevenient from residences, institutions, commercial and industrial establishments, as well with groundwater, surface

water and/or stormwater (Metcalf & Eddy, 2003). It is constituted by suspended solids, biodegradable organics, such as proteins, carbohydrates and fats that are measured in terms of chemical oxygen demand (COD) and biochemical oxygen demand (BOD), pathogens, heavy metals and nutrients such as phosphorus, nitrogen and carbon that are indispensable for plant and microorganisms growth, and when discharged in water bodies in excessive amounts can lead to water pollution problems such as eutrophication (Metcalf & Eddy, 2003). Effluent organic matter (EfOM) comprises dissolved and particulate organic substances. This includes natural organic matter (NOM) from surface waters, dissolved inorganics such as sodium, calcium and sulphate, priority pollutants and refractory organics that resist the conventional WWTPs unit processes upstream and are not removed effectively. Includes also soluble microbial products (SMP) that are derived from biological wastewater treatment (Metcalf & Eddy, 2003; Shon et al., 2006).

Conventional wastewater treatment comprises physicochemical and biological steps. It is divided in 4 main steps: preliminary treatment, primary treatment, secondary treatment, and tertiary (or advanced) treatment.

In preliminary treatment, the main objective is protecting the downstream treatment steps by implementation of physical operations. First, it is performed the screening of the larger objects present in the influent that arrive the wastewater treatment plant. This operation can be followed by a step for removal of sand, greases and floatables (Metcalf & Eddy, 2003).

Primary treatment has the objective of removing settleable suspended solids, and biological oxygen demand (BOD) associated with these solids. This is performed in settling tanks under the influence of gravity (Metcalf & Eddy, 2003). After this stage, according to the Portuguese law (D.L 152/97 of 19 June), CBO must be reduced at least in 20%, and the total suspension and dissolved solids must be removed at least in 50%.

Secondary treatment is designed to promote further removal of biodegradable organic matter (in suspension and soluble) that the primary treatment could not perform. Conventional Activated Sludge (CAS) has been referred as the most common biological treatment implement in urban WWTPs (Ensano et al., 2016; Gonzalez-Martinez et al., 2018). Other variants of biological treatment include fixed biomass systems, such as trickling filters or rotating biological contactors, that rely on the development of biofilm (Naidoo & Olaniran, 1990; Metcalf & Eddy, 2003).

CAS biological treatment is a suspense biomass system that utilizes a taxonomically diverse microbial community, consisting of archaea, bacteria, protists, and fungi that are able to metabolize, degrade and mineralize organic matter. Removal of nutrients such as nitrogen, is done by nitrifying microorganisms (mostly nitrifying bacteria and archaea), that oxidize ammonia to nitrite, and then nitrite-oxidizing bacteria oxidize nitrite to nitrate. Under anaerobic conditions, denitrifying bacteria reduce nitrate to gaseous forms. The efficiency of the process depends on solids retention time and on HRT. Oxygen needs to be supplied to

the system and sewage recirculated, in order to develop and to keep the microbial community (Johnston et al., 2019).

The fate of MPs along wastewater treatment stages is complex. PPCPs adsorption to sewage sludge microbial flocs in CAS also constitutes a removal pathway as the sludge suffer sedimentation and goes to further stabilization and disposal. Compounds with high Log Kow can absorb to the lipidic fraction of the sewage sludge and on bacterial lipidic cells through hydrophobic interactions (Radjenović et al., 2009). However, most PPCPs are polar and present hydrophilic characteristics so the removal in CAS systems is therefore limited (Ebele et al., 2017).

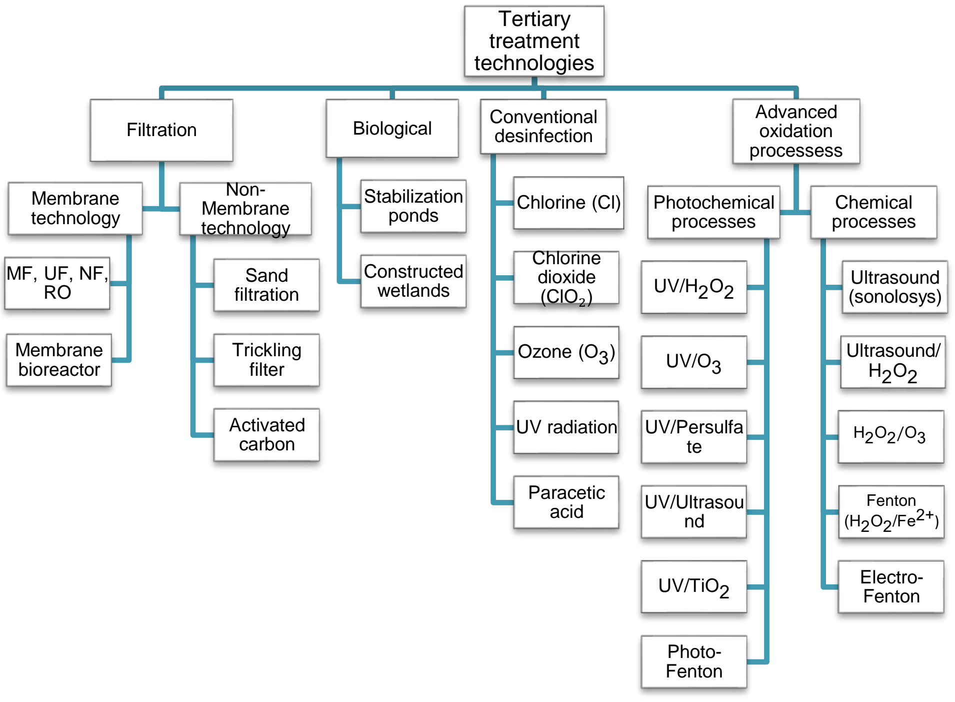
The discharge requirements of secondary effluent set by the Portuguese law (D.L 152/97 of 19 June) imply a minimum reduction of BOD between 70%- 90%, and for COD 75% minimum. In the case of the discharge of secondary effluent being made into sensitive areas such as estuaries and coastal areas susceptible to eutrophication, phosphorus and nitrogen must present a reduction in the secondary effluent of 80% and between 70-80%, respectively.

Tertiary treatment must be applied in the case of agglomerations with ≥ 10 000 p.e that discharge into sensitive areas. Appropriate treatment is required if the discharge of urban wastewater from agglomerations of less than 2000 p.e.is made into sensitive areas and if the discharge from agglomerations with less than 10 000 p.e is made into coastal areas (APA, 2019). Tertiary treatment is performed in order to improve the final quality of the effluent according with the requirements of the receiving water body, or for reclamation purposes by implementation of a polishing step that allows the removal of phosphorus and nitrogen, removal the remaining biodegradable organics, heavy metals, and pathogenic microorganisms (Metcalf & Eddy, 2003). According the European Environmental Agency latest data available on the level of wastewater treatment implemented in urban WWTPs (data from 2014) in cities with more than 150 000 p.e about 75.4% of WWTPs have implemented tertiary treatment (for nutrient removal) and more stringent treatment such as filtration and UV disinfection. Portugal present values below the European mean, with less than 20% of population connected to tertiary treatment (EEA, 2019b). In Portugal, most agglomerations are between 2 000 p.e and 10 000 p.e. and WWTPs that serve more than 150 000 p.e in Portuguese territory are only 14, with a representation of 40% of the total generated load (APA, 2019b).

2.5 Tertiary treatment

Several disinfection technologies are good candidates capable of meeting legal standards for effluent discharge and in reducing the concentration of pathogenic microorganisms, but having water reclamation in mind, only in some cases, these technologies go beyond compliance by being capable of achieving high removal of MPs such as PPCPs (Kehrein et al., 2020). In Figure 2.2 there are represented some of the principal tertiary treatment

technologies reviewed. Filtration and the conventional disinfection systems, divided in chemical (chlorine, chlorine dioxide, ozone and paracetic acid), photochemical (UV radiation), biological systems such as stabilization ponds and constructed wetlands are the most used technologies in full-scale WWTPs. Advanced oxidation processes (AOPs), are not still implemented in full scale but represent an emergent category for effluent treatment.



MF- Microfiltration, UF- Ultrafiltration, NF – Nanofiltration, RO – Reverse osmosis

Figure 2.2 - Tertiary treatment technologies (Adapted from Kehrein et al., 2020; Collivignarelli et al., 2017)

2.5.1 Removal of microorganisms and PPCPs in tertiary treatment systems

In addition to the conventional wastewater parameters monitored in WWTPs (e.g. COD, BOD), indicator microorganisms (e.g. total coliforms, fecal coliforms, enterococci, *Clostridium perfringens* and F-specific coliphages) are used as surrogates of pathogenic microorganisms such as enteric viruses and enteric protozoa (Giardia and *Cryptosporidium*) (Harwood et al., 2005). An indicator microorganism should be easy to detect in water samples, with a rapid and low-cost procedure, being present in higher concentrations than pathogens. These microorganisms should also be able to multiply in the environment and along the treatment system and must have survival characteristics of the pathogens. The choice of the indicator microorganisms to monitor, depends on the type of treatment, the quality of the influent and mostly, in the intended application of the effluent and sensitivity of the receiving waters (Momba et al., 2019). In other to ensure effluent microbiological safety, evaluation of the disinfection efficiency of a tertiary treatment system in the removal or inactivation of microorganisms is done by reporting the logarithmic (Log_{10}) reduction of microorganisms assessed by knowing the initial influent microorganisms concentration and the final

concentration after the unit process. Thus, 1 Log_{10} corresponds to 90% reduction, 2 Log_{10} to 99% reduction, 3 Log_{10} to 99.9%, 4 Log_{10} to 99.99%, and so on. The best tertiary treatment technologies available in WWTPs are only effective in ensuring microbial reduction according to the legislation standards (Momba et al., 2019; von Sperling et al., 2020).

More recent research on wastewater disinfection and due to new molecular-based microbial detection techniques revealed evidences related with a new range of emergent pathogens in effluent, that are not regulated. As an example, antibiotic resistant bacteria (ARB) are under the spotlight due to the risk in the decrease of antibiotics effectiveness on human and animal pathogens, as genetic material is not efficiently removed from WWTPs effluents (Rizzo et al., 2020; Ferro et al., 2015). From all eukaryotic microorganisms, fugal microbial communities are less studied after tertiary treatment, in comparation with secondary treatment done with a CAS system, were phylogenetic taxonomy studies have been performed with the interest of assessing the functional microbial communities, in which fungi are known to compete with bacteria for carbon sources, subtract decomposition and nutrient recycling by sorption and by production of intra and extracellular enzymes. Filamentous *Ascomycetes* from the genera *Fusarium, Aspergillum and Penicillium* produce toxic secondary metabolites called mycotoxins. These phytopathogenic fungi have been identified to survive secondary treatment *(*Viegas et al., 2014; Assress et al., 2019).

Many single and hybrid tertiary treatment systems have been applied in WWTPs. Filtration is usually implemented as an upstream step in tertiary treatment and can be performed alone or in combination with other disinfection technologies. Membrane technology are pressure driven technologies, in which disinfection is performed by physical barriers that are used to selectively restrict the passage of pollutants and microorganisms (Shon et al., 2009). Microfiltration (MF, pore diameter of 0.1 to 10 µm) and ultrafiltration (UF, pore diameter of 0.001 to 0.1 µm) can remove colloidal particles, proteins, polysaccharides, most bacteria and some virus, allowing to deliver higher quality effluent. Nanofiltration (NF, pore diameter of 0.001–0.01 µm) and reverse osmosis (RO, pore diameter < 0.001) have been used for water reclamation (Collivignarelli et al., 2017; Kehrein et al., 2020; Shon et al., 2009). Interaction of EfOM with membranes happens by adsorption (fouling) and due to electrostatic and steric exclusion (rejection) (Shon et al., 2006). Membrane technologies require high energy demand, in which fouling, and clogging adds up to the operational costs, due to the need for further clean-up. Process costs are however dependent on the membrane type used and on the influent quality. Disposal of the membrane retentate is performed into water bodies, whose discharge is currently not regulated, consisting in a potential environmental hazard (Justo et al., 2013). The membrane retentate has also potential to be a viable source of scarce elements that can be recovered, such as phosphorus (Couto et al., 2013; Kehrein et al., 2020). RO is highly effective in removing salts and low molecular weight contaminants, being capable of achieving a reduction up to 6 Log_{10} for the main indicator microorganisms and enteric pathogens in effluent. In Table 2.4 there are represented Log_{10} reduction units of the standard tertiary treatment systems.

Table 2.4 – Indicative Log_{10} removal of indicator microorganisms and pathogens in wastewater (Adapted from Australian guidelines for water recycling, 2006)

Tertiary treatment	*E. coli*	Coliphages[1]	Bacterial Pathogens (including *Campylobacter*)	Viruses (Including Adenoviruses, Rotaviruses and Enteroviruses)	*Giardia*	*Cryptosporidium*	*Clostridium perfringens*	Helminths
UV radiation	2.0 - > 4.0	3.0-6.0	2.0 ->4.0	>1.0 Adenovirus, >3.0 Enterovirus, hepatitis A	>3.0	>3.0	N/A	N/A
Chlorination	2.0 - 6.0	0.0-2.5	2.0-6.0	1.0 – 3.0	0.5 – 1.5	0.0 – 0.5	1.0 – 2.0	0.0 – 1.0
Ozonation	2.0 - 6.0	2.0-6.0	2.0 – 6.0	3.0 – 6.0	N/A	N/A	0.0 – 0.5	N/A
Membrane filtration	3.5 – >6.0	3.0->6.0	3.5 - >6.0	2.5 - >6.0	>6.0	>6.0	>6.0	>6.0
Reverse osmosis	> 6.0	>6.0	>6.0	>6.0	>6.0	>6.0	>6.0	>6.0
Stabilization ponds	1.0 – 5.0	1.0-4.0	1.0 – 5.0	1.0 – 4.0	3.0 – 4.0	1.0 – 3.5	N/A	1.5 - >3.0
Constructed wetlands – surface flow	1.5 – 2.5	N/A	1.0	N/A	0.5 – 1.5	0.5 – 1.0	1.5	0.0 – 2.0
Constructed wetlands – subsurface flow	0.5 – 3.0	N/A	1.0 – 3.0	N/A	1.5 – 2.0	0.5 – 1.0	1.0 – 3.0	N/A

[1]Momba et al., 2019.

N/A: Not available

Membrane processes, when performed before chemical disinfection processes (chlorine or ozone) or before UV radiation, increases synergistically the disinfection efficacy (Rizzo et al., 2020). Membrane bioreactors (MBR) are based on a CAS system but are coupled with membrane technology in order to achieve higher effluent quality. In these systems, biotic (biodegradation and biotransformation) and abiotic (adsorption to the membrane) are the main removal pathway of organic contaminants (Shon et al., 2009; Wang et al., 2018). Activated carbon (AC) is the most used adsorbent in wastewater treatment. Packed bed adsorption reactors with granular activated carbon have been used in drinking water treatment plants, and more recently in the treatment of wastewater effluents (Rizzo et al., 2020). Unlike chemical and photochemical disinfection methods, filtration technology does not produce potential harmful disinfection by-products. AC coupled to ozonation has been indicated to improve the degradation of many class of organic contaminants, as the activated carbon acts as a catalyst and the ozone increases the active surface area of the activated carbon (Kehrein et al., 2020).

Tertiary treatment can be performed using biological technologies such as polishing ponds or constructed wetlands. Constructed wetlands (CW) possess potential for phytoremediation of many organic contaminants such as PPCPs and are effective in the removal of suspended solids but might not be adequate to reduce effluent salinity (Becerra-Castro et al., 2015; Von Sperling et al., 2010). In these systems, HRT and the type of vegetation cover used play a great role in the efficiency of the system (Ghermandi et al., 2007). CW represent a more sustainable approach due to the lower maintenance and lower costs because they do not require an external source of energy and offer the benefit of landscape integration. However, these systems present the downside of requiring more land space and higher treatment time (Ferreira, et al., 2017b; Matamoros & Salvadó, 2012). CW appear reviewed as secondary treatment systems and more recently, as wastewater polishing treatment systems but in this case its application is still less implemented. Li et al (2014) reviewed the removal efficiencies of PPCPs obtained by several authors using different design parameters in CW systems. Based on the results, the following classification was suggested: readily removed compounds (mean removal >70%) such as caffeine, atenolol, metoprolol, furosemide, acetaminophen, tetracycline, salicylic acid, trimethoprim and sulfonamides; moderately removed compounds (mean removal between 50 and 70%), include naproxen, ibuprofen, gemfibrozil and doxycydine; low removed compounds (mean removal between 20 and 50%), such as diclofenac, ketoprofen, amoxicillin, clarithromycin, triclosan, clofibric acid and carbamazepine.

Conventional tertiary treatment technologies include chemical methods that rely on the addiction of reagents such as chlorine, chlorine dioxide, ozone and peracetic acid. However, these oxidants are selective and react preferentially with compounds containing electro-donating groups or electron-rich organic moieties (ERMs). In the case of free chlorine, only PPCPs containing aromatic compounds (e.g. phenol, aniline) or reduced sulfur groups are oxidized. Ozone reacts with PPCPs containing double bonds and monoprotonated amines. Chlorine dioxide has been reported only to oxidize sulfonamines, estrogens and macrolides (Guo et al., 2019; Rizzo et al., 2020). Chlorination is considered the most common disinfection method in WWTPs and in

drinking water treatment plants (Dodd, 2012; Kehrein et al., 2020), but the formation of harmful disinfection by-products has been identified as a major drawback (Kothny, 1992; Umar et al., 2019). Photochemical processes such as using ultraviolet radiation, especially at the germicidal wavelength (254 nm) have been gaining interest (Collivignarelli et al., 2017). Disinfection is usually the last step in treatment of effluent prior discharge into the receiving media, and it is a requirement for wastewater reuse (Zewde et al, 2019).

2.5.2 UV Disinfection of wastewater

Ultraviolet (UV) radiation for water and wastewater disinfection is a promising alternative to the prevalent chemical disinfection methods (namely chlorine) and has been used for broad application for inactivation of waterborne pathogenic microorganisms and leading to oxidation of organic pollutants, such as PPCPs (de Vidales et al., 2015; Guo et al., 2019; Rodríguez-Chueca et al., 2018). UV radiation has been referred to be able to kill *Cryptosporidium* and *Giardia,* that are considered chlorine resistant pathogens (Chen et al., 2006; Umar et al., 2019). Conventional UV disinfection is performed using a mercury arc lamp, that transfers electromagnetic energy to the target media (Chen et al., 2006). The most effective germicidal wavelength is comprised between 200 and 280 nm, corresponding to the UV-C radiation. Monochromatic mercury low pressure lamps are widely considered due to approximately 85% of the peak lamp output being in the 253.7 nm (Chen et al., 2006).

Efficiency of UV disinfection is dependent of the UV dose (or fluence) applied. The determination of the UV dose is widely performed in bench-scale UV apparatus called “collimated beam”. This apparatus consists of a petri dish which contains the water sample, that is irradiated with a lamp of known intensity. The irradiance it typically measured with a radiometer on a planar surface. It is possible to calculate UV dose-response curves, to different exposure times (Urban et al., 2011). In order to minimize errors, and increase the confidence in the comparison among analogous experiments, Bolton & Linde (2003) developed a detailed standardized method, addressing some of the main issues: design of the UV apparatus, determination of UV irradiance for calculation of UV dose, and suggestions on microbial testing.

UV radiation acts in microorganisms inactivation by inducing photochemical damage in the nucleic acids (DNA and RNA), as well as in proteins, inhibiting genome replication and transcription, resulting in cell death (Qiu et al., 2018). This in the most part happens due to the formation of cyclobutene pyrimidine dimer (CPD), a photoproduct in the DNA (Umar et al., 2019). When UV radiation is in contact with wastewater, chemical oxidation takes place and oxidizing species such as hydroxyl radicals (•OH) and hydrogen peroxide are formed in the aqueous media, contributing in some extent to the degradation of recalcitrant organic compounds (Chen et al., 2006; Collivignarelli et al., 2017). Production of free radicals, in the bulk solution, comprising indirect photolysis has been indicated as a possible pathway for microbial inactivation (Chen et al., 2006). If the target compound has a maximum absorption near the UV-C wavelength,

photodegradation can occur. UV radiation in combination with reagents can successfully increase the degradation efficiency.

2.5.3 Advanced oxidation processes

Advanced oxidation processes (AOPs) are a new competitive category for wastewater treatment. AOPs are based on the production of *in situ* •OH radicals. In comparation with conventional chemical oxidants (e.g. chlorine, ozone, chlorine dioxide), •OH radicals present low selectivity and high reactivity with almost all organic moieties, including aliphatic carbon hydrogen bonds, presenting a standard oxidation potential of 2.80 V (Khan et al., 2020; Lee & von Gunten, 2010).

These processes are classified as *homogeneous,* in the case when there is interaction of a chemical reagent with a target compound, and as *heterogenous* processes when rely in the addiction of a catalyst to a chemical reagent. Among the most studies AOPs, to highlight UV-enhanced photolysis, photocatalysis, H_2O_2 – enhanced photolysis. Fenton reaction is a usually a well-reviewed AOPs for PPCPs abatement from wastewater. However, secondary effluents have around neutral pH values, so fenton based processes will have to require pH adjustment (Catrinescu et al., 2017).

2.6 Electro-based technologies for wastewater treatment

Interest in electrochemical methods for wastewater treatment has grown in recent years (Sillanpää & Shestakova, 2017). In comparation with the previously described methods, electrochemical process presents the advantage of being robust and easy to operate, without addictions of reagents being efficient in the degradation of a wide range of organic compounds but presenting the downside of requiring electric power. Hybrid technologies for wastewater treatment and energy production have been developed (Muddemann et al., 2019; Magro et al., 2019). Microbial fuel cells are an example of an environmental friendly process, that combine electrochemical cells with metabolic activity of microorganisms allowing production of electric energy at the anode together with oxidation of organic contaminants (Modin & Aulenta, 2017). Recently, a proof-of-concept presented by Magro et al (2019) proved that the hydrogen produced during water electrolysis at the cathode can the stored with high levels of purity and reused in an proton-exchange membrane fuel cell, allowing to decrease external energy consumption.

Electrochemical reactors induce physicochemical transformations forced by the application of electric current. In most cases, an external current is supplied to the system (electrolysis), instead of being generated by elements present in water (galvanic cell). Processes under technical implementation are based in electrolysis systems (Muddemann et al., 2019).

2.7 Electrokinetic process

Electrokinetic (EK) process also referred as electrokinetic remediation (EKR) or electrochemical process, has been used as a clean-up technique, for remediation of both polar and non-polar organic contaminants, inorganic species and mixtures of contaminants from porous media such as soils, sediments and sludges, extensively reviewed by several authors (Reddy & Cameselle, 2009; Ribeiro et al., 2005; Ribeiro & Rodríguez-Maroto, 2006; Virkutyte et al., 2002; Gomes et al., 2013; Guedes et al., 2019).

The technique is based on the application of a low level direct current (DC), in the order of mA/cm² between working electrodes (two or more), in a porous matrix containing a fluid or a high fluid suspension / slurry, in order to induce migration of ionic species and surface charged particles. Mobilization of contaminants to near electrodes is induced by the electric field, where they can be extracted and treated (Cameselle et al., 2013; Pamukcu et al., 2014).The application of a low current intensity allows to minimize electric consumption, making the process a sustainable technology, whose proved feasibility have driven its further application in targeting remediation of different compound classes and in other types of environmental matrices. More recently, EK process have been studied in the field of wastewater treatment, for the removal of EOCs from wastewater matrices (Magro et al., 2020; Guedes, 2015; Ferreira et al., 2018).

2.7.1 Transport mechanisms under an electric field

In a classical EK set-up, a contaminated matrix under treatment is placed between electrodes in which the anode and cathode are connected to a DC power supply. The contaminants present in ionic form are transported to the electrode with opposite electrical charge. Acar & Alshawabkeh (1993) made a comprehensive review of the process, focusing in the removal of heavy metals from contaminated soils. The main identified transport mechanisms in EK process are: electromigration, electroosmosis and electrophoresis, represented in Figure 2.3. Difusion and hydraulic advection also have significance, in smaller extent (Paz-García et al., 2012).

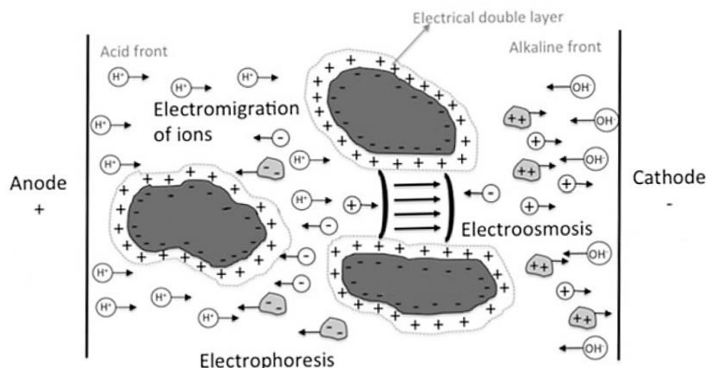


Figure 2.3 - Main transport mechanisms in EK (Adapted from Nunes et al., 2016)

Separation of charges can originate several EK phenomena depending if is the solid or the liquid fraction that moves.

Electromigration, also defined as ion migration, consists in the movement of ions and ionic species under an electric field. In aqueous solutions, ions move directly to the electrode with opposite charge by the shortest route (Hassen et al., 2016). For soluble polar organic compounds and/or ionic species, electromigration has been identified as the main transport mechanism. The mass flux is dependent on the electric potential applied, pH gradient and on the concentration of the specific ion (Cameselle et al., 2013; Ribeiro & Rodríguez-Maroto, 2006). Electromigration transport is described according to equation (1.1).

$$J_m = - u^* c\phi_e \quad (1.1)$$

In which J_m is the electromigration transport, u* is the ionic mobility, c is the concentration of ionic species and ϕ_e the gradient of electrical potential.

Electroosmosis is the mass flux of water or interstitial fluid relative to soil particles, induced by the application of an electric potential. In electroosmotic flux, cations in solution go from anode to cathode as this species are usually positively charged in the electric double layer (Guedes, 2015: Cameselle et al., 2013). The electrical double layer plays a great role as the interface of the colloidal system (Pamukcu et al., 2014). Electroosmosis is the main mechanisms for removal of weakly dissoaciated species and/or uncharged (Ribeiro & Rodríguez-Maroto, 2006) (e.g. when solution or media pH is below acid dissociation constant of a compound). The electroosmotic flux is described by equation (1.2)

$$J_{eo} = - k_e c\phi_e \quad (1.2)$$

In which k_e represents the electroosmotic permeability.

Electrophoresis is the transport of charged colloids (e.g. dissolved, suspended particles) in a stationary fluid (Cameselle et al., 2013). In slurry, electrophoresis is an important transport mechanism (Guedes, 2015). Most colloids and microorganisms in wastewater carry a negative charge and under the application of an DC electric field will move towards the anode (Alshawabkeh et al., 2004; Shon et al., 2006).

Diffusion is the movement of charged particles under a chemical concentration gradient, from regions of higher concentration to regions of lower concentration (Ribeiro & Rodríguez-Maroto, 2006). For higher current intensities applied, diffusion is negletible when compared with other transport phenomena (e.g. electromigration) (Magro et al, 2019). Chemical gradients are however important in the zone where the acid front from the anode meets the alkaline front formed from the cathode. Diffusive flux is described by equation (1.3)

$$J_{d} = -D^* \nabla_c \quad (1.3)$$

In which D* represents the effective diffusion coefficient and ∇_c is the concentration gradient. Models based in the Nernst-Planck-Poisson equations, have been presented as an efective way

of modeling the electro-diffusion transport of multi-species under the aplication of an electric field (Paz-García et al., 2012).

Alongside with transport mechanisms, several electrochemical reactions such as complexation, desorption/adsorption, precipitation/dissolution, electrodegradation, redox reactions can take place in bulk solution (Hassen et al., 2016).

Water electrolysis and electrodeposition take place at the electrodes surface (Cameselle et al., 2013). In electrodes surface, decomposition of water occurs. At the anode, water oxidation happens and an acid front is formed due to H^+ ions generation, according to equation (1.4), and at the cathode surface, water reduction happens and an alkaline front is formed by hydroxide ions OH^- according to equation (1.5). Oxygen and hydrogen gases are produced at the anode and cathode, respectively (Cameselle et al., 2013).

$$\text{At the anode: } 2H_2O - 4e^- \rightarrow O_2 + 4H^+, \quad E_0 = -1.229 \qquad (1.4)$$

$$\text{At the cathode: } 2H_2O + 2e^- \rightarrow H_2 + 2OH^-, \quad E_0 = -0.828 \qquad (1.5)$$

In consequence of water redox reactions, pH of the media is expected to change, inducing potential effects in the speciation of the contaminants. Protons are almost twice as mobile as hydroxide ions, and the acid front formed at the anode advances to the cathode, conductivity increases and the zeta potential decreases (Cameselle et al., 2013; Acar & Alshawabkeh, 1993).

Secondary reactions can also take place in electrodes surface, depending on the concentration of ions in bulk solution. Metals (Me) can suffer deposition in the cathode surface, following the reactions presented in equations (1.6) and (1.7), in which n correspond to the number of positive charges (Ribeiro & Rodríguez-Maroto, 2006). This phenomena has been identified to cause the decline of the electrochemical efficiency in transport mechanisms (Cerqueira et al., 2014).

$$\text{At the cathode: } Me^{n+} + ne^- \rightarrow Me \qquad (1.6)$$

$$\text{At the cathode: } Me(OH)_n + ne^- \rightarrow Me + nOH^- \qquad (1.7)$$

Also, if chlorines are present in solution, according to equation (1.8), chlorine gas can be produced.

$$\text{At the anode: } 2Cl^- \rightarrow Cl_2 + 2e^- \qquad (1.8)$$

2.7.2 EK remediation of PPCPs

EK process has proved to be a viable technology for the removal of PCPPs from several environmental matrices. In a lab-scale study carried out by Guedes et al (2014) the removal of six emerging contaminants: two estrogenic steroid hormones (17β-oestradiol and 17α-ethiniloestradiol) three industrial reagents (bisphenol A, nonylphenol and octylphenol) and one antimicrobial agent (triclosan) from two types of soils: silty loam soil and sandy soil were studied. The EK remediation was conducted in a 3-compartment cell, tested with different current intensities ranging from 0 to 10 mA, during a period of 4 days. Percentage of contaminants that remained in the soil was between 17% and 50% for sandy silty loam and 27% to 48% for sandy soil. Main transport mechanisms identified were electroosmotic flow and electrodegradation. pH control allowed the enhancement of the electroosmotic flow, that is known to be dependent of the pH of the medium as previously identified by other authors (Cameselle et al., 2013).

Lopes (2018) studied the application of the EK process in clay-soils, for the removal of five emergent contaminants: caffeine, sulfamethoxazole, ibuprofen, triclosan and atenolol. Different operational parameters were tested (current intensity, alternate and direct current). The application of a direct current of 50 mA allowed the best electrodegradation of the compounds with the highest removal being achieved for sulfamethoxazole: 97 ± 8% after 7 days.

Dionísio (2019) studied the EK process also in agricultural soils, with a current intensity of 20 mA, in a regime of alternate current (ON/OFF cycles of 12 h for 4 days). In this study, EK was performed in non-sterile soil and in sterile soil in in order to assess the influence of environmental factors in PPCPs degradation. In this study, EK experiments performed in sterile soil, without microbial community, showed a decrease in the removal of contaminants, when compared to non-sterile soil. From the 10 EOCs studied: 17β-oestradiol*,* 17α-ethiniloestradiol, caffeine, sulfamethoxazole, carbamazepine, oxybenzone, triclosan, diclofenac, bisphenol A, ibuprofen, the most biodegradable compounds were: 17β-oestradiol, sulfamethoxazole, and bisphenol A.

Other studies aiming removal of PPCPs from soil slurry were made by Ferreira et al (2017a). Aiming resource recovery from wastewater matrices, EK process was applied for the degradation of PPCPs and also for phosphorus recovery by introduction of an ion exchange membrane in the cell design by Guedes (2015), Pinto (2015) and Almeida (2015).

2.8 Analytical techniques

Due to the complexity and heterogeneity of environmental matrices such as effluent, or other biological samples (e.g. plasma or urine), the samples cannot be directly introduced into the analytical system. In order to avoid damage of the chromatographic equipment, a suitable sample preparation technique must be employed prior chromatographic analysis.

2.8.1 Solid-phase extraction

Solid Phase Extraction (SPE) is a versatile extraction technique, commonly considered a clean-up technique in complex matrices. When compared with other sample extraction methods such as liquid phase extraction (LPE), SPE has the advantage of requiring a small solvent amount, good recovery in just one extraction step, reduced sample preparation time and allow better reproducibility, specificity and selectivity of the method (Patel et al., 2019).

The SPE procedure comprises 5 main steps (Guedes, 2015): 1) Selection of a sorbent that allow extraction of the interest analyte(s), 2) Cartridge conditioning and equilibration; 3) Sample load (enrichment); 4) Washing and 5) Elution.

The choice of the most adequate sorbent is key to ensuring good extraction and recovery results. There are three classes of sorbents available in column or in cartridges: normal phase, reverse phase and mixed-mode sorbents. When choosing a sorbent, the target analyte(s) physicochemical properties should be considered. For polar PCPPs, the Oasis® HLB sorbent (Hydrophilic Lipophilic Balance) is considered the main choice, to perform extraction and elimination of interfering species (Martín-Pozo et al., 2019; Salgado et al., 2010). In reverse phase cartridges, the extraction mechanism is based on weak interactions (Wan der walls force) of non-polar functional groups of the sorbent material with the non-polar groups of the analyte(s) (Guedes, 2015). Most PPCPs are acidic, and at neutral pH are in an ionized form. In other to increase the analyte(s) retention to the sorbent, samples pre-treatment can be performed and samples acidified to pH 2 (Patel et al., 2019).

When performing conditioning of the cartridges, a suitable solvent must be chosen. In the case of extracting polar compounds such as PPCPs, a strong polar solvent should be used. The most used equipment to perform SPE is a Manifold, operated under negative pressure. The cartridge conditioning step will activate the stationary phase. An equilibration steps follows, in which is added an aqueous matrix compatible with the sample. The sample loading in the cartridges must be performed under a constant flow and pressure changes along the process might require new adjustment of the Manifold pressure. In this step, is crucial that the analytes in the sample have time to interact with the stationary phase. During the sample loading, the stationary phase must not dry out otherwise the extraction efficiency can be compromised. The cartridge filling must be set according with the sample volume to load to do not overcome the cartridge breakthrough volume. By the end of step 3), interest analyte(s) must be retained in filling of the cartridge, but it is still needed to remove interfering species, by washing the cartridges. By this point, the cartridges must be open and let to dry. The goal is to dry out the most water, with the aim to optimize the following step, the analyte(s) elution. Analytes elution is done with the solvent used in conditioning. A schematic procedure is represented in Figure 2.4.

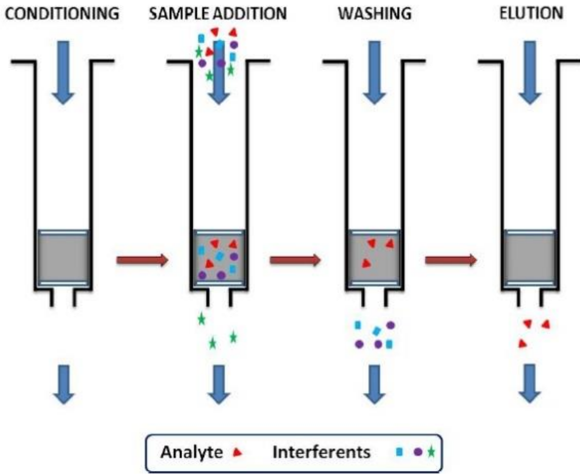


Figure 2.4 - Schematic SPE procedure (Source: Lucci et al., 2012)

2.8.2 High-Performance Liquid Chromatograph (HPLC)

High Pressure Liquid Chromatography (HPLC) is a column chromatography technique that uses high pressure to generate the flow necessary to perform liquid chromatography in a packed column.

In its simplest form, as in any other chromatography technique, performs separation of compounds, between a mobile phase and a stationary phase. Compounds that have affinity with the mobile phase, move more rapidly, and are eluted first, than the compounds that are distributed in the stationary phase (Waters, 2020; Guedes, 2015).

Liquid chromatography (LC) and Gas Chromatography (GC) coupled to mass spectrometry are considered the preference technique for PPCPs determination from different matrices. Due to the characteristics of PPCPs, LC is more adequate that GC. Some of the main advantages of an HPLC system are the easy maintenance, relatively inexpensive system, robustness and in comparation with a GC equipment, is a non-destructive technique for the sample and the sample does not require derivatization (Martín-Pozo et al., 2019).

There are three types of liquid chromatographic separation: Reverse phase liquid chromatographic (RPLC), normal phase liquid chromatography (NPLQ) and hydrophilic phase liquid chromatography. RPLC is the most common HPLC separation technique. The most used column is a non-polar column, packed with C18, with wide application in environmental analysis, such as polycyclic aromatic hydrocarbons, PPCPs, pesticides (Snyder et al., 2012). The main components of a HPLC system are represented in Figure 2.5.

The solvent reservoir corresponds to the mobile phase that is going to be controlled by an high-pressure pump that controls flow. The solvent can be carried by gradient, in which different solvents are sequentially and in different proportions used along the chromatographic run to carry the sample separation, or in isocratic mode (less used), with a single solvent. The injector is

programed to inject the samples into the chromatographic column. A column that is packed with sorbent, corresponds to the stationary phase. After the sample passes the detector, is sent to waste or in alternative could be collected. The data received by the detector is presented in a computer (Waters, 2020).

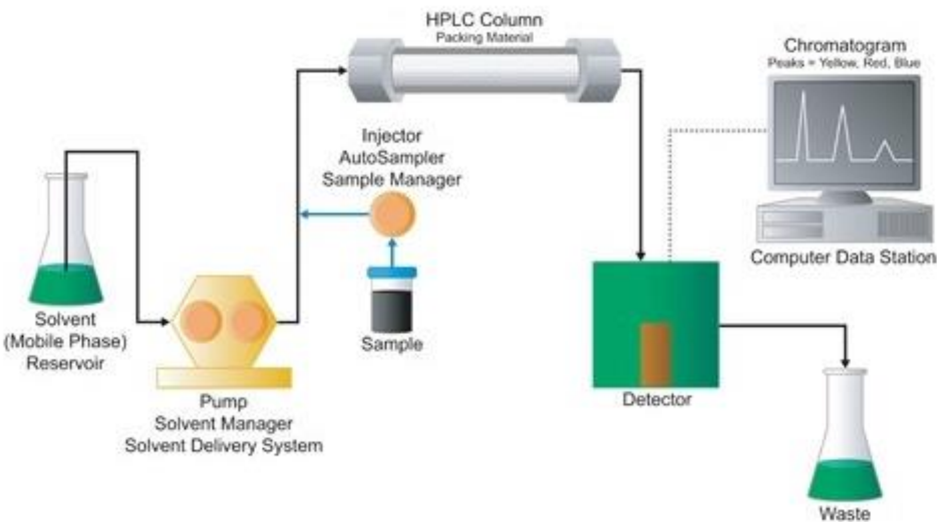


Figure 2.5 - Basic components of an HPLC system (Source: Waters, 2020)

The detector type should be chosen in accordance with the compounds in analysis. As compounds physicochemical properties vary significatively, HPLC can be coupled to different detectors according to the proprieties of the compounds in analysis. Some of the commercially available detectors are Ultraviolet-visible (UV-vis) that include the diode array detectors (DAD), Fluorescence (FLD), Evaporative Light Scattering, Refractive Index an Electrochemical (Waters, 2020). The coupling of a Diode Array Detectors (DAD) and a Fluorescence Detector (FLD) have been reviewed as a method for PPCPs determination due to its high sensitivity and wider and multiwavelength detection (Gumustas et al., 2018; Lucci et al., 2012). The quality of an HPLC separation can be adequately described in terms of critical resolution and run time (Snyder et al., 2012).

3. Materials and Methods

3.1 Effluent sampling

The effluent used in the experiments of the present dissertation was collected in Quinta do Conde WWTP (N38°34'14.77" W9°02'04.71"), located in Sesimbra, Portugal. This WWTP belongs to the multimunicipal system of Simarsul – Saneamento da Península de Setubal S.A (Simarsul, 2019). Location of the WWTP is represented in Figure 3.1.

Three discrete effluent samples were collected after secondary treatment, at the outlet of the secondary settling tank in the month of September and December 2019. The effluent was collected to plastic bottles (PET) of 5 L capacity. A total of 20 L (4 bottles) were collected from each effluent sample, and immediately brought to the RESOLUTION LAB (FCT NOVA). Once in the lab, effluent was firstly filtered through conventional filter paper, in order to speed up the filtration process by removing larger particles, and then by 2.7 µm microfiber glass filter (MFV4), purchased from Filter Lab (Barcelona, Spain). The filtrated effluent was then transferred to two 10 L clear Schott borosilicate glass laboratory bottles purchased from Duran (Germany), and kept stored away from direct light until the beginning of the experiments.

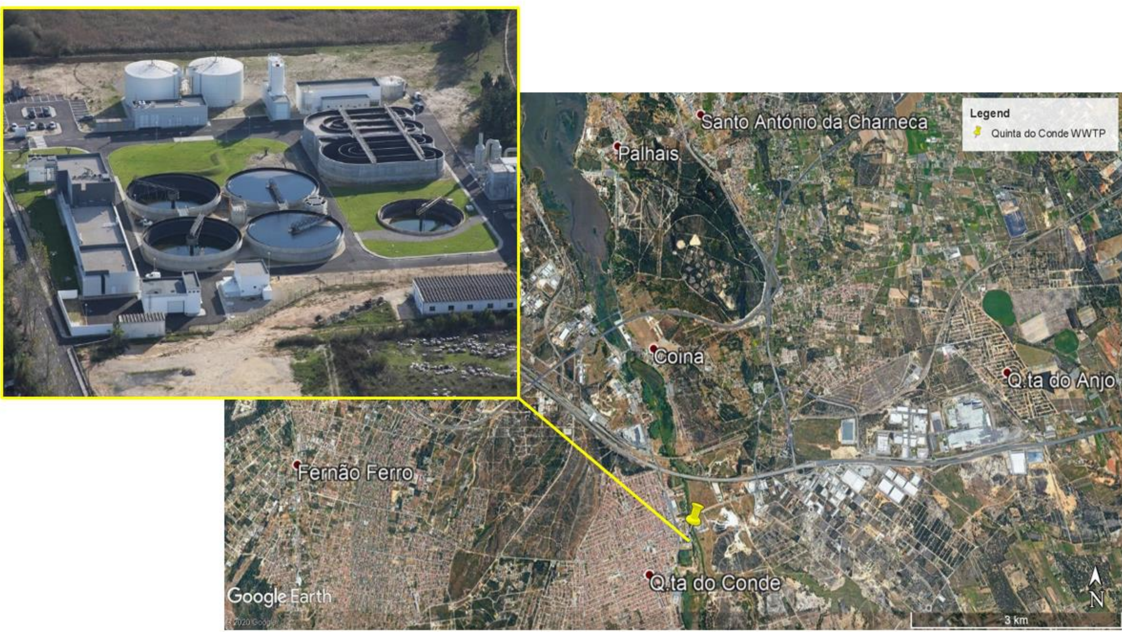


Figure 3.1-Location of Quinta do Conde WWTP (Source: Google Earth, 2020; Simarsul, 2020)

Regarding the characterization of the wastewater treatment system implemented in Quinta do Conde WWTP, the level of treatment installed is tertiary, performed with UV disinfection. This WWTP has the capacity to treat 19 300 m³/day of urban wastewater (mostly wastewater from domestic households, without relevant industrial wastewater contributions), corresponding to 94 000 equivalent inhabitants (Simarsul, 2020). A simplified flow diagram of the wastewater treatment processes is represented in Figure 3.2, alongside with the sampling point in the treatment system, represented in red.

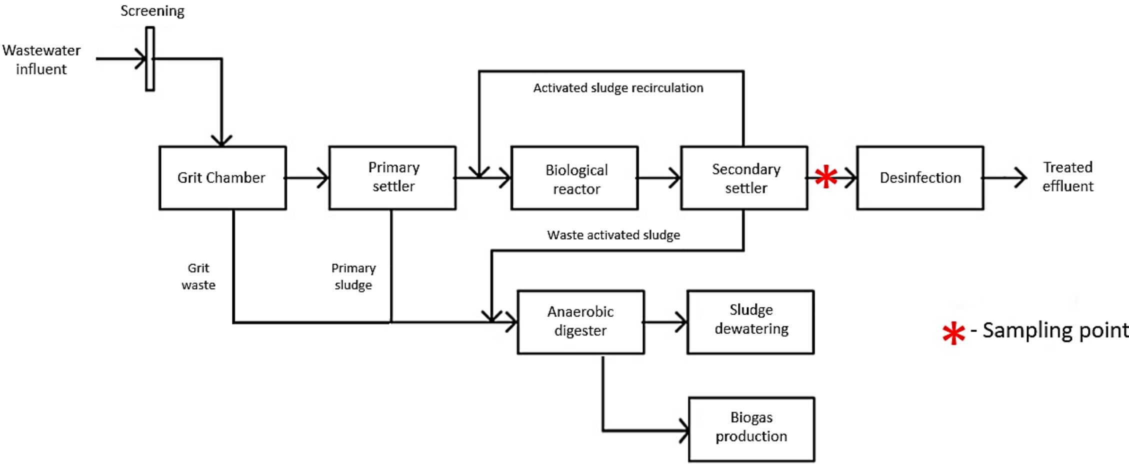


Figure 3.2 - Simplified flow diagram of Quinta do Conde WWTP unit processes

The Quinta do Conde wastewater treatment comprises different steps: In the liquid phase treatment, the raw influent passes through a step of screening, with two different mesh sizes, first by a 50 mm mesh and the by a 10 mm mesh grid. Grit removed is sent to the biological stabilization, performed by an anaerobic digester in the solid phase treatment.

Primary treatment is performed in settling lamellar tanks. The primary sludge produced is sent to the solid phase to biological stabilization. The primary effluent is sent to the secondary treatment, that is carried out in a biological anoxic/aerobic reactor with aeration. Part of the sludge is recirculated, and the excess is purged and goes to the anaerobic digester. Tertiary treatment is done in UV disinfection chambers. Part of the treated effluent is reused by the WWTP, and part is discharged into the receiving waters (Almeida, 2015).

3.2 Experimental design

The work was carried out in two experimental Sets according to Figure 3.3. In the experimental Set 1, the effluent was subjected to UV-C radiation before applying the EK treatment, whereas in the Set 2 effluent was only subjected to EK treatment. All experimental procedures are further detailed below.

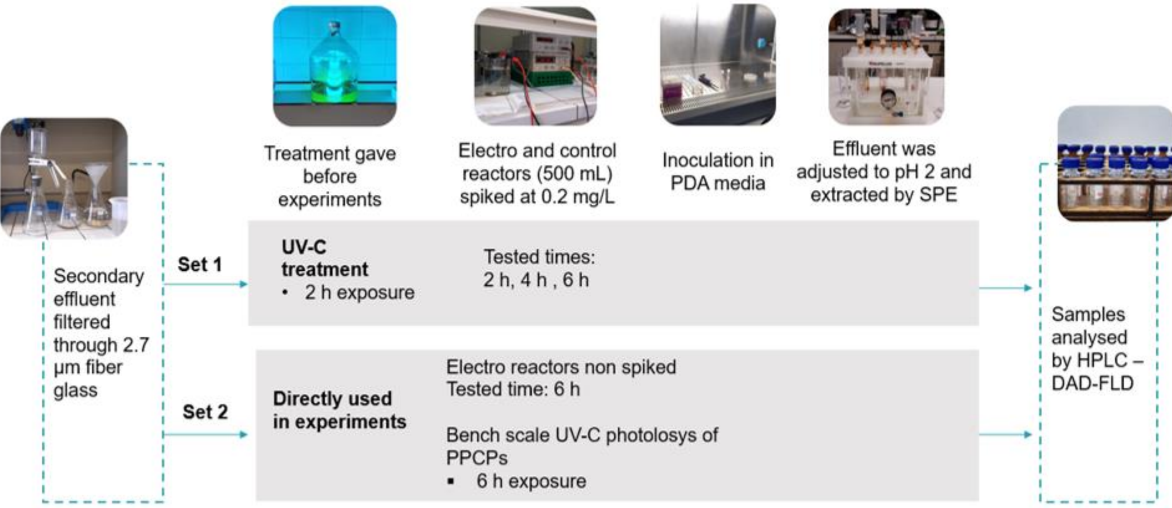


Figure 3.3 - Overview of the experimental design

The dates of the effluent sampling campaigns, and the type of treatment gave to the effluent are represented in Table 3.1. Set 1 experiments were done between the month of October and November. Set 2 experiments were done during the month of December.

Table 3.1 - Secondary effluent sampling dates and experimental treatment gave to the effluent prior EK assay

	Experiment Code	Sampling date	Type of experiment
Set 1	**UV**	24/09/2019	UV-C treated effluent followed by EK
Set 2	**F1**	3/12/2019	Fresh secondary effluent directly used in EK assays.
	F2	17/12/2019	

3.3 Effluent disinfection with UV-C radiation – sample treatment

In Set 1, effluent was exposed to UV-C radiation, using a UVP™ XX-15 Series UV Bench Lamp, 502 x 152 x 108 mm (L x W x H). A distance of 15 ± 5 cm was kept between the UV lamp and the bottle containing the effluent. From 29/9 to 5/11, the UV-C disinfected effluent was used to perform different assays and replicates. Assays with different remediation times were carried out

in different days, and prior assays, the 10 L Schott bottles were exposed for 2 h to UV-C radiation, with aluminum foil covering the lid of the bottle. The bottles and the lamp were set in a lab bench exposing the effluent to UV-C radiation.

3.4 Electro-reactor set up

The electro-reactor used in the experiments consists in a one-compartment glass cell (1c-cell), with an internal diameter of 8 cm, and height of 12 cm. As working electrodes, a pair of mixed metal oxide (MMO) mesh electrodes were purchased from FORCE Technology (Brøndby, Denmark) and were used both as anode and cathode. Electrodes had 9 cm of height and 2 cm of length and were in a fixed position in the reactor (see Figure 3.4), 5 cm from the bottom and parallel to each other (6 cm apart).

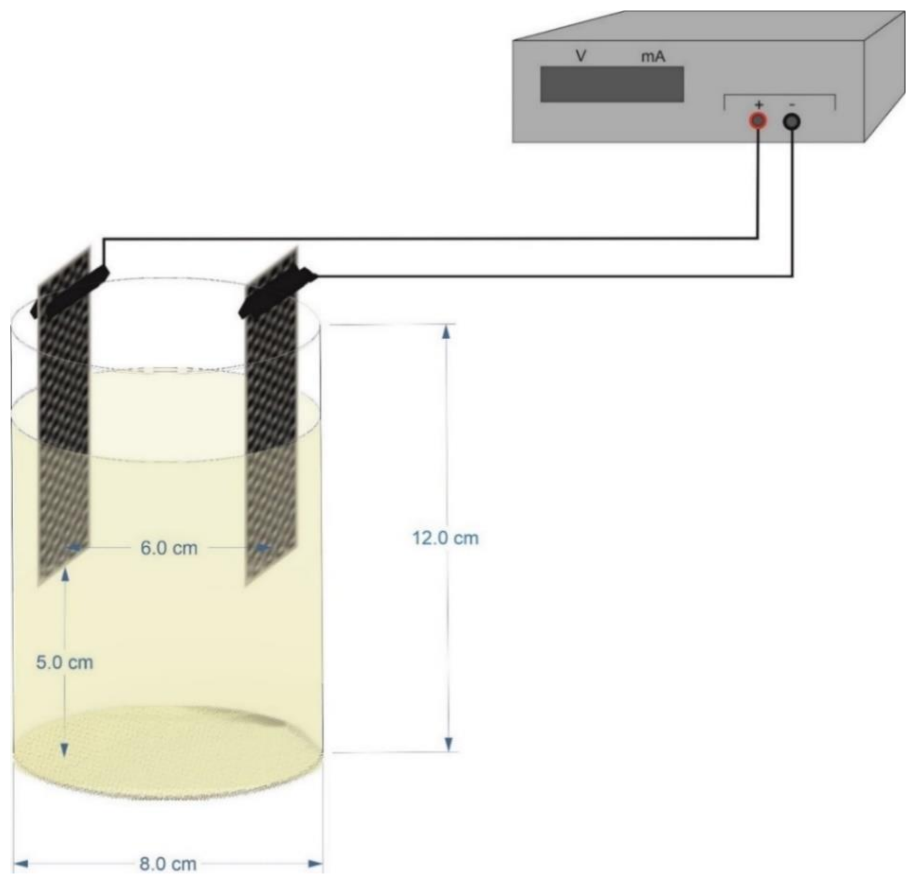


Figure 3.4 - Microcosmos of the electro-reactor

The working surface area of the electrodes in contact with the effluent was 6 cm². All assays were performed with constant DC (50 mA), using a power supply Hewlett Packard E3612A (Palo alto, USA).

3.5 EK experiments

To test the best PPCPs removals according to the applied remediation time (2 h, 4 h and 6 h), the EK process was applied after UV-disinfection. In this case, the best compound remediation time according to the operational characteristics set for the electro-reactor, was chosen for the following experiments (Set 2). Based on the electro-reactor dimensions, represented in Figure 3.4, a fixed volume of 500 mL was set for all experiments (both Set 1 and Set 2). A fixed current density of the electro reactor was set at 8 mA/cm². Two model reactors were used: electro-reactor (EK) and control (C). In the case of Set 2 operational conditions used in each experiment type are described in Table 3.2

Table 3.2 - Main operational parameters used in each assay

	Experiment code	Type of experiments performed	Time (h)	Current intensity (mA)	Number of replicas (n)	Description
Set 1	**UV[1]**	EK	2	50	2	EK experiments Effluent spiked (0.2 mg/L)
			4	50	2	
			6	50	2	
		C	2	0	2	Control. Effluent spiked (0.2 mg/L)
			4	0	2	
			6	0	2	
		t0	0	0	2	Effluent recovery
		t0*	0	0	2	Recovery in non-spiked effluent
Set 2	**F1[2]**	EK6	6	50	3	EK done with spiked effluent (0.2 mg/L)
		EK6*	6	50	3	EK done in effluent non spiked effluent
		C	6	0	3	Control. Effluent spiked (0.2 mg/L).
		UV6	6	0	3	Photolysis experiments Effluent spiked and exposed for 6 h to UV light
		T0	0	0	3	Effluent recovery
		T0*	0	0	3	Recovery in non-spiked effluent
	F2[3]	EK6	6	6	2	EK done with spiked effluent (0.2 mg/L)
		EK6*	6	6	2	EK done in effluent non-spiked
		C	6	6	2	Control. Effluent spiked (0.2 mg/L)
		UV6	6	6	2	Photolysis experiments Effluent spiked and exposed for 6 h to UV-C light
		T0	0	0	2	Effluent recovery
		T0*	0	0	2	Recovery in non-spiked effluent

[1]Bench scale UV-C treated effluent, [2]Fresh effluent sample #1, [3]Fresh effluent sample #2, * Non-spiked assays

All the material had to be previously set before the beginning the experiments. In Set 1, the effluent had to be previously disinfected by UV-C radiation, electrodes put in position, and 500 mL of effluent was poured into the reactors. Before turning the power supply, the effluent in the reactors were spiked, for further calculation of compounds degradation and recovery. Using a

micropipette, 50 µL was taken from a stock solution previously prepared with a concentration of 2 000 mg/L containing the five target compounds. The effluent was spiked at 0.2 mg/L and stirred in order to promote homogenization, using a glass rod. Effluent in electro-reactors during assays was stirred every 30 minutes, and before stirring the voltage value was taken, until the end of the assay.

Set 1 experiments aimed to set the best remediation time for the fresh effluent experiments (Set 2 experiments). To do so, different remediation times were performed in different days. The first assay from Set 1 was carried out in 1/10 and the last in 5/11. Between this period, a total of twelve UV-EK assays (including respective controls) were performed. Recovery experiments with spiked effluent (t0) and non-spiked (t0*) were done in the first day of assays, and one replicate of t0 was done after the end of UV-EK assays, in 11/11. Set 2 experiments were conducted for 6 h, the best remediation time obtained in Set 1 for all compounds. In Set 2 (F1 sample), fifteen assays were performed with fresh effluent (in a maximum of 24 h after collection) (5/11). This was possible with a total of 6 electro-reactors in parallel. In both sets, the PPCPs analyzed were CAF, CBZ, DCF, OXY. In Set 2, it was also performed photolysis in spiked reactors (in a maximum of 48 h after collection), being exposed for 6 h to UV-C radiation. The same procedure done with F1 was applied to F2.

Electro-reactors in Set 1 and Set 2 had the same operational parameters (CI: 50 mA; voltage registered and effluent stirred every 30 minutes) only changing the treatment time or the collected matrix (with or without UV-C pre-treatment, conserved or fresh effluent). In the end of assays, in Set 2, an aliquot of 100 mL was collected to three 50 mL Falcon tubes from the reactors for physicochemical analysis and an aliquot of 0.5 mL was taken for microbiology analysis. All experiments were made in controlled temperature of 22 ± 1 °C.

In the end of each assay from Set 1, a 1 mL aliquot was taken from each reactor for a 5 mL polypropylene tube purchased from Labcon (USA) to perform microbiology analysis. During assays, the laminar flow chamber was turned on and the UV lamp and pointed to the interior of the chamber for approximately two hours, to ensure sterile conditions prior microbiology experiments.

After experiments, electrodes were washed with soap and deionized water to remove any contaminants and polarity was reversed. The procedure was carried out in water, and the reversal duration was equivalent of the last assay in which the electrodes have been used.

Initial concentration of PPCPs in effluent (time zero) was determined and the calculation of recovery percentage as calculated, using equation 3.1.

$$\text{Recovery (\%)} = \frac{\text{Concentration of PPCPs detected (mg/L)}}{\text{Concentration of PPCPs spiked (mg/L)}} \tag{3.1}$$

PPCPs percentage of degradation in relation with control, after experiments was calculated using equation 3.2

$$\text{PPCPs degradation (\%)} = 1 - \frac{\text{PPCP concentration after experiments (mg/L)}}{\text{PPCP concentration obtained in control}} \tag{3.2}$$

3.6 Chemicals and materials

The organic compounds used were CAF (≥90%), SMX (analytical standard), CBZ (≥99%) , DCF (≥97%) all purchased form Sigma Aldrich (Steinhem, Germany) and MBPh (≥98) purchased from Alfa Aesar (Massachusetts, EUA). Individual stock solutions for calibration purposes were prepared dissolving 2000 mg/L for all organic compounds in MeOH:ACE (1:1) and stored at 6 °C.

Solvents used were from Sigma Aldrich (Steinhem, Germany), Merck (Darmstandt, Germany), Panreac (Barcelona, Spain), Carlo Erba (USA), Fluka (USA) and J.T Barker (Germany).

Methanol (MeOH), acetonitrile (ACN), acetone (ACE) and formic acid were acquired from Sigma Aldrich (Steinhem, Germany), J.T Baker (Germany), Carlo Erba (USA) and Fluka (USA) and were gradient grade type for HPLC. The water used was deionized and purified using a Millipore system (Bedford, MA, USA). Nitric acid was purchased from Sigma-Aldrich.

3.7 Analytical methodologies

3.7.1 pH and Conductivity

In the beginning of all assays, for both Set 1 and Set 2, pH and conductivity were measured using a pH meter by taking and aliquot from the middle of the reactors (Metrohmm-Solitrode with Pt1000) and a conductivity meter (Horiba-LAQUAtwin).

3.8 Culturable microorganisms

For all experiments from Table 3.2 (except for t0 spiked), an aliquot (1 mL in Set 1 and 0.5 mL in Set 2), was taken as previously described in section 3.7 and used to perform enumeration of the colony forming units (CFUs) in Potato Dextrose Aga (PDA) (composition: 5 g/L of potato extract, 20.0 g/L of glucose and 17.0 g of agar) purchased from Biolife, prepared according to the manufacturer instructions into sterile 9 cm diameter petri dishes. Using a proportion of 42 g of powder suspended in 1000 mL of sterile water, the mixture is heated to boiling and agitated in order to dissolve completely. The media is then sterilized by autoclaving at 121 °C for 15 minutes. Then, the media is waited to cool down to 45 °C – 50 °C and poured into sterile petri dishes. When completely dried, it is ready to use.

Procedure

An adapted spread plate technique was used to perform colony counts. This is an easy and direct method that required few steps and allows to achieve isolated colonies. The procedure was made inside the laminar flow chamber (Figure 3.5), in order to guarantee aseptic conditions and to avoid contamination. Before starting the microbiology procedure, all surfaces inside of the laminar flow chamber was cleaned with the solution of 70% ethanol, and subject to UV light for 30 minutes and then re-cleaned with the solution of 70% ethanol. All used material was previously sterilized. All the material used e.g. glassware, tubes with samples, micropipettes,"L" spreaders, agar plates, micropipettes points and hands as well, were sprayed with 70% ethanol before entering the laminar flow chamber.

Figure 3.5 - Laminar flow chamber used in microbiology experiments and elements used: 1- Sterile micropipettes points, 2- 70% ethanol, 3 - "L" spreader, 4 -500 µl micropipette, 5 – Effluent aliquots, 6 – PDA plate; 7 – Discard bag

Inside the laminar flow chamber, the spread plate procedure was conducted as follow:

1) Opening of the tube containing effluent aliquot
2) Sterile micropipette point was removed from the container and attach to the micropipette.
3) Lid of the agar plate was lifted (special attention to not pass hands or material over the open agar plaque and lead to minimize contamination).
4) 100 µl aliquot of sample is taken with the micropipette from the sample tube;
    a. Direct sample (without dilution) is taken in the following cases: all assays performed with previously UV disinfected effluent (Set 1), from Set 2 all EK assays, and assays made with effluent spiked and exposed to UV radiation.
    b. Sample diluted with sterile water (1:5) in which to 500 µl of sample inside the tube are added 2 mL of sterile water. This was done for control and recovery samples in Set 2.
5) The sample is plated in the center of the agar plate.

6) The micropipette point is discarded in the discard bag, and the micropipette is put down.
7) With the “L” spreader in the right hand and in fixed position, and with the left hand gently twisting the plaque in the same direction to ensure the sample is evenly spread in all agar surface until complete absorption.
8) Point of the “L” spreader that was in contact with the agar surface, is immersed in the solution of 70% ethanol to disinfect and are ready to be re-used between replicates and discarded.
9) Procedure from 1) to 7) is repeated until there are no more samples to inoculate.
10) Agar plates are covered with the lid and inverted.
11) Identification of agar plates with date, and assay name.
12) All material is taken out the laminar flow chamber

All the procedure was made the quickest as possible in order to avoid contamination. A negative control was performed, on a plaque, to verify the sterile conditions of the laminar flow chamber. At the end of the microbiology procedure and the flow chamber was subject to UV light for 30 minutes. Plates were covered with aluminum foil and taken to the incubator at 37 ± 2 °C. With 70% ethanol all surfaces were cleaned.

In Set 1 experiments (UV-C treated effluent), only one PDA plaque was prepared from each replicate. In Set 2 (fresh secondary effluent) from each replicate, plaques were done in triplicate, except for t0 and C6 spiked that were done in quadruplicate. After an incubation time of 24 ± 4 h, isolate colonies were count to the naked eye, and results were reported in colony forming units per 100 mL. Arithmetic mean of the triplicate (or quadruplicate) plates was done to assess variability of the method. Final mean of the CFUs values per assay type, was done by calculation mean of all replicas.

The main goal with the CFUs counts is to calculate Log Value Reduction (LVR). Based on the CFU counts (CFU/0.1 mL), CFU/100 mL concentration was estimated using the equation (3.3).

$$\text{CFU/ 100 mL} = \frac{B \times D_f}{V} \times 100 \qquad (3.3)$$

In which B is the number of colonies counted after 24 h (CFU/0.1 mL), D_f is the dilution factor, that indicates how much of the original samples is diluted. Two dilution were considered according to the expected microorganism’s concentration in samples of different assays: 10^0 in the case of no dilution and 2×10^{-1} in the case of dilution. V represents the inoculation volume. Removal efficiency or reduction (%) was calculated by application of equation (3.4) and based on the adaptation of the procedure described by Von Sperling et al (2020).

$$\text{Reduction (\%)} = \frac{A - B}{A} \times 100 \qquad (3.4)$$

In which A corresponds to the mean initial CFUs count expressed in CFU/100 mL and B corresponds to the mean final CFUs count in CFU/100 mL after the applied process. Equation (3.5) was applied to calculate LVR:

$$LVR = Log_{10}A - Log_{10}B = Log_{10}\left(\frac{A}{B}\right) \quad (3.5)$$

A general overview of the procedure is represented in Figure 3.6.

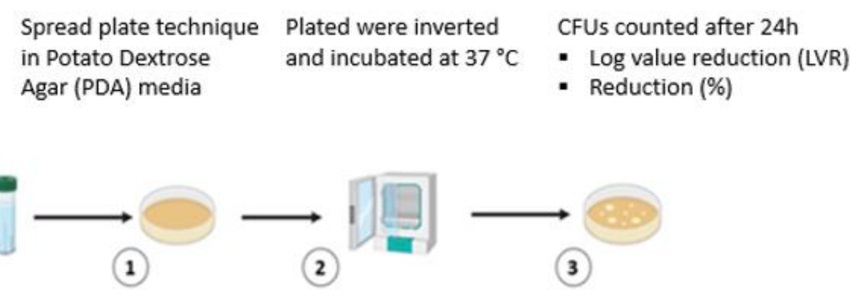


Figure 3.6 - Overview of the microbiology procedure for CFUs enumeration

3.9 PPCPs analysis

3.9.1 Solid phase extraction

All the effluent samples were extracted by solid phase extraction (SPE) in a Visiprep™ SPE Vacuum Manifold, purchased from Sigma Aldrich (Steinhem, Germany) (Figure 3.7).

The cartridges used were Oasis® HLB (500 mg, 6mL) purchased from Waters (Saint-Quentin En Yvelines). Before SPE, all samples were adjusted to pH 2 with nitric acid (HNO_3, 1:1 v/v).

The cartridges were conditioned by adding 3 x 6 mL Milli-Q water, followed by 3 x 6 mL MeOH to cartridge activation of the stationary phase, followed by re-equilibration with 3 x 6 mL 500 mL of sample were passed, at constant flow (aprox. 5 mL/min) through the cartridges. Then, the cartridges were let to dry for approximately 5 minutes under vacuum. The last step was the elution with 12 mL of MeOH to 15 mL glass vials, and stored at 6 °C, until further analysis.

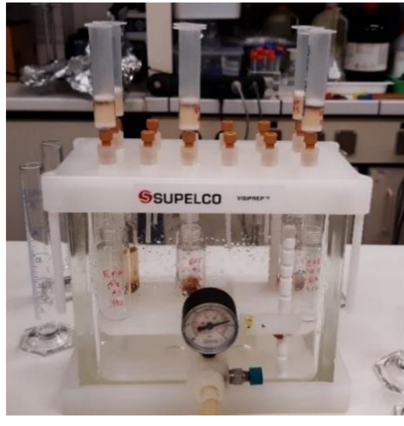


Figure 3.7 - Manifold SPE. Detail of the elution step

3.9.2 High pressure liquid chromatography (HPLC)

PPCPs concentration were determined using High Pressure Liquid Chromatography (HPLC). The chromatograph was equipped with a Diode Array Detector (DAD) (G1315B) and with a Fluorescence Detector (FLD) (1321A), all from Agilent 1 100 Series. Additional equipment includes a 1260 Infinity Quaternary Pump (G7111B) and an automatic sampler 1 260 (G7129A) (Figure 3.8).

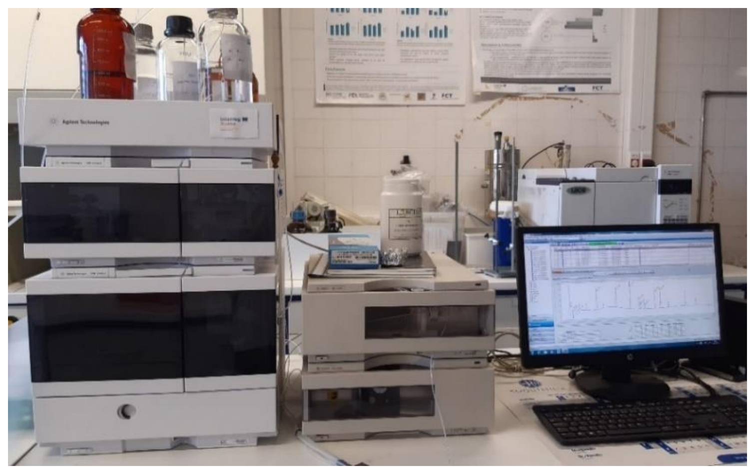


Figure 3.8 - HPLC-DAD-FLD

The column used was a Poroshell 120 EC - C18 2.7 µm (dimensions: 4.6 x 100 mm), acquired from Agilent (California, USA) with a precolumn Onyx SecurityGuard C18 cartridges (dimensions: 5x4.6), from Phenomenex (Torrance, USA).

The chromatographic method used in all HPLC chromatographic runs was a RESOLUTION Lab Internal method, adapted from Guedes et al (2019). HPLC runs were performed in gradient mode at constant flow (1 mL/min) with the oven set at 36 ºC and post equilibrium was carried for 2 min. Eluent A was a mixture of ACN/Milli-Q/formic acid (A: 5/94.5/0.5%), and eluent B was composed by the same mixture with B: 94.5/5/0.5%. Eluents were also filtrated using a 0.45 µm nylon membrane (Bellefonte, USA). The chromatographic runs were performed at a flow rate of 1.5 mL/min, in gradient mode described in Table 3.3, followed by 2 min post-run. Data processing were done using the LC OpenLab software

Table 3.3 - Gradient mode used in HPLC run, at a constant flow of 1.5 mL/min

Time (min)	A (%)	B (%)
1.0	95.0	5.0
9.0	5.0	95.0
10.0	3.0	97.0
12.0	3.0	97.0
13.0	95.0	5.0

Calibration curve was carried out with seven different concentration of the mixed PPCPs standard - 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5 mg/L, in triplicate along different days. Samples were injected in a 2:1 (effluent: eluent A) and injected into the HPLC. Integration of the peak height of the target compounds were measured at 282 nm in channel DAD B.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated for each compound under study. This value is obtained by multiplying the residual standard deviation S_x of the calibration function of the blank by 3. In order to assess the minimum quantified analyte concentration, the LOQ is calculated by multiplying the LOD by 3 (Guedes, 2015).

Based on the LOD value, the MDL value was calculated by applying all the dilutions and concentrations that the sample suffered until being analysed into the HPLC. The MLQ was calculated in an analogous way, but considering as the base value the LOQ.

3.10 Statistical Analysis

Statistically significant differences were analysed using one way-ANOVA with a significance set at 5%, followed by Tuckey test, performed in SPSS Statistics 20.0 (SPSS Inc., Chicago, USA).

The comparations were made the following way for:

In Set 1: for the same compound, different remediation times both electrodegradation assays and for natural attenuation assays at times 2 h, 4 h and 6 h; for the same time, electrodegradation and natural attenuation, different compounds (CAF, SMX, CBZ, DCF, OXY).

In Set 2: for the same compound, electrodegradation, natural attenuation and UV-C photolosys at 6 h; for the same treatment (electrodegradation, natural attenuation or UV-C photolysis) different compounds (CAF, SMX, CBZ, DCF and OXY).

General parameters were compared the following way: For both pH, conductivity and culturable microorganisms (CFU/100 mL), initial and final values within the same assay.

4. Results and discussion

4.1 HPLC calibration

An external calibration method was used to plot the calibration function. For all the target compounds under study, standard solutions were prepared with known concentrations as described previously in section 3.4.2. From the lowest to the highest concentration, the same volume of stock solution was injected into the HPLC system. Injection of stock solutions along time helped to identify the PPCPs retention time into the chromatographic system. In Figure 4.1 there is represented as an example, a chromatogram prepared with a stock concentration of the target PPCPs of 7.5 mg/L.

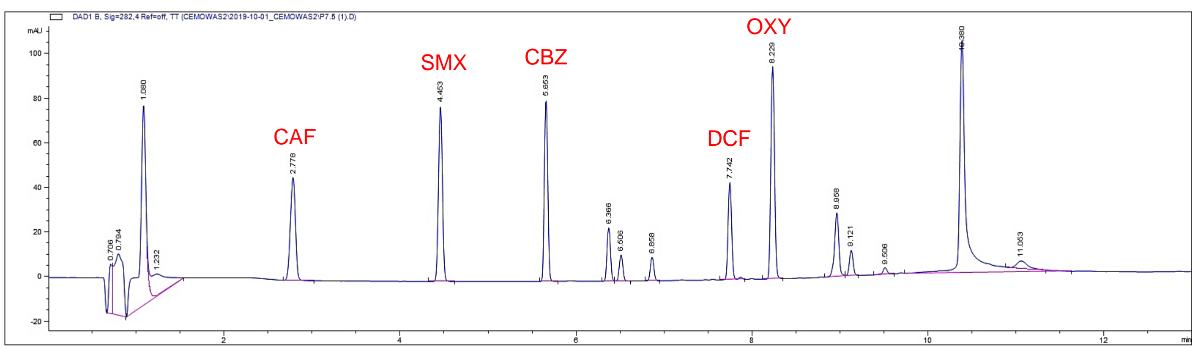


Figure 4.1 - Chromatogram with stock solution with a mixture of the target PCPPs in a concentration of 7.5 mg/*L*

CAF, SMX, CBZ, DCF and OXY had the following retention times: 2.778, 4.453, 5.653, 7.742, 8.229, respectively. Retention times of the PPCPs in spiked effluent samples were always confirmed by an injection of standard solutions. Retention time is a characteristic of a chromatographic run, and so may change with HPLC condition such as temperature, mobile-phase composition, pH and flow rate (Snyder et al., 2012).

During the experimental plan, several chromatographic runs were performed in order to inject different samples from different assays. Blank samples were injected prior samples. Compounds retention time suffered minimal shifts along time. that can be explained by the composition of the eluents of the mobile phase that had to be prepared (preparation was made by different operators might have influenced the solvents strength) and refilled into the HPLC system.

For the range of concentrations used (Table 4.1), a linear dependence was verified, and an adjustment to a linear regression according to the least squares' method was made. The calibration curve represents the dependence of the signal measured by the equipment on compound concentration within the working range (Huebschmann, 2015). All compounds were adjusted to a linear regression model according to the function y= mx+ b, in which m is the slope of the calibration equation and represents the sensitivity of the equipment. The sensitivity is the detector response to a certain analyte concentration per unit of volume or time (Huebschmann,

2015). The calibration curve equations, and respective coefficient of correlation and coefficient of variation are represented in table 4.1

Linearity of the calibration curve can be evaluated by the coefficient of determination (r^2). The coefficient of variation (CV) measures the values dispersion associated to the calibration curve. In this case, for all compounds the value of the determination coefficient was equal to the unit and the coefficient of variation is < 5%, displaying an excellent linearity for the range of concentrations considered.

Table 4.1 – Comparation of curve equations and calculate linearity parameters for every compound under study, measured in the DAD-B channel ((λ = 282 nm).

Compound	Wavelength measured (nm)	Regression equation	r^2	Working range (mg/L)	CV (%)
CAF	282	y = 24.026x – 1.0193	1.0000	[0.51 – 12.68]	2.2
SMX		y = 30. 44x – 0.8537	1.0000	[0.53 – 13.31]	2.9
CBZ		y = 27.724x – 0.1029	1.0000	[0.52 - 13.12]	3.1
DCF		y = 15.554x – 1.4843	1.0000	[0.52 – 12.88]	4.9
OXY		y = 36.205x – 1.3903	1.0000	[0.51 – 12.62]	2.6

4.2 Limits of detection and quantification

4.2.1 HPLC

The limit of detection (LOD), limit of quantification (LOQ), method limit of detection (MLD), and method limit of quantification (MLQ) calculated values are displayed in Table 4.2. The LOD is defined as the lowest concentration of analyte that can be detected (within the signal domain) but not necessarily quantified in the analytical instrument (Huebschmann, 2015). Some strategies to lower the values of LOD and LOQ could be using a more sensitive equipment, such as LC-MS/MS system, that has ability to detect an analyte at lower concentrations. The method of calculation of these parameters is described in section 3.4.2.

Table 4.2 – Instrument and method limit of detection of quantification

Compound	LOD (mg/L)	LOQ (mg/L)	MLD (mg/L)	MLQ (mg/L)
CAF	0.37	1.10	0.03	0.11
SMX	0.52	1.56	0.03	0.08
CBZ	0.55	1.64	0.04	0.12
DCF	0.84	2.53	0.05	0.15
OXY	0.44	1.32	0.03	0.08

4.2.2 Colonies enumeration

The microbiology method was validated by incubating a negative control, alongside with all experiments. No colonies have grown in this petri dish in any case. In Table 4.2 is represented the MLD of the spread plate procedure used.

Table 4.2 - Method detection limit of the microbiology method

Set	Dilution	Volume plated (mL)	MLD (CFU/100 mL)
1	10^0	0.1	1×10^3
2	10^0	0.1	1×10^3
	$2x10^{-1}$	0.1	5×10^3

4.2.3 Method recovery

Recovery assays were performed by spiking the effluent (0.2 mg/L) with the PPCPs under study and perform further extraction, making the time zero control. A contact time of 30 ± 10 minutes after spiking and before extraction by SPE was kept. Effluent was only agitated in the moment of the spiking in order to ensure homogenization.

Method recovery for effluent samples in both Set 1 and Set 2 revealed that for CAF, CBZ and DCF recoveries were above 100%. SMX and OXY in opposition, showed low recoveries (below 80%) appearing to have suffered signal suppression. In this case, it is expected that analytes could have stayed retained in the SPE cartridge.

In Table 4.3, values of different recoveries testes are represented. Compounds recoveries can be affected by the type of SPE cartridge used, solvent strength, matrix properties such as salinity and colloidal content. Effluent is a complex matrix, and humic acids have functional groups such as hydroxyl, carboxyl and amino groups known to have complexation and adsorption properties (Borecka et al., 2014). Compounds hydrophobicity and molecular size plays a great role in extraction efficiency in SPE as well. Vanderford et al (2003) denoted that the extraction procedure of PPCPs, steroids and EDCs in surface water containing wastewater highly affects the recovery of the compounds, contributing to matrix suppression. High polar compounds, presenting Log Kow < 3, have been indicated in the literature to being more susceptible to suffer signal suppression (Borecka et al., 2014). SMX (Log kow = 0.84), experienced an increased signal reduction along time in Set 1.

Besides the low recoveries obtained for OXY in all sets, RSD is ≤ 10%, ensuring robustness to the method

.

Table 4.3 - Method recoveries obtained in spiked effluent

Compound	Set 1 - Mean ± STD (%)	Set 2 - Mean ± STD (%)	
	EEF UV (0.2 mg/L) (n=2)	EFF F1 (0.2 mg/L) (n=3)	EFF F2 (0.2 mg/L) (n=2)
CAF	120 ± 5	150 ± 15	143 ± 4
SMX	44 ± 7	120 ± 9	89 ± 2
CBZ	123 ± 3	148 ± 10	123 ± 4
DCF	163 ± 21	140 ± 12	148 ±10
OXY	77 ± 4	85 ± 8	72 ± 0

In Figure 4.2 it is represented two chromatograms from assays made one month after the effluent sample from Set 1 had been kept in the lab and sequentially exposed to UV-C radiation. It is possible to see that for the same concentration (in this case was 0.3 mg/L), in different matrices – chromatogram a) represents water type II (a lower complexity matrix) and b) represents UV-C treated effluent used in Set 1. Both assays were done in the same day. SMX in this case showed no analytical signal in the effluent matrix in opposition to recovery in water type II.

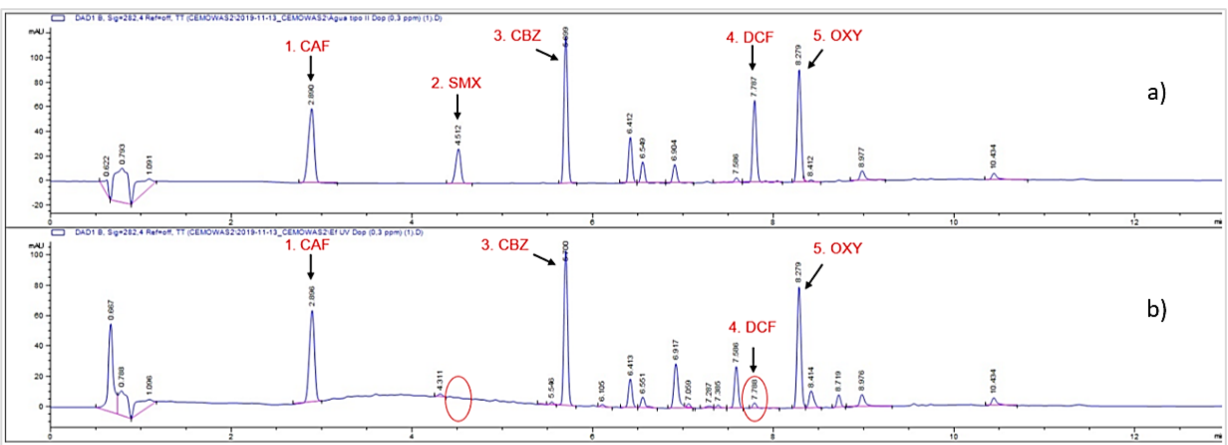


Figure 4.2 - Chromatograms of recovery assays Set 1 - 0.3 mg/L. a) Water type II spiked at 0.3 mg/L b) EFF UV spiked at 0.3 mg/L

Based on Set 1 experiments, Set 2 introduced some improvements. In Set 2, after adjustment to pH 2 prior SPE, effluent samples were filtrated using a 0.45 μm filter to remove colloidal particles in order to minimize the interferences of dissolved organic matter. All assays were done in the same day and reactors were covered with aluminium foil and protected from light.

4.3 Initial effluent characterization

All experiments were conducted using real effluent, corresponding to the liquid fraction collected form the secondary settler. The knowledge of initial chemical characteristics of the effluent helps

to assess the complexity and heterogeneity of the aqueous environmental matrix that served as basis of experiments.

Initial sample characterization is represented in table 4.4. Data were provided by the laboratory of control and processes of Quinta do Conde WWTP. Effluent characterization of 24/09/2019 corresponds to the effluent sample that was used Set 1 of experiments. Water quality analysis of the secondary effluent are performed every 15 days, so regarding the third effluent sampling (17/12/2019), the reported characterization is from a week later (24/12/2019), when the analysis was made.

Table 4.4 - Initial characteristics of the secondary effluent samples collected in Quinta do Conde WWTP

Date	pH	TSS (mg/L)	BOD_5 (mg/L)	COD (mg/L)	TN (mg/L)	TP (mg/L)	O&G (mg/L)	HC (mg/L)	NO_3 (mg NO_3/L)
24/09/2019	ND	13	9	63	65	6	ND	ND	<4
05/12/2019	7.6	16	20	97	66	5	<0.2	<0.1	<4
24/12/2019	7.6	26	13	67	43	8	<0.2	<0.1	<4

TSS – total suspended solids; COD_5 – biological oxygen demand determined after 5 days; COD – chemical oxygen demand; TN- total nitrogen; TP – total phosphorus; O&G – oils and greases; HC – hydrocarbons; NO_3 – nitrate; ND - Not determined

All the values respect the Portuguese law (DL nº 152/97 of 19 June) regarding the minimum discharge requirements for the receiving medium. Rainfall events during the month of December might have had impact in the removal rates of the WW quality parameters presented in table 4.4. The high value of total suspended solids (TSS) in 24/12/2019 might be due to low sedimentation of activated sludge as consequence of the perturbance caused by rainfall.

Regarding the parameters characterized, COD values represent the amount of oxygen equivalent that is required to mineralize a given substance to carbon dioxide and water, by a strong oxidation agent, including both organic and inorganic species. For these reasons, these values are higher that BOD_5, that only include the biodegradable fraction or information about the biodegradability of the effluent (Metcalf & Eddy, 2003).

4.4 Electrokinetic experiments

4.4.1 pH, conductivity, and voltage

The initial pH of the effluent samples used varied between 7.8 ± 0.6 (Set 1) to 8.20 ± 0.01 (Set 2, sample F2). Effluent is a matrix that has enough ionic conductivity to carry the electric current applied, due to natural occurring inorganic compounds and dissolved salts. With the application of electric current in EK reactors, oxidation and reduction of species present in the effluent occurs (Magro et al., 2019).

Water electrolysis leads to the reduction of water and production of OH^- in the cathode surface that causes the effluent to become more alkaline and at the same time, production of H^+ takes place in the anode surface. Also, the low-level current density applied of 8 mA/cm² controls the pH evolution. Stirring was performed each 30 minutes before voltage was registered and helped to ensure homogenization. This was a fundamental step to minimize regional differences in the reactor (Sillanpää & Shestakova, 2017).

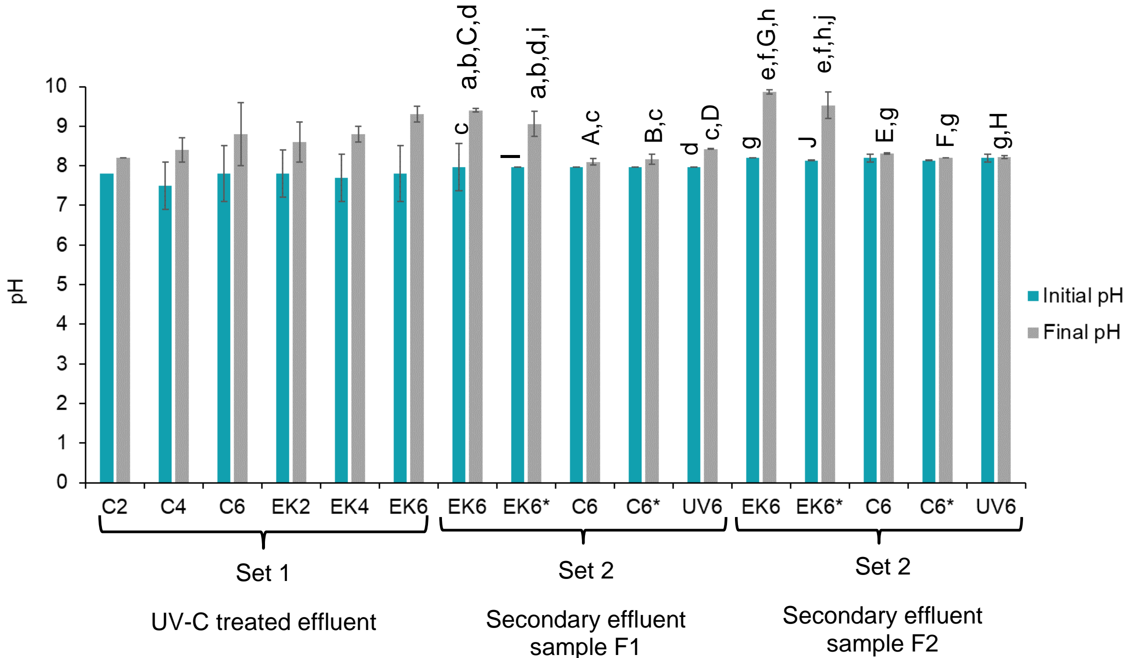


Figure 4.3 - Mean initial and final pH in Set 1 and Set 2. Error bars represent standard deviation. Lower case letters are statistically significative from the values with the same letter (*p*<0.05, 95% confidence interval) (UV-C n=2; F1 n=3 and F2 n=2). "*" represents non-spiked samples

In fresh effluent experiments, in all EK6 experiments the final pH increased significantly from the initial pH (*p*< 0.05) in both spiked and non-spiked EK reactors. This is mostly a result of the cell design used (1c-cell), as the electrodes are inserted in one single compartment directly in the effluent.

The highest pH value was obtained in EK6 (Set 2, sample F2) from an initial value of 8.20 ± 0.01 to 9.86 ± 0.05 after 6 h of electrolysis (complete values can be seen in Appendix).

Regarding controls (reactors without current applied), final pH and conductivity shown no statistically significative differences (*p*>0.05) in comparation with initial values, in any case. Effluent microbial community is affected by the neutral to alkaline pH shifts, as the application of the low-level DC creates selective conditions in the electrochemical cell (Alshawabkeh et al., 2004). In a complementary way, removal of PPCPs is pH dependent as it affects the speciation of the compounds, and its bioavailability for biodegradation (Nguyen et al., 2018). Figure 4.4 displays the final and initial conductivity measured in Set 2. No differences (*p*> 0.05) were found between initial and final conductivity in any experiment.

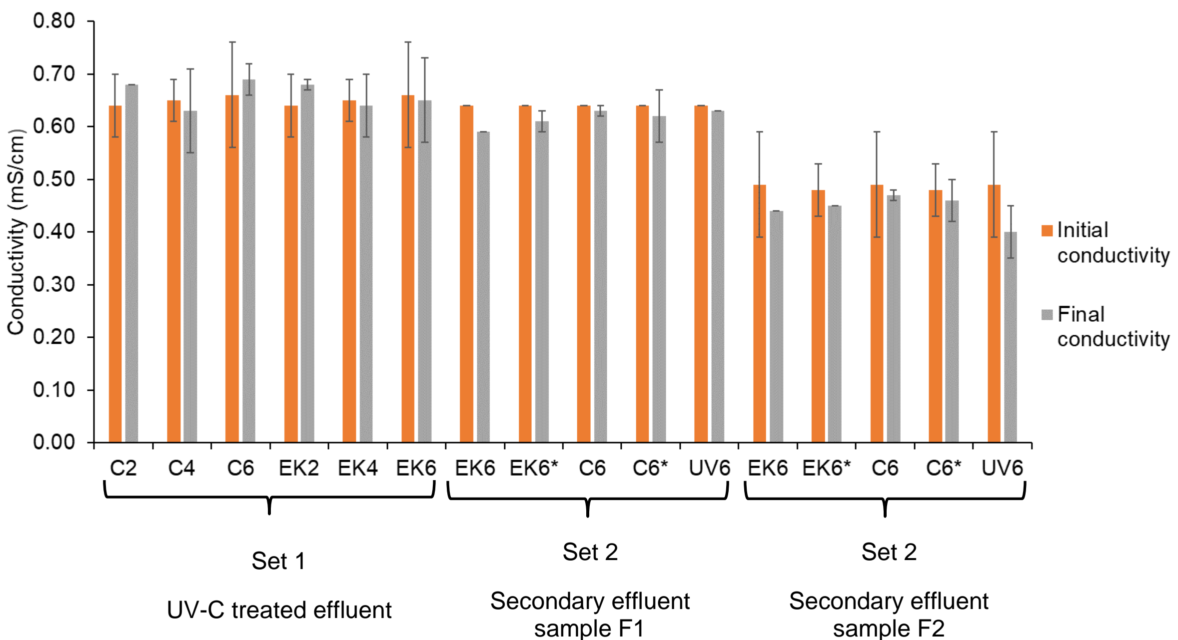


Figure 4.4 – Mean and standard deviation of conductivity in Set 1 and in Set 2. Error bars represent standard deviation. Lower case letters are statistically significative from the values with the same letter (p<0.05, 95% confidence interval) (UV-C n=2; F1 n=3 and F2 n=2). "*" represents non-spiked samples

The results of voltage measurements taken during experiments every 30 minutes are plotted in Figure 4.5. With the increase of treatment time, in all experiments the voltage between working electrodes followed an overall increasing trend, being higher at the end of experiments for all cases. Voltage drop (ΔV = final voltage - initial voltage) was the highest in experiment F2 EK6* with a ΔV of + 2.70 V, after 6 h of electrolysis. The lowest voltage drop was achieved in experiment F1 EK6 with a value of +1.03 V.

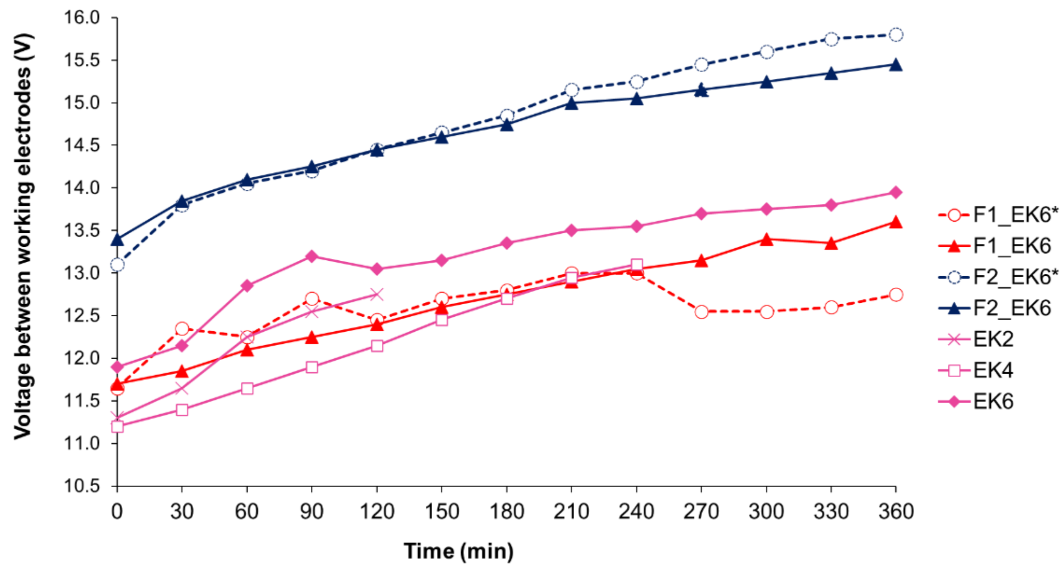


Figure 4.5 - Voltage evolution between a pair of electrodes. DC electric field applied of 50 mA. "*" represents non-spiked samples

Different voltage values registered at time 0 was a result of the use of different effluent samples, collected in different days from the WWTP.

In effluent sample F1, two peaks of voltage are observed after 30 and after 90 minutes. The fact that these assays were not spiked with the target PPCPs under study, explains the high potential observed as the effluent has less ionic species and this reflects in the resistance of the system. After 270 minutes of electrolysis for F1 EK6* sample, a sudden decrease of ≈ 0.5 V was observed. In general, the shifts in the linear behaviour might be due to oxidation and reduction of organic species taken place in the bulk solution.

According to the Ohm's law and at a fixed current intensity (e.g. 50 mA), voltage changes are due to an increase or decrease in the resistance (R), acording to the equation $V = I \times R$ (Bard et al., 2012). Voltage behaviour is related with the conductivity of the electrolyte. The increase in resistance indicates that the depletion of ions is happening faster (Guedes, 2015).

Agitation energy requirements takes a great part of the energy consumptions in an electrochemical reactor. By switching to non-continuous agitation, energy savings can be introduced, but the efficiency of the system need to be carefully assessed in order not to be compromised. Energy power consumption (P) is directly proportional to current intensity and voltage, according to the formula $P = I \times V$ (Modin & Aulenta, 2017). Lower resistance, due to natural high conductivity of effluent, makes the energy requirements lower. Working with low current intensities and low treatment durations aims to minimizes energy consumption. Designs and operational conditions of the electrochemical cells are diverse and are affected by the choice of voltage and current level, as for higher voltage gradients, higher conductivity of the media is required and energy consumption increases (Sillanpää & Shestakova, 2017).

As general observations, it was noted that during electrolysis, for all EK experiments performed, effluent apparent colour in the electro-reactor changed from light yellow to colourless by the end of treatment. Determination of species such as chlorine was not performed, but after the EK treatment a slight smell of chlorine gas was felt, indicating that most probably the effluent samples had chlorine.

4.5 PPCPS degradation

Application of the DC electric field controls the formation of •OH radicals at the anode surface, that are responsible for the oxidation of the organic species. PPCPs degradation pathways mediated by •OH radicals include dehydrogenation and hydroxylation reactions and charge transfer by redox reactions (Cuerda-Correa et al, 2019).

Other species generated by the oxidation of wastewater such as chlorine, hypochlorite, peroxosulphates, hydrogen peroxide also contribute to indirect oxidation of PPCPs (de Vidales et al., 2015; Magro et al., 2019).

The concentration not detected (below LOD or LOQ) in HPLC was considered to be totally removed by EK and/or to have suffered natural attenuation (biotic, such as bioremediation, and abiotic factors such as adsorption). In the case of Set 1 experiments, reactors were not protected

from visible light, so photodegradation may also be a degradation mechanism for PPCPs degradation. Application of a DC electric field of 50 mA has already proved to improve degradation of PPCPs namely hormones such as E2 and EE2 from sewage sludge by Almeida (2015). Alongside, the effect of the applied current intensity adversely affects the effluent microbial community (Li et al., 2003).

4.5.1 PPCPs degradation in Set 1 – UV treated effluent

In the first Set of experiments, effluent was previously exposed to UV-C radiation (254 nm) in order to ensure inactivation of effluent microbial community. In this set, EK process acted as an effluent polishing step. Results of the target PPCPs in comparation with control, are presented in table 4.5

PPCPs degradation performed in *post*-disinfected effluent allowed to obtain for the three tested times – 2 h, 4 h and 6 h removals in the range of 17 ± 6%, for CAF after 2 h of EK treatment and to complete degradation (below LOD) in the case of SMX after 4 h of EK treatment. In this Set, for SMX, no statistical differences ($p < 0.05$) were found between electrodegradation experiments and control experiments in disinfected effluent experiments. This could be related with the low recovery obtained. Regarding electrodegradation, the trend differed according to the electrolysis time tested, as for EK2 the degradation tend was: SMX > OXY > DCF > CBZ > CAF. In EK4 experiments, degradation of DCF increased by 2.7 fold when compared with the value of EK2. From EK4 to EK6, OXY become the second most degraded compound, with a removal of ≈ 95%.

Table 4.5 - PPCP degradation from UV-C treated effluent

Set	Type of assay	PPCPs degradation – Mean ± SDT (%)				
		CAF	SMX³	CBZ	DCF	OXY
1	EK2	17 ± 6	94 ± 8[1]	24 ± 3	21 ± 20	28 ± 2
	C2	3 ± 2[d]	95 ± 6[1]	3 ± 4[E]	54 ± 50	3 ± 1[g]
	EK4	31 ± 11	99 ± 0[2]	43 ± 9[e,f]	57 ± 35	41 ± 5
	C4	3 ± 8[d]	93 ± 8[1]	5 ± 8 [F]	51 ± 35	9 ± 6[g]
	EK6	41 ± 7[D]	99 ± 0[2]	56 ± 9[b,e,f]	63 ± 28	95 ± 6[G]
	C6	9 ± 3[A,d]	99 ± 0[2,a,b,]	13 ± 5[B]	60 ± 44	21 ± 4[C]

PPCPs EK (%) degradation = [1- ∑(Concentration after treatment/Concentration recovered in control)]*100; At least of the replicates is under LD or LQ¹, Totally removed below LQ or LQ², Degradation normalized in relation to the recovery³, ND – Not determined; Data with capital letters is compared with the lower case letter. $p < 0.05$, ANOVA

The lower treatment time (2 h), was the most promising in terms of costs minimization, but resulted in the lower electrodegradation of the PPCPs under study. In general, the higher the effluent electrolysis time, the higher the PPCPs degradation from UV-C treated effluent by EK. In general,

the 6 h treatment time was considered the best option for PCPPs electro degradation for further experiments in Set 2.

In figure 4.6, is possible to see that for DCF, the lack of symmetry of the peak, that does not happen in the respective controls, leading to indicate a possibility of a degradation by-product of DCF might be forming and co-eluting with similar retention time. In the first replicate of EK4 and EK6, OXY was below LOD.

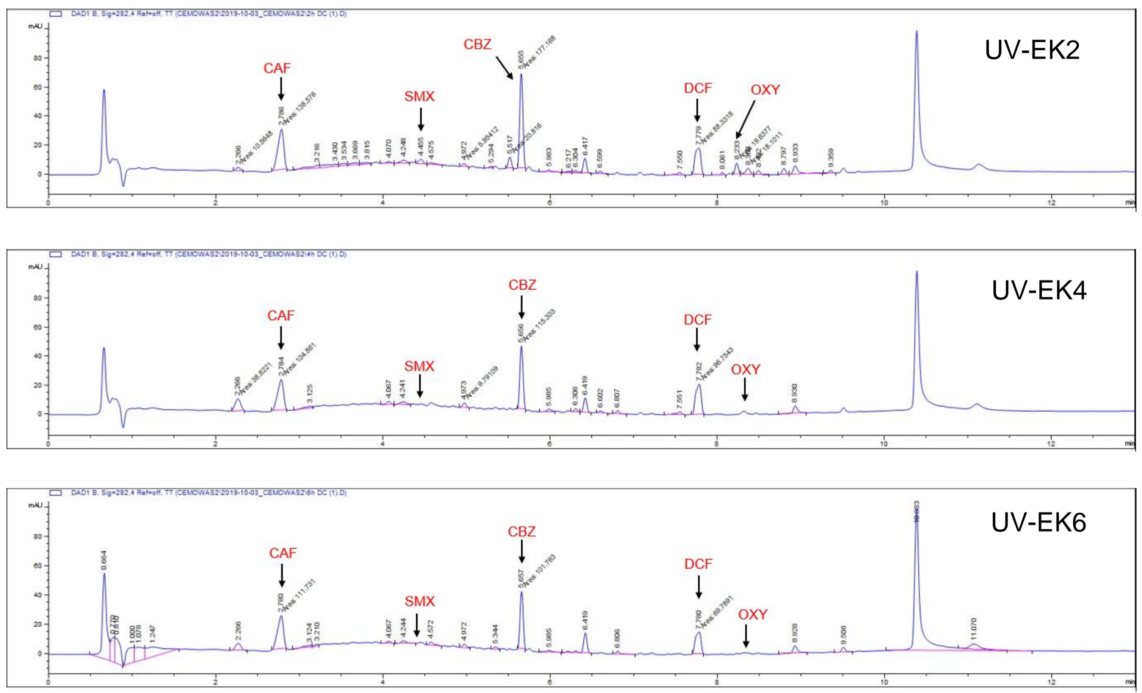


Figure 4.6 - Chromatograms from EK process in UV-C treated effluent

By comparing the concentration obtained in control experiments, with the concentration of spiked recovery assays (time zero controls), natural attenuation can also be assessed.

In wastewater, pathogens can be dispersed in the media or embedded within solids in suspension. In the last case, the UV radiation is less effective as particulate material in wastewater scatters and absorbs UV light, reducing the UV dose that reach target organisms. Other factors that affect UV efficiency are the dissolved organic matter (DOM) content of the water sample (Chen et al., 2006). Liang et al (2013) experimentally verified that the effect of the total suspended solids in tailing. Using *e-coli* as a model organism, the UV inactivation rate followed a first-order kinetic, and was shown to decreased with the increase of total suspended solids. In some circumstances, DNA repair can happen by photoreactivation (light dependent) or by dark repair (light independent). Photoreactivation can happen when the cyclobutene pyrimidine dimer Is disintegrated, in result of exposure to UV-A light or visible light by cleavage of the pyrimidine dimer (Wen et al., 2019). Such process however varies from microorganisms type, microorganisms specie and strain (Chen et al., 2006).

To highlight that in Set 1 experiments, the effluent was not filtered prior experiments and different replicates were made in different days, with days in which the effluent did not receive UV-C radiation. This also helps to explain the high standard deviation among replicates within the same experiment type. Photoreactivation also most likely happened in some extent.

For the three control times, 2 h, 4 h and 6 h, natural attenuation did not showed statistically differences among experiments ($p > 0.05$). However, the main issue with experiments done with effluent, disinfected in a lab set up, was that the experiments for different replicas and for different remediation times were done along different days with the same effluent stored and sequentially exposed to UV-C radiation. This induced changes in an already heterogeneous matrix, so any time EK treatment was applied, the EK process lead to variable results within the same compound, not allowing a proper study as happened in the case of SMX and DCF, due to the wide variability among replicates and also due to the low recovery in the case of SMX and high recovery, in the case of DCF. High natural attenuation values in DCF can be overestimating the real degradation and in EK experiments, due the high recovery (≈ 163%).

With the application of the electric field, speciation of PPCPs and the pH evolution of the effluent during electrolysis are key aspects in order to understand the degradation of the compounds. In UV-C treated effluent experiments, the pH of the effluent started being slightly neutral (7.7 ± 0.7) achieving in the case of the EK6 experiment the higher pH value of this set, 9.3 ± 0.3. For this pH range, the acid ionization constant in the case of CAF and CBZ was always higher than the solution pH (pKa_{Caf} = 14 and pKa_{CBZ} = 13.9) which means that compounds are in the nonionized form, having no electric charge. OXY (pKa_{MBPh}= 8.07) and SMX (pKa_{SMX}= 5.7) are presented in the ionized form. All experiments were performed at constant temperature of 22 ± 1°C. Compounds in ionized state are known to be more soluble and to be easier degraded than when presented in molecular form (Magro et al., 2019). This might be an explanation for SMX and OXY achieving the highest electrodegradation when compared with CAF, CBZ and DCF.

4.5.2 PPCPs degradation in Set 2 – Fresh effluent

In fresh secondary effluent experiments (Set 2), electrolysis time was further performed for the best PPCPs degradation time for all compound, in the previous experiments – 6 h. Two effluent samples were used in EK experiments, performed in different days with all replicas done in the same day for each fresh secondary effluent samples. For the two samples (F1 and F2), the same exact experimental procedure was followed, showing that remediation of PPCPs is dependent of physicochemical composition of effluent, that is known to vary with weather conditions and with influent wastewater characteristics that arrive the WWTP.

A new type of experiment was also introduced in parallel to EK treatment and aimed to establish a relative comparation with a typical disinfection step used in WWTP (UV-C photolosys). In opposition with the previous usage of UV-C radiation in experiments (Set 1), in which the effluent was exposed to UV-C radiation prior EK, in Set 2, the UV-C radiation was used as a treatment

technology for assessing PPCPs degradation and microorganisms inactivation from fresh effluent with spiked PPCPs.

In the simplest form, for all experiments types, EK and UV-C, the same treatment time was considered. For both fresh effluent samples (F1 and F2) an increment of concentration happened in comparation with the initial known concentration spiked, resulting in a negative removal of the parent compound, as can be seen in Table 4.6.

Table 4.6 - PPCPs degradation from Set 2

Set	Effluent code	Type of assay	PPCPs degradation – Mean ± SDT (%)				
			CAF	SMX	CBZ	DCF	OXY
2	F1	EK6	19 ± 7	95 ± 7^{1,C}	40 ± 8^{F}	27 ± 17	95± 4^{m,N,o,r}
		C6	19 ± 9	19 ± 9^{c,d}	16± 10^{E,h}	8 ± 18^{I,j,l}	30 ± 13^{M,n,o,p,q,r}
		UV6	-11 ± 12^{A}	26 ± 19^{c,d}	-14 ± 23^{f,h}	53 ± 15^{i,J,k}	-26 ± 13^{m,n,O,q}
	F2	EK6	38 ± 5^{a,B}	99 ± 0^{2,D}	61 ± 5^{e,g,H}	56 ± 1^{i,L}	99 ± 0^{2,m,o,p,Q,r}
		C6	6 ± 2	6± 2^{c,d}	6 ± 0^{G,h}	5 ± 4^{j,K,}	7 ± 1$^{n.P,q,r}$
		UV6	-4 ± 1^{b}	-4 ± 14^{c,d}	-10 ± 4^{f,h}	67 ± 5^{i,k}	-19 ± 12^{m,n,q,R}

PPCPs EK (%) degradation = [1- ∑(Concentration after treatment/Concentration recovered in control)]*100; At least one of the replicates is under LOD or LOQ[1], Totally removed below LOD or LOQ[2], Degradation normalized in relation to the recovery[3], ND – Not determined; Data with capital letters is compared with the lower case letter. $p < 0.05$, ANOVA

In EK6 assays, regarding F1 samples, CAF is statistically different ($p< 0.05$) from SMX and from OXY. In F1 EK6 removal among compounds is statistically different, except for CAF and SMX, and from CBZ and DCF, and CAF and DCF.

Higher final concentration in comparation with the initial concentration values have been reported in the literature. According to the process applied, re-transformation of metabolites to the parent compound might happen, in particular to pharmaceuticals such as sulfamethoxazole and carbamazepine (Nguyen et al., 2018). Only DCF presented a positive removal in this experiment type alongside with SMX in F1-UV6. In fact, when UV6 is compared with electrodegradation assays (EK), DCF showed no statistical differences when comparing F1-EK6 to F1-UV6 and F2-UV6 to F2-EK6 ($p> 0.05$). In this case, and mostly due to the complex nature of effluent samples, the application of UV-C radiation might have lead to transformation of natural occurring species present in effluent, that are co-eluting with the same retention time of the target PPCPs.

Overall results of PCPPs degradation *per* experiment, are represented in Figure 4.7. Photolysis happens when radiant energy is absorbed by molecules leading to reach excited states and in result chemical reactions take place. Oxidation reactions happens and are mediated by free radicals. Indirect photolysis can take place by the presence of dissolved organic matter - humic and fluvic acids that act as photosensitizers, and in a UV-C light mediated process absorbed radiation also generate free radicals. Humic acids (HA), in its photo induced excited state, known as triplet state can form reactive oxygen species (ROS) that contribute to indirect

photodegradation of PPCPs in bulk solution. A competing mechanism in also found in HA, where can even inhibit PPCPs degradation by absorbing photons in the same emission wavelength (Biancullo et al., 2019; Cuerda-Correa et al., 2019). As an hypothesis, the content of EfOM of the effluent sample, might have scavenge photons, not enabling proper degradation of the compounds.

The different speciation states also affect the light absorption properties of the PPCPs under UV-C radiation (Zhu et al., 2019). As effluent pH in Set 2 experiments is on the alkaline side (above pH 8), SMX, DCF and OXY are always in the ionized form, while CAF and CBZ remain in the molecular form. Regarding CAF, SMX, CBZ and OXY statistical differences were found between F1-UV6 and F2-EK6 and with F2-UV6 and F2-EK6 experiments. For DCF, both electrodegradation and photodegradation resulted in similar degradations, being the only compound whose degradation between these two types of experiments were not statistically significative ($p>0.05$), with a photodegradation of 67 ± 5% in F2-UV6 and electrodegradation of 56 ± 1% in F2-EK6. UV-C radiation is effective in photosensitive compounds, and in effluents with low COD. Paredes et al (2018) referred that the photon energy emitted by the UV-C lamp at 254 nm (472 kJ/mol) can attack compounds with bonds with low dissociation energy such as phenyl-Cl (406 kJ/mol), present in compound such as DCF and phenyl-NH_2 (436 kJ/mol) present in SMX. OXY, on the other hand is an UV-filter, so its formulated to be photostable, making this compound more recalcitrant to photodegradation processes.

Removal of PPCPs is both performed by biotic (e.g. biodegradation) and abiotic (e.g. biorremediation, adsorption) processes. The molecular structure and physicochemical properties affect the biodegradability of the compounds. DCF, has the lowest natural attenuation value in fresh effluent experiments from Set 2. When compared with the other molecular structures of other PPCPs under study, is the only compound having a chlorine group, identified to be more recalcitrant to biodegradation (Guedes et al., 2019). In Set 1, removal of DCF might have been due to visible light photodegradation as these reactors were not covered.

Lee & von Gunten (2010) studied the application of selective oxidant species such as chlorine, chlorine dioxide, ferrate VI an ozone and non-selective oxidant species such as •OH radicals for the removal of different MPs from WWTPs effluents. It was shown that •OH radicals react with almost all organic species, presenting high reactivity in comparation with the selective oxidants that react only in some electro rich organic moieties (ERMs). In this case, the presence of EfOM, nitrite, ammonia and bromide affected the removal efficiency when chlorine and ozone is applied.

Electrodegradation of PPCPs with two different effluent samples, resulted in overall higher values for the last secondary effluent sample collected (F2) from Quinta do Conde WWTP. Besides COD values were not experimentally determined, a lower COD content in this sample in comparation with F1 sample, might be a possible reason for the higher PCPPs degradation. In Figure 4.7 there is represented the overall PPCPs trend in all Sets.

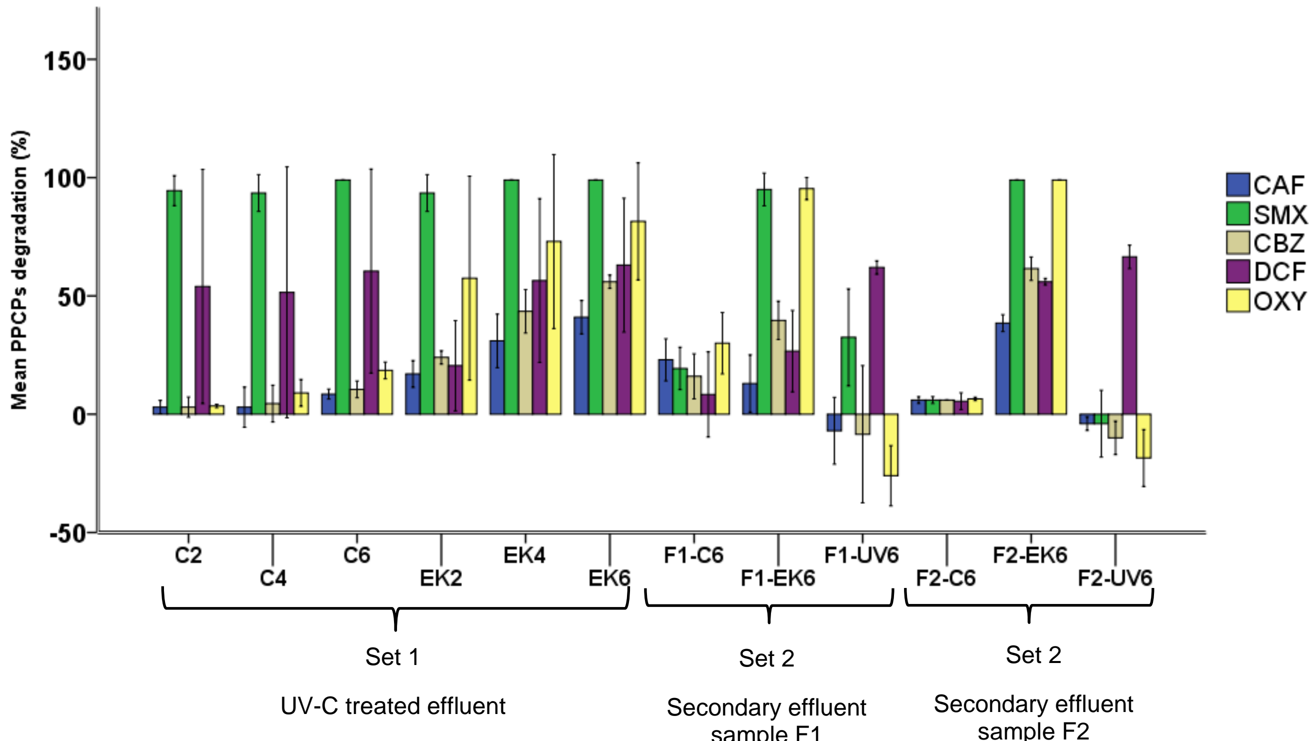


Figure 4.7 – Overall PPCPs mean degradation from UV-C treated effluent and from fresh effluent Deviation bars represent standard deviation

PPCPs electrodegradation trend obtained for fresh effluent samples was SMX ≈ OXY>CBZ > DCF > CAF, for both F1 and F2 effluent samples. Results indicate the highest removal of SMX and OXY in F1, in which in two of the three replicas done SMX was removed below LOD. In F2, SMX was totally removed bellow LOD, not being detected. For OXY, the same degradation trend was verified.

Complete degradation of SMX was reported by Hussain et al (2015), after 30 minutes of electrolysis using a support electrolyte of NaCl and $Ti/RuTiO_2$ anode. The main factor identified for the higher SMX degradation was the increase in the applied current density (20 mA/cm² to 40 mA/cm²), the concentration and type of electrolyte. In this case, the increase in concentration of NaCl lead to enhance the formation of oxidation species. When using H_2SO_4 as support electrolyte under the same operational conditions, no significative differences were obtained from the control (no applied current), showing that SMX was not degraded under these circumstances. The degradation of SMX occurred by indirect oxidation and under acidic conditions. Experiments were performed under acidic conditions (initial pH adjusted to 3), compound removal was higher at pH in the range of 2.5 and 5, that in the range of 7 and 9. Experimental conditions have a strong influence in the results obtained for the degradation of compounds.

The comparation between electrodegradation values and degradation trends has to be made in relative terms, as technologies and operating conditions vary widely among studies. According to

Cotillas et al (2016), whenever electrochemical oxidation is performed, disinfection of effluent also takes place. It was studied the feasibility of an one-step, photo-electrolysis system for oxidation of persistent organic contaminants from secondary effluent aiming reclamation purposes. In this type of photo-electro reactor, the anode used was a Boron Doped Diamond (BDD) plate, and the cathode a stainless-steel plate placed with a distance of 6 mm and current density of 30 mA/cm². Electrolysis and photo-electrolysis were tested and for sulfamethoxazole and dimethyl phthalate, UV-C radiation enhanced the remediation of the parent molecule, but the combined photo-electrolysis did not contribute for further mineralization. Main outcomes further include that the UV-C radiation does not necessarily enhance electrolysis, in comparation with electrolysis alone (Cotillas et al., 2016). Coupling electrochemical oxidation with UV radiation has been tested for the removal of dyes from the textile industry, for effluent decolourization and further reuse for the same industrial process (ECUVAL, 2017).

4.6 Culturable organisms

Alongside with PPCPs electrodegradation and UV-C photolosys, the microbial community present in effluent was also affected by the applied treatment. EK phenomena is known to cause change in the physicochemical characteristics of the effluent matrix and in the microbial community (Gill et al., 2014). In both Sets, the effect of the electric current in the total culturable microbial community was also studied.

Besides microorganisms being capable of degrading complex carbon sources, including PPCPs at high concentrations, they can be sensitive to many of them (Nguyen, 2018). Cell response to the environmental stress, in the case of the addiction of a mixed carbon sources and in the case of the application of a low-level current density (8 mA/cm²), showed to have an inhibition effected in the total culturable microbial community from the secondary effluent.

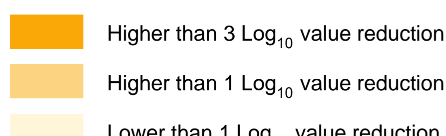
In Table 4.7 there are represented the initial and after treatment reduction values. The total culturable microorganisms were at its highest in the initial sample (t0*) for all cases.

Table 4.7 - Total culturable microorganisms obtained in Set 1 and Set 2 experiments

Set	Assay	n	Dilution	Spread plate - Potato dextrose agar			
				Number of plaques below detection limit (<1 CFU/plate)	Mean Concentration (CFU/100 mL)	Mean Reduction (%)	Log_{10} Value Reduction (LVR)
1	**t0***	2	10^0	0	1.48×10^5	-	-
	C2	2	10^0	0	5.95×10^4	-	-
	C4	2	10^0	0	3.35×10^4	-	-
	C6	2	10^0	0	4.30×10^4	-	-
	EK2	2	10^0	0	5.50×10^3	91[1]	1.05[1]
	EK4	2	10^0	0	6.00×10^3	82[1]	0.87[1]
	EK6	2	10^0	0	5.50×10^3	87[1]	0.91[1]
2	**t0***	4	2×10^{-1}	0	1.82×10^6	-	-
	F1-C6	4	2×10^{-1}	0	4.04×10^5	77.72	0.65
	F1-C6*	9	2×10^{-1}	0	1.65×10^6	8.88	0.04
	F1-EK6	9	10^0	3	1.11×10^3	99.94	3.21
	F1-EK6*	9	10^0	8	3.33×10^2	99.98	3.74
	F1-UV6	9	10^0	0	1.84×10^4	98.98	1.99
	t0*	4	2×10^{-1}	0	1.48×10^6	-	-
	F2-C6	4	2×10^{-1}	0	1.09×10^6	26.5	0.13
	F2-C6*	9	2×10^{-1}	0	1.45×10^6	2.28	0
	F2-EK6	9	10^0	3	5.00×10^2	99.98	3.47
	F2-EK6*	9	10^0	2	1.33×10^3	99.91	3.04
	F2-UV6	9	10^0	0	1.03×10^4	99.30	2.16

*Non-spiked assays
[1] Normalized to control with the same treatment time

Color scheme:



Many microorganisms can remain viable in effluent and the inactivated fraction can also suffer photoreactivation during the time the effluent did not received UV-C radiation (Kauser et al., 2019). Filtration in fresh effluent samples also physically removed the microorganisms associated in the TSS particles. pH variations can affect the speciation of the ionizable compounds and subsequently their tendency of sorption to particles or bioavailability for biodegradation.

The presence of primary substrates for microbial growth (e.g. organic carbon or ammonia) has been reported to enhance or inhibit removal of PPCPs due to competition for non-specific enzyme active sites (Rossmassler et al., 2019; Nguyen, 2018).

Zhang et al (2015) focused on the viable but not cultivable (VBNC) state of *Pseudomonas aerugiosa* and *e-coli* after UV disinfection in water samples. UV radiation induced effect in the reduction of culturable target microorganism's determination using heterotrophic plate count. The results comparasion with a molecular based approach, targeting virulence genes using PCR amplification, showed that similar VBNC states were induced for both strains, and that cell integrity was kept and analysis of the expression genes 16S rRNA was equivalent to controls. Due to the VBNC state present in many microorganisms, culturable methods can underestimate the total number of microorganisms, representing risks for public health, when UV radiation is used. Qiu et al (2018) studied the inactivation of enteric virus in two full scale WWTPs in Calgary, Canada, for a period of two years. Virus have been reported to be less susceptible to the effect of UV radiation and are common pathogens in WWTPs, that in terms of effluent reuse classes require the stricter removal, when compared to bacteria and protozoa. In this study, a total of 51 pre-UV samples and 50 Post-UV samples analyzed, and using integrated cell culture with real time polymerase chain reaction (ICC-qPCR)), results showed that reovirus was the most detected virus in affluent (92%), an in effluent, post a UV step (48%) being more resistant to UV-C radiation.

In order to assess the disinfection efficiency of EK treatment, CFUs counts were done based on aliquots inoculated in PDA media, left incubating for 24 h at 37 °C. The method used required few steps, was easy to perform and allowed results in 24 h.

In UV-C treated effluent samples, no dilution was performed as the EK treatment performed in already disinfected effluent was expected to have minimum density of microorganisms, in result to inactivation due to UV-C radiation. In Set 1, the mean CFUs counts (CFU/0.1 mL) ranged between 6 CFU/0.1 mL in EK treatments to 60 CFU/0.1 mL in C2. With the increasing of control time, the CFU density tended to diminish ($p > 0.05$). As different replicas were performed in different days in this Set, microorganism's enumeration was also performed alongside, resulting, in similarity with the electrodegradation results in wide standard deviation among replicas due to a wide effluent variability.

In Set 1 experiments, after a first UV fluence, by analysing the t0* (time zero non spiked samples), the total microbial community from effluent had decreased one order of magnitude in comparation with t0* with fresh effluent (F1 and F2 samples). It can also be seen in Set 1 experiments, that by testing different electrolysis times, from EK4 to EK6, total culturable microorganisms were inactivated, independently of electrolysis time, achieving ≈ 1 Log_{10} reduction in comparation with control samples. In this Set, as controls were performed at the same time of EK assays, were used as the "initial" reference for inactivation assessment. t0* replicates in Set 1, were done in different days. This was corrected in Set 2 experiments, by collecting an aliquot in the beginning of the experiments and inoculate a PDA petri dish using the spread plate method, and further considering these CFUs counts for LVR calculations.

The microbiology data treatment followed the EPA (2014) recommendations on interpreting microbiology environmental data from low counts. Due to the lack of previous information about the expected microbial concentration from EK experiments in effluent samples, the protocol used

into the microbiology procedure allowed only a first assessment, so high variability and uncertainty are acceptable. Zero counts were obtained after EK treatment in fresh secondary effluent samples. This represent a counting below detection limit of the method. There are many methods for dealing with zero values, or "non detected" counts, and the choice of the method should be evaluated case by case according with the objective of the experiment. Some strategies include imputation and substitution methods, maximum likelihood estimation and non-parametric statistical methods such as Kaplan-Meier (EPA, 2014).

The approach used in the present dissertation chooses to include the zero counts as detected values, and the reported microbial concentration as the mean of all spread plates. In fact, a zero count is a relative value obtained after a specific time period of incubation and does not imply that the true microorganism's concentration in a sample is zero. VNBC can be induced by the presence of disinfectant species, by osmotic pressure, adverse nutrition (e.g. addition of PPCPs that act as a carbon source for metabolic activity) and temperature changes (Viegas et al, 2015).

In fresh effluent experiments, both with F1 and F2 samples, low plated counts were obtained, as the disinfection potential of EK allowed inactivation of total culturable microorganisms, but the error in the method increases, with the possibility of false negatives. In fact, it was possible to identify after 72 h of incubation the appearing of a new colony in an EK non-spiked effluent petri dish, from secondary effluent (F1), as can be seen in Figure 4.8. High CFUs counts were also verified (> 300 CFU/plate) in fresh secondary effluent samples, for time zero controls. Both effluent samples were in the order of magnitude of 10^6 CFU/100 mL. In the non-spiked EK6 assay of F1, microorganisms were inactivated below the MLD (MDL = 1 x 10^3). Overall microorganism's reduction happened in a more accentuated extent in F1 sample, namely from time zero control, to spiked 6 h control ($p<0.05$). For F2 sample, this reduction also happened but was not so abrupt ($p>0.05$).

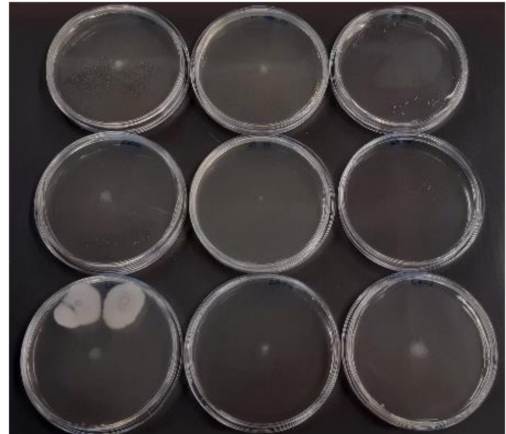
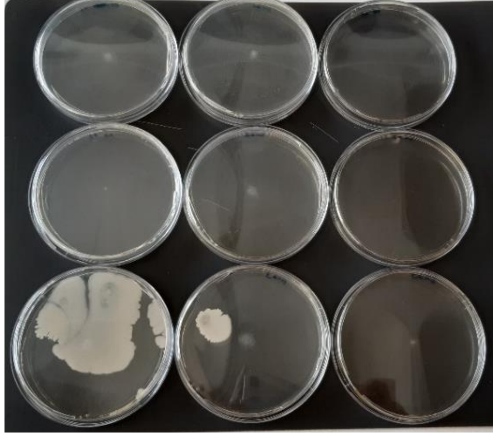



Figure 4.8 - False positive in F1 - EK6*, after 72 h of incubation at 37 °C (picture at the right). Photos were taken with 24 h of difference

Contrary to the PPCPs remediation trend in UV-C treated effluent, with the increase of EK treatment time, microorganism's inactivation did not increase. In electrochemical reactors, depending on the current density applied, the electric field can induce growth or inactivation. In spiked controls, the spiking procedure may constitute a (readly) available carbon substrate (McLain & Gachomo, 2019). In the spiked controls, seems that for the PPCPs concentration, growth inhibition occurred, indicating that either the MeOH:ACE present in the stock solution with PPCPs might have induced toxicity for the effluent microbial community.

In EK6 samples in both F1 and F2 effluent, the addiction of PPCPs did not showed statistical significative differences ($p > 0.05$) that in the non-spiked sample.

Different pathogens are known to have different UV susceptibilities and many studies have been performed targeting the repair of bacteria after disinfection. Wen et al (2019) studied the photoreactivation and dark repair mechanisms of 3 fungal spores, with common occurrence in groundwater in northwest china: *Trichoderma harzianum, Aspergillus niger* and *Penicillium polonicum*. Spores inactivation results were compared with results of photoreactivation of *E.coli*, under the same experimental conditions. Regarding the inactivation experiments, the inactivation rates for fungal spores were lower than for *E.coli*. The order of UV resistance based on kinetic data, proved with exposure to several UV dosages were higher for *Aspergilus niger*, followed by *Trichoderma harzianum, Penicillium polonicum and e-coli.* In photoreactivation, it was used a 2-Log reduction for all microorganisms, and during 8 h of exposure to UV-A (different intensities 0.10, 0.17 and 0.25 mw/cm^2), *Trichoderma harzianum* had the higher photoreactivation (51.35%), that was mostly achieved at a higher rate during the first two hours. When the UV intensity changed from 0.10 to 0.25 after 2 h, for *Penicillium polonicum* a 3-fold increase in the inactivation was achieved. Photoreactivation was different among different fungal species that could be explained due to different cell structure (e.g. membrane composition) and repair systems, and overall between fungi and bacteria. For dark repair experiments, reactivation of fungal spores was less than 1% after 8h. It was referred that dark repair mechanisms, specially the Nucleotide Excision Repair (NER), in fungal species might not be a reactivation pathway

Guedes et al (2018) studied the influence of a low intensity electric direct current application in microbial communities of sewage sludge, and assessed that for a electrochemical reactor operated with current intensities above 50 mA, protozoa and metazoan diversity decreased with shelled amoeba being the more resistant organisms.

Electrolysis operational conditions plays a great role in inactivating and/or in the growth of microbial communities. Negatively charged microorganisms inactivation in the electro-reactor can occur due to 1) Direct anodic oxidation, in electrode surface; 2) Indirect oxidation, in bulk solution due to presence of •OH radicals or •Cl (in the case of chlorine being present in the effluent) ; 3) H_2O_2 produced in the cathode surface (Zhang, 2016). By changing electrochemical parameters, microorganisms reduction have been referred to increase with the increase of current intensity (Celeste et al., 2010; Guedes et al., 2018).

In UV-C treated effluent, highest microorganism inactivation was achieved in UV-EK2, without statistical differences from UV-EK4 and UV-EK6 ($p>0.05$), with a 1.06-Log reduction. EK applied in disinfected effluent might have had effect in reactivation of microorganism that were inactivated by UV-C radiation. For all the EK experiments, the assays with UV-C treated effluent resulted in the lower microorganism reductions, but comparing with control, a reduction was made in one order of magnitude. When comparing EK applied after a disinfection step - effluent UV-C treated, in comparation with EK applied after secondary treatment, an increase of ≈ 2 fold Log_{10} reduction was achieved. In fresh secondary effluent, microorganism's inactivation was higher. In F1 sample, both EK assays (spiked and non-spiked), resulted in 3.21 and 3.74 Log reduction. This represents a reduction of 99.94% and 99.98%. Photolysis experiments lead to a lower reduction, of 98.98% in F1 and 99.30% in F2. Log reduction values resulted from electrodegradation experiments achieved higher values, in fresh effluent F2 sample. By crossing the natural attenuation (control experiments) values with microorganism's inactivation, the higher natural attenuation for the PPCPs under study in fresh secondary effluent was achieved in F1 sample, but the microbial density of this effluent sample suffered a significative reduction ($p>0.05$) in comparation with time zero control non spiked (t0). Regarding PPCPs electrodegradation, in F2-EK6 samples, the remediation values were the highest and microorganisms Log reduction also achieved the highest values.

.

5. Conclusions

This dissertation studied the electrokinetic (EK) process applied for PPCPs degradation in a secondary effluent and the effect of the process in its microbial community. All experiments were performed directly using a real environmental matrix - effluent and for PPCPs electro and photodegradation experiments, reactors were spiked aiming a closer environmental detected concentration (0.2 mg/L).

In Set 1 experiments, EK treatment applied as a polishing step in UV-C treated effluent, for the three electrolysis the higher overall electrodegradation was achieved in the 6 h experiments. In these conditions, for all tested times, EK treatment lead to an overall ≈ 1 Log_{10} reduction of total microorganisms from effluent. In this Set, wide variability was observed. Variability was due to prolongated storage of effluent in order to keep sterile conditions to assess the best operational conditions for the following Set (best treatment time). By working directly with an environmental matrix, it was possible to assess that interactions between PPCPs and effluent natural organic matter might have had influenced the overall results, resulting in a wide range of recoveries. This was constant, in both UV-C treated effluent and in fresh effluent experiments.

For the operational conditions used - electrode spacing: 6 cm, working volume of 500 mL, fixed current density used 8 mA/cm², inactivation in the total culturable microorganisms was achieved in all EK experiments. In the case of Set 2, by testing also the effect of UV-C photolysis is effluent microbial community, inactivation extension was inferior in comparation with the inactivation with EK process. Highest inactivation was obtained in fresh secondary effluent sample F2, with a 3.7 Log_{10} reduction in EK6 non-spiked sample. In fresh effluent experiments, between sample F1 and F2, no trend regarding the effect of PPCPs spiking in the influence of inactivation of microorganisms during EK process was observed, as between samples for the same assay type, statistically different values were obtained. In Set 2, when UV-C photolysis was compared with the EK process, electrodegradation allowed to achieve overall higher PPCPs degradation from effluent ($p > 0.05$). DCF was the exception, and no statistically difference was found between EK process and UV-C photolysis. Overall degradations were higher when EK process was applied.

It was possible based on two different sampling campaigns during the month of December with two fresh secondary effluent samples, to take a closer look in the variability of the effluent matrix and in which way certain physicochemical composition (e.g. COD) might affect the obtained results. In controls (spiked and without electric current), used to study PPCPs natural attenuation, the highest total microorganisms concentration (CFU/100 mL) values were obtained in F1 sample, indicating that it was not in the reactor with the highest microorganisms concentration that had the higher natural attenuation. PPCPs degradation trend obtained in fresh effluent samples after 6 h of electrolysis was SMX ≈ OXY>CBZ > DCF > CAF. pH increase ($p < 0.05$), due to the current density applied (8 mA/cm²) for a period of 6 h.

The two main possible degradation pathways identified for PPCPs and microorganisms inactivation are: 1) direct oxidation at the anode surface and/or 2) Indirect oxidation in bulk solution by intermediate species of oxygen evolution.

Overall, the best effluent treatment in which to apply EK process remain a question that needs to continue to be further investigated. Further studies should be carried out in order to optimize operational conditions of the electro-reactor.

6. Future Perspectives

By the end of this dissertation, many questions arise and some indications for future research are suggested.

- **Determination of complementary parameters** such as chemical oxygen demand and total organic carbon in the beginning and at the end of experiments in order to assess PPCPs mineralization extent. Determination of species such as chlorine, sulphate, and iron in the effluent samples, as these species can naturally occur in the effluent and synergistically increase indirect oxidation of organic compounds.
- When applying EK process in effluent or in any other environmental matrix, complementary evaluation of the effect of the electric field in the effluent microbial community should also be performed by choosing an adequate microorganism enumeration and/or identification technique. In the case of technique used (spread plate technique) serial dilutions should be implemented in control experiments in order to consider only the petri dishes in the optimum range of the spread plate method (30 – 300) to minimize errors associate with counts. The macroscopic colony counting was the most time-consuming part, and it is recommended the use of colony counting software that have been developed to help manage single colony identification.
- **Increase the sustainability of the electro-reactor**. One of the biggest constrains of the EK process is the dependence of an external energy source, so future work developed should tackle the feasibility of renewable energy sources such as solar or wind power.
- Evaluate the **kinetics of the degradation of the target PPCPs along treatment time**, by removing aliquots from the reactor during a defined time interval until the end of the experiment to analyse intermediate PPCPs concentrations.
- As metabolites might be present in higher concentrations than the parent compounds in the effluent, an **initial exploratory effluent screening** must be performed using a sensitive technique with a mass detector, in order to identify possible metabolites or interfering species that might be present in the effluent matrix and during assays can interfere with the final removal results. In the same perspective, after EK experiments, another effluent assessment in the search for possible degradation products should be carried out.
- In order to assess if the EK process degrades PPCPs to less harmful compounds, evaluation of the toxicity of the effluent after treatment by performing **acute and chronic toxicity bioassays** are also an important complement for this type of study, as will help to understand the impact into the receiving ecosystems (e.g soil, aquatic environment).
- Further explore the potential of electro-reactor in secondary effluent in other perspectives e.g. aiming to reduce antibiotic resistant bacteria and antibiotic resistant genes from effluent, due to the environmental and public health issues that antimicrobial resistance brings to society.

- Need to further understand the metabolism and optimal inhibition conditions of the microorganisms in the presence of a low-level DC electric field. DNA-based microbial techniques might be key to perform this assessment.
- Upscale of the technology readiness level. The electro-reactor was designed at bench-scale, and it is suggested to pursue to pilot-scale, in order to allow a higher effluent volume under treatment, to get close to real conditions of effluent reclamation. However, by changing the working volume, re-optimization of the operational conditions should be carefully assessed.
- A continuous mixing system is needed to ensure a proper supply of oxygen to the system. An alternated design with a flow reactor could be applied.
- In order to determine the PPCPs electrodegradation pathways, experiments with a lower complexity matrix e.g. water type II, instead of the EK process being directly applied in effluent, might be necessary in order to deeply understand PPCPs degradation in aqueous media.

7. References

Acar, Y. B., & Alshawabkeh, A. N. (1993). Principles of electrokinetic remediation. *Environmental Science and Technology*, 27(13): 2638–2647. https://doi.org/10.1021/es00049a002.

Águas do Tejo Atlântico (2018) Revista Tejo Atlântico, 2. https://www.aguasdotejoatlantico.adp.pt/sites/aguasdotejoatlantico.adp.pt/files/publicacoes/revista_adta_18abr.pdf. Accessed in February 2020.

Alcalde-Sanz, L., Gawlik, B. M. (2017). Minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge - Towards a legal instrument on water reuse at EU level. EUR 28962EN, Publications Office of the European Union, Luxembourg, 2017, ISBN 978-92-79-77175-0. http://doi.org/10.2760/804116.

Almeida, J. (2015) Electrodialytic recovery of phosphorus and organic contaminans removal from sewage sludge. Dissertação de Mestrado em Engenharia do Ambiente. FCT NOVA.

Alshawabkeh, A. N., Shen, Y., & Maillacheruvu, K. Y. (2004). Effect of DC Electric Fields on COD in Aerobic Mixed Sludge Processes. *Environmental Engineering Science*, 21(3): 321–329. https://doi.org/10.1089/109287504323066969.

APA (2019a). Guia para a reutilização de água para fins não potáveis. https://apambiente.pt/_zdata/Politicas/Agua/Licenciamento/ApR/APA_Guia_Reutilizacao_v1.pdf. Accessed in February 2020.

APA (2019b). Relatório de Estado do Ambiente – Águas Residuais Urbanas. https://rea.apambiente.pt/content/%C3%A1guas-residuais-urbanas. Accessed in February 2020.

Archer, E., Petrie, B., Kasprzyk-Hordern, B., & Wolfaardt, G. M. (2017). The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. *Chemosphere*, 174: 437–446. https://doi.org/10.1016/j.chemosphere.2017.01.101.

Aristizabal-Ciro, C., Botero-Coy, A. M., López, F. J., & Peñuela, G. A. (2017). Monitoring pharmaceuticals and personal care products in reservoir water used for drinking water supply. *Environmental Science and Pollution Research*, 24(8): 7335–7347. https://doi.org/10.1007/s11356-016-8253-1.

Assress, H. A., Selvarajan, R., Nyoni, H., Ntushelo, K., Mamba, B. B., & Msagati, T. A. M. (2019). Diversity, co-occurrence and implications of fungal communities in wastewater treatment plants. *Scientific Reports*, 9:14056. https://doi.org/10.1038/s41598-019-50624-z.

Bard, A. J., Inzelt, G., & Scholz, F. (2012). Electrochemical dictionary. 2nd ed. Springer International Publishing. 647-667. http://doi.org/10.1007/978-3-642-29551-5.

Becerra-Castro, C., Lopes, A. R., Vaz-Moreira, I., Silva, E. F., Manaia, C. M., & Nunes, O. C. (2015). Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health. *Environment International*, 75: 117–135. https://doi.org/10.1016/j.envint.2014.11.001.

Biancullo, F., Moreira, N. F. F., Ribeiro, A. R., Manaia, C. M., Faria, J. L., Nunes, O. C., Castro-Silva, S. M., Silva, A. M. T. (2019). Heterogeneous photocatalysis using UVA-LEDs for the removal of antibiotics and antibiotic resistant bacteria from urban wastewater treatment plant effluents. *Chemical Engineering Journal.* 367: 304 – 313. https://doi.org/10.1016/j.cej.2019.02.012.

Biel-Maeso, M., Corada-Fernández, C., & Lara-Martín, P. A. (2019). Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. *Water Research*, 150: 129–139. https://doi.org/10.1016/j.watres.2018.11.045.

Bolong, N., Ismail, A. F., Salim, M. R., & Matsuura, T. (2009). A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination*, 239(1–3): 229–246. https://doi.org/10.1016/j.desal.2008.03.020.

Bolton, J. R., & Linden, K. G. (2003). Standardization of Methods for Fluence (UV Dose) Determination in Bench-Scale UV Experiments. *Journal of Environmental Engineering*, 129(3): 209–215. https://doi.org/10.1061/(ASCE)0733-9372(2003)129:3(209).

Borecka, M., Białk-Bielińska, A., Siedlewicz, G., Stepnowski, P., & Pazdro, K. (2014). The Influence of Matrix Effects on Trace Analysis of Pharmaceutical Residues in Aqueous Environmental Samples. *GeoPlanet: Earth and Planetary Sciences*, 14: 1–16. https://doi.org/10.1007/978-3-319-03683-0_1.

Boretti, A., & Rosa, L. (2019). Reassessing the projections of the World Water Development Report. *Npj Clean Water*, 2(1): 1–6. https://doi.org/10.1038/s41545-019-0039-9.

Buerge, I. J., Poiger, T., Müller, M. D., & Buser, H. R. (2003). Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environmental Science and Technology*, 37(4): 691–700. https://doi.org/10.1021/es020125z.

Cameselle, C., Gouveia, S., Eddine, D., & Belhadj, B. (2013). Advances in Electrokinetic Remediation for the Removal of Organic Contaminants in Soils. In: Rashed, M. N (Ed) Organic Pollutants - Monitoring, Risk and Treatment. IntechOpen, 209 -229. https://doi.org/10.5772/54334.

Catrinescu, C., Chelba, A., Teodosiu, C., & Apopei, P. (2017). Removal of diclofenac from secondary wastewater effluents by Fenton based processes. *Environmental Engineering and Management Journal*, 16(4): 765–777. https://doi.org/10.30638/eemj.2017.078.

Celeste, I., Pereira Gusmão, C., Bueno Moraes, P., & Bidoia, E. D. (2010). Studies on the electrochemical disinfection of water containing Escherichia coli using a dimensionally stable

anode. *Brazilian Archives of Biology and Technology*. 53(5): 1235–1244. https://doi.org/10.1590/S1516-89132010000500029.

Cerqueira, A. A., Souza, P. S. A., & Marques, M. R. C. (2014). Effects of direct and alternating current on the treatment of oily water in an electroflocculation process. *Brazilian Journal of Chemical Engineering*, 31(3): 693–701. https://doi.org/10.1590/0104-6632.20140313s00002363.

Chen, J. P., Yang, L., Wang, L. K., & Zhang, B. (2006). Ultraviolet Radiation for Disinfection. In: Wang, K. L., Hung, T., Shammas, N, K (Eds) Advanced Physicochemical Treatment Processes. Handbook of Environmental Engineering. Humana Press. 317–366. https://doi.org/10.1007/978-1-59745-029-4_10.

Cizmas, L., Sharma, V. K., Gray, C. M., & McDonald, T. J. (2015). Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. *Environmental Chemistry Letters*, 13: 381–394. https://doi.org/10.1007/s10311-015-0524-4.

Collivignarelli, M., Abbà, A., Benigna, I., Sorlini, S., & Torretta, V. (2017). Overview of the Main Disinfection Processes for Wastewater and Drinking Water Treatment Plants. *Sustainability*, 10(2): 86. https://doi.org/10.3390/su10010086.

Collivignarelli, M., Abbà, A., Frattarola, A., Miino, M., Padovani, S., Katsoyiannis, I., & Torretta, V. (2019). Legislation for the Reuse of Biosolids on Agricultural Land in Europe: Overview. *Sustainability*, 11(21): 6015. https://doi.org/10.3390/su11216015.

Cotillas, S., de Vidales, M. J. M., Llanos, J., Sáez, C., Cañizares, P., & Rodrigo, M. A. (2016). Electrolytic and electro-irradiated processes with diamond anodes for the oxidation of persistent pollutants and disinfection of urban treated wastewater. *Journal of Hazardous Materials*, 319: 93–101. https://doi.org/10.1016/j.jhazmat.2016.01.050.

Couto, N., Guedes, P., Mateus, E. P., Santos, C., Ribau Teixeira, M., Nunes, L. M., Hansen, H. K., Gutierrez, C., Ottosen, L. M., & Ribeiro, A. B. (2013). Phosphorus recovery from a water reservoir-potential of nanofiltration coupled to electrodialytic process. *Waste and Biomass Valorization*, 4(3): 675–681. https://doi.org/10.1007/s12649-012-9194-7.

Cuerda-Correa, E. M., Alexandre-Franco, M. F., & Fernández-González, C. (2019). Advanced Oxidation Processes for the Removal of Antibiotics from Water. An Overview. *Water*, 12(1): 102. https://doi.org/10.3390/w12010102.

Daughton, C. G. (2004). Non-regulated water contaminants: Emerging research. *Environmental Impact Assessment Review*, 24(7–8): 711–732. https://doi.org/10.1016/j.eiar.2004.06.003.

Daughton, C. G., & Ternes, T. A. (1999). Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environmental Health Perspectives*, 107: 907–938. https://doi.org/10.1289/ehp.99107s6907.

de Oliveira, M., Frihling, B. E. F., Velasques, J., Filho, F. J. C. M., Cavalheri, P. S., & Migliolo, L. (2020). Pharmaceuticals residues and xenobiotics contaminants: Occurrence, analytical techniques and sustainable alternatives for wastewater treatment. *Science of the Total Environment*, 705: 135568. https://doi.org/10.1016/j.scitotenv.2019.135568.

de Vidales, M. J. M., Sáez, C., Pérez, J. F., Cotillas, S., Llanos, J., Cañizares, P., & Rodrigo, M. A. (2015). Irradiation-assisted electrochemical processes for the removal of persistent organic pollutants from wastewater. *Journal of Applied Electrochemistry*, 45(7): 799–808. https://doi.org/10.1007/s10800-015-0825-0.

Decreto lei nº 119/2019 de 21 de Agosto do Ministério do Ambiente e da Transição Energética. Diário da República. Série I nº 159 (2019).

Decreto lei nº 152/97 de 19 de Junho do Ministério do Ambiente. Diário da República. Série I – A nº 139 (1997).

Deviller, G., Lundy, L., & Fatta-Kassinos, D. (2020). Recommendations to derive quality standards for chemical pollutants in reclaimed water intended for reuse in agricultural irrigation. *Chemosphere*, 240: 124911. https://doi.org/10.1016/j.chemosphere.2019.124911.

Dhodapkar, R. S., & Gandhi, K. N. (2019). Pharmaceuticals and personal care products in aquatic environment: chemicals of emerging concern? In Prasad, M. N. V., Vithanage, M., & Kapley, A (Eds) Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology. Butterworth-Heinemann Publishing, 63–85. https://doi.org/10.1016/b978-0-12-816189-0.00003-2.

Díaz, A., & Peña-Alvarez, A. (2017). A Simple Method for the Simultaneous Determination of Pharmaceuticals and Personal Care Products in River Sediment by Ultrasound-Assisted Extraction Followed by Solid-Phase Microextraction Coupled with Gas Chromatography–Mass Spectrometry. *Journal of Chromatographic Science*, 55(9): 946–953. https://doi.org/10.1093/chromsci/bmx058.

Dionísio, J. (2019). Electrokinetic remediation of PPCPs in soil: influence of soil biota and environmental factors. Dissertação de Mestrado em Engenharia do Ambiente. FCT NOVA.

Dodd, M. C. (2012). Potential impacts of disinfection processes on elimination and deactivation of antibiotic resistance genes during water and wastewater treatment. *Journal of Environmental Monitoring*, 14: 1754–1771. https://doi.org/10.1039/c2em00006g.

Dodgen, L. K., Ueda, A., Wu, X., Parker, D. R., & Gan, J. (2015). Effect of transpiration on plant accumulation and translocation of PPCP/EDCs. *Environmental Pollution*, 198, 144–153. https://doi.org/10.1016/j.envpol.2015.01.002.

Dubowski, Y., Alfiya, Y., Gilboa, Y., Sabach, S., & Friedler, E. (2020). Removal of organic micropollutants from biologically treated greywater using continuous-flow vacuum-UV/UVC

photo-reactor. *Environmental Science and Pollution Research*, 27(7): 7578–7587. https://doi.org/10.1007/s11356-019-07399-7.

Ebele, A. J., Abou-Elwafa Abdallah, M., & Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants*, 3: 1–16. https://doi.org/10.1016/j.emcon.2016.12.004.

ECUVAL (2017). ElectroChemical technique combined with UV irradiation for the treatment and reuse of dyeing textile wastewaters: Industrial marketing - Eco-Innovation - European Commission. https://ec.europa.eu/environment/eco-innovation/projects/en/projects/ecuval. Accessed in March 2020.

EEA (2019a). Use of freshwater resources in Europe. https://www.eea.europa.eu/data-and-maps/indicators/use-of-freshwater-resources-3/assessment-4. Accessed in February 2020.

EEA (2019b). Urban wastewater treatment. https://www.eea.europa.eu/data-and-maps/indicators/urban-waste-water-treatment. Accessed in February 2020.

EIA (2018). EU Commission proposal for a Regulation on Minimum Requirements for Water Reuse. EIA Position and Further steps in the legislative process (Draft V2).

Ensano, B. M. B., Borea, L., Naddeo, V., Belgiorno, V., de Luna, M. D. G., & Ballesteros, F. C. (2016). Combination of electrochemical processes with membrane bioreactors for wastewater treatment and fouling Control: A review. *Frontiers in Environmental Science*, 4: 1-17. https://doi.org/10.3389/fenvs.2016.00057.

EPA (2014). Evaluation of Options for Interpreting Environmental Microbiology Field Data Results having Low Spore Counts. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-14/331, 2014.

European Comission (2016a). EU Common Implementation Strategy for the Water Framework Directive and the Floods Directive: Guidelines on Integrating Water Reuse into Water Planning and Management in the context of the WFD.

European Comission (2016b). EU-level instruments on water reuse. Final report to support the Commission's Impact Assessment. http://doi.org/10.2779/974903.

European Comission (2019). Proposal for a Regulation of the European Parliament and of the Council on minimum requirements for water reuse – Analysis of the final compromise text with a view to agreement. Brussels, 18.12.2019, COM (2018) 337 final, 2018/0169(COD).

European Union (2020). Water reuse. https://ec.europa.eu/environment/water/reuse.htm. Accessed in February 2020.

FAO (2017). Water for Sustainable Food and Agriculture. A Report produced for the G20 Presidency of Germany. http://www.fao.org/3/a-i7959e.pdf. Accessed in February 2020.

Ferreira, A. R., Couto, N., Guedes, P., Pinto, J., Mateus, E. P., & Ribeiro, A. B. (2018). Electrodialytic 2-compartment cells for emerging organic contaminants removal from effluent. *Journal of Hazardous Materials*, 358: 467–474. https://doi.org/10.1016/j.jhazmat.2018.04.066.

Ferreira, A. R., Guedes, P., Mateus, E. P., Couto, N., & Ribeiro, A. B. (2017a). Influence of the cell design in the electroremoval of PPCPs from soil slurry*. Chemical Engineering Journal*, 326: 162–168. https://doi.org/10.1016/j.cej.2017.05.148.

Ferreira, A. R., Ribeiro, A., & Couto, N. (2017b). Remediation of pharmaceutical and personal care products (PPCPS) in constructed wetlands: Applicability and new perspectives. In: Ansari, A. A., Gill, S. S., Gill, R., Lanza, G. R., & Newman, L (Eds) Phytoremediation: Management of Environmental Contaminants. Springer International Publishing, 5: 277–292. https://doi.org/10.1007/978-3-319-52381-1_9.

Ferro, G., Polo-López, M. I., & Fernández-Ibáñez, P. (2015). Conventional and New Processes for Urban Wastewater Disinfection: Effect on Emerging and Resistant Microorganisms. In: Fatta-Kassinos D., Dionysiou D., Kümmerer K (Eds) Advanced Treatment Technologies for Urban Wastewater Reuse. Springer International Publishing. 45: 107 - 128. https://doi.org/10.1007/698_2015_390.

Gawlik, B. M. (2017). Minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge Towards a legal instrument on water reuse at EU level. https://doi.org/10.2760/887727.

Ghermandi, A., Bixio, D., Traverso, P., Cersosimo, I., & Thoeye, C. (2007). The removal of pathogens in surface-flow constructed wetlands and its implications for water reuse. *Water Science and Technology*, 56(3): 207–216. https://doi.org/10.2166/wst.2007.511.

Gibson, K. (2010). Pharmaceuticals and personal care products (PPCPs): Disposal, scientific, and regulatory challenges. *Environmental Quality Management*, 20(1): 39–48. https://doi.org/10.1002/tqem.20270.

Gill, R. T., Harbottle, M. J., Smith, J. W. N., & Thornton, S. F. (2014). Electrokinetic-enhanced bioremediation of organic contaminants: A review of processes and environmental applications. *Chemosphere*, 107: 31–42. https://doi.org/10.1016/j.chemosphere.2014.03.019.

Gomes, H. I., Dias-Ferreira, C., & Ribeiro, A. B. (2013). Overview of in situ and ex situ remediation technologies for PCB-contaminated soils and sediments and obstacles for full-scale application. *Science of the Total Environment*, 445–446: 237–260 https://doi.org/10.1016/j.scitotenv.2012.11.098.

Gonzalez-Martinez, A., Sihvonen, M., Muñoz-Palazon, B., Rodriguez-Sanchez, A., Mikola, A., & Vahala, R. (2018). Microbial ecology of full-scale wastewater treatment systems in the Polar Arctic Circle: Archaea, Bacteria and Fungi. *Scientific Reports*, 8(1): 2208. https://doi.org/10.1038/s41598-018-20633-5.

Guedes, P. (2015). Electrokinetic treatment of environmental matrices. Contaminants removal and phosphorus recovery. Tese para obtenção do Grau de Doutor em Ambiente. FCT NOVA.

Guedes, P., Couto, N., Almeida, J., Rodrigues, A. M., Mateus, E. P., & Ribeiro, A. B. (2018). Electrodialytic treatment of sewage sludge: influence on microbiological community. International *Journal of Environmental Science and Technology*, 15(5): 1103–1112. https://doi.org/10.1007/s13762-017-1459-z.

Guedes, P., Lopes, V., Couto, N., Mateus, E. P., Pereira, C. S., & Ribeiro, A. B. (2019). Electrokinetic remediation of contaminants of emergent concern in clay soil: Effect of operating parameters. *Environmental Pollution*, 253: 625–635. https://doi.org/10.1016/j.envpol.2019.07.040.

Guedes, P., Mateus, E. P., Couto, N., Rodríguez, Y., & Ribeiro, A. B. (2014). Electrokinetic remediation of six emerging organic contaminants from soil. *Chemosphere*, 117(1): 124–131. https://doi.org/10.1016/j.chemosphere.2014.06.017.

Gumustas, M., Uslu, B., & Ozkan, S. A. (2018). Novel Validated UHPLC Method for the Estimation of Drug Active Compounds. *Bentham Science Publishers*. 2: 44-97. https://doi.org/10.2174/9781681085746118020004.

Guo, K., Wu, Z., & Fang, J. (2019). UV-based advanced oxidation process for the treatment of pharmaceuticals and personal care products. In: Hernández-Maldonado, A. J., & Blaney, L (Eds) Contaminants of Emerging Concern in Water and Wastewater: Advanced Treatment Processes. Butterworth-Heinemann Publishing. 367–408. https://doi.org/10.1016/B978-0-12-813561-7.00010-9.

Hansen, H.K, Ottosen, L.M., Ribeiro, A.B. (2016). Chapter 1. Electrokinetic soil remediation – an overview. In: A.B. Ribeiro, E.P. Mateus, N. Couto (Eds) Electrokinetics Across Disciplines and Continents. New Strategies for Sustainable Development. Springer International Publishing. 3-18. http://:doi.org/10.1007/978-3-319-20179-5_1.

Harwood, V. J., Levine, A. D., Scott, T. M., Chivukula, V., Lukasik, J., Farrah, S. R., & Rose, J. B. (2005). Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology*, 71(6): 3163–3170. https://doi.org/10.1128/AEM.71.6.3163-3170.2005.

He, K., Echigo, S., Asada, Y., & Itoh, S. (2018). Determination of caffeine and its metabolites in wastewater treatment plants using solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Analytical Sciences*, 34(3): 349–354. https://doi.org/10.2116/analsci.34.349.

Helmecke, M., Fries, E., & Schulte, C. (2020). Regulating water reuse for agricultural irrigation: risks related to organic micro-contaminants. *Environmental Sciences Europe*, 32(1): 4. https://doi.org/10.1186/s12302-019-0283-0.

Huebschmann, H. J. (2015). Handbook of GC-MS: Fundamentals and Applications, 3rd Edition. Wiley-VCH Verlag GmbH & Co. KGaA.

Hussain, S., Gul, S., Steter, J. R., Miwa, D. W., & Motheo, A. J. (2015). Route of electrochemical oxidation of the antibiotic sulfamethoxazole on a mixed oxide anode. *Environmental Science and Pollution Research*, 22(19): 15004–15015. https://doi.org/10.1007/s11356-015-4699-9.

IWA (2018). Action Agenda for Basin-Connected Cities. Influencing and Activating Urban Stakeholders to be Water Stewards in their Basins. https://iwa-network.org/wp-content/uploads/2018/09/Basin-Action-Agenda_SCREEN.pdf. Accessed in January 2020.

Johnston, J., LaPara, T., & Behrens, S. (2019). Composition and Dynamics of the Activated Sludge Microbiome during Seasonal Nitrification Failure. *Scientific Reports*, 9(1): 1–15. https://doi.org/10.1038/s41598-019-40872-4.

Justo, A., González, O., Aceña, J., Pérez, S., Barceló, D., Sans, C., & Esplugas, S. (2013). Pharmaceuticals and organic pollution mitigation in reclamation osmosis brines by UV/H2O2 and ozone. *Journal of Hazardous Materials*, 263: 268–274. https://doi.org/10.1016/j.jhazmat.2013.05.030.

Kauser, I., Ciesielski, M., Poretsky Corresp, R. S., Author, C., & Poretsky, R. S. (2019). Ultraviolet disinfection impacts the microbial community composition and function of treated wastewater effluent and the receiving urban river. *PeerJ*. 7: e7455. 1-26. https://doi.org/10.7717/peerj.7455.

Kehrein, P., van Loosdrecht, M., Osseweijer, P., Garfí, M., Dewulf, J., & Posada, J. (2020). A critical review of resource recovery from municipal wastewater treatment plants – market supply potentials, technologies and bottlenecks. *Environmental Science: Water Research & Technology*, 6(4): 877–910. https://doi.org/10.1039/c9ew00905a.

Khan, J. A., Sayed, M., Khan, S., Shah, N. S., Dionysiou, D. D., & Boczkaj, G. (2020). Advanced oxidation processes for the treatment of contaminants of emerging concern. In: Hernández-Maldonado, A. J., & Blaney, L (Eds) Contaminants of Emerging Concern in Water and Wastewater: Advanced Treatment Processes. Butterworth-Heinemann Publishing. 299–365. https://doi.org/10.1016/b978-0-12-813561-7.00009-2.

Kothny, E. L. (1992). UV disinfection of water. *Chemical and Engineering News*, 70: 5. https://doi.org/10.1016/b978-0-7506-6843-9.00019-6.

Kovacic, M., Juretic Perisic, D., Biosic, M., Kusic, H., Babic, S., & Loncaric Bozic, A. (2016). UV photolysis of diclofenac in water; kinetics, degradation pathway and environmental aspects. *Environmental Science and Pollution Research*, 23(15): 14908–14917. https://doi.org/10.1007/s11356-016-6580-x.

Lakshmi, V., & Das, N. (2013). Removal of caffeine from industrial wastewater using Trichosporon asahii. *Journal of Environmental Biology*, 34(4): 701–708.

Lapworth, D. J., Baran, N., Stuart, M. E., & Ward, R. S. (2012). Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environmental Pollution*, 163: 287–303. https://doi.org/10.1016/j.envpol.2011.12.034.

Lee, Y., & von Gunten, U. (2010). Oxidative transformation of micropollutants during municipal wastewater treatment: Comparison of kinetic aspects of selective (chlorine, chlorine dioxide, ferrate VI, and ozone) and non-selective oxidants (hydroxyl radical). *Water Research*, 44(2): 555–566. https://doi.org/10.1016/j.watres.2009.11.045.

Li, J., Dodgen, L., Ye, Q., & Gan, J. (2013). Degradation kinetics and metabolites of carbamazepine in soil. *Environmental Science and Technology*, 47(8): 3678–3684. https://doi.org/10.1021/es304944c.

Li, S., He, B., Wang, J., Liu, J., & Hu, X. (2020). Risks of caffeine residues in the environment: Necessity for a targeted ecopharmacovigilance program. *Chemosphere*, 243: 125343. https://doi.org/10.1016/j.chemosphere.2019.125343.

Li, Y., Zhu, G., Ng, W. J., & Tan, S. K. (2014). A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: Design, performance and mechanism. *Science of the Total Environment*, 468–469: 908–932. https://doi.org/10.1016/j.scitotenv.2013.09.018.

Liu, J. L., & Wong, M. H. (2013). Pharmaceuticals and personal care products (PPCPs): A review on environmental contamination in China. *Environment International*, 59: 208–224. https://doi.org/10.1016/j.envint.2013.06.012.

Lopes, V. (2018). Electrokinetic Remediation of PPCPs in Soil: Effect of operating parameters. Dissertação de Mestrado em Engenharia do Ambiente, FCT NOVA.

Lucci, P., Pacetti, D., Nunez, O., & G., N. (2012). Current Trends in Sample Treatment Techniques for Environmental and Food Analysis. In: Calderon, L, A (Ed) Chromatography - The most versatile method of chemical analysis. IntechOpen. https://doi.org/10.5772/47736.

Luo, Y., Guo, W., Hao Ngo, H., Duc Nghiem, L., Hai, I., Zhang, J., Liang, S., & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*, 473–474: 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065.

Magro, C. (2019). Advances in applied electrokinetics: Treatment, by products reuse and sensors system. Tese de Doutoramento em Ambiente e Sustentabilidade. FCT NOVA.

Magro, C., Almeida, J., Paz-Garcia, J. M., Mateus, E. P., & Ribeiro, A. B. (2019). Exploring hydrogen production for self-energy generation in electroremediation: A proof of concept. *Applied Energy*. 255: 113839. https://doi.org/10.1016/j.apenergy.2019.113839.

Magro, C., Mateus, E. P., Paz-Garcia, J. M., & Ribeiro, A. B. (2020). Emerging organic contaminants in wastewater: Understanding electrochemical reactors for triclosan and its by-

products degradation. *Chemosphere,* 247: 125758. https://doi.org/10.1016/j.chemosphere.2019.125758.

Manuel Paz-García, J., Johannesson, B., Ottosen, L. M., Alshawabkeh, A. N., Ribeiro, A. B., & Miguel Rodríguez-Maroto, J. (2012). Modeling of electrokinetic desalination of bricks. *Electrochimica Acta*, 86: 213–222. https://doi.org/10.1016/j.electacta.2012.05.132.

Marecos, H., & Albuquerque, M. A. (2010). Reutilização de Águas Residuais. Guia técnico nº 14. ERSAR.

Martín-Pozo, L., de Alarcón-Gómez, B., Rodríguez-Gómez, R., García-Córcoles, M. T., Çipa, M., & Zafra-Gómez, A. (2019). Analytical methods for the determination of emerging contaminants in sewage sludge samples. A review. *Talanta*, 192: 508–533. https://doi.org/10.1016/j.talanta.2018.09.056.

Matamoros, V., & Salvadó, V. (2012). Evaluation of the seasonal performance of a water reclamation pond-constructed wetland system for removing emerging contaminants. *Chemosphere*, 86(2): 111–117. https://doi.org/10.1016/j.chemosphere.2011.09.020.

McLain, N. K., & Gachomo, E. W. (2019). Chemicals of Emerging Concern in Treated Wastewater Impact Microbial Growth. *Frontiers in Environmental Science*, 7: 156. https://doi.org/10.3389/fenvs.2019.00156.

Mekonnen, M. M., & Hoekstra, A. Y. (2016). Sustainability: Four billion people facing severe water scarcity. *Science Advances*, 2(2): e1500323. https://doi.org/10.1126/sciadv.1500323.

Metcalf & Eddy (2003). Wastewater engineering: Treatment and Reuse. 4th ed. McGraw-Hill, New York.

Miao, X. S., & Metcalfe, C. D. (2003). Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography - Electrospray tandem mass spectrometry. *Analytical Chemistry*, 75(15): 3731–3738. https://doi.org/10.1021/ac030082k.

Modin, O., & Aulenta, F. (2017). Three promising applications of microbial electrochemistry for the water sector. *Environmental Science: Water Research and Technology*, 3: 391–402. https://doi.org/10.1039/c6ew00325g.

Momba, M., Edbon, J., Kamika, I., & Verbyla, M. (2019). Using Indicators to assess microbial treatment and disinfection efficacy. In: Rose, J. B., & Jiménez-Cisneros, B. (Eds.), Global Water Pathogen Project. https://doi.org/10.14321/waterpathogens.9.

Morone, A., Mulay, P., & Kamble, S. P. (2019). Removal of pharmaceutical and personal care products from wastewater using advanced materials. In: Prasad, M. N. V., Vithanage, & Kapley, A (Eds) Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology. Emerging contaminants and Micro pollutants. Butterworth-Heinemann Publishing. 173–212. https://doi.org/10.1016/b978-0-12-816189-0.00008-1.

Muddemann, T., Haupt, D., Sievers, M., & Kunz, U. (2019). Electrochemical Reactors for Wastewater Treatment. *ChemBioEng Reviews*, 6(5): 142–156. https://doi.org/10.1002/cben.201900021.

Naidoo, S., & Olaniran, A. O. (2014). Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources. *International Journal of Environmental Research and Public Health*, 11: 249-270 https://doi.org/10.3390/ijerph110100249.

National Guidelines for Water Recycling: Managing Health and Environmental Risks Natural Resource Management Ministerial Council Environment Protection and Heritage Council Australian Health Ministers' Conference (2006). https://www.awa.asn.au/Documents/water-recycling-guidelines-health-environmental-21.pdf. Accessed in March 2020.

Nguyen, P. Y., (2018). Bioaugmentation for the removal of the antibiotic sulfamethoxazole in wastewater treatment plants. Tese de Doutoramento em Química Sustentável. FCT NOVA.

Nguyen, P. Y., Carvalho, G., Polesel, F., Torresi, E., Rodrigues, A. M., Rodrigues, J. E., Cardoso, V. V., Benoliel, M. J., Plósz, B., Reis, M. A. M., Oehmen, A. (2018). Bioaugmentation of activated sludge with Achromobacter denitrificans PR1 for enhancing the biotransformation of sulfamethoxazole and its human conjugates in real wastewater: Kinetic tests and modelling. *Chemical Engineering Journal*, 352: 79–89. https://doi.org/10.1016/j.cej.2018.07.011.

Nunes, L. M., Gomes, H. I., Teixeira, M. R., Dias-Ferreira, C., & Ribeiro, A. B. (2016). Life cycle assessment of soil and groundwater remediation: groundwater impacts of electrokinetic remediation. In: Ribeiro, A. B., Mateus, E, P., Couto, N (Eds) Electrokinetics Across Disciplines and Continents: New Strategies for Sustainable Development. Springer International Publishing. 173-202. https://doi.org/10.1007/978-3-319-20179-5_10.

Oaks, J. L., Gilbert, M., Virani, M. Z., Watson, R. T., Meteyer, C. U., Rideout, B. A., Khan, A. A. (2004). Diclofenac residues as the cause of vulture population decline in Pakistan. *Nature*, 427(6975): 630–633. https://doi.org/10.1038/nature02317.

Pamukcu, S., Ghazanfari, E., & Wittle, K. (2014). Reduction of Contaminants in Soil and Water by Direct Electric Current. In: Chilingar, G. V., & Haroun M (Eds) Electrokinetics for Petroleum and Environmental Engineers. Scrivener Publishing, 33–101. https://doi.org/10.1002/9781118842805.ch2.

Paredes, L., Omil, F., Lema, J. M., & Carballa, M. (2018). What happens with organic micropollutants during UV disinfection in WWTPs? A global perspective from laboratory to full-scale. *Journal of Hazardous Materials*, 342: 670–678. https://doi.org/10.1016/j.jhazmat.2017.08.075.

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C. U., & Mohan, D. (2019). Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods. *Chemical Reviews*, 119(6): 3510-3673. https://doi.org/10.1021/acs.chemrev.8b00299

Pinto, J. (2015) Removal of organic contaminants from wastewater using the electrodiaytic process. Dissertação de Mestrado em Engenharia do Ambiente. FCT NOVA.

Prasannamedha, G., & Kumar, P. S. (2020). A review on contamination and removal of sulfamethoxazole from aqueous solution using cleaner techniques: Present and future perspective. *Journal of Cleaner Production*, 250: 119553. https://doi.org/10.1016/j.jclepro.2019.119553.

Pubchem (2020). https://pubchem.ncbi.nlm.nih.gov/. Accessed in February 2020.

Qiu, Y., Li, Q., Lee, B. E., Ruecker, N. J., Neumann, N. F., Ashbolt, N. J., & Pang, X. (2018). UV inactivation of human infectious viruses at two full-scale wastewater treatment plants in Canada. *Water Research*, 147: 73–81. https://doi.org/10.1016/j.watres.2018.09.057.

Radjenović, J., Petrović, M., & Barceló, D. (2009). Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water Research*, 43(3): 831–841. https://doi.org/10.1016/j.watres.2008.11.043.

Reddy, K. R., & Cameselle, C. (2009). Electrochemical Remediation Technologies for Polluted Soils, Sediments and Groundwater. John Wiley & Sons, Inc. https://doi.org/10.1002/978047052365.

Ribeiro, A. B., & Rodríguez-Maroto, J. M. (2006). Electroremediation of heavy metal-contaminated soils. Processes and applications. In: Prasad, M. N. V., Sajwan, K. S., Naidu, R (Eds) Trace elements in the environment: biogeochemistry, biotechnology, and bioremediation. Taylor & Francis, 341-368.

Ribeiro, A. B., Rodríguez-Maroto, J. M., Mateus, E. P., & Gomes, H. (2005). Removal of organic contaminants from soils by an electrokinetic process: the case of atrazine. Experimental and modeling. *Chemosphere*, 59(9): 1229-1239. https://doi.org/10.1016/j.chemosphere.2004.11.054.

Rizzo, L., Gernjak, W., Krzeminski, P., Malato, S., McArdell, C. S., Perez, J. A. S., Schaar, H., & Fatta-Kassinos, D. (2020). Best available technologies and treatment trains to address current challenges in urban wastewater reuse for irrigation of crops in EU countries. *Science of the Total Environment*, 710: 136312. https://doi.org/10.1016/j.scitotenv.2019.136312.

Rodríguez-Chueca, J., Laski, E., García-Cañibano, C., Martín de Vidales, M. J., Encinas, Kuch, B., & Marugán, J. (2018). Micropollutants removal by full-scale UV-C/sulfate radical based Advanced Oxidation Processes. *Science of the Total Environment*, 630: 1216–1225. https://doi.org/10.1016/j.scitotenv.2018.02.279.

Rossmassler, K., Kim, S., Broeckling, C. D., Galloway, S., Prenni, J., & De Long, S. K. (2019). Impact of primary carbon sources on microbiome shaping and biotransformation of pharmaceuticals and personal care products. *Biodegradation*, 30(2–3): 127–145. https://doi.org/10.1007/s10532-019-09871-0.

Salgado, R., Noronha, J. P., Oehmen, A., Carvalho, G., Reis, M. A. M., & Salgado, A. R. (2010). Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. *Water Science & Technology*, 62(12): 2862–2871. https://doi.org/10.2166/wst.2010.985.

SCHEER (Scientific Committee on Health, Environmental and Emerging Risks) (2017). Scientific advice on Proposed EU minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge. http://ec.europa.eu/health/scientific_committees/experts/declarations/scheer_wg_en. Accessed in February 2020.

Schneider, S. L., & Lim, H. W. (2019). Review of environmental effects of oxybenzone and other sunscreen active ingredients. *Journal of the American Academy of Dermatology*, 80: 266–271. https://doi.org/10.1016/j.jaad.2018.06.033.

Shon, H. K., Vigneswaran, S., & Snyder, S. A. (2006). Effluent organic matter (EfOM) in wastewater: Constituents, effects, and treatment. *Critical Reviews in Environmental Science and Technology*, 36(4): 327–374. https://doi.org/10.1080/10643380600580011.

Shon, H. K., Vigneswaran, S., Kandasamy, J., Cho, J., & Shon, H. K. (2009). Water and wastewater treatment technologies-Membrane Technology for Organic Removal in Wastewater. In Vigneswaran, S (Ed) Waste water treatment technologies. EOLSS Publications, 1: 128-172.

Sillanpää, M., & Shestakova, M. (2017). Electrochemical Water Treatment Methods: Fundamentals, Methods and Full Scale Applications. Butterworth-Heinemann Publishing. 131–225. https://doi.org/10.1016/B978-0-12-811462-9.00003-7.

Simarsul (2020) https://simarsul.adp.pt/content/quinta-do-conde. Accessed in February 2020.

Snyder, L. R., Kirkland, J. J., & Glajch, J. L. (2012). Non-Ionic Samples: Reversed- and Normal-Phase HPLC. In Snyder, L. R., Kirkland, J. J., & Glajch, J. L (Eds) Practical HPLC Method Development. 2nd Ed. John Wiley & Sons. 233–291. https://doi.org/10.1002/9781118592014.ch6.

Sousa, J. C. G., Ribeiro, A. R., Barbosa, M. O., Ribeiro, C., Tiritan, M. E., Pereira, M. F. R., & Silva, A. M. T. (2019). Monitoring of the 17 EU Watch List contaminants of emerging concern in the Ave and the Sousa Rivers. *Science of the Total Environment*, 649: 1083–1095. https://doi.org/10.1016/j.scitotenv.2018.08.309.

Ternes, T. A., Bonerz, M., Herrmann, N., Löffler, D., Keller, E., Lacida, B. B., & Alder, A. C. (2005). Determination of pharmaceuticals, iodinated contrast media and musk fragrances in sludge by LC tandem MS and GC/MS. *Journal of Chromatography A*, 1067(1–2): 213–223. https://doi.org/10.1016/j.chroma.2004.10.096.

Umar, M., Roddick, F., & Fan, L. (2019). Moving from the traditional paradigm of pathogen inactivation to controlling antibiotic resistance in water - Role of ultraviolet irradiation. *Science of the Total Environment*, 662: 923–939. https://doi.org/10.1016/j.scitotenv.2019.01.289.

Urban, M., Motteram, J., Jing, H.-C., Powers, S., Townsend, J., Devonshire, J., Pearman, K., Kanyuka, j. Franklin, K.E., Hammond-Kosack, K. E. (2011). Inactivation of plant infecting fungal and viral pathogens to achieve biological containment in drainage water using UV treatment. *Journal of Applied Microbiology*, 110(3): 675–687. https://doi.org/10.1111/j.1365-2672.2010.04917.x.

Vanderford, B. J., Pearson, R. A., Rexing, D. J., & Snyder, S. A. (2003). Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in Water Using Liquid Chromatography/Tandem Mass Spectrometry. *Analytical Chemistry*, 75(22): 6265–6274. https://doi.org/10.1021/ac034210g.

Viegas, C., & Pinheiro, A. C. (2015). Processing Methodologies. In Viegas, C., Pinheiro, C. A., Sabino, R., Viegas, S., Brandão, J., Veríssimo, C (Eds) Environmental Mycology in Public Health: Fungi and Mycotoxins Risk Assessment and Management. Academic Press, 415–419. https://doi.org/10.1016/B978-0-12-411471-5.00025-9.

Viegas, C., Faria, T., Gomes, A. Q., Sabino, R., Seco, A., & Viegas, S. (2014). Fungal contamination in two portuguese wastewater treatment plants. *Journal of Toxicology and Environmental Health - Part A: Current Issues,* 77(1–3): 90–102. https://doi.org/10.1080/15287394.2014.866925.

Virkutyte, J., Sillanpää, M., & Latostenmaa, P. (2002). Electrokinetic soil remediation - Critical overview. *Science of the Total Environment*, 289(1–3): 97–121. https://doi.org/10.1016/S0048-9697(01)01027-0.

Von Sperling, M., Dornelas, F. L., Assunção, F. A. L., De Paoli, A. C., & Mabub, M. O. A. (2010). Comparison between polishing (maturation) ponds and subsurface flow constructed wetlands (planted and unplanted) for the post-treatment of the effluent from UASB reactors. *Water Science and Technology*, 61(5): 1201–1209. https://doi.org/10.2166/wst.2010.961.

Von Sperling, M., Verbyla, M. E., & Oliveira, S. M. A. C. (2020). Removal efficiencies. In: Assessment of Treatment Plant Performance and Water Quality Data: A Guide for Students, Researchers and Practitioners. IWA Publishing, 181–206. https://doi.org/10.2166/9781780409320_0181.

Voulvoulis, N. (2018). Water reuse from a circular economy perspective and potential risks from an unregulated approach. *Current Opinion in Environmental Science and Health*, 2: 32–45. https://doi.org/10.1016/j.coesh.2018.01.005.

Wang, Y., Wang, X., Li, M., Dong, J., Sun, C., & Chen, G. (2018). Removal of pharmaceutical and personal care products (PPCPs) from municipal wastewater with integrated membrane

systems, MBR-RO/NF. *International Journal of Environmental Research and Public Health*, 15(2): 269. https://doi.org/10.3390/ijerph15020269.

Wang, Z., Zhang, X. H., Huang, Y., & Wang, H. (2015). Comprehensive evaluation of pharmaceuticals and personal care products (PPCPs) in typical highly urbanized regions across China. *Environmental Pollution,* 204: 223–232. https://doi.org/10.1016/j.envpol.2015.04.021.

Waters (2020) Guide to Liquid Chromatography. https://www.waters.com/watersBeginners. Acessed in February 2020.

Wen, G., Deng, X., Wan, Q., Xu, X., & Huang, T. (2019). Photoreactivation of fungal spores in water following UV disinfection and their control using UV-based advanced oxidation processes. *Water Research*, 148: 1–9. https://doi.org/10.1016/j.watres.2018.10.028.

WWAP (United Nations World Water Assessment Program) (2019). United Nations Global Water Report 2019. Leaving No One Behind. Paris, UNESCO.

Yan, J., Zhang, X., Lin, W., Yang, C., & Ren, Y. (2019). Adsorption behavior of diclofenac-containing wastewater on three kinds of sewage sludge. *Water Science and Technology*, 80(4): 717–726. https://doi.org/10.2166/wst.2019.315.

Yang, Yi, Lu, X., Jiang, J., Ma, J., Liu, G., Cao, Y., Liu, W., Li, J., Pang, S., Kong, X., & Luo, C. (2017). Degradation of sulfamethoxazole by UV, UV/H2O2 and UV/persulfate (PDS): Formation of oxidation products and effect of bicarbonate. *Water Research*, 118: 196–207. https://doi.org/10.1016/j.watres.2017.03.054.

Yang, Yongkui, Wang, L., Xiang, F., Zhao, L., & Qiao, Z. (2020). Activated sludge microbial community and treatment performance of wastewater treatment plants in industrial and municipal zones. *International Journal of Environmental Research and Public Health*, 17(2): 436. https://doi.org/10.3390/ijerph17020436.

Yin, L., Wang, B., Yuan, H., Deng, S., Huang, J., Wang, Y., & Yu, G. (2017). Pay special attention to the transformation products of PPCPs in environment. *Emerging Contaminants*, 3: 69–75. https://doi.org/10.1016/j.emcon.2017.04.001.

Zewde, A. A., Li, Z., Zhang, L., Odey, E. A., & Xiaoqin, Z. (2019). Utilization of appropriately treated wastewater for some further beneficial purposes: A review of the disinfection method of treated wastewater using UV radiation technology. *Reviews on Environmental Health.* 35(2): 139-146. https://doi.org/10.1515/reveh-2019-0066.

Zhang, D. (2019). The role of microorganisms in the removal of pharmaceutical and personal care products. In: Prasad, M. N. V., Vithanage, M., & Kapley, A (Eds) Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology Emerging Contaminants and Micro Pollutants. Butterworth-Heinemann Publishing, 341–382. https://doi.org/10.1016/B978-0-12-816189-0.00015-9.

Zhang, Q. (2016). Electrokinetic and electrochemical methods for microbial and organic fouling mitigation in liquid-solid interfaces. http://nrs.harvard.edu/urn-3:HUL.InstRepos:33493487 Accessed in March 2020.

Zhang, Y., Geißen, S. U., & Gal, C. (2008). Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere*, 73: 1151–1161. https://doi.org/10.1016/j.chemosphere.2008.07.086.

Zheng, S., Sun, J., & Han, H. (2011). Effect of dissolved oxygen changes on activated sludge fungal bulking during lab-scale treatment of acidic industrial wastewater. *Environmental Science and Technology*, 45(20): 8928–8934. https://doi.org/10.1021/es2018169.

Zhu, G., Sun, Q., Wang, C., Yang, Z., & Xue, Q. (2019). Removal of sulfamethoxazole, sulfathiazole and sulfamethazine in their mixed solution by UV/H2O2 process. International *Journal of Environmental Research and Public Health*, 16(10): 1797. https://doi.org/10.3390/ijerph16101797.

8. Appendix

Table 8.1 – Voltage measurements in EK experiments

Time (min)	Voltage (V) Mean (%) ± STD						
	UV-EK2 (n=2)	UV-EK4 (n=2)	UV-EK6 (n=2)	F1-EK6 Non-spiked (n=3)	F1-EK6 (n=3)	F2-EK6 Non-spiked (n=2)	F2-EK6 (n=2)
0	11.30 ± 0.57	11.20 ± 0.14	11.90 ± 0.42	11.30 ± 0.70	11.63 ± 0.42	13.10 ± 0.14	13.40 ± 0.14
30	11.65 ± 0.42	11.40 ± 0.42	12.15 ± 0.64	11.90 ± 0.82	11.77 ± 0.47	13.80 ± 0.14	13.85 ± 0.21
60	12.25 ± 0.14	11.65 ± 0.35	12.85 ± 1.48	11.87 ± 0.75	12.07 ± 0.40	14.05 ± 0.07	14.10 ± 0.42
90	12,55 ± 0.00	11.90 ± 0.28	13.20 ± 1.70	12.17 ± 0.92	12.20 ± 0.36	14.20 ± 0.14	14.25 ± 0.64
120	12.75 ± 0.28	12.15 ± 0.35	13.05 ± 1.20	12.00 ± 0.85	12.37 ± 0.40	14.45 ± 0.07	14.45 ± 0.64
150		12.45 ± 0.21	13.15 ± 1.06	12.17 ± 0.92	12.53 ± 0.42	14.65 ± 0.07	14.60 ± 0.71
180		12.70 ± 0.28	13.35 ± 1.06	12.27 ± 0.93	12.70 ± 0.36	14.85 ± 0.07	14.75 ± 0.78
210		12.95 ± 0.35	13.50 ± 0.85	12.43 ± 0.99	12.83 ± 0.42	15.15 ± 0.07	15.00 ± 0.85
240		13.10 ± 0.42	13.55 ± 1.34	12.47 ± 0.93	12.97 ± 0.47	15.25 ± 0.07	15.05 ± 0.78
270			13.70 ± 1.27	12.13 ± 0.91	13.07 ± 0.47	15.45 ± 0.21	15.15 ± 0.78
300			13.75 ± 1.34	12.13 ± 0.97	13.27 ± 0.55	15.60 ± 0.14	15.25 ± 0.78
330			13.80 ± 1.56	12.20 ± 0.92	13.27 ± 0.47	15.75 ± 0.21	15.35 ± 0.78
360			13.95 ± 1.48	12.33 ± 0.85	13.47 ± 0.55	15.80 ± 0.14	15.45 ± 0.78

Table 8.2 – pH and conductivity measurements in both Set 1 and Set 2 experiments

	Experiment	pH Mean ± STD (%)		Conductivity (mS/cm) Mean ± STD (%)	
		Initial	Final	Initial	Final
Set 1 EFF-UV (n=2)	t0*	7.7 ± 0.7	ND	0.71 ± 0.04	ND
	t0	ND	ND	ND	ND
	C2	7.8 ± 0.6	8.2 ± 0.0	0.64 ± 0.06	0. 68 ± 0.01
	C4	7.7± 0.7	8.4 ± 0.3	0.65 ± 0.04	0.63 ± 0.06
	C6	7.8 ± 0.6	8.8 ± 0.8	0.66 ± 0.10	0.69 ± 0.08
	EK2	7.8 ± 0.6	8.6 ± 0.5	0.64 ± 0.06	0.68 ± 0.00
	EK4	7.7 ± 0.7	8.8 ± 0.2	0.65 ± 0.04	0.64 ± 0.08
	EK6	7.8 ± 0.6	9.3 ± 0.2	0.66 ± 0.10	0.65 ± 0.03
Set 2 – EFF-F1 (n=3)	t0*	7.97 ± 0.00	ND	0.64 ± 0.00	ND
	t0	7.97 ± 0.00	ND	0.64 ± 0.00	ND
	C6	7.97 ± 0.00	8.10 ± 0.08	0.64 ± 0.00	0.63 ± 0.01
	C6*	7.97 ± 0.00	8.16 ± 0.13	0.64 ± 0.00	0.62 ± 0.05
	EK6	7.97 ± 0.00	9.40 ± 0.05	0.64 ± 0.00	0.59 ± 0.00
	EK6*	7.97 ± 0.00	9.06 ± 0.32	0.64 ± 0.00	0.61 ± 0.02
	UV6	7.97 ± 0.00	8.42 ± 0.01	0.64 ± 0.00	0.63 ± 0.00
Set 2 – EFF F2 (n=2)	t0*	8.13 ± 0.10	8.13 ± 0.11	0.48 ± 0.51	ND
	T0	8.20 ± 0.01	ND	0.49 ± 0.10	ND
	C6	8.20 ± 0.01	8.31 ± 0.01	0.49 ± 0.10	0.47 ± 0.01
	C6*	8.13 ± 0.10	8.21 ± 0.00	0.48 ± 0.05	0.46 ± 0.04
	EK6	8.20 ±0.01	9.86 ± 0.05	0.49 ± 0.10	0.44 ± 0.00
	EK6*	8.13 ± 0.10	9.53 ± 0.33	0.48 ± 0.05	0.45 ± 0.00
	UV6	8.20 ± 0.01	8.22 ± 0.03	0.49 ± 0.10	0.4 ± 0.05

* Non-spiked samples
ND – Not determined

Electro-reactor as a polishing step for the removal of emerging organic contaminants from wastewater: microcosm scale

Nazaré Couto[1], Cristiana Gonçalves[1], Joana P. Dionísio[1], Eduardo Mateus[1], Alexandra B. Ribeiro[1], Paula Guedes[1,2]

[1] *CENSE, Departamento de Ciências e Engenharia do Ambiente, Faculdade de Ciência e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal*

[2] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal*

Email of presenting author: md.couto@fct.unl.pt

The use of electro-based technologies to remediate contaminated matrices may be of great interest to public and the environmental health as it presents itself as a versatile and promising technology with potential for reducing the environmental and human risks associated with the spread of contamination. Emerging organic contaminants (EOC) are a large group of unregulated compounds, which decrease in effluents is needed aiming a safer discharge to the receiving water bodies and/or to promote a safer water re-use in agriculture. Electrokinetic process may be a cost-effective key solution for this situation.

The *ex situ* effluent remediation was tested in one electro-chemical cell and their controls (without electric current). The target EOC were: caffeine, sulfamethoxazole, carbamazepine, diclofenac, oxybenzone, bisphenol A, estradiol, ethinylestradiol, ibuprofen, as they present different physical and chemical characteristics, belonging to different categories and were already detected in various environmental compartments. The effect of electrode material (mixed metal oxide electrodes) and different treatment times (2, 4 and 6 hours of treatment) with a fixed current density (50 mA) was assessed in terms of EOCs removal. The organic component was extracted by solid phase extraction (SPE) and analysed by high-performance liquid chromatography (HPLC) with a diode array and fluorescence detectors (HPLC–DAD-FLD). A Poroshell column was used for analytes separation. A mixture of ACN/Mili-Q water/formic acid was used as eluent. Calibration curve was performed in the range between 0.5 and 8.0 mg L^{-1}. The limits of detection in this work were between 0.55 and 3.0 µg L^{-1}, and the quantification limits were between 1.7 and 9.0 µg L^{-1}. The recovery percentages were between 80 and 120% in all cases.

For all the cases, the electric current enhanced contaminants removal (up to 55% in 2 hours and 70% in 6 hours). This treatment does not require the addition of reagents and represents low energetic costs, making it more environmentally friendly.

Acknowledgements: Fundação para a Ciência e a Tecnologia is also acknowledged for N. Couto Contract established under Individual Call to Scientific Employment Stimulus (CEECIND/04210/2017) and P. Guedes (SFRH/BPD/114660/2016) fellowship.

Funding: This work was financially supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 778045; project CEMOWAS2 (SOE2/P5/F0505), co-financed by the European Regional Development Fund (FEDER); and from Fundação para a Ciência e Tecnologia, I.P., Portugal, UID/AMB/04085/2019 (Research unit CENSE "Center for Environmental and Sustainability Research"