The Handbook of Environmental Chemistry 108
Series Editors: Damià Barceló · Andrey G. Kostianoy

Sara Rodriguez-Mozaz
Paqui Blánquez Cano
Montserrat Sarrà Adroguer *Editors*

Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment

Springer

The Handbook of Environmental Chemistry

Volume 108

Founding Editor: Otto Hutzinger

Series Editors: Damià Barceló • Andrey G. Kostianoy

In over four decades, *The Handbook of Environmental Chemistry* has established itself as the premier reference source, providing sound and solid knowledge about environmental topics from a chemical perspective. Written by leading experts with practical experience in the field, the series continues to be essential reading for environmental scientists as well as for environmental managers and decision-makers in industry, government, agencies and public-interest groups.

Two distinguished Series Editors, internationally renowned volume editors as well as a prestigious Editorial Board safeguard publication of volumes according to high scientific standards.

Presenting a wide spectrum of viewpoints and approaches in topical volumes, the scope of the series covers topics such as

- local and global changes of natural environment and climate
- anthropogenic impact on the environment
- water, air and soil pollution
- remediation and waste characterization
- environmental contaminants
- biogeochemistry and geoecology
- chemical reactions and processes
- chemical and biological transformations as well as physical transport of chemicals in the environment
- environmental modeling

A particular focus of the series lies on methodological advances in environmental analytical chemistry.

The Handbook of Environmental Chemistry is available both in print and online via https://link.springer.com/bookseries/698. Articles are published online as soon as they have been reviewed and approved for publication.

Meeting the needs of the scientific community, publication of volumes in subseries has been discontinued to achieve a broader scope for the series as a whole.

Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment

Volume Editors: Sara Rodriguez-Mozaz ·
Paqui Blánquez Cano ·
Montserrat Sarrà Adroguer

With contributions by

A. Agüera · L. Baptista-Pires · D. Barceló · M. Bilal · P. Blánquez · A. M. Botero-Coy · M. Brienza · G. Buttiglieri · M. Escolà Casas · M. Castaño-Trias · S. Esplugas · P. Gago-Ferrero · J. Giménez · A. Ginebreda · B. Guieysse · F. I. Hai · F. Hernández · A. Hom-Díaz · H. M. N. Iqbal · J. Lee · S. Malato · J. Marugán · V. Matamoros · J. A. Mir-Tutusaus · I. Oller · W. E. Price · J. Radjenovic · J. Rodríguez-Chueca · S. Rodríguez-Mozaz · J. A. Sánchez Pérez · C. Sans · M. Sarrà · E. A. Serna-Galvis · M. C. Tomei · R. A. Torres-Palma · P. Verlicchi · T. Vicent · F. Zietzschmann

Springer

Editors
Sara Rodriguez-Mozaz
Catalan Institute for Water Research (ICRA)
University of Girona
Girona, Spain

Paqui Blánquez Cano
Universitat Autònoma de Barcelona
Barcelona, Spain

Montserrat Sarrà Adroguer
Universitat Autònoma de Barcelona
Barcelona, Spain

ISSN 1867-979X ISSN 1616-864X (electronic)
The Handbook of Environmental Chemistry
ISBN 978-3-030-77508-7 ISBN 978-3-030-77509-4 (eBook)
https://doi.org/10.1007/978-3-030-77509-4

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Series Editors

Prof. Dr. Damià Barceló

Department of Environmental Chemistry
IDAEA-CSIC
C/Jordi Girona 18–26
08034 Barcelona, Spain
and
Catalan Institute for Water Research (ICRA)
H20 Building
Scientific and Technological Park of the University of Girona
Emili Grahit, 101
17003 Girona, Spain
dbcqam@cid.csic.es

Prof. Dr. Andrey G. Kostianoy

Shirshov Institute of Oceanology
Russian Academy of Sciences
36, Nakhimovsky Pr.
117997 Moscow, Russia
and
S.Yu. Witte Moscow University
Moscow, Russia
kostianoy@gmail.com

Editorial Board Members

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last four decades, as reflected in the more than 150 volumes of *The Handbook of Environmental Chemistry*, there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry*, the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of

"pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via https://link.springer.com/bookseries/698. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Series Editors

Preface

Among organic micropollutants, pharmaceutically active compounds (PhACs) have been extensively investigated over the last few years due to their potential adverse ecological and/or human health effects. After administration, PhACs are excreted and delivered with wastewater to the wastewater treatment plants (WWTPs). Wastewater treatment plants thus become one of the first barriers in preventing them from reaching the ecosystems but also one of the main sources, since a significant proportion of PhACs is not properly eliminated in conventional WWTPs. Therefore, advances in wastewater treatment technologies are crucial to minimize the load of micropollutants, and thus to the optimal protection of both the environment and human health. In this context, this volume aims at providing a snapshot of selected wastewater treatment strategies, each of which is discussed in the corresponding chapter.

The efficiency of the wastewater treatment of choice can be evaluated by considering the overall removal of pollutants, although a better assessment is achieved if its environmental relevance is considered. Chapter One is specifically devoted to environmental risk assessment of pharmaceuticals in wastewater treatment, and different approaches are discussed and suggested as tools to evaluate the removal efficiency of a particular treatment. In Chapter Two, an overview of the fate of PhACs in conventional activated sludge (CAS) systems (the most standard practice in conventional WWTPs) is presented. Several parameters and constraints affecting PhAC removal in the water line of CAS systems are given and discussed, while for the sludge line different treatment options are also considered. Special attention is paid in these two chapters to the risks of pharmaceuticals in water reclamation practices. Even though most of the literature (and the chapters of this volume) deal with urban wastewater treatment, decentralized treatment of hospital effluents is becoming an interesting approach to reduce the impact on the aquatic environment of hazardous substances that are routinely administered or used in healthcare facilities. Chapter Three is entitled "New Insights into the Occurrence of Micropollutants and the Management and Treatment of Hospital Effluent," which

deals with this type of effluents and reviews promising new technologies proven to improve the removal of emerging pollutants such as PhACs.

Another critical issue addressed in this volume is the development of appropriate analytical methods to determine the presence of PhACs and transformation products in the environment. This is of utmost importance for the proper evaluation of its disposal in the wastewater treatment process of choice. Target analytical methodologies are the established analytical approach for evaluating pollutant removal, but they can only cover a small proportion of organic pollutants. Consequently, some compounds remain uncharacterized despite their potential relevance. New analytical approaches are reported in Chapter Four (Suspect and Non-target Screening Methodologies for the Evaluation of the Behaviour of Polar Organic Micropollutants and Changes in the Molecule Fingerprint During Water Treatment). These strategies allow to provide information on a broader set of contaminants (including PhACs) to understand their behavior and their corresponding transformation products during wastewater treatment processes. The chapter also discusses the novel non-target workflows to characterize dissolved organic matter (DOM) during water treatment.

After these four chapters that are dedicated to general aspects, the core of the book is dedicated to report on different wastewater treatment strategies and their efficiency with respect to the removal of PhACs from the waste. Physical, chemical, and biological processes have been tested with varying degrees of success. Biological-based treatments considered in this book are enzymes, fungal reactors, nature-based solution (NBS) like wetlands and microalgae-based processes (Chapters Five to Eight). Chapter Nine reviews the application of membrane bioreactors, which combine biodegradation and membrane separation in a single step. Finally, the removal and degradation of PhACs in wastewater are considered using physicochemical treatment processes such as low-cost adsorbents, ozone and advanced oxidation processes (AOPs), and other advanced processes based on solar oxidation, electrochemical and sonochemical processes (Chapters Ten to Fourteen). Many of these treatments can be applied as single treatment steps but also as polishing treatment after conventional WWTP, or just as a combination of some of them in the treatment train of choice.

We hope that readers will be able to find in this volume the latest information on the topic that can help them to further develop water treatment and reuse projects. We would also like to acknowledge all the colleagues who agreed to join this project and share their knowledge, as well as the editor and publisher of the series for inviting us to prepare this book.

Girona, Spain — Sara Rodriguez-Mozaz
Barcelona, Spain — Paqui Blánquez Cano
Barcelona, Spain — Montserrat Sarrà Adroguer

Contents

Environmental Risk Assessment of Pharmaceuticals in Wastewater Treatment

Antoni Ginebreda, Damià Barceló, and Sara Rodríguez-Mozaz

Contents

A. Ginebreda
Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

D. Barceló
Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Catalan Institute for Water Research (ICRA), Girona, Spain

University of Girona, Girona, Spain

S. Rodríguez-Mozaz (✉)
Catalan Institute for Water Research (ICRA), Girona, Spain

University of Girona, Girona, Spain
e-mail: srodriguez@icra.cat

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 1–22, DOI 10.1007/698_2020_694,

Abstract The presence of pharmaceutically active compounds (PhACs) in the environment has gained increasing attention the past two decades. After administration, PhACs are excreted and thus delivered with wastewater to the wastewater treatment plants (WWTPs). WWTP effluents are usually discharged into receiving environment and, therefore, a high proportion of pollutants (including pharmaceuticals) can enter water bodies (surface or sea water), but also soils and eventually groundwater. The risk that pharmaceuticals can pose to the environment depends not only on compound concentration but also on its toxicity. In this chapter, different existing risk assessment approaches are presented, applied to the effluents from conventional WWTPs but also from alternative and advance wastewater treatment processes. Environmental Risk assessment (ERA) can also be applied as a tool to evaluate the removal efficiency of a wastewater treatment. Moreover, special focus is paid to assess the risk of pharmaceuticals in water reclamation practices such as crop irrigation. Finally, some research lines or topics that demand greater attention related to ERA in wastewater treatment are addressed such as toxicogenomics, antibiotic resistance, and evaluation of impact of chemical mixtures.

Keywords Environmental hazard, Pharmaceutically active compounds (PhACs), Toxicity, Wastewater effluent

1 Introduction

1.1 Why Pharmaceuticals?

Our continuously growing technological society has given rise to extensive and intensive use of chemicals on a global scale. More than 140,000 chemicals are routinely used by both industry and household [1]. Depending on their properties, the amounts produced, and the mode of use, a substantial fraction of these chemicals may potentially reach the environment. Pharmaceuticals are recognized as one of the pillars of our health system. More than 4,000 active compounds are thus globally and increasingly used worldwide, a fact that goes in parallel with their ubiquitous environmental occurrence. After the patient's medication with a pharmaceutically active compound (PhAC), it is metabolized and subsequently excreted through the body fluids as a mixture of the original parent compound and the metabolites generated in the treated organism. Finally, the complex mixture is evacuated through the sewage system and eventually treated in a wastewater plant, before the final effluents are released to the environment. Roughly, 80% of the world's wastewater is discharged without any treatment [1, 2]. Furthermore, even when it is properly treated, leaks from sewage pipes, storm overflows, and runoff during heavy rain events constitute non-negligible sources of untreated water as well [2]. Finally, because conventional WWTPs are not specifically designed for that purpose, some PhACs are simply not degraded and require advanced tertiary processes not easily

affordable. Whereas excretion is the predominant source of PhACs into the environment, other pathways like inappropriate disposal, industrial spills, or manure spread (particularly for veterinary drugs) should not be overlooked. Once effluents reach the receiving waterbody PhACs and their metabolites may undergo further biotic and abiotic degradation processes such as sorption, photolysis, hydrolysis, and biodegradation potentially giving rise to new transformation products. Parent compounds plus the resulting transformation products (TPs) constitute a "chemical universe" whose long-term interactions with the different levels of biological organization (species, individuals, populations, and ecosystems) are still poorly known. The environmental concerns associated with PhACs are summarized in Box 1.

Box 1 Environmental Concerns Associated to Active Pharmaceutical Ingredients (APIs) and Their Transformation Products

- Drugs are regularly taken by the population with a clear growing trend. The process is ubiquitous on space and continuous along time and extends to some thousands of available APIs.
- Water treatment systems (if any) are not specifically designed to eliminate pharmaceuticals. Depending on each specific compound and characteristics of the treatment, their elimination maybe not complete. Further tertiary specific treatment steps may be necessary, but at a certain economic cost not always affordable.
- Even though many APIs are degraded, their continuous input into the environment makes them behave as "pseudo-persistent."
- Drugs are, by design, biologically active compounds, targeted to specific organisms (typically humans) and having precise modes of action. Possible side effects on other non-targeted unintentionally exposed organisms found in the receiving ecosystem biological communities are largely unknown.
- In addition to long-term ecotoxicity effects, there is a special human health concern for the specific case of antimicrobials (antibiotics, antifungals), whose environmental occurrence has been proved to promote the development of resistance genes (Antimicrobial Resistance Genes, ARG).
- The complex "cocktail" of many APIs, metabolites, and transformation may have unexpected and hardly predictable mixture interaction effects (i.e., synergistic, antagonistic, etc.).

Last but not least, the anthropogenic water cycle includes the safe supply of drinking water to the population. The occurrence of API residues in drinking water may pose a risk to human health, therefore their absence must be prevented in any case.

2 Regulatory Aspects

Altogether, it is recognized that the occurrence of drugs in the environment is not exempt from risk and there is a need of incorporating environmental aspects into the existing regulatory framework to assure the environmental safety of drugs. In that respect, two areas can be highlighted related to legislation (1) the registration process of drugs, and (2) the environmental occurrence of drugs in the environment, and more specifically in the aquatic environment. The current status of both aspects concerning drug legislation in the European Union is briefly reviewed below:

2.1 Environmental Risk Assessment Aspects in the Registration of Pharmaceuticals

The legal basis for the registration, production, distribution, and use of medicinal products in the EU member states is governed by the Directive 2001/83/EC. Following their provisions, any medical product should get an official authorization previous to its marketing. This is issued by the European Medicines Agency (EMA) after the proper completion of a registration process. According to article 8(3) of the directive, applicants must submit a dossier containing the necessary information of the pharmaceutical product to ensure its safety and therapeutic and clinical efficacy. As part of this authorization file, information regarding the potential risks of the PhAC to the environment is required. Therefore, companies wishing to register a new PhAC have to provide an Environmental Risk Assessment (ERA) [3, 4].

According to the EMA guideline [5], the overall process is depicted in Fig. 1 and includes a risk assessment and a specific hazard assessment. The risk assessment is focused on the environmental occurrence (exposure) and ecotoxic potential effects of the product on the exposed organisms. For some specific biological effects and substances (i.e., endocrine disruptors, antibiotics, etc.) additional aspects have to be considered. In turn, the hazard assessment refers to intrinsic properties of the products considered harmful for the living organisms exposed regardless of the concentration and specifically to the persistence, bioaccumulation, and toxicity (PBT) characteristics. Briefly, the procedure includes two phases (Fig. 1). In general, Phase I consists of a decision tree mostly addressed to differentiate among products that require a further assessment (Phase II) or those that not. This is done based on the Predicted Environmental Concentration in surface water (PEC) of the product estimated from its predicted use. If PEC $\geq$ 0.01 μg/L, the product enters Phase II, otherwise the process is finished. Phase II is a tiered process, starting with the study of physicochemical properties, environmental fate, and ecotoxicity and a predicted non-effect concentration (PNEC). Amongst other aspects like the potential risk to the groundwater and the soil, or the possibility of secondary poisoning in across the trophic chain, Tier A examines the risk ratio PEC/PNEC in surface water, and if it exceeds 1, a Tier B with PNEC refinement is performed. The PBT hazard

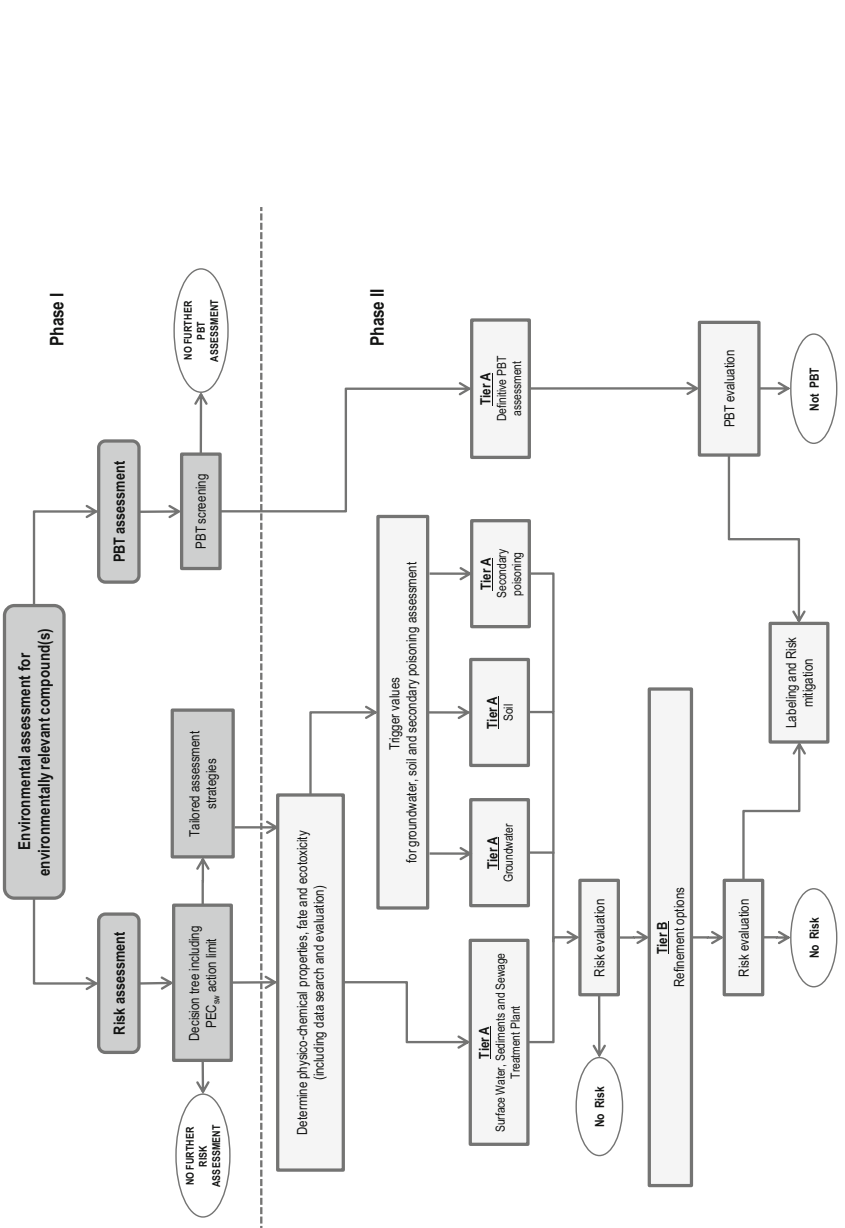


Fig. 1 Flow-chart of the environmental risk assessment process prescribed by the European Medicines Agency (EMA) to be used in the registration of new medicines (adapted from [5])

assessment, carried out (if necessary) in parallel to the risk assessment, aims at evaluating the potential long-term effects of the product in the environment, regardless of its environmental exposure concentration. Depending on the results obtained in Phase I (screening phase), a more detailed and definitive assessment is performed in Phase II.

Despite the implementation of the ERA procedures is a relevant advance to prevent the undesirable effects of pharmaceuticals in the environment, some limiting aspects are worth to be mentioned:

- Even though the inclusion of an ERA is mandatory in the registration of an PhAC, the final authorization or refusal does not depend on the ERA itself.
- ERAs are compulsory for new PhACs, but not for those authorized before the approval of the Directive 2001/83/EC.
- ERAs are conducted with pharmaceutical products rather than with PhACs.

2.2 The Occurrence of Drugs in the Aquatic Environment

The preservation of the aquatic environment, either surface (marine and fresh) or groundwater in Europe is essentially regulated by the Water Framework Directive (WFD) (Directive 2000/60/EC) and derived pieces of legislation. In that context, to achieve the good status of European water bodies both the good ecological and chemical status requirements must be fulfilled. For the latter, environmental levels must comply with the Environmental Quality Standards (EQS) of the so-called priority substances, whose EQS's were set up in the WFD daughter directives (Directive 2008/105/EC amended by Directive 2013/39/EU). Currently, the list of priority substances is constituted 45 chemical species. However, the "list of priority substances" is subjected to periodic revision, meaning that it is open to the incorporation of new candidate substances. To do so Directive 2013/39/EU (article 8b) foresees establishing a "watch list" of new substances of concern for which new monitoring data need to be gathered (sic) *"for the purpose of supporting future prioritization exercises in accordance with article 16(2) of the WFD."* Hence, the inclusion of a substance in the "watch list" might be regarded as a necessary (but not sufficient) condition to its final incorporation into the list of priority substances. On the other hand, the article 8c of the aforementioned Directive 2013/39/EU entitled "Specific provisions for pharmaceutical substances," the European Commission takes the commitment of carrying out a specific study about the risks posed by medicinal products in the environment and developing within the next 2 years a strategic approach to pollution of water by pharmaceutical substances. The outcomes of such an approach are available in the corresponding document issued by the European Commission [6].

Up to now the list of priority substances does not include any pharmaceutical substance. In contrast, several of them have been already added in the "watch list," in

their three successive versions [7–9]. The compounds included in the latest are the antibiotics ciprofloxacin, amoxicillin, sulfamethoxazole, trimethoprim, and the psychiatric drugs venlafaxine and o-desmethylvenlafaxine (venlafaxine human metabolite).

3 Overview of the Environmental Risk Assessment process

Risk may be generally defined as the combination (i.e., product) of a probability of occurrence of a certain event by its hazard effects [10]:

$$\text{Risk} = \text{Occurrence} \times \text{Hazard effects} \tag{1}$$

Different existing risk assessment approaches have been developed in order to identify and rank compounds of environmental concern for both regulatory and monitoring purposes. Most of the existing schemes share the above risk assessment concept, however, they differ on how risk, occurrence and effects, are defined and hence quantified. Occurrence (exposure) data can be expressed in the form of measured environmental concentration (MEC) or predicted environmental concentrations (PEC) which can be indirectly estimated on the basis of, e.g. the data of annual production, sales rate, number of prescriptions, number of inhabitatants, etc. Hazard effects are usually based on ecotoxicity data of chemicals that can be either available from experimental measurements or predicted by the so-called in silico models (QSAR), such as ECOSAR.

Typically, for a single compound *i* risk is quantified using Eq. (2):

$$\text{Risk}\ (i) = \frac{C_i}{C_i(\text{ref})} \tag{2}$$

where c_i is the MEC or PEC of a compound in the water phase and $c_i(ref)$ is an ecotoxicity reference concentration for the same compound. The "reference concentration" can be an acute toxicity (EC50 or LC50), a chronic (long term) ecotoxicty (predicted no-effect concentration, PNEC) or even a legal value. In the former case Risk is expressed in "toxic units" (TU) [11], while the second is usually referred to as a "hazard or risk quotient" (HQ, RQ).

A review of different risk-based prioritization schemes for pharmaceuticals can be found at [12].

When dealing with multicomponent mixtures, three basic types of combined action models are typically taken into consideration [13, 14]: (a) concentration (or dose) addition (CA), for chemicals acting through a similar mode of action; (b) independent action (IA) for chemicals acting through a dissimilar mode of action; and (c) interactions between the chemicals present in the mixture, which includes any deviation from additivity either greater (synergistic) or lesser (antagonistic) than that predicted by CA or IA. It is worth noting that both CA and IA models rely on the

assumption that the chemicals present in the mixture do not interact at the biological target site and hence do not influence each other's toxicity. On the other hand, at the environmental typically low concentrations and effect levels, the concentration-effect curves become linear and the two models converge. In turn, and for the same reasons, synergistic interactions are generally overlooked and their relevance is generally limited to mixtures with a small number of components at high concentrations [15, 16]. Altogether, the application of CA is generally an acceptable approach for mixture toxicity regardless of the components' similar or dissimilar mode of action. Hence, CA is usually the first tier approach used in several international recommendations and regulatory frameworks [13]. CA is usually expressed in Eq. (3):

$$\text{Risk} = \sum_{i=1}^{n} \frac{c_i}{c_i(\text{ref})} \tag{3}$$

where the index i extends over all the mixture components.

It is worth noting that both Eqs. (2) and (3) are referred to a specific ecotoxic assay or bioassay (organism, end-point, exposure time, etc.), and so it is the quantified risk. Therefore, it must be clearly specified.

4 Environmental Risk Assessment of Wastewater Effluents

There is a vast number of papers evaluating the risk of pharmaceuticals for the environment related to wastewater treatment and treated wastewater discharge. Treated wastewater effluents can be discharged in freshwater systems (lakes, rivers, and even groundwater through reclaimed water recharge) or directly in the ocean (in the case of coastal urban areas). In Table 1 some examples of different studies on the environmental risk of pharmaceuticals in different wastewater treatment scenarios are gathered. Due to water reuse activities, treated wastewater can also be applied for some activities, which can lead also to environmental and human impact, such as the use for irrigation of fields.

Most of the studies found in the literature are those focus on conventional activated sludge (CAS) which is the usual treatment in conventional WWTPs, and therefore most of publications are monitoring studies in full-scale WWTPs in different countries all over the world such as, South Africa, Kenya, Tunisia, Turkey, China, Iran, Brazil, Greece, Spain, Portugal, France, Sweden, Faroe Islands, Iceland, and Greenland as it is reported in Table 1 [19, 20, 22–35]. In these studies, risk or hazard quotients were calculated for the target pharmaceuticals found in the effluents of the selected WWTPs, whereas in some others the hazard posed in the receiving environment was directly calculated.

In addition, different types of alternative advanced technologies for treating emerging contaminants have been tested in the last years including physical-

Table 1 Selected environmental risk assessment studies related to the presence of PhACs in wastewater treatment processes

Ref.	Number of target compounds	Wastewater treatment	Receiving water body	Country	Highlighted compounds in WWTP effluents (high risk)
Guedes-Alonso et al. [17]	15 (steroid hormones)	Macrophyte pond-CW system	N.I.	Spain	No high risk
Lucas et al. [18]	81 PhACs	Fungal treatment	N.I.	Spain	N.I.
Afonso-Olivares et al. [19]	23 PhACs	CAS + reverse osmosis	N.I.	Spain	Ibuprofen, gemfibrozil, and ofloxacin
Díaz-Garduño et al. [20]	78 PhACs	CAS + photobioreactor	N.I.	Spain	No high risk
		CAS + multi-barrier treatment	N.I.		No high risk
Auvinen et al. [21]	12 PhACs	Constructed wetlands	River	France	No high risk
Ben et al. [22]	26 PhACs	14 WWTP (some tertiary treatment)	N.I.	China	Sulfamethoxazole clarithromycin erythromycin, ofloxacin, and ciprofloxacin
Kosma et al. [23]	16 PhACs	8 WWTP	N.I.	Greece	Diclofenac, trimethoprim, and sulfamethoxazole
Archer et al. [24]	31 PhACs	1 WWTP	River	South Africa	Carbamazepine, naproxen, diclofenac, and ibuprofen
Gros et al. [25]	73 PhACs	7 WWTP	River	Spain	No high risk
Kairigo et al. [26]	7 antibiotics	4 WWTP	River	Kenya	Amoxicillin, ciprofloxacin, trimethoprim, norfloxacin, and sulfamethoxazole
Mirzaei et al. [27]	7 antibiotics	2 WWTP	River	Iran	Cefixime, and azithromycin and erythromycin amoxicillin, penicillin, and ciprofloxacin
Pereira et al. [28]	10 psychiatric drugs	5 WWTP	River	Brazil	No high risk
Huber et al. [29]	36 PhACs	WWTP and untreated WW	Rivers, lakes, or the sea	Faroe Islands, Iceland, and Greenland	No high risk in water recipients

(continued)

Table 1 (continued)

Ref.	Number of target compounds	Wastewater treatment	Receiving water body	Country	Highlighted compounds in WWTP effluents (high risk)
Aydin et al. [30]	10 antibiotics	1 WWTP	Lake	Turkey	Azithromycin and clarithromycin
Afsa et al. [31]	40 PhACs	1 WWTP	Coastal seawater	Tunisia	No high risk in water recipients
Biel-Maeso et al. [32]	79 PhACs	1 WWTP	Coastal and oceanic water	Spain	Gemfibrozil and ofloxacine
Zhang et al. [33]	31 antibiotics	12 WWTP	NI	China	Erythromycin and clarithromycin
Pereira et al. [28]	11 PhACs	15 WWTP	NI	Portugal	Ciprofloxacin, bezafibrate, gemfibrozil, simvastatin, and diclofenac
Sörengård et al. [34]	78 PhACs	1 WWTP	River and Lake	Sweden	No high risk

N.I. Not Indicated

chemical and biological treatments, in order to find a proper alternative to conventional activated sludge (CAS) or even to be added as a tertiary treatment after CAS treatment. The use of this kind of technologies could be especially important in the treatment of wastewaters with a high PhACs concentration, such as hospital and to a lesser degree, veterinary hospital wastewaters [36]. Among all the publications reporting the presence, fate and removal of PhACs in these alternative wastewater systems, some have also considered the assessment of environmental risks. Lucas et al. [18] studied the impact of wastewater treatment based on fungi on the removal and environmental impact of a broad set of PhACs in different types of wastewater, whereas some other authors studied other biological treatments such as wetlands Guedes-Alonso et al. [17], who studied the occurrence of 15 steroid hormones along a waste-stabilization pond combined with a constructed wetlands (CWs). Final effluents showed a low ecological risk associated with steroid hormones in contrast to the medium-high ecological risks found in the influent samples.

5 Environmental Risk Assessment as a Tool to Evaluate the Removal Efficiency of Wastewater Treatment

Removal efficiency of wastewater treatment is usually assessed comparing the concentration of contaminants before and after the treatment under evaluation, i.e. as the percentage of reduction between the concentration in the initial untreated wastewater influent and the concentration in the treated wastewater effluent. However, as discussed in the previous section, actual risks of contaminants depend not only on their concentration but also on their hazardous effects. Therefore, some authors have proposed to evaluate the performance of water treatment in terms of reduction of total HQ. In this section some examples are discussed:

In the study by Verlicchi et al. [37], the environmental risk assessment was performed on the effluent from the hospital and the influent and effluent from the WWTP treating the effluents of the hospital among other urban wastewater contribution. The quotient between the maximum MEC and the PNEC (referring to acute toxicity) of each of the compounds detected was used as a marker of risk. 9 pharmaceuticals were contributing the most to the potential risk for the environment in all types of water although only 5 of them (the four antibiotics clarithromycin, erythromycin, ofloxacin, and sulfamethoxazole and the psychiatric drug fluoxetine) were posing a high risk for the environment both in the influent wastewater and the effluent of the municipal WWTP. The study points out antibiotic family as one of the most critical therapeutic classes, being highly recalcitrant to degradation and removal.

The elimination of broad set of pharmaceuticals (more than 80 compounds) by means of a biological treatment based on the fungus *Trametes versicolor* was evaluated in the study by Lucas et al. [18]. PhACs removal studied in different types of wastewaters (urban, reverse osmosis concentrate, hospital, and veterinary

hospital wastewaters) were reviewed and compared with conventional activated sludge (CAS) treatment. Hazard indexes were calculated based on the ecotoxicity value for each compound and used for the evaluation of environmental risk. PhACs elimination achieved with the fungal treatment (mean value 76%) was similar to that achieved in the CAS treatment (85%). However, the fungal reactor was superior in removing, among all pharmaceutical compounds analyzed, those compounds that pose higher environmental risk (antibiotics and psychiatric drugs): concentration removal achieved with fungal treatment was 56% for antibiotics and 59% for psychiatric drugs whereas only 36% and 39% for antibiotics and psychiatric drugs, respectively, was achieved by CAS. This led to a reduction of environmental risk up to 93% in fungal treatment whereas only 53% of hazard reduction was achieved by CAS. Fungal treatment can thus be considered as a good alternative to conventional treatment technologies for the elimination of PhACs from wastewaters.

The monitoring of the health risk associated with the presence of pharmaceuticals along wastewater treatment was also performed by Ma et al. [38] in the frame of water reuse activities to assure reclaimed water safety. In their study, domestic wastewater was treated using an anaerobic-anoxic-oxic unit followed by a membrane bioreactor (A2O-MBR), and the reclaimed water was used for replenishing a landscape lake. A total of 58 organic micropollutants were detected in the system including 13 polycyclic aromatic hydrocarbons (PAHs), 16 phenols, 3 pesticides, and 26 pharmaceuticals and personal care products (PPCPs). In this case the authors used another approach using the models proposed by the US EPA [39] and calculated the health risks associated with the target micropollutants in the water reclamation and ecological reuse system, including carcinogenic risks (CRs) and noncarcinogenic risks (non-CRs) assuming that people are exposed to micropollutants in reclaimed water mainly by ingestion. Although carcinogenic and noncarcinogenic risks associated with all the detected micropollutants were at negligible levels, the HQ of PPCPs accounted for more than 90% of the total HQ. Authors conclude that this approach is useful for an effective evaluation of health risk reduction into the water reclamation and reuse systems and therefore to be used to improve strategies for guaranteeing the safety of reclaimed water reuse related to micropollutants.

Guedes-Alonso et al. [17] studied the occurrence of 15 steroid hormones along a waste-stabilization pond combined with constructed wetlands (CWs), which are natural wastewater treatment systems used in small communities because of their low cost and easy maintenance. The pond-CW system showed high elimination rates of the 8 steroid hormone residues detected with average removal efficiencies higher than 77%. This efficacy was confirmed in the ecological risk assessment performed. Final effluents showed a low ecological risk associated with steroid hormones in contrast to the medium-high ecological risks found in the influent samples.

The latest application of the hazard quotient (HQ) approach to evaluate wastewater treatment is the one carried out by Ramírez-Morales et al. [40], who monitored the occurrence of 70 pharmaceutical active compounds (PhACs) in 11 WWTPs across Costa Rica. Among the 33 PhACs found, 20 compounds in influents and 15 in the effluents showed high HQ. The top HQ values were obtained for risperidone,

lovastatin, diphenhydramine and fluoxetine, caffeine and trimethoprim, which should be considered in prioritization lists of pharmaceuticals in the context of environmental hazard/risk. Likewise, almost all wastewater analyzed (both influents and effluents) seemed to pose a high hazard towards aquatic organisms.

6 Discharge of Wastewater and Sludge in the Receiving Environment; Risk Assessment

In their study Mirzaei et al. [27] pointed out six antibiotics cefixime, and azithromycin and erythromycin amoxicillin, penicillin and ciprofloxacin, as high risk to algae or bacteria in 2 WWTP effluents whereas only the 3 latest were posing a high risk in 2 rivers in Teheran (Iran). Kairigo et al. [26] also investigated 6 antibiotics for their occurrence and environmental risk assessment in 4 WWTPs and the receiving water bodies (river waters) in Kenya. The risk quotient for bacterial resistance selection in effluent and surface water ranged between <0.1 and 53, indicating a medium to high risk of antibiotic resistance developing within the study areas. The antibiotics posing medium to high risk in this respect were amoxicillin, ciprofloxacin, trimethoprim, norfloxacin, and sulfamethoxazole. In the work by Pivetta et al. [35], 10 psychotropic drugs widely consumed in Brazil were monitored at 5 WWTPs in the influents, at different stages of the treatments and in the surface waters from the Atibaia River and the Anhumas Creek, though he levels of the psychotropic compounds detected did not appear to present risks to the aquatic biota.

In the study by Afsa et al. [31], the occurrence of 40 pharmaceuticals belonging to several therapeutic groups was investigated in hospital effluent, wastewater treatment plant influent and effluent, and seawater in Mahdia, Tunisia. Measured environmental concentrations (MECs) detected in seawater samples seemed not pose a toxic effect to the aquatic organisms (except for salicylic acid, sulfamethoxazole, and fluoxetine). In another study in Spain Biel-Maeso et al. [32] also evaluated the impact of WWTP effluent discharges in sea water of the Gulf of Cadiz. Risk quotients were calculated for the 78 PhACs targeted and these were higher than 1 only for gemfibrozil and ofloxacin in one WWTP, whereas no high environmental risk was detected in both coastal and oceanic sampling areas.

To this respect it is interesting to highlight some studies such as the one by Rodriguez-Mozaz et al. [41] who were applying a specific dilution factor (the ratio between the volume of receiving water available and the domestic sewage discharge) to the PhACs concentration in the wastewater effluent of the corresponding country. This dilution factor or "National annual median dilution factor" was calculated for domestic effluents for approximately 100 countries by Keller et al. [42].

Sludge originated during wastewater treatment may contain a wide spectrum of organic and inorganic substances as well as microorganisms and viruses which are separated from the liquid phase during treatments. The main disposal routes are incineration, landfill, land application, and composting, with the specific percentages

varying from country to country, although 53% of sludge in EU is estimated to be reused in agriculture either directly or after composting [43, 44]. Disposal routes of sewage sludge must fulfill specific regulations although no limits have been set for pharmaceuticals and other contaminants of emerging interest. However, there is ongoing debate within the scientific community in order to evaluate potential (environmental) risks in this kind of practice, due to the occurrence of toxic and persistent substances in sludge, such as aquifer contamination, the accumulation of pollutants in soil, and their transfer into the food chain [44]. Due to the lack of data regarding chronic and acute toxicity for terrestrial organisms with regard to PhACs, some authors [44, 45] evaluate the corresponding PNEC for sludge and soil on the basis of the PNEC for water and partition coefficient Kd of the selected compound according to Eq. (4).

$$\mathrm{PNEC}_{\mathrm{sludge}} = \mathrm{PNEC}_{\mathrm{water}} \times \mathrm{Kd} \times 1,000 \tag{4}$$

This approach was adopted by Verlicchi and Zambello [44], to evaluate the environmental risk posed by the spreading of treated sludge in agriculture. The authors first reviewed concentrations for 152 pharmaceuticals and 17 personal care products in sludge reported in 59 papers and further calculated the corresponding RQ. The most critical compounds found in the sludge-amended soil were the 3 antibiotics ciprofloxacin, ofloxacin, tetracycline, and other related compounds such as estradiol, caffeine, triclosan, and triclocarban.

7 Environmental and Human Health Risks of Reclaimed Water Irrigation

Uses of reclaimed water include the irrigation of landscapes, forests, and agricultural fields; the use by industries as an alternative to freshwater and the supplementation or augmentation of stream flows and groundwater aquifers [46]. Some reuse applications might imply a great potential for human exposure and some studies have assessed the corresponding risks for human health. In this section some examples of such studies are presented related to irrigation with reclaimed water. Reclaimed wastewater (RG) is increasingly used to irrigate agricultural land and to alleviate agricultural water shortages worldwide. This use has led to also growing concerns about soil contamination by pharmaceuticals and other contaminants and the human health risks associated with dietary crop intake [47]. As in the case of environmental risk assessment, specific hazard quotients are calculated to evaluate the risks of accumulated pharmaceutical residues to humans through the consumption of edible part of the plants irrigated by reclaimed water. An increasing number of studies are being published in the last few years to evaluate those risks and it is a topic of growing concern. Liu et al. [47] evaluated the occurrence and accumulation of 11 PPCPs and one active metabolite in soils and various crops (cucumber, eggplant,

long bean, and wheat) from RW irrigation fields with different irrigation histories in Beijing and evaluated the human health risks associated with the consumption of these crops. The risk associated with the consumption of treated RW-irrigated crops was assessed using the threshold of toxicological concern (TTC) approach based on the Cramer classification tree [48, 49] and the hazard quotient (HQ) based on the description by Prosser and Sibley [50]. Cramer structural decision tree was constructed using Toxtree software (Toxtree V2.6.13) to classify substances as having genotoxic potential or into one of the three classes (I, II, III). The HQ was determined by dividing the estimated daily intake (EDI) by the acceptable daily intake (ADI), which is the amount that can be consumed daily over a person's lifespan without adverse effects. Humans are considered to be exposed to a potential hazard if the HQ value is >0.1 rather than >1, which is used when we recognize that humans may be exposed through other pathways. The estimated TTC and HQ values showed that the consumption of crops irrigated long term with RW presents a minimal risk to human health.

In another field study in Israel, Malchi et al. [51] evaluated the uptake of 15 PhACs by root crops (carrots and sweet potatoes) treated by wastewater and evaluated the potential risks for human health using the using the TTC approach. The TTC is a useful tool to estimate the safety of exposure to chemicals found at low concentration in foods and drinking water. Consumption of a PhACs above the corresponding TTC value indicates a possible risk of exposure. Authors show the extent of PhACs uptake is influenced by the physicochemical properties of the compound, the crop, and soil. The daily consumption of root crops irrigated with treated wastewater did not pose a health threat except for lamotrigine and 10,11-epoxycarbamazepine with concentrations above TTC value in edible organs.

Finally, Semerjian et al. characterized human health risks posed by 10 pharmaceuticals quantified in WWTP effluents used for landscape irrigation in United Arab Emirates, where wastewater reuse applications play an integral role in meeting water demands [46]. The human health risk was assessed and in none of the cases pharmaceutical exposure posed a high risk (RQ $<$ 1) neither through dermal nor through ingestion exposure pathways. RQs were highest for the landscape worker followed by children playing in green areas and then adult using the athletic fields and golf courses irrigated by treated wastewater.

8 Future Perspectives in Environmental Risk Assessment of PhACs

8.1 Emphasis on Environmental Mixtures

Up to now, the risk assessment of chemicals mainly relies on the assessment of individual chemicals (Fig. 2). Methodologies and guidance for assessing risks from combined exposure to multiple chemicals have been developed for different

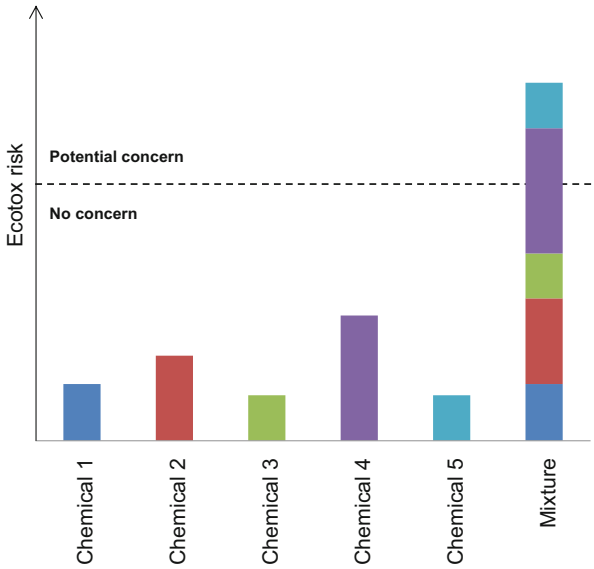


Fig. 2 Single compound vs. mixture risk, assuming concentration addition (CA) joint action. Even though every single component in the mixture is below the safety risk threshold, it may be exceeded by the whole mixture (Figure adapted from [52])

regulatory sectors, however, a harmonized, consistent approach for performing mixture risk assessments and management across different regulatory sectors is still lacking. Recent EU funded projects, such as SOLUTIONS (Grant no. 603437-2) proposed and developed an advanced methodological framework for identifying both priority pollutants and priority mixtures of chemicals posing the risk in European freshwaters [53]. The proposed framework aims at integrating different lines of evidence (LOE) regarding mixture risks. This encompasses evidence from different sources, namely, (1) ecological monitoring (field observations on so-called BQEs), (2) effect-based monitoring (in vitro or in vivo testing in the lab or on-site), (3) chemical monitoring in combination with component-based mixture risk assessment approaches, and (4) integrated modeling of co-exposure and resulting mixture risks. Where one or more lines of evidence identify groups of chemicals presenting a significant risk, these should be subject to prioritization for risk reduction measures. The above concerns strongly advocate for new legislation focusing on environmental mixtures rather than on single compounds [52].

8.2 Bioanalytical Tools to Capture Mixture Effects

Conventional ecotoxicological approaches make use of whole organism in in vivo bioassays to assess the toxicity of wastewater effluents. However, such bioassays exhibited some important drawbacks [14], as they are: (1) a limited sample throughput processing capability; (2) the inability to distinguish the effects of pollutants from those of the matrix (i.e., salinity, pH, etc.), and (3) they make unnecessary use

of living organisms. This limitation was overcome with the development of cell-based in vitro bioassays with high-throughput processing capacity [54]. The application of such in vitro bioassays in ecological risk assessment is just at its beginnings but appears a promising tool for routine environmental surveys and reduces animal testing [55].

8.3 Toxicogenomics

The interrelation between chemical exposure (chemosphere) with the surrounding living organisms (ecosphere) and their adverse biological effects at different levels of biological complexity (including humans) compose altogether a chain of events known in the current environmental toxicology literature as Adverse Outcome Pathways (AOP) [56–58]. The investigation of the effects of the totality of exposure on all levels of biological organization and complexity, from the ecosystems to human health (i.e. the processes involved across the AOP chain) appears crucial.

Exposure and adverse effects can be regarded as the two extremes of the AOP causal chain of events [56, 58]. Whole organism assays provide useful information on adverse effects, but they cannot allow establishing a causal link between exposure and effects. Effect-driven cell assays can link exposure and effects but for a limited number of toxicological mechanisms and they also suffer from false positive or negative results. Alternatively, toxicogenomics – the combination of omic/system biology technologies with toxicity assays – offers the possibility to assess expected and unknown molecular toxicity mechanisms of individual and more complex chemical mixtures in exposed whole organisms or cells [59]. Furthermore, by combining toxicogenomics with AOPs it is feasible to progress on establishing causal links between exposure and adverse effects [60].

8.4 Antibiotic Resistance

Antibiotics were identified as the main contributors to the high environmental risk of wastewater effluents and ciprofloxacin, sulfamethoxazole, clarithromycin, and erythromycin are pointed out in several of the studies reviewed in this chapter as those posing a high risk for the environment (Table 1). In addition, antibiotics is the therapeutic group that provokes the greatest concern among pharmaceuticals due to their potential contribution to the development and spread of antibiotic resistance. Overuse and misuse of antibiotics has led to the emergence of antibiotic resistance bacteria, compromising the effectiveness of antimicrobial therapy since the infectious organisms are becoming resistant to commonly prescribed antibiotics. Emergence and spread of antibiotic resistance bacteria have indeed been classified by the World Health Organization [61] as one of the biggest threats to public health in the twenty-first century. In this line, the capacity of antibiotics to promote antimicrobial

resistance spread has been considered in some ERA studies of antibiotics by using specific PNEC related to antibiotic resistance selection. [62, 63]. This approach was recently applied by Kairigo et al. [26] and Rodriguez-Mozaz et al. [41] to evaluate the risk posed by antibiotics present in wastewater effluents in the aquatic environment.

References

1. Kümmerer K, Clark JH, Zuin VG (2020) Rethinking chemistry for a circular economy. Science 367(6476):369–370. https://doi.org/10.1126/science.aba4979
2. Vermeulen R, Schymanski EL, Barabási A-L, Miller GW (2020) The exposome and health: where chemistry meets biology. Science 367:392–396
3. Lee D, Choi K (2019) Comparison of regulatory frameworks of environmental risk assessments for human pharmaceuticals in EU, USA, and Canada. Sci Total Environ 671:1026–1035
4. Walter S, Mitkidis K (2018) The risk assessment of Pharmaceuticals in the Environment: EU and US regulatory approach. Eur J Risk Regul 9:527–547
5. EMA (2018) Guideline on the environmental risk assessment of medicinal products for human use (draft). https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1_en.pdf. Accessed 20 Mar 2019
6. European Commission (2019) European Union strategic approach to pharmaceuticals in the environment. Brussels, 11.3.2019 COM(2019) 128 final
7. Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under document C(2015) 1756) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2015.078.01.0040.01.ENG
8. Commission Implementing Decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Implementing Decision (EU) 2015/495 (notified under document C(2018) 3362) https://eur-lex.europa.eu/eli/dec_impl/2018/840/oj
9. Commission Implementing Decision (EU) 2020/1161 of 4 August 2020 Establishing a watch list of substances for union-wide monitoring in the field of water policy pursuant to directive 2008/105/EC of the European Parliament and of the council. Off J Eur Union L 257 (2020):32–35. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2020.257.01.0032.01.ENG&toc=OJ:L:2020:257:TOC
10. Guillén D, Ginebreda A, Farré M, Darbra RM, Petrovic M, Barceló D (2012) Prioritization of chemicals in the aquatic environment analytical, modeling and regulatory perspective. Sci Total Environ 440:236–252
11. Sprague JB (1970) Measurement of pollutant toxicity to fish, II-utilizing and applying bioassay results. Water Res 4:3–32
12. Kuzmanovic M, Banjac Z, Ginebreda A, Petrovic M, Barcelo D (2013) Prioritization: selection of environmentally occurring pharmaceuticals to be monitored. Analysis, removal, effects and risk of pharmaceuticals in the water cycle occurrence and transformation in the environment. In: Petrovic M, Barcelo D, Pérez S (eds) Comprehensive analytical chemistry, vol 62, pp 71–90
13. Bopp SK, Barouki R, Brack W, Dalla Costa S, Dorne J-LCM, Drakvik PE, Faust M, Karjalainen TK, Kephalopoulos S, van Klaveren J, Kolossa-Gehring M, Kortenkamp A, Lettieri T, Nørager S, Rüegg J, Tarazona JV, van de Water B, van Gils J, Bergman A,

Lebret E, Trier X (2018) Current EU research activities on combined exposure to multiple chemicals. Environ Int 120:544–562
14. Escher BI, Stapleton HM, Schymanski EL (2020) Tracking complex mixtures of chemicals in our changing environment. Science 367:388–392
15. Boobis A, Budinsky R, Collie S, Crofton K, Embry M, Felter S, Hertzberg R, Kopp D, Mihlan G, Mumtaz M, Price P, Solomon K, Teuschler L, Yang R, Zaleski R (2011) Critical analysis of literature on low-dose synergy for use in screening chemical mixtures for risk assessment. Crit Rev Toxicol 41:369–383
16. Cedergreen N (2014) Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. PLoS One 9(5):e96580
17. Guedes-Alonso R, Montesdeoca-Esponda S, Herrera-Melián JA, Rodríguez-Rodríguez R, Ojeda-González Z, Landívar-Andrade V et al (2020) Pharmaceutical and personal care product residues in a macrophyte pond-constructed wetland treating wastewater from a university campus: presence, removal and ecological risk assessment. Sci Total Environ 703:135596
18. Lucas D, Barceló D, Rodriguez-Mozaz S (2016) Removal of pharmaceuticals from wastewater by fungal treatment and reduction of hazard quotients. Sci Total Environ 571:909–915
19. Afonso-Olivares C, Sosa-Ferrera Z, Santana-Rodríguez JJ (2017) Occurrence and environmental impact of pharmaceutical residues from conventional and natural wastewater treatment plants in gran Canaria (Spain). Sci Total Environ 599-600:934–943
20. Díaz-Garduño B, Pintado-Herrera MG, Biel-Maeso M, Rueda-Márquez JJ, Lara-Martín PA, Perales JA et al (2017) Environmental risk assessment of effluents as a whole emerging contaminant: efficiency of alternative tertiary treatments for wastewater depuration. Water Res 119:136–149
21. Auvinen H, Gebhardt W, Linnemann V, Du Laing G, Rousseau DPL (2017) Laboratory- and full-scale studies on the removal of pharmaceuticals in an aerated constructed wetland: effects of aeration and hydraulic retention time on the removal efficiency and assessment of the aquatic risk. Water Sci Technol 76(6):1457–1465
22. Ben W, Zhu B, Yuan X, Zhang Y, Yang M, Qiang Z (2018) Occurrence, removal and risk of organic micropollutants in wastewater treatment plants across China: comparison of wastewater treatment processes. Water Res 130:38–46
23. Kosma CI, Lambropoulou DA, Albanis TA (2014) Investigation of PPCPs in wastewater treatment plants in Greece: occurrence, removal and environmental risk assessment. Sci Total Environ 466-467:421–438
24. Archer E, Petrie B, Kasprzyk-Hordern B, Wolfaardt GM (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
25. Gros M, Petrović M, Ginebreda A, Barceló D (2010) Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ Int 36(1):15–26
26. Kairigo P, Ngumba E, Sundberg LR, Gachanja A, Tuhkanen T (2020) Occurrence of antibiotics and risk of antibiotic resistance evolution in selected Kenyan wastewaters, surface waters and sediments. Sci Tot Environ 720
27. Mirzaei R, Mesdaghinia A, Hoseini SS, Yunesian M (2019) Antibiotics in urban wastewater and rivers of Tehran, Iran: consumption, mass load, occurrence, and ecological risk. Chemosphere 221:55–66
28. Pereira AMPT, Silva LJG, Meisel LM, Lino CM, Pena A (2015) Environmental impact of pharmaceuticals from Portuguese wastewaters: geographical and seasonal occurrence, removal and risk assessment. Environ Res 136:108–119
29. Huber S, Remberger M, Kaj L, Schlabach M, Jörundsdóttir HT, Vester J et al (2016) A first screening and risk assessment of pharmaceuticals and additives in personal care products in waste water, sludge, recipient water and sediment from Faroe Islands, Iceland and Greenland. Sci Total Environ 562:13–25

30. Aydin S, Aydin ME, Ulvi A, Kilic H (2019) Antibiotics in HOSPITAL effluents: occurrence, contribution to urban wastewater, removal in a wastewater treatment plant, and environmental risk assessment. Environ Sci Pollut Res 26(1):544–558
31. Afsa S, Hamden K, Lara Martin PA, Mansour HB (2020) Occurrence of 40 pharmaceutically active compounds in HOSPITAL and urban wastewaters and their contribution to Mahdia coastal seawater contamination. Environ Sci Pollut Res 27(2):1941–1955
32. Biel-Maeso M, Baena-Nogueras RM, Corada-Fernández C, Lara-Martín PA (2018) Occurrence, distribution and environmental risk of pharmaceutically active compounds (PhACs) in coastal and ocean waters from the Gulf of Cadiz (SW Spain). Sci Total Environ 612:649–659
33. Zhang X, Zhao H, Du J, Qu Y, Shen C, Tan F et al (2017) Occurrence, removal, and risk assessment of antibiotics in 12 wastewater treatment plants from Dalian, China. Environ Sci Pollut Res 24(19):16478–16487
34. Sörengård M, Campos-Pereira H, Ullberg M, Lai FY, Golovko O, Ahrens L (2019) Mass loads, source apportionment, and risk estimation of organic micropollutants from HOSPITAL and municipal wastewater in recipient catchments. Chemosphere 234:931–941
35. Pivetta RC, Rodrigues-Silva C, Ribeiro AR, Rath S (2020) Tracking the occurrence of psychotropic pharmaceuticals in Brazilian wastewater treatment plants and surface water, with assessment of environmental risks. Sci Total Environ 727:138661
36. Badia-Fabregat M, Lucas D, Gros M, Rodríguez-Mozaz S, Barceló D, Caminal G, Vicent T (2015) Identification of some factors affecting pharmaceutical active compounds (PhACs) removal in real wastewater. Case study of fungal treatment of reverse osmosis concentrate. J Hazard Mater 283:663–671
37. Verlicchi P, Al Aukidy M, Galletti A, Petrovic M, Barceló D (2012) HOSPITAL effluent: investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. Sci Total Environ 430:109–118
38. Ma XY, Li Q, Wang XC, Wang Y, Wang D, Ngo HH (2018) Micropollutants removal and health risk reduction in a water reclamation and ecological reuse system. Water Res 138:272–281
39. U.S. EPA (1989) Risk assessment guidance for superfund volume I human health evaluation manual (part A)
40. Ramírez-Morales D, Masís-Mora M, Montiel-Mora JR, Cambronero-Heinrichs JC, Briceño-Guevara S, Rojas-Sánchez CE, Méndez-Rivera M, Arias-Mora V, Tormo-Budowski R, Brenes-Alfaro L, Rodríguez-Rodríguez CE (2020) Occurrence of pharmaceuticals, hazard assessment and ecotoxicological evaluation of wastewater treatment plants in Costa Rica. Sci Total Environ 746:141200
41. Rodriguez-Mozaz S et al (2020) Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment. Environ Int 140:105733
42. Keller VDJ, Williams RJ, Lofthouse C, Johnson AC (2014) Worldwide estimation of river concentrations of any chemical originating from sewage treatment plants using dilution factors. Environ Toxicol Chem 33(2):447–452
43. Kelessidis A, Stasinakis AS (2012) Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste Manag 32(6):1186–1195
44. Verlicchi P, Zambello E (2015) Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence and environmental risk in the case of application on soil - a critical review. Sci Total Environ 538:750–767
45. Martín J, Camacho-Muñoz MD, Santos JL, Aparicio I, Alonso E (2012) Distribution and temporal evolution of pharmaceutically active compounds alongside sewage sludge treatment. Risk assessment of sludge application onto soils. J Environ Manag 102:18–25
46. Semerjian L, Shanableh A, Semreen MH, Samarai M (2018) Human health risk assessment of pharmaceuticals in treated wastewater reused for non-potable applications in Sharjah, United Arab Emirates. Environ Int 121:325–331

47. Liu X, Liang C, Liu X, Zhao F, Han C (2020) Occurrence and human health risk assessment of pharmaceuticals and personal care products in real agricultural systems with long-term reclaimed wastewater irrigation in Beijing, China. Ecotoxicol Environ Saf 190:110022
48. Cramer GM, Ford RA, Hall RL (1978) Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol 16(3):255–276
49. Tomer M, Yehoshua M, Galit T, Moshe S, Benny C (2014) Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. Environ Sci Technol 48(16):9325–9333
50. Prosser RS, Sibley PK (2015) Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation. Environ Int 75:223–233
51. Malchi T, Maor Y, Tadmor G, Shenker M, Chefetz B (2014) Irrigation of root vegetables with treated wastewater: evaluating uptake of pharmaceuticals and the associated human health risks. Environ Sci Technol 48(16):9325–9333
52. Kortenkamp A, Faust M (2018) Regulate to reduce chemical mixture risk. Science 361 (6399):224–226
53. Faust M, Backhaus T, Altenburger R, Dulio V, van Gils J, Ginebreda A, Kortenkamp A, Munthe J, Posthuma L, Slobodnik J, Tollefsen KE, van Wezel A, Brack W (2019) Prioritisation of water pollutants: the EU Project SOLUTIONS proposes a methodological framework for the integration of mixture risk assessments into prioritisation procedures under the European Water Framework Directive. Environ Sci Eur 31:66
54. Attene-Ramos MS, Miller N, Huang R, Michael S, Robert M, Kavlock J, Austin CP, Shinn P, Simeonov A, Tice RR, Xia M (2013) The Tox21 robotic platform for the assessment of environmental chemicals – from vision to reality. Drug Discov Today 18:716–723
55. Villeneuve DL, Coady K, Escher BI, Mihaich E, Murphy CA, Schlekat T, Garcia-Reyero N (2019) High-throughput screening and environmental risk assessment: state of the science and emerging applications. Environ Toxicol Chem 38:12–26
56. Edwards SW, Tan Y-M, Villeneuve DL, Meek M, McQueen CA (2016) Adverse outcome pathways – organizing toxicological information to improve decision making. J Pharmacol Exp Ther 356(1):170–181
57. Escher BI, Hackermüller J, Polte T, Scholz S, Aigner A, Altenburger R, Böhme A, Bopp SK, Brack W, Busch W (2017) From the exposome to mechanistic understanding of chemical-induced adverse effects. Environ Int 99:97–106
58. Groh KJ, Carvalho RN, Chipman JK, Denslow ND, Halder M, Murphy CA, Roelofs D, Rolaki A, Schirmer K, Watanabe KH (2015) Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: I. Challenges and research needs in ecotoxicology. Chemosphere 120:764–777
59. Waters MD, Fostel JM (2004) Toxicogenomics and systems toxicology: aims and prospects. Nat Rev Genet 5(12):936
60. Ortiz-Villanueva E, Navarro-Martín L, Jaumot J, Benavente F, Sanz-Nebot V, Piña B, Tauler R (2017) Metabolic disruption of zebrafish (Danio rerio) embryos by bisphenol A. An integrated metabolomic and transcriptomic approach. Environ Pollut 231:22–36
61. WHO (2016) Antimicrobial resistance: global report on surveillance 2014. WHO
62. Bengtsson-Palme J, Larsson DGJ (2016) Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environ Int 86:140–149
63. Tell J, Caldwell DJ, Häner A, Hellstern J, Hoeger B, Journel R et al (2019) Science-based targets for antibiotics in receiving waters from pharmaceutical manufacturing operations. Integr Environ Assess Manag 15(3):312–319

Fate and Removal of Pharmaceuticals in CAS for Water and Sewage Sludge Reuse

Marc Castaño-Trias, Monica Brienza, Maria Concetta Tomei, and Gianluigi Buttiglieri

Contents

Abstract Among organic micropollutants, pharmaceutical active compounds (PhACs) have been extensively investigated in the last decades due to their potential adverse ecological and/or human health effects. Wastewater treatment plants are one of the first barriers in preventing them from reaching sensible ecosystems. In

M. Castaño-Trias and G. Buttiglieri (✉)
Catalan Institute for Water Research (ICRA), Girona, Spain

Universitat de Girona, Girona, Spain
e-mail: mcastano@icra.cat; gbuttiglieri@icra.cat

M. Brienza
Department of Science, University of Basilicata, Potenza, Italy
e-mail: monica.brienza@unibas.it

M. C. Tomei
Water Research Institute, C.N.R., Rome, Italy
e-mail: tomei@irsa.cnr.it

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 23–52, DOI 10.1007/698_2020_666,

particular, conventional activated sludge (CAS) systems may be only partially effective in PhAC removal or degradation. The present work presents an overview of the fate of PhACs in CAS. Several parameters and constraints affecting PhAC removal are given and discussed including the physicochemical properties of the compounds and the operating parameters like the influent concentration, its seasonal variation, CAS hydraulic retention time and sludge retention time. A detailed data analysis is dedicated to selected representative compounds (i.e. carbamazepine, ibuprofen and sulfamethoxazole) in the water line of CAS systems, while for the sludge line, different treatment options are discussed, with special focus on the sequential anaerobic-aerobic digestion, a promising alternative to improve the removal of organic micropollutants, including PhACs, in the sludge matrix. Finally, the criteria for safe reuse of treated effluent and agricultural application of the produced sludge, related to PhACs, are discussed and analysed in the frame of EU and other regulations.

Keywords HRT, Regulation, Seasonal variability, Sludge treatment, SRT, Transformation products

1 Introduction

The presence of xenobiotics in the aquatic environment has raised environmental concern due to their high toxicity, at times even at low concentrations. These so-called micropollutants have been detected in all water matrices, and their removal in wastewater treatment plants (WWTPs) is known to be incomplete and challenging for several classes of compounds.

The focus of environmental research has extended in the last two decades beyond more established and known wastewater xenobiotics, such as some polychlorinated biphenyls, dioxins and pesticides, towards other classes of emerging organic micropollutants. Among the latter ones, pharmaceutical active compounds (PhACs) are of great interest, and they are getting more and more attention in the literature, as it can be seen in *bibliometric studies*. To give a figure of the relevance of these compounds, it is worth noting that in the European Union (EU), around 3,000 different PhACs are being used in medicine such as painkillers, antibiotics, contraceptives, beta-blockers, lipid regulators, tranquilizers and impotence drugs [1]. During and after medical treatment, humans and animals excrete a combination of intact and metabolized PhACs. Kümmerer and Henninger, for instance, reported that the metabolization rate of all antibiotics used in Germany was estimated to be around 30% [2]. In other words, approximately 70% of the total amount is excreted unchanged into raw wastewater. PhACs are continuously entering the environment via non-point sources (i.e. run-off or drainage from land surfaces) but mainly via point sources (i.e. both municipal and pharmaceutical factory WWTPs). Their

harmful effects on the ecosystem and on the human health are well recognized and currently under extensive evaluation [3]. To this aim, the *characterization of WWTPs* in terms of PhAC concentration is mandatory.

Many advanced and more efficient wastewater treatment schemes are being proposed and tested, even at full scale. Nonetheless, the most common WWTPs in Europe, and worldwide, are anyway still based on *conventional activated sludge* (CAS) *systems*, to be noted that CAS are usually designed to remove organic carbon and nutrient loads but, generally, they are not designed to remove trace organics [4]. Consequently, the operative parameters that most affect *PhAC removal in CAS* need to be evaluated in order to improve the plant performance. Three main removal pathways are usually accounted in CAS: microbial processes (biodegradation, either metabolic or co-metabolic), sorption onto sludge flocs and volatilization (mainly during aeration). However, volatilization can be considered negligible for most PhACs, because of Henry's constant value of such molecules [5]. It must be generally remarked that extremely variable removal was reported in CAS treatments, even for the same compound. For instance, removal rates in the liquid fraction from 17% [6] to 89% [7] were reported for sulfamethoxazole (SMX), from 12% [8] to 80% [9] for tetracycline (TC), and from 4.3% to 72% [6] for erythromycin-H_2O. Other compounds, conversely, are usually reported as more recalcitrant: for example, less than 30% removal for carbamazepine (CBZ) [10] and 17% for lincomycin (LCM) [11]. Persistence has often been linked to their molecular properties, but also to process-specific factors such as sludge retention time (SRT), or hydraulic retention time (HRT), biomass concentration, cation-exchange properties as well as seasonal parameters such as temperature, precipitation rate and solar radiation [12].

For a comprehensive analysis of the fate of PhACs, it is vital to consider not only the parent compounds, or the metabolites that can be excreted, but also the so-called transformation products (TPs), generated from biotic/non-biotic processes. TPs and parent compounds in CAS can behave with a different pattern [13], and TPs can be less biodegradable, more toxic and inhibitory compared to the parent compound. Even though more and more information is now available on the occurrence and fate of TPs of PhACs in WWTPs, there are still many knowledge gaps on TP occurrence, fate, exposure, toxicity and risks.

PhACs that are inefficiently or not adequately eliminated may pass through CAS biological treatment processes. Consequently, they may enter the receiving waters as dissolved pollutants, via the WWTP effluent. Moreover, *water reuse*, e.g. agriculture reuse of treated wastewater, may be applied, also based on the recent EU law on minimum requirements for water reuse and/or other legislations and/or individual national regulations [14].

In parallel, also PhAC concentration in the solid phase should be considered. Pharmaceutical residues are, in fact, frequently detected in biosolids at μg kg^{-1} to mg kg^{-1} levels. For example, an average concentration of 68 μg kg^{-1} dry weight (dw) for SMX in CAS was reported by Göbel et al. [15], and about 15 μg kg^{-1} dw for TC in biosolids was shown by Spongberg and Witter [8]. Different *sludge treatment alternatives* are available. Among them, anaerobic digestion treatment

has long been practiced and commonly applied as a renewable energy source in integrated waste management, but other ones may be available. Treated sludge may be used in agriculture, and there may be health constraints on the agricultural recycling of wastewater sludge [16]. Quite extensive information is available in terms of metals in sludge and sludge treatments. Less information is available in terms of micropollutants, and in particular of PhACs, thus requiring much more insights.

2 Bibliometric Study Regarding Micropollutants and PhACs

The interest of the scientific community on PhACs and the corresponding studies have been increasing in the last decades. In order to quantify the evolution and the past and current trends of investigations on the occurrence of pharmaceuticals compounds in wastewater, a bibliometric study has been carried out on Scopus database. This way both historical progress and quantitative trends of research publications on a specific topic can be effectively considered [17].

The analysis has been structured as follows: first, identification of research historical trends on micropollutants, analyses, fate and removal; second, country-specific distribution of paper productivity; and third, definition of most evaluated PhAC therapeutic classes.

The first analysis was performed in *SCOPUS* searching in the string "micropollutants" *OR* "emerging pollutants" *OR* "trace pollutants", with the applied filters "Chemistry" *OR* "Environmental Science" *OR* "Chemical Engineering", between 1980 and 2019. A total of 17,780 publications, in the whole period 1980–present, related to the micropollutants were identified, and the number of publications per year over the past 30 years is displayed in Fig. 1. Application of tandem mass analysers, in fact, started in the late 1970s, and they spotted a new possible challenge and corresponding treat for human health and the environment: the environmental occurrence of micropollutants, including PhACs.

In the decade 1980–1989, few publications over the total, only 576, are reported, also due to limitations in full commercialization of MS/MS equipment for micropollutants analyses. Subsequently, the increase in the attention of the research community was more and more sustained, and, for example, 266 research papers were remarkably published only in the year 2000. Then, 1,112 articles per year were published, on average, in the period 2010 to 2019. Most of the compounds themselves are not new in environmental matrices. Nonetheless, the increase in the number of publications is intrinsically linked to the extreme increase in the sensitivity provided by new analytical instruments and tools.

The second search aimed to evaluate if the paper title, abstract or keywords included also the words "fate", *OR* "monitoring", *OR* "removal", *OR* "analyses" *OR* "occurrence" that can be related, from different perspectives, to studies on

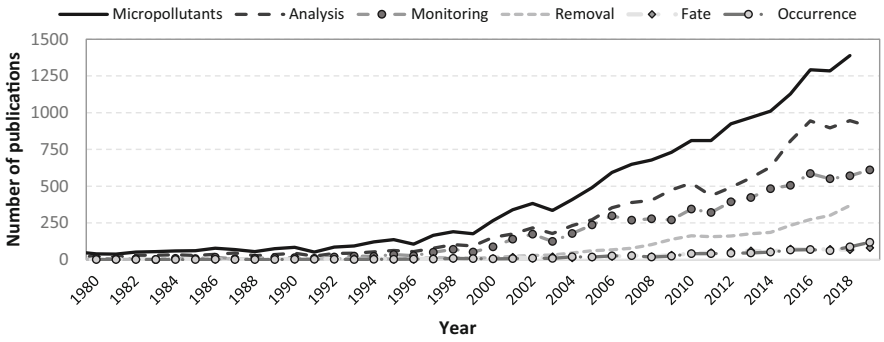


Fig. 1 Bibliometric search of publications about micropollutants and analysis, or monitoring, or removal, or fate or occurrence using *SCOPUS*. The series "micropollutants" was obtained searching in Title-abstract-keywords for the string "micropollutants" *OR* "trace pollutants" *OR* "emerging pollutants". The other series were obtained adding to the previous one the string *AND* "monitoring" *OR* "analyses" *OR* "removal" *OR* "occurrence" *OR* "fate". In all the series, also the filters "Chemistry" *OR* "Environmental Science" *OR* "Chemical Engineering" *AND* "PUBYEAR>1980" to "present" were added

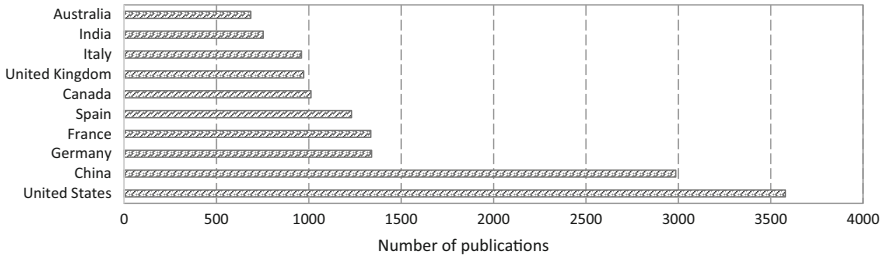


Fig. 2 Bibliometric search of number of publications on micropollutants, per country. The string is the same as the "micropollutants" series of the previous figure

micropollutants (Fig. 1). In 1980, only 1 publication was dealing with "fate", 2 with "removal" and 8 and 19 publications with "monitoring" and "analysis", respectively. A considerable increase can be observed, also in this case, in the last 20 years. In 2019 the published papers were 906, 611, 406, 118 and 81 for "analysis", "monitoring", "removal", "occurrence" and "fate", respectively. The increasing trends are similar, even though at different levels, demonstrating the interest of the scientific community in this topic, in particular as regards "analysis" and "monitoring". It might be related, on one hand, to the development of new methods of analysis and, on the other hand, to the research demand of reliable and accurate analytical and monitoring tools to evaluate contaminant removal and fate.

A complementary bibliometric search was performed to determine which country was leading the investigation. The result shows in Fig. 2 that the USA and China

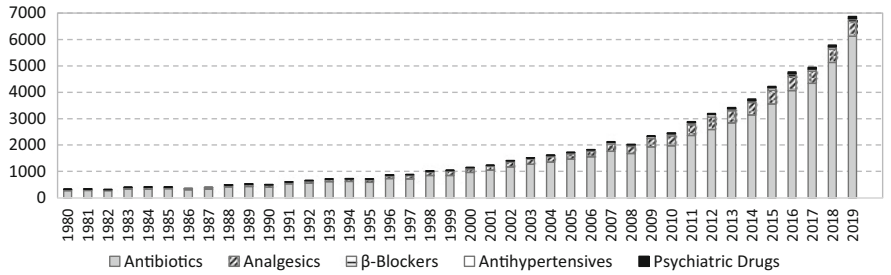


Fig. 3 Bibliometric search of publications about different pharmaceutical therapeutic classes using *SCOPUS* with the string "antibiotics" *OR* "analgesics" *OR* "β-blockers" *OR* "antihypertensives" or "psychiatric drugs" in Title-abstract-keywords with the filters Chemistry *OR* Environmental Science *OR* Chemical Engineering *AND* "*PUBYEAR*>1980" to "present"

have the highest research outputs on the topic on micropollutants with around 3000/3500 publications. Afterwards, Germany, France and Spain are the other countries in the top five. A noticeable difference is spotted between the first two and the other ones, with German manuscript production being only 37% and 45% with respect to the USA and China, while smaller difference is shown among the other countries, from Germany to Australia. Finally, five out of the ten countries with more publication on this topic are EU countries (39% of the total production, Fig. 2).

An even larger amount of studies is available in the literature reporting PhACs in their title, keywords, or abstract. Around 4,000 manuscripts were published in the decade 1980–1989 and more than 43,000 papers only in the last decade (Fig. 3), with an increasing trend comparable to previous Figures.

PhACs can be classified based on their therapeutic class. Among them, *antibiotics* are applied to stop bacterial growth in an organism (e.g. ciprofloxacin or amoxicillin); *analgesics* (usually extremely highly consumed) to relieve pain without affecting the conduction of nerve impulses, sensory perception or consciousness (e.g. naproxen); *β-blocking agents* to control abnormal heart beating rhythms and to control blood pressure (blocking epinephrine hormone, e.g. metoprolol, atenolol or sotalol); *antihypertensives* to prevent, control or treat hypertension (e.g. losartan); and *psychiatric drugs* to generate a change on consumers' mind, emotions and behaviours (to treat mental health diseases, e.g. venlafaxine to treat depression).

Bibliometric search highlights that antibiotics, out of the above-indicated categories, are the group with more scientific publications (from 3,301 in the decade 1980–1989 to 36,111 in the decade 2010–2019) with a percentage of around 83% over the total (Fig. 3). This large attention is also related to the thread that continuous exposure to antibiotics may spread antibiotic resistance in the environment and new antibiotics are eventually to be developed because old ones are not effective anymore [18].

3 Influent PhAC Characterization in Municipal WWTP

The development of effective strategies to remove organic micropollutants, and PhACs among them, from wastewater is critical. Whatever the applied strategy is, first it is mandatory to identify which compounds can be found in the influent, at which concentration they are and if significant variabilities are to be accounted for. The literature regarding PhACs in municipal WWTP influents is quite extensive. The data reported and treated in this section refer mainly to full-bodied reviews considering a large number of compounds and WWTPs (e.g. [9, 19–21]).

Among the PhACs, 18 compounds have been selected here, as representatives of different therapeutic classes, and their occurrences are compared. It is well known and reported that the concentration of these compounds in influent wastewater usually has a huge variability, as it can be observed in Fig. 4.

The mean concentration of these compounds in influent wastewater may be roughly calculated around 1 μg/L but heavily depending on the compound, the country and the WWTP. For most compounds, in fact, many orders of magnitude of difference are reported in real influent wastewater (e.g. ibuprofen can be found between 28 ng/L [21] and 372,000 ng/L [20]). Minimum and maximum concentrations may be representative of extremely low or high occurrence of these compounds in influent WWTP. Nonetheless, it is apparent that huge variability must be considered when evaluating the presence of PhACs in WWTPs.

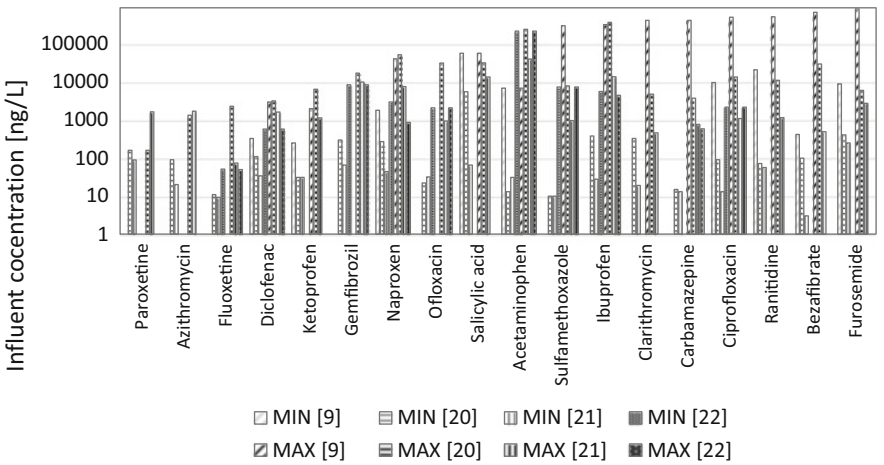


Fig. 4 Representation of the minimum and maximum influent concentrations to the WWTP included in [9, 20–22]

3.1 Seasonal Variability in Influent WWTP PhAC Occurrence

Seasonal/temporal variability is among the main variables that must be considered when evaluating micropollutant WWTP influent content. Some compounds may be present or not, based on the considered WWTP and/or season. For example, UV filter compounds are representative of extremely seasonal organic micropollutants because their use increases in the high touristic season [23]. Illicit drug use is reported to increase during the weekend, festivals and public holidays [24].

As regards PhACs, relevant seasonal variations were also reported for some therapeutic classes of compounds (i.e. analgesics/anti-inflammatory compounds or histamine receptor antagonists in the flowering period) based on their consumption [25]. Three representative PhACs of different therapeutic classes (the psychiatric drug venlafaxine, the analgesic ibuprofen and the antibiotic sulfamethoxazole) are here evaluated in more detail, reporting the average of WWTP influent PhAC concentrations in sampling campaigns performed in different seasons (Fig. 5; [21], info from ten papers).

Ibuprofen was confirmed as the compound reported at the highest concentration throughout the year. Moreover, a large seasonal variability was also confirmed between the winter-spring and the summer-autumn periods (on average 20,045 ng/L and 7,979 ng/L, respectively). Much smaller seasonal variability can be observed for the other two compounds, even though the minimum for both compounds was reported in autumn and the maximum in winter.

A strong seasonal effect can be confirmed also considering a larger list of 61 PhACs, from different therapeutic classes. It was confirmed, similarly to ibuprofen, a larger concentration in winter-spring than in summer-autumn (on average 181,311 ng/L and 62,256 ng/L, respectively), as it is visible in Fig. 5b. Likewise, the season with higher total PhAC concentrations was winter. Moreover, it is apparent that the analgesics are the analysed therapeutic class that most contribute to the total

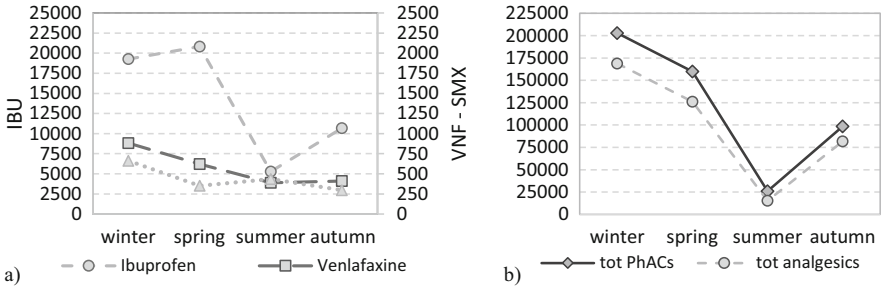


Fig. 5 (**a**) Average concentration, in ng/L, of three selected PhACs (IBU, ibuprofen; VNF, venlafaxine; SMX, sulfamethoxazole) throughout the year. (**b**) Total average concentrations of 61 PhACs and total average of analgesics, in ng/L. The reported data are obtained as average of ten papers [21]

influent PhAC concentration, with a much smaller contribution in summer (i.e. 83%, 79%, 59% and 83% of analgesics over the total PhAC concentration in winter, spring, summer and autumn, respectively). That the differences in season temperature may affect influent WWTP PhAC content for different reasons: from one side the consumption may change remarkably based on the season and the countries, and from the other one, higher temperature may lead to higher removal even in the sewer systems, before entering the WWTP [26].

4 Pharmaceutical Removal in Conventional Activated Sludge Systems

WWTPs were first designed only for biological oxygen demand removal. Through the decades they were upgraded with chemical phosphate precipitation, to reduce the phosphorus load discharge, to convert ammonia to nitrate and to partially convert nitrate to molecular nitrogen. CAS systems are still the most commonly applied in the world, but they are not designed to remove organic micropollutants, including PhACs [4]. Nonetheless, a full understanding of removal mechanisms can upgrade CAS capabilities and possibly reduce additional/alternative treatments and their cost of application.

PhACs comprehend an extremely large range of compounds with different chemical structures, persistence and effects in the environment. The most evaluated PhAC therapeutic classes in terms of occurrence and removal are usually the antibiotics, also due to their possible consequences in the environment, and the nonsteroidal anti-inflammatory drugs (NSAIDs), due to their high consumption [27]. These PhAC therapeutic classes exemplify different, and sometimes complementary, needs in terms of removal evaluation in treatment technologies. Some PhACs, in fact, may have influent WWTP concentrations lower than other compounds but with higher demonstrated or suspected effects on the environment [18] and/or more recalcitrant (e.g. some antibiotics). Some other PhACs, on the other hand, may usually exhibit higher removal rates, but as they have higher influent and effluent concentrations, they continuously enter the environment, e.g. NSAIDs [28]. Both classes of compounds, and the corresponding behaviours, are relevant. The topic of PhACs is clearly a remarkably interdisciplinary research area. The topic of PhACs is clearly a remarkably interdisciplinary research area *AND* IT poses obvious challenges for researchers across numerous disciplines (i.e. ecotoxicology, antimicrobial resistance genes, bioaccumulation, etc.) which are required in this field [29].

In the literature, there are several reviews presenting in detail and comparing the efficiency of CAS and of several advanced treatment technologies as regards the removal of several PhACs [30], among them the recent detailed review of Patel et al. [9] and Verlicchi et al. [20]. Analgesics and anti-inflammatory drug (e.g. naproxen, ibuprofen and acetaminophen) removal is generally high (over 75%) even though in some cases also lower removal rates are reported [9]. Among the broadly studied

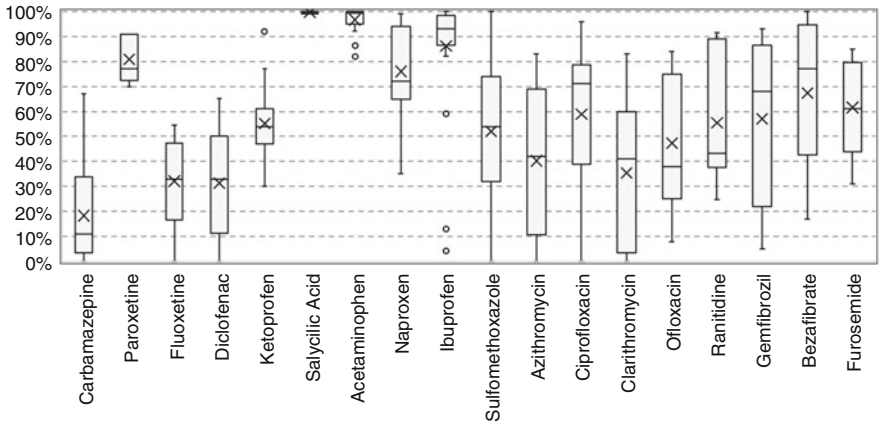


Fig. 6 Representation of reported PhAC removal in CAS (data from [9, 20, 36–38])

class of antibiotics, fluoroquinolones are a subclass of the most frequently investigated compounds. Similar results are reported about the removal rate of ciprofloxacin and ofloxacin in different countries such as Sweden [31], Italy [32], Finland [33] and Switzerland [34], with a higher percentage for ciprofloxacin than ofloxacin. The fluoroquinolone removal rate in CAS is associated also with the sorption on activated sludge, due to the relatively significant measured amount of these compounds in sludge, as reported by various authors [34, 35]. Other compounds, finally, are characterized by inconsistent results in terms of occurrence and fate like diclofenac, with removal rates ranging between 0% and 90% and, in some cases, with higher effluent concentration, compared with the influent.

In order to collect and combine the information from several reviews at the same time, the data from 40 papers are represented in Fig. 6. As discussed above, a large variability in terms of removal of the considered PhAC compounds can be observed.

4.1 Compound Characteristics and Parameters Affecting PhAC Removal in CAS

The removal of PhACs, like any other pollutant, is directly related to their chemical structure. For example, the presence of chloro, nitro and fluoro functional groups attached to aromatic rings may cause persistence and resistance to removal. On the other hand, electron-withdrawing groups, such as amines or alcohols, may enhance the removal in CAS [39–42].

As previously mentioned, the main removal pathways for PhACs in CAS are microbial processes (biodegradation, either metabolic or co-metabolic) and sorption onto sludge flocs and suspended material, being volatilization usually negligible. As regards sorption, different removal pathways can be observed if the compound has

hydrophobic or hydrophilic molecular structure. High polarity is usually linked to higher water solubility, lowering adsorption, as it is reported for carbamazepine (7–10%). Compounds with medium polarity may have instead higher average removal percentages. As regards degradation, it is reported that PhACs with simpler molecular structure are more prone to biodegradation. Instead, the drugs with saturated aliphatic and aromatic rings (such as iomeprol and diatrizoate) exhibit resistance to degradation in CAS [43].

Nonetheless, whatever the chemical structure and characteristics are, also operative conditions of the chosen treatment technology, in this case CAS, must be considered and carefully evaluated case by case. As detailed in the next paragraph for selected PhACs, in fact, extremely different removal rates have been reported in the literature, even though CAS was the technology of choice in all the cases. A careful evaluation of both aspects, chemical and operative parameters, must be promoted in future studies. Among the main operative conditions and variables that can affect PhAC removal in CAS, there are the sludge retention time (SRT), the hydraulic retention time (HRT), the presence of aerobic/anoxic treatment, the seasonality effects and the influent concentration. These variables may lead to a much wider range of PhAC removals than those foreseen only based on their chemical properties only.

As regards the biology of the activated sludge, one of the most relevant parameters is SRT, representing the mean residence time of microorganisms in CAS systems. Several studies state that high SRT, allowing the development of slow-growing bacteria, is required for PhAC biodegradation/biotransformation. In addition, the enrichment of the microbial culture with new species may favour the development of a wider spectrum of metabolic pathways useful for PhAC degradation. This may explain the higher removal of some PhACs observed in WWTP removing nitrogen (i.e. at higher SRT and including slow-growing autotrophic species like nitrifiers) in comparison to the ones removing only organic matter [44–47]. Concerning the microbial diversity and the SRT effect, three hypotheses have been proposed: (1) microbial diversity is proportional to SRT; so higher SRT is related to higher microbial diversity; (2) activate sludge with high microbial diversity have more functional traits than those with low diversity; (3) high microbial diversity may result in higher PhAC removal potential [48]. It is reported that a SRT of 2–5 days is usually enough for the degradation of sulfamethoxazole, bezafibrate, aspirin and ibuprofen. Conversely, longer SRTs (5–15 days) were required for substantial degradation of iopromide, roxithromycin and diclofenac [49]. Finally, some other PhACs, such as carbamazepine, are usually not affected by SRT [50]. Nevertheless, even though SRT has been reported as influential for PhAC biodegradation, a clear correlation between SRT and biodegradation has not been completely elucidated.

In terms of hydraulics, the HRTs quantify the contact time between the microorganisms and the pollutants during the treatment. As it is for SRT, contrasting results are reported in the literature with similar HRT and other parameters, but dissimilar biodegradation efficiencies [51, 52]. Nonetheless, usually higher HRTs may result in longer contact/reaction time and higher PhAC removals.

Another important parameter that affects the biological activity is the temperature and, consequently, also PhAC degradation. In summer, for example, the increase in the temperature may favour the microbial activity, fasten bacterial metabolism and increase PhAC biodegradation. During the winter, on the other hand, a drop in the removal efficiencies can be sometimes anticipated. For instance, Vieno et al. [12] showed that low removal was most probably linked to the low water temperature in wintertime (~7°C). Adversely, Göbel et al. [46] reported no correlation between the water temperature and the elimination of sulphonamides, macrolides and trimethoprim in the range of 12 to 19°C. These data demonstrate that, as observed for SRT, the temperature effect is compound-specific and not of general validity.

Nonsteroidal anti-inflammatory drugs with low *pK*a, such as clofibric acid and bezafibrate, are ionized to their conjugate base at neutral pH *AND*, *THEREFORE*, they will have a *HIGH* affinity for the aqueous phase, reducing their adsorption on negatively charged sludge surface. *CONVERSELY*, zwitterionic and basic pharmaceuticals (e.g. the fluoroquinolone antibiotics) show significant adsorption [43].

Finally, anaerobic or aerobic conditions will also lead to different results in terms of removal. This factor is particularly critical for some compounds, such as the fluoroquinolone antibiotics [53]. These compounds are better removed under aerobic conditions, while their biodegradation under anoxic environments is almost negligible. This fact can be due to the higher oxidation potential of oxygen, compared to nitrate [43, 53].

4.2 Removal Rates of Selected Representative PhACs

As commented in the previous paragraph, operative parameters such as, HRT, SRT and temperature may affect directly or indirectly PhAC removal. In this paragraph a detailed analysis is reported on the effects of these parameters on the removal of carbamazepine, ibuprofen and sulfamethoxazole.

The psychiatric drug carbamazepine is generally scarcely or not removed in CAS systems (Table 1). This fact can be explained by its structure: the presence of heterocyclic N-containing aromatic rings makes difficult its biodegradation whatever the conditions are [54, 55].

Nonetheless, there are some exceptions (Fig. 7). In some cases, for sampling campaigns performed in winter times, a higher removal (i.e. 35%) was observed even though other parameters like HRT and SRT are in the same range (Fig. 7).

Based on the provided HRT and SRTs, when available, it can be confirmed the recalcitrance of the compound: the increase of HRT or SRT does not affect its removal in CAS (Fig. 8).

Table 1 Removal of carbamazepine in CAS systems

Season	Removal (%)	HRT (h)	SRT (days)	Country	Reference
Winter	−25	9–17	11–15	China	[56]
Winter	0	Non-specified	Non-specified	Italy	[32]
Winter	0	1.5–20	2–20	Finland	[33]
Winter	0	7.3	10	Switzerland	[5]
Winter	15	7.1–9.4	3.8–8.4	Japan	[57]
Winter	60	Non-specified	Non-specified	Spain	[58]
Winter	73	27	Non-specified	Spain	[59]
Winter	95	Non-specified	3–5	Turkey	[60]
Spring/autumn	−28	7.1–9.4	3.8–8.4	Japan	[57]
Spring/autumn	−21	Non-specified	Non-specified	Spain	[59]
Spring/autumn	−12	5	18	Germany	[61]
Spring/autumn	−1	27–48	Non-specified	Spain	[62]
Spring/autumn	10	12	3	Spain	[30]
Spring/autumn	10	11.5	10	Spain	[51]
Spring/autumn	10	23	7.5	Canada	[63]
Spring/autumn	32	Non-specified	Non-specified	Spain	[58]
Summer	−67	12–16	1.5–2.7	Spain	[64]
Summer	−4	12–16	1.5–2.7	Spain	[64]
Summer	0	16–24	15–25	USA	[65]
Summer	11	7.1–9.4	3.8–8.4	Japan	[57]
Summer	25	11	6–8	Canada	[66]
Summer	35	Non-specified	1.5–26	Austria	[67]
Mean year	11	12–17	1.5–5.1	Spain	[68]
Mean year	7	12–17	1.5–5.1	Spain	[68]
Non-specified	−47	24–48	1–26	Austria	[47]
Non-specified	−43	1.9–326	2–237	Austria	[47]
Non-specified	−43	24–48	1–26	Austria	[47]
Non-specified	−35	24–48	1–26	Austria	[47]
Non-specified	−11	1.9–326	2–237	Austria	[47]
Non-specified	−3	24–48	1–26	Austria	[47]
Non-specified	0	Non-specified	52–237	Austria	[69]
Non-specified	0	5.5	8	USA	[70]
Non-specified	10	24	60	Germany	[71]

The collected data regarding ibuprofen are shown in the following Table 2. Generally, high removals are reported in most of the studies, and biodegradation is usually accounted.

As it can be observed from Fig. 7, the removal of ibuprofen is generally not affected by the season, nor by operating parameters like SRT or HRT. Most of the considered papers reported high removal for this analgesic compound. Nonetheless, a lower removal was observed in few cases and most of them in the winter seasons.

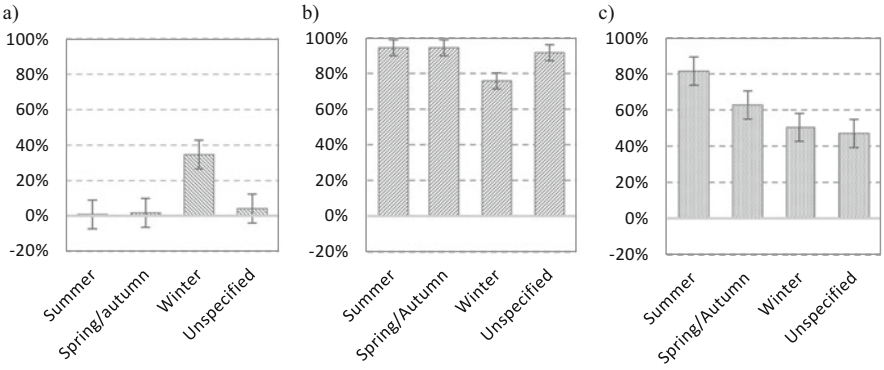


Fig. 7 CAS removal (%) in different seasons for carbamazepine (**a**), ibuprofen (**b**) and sulfamethoxazole (**c**). The reported data corresponds to averages of the references reported in Tables 1, 2 and 3

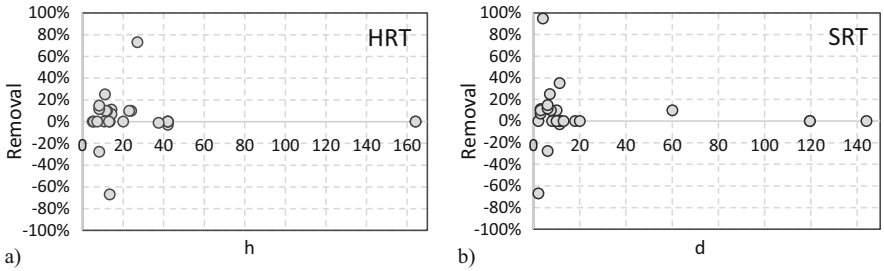


Fig. 8 CAS removal (%) of carbamazepine as a function of HRT (**a**) and SRT (**b**). The reported data corresponds to the references provided in Table 1

The lowest removal in winter, with the lowest temperature, jointly with the evaluation of its chemical structure, confirms that the main removal mechanism for ibuprofen in CAS is usually biodegradation, as also reported in the next paragraph. On the other hand, based on the provided HRT and SRTs, when available, it can be confirmed that ibuprofen is effectively removed also at low HRT and low SRT, thus confirming, even though with some exception, that it is one of the most easily removed analgesics in CAS (Fig. 9).

Finally, the collected data for sulfamethoxazole are reported in Table 3 and Fig. 7.

The removal of this compound is strongly variable in the sampled WWTPs. The variability is influenced by the season with higher removal in summer (on average 80%) and lower, on average, in winter. HRT and SRT confirmed variable removal results under a wide range of operating conditions (Fig. 10).

In the literature, HRT and SRT are recognized to be important parameters to be considered in this context [86]. Tailored adjustment of these parameters may have a

Table 2 Removal of ibuprofen in CAS systems

Season	Removal (%)	HRT (h)	SRT (days)	Country	Reference
Winter	48.5	Non-specified	Non-specified	Italy, France, Greece and Sweden	[72]
Winter	50	Non-specified	Non-specified	Italy, France, Greece and Sweden	[24]
Winter	65	24	Non-specified	Spain	[73]
Winter	66	Non-specified	Non-specified	Finland	[74]
Winter	84	27	Non-specified	Spain	[75]
Winter	95	8–15	4–21	China	[56]
Winter	98	Non-specified	Non-specified	Switzerland	[76]
Winter	100	Non-specified	Non-specified	Spain	[58]
Spring/autumn	83	12	3	Spain	[30]
Spring/autumn	86	13.5	1.5–2.7	UK	[77]
Spring/autumn	94	27	Non-specified	Spain	[75]
Spring/autumn	99	35	6–10	Sweden	[31]
Spring/autumn	99	11.5	10	Spain	[51]
Spring/autumn	100	36	20	Finland	[12]
Spring/autumn	100	Non-specified	Non-specified	Spain	[58]
Spring/autumn	100	27–48	Non-specified	Spain	[62]
Summer	−13	12.5	Non-specified	England	[78]
Summer	88	12–16	1.5–2.7	Spain	[64]
Summer	90	12–16	1.5–2.7	Spain	[64]
Summer	92	Non-specified	1.5–26	Austria	[67]
Summer	98	12	15–65	Japan	[79]
Summer	99	23	18	Greece	[5, 80]
Mean year	84	12–17	1.5–5.1	Spain	[68]
Mean year	87	12–17	1.5–5.1	Spain	[68]
Non-specified	−4.4	24–48	1–26	Austria	[47]
Non-specified	82	24	60	Germany	[71]
Non-specified	87	Non-specified	Non-specified	USA	[81]
Non-specified	92	24–48	1–26	Austria	[47]
Non-specified	97	Non-specified	52–237 days	Austria	[69]
Non-specified	98	1.9–326	2–237	Austria	[47]
Non-specified	98	24–48	1–26	Austria	[47]
Non-specified	99	24–48	1–26	Austria	[47]

beneficial influence on the removal of some PhACs and should be carefully considered. Moreover, prolonged SRT may favour nitrifying biomass and/or other slowly growing bacteria, which may have better xenobiotic removal capabilities.

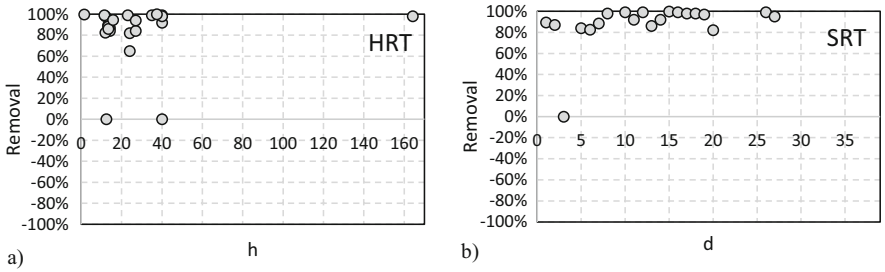


Fig. 9 CAS removal (%) of ibuprofen as a function of HRT (**a**) and SRT (**b**). The reported data corresponds to the references provided in Table 2

Table 3 Removal of sulfamethoxazole in CAS systems

Season	Removal (%)	HRT (h)	SRT (days)	Country	Reference
Winter	4.5	Non-specified	Non-specified	Switzerland	[46]
Winter	25	16.5	12.5	Australia	[82]
Winter	26	2.8–14	13–19	Japan	[83]
Winter	39	2.8–14	13–19	Japan	[83]
Winter	42	Non-specified	Non-specified	Italy	[32]
Winter	42	8–24	Non-specified	Sweden	[35]
Winter	61	15.8	21.4	China	[56]
Winter	74	Non-specified	Non-specified	Spain	[58]
Winter	94	27	Non-specified	Spain	[59]
Winter	97	10–17	7–12	China	[52]
Spring/autumn	10	11.5	10	Spain	[51]
Spring/autumn	56	12	3	Spain	[30]
Spring/autumn	62	Non-specified	Non-specified	Spain	[58]
Spring/autumn	71	27	Non-specified	Spain	[62]
Spring/autumn	71	27	Non-specified	Spain	[59]
Spring/autumn	75	10–17	7–12	China	[52]
Spring/autumn	94	10–17	7–12	China	[52]
Summer	51.9	7.9–15	16.8–43	South Korea	[84]
Summer	93	10–17	7–12	China	[52]
Summer	100	8–24	Non-specified	Sweden	[35]
Non-specified	32	Non-specified	52–237	Austria	[47]
Non-specified	62	14–16	2–4	Israel	[85]

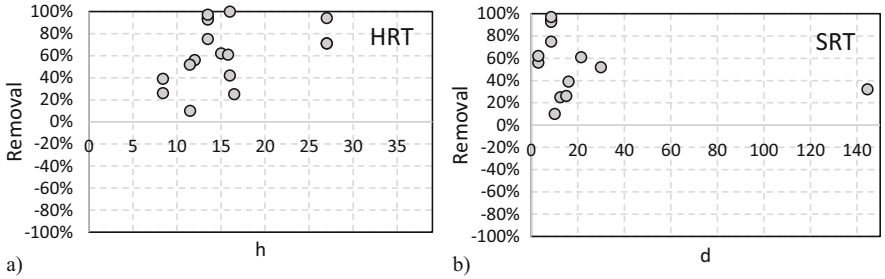


Fig. 10 CAS removal (%) of sulfamethoxazole as a function of HRT (**a**) and SRT (**b**). The reported data corresponds to the references provided in Table 3

5 Metabolites, Conjugates and Transformation Products

To fully understand the fate of PhACs, as for any other organic (micro)pollutant, in CAS it is crucial to consider also micropollutant metabolites (including conjugates) and transformation products.

Upon consumption, a variable amount of the ingested compound is excreted as a metabolite. Metabolites can be separated based on the undergone transformation: phase I metabolites and phase II metabolites. Phase I metabolites are originated through in vivo biochemical oxidation, reduction and hydrolysis reactions increasing aqueous solubility and facilitating the elimination from the body. On the other hand, phase II metabolites (or conjugated metabolites) are the result of the addition of a molecule to the parent compound (i.e. glucuronic acid). Phase II metabolites may deconjugate back to the parent compound in wastewater [87]. Afterwards, when PhACs enter WWTPs and/or in the environment, they can be exposed to biotic (biodegradation by bacteria and fungi)/non-biotic (hydrolysis or photolysis) processes [13].

The term "metabolite" is sometimes applied for compounds originated from both human, microbial and environmental sources. Nevertheless, in general it is recommended to only call "metabolites" those molecules that result from changes in human and animals and to call "transformation products" (TPs) to those generated from biotic/non-biotic processes [88]. In some cases, the same human metabolites can also be generated by microbial biodegradation (e.g. ibuprofen, [75]), but it is not the general case, and attention needs to be paid in this context.

The fraction of the parent compound to be excreted in an unchanged form depends on the structure of the micropollutant. For example, it may range from 20 to 75% for antibiotics. In the case of metoprolol, three main metabolites are formed by mammals (metoprolol acid, a-hydroxymetoprolol and O-desmethyl metoprolol), accounting for 85% of the urinary excretion [89]. Information on metabolites is increasing rapidly but still lacking for many compounds. The load of organic micropollutants entering CAS, hence, is greatly underestimated, if metabolites (and conjugates) are not accounted for.

The parent compound may be transformed, if it is degradable, into a more hydrophilic form (and usually of a lower molecular weight) through different degradation pathways. For example, ibuprofen biodegradation may lead, among other ones, to the formation of three main transformation products (1-OH-ibuprofen, 2-OH-ibuprofen and carboxy-ibuprofen [75]). TPs can be less or more biodegradable, and, generally speaking, they are found at a lower concentration than the corresponding parent compound in CAS effluent, except for persistent compounds (e.g. metoprolol acid, in some conditions, [89]). In just limited cases, the complete (bio)degradation pathway has been clarified and verified in real matrixes, concentrations (i.e. μg/L or ng/L levels) and conditions other than laboratory-controlled ones [13]. Each micropollutant can originate many TPs, and these TPs can also be transformed into different TPs; therefore their monitoring is one of the most challenging objectives.

Whatever the case, if they are not completely removed, TPs may pass through CAS and end up in the receiving waters. In some cases, the originated compound can even be more toxic than the parent compound [90]. On the other hand, some large organic micropollutants quickly lose their toxic features after the first degradation steps, making following treatment efforts counterproductive [21].

6 Regulation Regarding Water Reuse and PhACs

PhACs are ubiquitously detected in wastewater, but also freshwater, seawater and even drinking water. PhACs are among the compounds that can be referred to as "pseudo-persistent" contaminants as their transformation and removal are compensated by their continuous introduction into the environment [91]. Potential harmful effects both on the ecosystem and on human health are currently under extensive evaluation [3]. Continuous input of low-level pharmaceuticals into water bodies from WWTPs can adversely affect a variety of aquatic organisms [92] and lead to many kinds of anomalies [93]. PhACs can also enter the food chain, possibly resulting in biomagnification. Direct intake of these pollutants is also possible when human beings and animals drink already contaminated water. Additionally, antibiotics are not completely removed in WWTPs; microorganisms in the environment can be therefore continuously exposed to them, favouring the spread of antimicrobial resistance genes. According to the World Health Organization (WHO), "By 2050 – if effective interventions against antimicrobial resistance are not made – 10 million deaths and an economic loss of US$ 100 trillion may occur annually as the result of such resistance" [94].

The adoption of new legislation concerning water reuse, a better understanding of organic micropollutant impacts on the environment and public awareness campaigns about their hazards are paramount to encourage public and private initiatives to attain high-quality water and wastewater treatment standards in the next years [95]. Nonetheless the regulations at EU level that restrict PhAC presence in wastewater and the environment are still very limited. In 2020, the Council of the European Union proposed a new regulation to reduce the risks of water shortages by improving the

availability of water and encourage its efficient reuse. The main focus was on water reuse in agricultural, while other possibilities (e.g. aquifer recharge) were finally disregarded. In this context, the removal of substances of emerging concern (mentioning in the risk assessment section also organic micropollutants, including PhACs) is fundamental for safe water reuse [14].

Moreover, micropollutants have been targeted with a Commission Implementing Decision in 2015 [96] and then updated through Decision 2018/480 [97]. The watch list is a list of micropollutants that may pose a significant risk to or via the aquatic environment but for which data are still insufficient to support their prioritization and must, therefore, be monitored Europe-wide by member states [13]. The PhACs included, for the first time in an EU Decision, were diclofenac in the 2015 watch List, amoxicillin and ciprofloxacin in the 2018 watch list and three macrolide antibiotics (erythromycin, clarithromycin and azithromycin) in both. Since the monitoring must be carried out in freshwater only, there is a missing link between the occurrence of such pollutants in wastewater and the risk associated with their presence when reclaimed wastewater is reused [98].

The situation outside the EU is different. Switzerland regulation established that WWTPs following certain criteria should be upgraded with advanced treatment by 2035 in order to ensure the removal of at least 80% of micropollutant loads (including amisulpride, carbamazepine, citalopram, clarithromycin, diclofenac, hydrochlorothiazide, metoprolol, venlafaxine, candesartan and irbesartan), tracking WWTPs efficiency by means of periodical monitoring campaigns [99]. Other countries (e.g. Australia, Singapore and the State of California) have implemented cutting water reuse policies, including specific regulations on grey water and direct or non-direct potable reuse. In some cases micropollutants, and PhACs among them, are also included to be monitored and/or considered in terms of potential stressors. The legislation of these countries might pave the way for implementation at EU levels [98].

Finally, the list of target compounds is typically created by selecting PhACs according to existing legislation, consumption, occurrence and available analytical methods. Nevertheless, the list of evaluated PhACs is frequently limited to previously studied compounds, while other PhACs are widely prescribed but overlooked. Moreover, new compounds are continuously introduced into the market and/or for emerging necessity (e.g. COVID-19). For this reason, other possibly relevant compounds should be also included in future legislation and studies.

7 PhAC Fate in Sludge Treatment Line: Focus on Agricultural Reuse

7.1 Agricultural Sludge Reuse: Advantages and Environmental Impacts

Nutrient contents and soil-conditioning properties of sewage sludge can be usefully exploited with its reuse in agriculture, especially for restoring overexploited land or

for improving the humus content and water-holding capacity of light-textured sandy soil [100]. Agricultural reuse (directly or after composting) is a sustainable practice in the frame of the circular economy; it is extensively applied in several countries accounting 53% of the produced sludge in EU-27 countries [101], but the potential risk associated with the release of toxic and persistent substances in aquifers and soils has to be adequately considered.

PhACs constitute one of the broadest groups of compounds present in the urban wastewater; they are often characterized by hydrophobic-lipophilic properties which can favour their sorption (adsorption or absorption) onto the sludge. This prevalent removal mechanism causes an enrichment of the solid fraction at concentrations of several orders of magnitude higher than in the original wastewater [102].

Release of sorbed compounds onto the sludge in agricultural reuse can have a strong environmental impact because of the possible direct uptake by crops and consequent transfer to the food chain with a serious concern for human health and negative effects on plant growth [103]. In the specific case of PhACs, it has been observed that WWTPs concentrate antibiotics in the produced sludge, especially for hospitals and industrial wastewater, and the activated sludge systems provide a favourable environment with high microbial biomass concentration and selective process for horizontal gene transfer of antibiotic-resistant genes [102]. Moreover, the persistence and recalcitrance of many PhACs amplify negative impacts over time with consequences difficult to predict for long-term scenarios.

PhACs are the most investigated class of micropollutants in WWTPs, but published studies are mainly devoted to the characterization of the different types of sludge produced by different treatment options more than on the sludge treatment solutions able to provide a more effective removal of the accumulated pollutants. This aspect has been considered in recent publications, where the performance of different sludge treatment lines is analysed, not only in terms of solid reduction and stabilization but also taking into account the "quality" of the digested sludge, for several classes of micropollutants including PhACs [104, 105]. Results of these studies are summarized in the next section.

7.2 *Sludge Treatment Alternatives to Enhance PhAC Removal*

For WWTPs of medium/high potentiality (i.e. $\geq$30,000 P.E.), the preferred sludge treatment option is the anaerobic digestion, which provides good solid removal efficiencies and allows energy recovery though methane production. Mesophilic process ($T = 35°C$) is more commonly applied than the thermophilic one (T in the range of 55–60°C) given the lower energy demand, but the one-step mesophilic digestion is less efficient for organic compound removal (including PhACs), methane production and hygienization [106]. Enhancement of mesophilic sludge digestion can be achieved with appropriate pre- or post-treatments (both chemical-physical and biological) or with the combined anaerobic-aerobic sequential process.

Braguglia et al. analysed the fate of 11 selected PhACs in an extended study on the thermophilic digestion coupled with thermal hydrolysis pretreatment (THT) and of a dual-stage mesophilic/thermophilic digestion with (UMT) and without (MT) ultrasound pretreatment applied to secondary and mixed sludge [104]. PhAC concentration was in the range of 100–160 and 110–130 mg/kg$_{dw}$ in the secondary and mixed sludge, respectively. They observed that THT process was not effective in PhAC removal for all investigated conditions, while better results were achieved with MT process resulting in ~57% removal efficiency with no substantial beneficial effect exerted by the ultrasound pretreatment (UMT). Better performance of the combined MT process was detected for PhAC removal in secondary sludge than in mixed sludge digestion, where no statistically significant removal was observed.

Enhanced performance of sequential anaerobic-aerobic (AA) digestion with respect to the single-step anaerobic or aerobic process was demonstrated in previous studies: the motivation behind this approach is that the combined process provides differentiated reaction environments (anaerobic and aerobic) and thus optimal biodegradability conditions for the different volatile solid sludge fractions and pollutants present in the sludge matrix. A further improvement of the AA can be achieved by operating the post-aerobic step under mesophilic conditions [107] with a low external energy supply well compensated by the improved efficiency. Tomei et al. investigated the fate of a selected group of PhACs on secondary sludge treated in two AA schemes conducted with the same operating conditions for the anaerobic step (SRT 15 days $T = 37°C$), while the aerobic phase was operated at SRT = 12 days, $T = 20°C$ (AA1) and $T = 37°C$ (AA2) [105]. Table 4 shows the removal efficiencies for PhACs detected at the highest concentration among the investigated ones. Negative values indicate accumulation due to poor removal and volatile solid concentration decrease in the digestion process.

Table 4 highlights a high variability of the removal efficiencies which are attributable to the variability of the fed sludge, which remarkably affects the anaerobic removals in the two series, especially for the less biodegradable compounds, i.e. carbamazepine and diclofenac. Additional removal was achieved, for both compounds, in the post-aerobic step at both temperatures but with better

Table 4 Removal efficiencies (RE) of sequential anaerobic-aerobic (AA) digestion observed for representative PhACs (modified from [105]). Negative values indicate accumulation in the solid phase. The average PhAC concentration in the fed sludge was 140 ± 40 mg/kg$_{dw}$

	RE AA1 (%)			RE AA2 (%)		
Compound	Anaerobic	Aerobic	Total	Anaerobic	Aerobic	Total
Carbamazepine	34	3	36	−13	28	19
Dihydro-dihydroxy-carbamazepine	38	4.5	41	−46	13	−28
Dihydro-hydroxy-carbamazepine	56	25	67	−21	46	34
Clarithromycin	90	−271	63	85	−137	63
Diclofenac	41	2	43	−31	45	28
Fluconazole	51	−10	46	−3	24	22
Venlafaxine	45	8	49	19	27	40

performance in AA2 (at higher aerobic temperature). Similar results were observed for the other compounds with the only exception of clarithromycin, which was mainly removed in the anaerobic phase (85–90%).

The reported study cases are good examples of the possibility of producing sludge of better quality, in terms of PhAC content, with more efficient digestion lines, even if the complexity and variability of the sludge matrix require additional research in the field.

7.3 EU Regulation for Agricultural Reuse

The EU reference document for the regulation of land disposal of sewage sludge is the Sewage Sludge Directive (SSD) 86/278/EEC [108], which focuses essentially on heavy metals but does not set any threshold value for organic compounds for sludge application. National regulations in EU member states are established with the option to fix more restrictive limit values than the ones suggested in the SSD [108]. A more recent document is the "Working Document on Sludge and Biowaste" [109] with maximum proposed permissible levels for heavy metals and polycyclic aromatic hydrocarbons but not yet for PhACs.

This short overview highlights the need for revision and integration of the EU and national legislation to establish additional limit values for sludge reuse in agriculture, including a wider spectrum of organic contaminants exerting detrimental effects for both the human health and the environment. In the specific case of PhACs, a serious concern is that the sludge might be a reservoir of antibiotic resistance genes (ARGs) and, when reused in agriculture, could contribute to the spreading of the "antibiotic resistance", which is recognized as one of the most important challenges of contemporary medicine and a serious public health problem. Future regulations should also consider this phenomenon and define accurate parameters to be monitored.

Acknowledgements Marc Castaño acknowledges the FI fellowship (2020 FI_B 00711) from the Catalan Government. Gianluigi Buttiglieri acknowledges the Ramon y Cajal research fellowship RYC-2014-16754 and the CLEaN-TOUR project (CTM2017-85385-C2-1-R) from the Spanish Ministry of Economy and Competitiveness. The authors thank Generalitat de Catalunya through Consolidated Research Group 2017-SGR-1318. ICRA researchers thank funding from the CERCA programme. Monica Brienza acknowledges the "Attraction and International Mobility Programme" for her competitive contract funded by the European Union.

References

1. aus der Beek T, Weber FA, Bergmann A, Hickmann S, Ebert I, Hein A, Küster A (2016) Pharmaceuticals in the environment-global occurrences and perspectives. Environ Toxicol Chem 35(4):823–835. https://doi.org/10.1002/etc.3339
2. Kümmerer K, Henninger A (2003) Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin Microbiol Infect 9(12):1203–1214. https://doi.org/10.1111/j.1469-0691.2003.00739.x

3. Majumder A, Gupta B, Gupta AK (2019) Pharmaceutically active compounds in aqueous environment: a status, toxicity and insights of remediation. Environ Res 176:108542. https://doi.org/10.1016/j.envres.2019.108542
4. Buttiglieri G (2008) Removal of emerging contaminants in wastewater treatment: conventional activated sludge treatment. In: Barceló D, Petrović M (eds) The handbook of environmental chemistry. Springer, Berlin, pp 1–35. https://doi.org/10.1007/698_5_098
5. Joss A, Zabczynski S, Göbel A, Hoffmann B, Löffler D, McArdell CS, Ternes TA, Thomsen A, Siegrist H (2006) Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. Water Res 40(8):1686–1696. https://doi.org/10.1016/j.watres.2006.02.014
6. Rosal R, Rodríguez A, Perdigón-Melón JA, Petre A, García-Calvo E, Gómez MJ, Agüera A, Fernández-Alba AR (2010) Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Res 44(2):578–588. https://doi.org/10.1016/j.watres.2009.07.004
7. Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S, Wang XC (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ 473–474:619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065
8. Spongberg AL, Witter JD (2008) Pharmaceutical compounds in the wastewater process stream in Northwest Ohio. Sci Total Environ 397(1–3):148–157. https://doi.org/10.1016/j.scitotenv.2008.02.042
9. Patel M, Kumar R, Kishor K, Mlsna T, Pittman CU, Mohan D (2019) Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chem Rev 119(6):3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299
10. Miao XS, Yang JJ, Metcalfe CD (2005) Carbamazepine and its metabolites in wastewater and in biosolids in a municipal wastewater treatment plant. Environ Sci Technol 39 (19):7469–7475. https://doi.org/10.1021/es050261e
11. Karthikeyan KG, Meyer MT (2006) Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. Sci Total Environ 361(1–3):196–207. https://doi.org/10.1016/j.scitotenv.2005.06.030
12. Vieno NM, Tuhkanen T, Kronberg L (2005) Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environ Sci Technol 39(21):8220–8226. https://doi.org/10.1021/es051124k
13. Carballa C, Alvarino T, Buttiglieri G, Choubert JM, Pons MN (2017) Innovative primary and secondary sewage treatment technologies for organic micropollutants abatement. In: Innovative wastewater treatment and resource recovery technologies: impacts on energy, economy and environment. https://doi.org/10.2166/9781780407876_0179
14. European Commission (2020) Regulation (EU) 2020/741 of the European Parliament and of the Council of 25 may 2020 on minimum requirements for water reuse
15. Göbel A, Thomsen A, McArdell CS, Joss A, Giger W (2005) Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ Sci Technol 39(11):3981–3989. https://doi.org/10.1021/es048550a
16. Godfree A (2003) Health constraints on the agricultural recycling of wastewater sludges. In: The handbook of water and wastewater microbiology, vol 1, pp 281–300. ISBN 0-12-470100-0
17. Chen H, Jiang W, Yang Y, Yang Y, Man X (2015) Global trends of municipal solid waste research from 1997 to 2014 using bibliometric analysis. J Air Waste Manage Assoc 65 (10):1161–1170. https://doi.org/10.1080/10962247.2015.1083913
18. Rodriguez-Mozaz S, Chamorro S, Marti E, Huerta B, Gros M, Sànchez-Melsió A, Borrego CM, Barceló D, Balcázar JL (2015) Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res 69:234–242. https://doi.org/10.1016/j.watres.2014.11.021

19. Couto CF, Lange LC, Amaral MCS (2019) Occurrence, fate and removal of pharmaceutically active compounds (PhACs) in water and wastewater treatment plants – a review. J Water Process Eng 32:100927. https://doi.org/10.1016/j.jwpe.2019.100927
20. Verlicchi P, Al Aukidy M, Zambello E (2012) Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment-A review. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2012.04.028
21. Castaño-Trias M, Rodríguez-Mozaz S, Buttiglieri G (2021) 10 years in-house prioritization method development for organic micropollutants and its application to Spanish and Greece real samples of wastewater, river and sea. Submitted
22. Wang J, Wang S (2016) Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: a review. J Environ Manage 182:620–640. https://doi.org/10.1016/j.jenvman.2016.07.049
23. Ekowati Y, Ferrero G, Farré MJ, Kennedy MD, Buttiglieri G (2019) Application of UVOX Redox® for swimming pool water treatment: microbial inactivation, disinfection byproduct formation and micropollutant removal. Chemosphere 220:176–184. https://doi.org/10.1016/j.chemosphere.2018.12.126
24. Castiglioni S, Borsotti A, Riva F, Zuccato E (2016) Illicit drug consumption estimated by wastewater analysis in different districts of Milan: a case study. Drug Alcohol Rev 35 (2):128–132. https://doi.org/10.1111/dar.12233
25. Bai X, Lutz A, Carroll R, Keteles K, Dahlin K, Murphy M, Nguyen D (2018) Occurrence, distribution, and seasonality of emerging contaminants in urban watersheds. Chemosphere 200:133–142. https://doi.org/10.1016/j.chemosphere.2018.02.106
26. Jelic A, Rodriguez-Mozaz S, Barceló D, Gutierrez O (2015) Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Water Res 68:98–108. https://doi.org/10.1016/j.watres.2014.09.033
27. Correia A, Marcano L (2015) Presence and elimination of pharmaceutical compounds in wastewater treatment plants. Worldwide review and national perspective. Boletín de malariología y salud ambiental, pp 1–18
28. Kot-Wasik A, Jakimska A, Śliwka-Kaszyńska M (2016) Occurrence and seasonal variations of 25 pharmaceutical residues in wastewater and drinking water treatment plants. Environ Monit Assess 188(12). https://doi.org/10.1007/s10661-016-5637-0
29. Daughton CG (2016) Pharmaceuticals and the environment (PiE): evolution and impact of the published literature revealed by bibliometric analysis. Sci Total Environ 562:391–426. https://doi.org/10.1016/j.scitotenv.2016.03.109
30. Radjenovic J, Petrovic M, Barceló D (2007) Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. Anal Bioanal Chem 387:1365–1377. https://doi.org/10.1007/s00216-006-0883-6
31. Zorita S, Mårtensson L, Mathiasson L (2009) Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. Sci Total Environ 407 (8):2760–2770. https://doi.org/10.1016/j.scitotenv.2008.12.030
32. Castiglioni S, Bagnati R, Fanelli R, Pomati F, Calamari D, Zuccato E (2006) Removal of pharmaceuticals in sewage treatment plants in Italy. Environ Sci Technol 40(1):357–363. https://doi.org/10.1021/es050991m
33. Vieno N, Tuhkanen T, Kronberg L (2007) Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Res 41(5):1001–1012. https://doi.org/10.1016/j.watres.2006.12.017
34. Golet EM, Alder AC, Giger W (2002) Environmental exposure and risk assessment of fluoroquinolone antibacterial agents in wastewater and river water of the Glatt Valley watershed, Switzerland. Environ Sci Technol 36(17):3645–3651. https://doi.org/10.1021/es0256212
35. Lindberg RH, Olofsson U, Rendahl P, Johansson MI, Tysklind M, Andersson BAV (2006) Behavior of fluoroquinolones and trimethoprim during mechanical, chemical, and active sludge treatment of sewage water and digestion of sludge. Environ Sci Technol 40 (3):1042–1048. https://doi.org/10.1021/es0516211

36. Sipma J, Osuna B, Collado N, Monclús H, Ferrero G, Comas J, Rodriguez-Roda I (2010) Comparison of removal of pharmaceuticals in MBR and activated sludge systems. Desalination 250(2):653–659. https://doi.org/10.1016/j.desal.2009.06.073
37. Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ (2009) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res 43(2):363–380. https://doi.org/10.1016/j.watres.2008.10.047
38. Petrovic M, Verlicchi P (2014) Water treatment plants and pharmaceutical residues in Catalonia and Italy. Contrib Sci 10(2):135–150. https://doi.org/10.2436/cs.v10i2.139201
39. Dutta K, Lee MY, Lai WWP, Lee CH, Lin AYC, Lin CF, Lin JG (2014) Removal of pharmaceuticals and organic matter from municipal wastewater using two-stage anaerobic fluidized membrane bioreactor. Bioresour Technol 165(C):42–49. https://doi.org/10.1016/j.biortech.2014.03.054
40. Monsalvo VM, McDonald JA, Khan SJ, Le-Clech P (2014) Removal of trace organics by anaerobic membrane bioreactors. Water Res 49:103–112. https://doi.org/10.1016/j.watres.2013.11.026
41. Musson SE, Campo P, Tolaymat T, Suidan M, Townsend TG (2010) Assessment of the anaerobic degradation of six active pharmaceutical ingredients. Sci Total Environ 408 (9):2068–2074. https://doi.org/10.1016/j.scitotenv.2009.11.042
42. Adrian NR, Suflita JM (1994) Anaerobic biodegradation of halogenated and nonhalogenated heterocyclic compounds in aquifer slurries. Environ Toxicol Chem 13(10):1551–1557. https://doi.org/10.1002/etc.5620131002
43. Alvarino T, Lema J, Omil F, Suárez S (2018) Trends in organic micropollutants removal in secondary treatment of sewage. Rev Environ Sci Biotechnol 17:447–469. https://doi.org/10.1007/s11157-018-9472-3
44. Kassotaki E, Buttiglieri G, Ferrando-Climent L, Rodriguez-Roda I, Pijuan M (2016) Enhanced sulfamethoxazole degradation through ammonia oxidizing bacteria co-metabolism and fate of transformation products. Water Res 94:111–119. https://doi.org/10.1016/j.watres.2016.02.022
45. Miège C, Choubert JM, Ribeiro L, Eusèbe M, Coquery M (2009) Fate of pharmaceuticals and personal care products in wastewater treatment plants – conception of a database and first results. Environ Pollut 157(5):1721–1726. https://doi.org/10.1016/j.envpol.2008.11.045
46. Göbel A, McArdell CS, Joss A, Siegrist H, Giger W (2007) Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci Total Environ 372 (2–3):361–371. https://doi.org/10.1016/j.scitotenv.2006.07.039
47. Clara M, Kreuzinger N, Strenn B, Gans O, Kroiss H (2005) The solids retention time – a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res 39(1):97–106. https://doi.org/10.1016/j.watres.2004.08.036
48. Bouju H, Buttiglieri G, Malpei F (2008) Perspectives of persistent organic pollutants (POPS) removal in an MBR pilot plant. Desalination 224(1–3):1–6. https://doi.org/10.1016/j.desal.2007.04.071
49. Ternes TA, Joss A, Siegrist H (2004) Scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environ Sci Tech 38(20):392A–399A. https://doi.org/10.1021/es040639t
50. Metcalfe CD, Koenig BG, Bennie DT, Servos M, Ternes TA, Hirsch R (2003) Occurrence of neutral and acidic drugs in the effluents of Canadian sewage treatment plants. Environ Toxicol Chem 22(12):2872–2880. https://doi.org/10.1897/02-469
51. 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 Res 43(3):831–841. https://doi.org/10.1016/j.watres.2008.11.043
52. Li B, Zhang T (2011) Mass flows and removal of antibiotics in two municipal wastewater treatment plants. Chemosphere 83(9):1284–1289. https://doi.org/10.1016/j.chemosphere.2011.03.002

53. Dorival-García N, Zafra-Gómez A, Navalón A, González-López J, Hontoria E, Vílchez JL (2013) Removal and degradation characteristics of quinolone antibiotics in laboratory-scale activated sludge reactors under aerobic, nitrifying and anoxic conditions. J Environ Manage 120:75–83. https://doi.org/10.1016/j.jenvman.2013.02.007
54. Boethling RS, Howard PH, Meylan W, Stiteler W, Beauman J, Tirado N (1994) Group contribution method for predicting probability and rate of aerobic biodegradation. Environ Sci Technol 28(3):459–465. https://doi.org/10.1021/es00052a018
55. Fernandez-Fontaina E, Gomes IB, Aga DS, Omil F, Lema JM, Carballa M (2016) Biotransformation of pharmaceuticals under nitrification, nitratation and heterotrophic conditions. Sci Total Environ 541:1439–1447. https://doi.org/10.1016/j.scitotenv.2015.10.010
56. Yan Q, Gao X, Huang L, Gan XM, Zhang YX, Chen YP, Peng X-Y, Guo JS (2014) Occurrence and fate of pharmaceutically active compounds in the largest municipal wastewater treatment plant in Southwest China: mass balance analysis and consumption back-calculated model. Chemosphere 99:160–170. https://doi.org/10.1016/j.chemosphere.2013.10.062
57. Nakada N, Tanishima T, Shinohara H, Kiri K, Takada H (2006) Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Res 40(17):3297–3303. https://doi.org/10.1016/j.watres.2006.06.039
58. Čelić M, Gros M, Farré M, Barceló D, Petrović M (2019) Pharmaceuticals as chemical markers of wastewater contamination in the vulnerable area of the Ebro Delta (Spain). Sci Total Environ 652:952–963. https://doi.org/10.1016/j.scitotenv.2018.10.290
59. García-Galán MJ, Díaz-Cruz MS, Barceló D (2011) Occurrence of sulfonamide residues along the Ebro river basin. Removal in wastewater treatment plants and environmental impact assessment. Environ Int 37(2):462–473. https://doi.org/10.1016/j.envint.2010.11.011
60. Komesli OT, Muz M, Ak MS, Bakirdere S, Gokcay CF (2015) Occurrence, fate and removal of endocrine disrupting compounds (EDCs) in Turkish wastewater treatment plants. Chem Eng J 277:202–208. https://doi.org/10.1016/j.cej.2015.04.115
61. Wick A, Fink G, Joss A, Siegrist H, Ternes TA (2009) Fate of beta blockers and psycho-active drugs in conventional wastewater treatment. Water Res 43(4):1060–1074. https://doi.org/10.1016/j.watres.2008.11.031
62. Gros M, Rodríguez-Mozaz S, Barceló D (2012) Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J Chromatogr A 1248:104–121. https://doi.org/10.1016/j.chroma.2012.05.084
63. Lajeunesse A, Smyth SA, Barclay K, Sauvé S, Gagnon C (2012) Distribution of antidepressant residues in wastewater and biosolids following different treatment processes by municipal wastewater treatment plants in Canada. Water Res 46(17):5600–5612. https://doi.org/10.1016/j.watres.2012.07.042
64. Santos JL, Aparicio I, Alonso E (2007) Occurrence and risk assessment of pharmaceutically active compounds in wastewater treatment plants. A case study: Seville city (Spain). Environ Int 33(4):596–601. https://doi.org/10.1016/j.envint.2006.09.014
65. Subedi B, Kannan K (2015) Occurrence and fate of select psychoactive pharmaceuticals and antihypertensives in two wastewater treatment plants in New York state, USA. Sci Total Environ 514:273–280. https://doi.org/10.1016/j.scitotenv.2015.01.098
66. Kim M, Guerra P, Shah A, Parsa M, Alaee M, Smyth SA (2014) Removal of pharmaceuticals and personal care products in a membrane bioreactor wastewater treatment plant. Water Sci Technol 69(11):2221–2229. https://doi.org/10.2166/wst.2014.145
67. Kreuzinger N, Clara M, Strenn B, Kroiss H (2004) Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater. Water Sci Technol 50(5):149–156. https://doi.org/10.2166/wst.2004.0322

68. Santos JL, Aparicio I, Callejón M, Alonso E (2009) Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). J Hazard Mater 164(2–3):1509–1516. https://doi.org/10.1016/j.jhazmat.2008.09.073
69. Clara M, Strenn B, Ausserleitner M, Kreuzinger N (2004) Comparison of the behaviour of selected micropollutants in a membrane bioreactor and a conventional wastewater treatment plant. Water Sci Technol 50(5):29–36. https://doi.org/10.2166/wst.2004.0305
70. Inyang M, Flowers R, McAvoy D, Dickenson E (2016) Biotransformation of trace organic compounds by activated sludge from a biological nutrient removal treatment system. Bioresour Technol 216:778–784. https://doi.org/10.1016/j.biortech.2016.05.124
71. Suárez S, Ramil M, Omil F, Lema JM (2005) Removal of pharmaceutically active compounds in nitrifying-denitrifying plants. Water Sci Technol 52(8):9–14. https://doi.org/10.2166/wst.2005.0214
72. Andreozzi R, Marotta R, Paxéus N (2003) Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere 50(10):1319–1330. https://doi.org/10.1016/S0045-6535(02)00769-5
73. Carballa M, Omil F, Lema JM, Llompart M, García-Jares C, Rodríguez I, Gómez M, Ternes T (2004) Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. Water Res 38(12):2918–2926. https://doi.org/10.1016/j.watres.2004.03.029
74. Lindqvist N, Tuhkanen T, Kronberg L (2005) Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. Water Res 39(11):2219–2228. https://doi.org/10.1016/j.watres.2005.04.003
75. Ferrando-Climent L, Collado N, Buttiglieri G, Gros M, Rodriguez-Roda I, Rodriguez-Mozaz-S, Barceló D (2012) Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment. Sci Total Environ 438:404–413. https://doi.org/10.1016/j.scitotenv.2012.08.073
76. Tauxe-Wuersch A, De Alencastro LF, Grandjean D, Tarradellas J (2005) Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment. Water Res 39(9):1761–1772. https://doi.org/10.1016/j.watres.2005.03.003
77. Jones OAH, Voulvoulis N, Lester JN (2007) The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilising activated sludge treatment. Environ Pollut 145(3):738–744. https://doi.org/10.1016/j.envpol.2005.08.077
78. Roberts PH, Thomas KV (2006) The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci Total Environ 356(1–3):143–153. https://doi.org/10.1016/j.scitotenv.2005.04.031
79. Kimura K, Hara H, Watanabe Y (2007) Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors. Environ Sci Technol 41(10):3708–3714. https://doi.org/10.1021/es061684z
80. Samaras VG, Stasinakis AS, Mamais D, Thomaidis NS, Lekkas TD (2013) Fate of selected pharmaceuticals and synthetic endocrine disrupting compounds during wastewater treatment and sludge anaerobic digestion. J Hazard Mater 244–245:259–267. https://doi.org/10.1016/j.jhazmat.2012.11.039
81. Yu JT, Bouwer EJ, Coelhan M (2006) Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent. Agric Water Manag 86(1–2):72–80. https://doi.org/10.1016/j.agwat.2006.06.015
82. Watkinson AJ, Murby EJ, Costanzo SD (2007) Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. Water Res 41(18):4164–4176. https://doi.org/10.1016/j.watres.2007.04.005
83. Ghosh GC, Okuda T, Yamashita N, Tanaka H (2009) Occurrence and elimination of antibiotics at four sewage treatment plants in Japan and their effects on bacterial ammonia oxidation. Water Sci Technol 59(4):779–786. https://doi.org/10.2166/wst.2009.067
84. Behera SK, Kim HW, Oh JE, Park HS (2011) Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest

industrial city of Korea. Sci Total Environ 409(20):4351–4360. https://doi.org/10.1016/j.scitotenv.2011.07.015
85. Sahar E, Ernst M, Godehardt M, Hein A, Herr J, Kazner C, Melin T, Cikurel H, Aharoni A, Messalem R, Brenner A, Jekel M (2011) Comparison of two treatments for the removal of selected organic micropollutants and bulk organic matter: conventional activated sludge followed by ultrafiltration versus membrane bioreactor. Water Sci Technol 63(4):733–740. https://doi.org/10.2166/wst.2011.300
86. Kassotaki E, Pijuan M, Rodriguez-Roda I, Buttiglieri G (2019) Comparative assessment of endocrine disrupting compounds removal in heterotrophic and enriched nitrifying biomass. Chemosphere 217:659–668. https://doi.org/10.1016/j.chemosphere.2018.11.012
87. Evgenidou EN, Konstantinou IK, Lambropoulou DA (2015) Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: a review. Sci Total Environ 505:905–926. https://doi.org/10.1016/j.scitotenv.2014.10.021
88. Langin A, Schuster A, Kummerer K (2008) Chemicals in the environment – the need for a clear nomenclature: parent compounds, metabolites, transformation products and their elimination. Clean-Soil Air Water 36:349–350
89. Rubirola A, Llorca M, Rodriguez-Mozaz S, Casas N, Rodriguez-Roda I, Barceló D, Buttiglieri G (2014) Characterization of metoprolol biodegradation and its transformation products generated in activated sludge batch experiments and in full scale WWTPs. Water Res 63:21–32. https://doi.org/10.1016/j.watres.2014.05.031
90. Majewsky M, Glauner T, Horn H (2015) Systematic suspect screening and identification of sulfonamide antibiotic transformation products in the aquatic environment. Anal Bioanal Chem 407(19). https://doi.org/10.1007/s00216-015-8748-5
91. Barceló D, Petrovic M (2007) Pharmaceuticals and personal care products (PPCPs) in the environment. Anal Bioanal Chem 22(3):194–203. https://doi.org/10.1007/s00216-006-1012-2
92. Fent K, Weston AA, Caminada D (2006) Ecotoxicology of human pharmaceuticals. Aquat Toxicol 76(2):122–159. https://doi.org/10.1016/j.aquatox.2005.09.009
93. Zenker A, Cicero MR, Prestinaci F, Bottoni P, Carere M (2014) Bioaccumulation and biomagnification potential of pharmaceuticals with a focus to the aquatic environment. J Environ Manage 133:378–387. https://doi.org/10.1016/j.jenvman.2013.12.017
94. Tangcharoensathien V, Sattayawutthipong W, Kanjanapimai S, Kanpravidth W, Brown R, Sommanustweechai A (2017) Antimicrobial resistance: from global agenda to national strategic plan, Thailand. Bull World Health Organ 95(8):599–603. https://doi.org/10.2471/BLT.16.179648
95. Rizzo L, Malato S, Antakyali D, Beretsou VG, Đolić MB, Gernjak W, Heath E, Ivancev-Tumbas I, Karaolia P, Lado Ribeiro AR, Mascolo G, McArdell CS, Schaar H, Silva AMT, Fatta-Kassinos D (2019) Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. Sci Total Environ 655:986–1008. https://doi.org/10.1016/j.scitotenv.2018.11.265
96. European Commission (2015) EUR-Lex – 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off J Eur Communities L78:40–42
97. European Commission (2018) EUR-Lex – Commission implementing decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Implementing Decision (EU) 2015/495. J Eur Union L141:9–12
98. Nika E, Gusmaroli L, Ghafourian M, Atanasova N, Buttiglieri G, Katsou E (2020) Nature-based solutions as enablers of circularity in water systems: a review on assessment methodologies, tools and indicators. Water Res 183:115988. https://doi.org/10.1016/j.watres.2020.115988

99. Swiss Confederation (2016) Ordinance of the Federal Law of introduction on water protection (Cantonal Ordinance on the water protection, OCPAc)
100. Verlicchi P, Zambello E (2015) Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence and environmental risk in the case of application on soil – a critical review. Sci Total Environ 538:750–767. https://doi.org/10.1016/j.scitotenv.2015.08.108
101. Kelessidis A, Stasinakis AS (2012) Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste Manag 32(6):1186–1195. https://doi.org/10.1016/j.wasman.2012.01.012
102. Mailler R, Gasperi J, Patureau D, Vulliet E, Delgenes N, Danel A, Deshayes S, Eudes V, Guerin S, Moilleron R, Chebbo G, Rocher V (2017) Fate of emerging and priority micropollutants during the sewage sludge treatment: case study of Paris conurbation. Part 1: contamination of the different types of sewage sludge. Waste Manag 59:379–393. https://doi.org/10.1016/j.wasman.2016.11.010
103. Smith SR (2009) Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. Phil Trans R Soc A Math Phys Eng Sci 367(1904):4005–4041. https://doi.org/10.1098/rsta.2009.0154
104. Braguglia CM, Coors A, Gallipoli A, Gianico A, Guillon E, Kunkel U, Mascolo G, Richter E, Ternes TA, Tomei MC, Mininni G (2015) Quality assessment of digested sludges produced by advanced stabilization processes. Environ Sci Pollut Res 22(10):7216–7235. https://doi.org/10.1007/s11356-014-3090-6
105. Tomei MC, Mosca Angelucci D, Mascolo G, Kunkel U (2019) Post-aerobic treatment to enhance the removal of conventional and emerging micropollutants in the digestion of waste sludge. Waste Manag 96
106. Riau V, De la Rubia MÁ, Pérez M (2010) Temperature-phased anaerobic digestion (TPAD) to obtain class A biosolids: a semi-continuous study. Bioresour Technol 101(8):2706–2712. https://doi.org/10.1016/j.biortech.2009.11.101
107. Tomei MC, Mosca Angelucci D, Levantesi C (2016) Two-stage anaerobic and post-aerobic mesophilic digestion of sewage sludge: analysis of process performance and hygienization potential. Sci Total Environ 545–546:453–464. https://doi.org/10.1016/j.scitotenv.2015.12.053
108. CEC (1986) Council Directive of 12 June 1986 on the Protection of the Environment, and in Particular of the Soil, when Sewage Sludge is used in Agriculture (86/278/EEC). Council of the European Communities. Off J Eur Communities No. L 181/6-12
109. EU Working Document on Sludge and Biowaste (2010) Retrieved from European Water Association: http://www.dwa.de/portale/ewa/ewa.nsf/home?readform&objectid=AC9B268C90BC9930C12577E00045E8E2; Last Accessed July 2020

New Insights into the Occurrence of Micropollutants and the Management and Treatment of Hospital Effluent

Paola Verlicchi

Contents

Abstract This chapter deals with investigations carried out over the last 5 years on hospital effluent in terms of the occurrence of micropollutants; new and promising technologies tested to improve the removal of key compounds (including emerging contaminants); the environmental and health risk assessments of pharmaceutical residues and pathogens; and, finally, some of the strategies adopted in hospital effluent management and treatments through the discussion of some case studies. It emerges that the occurrence and treatment of hospital effluent are becoming issues of increasing concern also for countries such as Morocco, Tunisia, Iran and Colombia, whose research groups had not actively participated in the worldwide debate thus far. Their interest in these topics highlights the shared, global awareness of the need to adopt safe, economic and technically feasible technologies for the treatment of hospital effluent to reduce the impact on the aquatic environment of hazardous substances typically administered or used in healthcare facilities. The experiences reported and discussed herein demonstrate the worldwide efforts that have been

P. Verlicchi (✉)
Department of Engineering, University of Ferrara, Ferrara, Italy
e-mail: paola.verlicchi@unife.it

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 53–96, DOI 10.1007/698_2020_677,

made and are still ongoing with the aim of reaching Sustainable Millennium Goal number 6 “Improve Clean Water and Sanitation” by 2030, as defined by the World Health Organization.

Keywords Environmental risk assessment, Hospital effluent, Management, Occurrence, Pharmaceuticals, Removal

Acronyms

AOP	Advanced oxidation process
AOX	Adsorbable organic halide
ARB	Antibiotic-resistant bacteria
ARG	Antibiotic-resistant gene
CPE	Carbapenemase-producing bacteria
CRE	Carbapenem-resistant *Enterobacteriaceae*
DOC	Dissolved organic carbon
DOX	Doxorubicin
EQS	Environmental quality standards
ESBL	Extended-spectrum β-lactam
FC	Faecal coliform
FIB	Fecal indicator bacteria
FS	Flat sheet
GAC	Granular activated carbon
HF	Hollow fibre
HWW	Hospital wastewater
LOD	Limit of detection
MBBR	Moving bed biofilm reactor
MBR	Membrane bioreactor
MDR	Multidrug resistance
MDRB	Multidrug-resistant bacteria
MEC	Measured environmental concentration
MLSS	Mixed liquor suspended solid
MLVSS	Mixed liquor volatile suspended solids
MTP	Metoprolol
MTPA	Metoprolol acid
NF	Nanofiltration
NSAID	Non-steroidal anti-inflammatory drug
O_3	Ozonation
OLR	Organic loading rate
PAC	Powder activated carbon
PNEC	Predicted no-effect concentration
RO	Reverse osmosis
RQ	Risk quotient
SMBR	Sponge membrane bioreactor

SVI Sludge volume index
VRE Vancomycin-resistant enterococci
WWTP Wastewater treatment plant

1 Introduction

Over the last 10 years, an increasing number of studies have dealt with the chemical, physical and microbiological characteristics of hospital wastewater (HWW), their variability over time (day, month and year), its treatability, the removal of macro- and micropollutants and the risks related to their residues once released into the environment. There have also been discussions among the scientific community about the options to treat hospital wastewater separately or in combination with domestic wastewater. In 2010, an initial comprehensive review on hospital effluent as a source of micropollutants was published [1] and gave a snapshot into what was already known and also underlined the need for further research in a range of fields related to the management and treatment of this effluent. At the same time, some EU-funded projects have shed light on specific issues related to the treatment of hospital effluent. Of these, the PILLS project (https://cordis.europa.eu/project/id/214599/reporting) investigated wastewater quality in terms of selected micropollutants and researched and tested removal methods in order to identify and suggest better wastewater treatment at the source (hospitals and households) [2]. In 2015, another review was published focusing on the lessons learned on the management and treatment of hospital effluent [3], and, in 2018, a book by Springer published a collection of contributions from different expert research groups on hospital wastewater in terms of the occurrence of micropollutants, treatment options and risk assessment [4].

This chapter gives an update on the discussion and aims to highlight the main results achieved worldwide in the management and treatment of hospital effluent over the last 5 years. What emerges is that attention to these topics has involved new countries (including Morocco, Tunisia, Iran, Colombia), which were not among those reported in the previous review. In addition, the scientific debate tends to focus on antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARG) and their correlation with antibiotics and the risks related to the use of the receiving surface water for different needs (mainly for agricultural and drinking purposes). The main results refer to the studies reported on the map in Fig. 1. It clearly shows the countries where the studies took place as well as "knowledge spheres" (occurrence, ARB/ARG, risk, treatment, management, prioritisation) which have contributed to this update. On the same map, it is possible to see the countries where previous investigations on HWW treatments have been conducted, according to the study by [3].

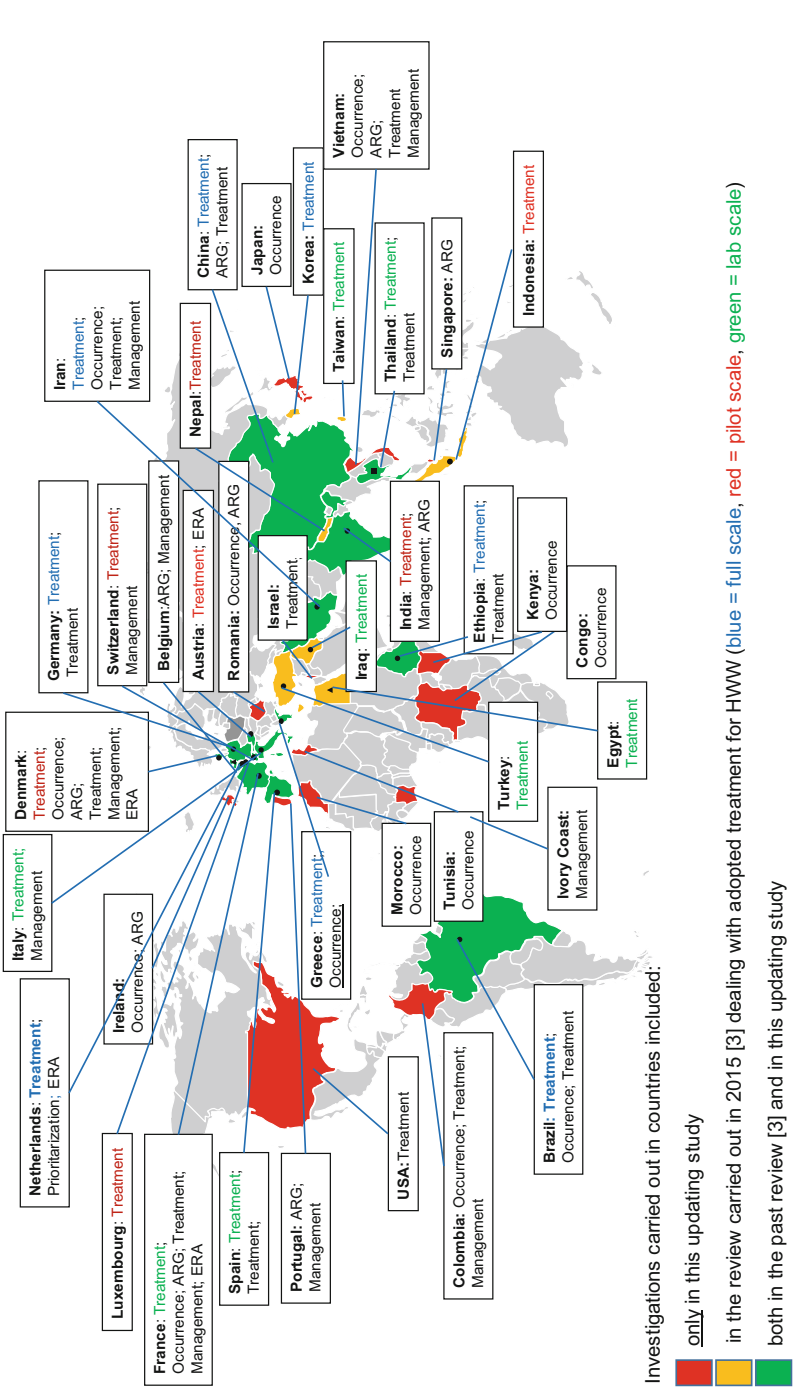


Fig. 1 Worldwide distribution of the studies on hospital effluent included in this chapter (red and green countries) and studies included in the review by [3] (yellow and green countries)

2 Investigations into the Occurrence of Pharmaceuticals in Hospital Effluent

Different classes of pollutants are present in HWW due to the different activities carried out within the healthcare structure. As noted in many studies, including [1, 5, 6], detergents and disinfectants are largely consumed in healthcare structures and represent classes of substances of concern. The occurrence of detergents and disinfectants in HWW was investigated in many cases in European countries [7]. Recently their consumption was analysed in the study conducted by [8] relating to the University Hospital of Marrakesh, Morocco (409 beds, 425 m^3/day). The study also analysed effluent in terms of common macropollutants (BOD, COD, nitrogen and phosphorus compounds), heavy metals and pathogens (bacteria and viruses) in order to evaluate the potential pollutant load and risk posed by the healthcare facility on the environment. It was found that, annually, around 17,100 kg of the detergents and disinfectants used included 25% sodium hypochlorite, 24% mild soap and 18% disinfectant glutaraldehyde. Administered pharmaceutical compounds (PhCs) over the same year exceeded 106,000 kg, with the class of anaesthetics at the top with more than 38,100 kg/year consumed, followed by the class of medicines for infectious diseases and parasitology which required 14,850 kg/year of PhCs. Water samples were taken at 12 different points within the hospital and underwent chemical and bacteriological analysis. Concentrations of copper (2.84 mg/L) and iron (3.38 mg/L) were higher than the standard limits in Morocco. This was due to the extensive consumption of drugs containing such minerals, but also to release from the old drainage pipes of the studied hospital, which was built in 1938. With reference to microorganisms, average concentrations were found of 3.9×10^7 CFU/100 mL for total coliforms, 2.6×10^7 CFU/100 mL for faecal coliform and 4.7×10^6 CFU/100 mL for intestinal enterococci. Regarding pathogens, the highest concentrations were found for *Staphylococcus aureus* (7.5×10^5 CFU/100 mL) and *Pseudomonas aeruginosa* (5.3×10^6 CFU/100 mL) in medical lab effluent and the presence of strains of *Salmonella* in intensive care unit, cardiology and gastroenterology surgery ward effluent, as well as in medical laboratory effluent and, finally, *Vibrio* in surgery and radiology ward effluent. They concluded that it is fundamental to know the characteristics of the hospital effluent in order to provide an adequate pre-treatment prior to its disposal into the public sewer system and to reduce the load directly discharged.

Investigations were carried out on the bacteriological contamination of hospitals and urban wastewaters in Kinshasa, which is the capital and largest city in the Democratic Republic of Congo [9, 10]. In this area, there are 20 hospitals, various medical centres and numerous polyclinics with different intrinsic characteristics. Big hospitals discharge their effluents in the urban drainage systems and then into the river without any treatment. Smaller healthcare structures and medical centres release their wastewater directly onto soil or directly into the river. Analysis carried out by [9] on the effluent of three hospitals showed that the concentrations of *E. coli*

were in the range 4.5×10^5 UFC/100/L and 1.0×10^6 UFC/100 mL and of enterococci between 2.3×10^4 UFC/100 mL and 1.0×10^5 UFC/100 mL.

In the same area, [10] instead investigated the occurrence of toxic metals, ARGs and faecal indicator bacteria (FIB) in the sediments upstream and downstream of hospital effluent release points in the receiving rivers. They found that, depending on the size and activities within the healthcare structure, directly discharged raw hospital effluent leads to an increment in FIB, ARGs and heavy metal concentrations. In some cases, higher values of FIB and ARGs were found in sediment samples upstream of the hospital discharge point. This could be related to the fact that the studied rivers go through the City of Kinshasa and thus other sources could be responsible for the deterioration of the bacteriological quality in the rivers, including open defecation, uncontrolled landfills, unregulated effluent discharges and inadequate sewage collection near the studied sites. The authors remarked that the rivers act as a reservoir of these contaminants and, in tropical conditions like in Congo, they could potentially favour the transfer of mobile genetic elements carrying ARGs to susceptible bacterial pathogens.

Both studies remark the need for the adequate management and treatment of hospital effluent. In Congo, as in most other developing countries, river water is commonly used for both human and animal consumption and is also used for irrigation for fresh urban products. High values of metals, FIB and ARGs in rivers represent a risk to human health and the environment.

Ibrahim et al. [11] investigated the occurrence and frequency of sapoviruses in wastewater from three clinics located in the residential and business area of El Menzeh I, in the centre of Tunis (Tunisia), as well as their removal in the case of rotating biodisks (25 m^3/day, 2 m^3 the volume of the biodisk tank) and in a series of five oxidising ponds (average flow rate 47 m^3/day, overall HRT 13.3 days). As the analysed treatments were able to reduce the content of viruses of only 1 log unit (90 94%), they emphasised the inadequacy of the sanitary quality of these treated effluents for safe discharge into the natural receiving environment and also for indirect reuse purposes. In addition, they also underlined the urgent need for a (chemical or physical) disinfection step for hospital wastewater. The results highlighted that treated effluent may represent a possible route for the transmission of opportunistic pathogenic bacteria and sapoviruses. Lastly, they highlighted the high risk of infection related to this kind of water being released into the environment, if then to be used for agricultural and recreational purposes.

In another area in Africa, the Lake Victoria Basin (Kenya), the detection of the level of common antibiotics (ampicillin, amoxicillin, sulphamethoxazole, chloramphenicol and ciprofloxacin) in hospital lagoon effluents and sludge, as well as in the effluent and sludge of ten local wastewater treatment plants (WWTPs) and receiving rivers and lakes, was the focus of dedicated monitoring campaigns [12]. With regard to hospital lagoon effluent, it was found that selected antibiotics ranged between <0.05 and 0.79 ± 0.05 μg/L (the highest concentrations were due to ampicillin). Average concentrations in the ten sampled WWTP effluents were in the range $0.050.36 \pm 0.04$ μg/L, and the average values found in the receiving water bodies were in the range $0.050.29 \pm 0.02$ μg/L. Antibiotics were found in higher

concentrations in sludges from hospital lagoons (<50 to 276 ng/g) rather than in WWTP sludge (<loq to 145 ng/g) and in sediments collected in the receiving streams (<50 to 88 ng/g).

These data show that the direct reuse of treated wastewater in agriculture could introduce antibiotics into the food chain after plant uptake, and the need to improve wastewater treatment in order to reduce the load of antibiotics released into surface water, sludge and soils is of urgent concern.

Composite samples of the raw hospital wastewater in Tumaco (Colombia) [13] were analysed in three different seasons (October 2016, April 2017 and July 2017). A high variability in concentrations was found, probably due to the different amount of medicines administered to patients during the three sampling campaigns. Acetaminophen was always the compound most present being in the range 10–78 μg/L; the other highest levels were found for five antibiotics, namely, azithromycin, ciprofloxacin, clarithromycin, clindamycin and norfloxacin, with concentrations above 10 μg/L in 8 out of 15 samples. The hospital effluent is directly discharged into surface water. The authors are well aware of the environmental risks related to this practice and comment on the urgent need to adopt adequate treatments for the removal of macro- and micropollutants. In this context, they suggest the adoption of adequate treatments which, according to Giannakis et al. [14], should be light-assisted advanced oxidation processes (AOPs): UV-based (UV, UV/H_2O_2) for developed countries and solar-supported ones for developing countries.

Research efforts have also led to the definition and validation of methods to analyse specific groups of compounds. In this context, Martins et al. [15] proposed an analytical method to analyse statin drugs and [16] proposed another method to analyse psychiatric drugs in water. Both studies then applied their developed methods to sample the effluent of the University Hospital (HUSM) of the Federal University of Santa Maria, RS Brazil**.** This is a medium-large hospital (403 beds, 475 m^3/day as the average flow rate), with a wide range of wards, including psychiatry, oncology, radiology, obstetrics, surgery, neurology and intensive care. The authors sampled the HWW after it had passed through a septic tank. The investigations by [15] on statins confirmed the presence of atorvastatin and simvastatin in all the (treated) hospital effluent samples: atorvastatin was in the range 18.8–35.3 μg/L, with an average of 28.8 μg/L, and simvastatin in concentrations between 30.3 and 38.5 μg/L, with an average of 36.7 μg/L. Compared to those reported by [17] in Norwegian hospitals, these ranges are much higher. This could be explained by the wide use of lipid regulator drugs not only by patients but also by hospital staff and visitors. The study outlined that an environmental risk assessment (on the basis of the risk quotient (RQ) approach that is the ratio between measured environmental concentration (MEC) and predicted no-effect concentration (PNEC)) highlights that atorvastatin and simvastatin concentrations in the treated effluent pose a high risk for the environment and further adequate treatment should be added to the existing septic tank.

Reichert et al. [16] compared the concentrations of six antipsychotic drugs (olanzapine, clozapine, haloperidol, risperidone, pimozide and chlorpromazine) at two sampling points within the hospital sewer network. Point A corresponds to the

psychiatric wing effluent after its passage through an anaerobic/anaerobic septic tank, and point B corresponds to the emergency ward effluent after treatment in the dedicated septic tank. On the basis of the 2-h water samples collected on the first day of the investigation, haloperidol and clozapine occurred at the highest concentrations. Between 4 a.m. and 8 p.m., the largest number of antipsychotics was found in the treated HUSM wastewater. While from 2 a.m. to 4 a.m., no antipsychotic was detected ($\leq$ limit of detection, LOD) at either sampling point. Pimozide was never detected due to its limited use in the hospital. The composite water samples collected during the whole sampling week, from 8 a.m. to 6 p.m., showed that occurrence frequency was around 54% at point A and 40% at point B. The most present compounds were haloperidol and clozapine at both points, and the ranges of occurrence were 0.31–0.52 μg/L for olanzapine, 0.56–0.97 μg/L for clozapine, 1.43–2.73 μg/L for haloperidol and 0.92–0.98 μg/L for risperidone. Chlorpromazine was found only once at a concentration of 0.52 μg/L.

The study also carried out an environmental risk assessment following two approaches: the persistence-bioaccumulation-toxicity (PBT) approach [18] (http://www.pbtprofiler.net/) which assigns a mark from 1 to 3 (3 being the highest value) to each compound on the basis of its persistence, bioaccumulation and toxicity and the risk quotient (RQ) approach. The risk may be low if $RQ < 0.1$, moderate when $0.1 \leq RQ \leq 1$ and high if $RQ > 1$ [19]. The analysis showed that, based on PBT values, the highest risks should be related to the presence of clozapine, pimozide and haloperidol. On the basis of the risk quotient approach, a high risk is correlated to risperidone, clozapine and chlorpromazine. Both sampling points displayed a very high risk, which can be attributed to clozapine, but the risk posed at point A was much higher, owing to the additional occurrence of chlorpromazine and risperidone.

In the South of Iran, at the Bushehr coastline of the Persian Gulf, Kafaei et al. [20] investigated the occurrence of six antibiotics (tetracycline, norfloxacin, azithromycin, anhydroerythromycin, cephalexin and amoxicillin) in the raw effluents of two 85-bed hospitals (H1 and H2) and after being treated only in a septic tank (hospital H1, flow rate equal to 8 m^3/day, HRT 5 days) and in a conventional activated sludge system (hospital H2, with a flow rate of 14.5 m^3/day; HRT 2 days). Composite samples were collected during the dry and wet seasons, by mixing wastewater taken at 7 a.m., 3 p.m. and 11 p.m. Moreover, samples of seawater and marine sediments were analysed in order to evaluate the impact of the release of the treated effluent into the aquatic environment. The results showed that in the raw HWW, norfloxacin had the highest values of concentrations (150 and 198 ng/L). Tetracycline was the second most abundant antimicrobial agent in hospital effluents with its average concentrations being equal to 68 and 144 ng/L. With respect to cephalexin and amoxicillin in the H2 effluent, their mean concentrations were >11-fold and 5-fold higher, respectively, than the corresponding average concentrations in the H1 effluent. These differences could be related to different treatment practices in the two hospitals. Finally, macrolides showed close concentrations in the two hospital effluents. The comparison between the occurrence in the two wastewaters and in the raw domestic wastewater of the town highlighted very lower concentrations of the selected compounds in domestic wastewater. When

investigating removal efficiency achieved by the two (modest) treatments, an average removal of 71% is shown in the septic tank, with concentrations of all selected antibiotics being in the range 2.9−42 ng/L and >84% in the conventional activated sludge system. They concluded that there should be further stages in the treatment of hospital effluent in order to enhance the removal of typical micropollutants.

Also the study carried out in two hospitals in the area of Hanoi (Vietnam) by Lien et al. [21] focused on antibiotics. Metronidazole, sulphamethoxazole, trimethoprim, ceftazidime, ciprofloxacin, ofloxacin and spiramycin were analysed in the raw effluents of a rural hospital (220 beds) and an urban hospital (520 beds). The investigations lasted 1 year and were based on continuous flow samples collected on weekdays of the last week of each month. These effluents are pre-treated on site and then subjected to a biological stage followed by a polishing step. With regard to raw wastewater, it was found that higher overall monthly concentrations of antibiotics were found for the rural hospital effluent where they were present in all the monthly samples. Concentrations ranged from 21.8 to 103.5 μg/L, with an average of 70.5 μg/L, resulting in a mean hospital bed contribution equal to 0.32 μg/(L bed). In the urban HWW, antibiotics were detected in 10 out of the 12 months with very high concentrations only in 3 months (September 151.1 μg/L, October 202.4 μg/L and December 337.3 μg/L). In the remaining months, concentrations were between 8.7 and 39.9 μg/L, with the average monthly value being 93.5 μg/L and a bed contribution of 0.18 μg/(L bed). The compounds most present were ciprofloxacin (on average 42.8 μg/L) in the raw rural hospital effluent and metronidazole (on average 36.5 μg/L) in the raw urban HWW.

The treatment applied to the two HWWs allowed an overall average removal of 49% in the rural and 67% in the urban site. The released amount of antibiotics was calculated for the rural hospital, and, on the basis of the provided water consumption within the structure, it was estimated at 61 g/month.

This result underlines the need to better investigate the risk posed by these residues in promoting antibiotic resistance due to the selective pressure placed on bacteria. Moreover, ARGs carried by bacterial contaminants can be transferred to other bacterial populations including pathogenic bacteria found in hospital wastewater.

In Japan, Azuma et al. [22] carried out an investigation into the occurrence of 41 compounds in HWW, including PhCs and their metabolites, and phytochemicals, of which the pollution status is unknown for the aquatic environment and, in particular, for hospital effluent. The selected target compounds belong to nine different classes: antivirals, antibacterials, anticancers, psychotropics, analgesic-antipyretics, bronchodilators, antipruritic, herbal medicines and phytoestrogens. The authors compared the effluent of a medium-large hospital (477 beds, average flow rate of 460 m^3/day) with the influent and effluent of the WWTP (420,000 people, flow rate of 134,000 m^3/day) to which the HWW is conveyed. From this study, it emerges that for many compounds, concentrations are in similar ranges in HWW and WWTP influent. Some results should be highlighted with regard to analgesics-antipyretics. In HWW, the top compounds are two conjugated metabolites of acetaminophen (acetaminophen glucuronide and acetaminophen sulphate),

the average concentrations of which were as high as over 24 μg/L and about one order of magnitude higher than that of the mother compound. In WWTP influent, acetaminophen was detected at the same order of magnitude as in HWW, whereas in the case of its metabolites, only acetaminophen sulphate was found at a lesser concentration (on average 6.4 μg/L). Loxoprofen and its metabolite loxoprofen alcohol were found at higher concentrations in HWW than in WWTP influent: on average 15 μg/L and 14 μg/L, respectively, in HWW vs 3.5 and 2.3 μg/L in WWTP influent. Finally the study reported that in the ozonated effluent (8.6 mg O_3/L and 100 min as contact time), some recalcitrant compounds still remained above 10 ng/L (on average): 47 ng/L the anticancer bicalutamide, 17 ng/L carbamazepine, 13 ng/L indomethacin, 34 ng/L caffeine and 16 ng/L the antipruritic crotamiton.

Papageorgiou et al. [23] remarked that monitoring campaigns remain a useful tool that is able to provide up-to-date information on the current situation, mainly in those areas where PhC consumption data for the different users (in hospital and in urban settlements) are scarce or difficult to obtain. They carried out a 1-year monitoring campaign (24-h composite samples) on the raw effluent of two medium-sized hospitals in the city of Larissa, in the rural Thessaly region, Greece. Out of the 135 monitored compounds belonging to 37 therapeutic classes (including not frequently monitored substances such as thyroid hormones, antineoplastics, steroid hormones, antivirals, antiparkinsonian agents, anti-vertigo inotropic agents and cardiovascular agents), only 35 compounds were detected in the range $<$ LOD 81.5 μg/L. The highest values were found for the stimulant caffeine (81.5 μg/L), the analgesic paracetamol (31,614 ng/L) and the antiseptic triclosan (10.5 μg/L). In addition, other compounds were detected at high concentration levels ranging from 1.2 to 9.8 μg/L. This is the case for the antibiotics levofloxacin and clindamycin, the antihypertensives irbesartan and valsartan, the non-steroidal anti-inflammatory drugs (NSAID) ibuprofen and diclofenac, the analgesic salicylic acid, the diuretic furosemide, the antidiabetic metformin and the anaesthetic lidocaine. They found that analgesics and anti-inflammatories, antibiotics and antihypertensives followed by antidiabetics, diuretics, psychiatrics, insect repellent and β-blockers seem to contribute more to the PPCP load in wastewaters. An investigation on the removal capacity of the existing WWTPs highlights that they are not able to remove all the key contaminants at a concentration corresponding to a low (acute and chronic) environmental risk.

Interesting results are reported by Szekeres et al. [24] who investigated the effluent of three hospitals, H1, H2 and H3 (with 535, 113 and 453 beds, respectively), in Cluj County in Romania. H2 and H3 currently release their raw effluent directly into the nearby river; H1 effluent instead is subjected to a dedicated treatment (conventional activated sludge followed by chlorination) and is then released into the public sewer network. The authors analysed the occurrence of 14 common antibiotics, 36 ARGs and the bacterial composition of the hospital effluents. They found that antibiotics occurred between 3.67 and 53.05 μg/L; the most abundant were β-lactams, glycopeptides and trimethoprim. Ampicillin was detected in all HWW samples, and the highest concentration was found in H1 (53.05 $\pm$ 0.08 μg/L), confirming the results found in other studies [12]. High

concentrations of trimethoprim were also detected in H1 (13.06 ± 0.09 μg/L) and H3 (13.06 ± 0.09 μg/L) wastewater samples, together with vancomycin (13.98 ± 0.41), imipenem (14.42 ± 0.39 μg/L) and tazobactam (10.26 ± 0.08 μg/L) in H3 wastewater samples. Ciprofloxacin and norfloxacin were never detected.

As for ARGs, out of the 36 genes initially investigated during the prescreening process, 16 genes were detected in hospital wastewater samples. Fourteen genes confer resistance to β-lactams, aminoglycosides, chloramphenicol, macrolide-lincosamide-streptogramin B antibiotics, sulphonamides and tetracyclines. Genes encoding quaternary ammonium resistance and a transposon-related element were also detected. The *sul*I and qacED1 genes, which confer resistance to sulphonamides and quaternary ammonium, had the highest relative abundance with values ranging from 5.33×10^{-2} to 1.94×10^{-1} and 1.94×10^{-2} to 4.89×10^{-2} copies/16 rRNA gene copies, respectively. The abundance of the investigated ARG types versus classes of antibiotics administered in the three hospitals shows an apparent similar pattern. This leads to the consideration that the three hospital effluents may contain enough antibiotics to be able to exert selective pressure on bacteria species.

Regarding bacterial composition in the HWW, the dominant phyla detected were *Proteobacteria, Bacteroidetes, Firmicutes* and *Actinobacteria*. Finally, after the treatment (activated sludge process + chlorination) moderate removal efficiency of the studied pollutants was demonstrated, with a 55–81% decrease in antibiotic concentrations, 13 orders of magnitude lower than the abundance of ARGs, but with a slight increase in *Proteobacteria* phyla, from 97.5 to 98.7%, and *Bacteroidetes* phyla from 0.1 to 0.5%. It was also found that after the treatment, abundance of *Enterobacteriaceae* increased from 1.1 to 10%.

A monitoring investigation carried out by Lerat-Hardy et al. [25] between 2013 and 2016 on the effluent of different wards of the 1,500-bed Pellegrin hospital in the Bordeaux conurbation (France) which focused on rare-earth element concentrations showed that their concentrations may vary among the departments and, for most of them, the concentrations are much higher than in the surface water. As for gadolinium (Gd, a paramagnetic contrast agent for medical imaging methods such as magnetic resonance imaging), a maximum concentration of 98 μg/L was measured in the effluent from the surgical departments, being almost four orders of magnitude greater than the concentrations in the Garonne River. In addition, the study analysed the trend of Gd fluxes over a 15-year period in Garonne River water and found a continuous increment (from 413 ng/L in 2003 to 820 ng/L in 2017) which is probably related to the growing regional population and the increased number of MRI scanners. Other rare-earth elements Ce, Gd, Tb, Er and Tm were detected at relevant concentrations in the hospital effluent. These results lead to the consideration that, as hospitals contribute significantly to the emission of these elements, in particular Gd, further studies should be carried out on the chronic effects they have on the aquatic organisms living in the receiving water bodies.

A complete characterisation of the effluent of the large Danish Herlev Hospital, near Copenhagen (700 beds), is provided in the report by DHI [26]. This covered 40 PhCs, largely consumed in the healthcare facility, heavy metals, pathogens, antibiotic-resistant bacteria and oestrogenicity (expressed as 17β-oestradiol

Table 1 Hazard profile of the raw effluent of Herlev Hospital, near Copenhagen, Denmark

Parameters	Reasons
Ciprofloxacin, clarithromycin, sulphamethoxazole, diclofenac, capecitabine	Their concentration was 10–300 times higher than the corresponding PNEC in fresh water for water living organisms
Iomeprol	High concentration (2.5–7 mg/L)
ARG Cefotaxime-resistant *E. coli* Ciprofloxacin-resistant *E. coli* Vancomycin-resistant enterococci	High concentrations 1.0×10^5 9.8×10^5 (mean 4.4×10^5) MPN/100 mL 4.6×10^5 1.7×10^7 (mean 7.1×10^5) MPN/100 mL 1.5×10^5 1.1×10^6 (mean 4.8×10^5) MPN/100 mL
Norovirus	High concentrations genome copies/L (211 6.2×10^5, mean 1.7×10^5)
Zebra fish	100% mortality within 96 h
Daphnia	All test animals died
Estrogenic activity	Oestrogen effects

equivalents, EEQ). The highest mean concentrations were found for iomeprol (iodinated contrast medium) found at 2.9 mg/L, acetaminophen at 352 μg/L and ciprofloxacin at 13 μg/L. As for heavy metals, zinc and copper occurred at the highest concentrations, 100 and 110 μg/L, respectively, and lead and nickel at 4.0 and 4.8 μg/L, and as for estrogenic activity, it was found to be in the range 0.16–44 ng EEQ/L in the effluent. Regarding microorganisms, *E. coli* were in the range $3.1 \cdot 10^6$ $1.0 \cdot 10^7$ MPN/100 mL (on average 5.5×10^6), enterococci in the range 6.8×10^6 1.5×10^7 MPN/100 mL (on average 9.5×10^6) and norovirus between 211 6.2×10^5 genome copies/L (on average 1.7×10^5).

Finally, cefotaxime-resistant *E. coli* were in the range 1.0×10^5 9.8×10^5 MPN/100 mL (on average 4.4×10^5 MPN/100 mL), ciprofloxacin-resistant enterococci 4.6×10^5 1.5×10^7 MPN/100 mL (on average 7.1×10^5 MPN/100 mL) and vancomycin-resistant enterococci 1.5×10^5 1.1×10^6 MPN/100 mL (on average 4.8×10^5 MPN/100 mL).

The hazard profile of the raw Herlev Hospital effluent was related to the compounds reported in Table 1.

Lasek et al. [27] investigated the occurrence of biocides in the effluent of the large (1,900 beds) hospital in Poitiers in France at different points and in the final outfall. Large quantities of biocides are used daily in hospitals for hygiene purposes, to disinfect surfaces and medical instruments and to clean linen (if a laundry is present [1]), and also as antiseptics in healthcare procedures. They found that biocides are continuously discharged into the hospital sewage network over the 24 h, with higher quantities discharged during the day because of the greater number of healthcare and facility cleaning activities taking place during this period. The monitored biocides include chlorhexidine digluconate (CHD), bis(aminopropyl)laurylamine (BAPLA) and didecyldimethylammonium chloride (DDAC); only the latter was found in concentrations higher than its PNEC in the hospital sewage outfall. Due to the continuous release, biomagnification phenomena are envisaged.

Finally, some research works discussed the possibility of predicting hospital concentrations on the basis of the consumption of PhCs within the healthcare structure [28–30]. Of these, Verlicchi [29] compared, measured and predicted concentrations and loads in HWW with a focus on the strengths and weaknesses of the two approaches and discussed the main factors responsible for inaccurate predictions and uncertain measures on the basis of some case studies.

3 Investigations on Occurrence of Antibiotic-Resistant Genes and Bacteria (ARG and ARB)

Monitoring campaigns confirm the presence of different antibiotics in HWW which are generally directly discharged into the public sewer systems without any pre-treatment or, in some countries, are even discharged directly into the surface water body without treatment. Many pharmaceuticals, radionuclides and antimicrobial agents may contain heavy metals in their formula. The capability of a microorganism to stay alive and replicate in a heavy metal polluted environment depends on genetic/or physiological alteration. In particular, bacteria can promote resistance to an antimicrobial agent, for instance, by the production of extracellular enzymes which are able to eliminate antibiotic and metal toxicity. The interesting studies carried out by [10, 31, 32] note that there "has been a significant hypothesis regarding conceivable hereditary relationship among microbial *resilience* for these heavy metals and multidrug resistance". For instance, penicillins, carbapenems, cephalosporins and monobactams are recognised synthetically as beta-lactam anti-infection agents, and numerous microorganisms become resistant to these drugs by producing different beta-lactamases that are fit to inactivate a few types of these anti-infection agents.

The fact that antimicrobials exert a continuous selective pressure upon ARB is worrying the scientific community. The World Health Organization has recently included some ARB (including *Acinetobacter baumannii*, carbapenem-resistant; *Pseudomonas aeruginosa*, carbapenem-resistant; *Enterobacteriaceae*, carbapenem-resistant, third-generation cephalosporin-resistant with priority 1 and *Enterococcus faecium*, vancomycin-resistant with priority 2) on the critically important priority list of pathogens for which new antibiotics are necessary [33].

Antimicrobial selective pressure favours the intraspecies and interspecies horizontal transfer of resistance genes [34]. Antibiotic resistance causes prolonged illness, excess mortality and higher costs for patients and health systems. This is why recently the United Nations General Assembly remarked that antibiotic resistance is among the greatest global health risks, requiring urgent attention [35]. This risk could be higher in low and middle developing countries where HWWs are not properly managed and where they are often directly released into surface water bodies which are then used for irrigation purposes, promoting the spread of ARB and

ARG into the environment, but also for domestic uses, sometimes without any treatment [36].

Samples taken from the effluent of the King George's Medical University, in Lucknow, India, and analysed by Alam et al. [31], showed an elevated number of one and more antimicrobial tolerant coliform bacterial species. Selected isolates of these bacteria were recorded to have variable plasmids which encoded with the metal and drug resistance. The study concluded by remarking that the hospital aquatic environment may act as a possible source of resistance transfer among the pathogens as well as to humans. The finding of this investigation shows a potential medical issue as the prevalent coliform species has progressively been related to the outbreak of contamination at doctors' facility. It is prescribed that healthcare effluent must be treated before its discharge into the environment.

Faecal coliforms (FC), carbapenem-resistant *Enterobacteriaceae* (CRE) or carbapenemase-producing *Enterobacteriaceae* (CPE) producing $bla_{\text{NDM-1}}$ and selected extended-spectrum β-lactam (ESBL)-resistant bacteria (bla_{CTX}, bla_{OXA}, bla_{TEM}) and genes (*int1*) were investigated in the effluent of 12 hospitals of different sizes and in five sewer drains (with no hospital) across New Delhi, India, over two seasons (winter and summer) [37]. A close association was found between faecal bacterial concentrations in HWW and concentrations of CRE and ESBL-resistant isolates and genes, which are released into the environment. Concentrations were higher in winter than in summer, with $bla_{\text{MDN-1}}$ abundance exhibiting the highest differences (from three to nine orders of magnitude). Larger hospitals were found to release significantly higher concentrations of FC, CRE and $bla_{\text{NDM-1}}$ on a mass-per-wastewater volume basis, confirming the findings by [10].

Finally, concentrations in HWW are higher than those found in the sewer network not receiving HWW, and the greatest difference refers to $bla_{\text{NDM-1}}$: almost nine orders of magnitude.

The literature survey by [32] reports that major multidrug-resistant bacteria (MDRB) recovered in hospital effluents are extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, vancomycin-resistant enterococci (VRE) and *Pseudomonas aeruginosa*. Moreover, they highlighted that conventional WWTPs are able to reduce the concentrations of *E. coli* but the proportion of ESBL-producing *E. coli* among *E. coli* increases significantly with treatment (0.30% in the inflow versus 0.61% in the outflow).

Attention should also be posed to the municipal treated sludge, often used as a fertiliser: it was found that a gram of the anaerobically digested sludge at the municipal WWTP in Besançon (France) contained on average 2.6×10^5 ESBL-producing *E. coli* [38].

The efficacy of wastewater treatment in reducing the content of multidrug-resistant bacteria is under discussion: some studies reported that the total load of enterococci decreases during wastewater treatment without a substantial change in species distribution, *E. faecium* being predominant [39]. Others found that there is an increment in the proportion of *E. faecalis* and *E. faecium* resistant to fluoroquinolone [40]. By contrast, the study carried out by Magiorakos et al. [41] reported that wastewater treatment efficiently reduced the concentration of VRE.

Table 2 Main results of the analysis of prevalence of antibiotic resistance in rural and urban raw and treated HWW [36] and of ARGs

Antibiotics	Raw rural HWW	Treated rural HWW	Raw urban HWW (%)	Treated urban HWW (%)	Overall, both hospitals
E. coli isolates (#)	84	74	60	47	265
Antibiotic with the highest prevalence of antibiotic resistance	Co-trimoxazole	Co-trimoxazole	Co-trimoxazole	Co-trimoxazole	Co-trimoxazole
Prevalence to at least 1 antibiotic (%)	94	74	88	68	
MDR (%)	44	26	32	21	32
ESBL-producing *E. coli* (#)	45	31	27	12	115
Ciprofloxacin-resistant *E. coli* (#)	21	26	14	8	69

Lien et al. [36] compared the antibiotic resistance of raw and treated effluents from a rural and an urban hospital in the area of Hanoi in Vietnam. They analysed the prevalence of resistance to at least one of the selected antibiotics (amoxicillin/clavulanic acid, ceftazidime, ceftriaxone, ciprofloxacin, co-trimoxazole (trimethoprim/sulphamethoxazole), fosfomycin, gentamicin and imipenem), to each studied antibiotic, and multidrug resistance (MDR) in *E. coli* isolates. The bacterial isolates that resulted as non-susceptible to at least one agent in three or more antibiotic categories (MDR $\geq$ 3) were considered multidrug-resistant, according to [42]. The collected results, summarised in Table 2, showed that in both HWWs (raw and treated), the highest prevalence of antibiotic resistance was for co-trimoxazole and the prevalence to at least one antibiotic was higher in percentage for rural HWW (raw as well as treated). MDR was found again in most cases for rural HWW.

Prevalence of resistance to the studied antibiotics in *E. coli* isolates from treated HWW was less common than raw HWW, with the only exception of ciprofloxacin and fosfomycin in the rural hospital, for which enrichment of resistant *E. coli* isolates after wastewater treatment was found. As for ARGs in the collected samples (ESBL-producing and ciprofloxacin-resistant *E. coli* strains), it was found that in the rural hospital, 76 *E. coli* isolates were ESBL-producing (48%) and, in particular, bla_{TEM} was detected in 97% of isolates and $\text{bla}_{\text{CTX-M}}$ in 76%. Both $bla_{\text{CTX-M}}$ and bla_{TEM} were detected in 75% of isolates. Quinolone-resistant gene (q_{epA}) was detected in 72% of ciprofloxacin-resistant isolates. All three genes (q_{epA}, $bla_{\text{CTX-M}}$ and bla_{TEM}) were detected in 51% of ciprofloxacin-resistant isolates. In the urban hospital, 39 *E. coli* isolates were ESBL-producing (36%), and, in particular, bla_{TEM} was detected in 95% of isolates and $\text{bla}_{\text{CTX-M}}$ in 41%. Both $bla_{\text{CTX-M}}$ and bla_{TEM} were detected in 41% of isolates. Quinolone-resistant gene (q_{epA}) was detected in 86% of ciprofloxacin-resistant isolates. All three genes (q_{epA}, $bla_{\text{CTX-M}}$ and bla_{TEM}) were detected in 36% of ciprofloxacin-resistant isolates. These results demonstrate the

need to better investigate the presence of ARB and ARG in the treated effluent of the hospital and their occurrence in the receiving environment.

A recent investigation carried out in Sichuan province in China [43] highlighted the prevalence of ESBL- and carbapenem-resistant *Enterobacteriaceae* and non-*Enterobacteriaceae* species (such as *Acinetobacter* and *Aeromonas*) in hospital sewage systems and in the receiving waters and concluded that hospital WWTP effluent could be an important source of ARB propagation into the environment. The study suggested that efficient disinfection techniques are expected to improve the elimination of ARB during wastewater treatment, and closer monitoring of the quality of treated effluents is needed to minimise the dissemination of antimicrobial resistance into the receiving environment.

In Singapore the presence of densely urbanised areas in close proximity to ambient water sources might facilitate the spread of antibiotic resistance. In order to shed light on this issue, Haller et al. [44] analysed the prevalence of carbapenem-resistant bacteria (CRB) and ESBL-producing strains in the effluent of two hospitals: H1 (1,597 beds) and H2 (1,500 beds). They then characterised the isolated strains and identified which strains are responsible for the resistance. They sampled two blocks in H1, block H1a (corresponding to an isolation ward and clinical specialty wards) and block H1b (corresponding to general wards), whereas in H2, they analysed the mixed water of the whole healthcare structure.

The results showed the relatively high prevalence of *Enterobacteriaceae* as well as non-*Enterobacteriaceae* species (such as *Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Aeromonas hydrophila*) resistant to modern extended-spectrum cephalosporins and carbapenems in the sampled hospital effluents. Moreover, it emerged that among the isolates resistant to these antibiotics, a high proportion of ESBL and carbapenemase producers were detected in all the hospital effluents and most of these strains exhibited a multidrug resistance phenotype. Non-disinfected hospital effluents carry significant quantities of antibiotic-resistant bacteria to municipal sewage. These findings lead to the identification of strategies able to control and limit the spread of antimicrobial resistance from hospital effluent and thus to the adequate treatment of this major source of antibiotic-resistant bacteria.

With regard to recent European investigations, there are interesting results within the SIPIBEL project at the pilot site of Bellecombe in France. In this site, in Scientrier, Haute-Savoie, France, the existing treatment plant receives and separately treats hospital effluent (146 m^3/day on average from a 450-bed hospital) and urban wastewater (5,355 m^3/day from a catchment area of 21,000 inhabitants) in two lines adopting the same conventional treatments (activated sludge processes). This configuration offers the possibility to carry on investigations regarding raw and treated HWW and urban wastewater characteristics, removal efficiencies in the two treatment trains and the environmental impact of the residual pollutant load in the two streams. In this context, Laquaz et al. [45] found that the relative abundance of the class 1 integrons was much higher in HWW than in the urban sample, from 0.5 to 4 in HWW and from 0.1 to 0.3 in UWW. The mixture of HWW and urban wastewater in the proportion of 1/3–2/3 did not show to have a synergetic or

antagonist ecotoxic phenomena, nor does it promote the spread of antibiotic resistance.

In Portugal, Vaz-Moreira et al. [46] analysed the antibiotic resistance prevalence in the effluent of a large hospital (1,120 beds) and in raw and treated wastewater generated in the surrounding catchment area. They found that the prevalence of resistance to at least one antibiotic of three or more distinct classes (MDR $\geq$ 3) ranged from 91.7 to 72.4%, with the highest value being for hospital effluent followed by raw wastewater and then treated wastewater. Moreover, the resistance to antibiotics belonging to six or more different classes (MDR $\geq$ 6) was significantly higher in hospital effluent than in municipal wastewater.

As for the detection of carbapenemase-producing bacteria and ARGs conferring resistance to carbapenems, monitoring campaigns involved non-clinical environments including WWTP effluent. Among them, in Ireland, Cahill et al. [47] investigated the occurrence of CPEs in the effluent of a 693-bed hospital as well as in municipal sewer system before and after the emission of the hospital effluent. They were present in all the HWW samples. They found that hospital effluent represents the major source of CPEs in municipal wastewater and remarked that the need for the monitoring of hospital wastewater for CPEs could have important applications in the detection and risk management of unrecognised dissemination of CPEs in both the healthcare network and the environment. Moreover, according to a recent study by EPA [48], there is evidence that there should be regulations with regard to the release of hospital effluent into the municipal waste system in order to prevent the encouragement of antimicrobial resistance dissemination. In Belgium, Proia et al. [49] investigated the prevalence and spread of carbapenemase genes (CGs: bla_{KPC}, bla_{NDM} *and* $bla_{OXA\text{-}48}$) from its source in hospital wastewater to the river water after passing through the two WWTPs and made a distinction between particle-attached bacteria and free-living bacteria as bacteria lifestyle (attached to a particle or free in the river water) influences the behaviour in the environment. They found that the absolute abundances were the highest in HWW and they can be significantly reduced in WWTP effluents, but the relative abundances (normalised per 16S rRNA) were never lowered through wastewater treatment. Particularly, for the particle-attached bacteria, the relative abundances were significantly higher in the effluents with respect to the WWTP influent for all the genes. They concluded that the levels of carbapenemase genes are significantly lower than other genes conferring resistance to more widely used antibiotics, but they could rise to the levels of high prevalent resistance genes.

4 Investigations on Hospital Effluent Management and Treatments

Over these last 5 years, treatments of hospital effluent have been investigated in many countries, by different research groups, testing consolidated and modified technologies as well as new systems. What appears is that research has also been carried out in countries for which scarce information had been available on this matter. This is the case for Columbia [13], Iran [50] and Thailand [51] among the collection discussed herein. The investigations referred to the whole hospital effluent, the effluent from specific wards/departments inside the hospital or effluent from specific healthcare structures, such as nursing homes specialised in psychiatric diseases [52], as well as service units, for instance, laundry [53]. Technologies were tested with regard to different key compounds: from macropollutants to micropollutants and pathogens, from antibiotics to ARB and ARG.

Vo et al. [51] investigated the removal of acetaminophen from hospital effluent by a pilot vertical flow constructed wetland in Pathum Thani, Thailand. This compound was investigated as it frequently emerges in hospital effluent and its consumption is very high all over the world. The peculiarity of their study is the attempt to find a correlation between acetaminophen and peroxidase enzymes which seem to have an important role in the removal of (micro)contaminants, acting as catalysts for their degradation reactions. It was in fact found that these enzymes are produced within the plant species in the case of (micro)contaminants accumulating in their body and then causing stress and altering their biochemical system. In these circumstances, the plants induce the generation of reactive oxygen species, such as H_2O_2. But in the case of an overproduction of these species, the macromolecules, including nucleic acids, proteins and lipids, may be damaged. For these reasons, the plants trigger the antioxidant systems with the production of different peroxidase enzymes which are catalysts in the oxidation reactions between H_2O_2 and organic contaminants, thus reducing the stress of the plants. In their investigation, the hospital effluent fed to the constructed wetland (vegetated with *Scirpus validus,* size, $1.5 \times 0.6 \times 0.6$; retention time, 5 days; flow rate, 7,585 L/day) was doped with a stock solution of acetaminophen up to 10 mg/L. On the basis of the 65-day investigation, it emerges that the peroxidase enzymes of *S. validus* planted in CW were feasible for monitoring acetaminophen. Further studies should evaluate if a correlation can be suggested for other micropollutants as this promising result could help reduce the costs for monitoring many micropollutants.

Other researchers [54] in South Ethiopia analysed the capacity of a subsurface flow bed filled with broken bricks in removing macropollutants (BOD, COD, nitrogen compounds and phosphorus) from a HWW. The bed was filled with broken bricks and fed with the effluent of Hawassa University Referral Hospital. The results showed that broken brick provides better removal of TKN, ammonia, nitrate and phosphate from wastewater than gravel and also provides better adsorption sites for ammonium, nitrate and phosphate. The results confirmed the possibility of using

Table 3 Main results collected by SeMPAC© technology

Compound	SBR	SBR + MF	SBR + MF/PAC (5 days after PAC addition)	SBR + MF/PAC (28 days after PAC addition)
Ibuprofen	>95%	>95%	>95%	>95%
Diclofenac	~45%	75%	>90%	87%
Carbamazepine	<0	<0	>90%	24%
Trimethoprim	<0	<0	>90%	75%
EE2	>95%	>95%	>90%	>90%

local materials (bricks) for filling the beds of constructed wetlands and to remove macropollutants from hospital effluent.

Apart from a few cases dealing with nature-based solutions for the treatment of HWW, most of the investigations referred to membrane technology: membrane bioreactor (MBR) coupled with other technologies acting as a polishing step; modified MBR, or MBR integrated with added materials; and pre-treatments to an MBR to improve the removal of target compounds. The main results are presented here and discussed.

Interesting results were achieved by the Spanish group of research [55] who tested the SeMPAC® process with HWW. SeMPAC® is a new hybrid system they developed (patents ES 2362298 B2, EP 2960214 A1) in 2012 and licensed to the Suez Group for the treatment of effluent containing recalcitrant compounds. It consists of a sequential batch reactor (SBR) followed by an external submerged microfiltration membrane compartment in which powdered activated carbon (PAC) is added to promote the removal of more persistent substances. The pilot plant used in the investigation included a homogenisation tank (to guarantee the homogenisation of the wastewater hydraulic and pollutant loads), an SBR (operating at 6 g VSS/L, HRT 16 h), a stand-by tank (to guarantee the continuous feed to the membrane compartment) and the chamber with the membrane module (pore size 0.2 μm, total surface 6.4 m^2, 7 min of permeation and 1 min of relaxation). Five PhCs were selected to investigate: ibuprofen, EE2, diclofenac, carbamazepine and trimethoprim.

The study lasted 150 days with the first 56 days with no PAC addition and the remaining 94 days with PAC (dosed at 1 g/L). Once added, the PAC particles integrated into the sludge flocks, strengthening their structure and increasing sludge settleability and filterability. In addition, they favour the development of a biofilm on the particle surface, and an enhancement of the microbial diversity was also found. At the same time, filamentous bacteria reduced.

An analysis of the biomass in the bioreactor with PAC revealed the presence of different species, including slowly growing species, such as worms and tardigrades, amoebae (related to nitrification), the protozoa *Carchesium polypinum* (indicators of high-quality effluent) and rotifers (able to improve flock growth because they can consume free cells, as well as small flocks).

As for the observed removal efficiencies for selected PhCs, the main results are summarised in Table 3. It emerges that without PAC addition, the readily

biodegradable compound (ibuprofen) is efficiently removed in the biological reactor (SBR) and the following step of microfiltration does not significantly increase its removal (always >95%). For EE2, the removal was 80% in the SBR followed by an additional 15% in the membrane chamber; diclofenac achieved an important removal efficiency in the whole process (around 70%). These overall removal values are not generally observed in biological processes [56]. As for persistent compounds (carbamazepine and trimethoprim), no removal was found in these conditions. The presence of PAC enhances their removal: 5 days after PAC addition, high removals were observed for all the key compounds, but after 28 days, a rapid decrement was observed for carbamazepine (down to 24%) and trimethoprim (75%) and a slight worsening for diclofenac (87%). These values suggest that PAC was saturated of the different compounds after different time intervals and further sorbent should be added to maintain and guarantee the high removal observed with fresh PAC. Based on these tests, PAC should be added every 20 days in order to guarantee a removal of 50% at least for all the selected compounds.

Finally, the study remarked that the main operational conditions might be optimised on the basis of the behaviour of the different key compounds: (1) the recalcitrant carbamazepine defines PAC dosage; (2) the easily degradable ibuprofen sets duration of the aerobic cycle in the SBR; and (3) the denitrification efficiency defines the correct time length of the anoxic period.

Lima et al. [57] investigated the efficiency of a particular activated carbon in removing a target pharmaceutical (acetaminophen) from synthetic water, simulating hospital wastewater containing different pharmaceuticals (acetaminophen, enalapril, nicotinamide, diclofenac and metformin) and organic and inorganic salts. The tested activated carbon was obtained from the residual biomass of Brazil nut shells, chemically activated with $ZnCl_2$. The lab tests evaluated the sorption capacity with feeding containing up to 80 mg/L of acetaminophen and 30 mg/L of the other PhCs. It was found that the chemical activation with $ZnCl_2$ leads to activated carbons with better textural characteristics and much higher sorption capacities with regard to the target compound if compared to commercial and commonly used activated carbons.

In addition, it was also found that the proposed adsorbent can be regenerated up to 74% with a mixture of NaOH 0.1 M + 20% EtOH solution and reused up to four cycles.

In China, the most adopted treatment train for hospital effluent includes an MBR followed by a chemical disinfection. This sequence complies with the Chinese standards for the release of the hospital treated effluent in surface water bodies. Investigations are still ongoing to optimise the operational conditions with regard to the characteristics of local hospital effluent. Recently, great efforts have been made to find technologies able to better control the dissemination of resistant bacteria, including extended-spectrum β-lactamase-and carbapenemase-producing bacteria, from hospital sewage into the environment [43].

Combining treatments including MBR and a further filtration step (nanofiltration (NF), or reverse osmosis (RO)) is becoming a strategy of increasing concern. In this context, at Purpan Hospital in Toulouse (France). Lan et al. [58] studied the combination MBR + NF and identified the key characteristics of the MBR effluent

which control fouling and flux decline. They also analysed the relationship between the content of salt (calcium and phosphate) in the permeate with the stabilised fluxes in the following NF step. In their 145-day experimental campaign, the lab scale MBR (500 mL), fed by raw hospital effluent, was equipped with immersed hollow fibre membranes (polysulfone 0.2 μm, surface of 7 m^2) and operated with an SRT of 40 days and an HRT of 24 h. The permeate was sent to a 10-L feeding tank which supplied the cross-flow nanofiltration unit where the flat feet membrane (NE 70 polyamide composite 1.4×10^{-2} m^2) was installed.

Filtrations were conducted at constant transmembrane pressures (535 bar) with a 0.3 m/s cross-flow velocity. Two types of fouling were observed depending on the pH range: silica and colloidal organic fouling when pH was in the range 6.05–6.8 and a combination of silica and colloidal organic fouling and calcium phosphate scaling when pH was between 7.20 and 8.27. The measured stable fluxes varied between 60 and 10 L/m^2 h depending on the type of compounds (calcium and phosphate) fouling the membrane and their distribution on the surface. Extremely low values appeared when calcium and phosphate scaling evenly occupied the entire membrane surface; greater stable fluxes (120–80 L/m^2 h) were obtained when only silica and organic fouling occurred. A correlation was found between the NF stable flux values for filtration of various MBR effluents on the basis of calcium and phosphate concentrations after judgement of the pH of the effluents.

At the Military Hospital in Ho Chi Minh (Vietnam), Tran et al. [59] investigated a similar combination. A pilot plant (MBR + NF) was in fact installed to treat the effluent (50 L/day) from the operating rooms of the healthcare facility. MBR characteristics: were: concentration of the mixed liquor volatile suspended solids (MLVSS) between 3.5 and 4.5 kg/m^3, hollow fibre microfiltration membrane equipped in the reactor, throughput of 12–18 L/m^2 h; pore size 0.1 mm, operating pressure $\leq$ 40 kPa, SRT of 40 days; as for NF modules: pore size equal to 1 nm, flux of 6–8 L/h. The system operated at three applied organic loading rates (OLR) of 0.5, 1.5 and 2.5 kgCOD/m^3 day. The excellent removal efficiencies observed for common organic pollutants (around 9,195% for COD), nutrients (9,098%for phosphorus, 9,598% for total iron) and microorganisms (4 log units for *E. coli* and 6 l.u. for coliforms) led the authors to think that the tested combination is a promising solution for the control of macropollutant release in the environment from hospital effluent. Nanofiltration was preferred to reverse osmosis due to the lower investment and operational costs, its capacity to retain high-valence ions while allowing monovalent ions to pass, the lower pressure required and the lower pressure requested and due to the fact that NF allows backwashing, which could significantly reduce the frequency of membrane fouling.

The combination MBR + ozonation (O_3) + granular activated carbon (GAC) filtration + UV irradiation was considered the best of the available technologies according to the results from the *Danish* project on hospital effluent treatment carried out between 2012 and 2015, which led to the construction of the full-scale treatment plant at Herlev Hospital (700 beds, 150 m^3/day). As stated in the report by DHI [26], the polishing sequence (O_3 + GAC + UV) was preferred to the GAC + O_3 + UV sequence, which was tested in parallel at the WWTP under construction, as it

allowed greater removal of the key compounds from the permeate and lower operational and maintenance costs. With the adopted sequence, more than 99% of iodinated contract media – the most recalcitrant compounds, such as iomeprol, iohexol and iopamidol, which are largely administered at the Danish hospital – were also removed. After the treatment, oestrogenic activity in the range 0.16–44 ng EEQ/L in the influent samples was significantly reduced to <0.076–1.1 ng EEQ/L. As for heavy metals, their concentrations in the polished effluent were found below the environmental quality standards (EQS) for inland surface water. Disinfection was efficiently guaranteed by the MBR and ozonation steps. The ozonated effluent did not contain *E. coli* and coliforms at 37°C, but only culturable bacteria at 22 and 37°C, which were therefore assumed to be related to regrowth of bacteria in the polishing lines after membrane filtration. The UV reactor worked with a UV dose of 6–10 mJ/cm^2, which is lower than the common dose adopted in water works (around 40 mJ/cm^2). It was found that an applied dose of 6–10 mJ/cm^2 was sufficient to remove culturable bacteria at 22 and 37°C.

Paulus et al. [60] demonstrated that the O_3 + GAC + UV sequence provides an effective barrier effect against ARG contained in the HWW. They investigated the removal of ARG from hospital effluent pre-treated in an MBR by means of the patented Pharmafilter system technology (reproducing the same treatment technologies adopted for the full-scale WWTP receiving the effluent of Herlev Hospital in Denmark). They found the treatment reduced the ARG from 13 to 4 and the number of quantified antibiotics from 17 to 7. They agreed with the consideration that on-site treatment of a high-risk source of effluent was proved to be advantageous with regard to antibiotic as well as ARG reduction. Thus, legislative guidelines and suggested best practices should move in the direction of implementing on-site wastewater treatment.

The good performance reported by Ngo et al. [61] regarding the application of sponge media with high porosity (polyester-urethane sponge) within the aerobic compartment of an MBR (SMBR) in order to improve macropollutant removal and reduce membrane fouling resulted in testing the performance of this modified MBR with regard to micropollutants. In this context, the removal of antibiotics from the effluent of Trung Vuong Hospital (Vietnam) was investigated by Nguyen et al. [62]. They compared the performance of lab-scale MBRs (two reactors of 8 L, operating with a concentration of biomass of around 5,000 mg/L, with a ratio between volatile and total suspended solids (MLVSS/MLSS) equal to 0.79 and a sludge retention time of 20 days) equipped with hollow fibre (HF) and flat sheet (FS) membranes with the same pore size (0.4 μm) and surface area (0.1 m^2), operating at different high fluxes (10, 15 and 20 L/m^2 h). The analysed antibiotics and the range of concentrations in the raw HWW fed to the systems were norfloxacin (6.305–43.610 μg/L), ofloxacin (7.634–40.261 μg/L), ciprofloxacin (1.926–23.841 μg/L), sulphamethoxazole (0.378–2.078 μg/L), erythromycin (0.135–2.407 μg/L), tetracycline (0.036–1.612) μg/L and trimethoprim (0.676–2.911 μg/L). The results are reported in Table 4 in terms of ranges of concentration in the permeate from the two SMBRs and ranges of removal efficiencies observed in the two small reactors.

Table 4 Ranges of concentrations in the permeate and removal efficiencies achieved in SMBR equipped with HF and FS membranes and operating with a flux in the range of 10–20 L/m^2 h [62]

Antibiotic	SMBR-FS permeate (μg/L)	SMBR-HF permeate (μg/L)	SMBR-FS removal (%)	SMBR-HF removal (%)
Norfloxacin	0.07–0.10	0.08–0.09	93–99	62–86
Ofloxacin	0.2–2.1	0.22–6.73	73–93	68–93
Ciprofloxacin	0.75–8.52	0.69–8.12	76–93	54–70
Trimethoprim	0.049–0.494	0.058–0.809	60–97	47–93
Tetracycline	0.0001–0.106	Not detected		
Erythromycin	0.085–0.647	0.137–1.274	67–78	22–48

The slightly higher removal efficiency observed in flat sheet SMBR seems to be due to the higher concentration of MLVSS within the MBR equipped with FS membranes (4,546 ± 777 mg/L) compared to HF-SMBR (3,794 ± 1,243 mg/L). As for erythromycin, a higher removal was achieved with the flat sheet membranes, probably due to the better adsorption of erythromycin on the biomass and/or on the flat sheets as the operating conditions were the same in both reactors. Sulphamethoxazole was the only antibiotic which was not removed in these systems, and its concentration in the permeate increased mainly with the highest values of flux (15 and 20 L/m^2 h) due to back conversion of N4-acetylsulphamethazole to sulphamethoxazole during degradation [63].

The hybrid and modified MBR, consisting of the sponge MBR-HF proposed by Nguyen et al. [62] coupled with an ozonation step, was investigated by Vo et al. [64] in order to enhance the removal of the most recalcitrant antibiotics from the effluent of the hospital in Ho Chi Minh (Vietnam). The sponge MBR was operating at the same conditions as the previous investigators, and it was fed by real hospital effluent where the concentrations of the monitored antibiotics were those reported in Table 5. The chemical and physical properties of the investigated compounds influence the total removal that was achieved and is reported in the second-to-last column of Table 5. In general, an increment after ozonation was observed, and this can be attributed to the presence of ozone and hydroxyl radicals that are able to oxide double-bond carbons, activated aromatic groups and non-protonated amines [65]. A clear example is given by ciprofloxacin, which shows a higher ozone affinity due to a higher electron density at functional groups in the molecular structure.

Zotesso et al. [52] in Germany analysed the efficiency of ozonation on the effluent of a nursing home (271 beds, specialised in psychiatric diseases) that was pre-treated in an UF-MBR (UF membrane pore size, 0.04 μm, 10 m^2 area). The interesting study also analysed the effect of ozonation on the dissolved organic carbon (DOC, around 9 mg/L in the influent) and bromide (Br^- and BrO_3^- were always below the limit of quantification of 0.1 mg/L) and the production of toxic substances (including transformation products). Selected micropollutants included analgesics (including ibuprofen, diclofenac and tramadol), psychiatric drugs (carbamazepine, bupropion), antidiabetics (metformin), antihypertensives (valsartan), beta-blockers (atenolol, metoprolol) and antibiotics (sulphamethoxazole). They tested different dosages of

Table 5 Data collected in the investigation by Vo et al. [64] in the system sponge MBR-HF + ozonation

Antibiotic	HWW influent (μg/L)	SMBR-HF permeate (μg/L)	SMBR-HF removal (%)	SMBR + O_3 effluent (μg/L)	O_3 removal (%)	SMBR-HF + O_3 removal (%)	Specific removal (μg/g O_3)
Norfloxacin	24.96 ± 18.65	3.51 ± 3.15	6,286	2.12 ± 0.31	46	92 ± 4	1.56
Ofloxacin	15.12 ± 7.49	4.77 ± 3.35	1,470	1.83 ± 0.25	1,321	88 ± 4	1.27
Ciprofloxacin	12.88 ± 10.96	3.82 ± 2.91	6,893	2.13 ± 0.29	1,321	83 ± 7	1.68
Trimethoprim	1.79 ± 1.12	0.13 ± 0.06	<0	0.05 ± 0.04	4–6	97 ± 2	0.11
Tetracycline	0.76 ± 0.73	n.d.±	2,460	n.d.		100	0.07
Erythromycin	2.15 ± 0.26	1.11 ± 0.17	4,793	0.21 ± 0.03	42	90 ± 1	0.46
Sulphamethoxazole	3.37 ± 0.54	1.86 ± 0.43	<0	1.14 ± 0.14	1,321	66 ± 1	0.37

ozone (2.5, 5, 10 and 15 mg O_3/L) and two HRTs (12.8 and 25 min). The results obtained showed that the removal was >90% for 12 out of 18 key compounds by applying 5 mg O_3/L and operating with HRT equal to 12.8 min. This dosage corresponds to a specific ozone consumption of 0.6 g O_3/(g DOC). As for DOC, only a slight (a few percentages) removal was found during the ozonation step. This fact confirmed the assumption by Bourgin et al. [66] that substances (micropollutants) are not completely mineralised after the ozonation step, but are turned into transformation products which need to be better investigated in terms of occurrence and toxicity, and a further treatment might be suggested. The study concluded that optimisation of the design of the ozonation stage might lead to a lower ozone dosage and lower specific ozone consumption, thus lower operational costs. Moreover, they highlighted that transformation products are expected to be generated as a low removal of organic content in the ozonation plant was found. These undesired compounds might require a further post-treatment. This issue was not considered and should be the subject of further research.

The pilot 100-L moving bed biofilm reactor (MBBR) investigated by Shokoohi et al. [67] fed with the effluent from Beasat Hospital in the town of Hamadan, Iran, showed a good removal of LAS (linear alkylbenzene sulfonic acid) anionic detergent. A removal of 92.3% of LAS was found with a filling rate (Kaldnes carriers) at 70% of the volume, biomass concentrations of 3,000 mg/L and HRT of 24 h.

In Denmark, interesting results were achieved by Tang et al. [68] who tested the removal of a selection of 22 PhCs at lab and pilot scale in the effluent of Aarhus University Hospital at Skejby, by means of the following two combinations: MBBR +ozonation and MBBR+ozonation+MBBR. In the MBBR, AnoxKaldnes™ K5 carriers (AnoxKaldnes, Lund, Sweden) were used with a filling ratio of 50%. The ozonation step was tested in order to reduce the concentrations of PhCs and toxicity and the second MBBR in order to remove biodegradable organic matter and toxicity generated from ozonation by-products. The ozone dosage able to guarantee 90% of the removal (DDO_3) of key compounds was normalised to the dissolved organic carbon (DOC) concentrations (Z_{90} = DDO_3/DOC). Propranolol, tramadol and trimethoprim had the lowest Z_{90}, between 0.72 and 0.88 (mg O_3/mg DOC), indicating that they are easily degraded by ozone. From the other side, iopromide had the highest Z_{90}, indicating that it reacted the slowest with ozone. They also found that ozone can reduce any toxicity by half (with regard to *Vibrio fischeri*) of MBBR effluent, and a subsequent polishing MBBR after ozonation can further reduce any residual toxicity in ozonated effluent.

In the large hospital of Valencia, Spain (1,000 beds, 6 oncology outpatient wards for adults and 2 for children), Ferre-Aracil et al. [69] evaluated and compared the efficacy of ozonation and ozonation combined with hydrogen peroxide as a pre-treatment for the hospital effluent before mixing it with the municipal wastewaters and thus avoiding the dilution of pharmaceuticals arriving at the WWTPs. Analyses of composite samples of the hospital effluent (taken every 4 h from 8 a.m. to 8 p.m. for 5 consecutive days) were processed to determine some cytostatics administered in the structure. Out of the 17 compounds investigated, only four were detected: irinotecan hydrochloride trihydrate, ifosfamide, cyclophosphamide and

capecitabine. Their measured concentrations decreased over the week with the highest values being on the Monday and the only exception of ifosfamide. This trend is correlated with the days on which chemotherapy treatment is administered. These treatments are usually applied on the day on which the experimental campaign started, and most patients returned to their homes, although some remain at the hospital and, consequently, the concentration diminishes as the week went by. The highest concentrations were found equal to 273 ng/L (irinotecan), 1,187 ng/L (cyclophosphamide) and 1,139 ng/L (capecitabine). They found in the tested operational conditions (under O_3 or O_3/H_2O_2) that cyclophosphamide was the most recalcitrant: it was still present when all the other 16 investigated cytostatics were completely removed. The results show that the removal efficiency was around 97% for cyclophosphamide and higher for the other cytostatics in relatively short reaction times (10 min) and for ozone gas concentrations in the order of 43.9 g/m^3. Moreover, they found that there was no apparent effect of hydrogen peroxide on ozone gas consumption.

Research has been carried out on the treatment of HWW with fungi which has highlighted promising results in the removal of specific compounds as reported in [3]. In this context Mir-Tutusaus et al. [70] evaluated the ability of *Trametes versicolor* in removing a wide spectrum of pharmaceuticals (PhCs) from real HWW as well as toxicity due to their residues occurring in the effluent. Samples taken from Sant Joan de Deu Hospital effluent (Barcelona, Spain) were first subjected to coagulation (by adding 95 mg/L or 190 mg/L of coagulant with 2 min of coagulation at 200 rpm), flocculation (by adding 10 mg/L and 20 mg/L of flocculant, respectively 15 min of flocculation at 20 rpm) and then settling (30 min). The pre-treated effluents were then subjected to biological treatment in the presence and absence of *T. versicolor*. They found that out of the 81 selected PhCs, 46 were detected in HWW. *Trametes versicolor* treatment consistently removed most of the detected PhCs, including the most recalcitrant ones (above all carbamazepine). In addition, the treated effluent did not exhibit any toxicity, and therefore the fungal treatment might have removed potential toxic metabolites.

Jaén-Gil et al. [71] instead investigated degradation, transformation and sorption of the β-blocker metoprolol MTP, and its recalcitrant metabolite metoprolol acid (MTPA), during a treatment based on the fungi *Ganoderma lucidum* – a batch aerobic fluidised bed reactor, fed with real HWW. They found a 33% removal of MTP and 64% of MTPA and a slight increase in toxicity, probably due to the presence of other contaminants in the HWW.

For the first time, Badia-Fabregat et al. [72] characterised *veterinary* hospital effluent. They found that it is quite similar to urban effluent in terms of macropollutants, whereas it presents inter-day variability for PhCs, with peaks of high concentrations for some antibiotics (metronidazole, cefalexin and ciprofloxacin), analgesics and anti-inflammatories (mainly salicylic acid). On the whole, antibiotics, analgesics and anti-inflammatories presented the highest concentrations. In general, the overall concentration levels of PhCs in veterinary hospital effluent were lower than those reported for hospital wastewaters [1, 73] probably due to the higher use of water (and therefore dilution of wastewater) for cleaning the veterinary

facilities and the fact that urine from large animals (e.g. horses) was collected with straw and disposed separately. They concluded that the concentrations of pharmaceuticals are between those found in urban wastewater and hospital wastewater and thus some dedicated treatment at the source might be recommended. They investigated the efficacy of a fungal treatment with *Trametes versicolor*, which has already been tested for the treatment of hospital wastewater with promising results [71, 74]. They first investigated the removal of a wide spectrum of PhCs in veterinary effluent in batch mode in non-sterile conditions and at the same time analysed the evolution of the bacterial and fungal population. They found a removal of 66% for many PhCs and identified some microbial interactions which encouraged them to investigate the treatment of the same effluent in continuous mode. The corresponding removal efficiency was around 44%.

With the aim to better focus on the strategy to improve the removal of antibiotics and ARGs from veterinary hospital effluent, Lucas et al. [75] analysed the removal of 47 antibiotics belonging to 7 different groups (β-lactams, fluoroquinolones, macrolides, metronidazoles, sulphonamides, tetracyclines and trimethoprim) and a selection of ARGs bla_{TEM}, bla_{SHV}, *ermB, qnrS, sulI, tetW* and 16S rRNA. The observed removal was 77% of antibiotics after the fungal treatment, which is higher than the removal obtained in conventional treatment plants. As for the removal of ARGs, they found that the fungal treatment resulted in a good removal of the *sul*I and bla_{TEM} genes (removal rates of 56% and 100%, respectively), as compared with data obtained from a conventional WWTP, in which the removal rates were negative, 156 and 58% [76]. The *qnrS* gene showed a negative removal rate equal to 163% (in conventional WWTP its removal was even worse and was equal to 302%, [76]). As for *ermB* and *tetW* genes, fungal treatment showed a complete removal for both species. Very good results were obtained in conventional WWTPs which exhibited slightly lower values (82% and 97% for the two ARGs, [76]). They concluded that fungal treatment may represent an alternative solution to conventional WWTP as it showed a good removal of certain compounds such as ciprofloxacin, enrofloxacin, marbofloxacin and ampicillin, which are recalcitrant in conventional WWTPs. They also remarked that not only the occurrence of antibiotic compounds may influence the fate of antibiotic resistance but also other factors, including operational parameters of bioreactors, the wastewater-associated bacterial communities and their interaction with fungi.

Badia-Fabregat et al. [77] reported that many indigenous fungi rather than bacteria can successfully compete with the inoculated fungi and that wastewater origin and operational conditions have a stronger impact on the diversity of microbial communities developed in the bioreactors than the inoculation or not with *T. versicolor*.

As already reported, in some countries, HWWs are directly discharged into the receiving water, without specific treatments. This practice is followed by the hospital of Tumaco (Nariño), as well as from the city of Florencia (Caquetá), in Colombia, whose effluents are directly released in two rich biodiversity regions (the Pacific and Amazonian regions) [13]. The authors well know that the direct release of untreated hospital effluent into the sea may pose serious risk to aquatic life and underline the

need to adopt adequate treatments to reduce such risk. With this aim, they investigated the efficacy in removing different pharmaceuticals from a real HWW by a combination of biological and sonochemical treatments (first sonochemical then sono-photo-Fenton). Fifteen pharmaceuticals (the analgesics acetaminophen and diclofenac; the psychiatric drugs carbamazepine and venlafaxine; the antihistamine loratadine; the antibiotics sulphamethoxazole (sulphonamide), trimethoprim, ciprofloxacin and norfloxacin (fluoroquinolones), erythromycin, azithromycin and clarithromycin (macrolides), and clindamycin (lincosamide); the β-blocker antihypertensives valsartan and irbesartan) were analysed in the raw effluent of the medium-sized (122 beds) hospital in Tumaco [78], after the two steps of the treatment trains. They found that, with the exception of acetaminophen and valsartan, all the other PhCs were not removed. If this effluent is then subjected to 90 min of sonochemical treatment, all the PhCs are removed with the exception of the two cited compounds. According to the authors, acetaminophen and valsartan could be sorbed onto the flocs formed during the biological step; ultrasonic waves would break them and cause the release of the two PhCs, thus justifying the observed increment of their concentration in the first 60 min of treatment. The modest decrement in the PhC concentration up to the end of the observation period (in the remaining 30 min) should be due to the chemical attack of the produced radicals ($HO^{\cdot}$) towards the PhCs, as well as to the chemical effects of ultrasound. In the sonochemical process, radicals are produced through acoustic cavitation phenomena. In order to enhance the degradation of the target pollutants, the authors also investigated a sono-photo-Fenton treatment that is an on-site photo-Fenton process where ferrous ions and UVC irradiation are added to the sonochemical component (volume of 350 mL; frequency equal to 375 kHz; power equal to 88 W/L; pH in the feeding equal to 7.9; $[Fe^{2+}]$ equal to 90 μmol/L, and UVC of 4 W). The system showed a higher ability in removing recalcitrant compounds, and it was able to reach an average removal equal to 85% for the whole PhC load in the biologically treated effluent, while in the sonochemistry treatment, the average removal was 51%.

The Iranian study by Karami et al. [50] investigated the application of high-frequency ultrasound (1.8 MHz) to a conventional activated sludge process for the treatment of hospital effluent. They analysed the effect of the modified activated sludge process on the removal of COD and turbidity and on the sludge volume index (SVI) in a bioreactor characterised by a high biomass concentration (8,000 mg/L). It was found that with and without sonication, the removal of COD was similar, but in the sonicated system, SVI and effluent turbidity were lower than the non-sonicated system.

Giannakis et al. [14] discussed the need to adopt specific strategies for HWW management and treatment, distinguishing between developed and developing countries. They evaluated the application of light-assisted AOP solutions for both situations: UV-based AOPs in developed countries and solar-based AOPs in developing countries (focusing on the main characteristics of Ivory Coast and Colombia). They underlined that developed countries more or less have the problem of microorganisms under control and mainly focus on micropollutants, while the priority of developing countries should be the acute risk caused by microorganisms in the

effluent which are often directly discharged in the surface water and thus indirectly reused for crop irrigation. In their study, they treated the effluent of the Vidy WWTP (Switzerland) by UV/H_2O_2 and photo-Fenton process and analysed the removal of microorganisms and a selection of micropollutants (carbamazepine, clarithromycin, diclofenac, metoprolol, venlafaxine, benzotriazole and mecoprop). They found UV-based treatment more efficient in the removal of microbiological and chemical contaminants: they achieved 100% removal after 10 min of exposure in the case of an effluent from an activated sludge system or an MBBR and after 30 min in the case of coagulation-flocculation treatment effluent. On the contrary, the photo-Fenton treatment achieved 20% of removal after 30 min of exposure. As for microorganisms, 5 min were sufficient for the UV treatments to complete their removal, whereas more than 3 h were needed for photo-Fenton. With regard to developing countries, they took as an example the conditions in Ivory Coast and Colombia and remarked that the application of solar photo-Fenton as a feasible AOP is suggested only after (the construction of) basic treatment (primary and secondary treatments), or as a possible implementation of a barrier of microbiological-related problems. They found photo-Fenton was able to achieve high bacterial removal, regrowth suppression and yeast and virus inactivation and was also a non-selective and effective system in removing micropollutants and organic matter. They concluded that light-assisted AOP application in hospitals wastewater is promising both in developed and developing countries.

The need to evaluate the health and environmental risks posed by reclaimed water in Israel led Petrovich et al. [79] to investigate (pre)treatments for the hospital effluent before its emission in the public sewer system. They tested the sequence, sedimentation + activated sludge process + secondary sedimentation on a pilot scale, and analysed microbial and viral communities and their antibiotic-resistant genes in raw hospital effluent and treated effluent. They found that ARGs, including many that confer resistance to antibiotics of high clinical relevance, were abundant and persistent throughout the pilot-scale system, including *aadA*, $bla_{\text{TEM-1}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-2}}$, bla_{OXA35}, $bla_{\text{OXA-10}}$, *mefA*, *mel*, *tetX* and *sul1*. They found an overall removal efficiency of ARGs throughout the pilot-scale system equal to 16% on a per genome equivalent basis. Moreover, based on their results, they did not identify strong linkages between ARGs and viruses in viral metagenomes, suggesting that the viral community may not play a significant role in promoting the horizontal gene transfer of ARGs in this hospital wastewater treatment system. They also commented on the lack of knowledge in this context, and the conflicting results from previous studies warrant further research on this topic.

The issue of the proper management and treatment of HWW is becoming of great concern for the scientific community of countries where attention has not yet been paid to this problem. For instance, in Iran, the recent study by Azizi et al. [80] remarks that in Kermanshah and Hamadan, there are 19 and 16 differently sized hospitals, respectively, with a capacity of 2,526 and 3,042 beds. The effluent of the healthcare facility is treated only in 7 out of 19 hospitals and 5 out of 16 hospitals in the 2 study areas. The adopted treatments generally include a biological process (activated sludge system). The study concludes with the need to urgently define

policy for the construction of wastewater treatment in every single hospital and to demand teams of experts at the various phases: design, construction, exercise and control.

In Brazil, with the intention to reduce the impact of hospital effluent, which is generally discharged into the public sewage network without any treatment, at the Regional University Hospital of Maringá, Zotesso et al. [53] investigated the possibility of treating the hospital laundry wastewater on site and eventually to reuse the treated effluent for the requirements of internal services. The fact that, in this hospital, laundry water consumption represents 50% of the overall hospital water consumption and contains organic substances at high concentrations (COD in the range 211–1,162 mg/L), the research group was commissioned to evaluate an adequate treatment able to reduce the pollutant discharge load and also the demand for fresh water. Bearing this in mind, they tested the sequence:

- Coagulation-flocculation (by the addition of 40–140 mg/L of the organic cationic polymer Tanfloc in order to remove suspended solids and organic compounds, mainly colour and detergents)
- Anthracite filtration for the removal of turbidity
- UV/H_2O_2 in order to complete the removal of COD (the influence of H_2O_2 was evaluated by means of different [H_2O_2]/COD ratios in relation to the COD value of the pre-treated effluent, 0.5, 1, 1.5, 2)

The results show that it is possible to achieve a complete removal of COD. Moreover, the increase in [H_2O_2]/COD ratio favours COD removal; however, because H_2O_2 acts as an additional pollutant, its concentration cannot be indefinitely increased. However, the investigations did not determine if the tested system is efficient in both the disinfection of raw hospital laundry wastewater and the reduction of its toxicity, as well as if treated effluents meet the requirements for reuse.

Several attempts have been made to promote the degradation and mineralisation of specific compounds by specific technologies. Of these, Moreno et al. [81] investigated the electrochemical degradation of the chemotherapy drug doxorubicin (DOX) in hospital effluent (where it often occurs at concentrations greater than 1 μg/L) by means of nanostructured graphite electrodes with metallic oxides (graphite, TiO2@graphite and AuO-TiO2@graphite electrodes). The study was carried out on a laboratory scale with cells of a capacity of 5 mL. The assays were conducted using 3 mL of DOX 1.25 mg/L in tap water and in Milli-Q water (Millipore S.A) with NaCl 10 mmol/L and with an applied voltage of 1.5, 2.5 and 5.0 V. It was found that the technology seems promising as no undesired compounds are formed. The complete degradation was found with AuO-TiO2@graphite electrode fed with DOX 1.45 mg/L over 40 min. It is important to highlight that the addition of NaCl improves degradation due to the formation of active chlorine with high oxidation power.

With regard to ecotoxicological issues, it was found that DOX treatment by electro-oxidation caused no effect on embryo-larval development of zebrafish, but damage was found in the DNA after 96 h of exposure. This underlines the

importance of an in-depth ecotoxicological evaluation during the development of electrochemical oxidation.

In Seifert et al. [82], a benchmark of the measures taken by 31 German hospitals registered in EMAS, regarding their environmental management, reports that there are no specific measures regarding hospital wastewater management. They generally are released into the public sewer systems and conveyed to a municipal WWTP. Some healthcare structures mention the renewal of certain devices: grease separators in the kitchen, light liquid separators, a water treatment plant or the rehabilitation of the drainage canals on the hospital site. However, no measures regarding the specific and dedicated treatment of wastewater or the removal of certain pollutants are mentioned.

Finally, it is also worth mentioning a recent study which had the main aim to suggest adequate treatments for HWW before its release into the public sewage in order to guarantee the safety to workers in the case of damage to the sewer network [83]. It was reported that in the USA, according to the guidance by the World Health Organization and Centers for Disease Control and Prevention, wastewater from patients undergoing treatment at hospitals for *Ebola* has to be managed as ordinary wastewater without specific pre-treatment. The authors underline that the potential for *Ebolavirus* transmission via liquid waste discharged into the wastewater environment is currently unknown. On the basis of this analysis, it was concluded that the results suggest that when sewer workers operate in a wastewater collection system downstream from a hospital treating patients with *Ebola*, they are exposed to a potential risk that requires more attention and that disposal into the sanitary sewer without further treatment may result in the insufficient protection of the safety of sewer workers.

To conclude, the issue of the treatment of emergency hospitals in zones affected by extreme conditions due to earthquakes, flooding and other natural disasters is addressed in Khan et al. [84]. In these areas, the first medical assistance is supplied by means of mobile hospitals where victims receive the initial medical care and can be transferred to other stationary hospitals. However, in many cases, these mobile hospitals can be transformed into field hospitals and have to provide definitive care right on the field [85]. The amount of antibiotics used for the treatment of patients in these healthcare structures is high, which in turn results in a high impact on the environment. For field hospital effluent, a treatment train is recommended that consists of pre-treatments followed by a membrane bioreactor and finally a photo-Fenton stage to complete the removal of the most recalcitrant micropollutants.

5 Prioritisation: Environmental Risk Assessment of PhCs in HWW

In Zillien et al. [86], an interesting modelling framework is presented and applied to two large *Dutch* academic hospitals in order to prioritise pharmaceuticals based on their relative risks for aquatic organisms, considering the three trophic levels, fish,

daphnia and algae, using purchase and prescription data from the individual hospitals. The framework consists of an emission prediction module and a risk prioritisation module (based on the risk quotient, i.e. the ratio between predicted concentrations and corresponding predicted no-effect concentrations). The study was based on a selection of 16 pharmaceuticals, including antibiotics, antineoplastics, iodinated contrast media, non-steroidal anti-inflammatory drugs, anticonvulsants, antidepressants, lipid regulators, antidiabetics and beta-blockers. Three ranking strategies were considered in evaluating the most critical compounds: the first is based on the risks associated with the total release of pharmaceuticals from the hospital due to inpatients and staff personnel, the second strategy is based on the risk due to only hospitalised patients, and the third is based on risks related to the environment by comparing the total predicted emissions based on hospital prescriptions (regardless if excretion occurs within the hospital or outside) to the emissions of only hospitalised patients in order to estimate the urban impact of the hospital emission. Results from prioritising pharmaceuticals indicate that azithromycin and ciprofloxacin represent the highest risk to aquatic organisms in relation to the substance selection of this study. Similarly, NSAIDs scored relatively high even though the release of this pharmaceutical group is underestimated in both cases. Emission mitigation strategies by hospitals could therefore focus their efforts primarily on reducing antibiotic and NSAID emissions. Both modules of the presented framework require a limited amount of relatively simple input data. Therefore, this study provides healthcare managers with a useful and practical tool to acquire sufficient exploratory information on hospital emissions for prioritising potential emission reduction measures.

In the frame of the EU project CytoThreat, Mišík et al. [87] carried out an environmental risk assessment due to four anticancer drugs (5-fluorouracil (5FU), cisplatin (CDDP), imatinib mesylate (IM) and etoposide (ET)) in hospital effluent in terms of acute, subacute and chronic effects (growth inhibition, reproduction inhibition and mortality testing), as well as genotoxic effects (DNA damage, chromosomal damage and gene mutation) with regard to many species of bacteria, algae, cyanobacteria, plants, Crustacea, Rotifera, freshwater mussels, fish and cell lines derived from humans and fish. The risk was assessed by means of the risk quotient RQ between the predicted concentration and the corresponding PNEC found in the investigation for the different cases (toxicity or genotoxicity). The results showed that in terms of toxicity, the highest RQs were obtained with IM, followed by 5FU, CDDP and ET. All values are below 1.0 indicating that none of these compounds represents a significant environmental risk. As for the genotoxic effects, they found that the $RQ_{genotox}$ value for 5FU is between 10 and 100, indicating significant potential for adverse effects. The $RQ_{genotox}$ values obtained with IM and CDDP are between 1 and 10, which indicates a small potential for adverse effects, while ET represents no significant risk.

In conclusion, their study highlighted that acute, subacute and chronic toxic effects of cytostatics in the aquatic environment are unlikely but may occur when the compounds are not removed from hospital wastewaters by efficient treatments and where the measured concentrations were always found higher than those in

urban wastewater. Furthermore, they indicate that certain species (e.g. Crustacea) are highly sensitive with regard to the induction of the genotoxic effects which were found with environmentally relevant concentrations of 5FU, CDDP and IM.

Within the SIPIBEL project, an investigation was carried out on ecotoxicity (*Daphnia magna* mobility, *Pseudokirchneriella subcapitata* growth, *Brachionus calyciflorus* reproduction and SOS chromotest) and antibiotic resistance (integron quantification) during the mixing of hospital and urban wastewater and the treatment steps they were subjected to Laquaz et al. [45]. The results showed that hospital effluent presents higher ecotoxicity than the mixture probably due to the high content of adsorbable organic halides (AOX, mainly including chloroform), ammonium, non-ionic detergents and certain pharmaceuticals. The observed variation in ecotoxicity over the day, week, month and year might be related to the specific activities at the local hospital and their variability over time. These include the pharmaceutical consumption pattern [28, 29] which depends on the treatment of various seasonal pathologies and some management factors such as disinfection campaigns that may also have led to the punctual release of high quantities of ecotoxic compounds [45]. The mixture of HWW and urban wastewater in the proportion of 1/3–2/3 did not show any promotion in the spread of antibiotic resistance.

Within the Danish project which led to the definition of guidelines for the management and treatment of HWW in Denmark [26], the health and environmental risk was assessed with reference to the treated effluent from Herlev Hospital in three different scenarios, which reflected the normal operating conditions of the WWTP receiving HWW, its partial failure (simulating the main failures occurring during the test periods of the plant) and its total breakdown. The WWTP effluent is discharged in the small Kangsa River, a tributary of the Harrestrup River which flows into the marine area of Kalveboderne, used for bathing and other recreational activities. As for the environmental risk, the study evaluated (modelled) the exceeding of the corresponding PNEC value in the marine area by the average concentrations of the most critical compounds (ciprofloxacin, clarithromycin, diclofenac and sulphamethoxazole), once the WWTP effluent has mixed with the receiving water and reached the sea (no decay rate of the key compounds is considered on the way to the final receptor). With regard to health risk, the risk of infection was assessed with norovirus, and the concentrations of FIB were estimated in the river and then in the final receptor (the sea). These values were then compared with the EU bathing water requirements (250 MPN/100 mL). The distribution of the expected concentrations of norovirus in the receiving environments were evaluated by means of Monte Carlo simulations; the risk of infection was calculated by applying the approximated β-Poisson norovirus dose-response model with immunity [88] and the distribution of the infection by using Monte Carlo simulations. The results of this complex risk assessment showed that even after a realistic incident leading to a decrease in treatment removal efficiency, the treated effluent will not affect safe bathing water quality in the marine area. A total breakdown of the WWTP over a period of 1 day will cause a breach of the bathing standards. In the same way, during normal operations, there will be no risk of infection with norovirus in the marine water.

The release of untreated wastewater will only have a small impact on the risk of norovirus infection in the final receptor. The discussion of the uncertainties reported in the report by DHI [26] is of great help in evaluating the reliability of the collected results and, if necessary, in which phase of the analysis an in depth evaluation could be carried out.

6 Strategies in HWW Management and Treatments: Lessons Learned from Singular Experiences

PhC concentrations in hospital effluents depend on the size of the hospital and wards/departments, the activities taking place inside the structure (laboratory, chemical analysis, kitchen, laundry, etc.) and water consumption. A reply to the question of how similar or different they are with respect to municipal wastewater involves a deep analysis of the characteristics of the healthcare structure and its surrounding area [17, 89, 90].

A quick look at the observed ranges of concentrations of the most common antibiotics in the two effluents reported in the review by Souza et al. [91] highlights that the concentration of antibiotics (including azithromycin, tetracycline, ofloxacin, ciprofloxacin, sulphamethoxazole, trimethoprim, metronidazole and lincomycin) in HWW is higher than in municipal wastewater, confirming the conclusion by Verlicchi et al. [1]. Moreover, they also found that small- and medium-sized hospitals tend to discharge higher mean concentrations than larger ones with regard to many analgesics and anti-inflammatories, antibiotics, lipid regulators and psychiatric drugs.

The occurrence of antibiotic-resistant bacteria in hospital effluent documented by the study by Haller et al. [44] suggests the need to improve their removal from this major source of immission in the environment and to discuss and share strategies to be put into practice. More effort should be made to monitor and further control the dissemination of resistant bacteria in the environment from such a major source.

The adoption of a separate treatment has benefits according to Alvarino et al. [55], for instance, the avoidance of dilution with domestic wastewater and the specific treatments of potential toxics. In 2014, in its updated report [92], the WHO remarked that although a large part of HWW could present similar characteristics to domestic wastewater, "a proportion of the generated wastewater from health-care facilities will pose a higher risk than domestic wastewater. Depending on the service level and tasks of the health-care facility, the wastewater might contain chemicals, pharmaceuticals and contagious biological agents, and might even contain radioisotopes". According to WHO [92], the effluent from a healthcare structure should be treated before its release into the environment. The best solution should be an on-site facility for the *pre-treatment* of the produced wastewater prior to its release into the public sewer network in order to remove the presence of hazardous components including microbiological pathogens, radioactive drugs, toxic chemicals and antibiotics. A

discussion of the WHO guidelines and the specific regulations in force in some countries (France, Germany, Spain, the UK, Italy, India, China and Vietnam) is reported and discussed in [93] and in the book [4].

In a previous study, Verlicchi et al. [94] already presented and discussed four scenarios for the management and treatment of HWW, and, in another one [95], the main factors to consider in planning the proper management and treatment of HWW were proposed. They suggest different strategies in the case of developed and developing countries. For developed countries, they proposed options on the basis of hospital and catchment area size. Recently, Mousel and Pinnekamp [52] confirmed the adequateness of this approach and in particular the suggestion that for healthcare structures with large capacities connected to small WWTPs, dedicated treatments or at least (pre-)treating the wastewater could be useful in order to convey lower PhC loads to the municipal WWTP. Full-scale treatment plants for the on-site (pre-)treatment of HWW have already been built [96], and this barrier can effectively act against the spread of ARB and ARG in the environment.

Two interesting experiences deserve particular attention, the French and the Danish experiences, and lessons can be learned from them.

At the Bellecombe site, France, as already mentioned, the existing treatment plant receives and separately treats hospital effluent in two lines adopting the same conventional treatments (activated sludge processes). SIPIBEL (SIte PIlote de BELlocombe) is the name of the project, the preparation and implementation of which was coordinated by GRAIE (http://www.graie.org/Sipibel/index.html)) [97]. Ongoing monitoring and research activities highlighted that HWW, compared to urban wastewater, is characterised by a higher concentrations of organic carbon, AOX, phosphates, gadolinium, acetaminophen, ketoprofen, sulphamethoxazole and ciprofloxacin [98], higher ecotoxicity, as well as the presence of bacteria with potentially higher antibiotic resistance. A higher variability was also found in PhC removal efficiencies in urban WWTP than in hospital WWTP. This could be explained by a larger variation of contamination sources and stronger seasonal fluctuations in urban wastewater [99]. The results also showed higher removal efficiencies in HWW treatment train for antibiotics, analgesics and beta-blockers, while concentrations of antibiotics and analgesics in the hospital treated effluent were higher than in urban treated effluent because of their higher influent concentrations [99]. An analysis of the potential environmental impact of the two treated effluents highlighted that due to the different content of pharmaceutical residues and nutrients, the two treated effluents cause different environmental adaptations of bacterial communities. In fact, they found that biofilms exposed to hospital treated effluent exhibited less developed biomass and lower diversity. Lessons learned from the collected studies have led to remark that, on the basis of the removal efficiency of (analysed) PhCs, separate treatment is beneficial. However, high PhC concentrations in the hospital treated effluent and the following adaptations of biofilm communities note the importance of adopting adequate wastewater technologies for the control of specific hospital pollutants.

In Denmark, since 2009, hospital effluent is managed in the same manner as industrial effluent, and a permit is required before it can be discharged into the public

sewer system. Around 10 years ago, the Danish authorities wanted to increase their knowledge on how HWW *can* be treated and whether suggested technologies are feasible from a technical and economic viewpoint. For this reason, in 2010–2011, pre-tests were carried out on a laboratory scale by DHI for the Danish Environmental Protection Agency on innovative technologies which were then followed by pilot and lab tests with the effluent from Herlev Hospital [26]. This first study phase showed that, with reference to the selected PhCs, the most adequate technologies were the membrane bio-reactor (MBR) technology combined with activated carbon, ozonation and UV. The most efficient sequence of treatment has not yet been clearly identified. It was during the private-public innovation project, which started in 2012, that this could be identified. The aim of the project was to acquire knowledge and define a "national" strategy in the management and treatment of HWW in the country. In this context, a full-scale WWTP was built (which has been in operation since 2014) for the effluent of Herlev Hospital (700 beds with a flow rate of 150,000 m^3/year, with plans for it to be increased to 900 beds and to 200,000 m^3/year by 2020). It includes an MBR (with nitrogen and phosphorus removal) followed by ozonation (2.5 and 3.4 mg O_3/mg DOC), GAC filtration and UV radiation (5–10 mJ/cm^2). This polishing sequence proved to be the most adequate after a period of tests on the field [26] which compared different sequences. The sequence O_3-GAC-UV was selected as it optimises the removal of key pharmaceuticals (a list of 40 compounds) and costs for GAC regeneration, most likely because the general organic matter is transformed into more water-soluble compounds by the ozonation. Moreover, no critical formation of ozone by-products, such as bromate or NDMA, was observed. The amount of GAC to be replaced yearly is around 3,000 kg. Based on the collected results, it was found that the treated effluent could be directly released into a small local stream (Kagså). It is also planned that part of the treated water will be reused as cooling water in the existing cooling tower at the hospital.

In the same time, a task group developed guidelines for the regulation of the management and treatment of hospital effluent. This document set a list of 40 pharmaceuticals and the corresponding guideline values (maximum values for discharge into the public sewer system) and a method to define a hospitals as a major, medium and minor point sources based on the amount of pharmaceuticals administered in the hospital and the hospital consumption of antibiotics with respect to the catchment area.

The study carried out a health and environmental risk assessment assuming (1) a complete breakdown of the hospital WWTP and the subsequent release of untreated wastewater, (2) a partially treated effluent and (3) normal operation of the WWTP. The main results and lessons learned during this investigation are well described and discussed in [26].

The French and the Danish strategies highlight how useful it can be to share the planning of research activities which involve decision-makers, administrators, technicians, practitioners, researchers and hospital administrative and technical staff in defining adequate strategies for the management and treatment of this particular effluent, also with a view to the ongoing European legislation, attention to the

occurrence of micropollutants in water compartments and the control and reduction of their emission into the environment.

An environmental audit similar to that discussed in Vaccari et al. [100] which referred to pharmaceuticals consumed and released in a healthcare facility as well as the adopted treatment could help optimise management and minimise the impact of hospital (treated) effluent in the environment.

7 The Need for Further Debate and Research

The experiences herein reported and discussed demonstrate the efforts that have been carried out worldwide and that are still ongoing in reaching Sustainable Millennium Goal number 6 "Improve Clean Water and Sanitation" defined by the World Health Organization by 2030.

However, they also underline the need for further research. In particular, it emerges that:

- The direct disposal of HWW to a natural water body without any kind of treatment is the common practice for many countries [101].
- Adequate treatments are necessary to reduce the emission of micropollutants in the environment. National guidelines could help in this aim and should be defined by a team of expert involving scientists, technicians, hospital technical and administrative staff, as well as policy makers and decision-makers.
- HWW management and treatment should be dealt with in a holistic view, including all the water cycle, in particular in developing countries. An improvement in sanitation systems will reflect on the water sector, as discussed in [102].
- With regard to some critical compounds, households could be the main source of such compounds, and the municipal WWTP should be updated. This was the case for 94% of anticancer drugs which were due to outpatients in the hospital and catchment area investigated by [103].
- Investigations should be carried out on the efficacy of new treatments in removing key pharmaceuticals and not only the parent compounds but also the intermediates generated during treatment [71].
- Environmental risk assessment should also focus on the mixture of substances [86].
- Improving the elimination of antimicrobial-resistant microorganisms from HWW would appear to be a reasonable policy to put into practice, and more effort should be made to monitor and further control the dissemination of resistant bacteria in the environment from such a major source [44].

To conclude: much is already known but much more is still to be discovered and even more is to be done.

References

1. Verlicchi P, Galletti A, Petrovic M, BarcelÓ D (2010) Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options. J Hydrol 389:416–428
2. Pills_Report. Pharmaceutical input and elimination from local sources
3. Verlicchi P, Al Aukidy M, Zambello E (2015) What have we learned from worldwide experiences on the management and treatment of hospital effluent? – An overview and a discussion on perspectives. Sci Total Environ 514:467–491
4. Verlicchi P (2018) Hospital wastewaters. 60:17–32. http://link.springer.com/10.1007/978-3-319-62178-4
5. Kümmerer K (2001) Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. Chemosphere 45(6–7):957–969
6. Weissbrodt D, Mcardell CS, Kovalova L, Hollender J, Siegrist H (2006) Hospital wastewater: mass flow analysis of pharmaceuticals and disinfectants. 1–2
7. Boillot C, Bazin C, Tissot-Guerraz F, Droguet J, Perraud M, Cetre JC et al (2008) Daily physicochemical, microbiological and ecotoxicological fluctuations of a hospital effluent according to technical and care activities. Sci Total Environ 403(1–3):113–129
8. El-Ogri F, Ouazzani N, Boraâm F, Mandi L (2016) A survey of wastewaters generated by a hospital in Marrakech city and their characterization. Desalin Water Treat 57 (36):17061–17074
9. Kilunga PI, Kayembe JM, Laffite A, Thevenon F, Devarajan N, Mulaji CK et al (2016) The impact of hospital and urban wastewaters on the bacteriological contamination of the water resources in Kinshasa, Democratic Republic of Congo. J Environ Sci Heal A Toxic/Hazardous Subst Environ Eng 51(12):1034–1042
10. Laffite A, Kilunga PI, Kayembe JM, Devarajan N, Mulaji CK, Giuliani G et al (2016) Hospital effluents are one of several sources of metal, antibiotic resistance genes, and bacterial markers disseminated in sub-saharan urban rivers. Front Microbiol 7:1–14
11. Ibrahim C, Hammami S, Chérif N, Mejri S, Pothier P, Hassen A (2019) Detection of Sapoviruses in two biological lines of Tunisian hospital wastewater treatment. Int J Environ Health Res 29(4):400–413. https://doi.org/10.1080/09603123.2018.1546835
12. Kimosop SJ, Getenga ZM, Orata F, Okello VA, Cheruiyot JK (2016) Residue levels and discharge loads of antibiotics in wastewater treatment plants (WWTPs), hospital lagoons, and rivers within Lake Victoria Basin, Kenya. Environ Monit Assess 188(9)
13. Botero-Coy AM, Martínez-Pachón D, Boix C, Rincón RJ, Castillo N, Arias-Marín LP et al (2018) An investigation into the occurrence and removal of pharmaceuticals in Colombian wastewater. Sci Total Environ 642:842–853. https://doi.org/10.1016/j.scitotenv.2018.06.088
14. Giannakis S, Rtimi S, Pulgarin C (2017) Light-assisted advanced oxidation processes for the elimination of chemical and microbiological pollution of wastewaters in developed and developing countries. Molecules 22(7)
15. Martins AF, Frank C, da S, Altissimo J, de Oliveira JA, da Silva DS, Reichert JF et al (2017) Determination of statin drugs in hospital effluent with dispersive liquid–liquid microextraction and quantification by liquid chromatography. J Environ Sci Heal A Toxic/Hazardous Subst Environ Eng 52(10):939–945. https://doi.org/10.1080/10934529.2017.1324706
16. Reichert JF, Souza DM, Martins AF (2019) Antipsychotic drugs in hospital wastewater and a preliminary risk assessment. Ecotoxicol Environ Saf 170:559–567. https://doi.org/10.1016/j.ecoenv.2018.12.021
17. Langford KH, Thomas KV (2009) Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works. Environ Int 35(5):766–770. https://doi.org/10.1016/j.envint.2009.02.007

18. Stockholm County Council (2014) Environmentally classified pharmaceuticals 2014-2015. 1–54. https://noharm-europe.org/documents/environmentally-classified-pharmaceuticals-2014-2015
19. Hernando MD, Mezcua M, Fernández-Alba AR, Barceló D (2006) Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta. 69(2 SPEC ISS):334–342
20. Kafaei R, Papari F, Seyedabadi M, Sahebi S, Tahmasebi R, Ahmadi M et al (2018) Occurrence, distribution, and potential sources of antibiotics pollution in the water-sediment of the northern coastline of the Persian Gulf, Iran. Sci Total Environ 627:703–712. https://doi.org/10.1016/j.scitotenv.2018.01.305
21. Lien LTQ, Hoa NQ, Chuc NTK, Thoa NTM, Phuc HD, Diwan V et al (2016) Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment, and the relationship with antibiotic use-a one year study from Vietnam. Int J Environ Res Public Health 13(6):1–13
22. Azuma T, Arima N, Tsukada A, Hirami S, Matsuoka R, Moriwake R et al (2016) Detection of pharmaceuticals and phytochemicals together with their metabolites in hospital effluents in Japan, and their contribution to sewage treatment plant influents. Sci Total Environ 548–549:189–197. https://doi.org/10.1016/j.scitotenv.2015.12.157
23. Papageorgiou M, Zioris I, Danis T, Bikiaris D, Lambropoulou D (2019) Comprehensive investigation of a wide range of pharmaceuticals and personal care products in urban and hospital wastewaters in Greece. Sci Total Environ 694:133565. https://doi.org/10.1016/j.scitotenv.2019.07.371
24. Szekeres E, Baricz A, Chiriac CM, Farkas A, Opris O, Soran ML et al (2017) Abundance of antibiotics, antibiotic resistance genes and bacterial community composition in wastewater effluents from different Romanian hospitals. Environ Pollut 225:304–315
25. Lerat-Hardy A, Coynel A, Dutruch L, Pereto C, Bossy C, Gil-Diaz T et al (2019) Rare earth element fluxes over 15 years into a major European Estuary (Garonne-Gironde, SW France): Hospital effluents as a source of increasing gadolinium anomalies. Sci Total Environ 656:409–420. https://doi.org/10.1016/j.scitotenv.2018.11.343
26. DHI (2016) Full scale advanced wastewater treatment at Herlev Hospital – treatment performance and evaluation. 87
27. Lasek F, Karpel Vel Leitner N, Rauwel G, Blanchier L, Castel O, Ayraud-Thevenot S et al (2019) Discharge of biocidal products from healthcare activities into a sewage system – a case study at a French university hospital. Environ Sci Pollut Res 26(5):4938–4951
28. Verlicchi P, Zambello E (2016) Predicted and measured concentrations of pharmaceuticals in hospital effluents. Examination of the strengths and weaknesses of the two approaches through the analysis of a case study. Sci Total Environ. 565
29. Verlicchi P (2018) Pharmaceutical concentrations and loads in hospital effluents: Is a predictive model or direct measurement the most accurate approach? Handbook of environmental chemistry, vol 60
30. Daouk S, Chèvre N, Vernaz N, Widmer C, Daali Y, Fleury-Souverain S (2016) Dynamics of active pharmaceutical ingredients loads in a Swiss university hospital wastewaters and prediction of the related environmental risk for the aquatic ecosystems. Sci Total Environ 547:244–253. https://doi.org/10.1016/j.scitotenv.2015.12.117
31. Alam M, Imran M, Ahmad SS (2019) Screening of metal and antibiotic resistance in Beta-lactamase producing coliform Bacteria from Hospital wastewater of northern India. Recent Pat Biotechnol 14(1):63–77
32. Hocquet D, Muller A, Bertrand X (2016) What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. J Hosp Infect 93(4):395–402. https://doi.org/10.1016/j.jhin.2016.01.010
33. WHO (2017) Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. WHO. 7. https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

34. Cantas L, Shah SQA, Cavaco LM, Manaia CM, Walsh F, Popowska M et al (2013) A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. Front Microbiol 4:1–14
35. IAGC (2019) Report to the secretary general of the United Nations, no time to wait: securing the future from drug-resistant infections. Artforum Int 54(10):113–114. https://www.who.int/docs/default-source/documents/no-time-to-wait-securing-the-future-from-drug-resistant-infections-en.pdf?sfvrsn=5b424d7_6
36. Lien LTQ, Lan PT, Chuc NTK, Hoa NQ, Nhung PH, Thoa NTM et al (2017) Antibiotic resistance and antibiotic resistance genes in *Escherichia coli* isolates from hospital wastewater in Vietnam. Int J Environ Res Public Health 14(7):1–11
37. Lamba M, Graham DW, Ahammad SZ (2017) Hospital wastewater releases of Carbapenem-resistance pathogens and genes in Urban India. Environ Sci Technol 51(23):13906–13912
38. Bréchet C, Plantin J, Sauget M, Thouverez M, Talon D, Cholley P et al (2014) Wastewater treatment plants release large amounts of extended-spectrum β-lactamase-producing *Escherichia coli* into the environment. Clin Infect Dis 58(12):1658–1665
39. Leclercq R, Oberlé K, Galopin S, Cattoir V, Budzinski H, Petit F (2013) Changes in enterococcal populations and related antibiotic resistance along a medical center-wastewater treatment plant-river continuum. Appl Environ Microbiol 79(7):2428–2434
40. Łuczkiewicz A, Jankowska K, Fudala-Ksiazek S, Olańczuk-Neyman K (2010) Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. Water Res 44 (17):5089–5097
41. Rosenberg Goldstein RE, Micallef SA, Gibbs SG, George A, Claye E, Sapkota A et al (2014) Detection of vancomycin-resistant enterococci (VRE) at four U.S. wastewater treatment plants that provide effluent for reuse. Sci Total Environ 466–467:404–411. https://doi.org/10.1016/j.scitotenv.2013.07.039
42. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG et al (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18(3):268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x
43. Zhang L, Ma X, Luo L, Hu N, Duan J, Tang Z et al (2020) The prevalence and characterization of extended-spectrum β-lactamase-and carbapenemase-producing bacteria from hospital sewage, treated effluents and receiving rivers. Int J Environ Res Public Health 17(4)
44. Haller L, Chen H, Ng C, Le TH, Koh TH, Barkham T et al (2018) Occurrence and characteristics of extended-spectrum β-lactamase- and carbapenemase-producing bacteria from hospital effluents in Singapore. Sci Total Environ 615:1119–1125. https://doi.org/10.1016/j.scitotenv.2017.09.217
45. Laquaz M, Dagot C, Bazin C, Bastide T, Gaschet M, Ploy MC et al (2018) Ecotoxicity and antibiotic resistance of a mixture of hospital and urban sewage in a wastewater treatment plant. Environ Sci Pollut Res 25(10):9243–9253
46. Vaz-Moreira I, Varela AR, Pereira TV, Fochat RC, Manaia CM (2016) Multidrug resistance in quinolone-resistant gram-negative Bacteria isolated from Hospital effluent and the municipal wastewater treatment plant. Microb Drug Resist 22(2):155–163
47. Cahill N, O'Connor L, Mahon B, Varley Á, McGrath E, Ryan P et al (2019) Hospital effluent: a reservoir for carbapenemase-producing Enterobacterales? Sci Total Environ 672:618–624. https://doi.org/10.1016/j.scitotenv.2019.03.428
48. Harris S, Cormican M (2016) Report No. 162 Hospital effluent: impact on the microbial environment and risk to human health Authors: Dearbháile Morris, Suvi Harris, Carol Morris, Enda Commins and Martin Cormican
49. Proia L, Anzil A, Borrego C, Farrè M, Llorca M, Sanchis J et al (2018) Occurrence and persistence of carbapenemases genes in hospital and wastewater treatment plants and propagation in the receiving river. J Hazard Mater 358:33–43. https://doi.org/10.1016/j.jhazmat.2018.06.058

50. Karami N, Mohammadi P, Zinatizadeh A, Falahi F, Aghamohammadi N (2018) High rate treatment of hospital wastewater using activated sludge process induced by high-frequency ultrasound. Ultrason Sonochem 46:89–98. https://doi.org/10.1016/j.ultsonch.2018.04.009
51. Vo HNP, Koottatep T, Chapagain SK, Panuvatvanich A, Polprasert C, Nguyen TMH et al (2019) Removal and monitoring acetaminophen-contaminated hospital wastewater by vertical flow constructed wetland and peroxidase enzymes. J Environ Manage 250:109526. https://doi.org/10.1016/j.jenvman.2019.109526
52. Mousel D, Pinnekamp J (2018) Ozonation of nursing home wastewater pretreated in a membrane bioreactor. Water Sci Technol 78(2):266–278
53. Zotesso JP, Cossich ES, Janeiro V, Tavares CRG (2017) Treatment of hospital laundry wastewater by UV/H2O2 process. Environ Sci Pollut Res 24(7):6278–6287. https://doi.org/10.1007/s11356-016-6860-5
54. Dires S, Birhanu T, Ambelu A (2019) Use of broken brick to enhance the removal of nutrients in subsurface flow constructed wetlands receiving hospital wastewater. Water Sci Technol 79 (1):156–164
55. Alvarino T, García-Sandá E, Gutiérrez-Prada I, Lema J, Omil F, Suárez S (2020) A new decentralized biological treatment process based on activated carbon targeting organic micropollutant removal from hospital wastewaters. Environ Sci Pollut Res 27(2):1214–1223
56. Verlicchi P, Al Aukidy M, Zambello E (2012) Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment-a review. Sci Total Environ 429
57. Lima DR, Hosseini-Bandegharaei A, Thue PS, Lima EC, de Albuquerque YRT, dos Reis GS et al (2019) Efficient acetaminophen removal from water and hospital effluents treatment by activated carbons derived from Brazil nutshells. Colloids Surf A Physicochem Eng Asp 583:123966. https://doi.org/10.1016/j.colsurfa.2019.123966
58. Lan Y, Groenen-Serrano K, Coetsier C, Causserand C (2018) Nanofiltration performances after membrane bioreactor for hospital wastewater treatment: fouling mechanisms and the quantitative link between stable fluxes and the water matrix. Water Res 146:77–87
59. Tran T, Nguyen TB, Ho HL, Le DA, Lam TD, Nguyen DC et al (2019) Integration of membrane bioreactor and nanofiltration for the treatment process of real hospital wastewater in Ho Chi Minh City, Vietnam. Processes 7(3):1–13
60. Paulus GK, Hornstra LM, Alygizakis N, Slobodnik J, Thomaidis N, Medema G (2019) The impact of on-site hospital wastewater treatment on the downstream communal wastewater system in terms of antibiotics and antibiotic resistance genes. Int J Hyg Environ Health 222 (4):635–644. https://doi.org/10.1016/j.ijheh.2019.01.004
61. Ngo HH, Guo W, Xing W (2008) Evaluation of a novel sponge-submerged membrane bioreactor (SSMBR) for sustainable water reclamation. Bioresour Technol 99(7):2429–2435
62. Nguyen TT, Bui XT, Luu VP, Nguyen PD, Guo W, Ngo HH (2017) Removal of antibiotics in sponge membrane bioreactors treating hospital wastewater: comparison between hollow fiber and flat sheet membrane systems. Bioresour Technol 240:42–49. https://doi.org/10.1016/j.biortech.2017.02.118
63. García Galán MJ, Díaz-Cruz MS, Barceló D (2012) Removal of sulfonamide antibiotics upon conventional activated sludge and advanced membrane bioreactor treatment. Anal Bioanal Chem 404(5):1505–1515
64. Vo TKQ, Bui XT, Chen SS, Nguyen PD, Cao NDT, Vo TDH et al (2019) Hospital wastewater treatment by sponge membrane bioreactor coupled with ozonation process. Chemosphere 230:377–383. https://doi.org/10.1016/j.chemosphere.2019.05.009
65. Von Gunten U (2003) Ozonation of drinking water: part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine. Water Res 37(7):1469–1487
66. Bourgin M, Beck B, Boehler M, Borowska E, Fleiner J, Salhi E et al (2018) Evaluation of a full-scale wastewater treatment plant upgraded with ozonation and biological post-treatments: abatement of micropollutants, formation of transformation products and oxidation by-products. Water Res 129:486–498. https://doi.org/10.1016/j.watres.2017.10.036

67. Shokoohi R, Torkshavand Z, Zolghadnasab H, Alikhani MY, Hemmat MS (2018) Study of the efficiency of moving bed biofilm reactor (MBBR) in LAS anionic detergent removal from hospital wastewater: determination of removing model according to response surface methodology (RSM). Water Sci Technol 2017(1):1–7
68. Tang K, Spiliotopoulou A, Chhetri RK, Ooi GTH, Kaarsholm KMS, Sundmark K et al (2019) Removal of pharmaceuticals, toxicity and natural fluorescence through the ozonation of biologically-treated hospital wastewater, with further polishing via a suspended biofilm. Chem Eng J 359:321–330. https://doi.org/10.1016/j.cej.2018.11.112
69. Ferre-Aracil J, Valcárcel Y, Negreira N, de Alda ML, Barceló D, Cardona SC et al (2016) Ozonation of hospital raw wastewaters for cytostatic compounds removal. Kinetic modelling and economic assessment of the process. Sci Total Environ 556:70–79. https://doi.org/10.1016/j.scitotenv.2016.02.202
70. Mir-Tutusaus JA, Parladé E, Llorca M, Villagrasa M, Barceló D, Rodriguez-Mozaz S et al (2017) Pharmaceuticals removal and microbial community assessment in a continuous fungal treatment of non-sterile real hospital wastewater after a coagulation-flocculation pretreatment. Water Res 116:65–75
71. Jaén-Gil A, Castellet-Rovira F, Llorca M, Villagrasa M, Sarrà M, Rodríguez-Mozaz S et al (2019) Fungal treatment of metoprolol and its recalcitrant metabolite metoprolol acid in hospital wastewater: biotransformation, sorption and ecotoxicological impact. Water Res 152:171–180
72. Badia-Fabregat M, Lucas D, Pereira MA, Alves M, Pennanen T, Fritze H et al (2016) Continuous fungal treatment of non-sterile veterinary hospital effluent: pharmaceuticals removal and microbial community assessment. Appl Microbiol Biotechnol 100(5):2401–2415
73. Kovalova L, Siegrist H, Singer H, Wittmer A, McArdell CS (2012) Hospital wastewater treatment by membrane bioreactor: performance and efficiency for organic micropollutant elimination. Environ Sci Technol 46(3):1536–1545
74. Cruz-Morató C, Lucas D, Llorca M, Rodriguez-Mozaz S, Gorga M, Petrovic M et al (2014) Hospital wastewater treatment by fungal bioreactor: removal efficiency for pharmaceuticals and endocrine disruptor compounds. Sci Total Environ 493:365–376. https://doi.org/10.1016/j.scitotenv.2014.05.117
75. Lucas D, Badia-Fabregat M, Vicent T, Caminal G, Rodríguez-Mozaz S, Balcázar JL et al (2016) Fungal treatment for the removal of antibiotics and antibiotic resistance genes in veterinary hospital wastewater. Chemosphere 152:301–308
76. Rodriguez-Mozaz S, Chamorro S, Marti E, Huerta B, Gros M, Sànchez-Melsió A et al (2015) Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res 69:234–242
77. Badia-Fabregat M, Lucas D, Tuomivirta T, Fritze H, Pennanen T, Rodríguez-Mozaz S et al (2017) Study of the effect of the bacterial and fungal communities present in real wastewater effluents on the performance of fungal treatments. Sci Total Environ 579:366–377. https://doi.org/10.1016/j.scitotenv.2016.11.088
78. Serna-Galvis EA, Silva-Agredo J, Botero-Coy AM, Moncayo-Lasso A, Hernández F, Torres-Palma RA (2019) Effective elimination of fifteen relevant pharmaceuticals in hospital wastewater from Colombia by combination of a biological system with a sonochemical process. Sci Total Environ 670:623–632. https://doi.org/10.1016/j.scitotenv.2019.03.153
79. Petrovich ML, Zilberman A, Kaplan A, Eliraz GR, Wang Y, Langenfeld K et al (2020) Microbial and viral communities and their antibiotic resistance genes throughout a Hospital wastewater treatment system. Front Microbiol 11:1–13
80. Azizi N, Kiomars S, Jila A, Kamaladdin K, Hooshmand S, Hossein A et al (2016) Management of collection, treatment and disposal of hospital wastewater. Case study: Hamadan and Kermanshah province hospitals. Int J Pharm Technol 8(2):12924–12933. www.ijptonline.com
81. Moreno EKG, Garcia LF, Lobón GS, Brito LB, Oliveira GAR, Luque R et al (2019) Ecotoxicological assessment and electrochemical remediation of doxorubicin. Ecotoxicol Environ Saf 179:143–150. https://doi.org/10.1016/j.ecoenv.2019.04.050

82. Seifert C, Guenther E (2019) Prevention is better than cure—environmental management measures in hospitals. Corp Soc Respon Environ Manag 26(4):781–790
83. Haas CN, Rycroft T, Bibby K, Casson L (2017) Risks from ebolavirus discharge from Hospitals to sewer workers. Water Environ Res 89(4):357–368
84. Khan NA, Ahmed S, Vambol S, Vambol V, Farooqi IH (2019) Field hospital wastewater treatment scenario. Ecol Quest 30(3):57–69
85. Bar-On E, Abargel A, Peleg K, Kreiss Y (2013) Coping with the challenges of early disaster response: 24 years of field hospital experience after earthquakes. Disaster Med Public Health Prep 7(5):491–498
86. Zillien C, van Loon C, Gülpen M, Tipatet K, Hanssen B, Beeltje H et al (2019) Risk-management tool for environmental prioritization of pharmaceuticals based on emissions from hospitals. Sci Total Environ 694:133733. https://doi.org/10.1016/j.scitotenv.2019.133733
87. Mišík M, Filipic M, Nersesyan A, Kundi M, Isidori M, Knasmueller S (2019) Environmental risk assessment of widely used anticancer drugs (5-fluorouracil, cisplatin, etoposide, imatinib mesylate). Water Res 164
88. Schmidt PJ (2015) Norovirus dose-response: are currently available data informative enough to determine how susceptible humans are to infection from a single virus? Risk Anal 35 (7):1364–1383
89. Verlicchi P, Galletti A, Petrovic M, Barceló D, Al Aukidy M, Zambello E (2013) Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed – analysis of their respective contributions. Sci Total Environ 454–455:411–425
90. Santos LHMLM, Gros M, Rodriguez-Mozaz S, Delerue-Matos C, Pena A, Barceló D et al (2013) Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: identification of ecologically relevant pharmaceuticals. Sci Total Environ 461–462:302–316. https://doi.org/10.1016/j.scitotenv.2013.04.077
91. Souza FS, Féris LA (2016) Hospital and municipal wastewater: identification of relevant pharmaceutical compounds. Water Environ Res 88(9):871–877
92. WHO (2014) In: Chartier Y et al. Safe management of wastes from health-care activities. World Heal Organ. p 329. http://www.searo.who.int
93. Carraro E, Bonetta S, Bertino C, Lorenzi E, Bonetta S, Gilli G (2016) Hospital effluents management: chemical, physical, microbiological risks and legislation in different countries. J Environ Manage 168:185–199. https://doi.org/10.1016/j.jenvman.2015.11.021
94. Verlicchi P, Galletti A, Masotti L (2010) Management of hospital wastewaters: the case of the effluent of a large hospital situated in a small town. Water Sci Technol 61(10)
95. Verlicchi P, Galletti A, Petrovic M, Barceló D (2012) Micro-pollutants in Hospital effluent: their fate, risk and treatment options. Handbook of environmental chemistry, vol 20
96. Beier S, Cramer C, Köster S, Mauer C, Palmowski L, Schröder HF et al (2011) Full scale membrane bioreactor treatment of hospital wastewater as forerunner for hot-spot wastewater treatment solutions in high density urban areas. Water Sci Technol 63(1):66–71
97. Chonova T, Lecomte V, Bertrand-Krajewski JL, Bouchez A, Labanowski J, Dagot C et al (2018) The SIPIBEL project: treatment of hospital and urban wastewater in a conventional urban wastewater treatment plant. Environ Sci Pollut Res 25(10):9197–9206
98. Wiest L, Chonova T, Bergé A, Baudot R, Bessueille-Barbier F, Ayouni-Derouiche L et al (2018) Two-year survey of specific hospital wastewater treatment and its impact on pharmaceutical discharges. Environ Sci Pollut Res 25(10):9207–9218
99. Chonova T, Keck F, Labanowski J, Montuelle B, Rimet F, Bouchez A (2016) Separate treatment of hospital and urban wastewaters: a real scale comparison of effluents and their effect on microbial communities. Sci Total Environ 542:965–975. https://doi.org/10.1016/j.scitotenv.2015.10.161

100. Vaccari M, Montasser W, Tudor T, Leone L (2017) Environmental audits and process flow mapping to assess management of solid waste and wastewater from a healthcare facility: an Italian case study. Environ Monit Assess 189(5)
101. Khan NA, Khan SU, Ahmed S, Farooqi IH, Yousefi M, Mohammadi AA et al (2020) Recent trends in disposal and treatment technologies of emerging-pollutants – a critical review. Trends Anal Chem 122
102. Verlicchi P, Grillini V (2020) Surface water and groundwater quality in South Africa and Mozambique-analysis of the most critical pollutants for drinking purposes and challenges in water treatment selection. Water 12(1)
103. Cristóvão MB, Torrejais J, Janssens R, Luis P, Van der Bruggen B, Dubey KK et al (2019) Treatment of anticancer drugs in hospital and wastewater effluents using nanofiltration. Sep Purif Technol 224:273–280. https://doi.org/10.1016/j.seppur.2019.05.016

Suspect and Non-target Screening Methodologies for the Evaluation of the Behaviour of Polar Organic Micropollutants and Changes in the Molecule Fingerprint During Water Treatment

Pablo Gago-Ferrero

Contents

Abstract The evaluation of water treatment systems in terms of presence, fate and removal of organic micropollutants is of paramount importance for the optimal protection of both the environment and human health. In this regard, there are significant gaps in the existing data for compounds present in treated water that remain uncharacterised but are still hazardous from both a toxicological and ecological point of view. This work aims to critically summarise the existing

P. Gago-Ferrero (✉)
Catalan Institute for Water Research (ICRA), Girona, Spain

University of Girona, Girona, Spain
e-mail: pgago@icra.cat

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 97–118, DOI 10.1007/698_2020_662,

information about the application of suspect and non-target screening approaches based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) to understand the behaviour of polar organic micropollutants and their corresponding transformation products during water treatment processes, focusing particularly on wastewater. Also, it includes a discussion about the novel non-target workflows whose objective is not the further structure elucidation of particular compounds but assessing changes in the molecular elemental compositions of the dissolved organic matter (DOM) during water treatment (without structural characterisation). These strategies can provide additional information and become a common step for a better understanding of treatment performance and transformation product formation.

Keywords Anthropogenic, Cheminformatics, Electrokinetic, Hydrophilic-lipophilic balance, Spectrometry

1 Introduction

Discharges from wastewater treatment plants (WWTPs) are one of the major sources of aquatic pollution. Commonly used WWTPs, primarily operating through biological processes, were developed and designed to protect natural aquatic systems and water resources mainly by removing loads of carbon, nitrogen and phosphorous present in the influent in the mg L^{-1} range. The increased detection of a wide range of emerging, not-regulated anthropogenic organic micropollutants (MPs) in the aquatic environment shows the limitations of conventional WWTPs in removing these compounds. Their continuous introduction into the environment, pseudo-persistence and intrinsic ability to interfere with organisms concern the scientific and public community because their potential toxic effects can threaten the ecological status of water bodies and human health via, e.g. the drinking water quality and/or long-term endocrine effects. Therefore, advances in wastewater treatment technologies are crucial in minimising the burden of wastewater-originated contaminants. In parallel to the technological developments, one of the major challenges remains the evaluation of novel and alternative treatment options regarding their potential to minimise the toxicological risks for both ecosystems and human health [1].

The global universe of chemicals is very complex and includes hundreds of thousands of substances in commercial use [2]. However, the effects of these compounds on the environment and human health and their combined effects are in most cases not at all or only partially investigated [3]. Within the last years, polar organic MPs (e.g. pharmaceuticals) became an increasing area of focus for environmental scientists and regulatory authorities encouraged by the advances in liquid chromatography coupled to mass spectrometry (LC-MS) technologies. The existing

target screening methods are based on the preselection of certain chemicals and the use of reference standards and can only cover a small proportion of organic pollutants. However, it should be stressed that toxicity and related unwanted effects can be associated with chemicals that can be identified and those that cannot be identified as a result of limitations in the repertoire of chemical analytical techniques. Therefore, important site-specific and potentially ecotoxicologically relevant compounds are systematically missed [4], and the use of *suspect* and *non-target* screening analytical approaches, where no particular chemical is being searched for, is necessary to obtain a broader and more realistic picture. Thus, the improvements in the characterisation of water and the study of the fate of MPs during processes (e.g. WWTPs), where the entire bulk of the dissolved organic matter (DOM) and not few selected substances are considered, are of paramount importance. For a holistic risk assessment, target-based environmental monitoring should be accompanied by non-target analysis. This is particularly important in order to evaluate the behaviour and removal of organic MPs during water treatment processes, where a large number of transformation products (TPs) are continuously generated, which can pose a higher threat than the corresponding parent compounds to both the environment and human health.

This chapter aims to critically summarise the existing information about the application of suspect and non-target screening approaches based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) to understand the behaviour of polar organic MPs and their corresponding TPs during water treatment processes, focusing particularly on wastewater. All this information is crucial to evaluate which are the potentially hazardous compounds that reach the environment through effluents and which improvements are urgently needed. Also, it is included a section describing novel non-target workflows whose objective is not the further structure elucidation of particular compounds but assessing changes in the molecular fingerprints of DOM during water treatment (without structural characterisation). These strategies can provide additional information and become a common step in research dealing with the evaluation of water treatment.

2 LC-HRMS-Based Analytical Methodologies

2.1 Sampling Strategy and Sample Pretreatment

The sampling strategy as well as the sample treatment procedure should be defined considering the research question that needs to be tackled as well as the resource availability. It can only be optimally designed when the objective and the methodology of a given study has been well formulated.

In order to evaluate the presence and behaviour of MPs during water treatment, it is necessary to collect different samples in time and space. For example, the studies focusing on assessing the removal of MPs in WWTPs usually need to collect at least influent and effluent (after secondary or tertiary treatment) water samples. Collection

of grab samples is probably the most used approach due to its simplicity. However, it only allows the obtaining of the chemical profile of the exact time point when the samples were collected. Composite samples (e.g. 24-h integrated samples) allow the obtaining of a more comprehensive picture, and this approach is highly recommended (if not totally necessary) when fluctuating concentrations or episodic pollution events are expected (as in the case of WWTP effluents) and average concentrations or profiles need to be determined ([5]; Gago-Ferrero et al., [6]). Composite sampling should be carefully designed to avoid the loss of compounds due to degradation processes during the collection period. In this regard, collectors with thermostatic control are highly useful. For studies comparing the levels of MPs in different steps of the water treatment process (e.g. comparing influent and effluent water), it is of paramount importance to use time and flow proportional samples. In this manner, the water that is being analysed in the influent corresponds to the same one that will be analysed in other treatment steps a certain time later (e.g. secondary effluent or tertiary effluent). Otherwise, results can lead to wrong conclusions. The use of time-integrated passive samples is not common to evaluate wastewater treatments, but it is regularly used to evaluate waters affected by wastewater effluents [7–9].

Until now, the studies conducted using generic suspect and non-target screening approaches for the evaluation of wastewater treatment have primarily focused on aqueous samples (mainly influent and effluent). In these cases, pretreatment consisted basically of a filtration step through glass fibre and/or cellulose filters and adjusting the pH to a value optimum for the subsequent extraction. Filtration of the samples should be done as fast as possible to prevent bacterial degradation. This is the same sample pretreatment that it is usually applied to natural waters. In cases where the formation of disinfection by-products (DBPs) needs to be evaluated, it is necessary the previous addition of a quencher (e.g. ascorbic acid) in order to stop the disinfection reactions.

2.2 Sample Extraction and Instrumental Analysis

In the case of target methodologies, the choice of the sample extraction procedure is based on the properties of the analytes that are to be determined as well as the matrix of interest. Non-target methodologies follow a different conceptual approach since the aim normally consists of the determination of a wide range of still unknown substances with different physicochemical properties. All organic compounds present in the samples are potentially relevant, and therefore losses during the extraction process must be minimised. In this regard, strategies avoiding sample treatment, like a direct injection of filtrated liquid samples without any enrichment, are great options since they are fast and allow a comprehensive analysis when the sensitivity of the MS is high enough [10]. However, interferences with the matrix are common in wastewater, and their applicability is limited. Therefore, the most common strategy so far consists of conducting solid-phase extraction (SPE) by using different sorbent

materials, depending on the specific objective of the study. There are studies using specific SPE sorbents for very specific compounds, like the use of coconut charcoal (Restek®) for the analysis of nitrosamines (in this case using GC-MS) [11, 12]. Other studies use stationary phases for a broader chemical enrichment, like the popular hydrophilic-lipophilic balance Oasis HLB which have been largely used for screening of pharmaceuticals, pesticides and other polar compounds [13]. For real wide-scope screening, the use of mixed-bed multilayered cartridges has become a very popular approach. These cartridges normally contain HLB (or equivalent) sorbents, anion- and cation-exchange resins (e.g. Strata XAW and Strata XCW, respectively) and the highly retentive nonpolar sorbent (e.g. Isolute ENV+), in order to enrich neutral, cationic and anionic species of a broad range of physicochemical properties [14, 15]. Using different cartridges stacked in series [16] or the combination of more specialised SPE sorbents to analyse substances with specific physicochemical properties (per- and polyfluoroalkyl substances (PFASs) or specific drugs) is also a valid option [16, 17]. Wide-scope SPE can lead to the extraction of a large number of substances that can significantly increase the matrix effect, causing serious difficulties in the analysis. Thus, it is always necessary to find a compromise to balance all the factors considering the analytical needs of the work. In some cases, procedures including more intense clean-up steps should be considered since the reduction of interferences can improve the outcome.

For wide-scope screening of water samples (both target and not target), most studies use LC coupled to HRMS, which provides high mass accuracy and high mass resolution. These features are necessary in order to identify suspected and unknown compounds. Hybrid instruments like linear ion trap/Orbitrap or quadrupole/TOF (qTOF) have shown high-performance detection capabilities, particularly for small molecules in complex matrices like wastewater. These technologies allow reliable identifications based on high-resolution accurate mass measurement of precursor and product ions. Electrospray (ESI) is by far the most used ionisation technique due to its good performance for polar organic MPs in aqueous matrices. However, it can be effectively complemented with other techniques suitable for less polar compounds like atmospheric pressure photoionisation (APPI) or atmospheric pressure chemical ionisation (APCI) [18, 19].

Ultra-high-performance liquid chromatography (UHPLC) increases the identification capability of MPs in complex matrices due to better separation power [20]. Also, it reduces the problem of co-eluting peaks and mass spectral overlap [21]. However, the shorter run times of this technique lead to less MS/MS data points per peak, which can negatively affect the identifications. Thus, the optimal solution involves finding a compromise, considering all the factors and the analytical needs for each specific study. The loss of MS/MS data points discouraged coupling UHPLC with Orbitrap type instruments in the past. However, the new generation of Orbitrap instruments, with shorter scan times, has solved this problem to an acceptable extent. Separation for HRMS-based screenings is mostly achieved using reverse-phase (RPLC) with C18-type columns, allowing good stability and performance for a very wide range of compounds. However, RPLC has important limitations, and highly polar compounds remain in many cases undetected. This is

particularly very important for the identification of TPs (often with high polarity) during water treatment. In this regard, hydrophilic interaction liquid chromatography (HILIC) is an attractive complementary technique due to its ability to separate hydrophilic compounds that are poorly retained on RP columns as it has been demonstrated in different studies performed in wastewater [14, 22–25]. Recently, ion mobility separation coupled to HRMS has become available and promises significantly improved instrumental performance by adding one additional dimension of separation to the analytical system, i.e. separation in an electric field based on the shape-dependent velocity of the molecule [26, 27].

3 Strategies for the Evaluation of the Behaviour of Organic Micropollutants During Wastewater Treatment

3.1 Data Preprocessing

HRMS instruments such as qTOFs and Orbitraps acquire accurate mass and high-resolution MS and MS/MS full-scan spectra leading to a large amount of data. In the margins of the analytical conditions (e.g. sampling, enrichment method and solvents used) and instrumental limitations (e.g. ionizability, selectivity, sensitivity and resolution), the full mass spectral information of detectable compounds are stored in raw data files [28]. At this point, preprocessing is necessary to improve data quality and reduce complexity. This step normally includes chromatographic retention time alignment and mass correction, peak detection, subtraction of compounds present in blanks as well as "componentisation" via a grouping of isotopes, adducts, multi-charged ions and in-source fragments to define components (i.e. grouping all signals that likely belong to one unique molecular structure) [29, 30]. Differences in algorithms between different software influence significantly the outcome of the data preprocessing. Therefore, a good optimisation of the preprocessing step is crucial for obtaining high quality and reliable ready-to-use data. Different vendor software is available for preprocessing the data, although in many cases they do not allow full control of the process but only minor adjustments [31]. However, there are open-source possibilities that have been used in the preprocessing step of wastewater samples including RMassBank [32], enviMass (Martin [33]), XCMS [34] or MZmine 2 [35], among others.

3.2 Prioritisation Strategies

There are significant gaps in the existent data for compounds present in treated water that remain uncharacterised but are still hazardous from both a toxicological and ecological point of view. However, their prioritisation and further identification is

not an easy task. Wastewater and other environmental samples are complex chemical mixtures containing tens of thousands of individual substances that produce a high number of peaks in LC-HRMS analysis. The suspect and non-target screening approaches are laborious, time-consuming and computationally challenging, especially when there is limited prior knowledge of the chemical classes likely to be present [36]. The complete elucidation of all the peaks present in the samples is not feasible since it would require extensive time and effort. Thus, prioritisation strategies (peak prioritisation) are necessary and a key step in any investigation involving non-target analysis to focus the efforts on relevant chemicals. Therefore, different prioritisation strategies should be applied to the set of obtained chromatographic peaks depending on the specific goals of the study and the research question to be answered [29, 30]. Prioritisation should also find an acceptable balance between the prioritised peaks and the number of false negatives caused by too strict procedures.

So far, many of the developed prioritisation strategies for environmental samples followed intensity-based criteria combined with the prioritisation of chemicals with a distinctive isotopic pattern (e.g. compounds containing halogens) [37–40], as these can be considered as relevant chemicals with reasonable identification changes. Other developed approaches used mass defect to focus the identification efforts on molecular formulas outside the matrix domain in complex samples [5, 10]. This strategy has also been proved useful when the objective was to find molecules with specific characteristics. An example can be found in the detection of perfluoroalkyl ether carboxylic acids and sulfonic acids in natural waters due to the negative mass defect of the multiple fluorine and oxygen atoms [41]. Peak prioritisation can be conducted based on effect-directed analysis (EDA), which is a valuable tool for understanding the link between the presence of mixtures of pollutants and their associated effects. EDA is useful for identifying predominant toxicants in complex environmental mixtures combining effect testing and fractionation [15, 18, 19, 42–46]. In EDA, sample fractionation includes adsorption, size exclusion and partition chromatography techniques, resulting in a significant simplification of the complex mixture, and facilitates linking the presence of a compound with their cause and effect. Other strategies that have been shown to be effective include time-series prioritisation (prioritising substances whose intensities varied substantially over a specific lapse of time in one sampling site [28, 47–50], are based on spatial variation [51] or use metabolomics logics [52]). In the field of trend analysis, Schlüsener et al. [47] used vendor software from SCIEX (MarkerView) to analyse long time-series LC-HRMS data coming from a specific site of Rhine river which was affected by effluents of WWTPs. Afterwards, they used open-source scripts to visualise the patterns and to perform autocorrelation to search and prioritise the features with high periodic variations. One of the main limitations in prioritisation efforts, particularly important for the evaluation of wastewater treatment, is that comparison between matrices (influent and effluent) is hampered by suppression of signals in matrix-rich samples [29, 30]. Isotopic-labelled internal standards are used for correction, but robust methods to correct for these influences for unknown compounds with various functional groups and thus varying ionisation efficiencies are needed [29, 30].

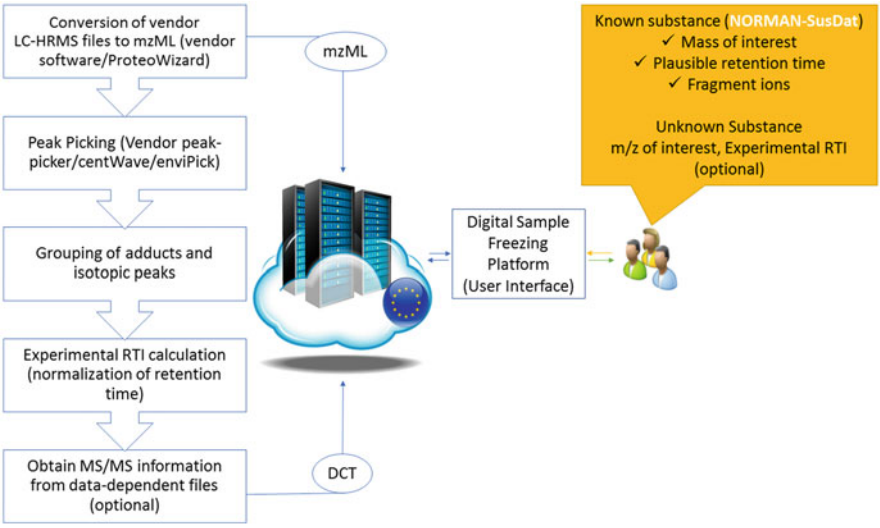


Fig. 1 Summary of the workflow used in the NORMAN Digital Sample Freezing Platform (DSFP) for automatic suspect screening. Adapted with permission from ref. Alygizakis et al. [28]. copyright (2019) Elsevier

Without a doubt the most commonly used conceptual approach to prioritise compounds is the so-called suspect screening that consists of the search for masses of compounds that are expected in the sample without the use of reference standards. Fragmentation information helps find structurally related compounds, and chromatographic retention time prediction models help in increasing the confidence in the identifications. Building the suspect list is a crucial step and should be done carefully in order to answer specific research questions. Suspect screening can include a large list of compounds (up to several thousands) for wide-scope screening. In this regard, a massive tool can be found in the NORMAN Digital Sample Freezing Platform (DSFP) [28], whose operation is summarised in Fig. 1. In this platform the layer-separated mzML files and their meta-data (instrumental, sample meta-data, matrix-specific meta-data and retention time of retention time index (RTI) calibration substances) are uploaded by the user. DSFP has integrated standard procedure to process the mzML files and store the files together with all meta-data for the generation of Data Collection Template (DCTs) databases. Uploading of files in DSFP enabled retrospective screening of >60,000 organic chemicals. Moreover, this will enable the possibility for future retrospective analysis when more advanced tools will be available. This tool also allows performing smaller and more specific suspect screening studies by selecting the compounds of interest or choosing smaller predefined suspect lists (e.g. pesticides, pharmaceuticals TPs, PFASs). In many cases when a specific research question has been clearly defined, it can be more efficient to create smaller smart suspect lists that allow a more time efficient management of the resources and efforts. Some applications of this type of more

concise suspect consist of focusing on known toxic chemicals that have been previously detected [53, 54], focusing on specific lists of potential TPs [23] or using regulatory databases (market data) based on exposure index to focus on previously non-reported compounds with high chances of being present [55–57].

In the specific case of wastewater treatment, the study of the TPs that are generated during these processes is especially important. TPs encompass a large proportion of unregulated chemicals present in the environment and generated during water treatments that may be equally, if not more, persistent and toxic than their precursor chemicals. Other toxic TPs include metabolites of pesticides or pharmaceuticals [36]. From the non-target point of view, it is necessary to find the best approaches to determine this specific group of compounds considering their specificities. One simple and useful approach consists of prioritising components across related samples, from spatial and time trends through to "before and after" comparison of treatment technologies [29, 30]. In this approach, a sample is used to create a reference method, containing a comprehensive list of peaks with accompanying retention time and mass spectra. The reference sample serves as the basis of comparison for other samples. There are types of software that compute pairwise comparison to determine peaks present only in the samples and not in the references (e.g. generated during the treatment). Other interesting strategies include using metabolic logic in combination with multivariate statistics in order to find unknown metabolites of certain substances [52].

Obviously, the use of suspect screening is a powerful tool to detect TPs. The main limitation is that in several cases, the potential TPs that can be formed during treatment are not present in any available database. However, provided that there is some a priori knowledge of the classes of compounds present in a complex chemical mixture (e.g. wastewater), cheminformatics can be used to predict the structure of potential toxic TPs. Several powerful tools have been developed in this regard like the EAWAG path prediction system (EAWAG PPS), enviPath [58], PathPred [59], Meteor Nexus (Lhasa Ltd., Leeds, UK), ADMET Predictor software (Simulation Plus Inc., Lancaster, USA) or Catalogic (Laboratory of Mathematical Chemistry). An extensive explanation of these approaches can be found elsewhere [36, 60, 61].

A representative example for the study of the formation of TPs during water treatment can be found in the work carried out by Beretsou et al. [22]. This work aimed at understanding the fate and transformation of citalopram (a worldwide highly consumed antidepressant which has demonstrated incomplete removal by conventional wastewater treatment) during the biological treatment process. For this purpose, biodegradation batch experiments under aerobic conditions were carried out to investigate its behaviour and the formation of TPs during activated sludge treatment. One of the main objectives of the study was the identification of the formed TPs by applying suspect and non-target strategies based on LC-HRMS. As a first step, a suspect database of plausible TPs was compiled by using two different in silico prediction tools: (I) the EAWAG-Biocatalysis/Biodegradation Database Pathway Prediction System (EAWAG-BBD/PPS), an artificial intelligence system, which predicts microbial metabolic reactions based on biotransformation rules set

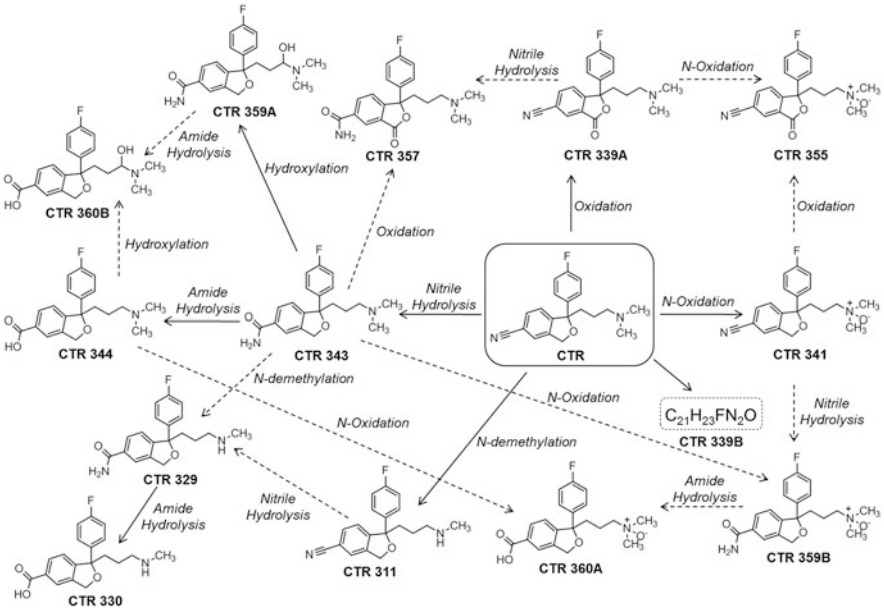


Fig. 2 Proposed biotransformation pathway for citalopram with transformation products identified following suspect and non-target screening strategies. Adapted with permission from Beretsou et al. [22]. copyright (2016) Elsevier

in the EAWAG-BBD and scientific literature and (II) the MetabolitePredict software (Metabolite Tools 2.0, Bruker Daltonics, Bremen, Germany), a rule-based expert system, which predicts metabolites from Phase I and Phase II and Cytochrome P450 reactions. As a second step, samples were also screened for additional TPs not present in the suspect database, following a non-target approach based on pairwise comparison to determine peaks present only in the biotic samples but absent in the control samples and that showed a meaningful time trend. After careful application of identification and confirmation strategies, 12 TPs of citalopram were identified through the use of suspect screening, and 2 additional TPs were detected by non-target screening. The pathway for the biotic transformation of citalopram is shown in Fig. 2. The performance of suspect screening and non-target screening as independent and complementary approaches resulted in comprehensive identification of the formed TPs within specific biotransformation systems. In this work, analyses were performed by both RPLC and hydrophilic interaction liquid chromatography (HILIC) to investigate their complementarity for the detection of additional compounds. In-house developed QSRR prediction models were also used to support identification in both chromatographic systems. The orthogonal confirmation by RPLC and HILIC analysis, along with chromatographic retention time prediction models, proved to be complementary strategies in the identification workflow. The importance of HILIC is emphasised in the detection of an additional TP (enabling the separation from its isomer), which could not be detected through RPLC analysis. In

this regard, HILIC is becoming an attractive alternative (or complement) to the commonly used reversed-phase liquid chromatography (RPLC), due to its ability to separate hydrophilic compounds which are poorly retained on RPLC columns [14, 41].

3.3 *Suspect/Non-target: Identification Approaches of Polar Organic Contaminants*

After the crucial prioritisation step, efforts should be focused on the identification of the selected components. This step involves the evaluation of all information available from MS and MS/MS (molecular ion, isotope pattern and fragments), spectral and compound databases, chromatographic retention time information as well as meta-information such as the specific water treatment context. In general, for suspect screening, an exact mass match is not sufficient for identification alone [29, 30]. Suspect screening approaches, apart from a strict mass accuracy threshold, require a detailed study of the MS/MS spectra. A powerful option is the use of MS/MS libraries, which are in constant expansion and contain >1 million of MS/MS for more than 30,000 chemicals. Popular libraries include MassBank (www.massbank.eu (Europe), www.massbank.jp (Japan) and www.mona.scripps.edu (North America)), NIST MSMS (https://www.nist.gov/srd/nist-standard-reference-database-1a-v17), METLIN (www.metlin.scripps.edu) or mzCloud (www.mzcloud.org). However, in many cases, it is not possible to find the MS/MS spectra for the compounds of interest in available libraries. This is a very common situation for TPs, with still a very low presence in public databases. In these cases, in silico fragmentation techniques (such MetFrag [62], CFM-ID [63] or CSI-FingerID/SIRIUS [64]) can be used to obtain predicted fragments and compare with the experimental ones (as well as the manual expert inspection of the MS/MS spectra if there is sufficient structural information). Apart from this, other useful additional data to support identification include literature references, patent data or functional uses.

The identification of *known unknowns*, compounds without prior knowledge but present in large compound databases, is a more challenging but still doable task. In these cases, the first step consists of the determination of the molecular formula. For this purpose, there are various calculations based on mass accuracy, presence/absence of certain elements and using the presence of isotope signals for certain elements (e.g. sulphur), isotopic pattern fit and fragment sub-formula assignment. Most vendor software includes useful tools to perform this task. Other advance software also considers MS/MS data. MOLGEN-MS [65] suggests candidate structures based on the mass spectral fragmentation patterns, and it has shown excellent performance in the formula assignments. Some studies have also added steric energy to make the search more accurate [45]. Once the molecular formula has been determined, a combination of in silico fragmentation techniques with MS/MS libraries and expert knowledge are the most used tools to tentatively elucidate the

structure of those unknown compounds. The most sophisticated in silico fragmentation approaches along with environmental context or metadata ranked the correct candidate to over 70%, showing a very good performance of these approaches [62, 66]. In water treatment, evaluation is often required to identify novel TPs and other unknown compounds. If these compounds have not been documented anywhere, the identification is much more challenging with no guarantees of success. However, some successful examples have been reported for small molecule and sufficient available structural information [67].

The evaluation of the chromatographic retention time (RT) plausibility for the compounds of interest using different developed models plays an important role in increasing confidence and eliminating less-plausible candidates in both suspect and non-target approaches. Tentatively identifications of the candidates are based on comparing how well the experimental RT (or retention index (RI)) matches the predicted RT (or RI) from computational models. Different RT prediction tools have been designed including quantitative structure retention relationships (QSRR) models [25, 68], artificial neural networks (ANNs) [69] or linear solvation energy relationship (LSER) [70]. It is noticeable that some of the platforms for automated suspect screening, like the NORMAN DSFP [28] or STOFFIDENT (https://www.lfu.bayern.de/stoffident), have implemented RT prediction models for a better identification performance. They include a step that calculates an experimental RTI for every feature detected based on the retention time observed and the calibration curve equation (RT ¼ f(RTI)), derived from the retention time of the standard calibration mixture [71].

Ion mobility (IM) separation coupled to HRMS (only QTOF systems) has recently been introduced to the market and promises significantly improved instrumental performance by adding one additional dimension of separation to the analytical system, i.e. separation in an electric field based on the shape-dependent velocity of the molecule [26, 27]. It significantly improves the identification of problematic compounds (e.g. isomers) and has been successfully applied to improve identification in both suspect and non-target methodologies [72].

The increasing interest in HRMS-based analysis has produced a very pronounced growth in the suspect and non-target identifications. This fact creates also a need to communicate the confidence in the identifications in a way that reflects all the evidence available. The varying levels of confidence are difficult to communicate concisely and accurately. So far, most of the studies report the confidence based on the hierarchical degrees of confidence described by Schymanski et al. [73]. In this classification, the compounds that have been confirmed with an available commercial standard are classified as Level 1 (maximum degree of confidence). The compounds identified without standard but by mass spectral library or database matching are reported with confidence Level 2 (probable structure). Level 3 (tentative candidate) describes a “grey zone”, where evidence exists for possible structure (s) but insufficient information for one exact structure only (e.g. positional isomers). Compounds for which structures cannot be elucidated are reported with Level 4 (if the molecular formula is unequivocally assigned) or Level 5, reporting only a mass of interest.

In many cases, although the aforementioned levels (published in 2014) are certainly useful, it is difficult to communicate the identification's confidence level in a concise manner. Therefore, a complementary system that allows the community to rapidly and concisely understand the identification proofs that have been obtained when a compound is reported is needed. In this regard, a system based on identification points (IPs), compatible between target and non-targeted approaches would be really valuable and would add accuracy to the field. Recently, a new system of identification points (IPs) based on the one described in the Commission Decision 2002/657/EC was applied to communicate the confidence level in the identification of the analytes for target analysis [74]. This system considers retention time, mass accuracy, isotopic fit and fragmentation, taking full advantage of the capacities of the HRMS instruments [74]. This system could be easily extended and encompass non-target approaches by including variables such as predicted retention time, determined MS/MS experimental ions and predicted fragment ions (both with mass accuracy) or ion mobility, assigning a specific value of IPs in each case.

3.4 Novel Non-target Strategies to Assess Changes in the Molecular Fingerprints Without Structural Elucidation

DOM is a heterogenic, complex mixture of proteins, lipids, polysaccharides, nucleic acids, soluble microbial products and also synthetic organic chemicals. In wastewater, these chemicals include thousands of compounds and related TPs, metabolites and, in effluents with tertiary treatment, also a wide range of disinfection by-products (DBPs). Wastewater is a matrix with high complexity, and HRMS analysis typically produces a large number of signals. Even after preprocessing steps, performing a manual structural identification of all the compounds is not a feasible task, and the same applies to other water treatment systems. Therefore, approaches are being developed to characterise the chemical composition of DOM in water, where previously most of the DOM components remained unidentified [75]. The objective is not the further structure elucidation of particular compounds but assessing changes in the molecular fingerprints during water treatments. It is possible to obtain relevant information from HRMS analysis on DOM if a large part of the detected features can be assigned a probable elemental composition (without focusing on structural elucidation of specific components). This is giving rise to a new field of study that allows the study of changes (e.g. the overall oxidation state, the number of sulfonated or chlorinated formed compounds or the average molecular weight) during wastewater treatment (as well as other treatments such as drinking water treatment and even soil remediation) by measuring shifts in the molecular fingerprint and other physicochemical changes.

The common protocol of a data-driven non-targeted analysis involves the same steps as described in the preprocessing section (Sect. 3.1) in order to obtain ready-to-

use data. Data mining from HRMS data on DOM analysis yields to large lists of molecular features, understood as the integration of all the different MS features such as accuracy mass, isotopic information or average retention time (when chromatography is used). Based on that data, algorithms can be constructed to create an artificial list of DOM molecule elemental compositions (e.g. $C_nH_nO_nN_nS_nCl_nBr_n$) and therefore obtain DOM fingerprints. Such non-targeted workflow aims to gain insights on wastewater DOM transformation processes based on changes in this large number of detected molecular features. This new manner of evaluating HRMS data reduces manual data treatment present in the conventional suspect and non-target strategies, and it has been adapted from other fields like the characterisation of natural organic matter (NOM) [76–79] or petroleomics [80]. It has been widely applied to evaluate the OM in processed water [81–83], and there are also few studies on wastewater using different instrumentation. Back in 2011, Gonsior et al. performed a molecular characterisation of effluent OM using Fourier-transform ion cyclotron resonance MS (FTICR-MS) [84]. Also, by using FTICR-MS, Mesfioui et al. characterised effluent organic nitrogen (EON) from wastewater treatment plants and study its reactivity [85]. The results obtained from these non-target-based approaches for the molecular characterisation of DOM are highly influenced by the type of HRMS analyser, due to differences in resolution of masses, which affect the number of substances and how accurately the HRMS features can be assigned with an elemental composition. The differences obtained with different instrumentation systems are not that crucial when screening for specific substances [86] but are more important in the analysis of unknown DOM constituents and should be carefully considered [87]. It has been demonstrated that FTICR-MS is capable of unravelling complex DOM mixtures across aquatic systems [88, 89] and might allow a comprehensive evaluation of changes in the molecular fingerprints. However, the high cost of this technology made it inaccessible for most research laboratories. Recently, Orbitrap-MS (a much more accessible technology) has proven to be a valid alternative, despite its lower resolution, as it is able to detect subtle changes in DOM profiles. Verkh et al. use LC-HRMS with an LTQ-Orbitrap Velos™ to evaluate a real wastewater treatment system with secondary and tertiary treatment identifying significant chances in the DOM during these processes [90]. This study detected a reduction in the number of large molecules, lower average masses as well as an increase in saturated molecular features of the effluent OM.

In the field of drinking water treatment, these approaches have great potential. There are studies that have applied it to the study of the formation of DBPs and to investigate how DOM contributes to their generation where, i.e. Postigo et al. investigated the chemodiversity of DBPs in different drinking water plants [91]. In a similar way, Sanchis et al. investigated how DOM contributes to the formation of DBPs aiming to understand and minimise their formation [92]. This study showed that sample chlorination primarily involved a decrease in the concentration of non-nitrogenised lignin-like features, and it was also related with changes in the trihalomethanes and haloacetonitrile formation potential. The N-nitrosodimethylamine (NDMA) formation potential in natural waters has also

been studied following a similar workflow, and a correlation was found with the number of compounds with high hydrogen saturation (H/C $\geq$ 1.5), which corresponded to reservoirs with higher background nutrient concentrations and wastewater indicators [93]. Other water processes like the KMnO4 oxidation have also been studied and linked with DOM [94]. These non-target approaches have shown to be also useful for other type of treatment processes. For example, a non-target workflow based on the evaluation of the molecular fingerprint without structural elucidation has been recently applied to an electrokinetic soil remediation process as a case study and allowed for an in-depth assessment of the chemical mechanistic processes, which has previously only been hypothesised and has contradictory results [95]. Many of these works use additional tools to evaluate changes to the DOM such as Van Krevelen diagrams, where the atomic ratio X/C, with X being an element of interest, is plotted against H/C. In other fields like NOM chemistry or petroleomics, the correlation between areas in these diagrams and functional classes of compounds provide important information to elucidate the chemical composition of OM [96–99]. In many studies, O/C ratio is plotted against H/C obtaining information about the oxidation of the DOM. Also, the monitoring of double bond equivalents (DBE) or DBE minus oxygen atoms (DBE-O) in wastewater treatment can be useful to estimate the quality of the process, to evaluate the hydrophobicity-altering reactions (e.g. oxidation or hydrolysis) and conceptually to even support the control of the process [90, 100].

These workflows for the assessment of changes in the molecular fingerprint can be very useful and become a common step in the research dealing with the evaluation of water treatments. They can be applied to tertiary treatments (e.g. UV irradiation, Fenton-like processes, ozonation, membranes or chlorination) and other innovative techniques. Overlooking potentially hazardous constituents in effluents might limit the understanding of the impact of the discharged organic matter on the environment. Overall changes in the elemental compositions can reveal changes in the degree of oxidation, the formation of TPs with specific elements like sulphur (e.g. using sulphate-based advanced oxidation processes) or halogens during disinfection, along with other valuable information. Therefore, their evaluation is crucial for a better understanding of the different treatments' performance and the TPs formation.

References

1. Prasse C, Stalter D, Schulte-Oehlmann U, Oehlmann J, Ternes TA (2015) Spoilt for choice: a critical review on the chemical and biological assessment of current wastewater treatment technologies. Water Res 87:237–270
2. Swedish EPA (2011) Swedish consumption and the global environment. http://www.naturvardsverket.se/Documents/publikationer/978-91-620-1285-4.pdf?pid=2687. Accessed 05 Apr 2020
3. EEA (2010) The European environment. State and outlook 2010

4. Hug C, Ulrich N, Schulze T, Brack W, Krauss M (2014) Identification of novel micropollutants in wastewater by a combination of suspect a nontarget screening. Environ Pollut 184:25–32
5. Moschet C, Piazzoli A, Singer H, Hollender J (2013) Alleviating the reference standard dilemma using a systematic exact mass suspect screening approach with liquid chromatography-high resolution mass spectrometry. Anal Chem 85:10312–10320
6. Menger F, Gago-Ferrero P, Wiberg K, Ahrens L (2021) Wide-scope screening of polar contaminants of concern in water: a critical review of liquid chromatography-high resolution mass spectrometry-based strategies. Trends Environ Anal Chem. (Online) https://www.sciencedirect.com/science/article/pii/S2214158820300301?via%3Dihub. https://doi.org/10.1016/j.teac.2020.e00102
7. Soulier C, Coureau C, Togola A (2016) Environmental forensics in groundwater coupling passive sampling and high-resolution mass spectrometry for screening. Sci Total Environ 563–564:845–854. https://doi.org/10.1016/j.scitotenv.2016.01.056
8. Gallen C, Heffernan AL, Kaserzon S, Dogruer G, Samanipour S, Gomez-Ramos MJ, Mueller JF (2019) Integrated chemical exposure assessment of coastal green turtle foraging grounds on the Great Barrier Reef. Sci Total Environ 657:401–409. https://doi.org/10.1016/j.scitotenv.2018.11.322
9. Menger F, Ahrens L, Wiberg K, Gago-Ferrero P (2021) Suspect screening based on market data of polar halogenated micropollutants in river water affected by wastewater. J Hazard Mat 401:123377. https://doi.org/10.1016/j.jhazmat.2020.123377
10. Vergeynst L, van Langenhove H, Joos P, Demeestere K (2014) Suspect screening and target quantification of multi-class pharmaceuticals in surface water based on large volume injection liquid chromatography and time-of-flight mass spectrometry. Anal Bioanal Chem 406 (11):2533–2547
11. Farré MJ, Insa S, Lamb A, Cojocariu C, Gernjak W (2020) Occurrence of: N -nitrosamines and their precursors in Spanish drinking water treatment plants and distribution systems. Environ Sci Water Res Technol 6(1):210–220
12. Farré MJ, Döderer K, Hearn L, Poussade Y, Keller J, Gernjak W (2011) Understanding the operational parameters affecting NDMA formation at advanced water treatment plants. J Hazard Mater 185(2–3):1575–1581
13. Leendert V, Van Langenhove H, Demeestere K (2015) Trends in liquid chromatography coupled to high-resolution mass spectrometry for multi-residue analysis of organic micropollutants in aquatic environments. TrAC Trends Anal Chem 67:192–208
14. Gago-Ferrero P, Schymanski EL, Bletsou AA, Aalizadeh R, Hollender J, Thomaidis NS (2015) Extended suspect and non-target strategies to characterize emerging polar organic contaminants in raw wastewater with LC-HRMS/MS. Environ Sci Technol 49 (20):12333–12341. https://doi.org/10.1021/acs.est.5b03454
15. Kern S, Fenner K, Singer HP, Schwarzenbach RP, Hollender J (2009) Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. Environ Sci Technol 43:7039–7046. https://doi.org/10.1021/es901979h
16. Wang Y, Yu N, Zhu X, Guo H, Jiang J, Wang X, Shi W, Wu J, Yu H, Wei S (2018) Suspect and nontarget screening of per- and Polyfluoroalkyl substances in wastewater from a fluorochemical manufacturing park. Environ Sci Technol 52:11007–11016. https://doi.org/10.1021/acs.est.8b03030
17. Casado J, Rodríguez I, Ramil M, Cela R (2014) Selective determination of antimycotic drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography quadrupole time-of-flight mass spectrometry. J Chromatogr A 1339:42–49. https://doi.org/10.1016/j.chroma.2014.02.087
18. Chiaia-Hernandez AC, Krauss M, Hollender J (2013) Environ Sci Technol 47:976e986
19. Gallampois CMJ, Schymanski EL, Krauss M, Ulrich N, Bataineh M, Brack W (2015) Multicriteria approach to select Polyaromatic River mutagen candidates. Environ Sci Technol 49(5):2959–2968
20. Terzic S, Ahel M (2011) Environ Pollut 159:557e566

21. Plumb R, Castro-Perez J, Granger J, Beattie I, Joncour K, Wright A (2004) Rapid Commun Mass Spectrom 18:2331e2337
22. Beretsou VG, Psoma AK, Gago-Ferrero P, Aalizadeh R, Fenner K, Thomaidis NS (2016) Identification of biotransformation products of citalopram formed in activated sludge. Water Res 103:205–214
23. Salas D, Borrull F, Fontanals N, Marcé RM (2017) Hydrophilic interaction liquid chromatography coupled to mass spectrometry-based detection to determine emerging organic contaminants in environmental samples. Trends Anal Chem 94:141–149. https://doi.org/10.1016/j.trac.2017.07.017
24. Bieber S, Greco G, Grosse S, Letzel T (2017) RPLC-HILIC and SFC with mass spectrometry: polarity-extended organic molecule screening in environmental (water) samples. Anal Chem 89(15):7907–7914. https://doi.org/10.1021/acs.analchem.7b00859
25. Aalizadeh R, Nika M-C, Thomaidis NS (2019) Development and application of retention time prediction models in the suspect and non-target screening of emerging contaminants. J Hazard Mater 363:277–285
26. Lanucara F, Holman SW, Gray CJ, Eyers CE (2014) The power of ion mobility-mass spectrometry for structural characterization and the study of conformational dynamics. Nat Chem 6:281–294. https://doi.org/10.1038/nchem.1889
27. Mollerup CB, Mardal M, Dalsgaard PW, Linnet K, Barron LP (2018) Prediction of collision cross section and retention time for broad scope screening in gradient reversed-phase liquid chromatography-ion mobility-high resolution accurate mass spectrometry. J Chromatogr A 1542:82–88. https://doi.org/10.1016/j.chroma.2018.02.025
28. Alygizakis NA, Oswald P, Thomaidis NS, Schymanski EL, Aalizadeh R, Schulze T, Oswaldova M, Slobodnik J (2019) NORMAN digital sample freezing platform: a European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in "digitally frozen" environmental samples. TrAC Trends Anal Chem 115:129–137. https://doi.org/10.1016/j.trac.2019.04.008
29. Hollender J, Schymanski EL, Singer HP, Ferguson PL (2017) Nontarget screening with high resolution mass spectrometry in the environment: ready to go? Environ Sci Technol 51:11505e11512
30. Hollender J, Schymanski EL, Singer HP, Ferguson PL (2017) Nontarget screening with high resolution mass spectrometry in the environment: ready to go? Environ Sci Technol 51:11505–11512. https://doi.org/10.1021/acs.est.7b02184
31. Rager JE, Strynar MJ, Liang S, McMahen RL, Richard AM, Grulke CM, Wambaugh JF, Isaacs KK, Judson R, Williams AJ, Sobus JR (2016) Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring. Environ Int 88:269–280. https://doi.org/10.1016/j.envint.2015.12.008
32. Stravs MA, Schymanski EL, Singer HP, Hollender J (2013) Automatic recalibration and processing of tandem mass spectra using formula annotation: recalibration and processing of MS/MS spectra. J Mass Spectrom 48:89–99. https://doi.org/10.1002/jms.3131
33. Loos M (2019) enviMass version 3.5 LC-HRMS trend detection workflow – R package. URL Zenodo. https://doi.org/10.5281/zenodo.1213098
34. Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G (2006) XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem 78:779–787. https://doi.org/10.1021/ac051437y
35. Pluskal T, Castillo S, Villar-Briones A, Orešič M (2010) MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinf 11:395. https://doi.org/10.1186/1471-2105-11-395
36. Chibwe L, Titaley IA, Hoh E, Simonich SL (2017) Integrated framework for identifying toxic transformation products in complex environmental mixtures. Environ Sci Technol Lett 4 (2):32–43. https://doi.org/10.1021/acs.estlett.6b00455
37. Bletsou AA, Jeon J, Hollender J, Archontaki E, Thomaidis NS (2015) Trends Anal Chem 66:32e44

38. Stasinakis AS, Gatidou G, Mamais D, Thomaidis NS, Lekkas TD (2008) Water Res 42:1796e1804
39. Schulze T, Weiss S, Schymanski EL, Carsten von der Ohe P, Schmitt-Jansen M, Altenburger R, Streck G, Brack W (2010) Environ Pollut 158(5):1461e1466
40. Escher BI, Fenner K (2011) Environ Sci Technol 45:3835e3847
41. Zonja B, Delgado A, Perez S, Barcelo D (2015) LC-HRMS suspect screening for detection-based prioritization of iodinated contrast media photodegradates in surface waters. Environ Sci Technol 49(6):3464–3472
42. Hernandez F, Ibanez M, Portoles T, Cervera MI, Sancho JV, Lopez FJ (2015) J Hazard Mater 282:86e95
43. Chibwe L, Geier MC, Nakamura J, Tanguay RL, Aitken MD, Simonich SLM (2015) Aerobic bioremediation of PAH contaminated soil results in increased genotoxicity and developmental toxicity. Environ Sci Technol 49(23):13889–13898
44. Lübcke-von Varel U, Streck G, Brack W (2008) Automated fractionation procedure for polycyclic aromatic compounds in sediment extracts on three coupled normal-phase high-performance liquid chromatography columns. J Chromatogr A 1185(1):31–42
45. Brack W, Ait-Aissa S, Burgess RM, Busch W, Creusot N, di Paolo C, Escher BI, Mark Hewitt L, Hilscherova K, Hollender J et al (2016) Effect-directed analysis supporting monitoring of aquatic environments an in-depth overview. Sci Total Environ 544:1073–1118
46. Fang M, Webster TF, Stapleton HM (2015) Effect-directed analysis of human peroxisome proliferator-activated nuclear receptors (PPARγ1) ligands in indoor dust. Environ Sci Technol 49(16):10065–10073
47. Schlusener MP, Kunkel U, Ternes TA (2015) Quaternary triphenylphosphonium compounds: a new class of environmental pollutants. Environ Sci Technol 49:14282–14291
48. Plassmann MM, Tengstrand E, Aberg KM, Benskin JP (2016) Non-target time trend screening: a data reduction strategy for detecting emerging contaminants in biological samples. Anal Bioanal Chem 408:4203–4208
49. Alygizakis NA, Gago-Ferrero P, Hollender J, Thomaidis NS (2019) Untargeted time-pattern analysis of LC-HRMS data to detect spills and compounds with high fluctuation in influent wastewater. J Hazard Mater 361:19–29. https://doi.org/10.1016/j.jhazmat.2018.08.073
50. Anliker S, Loos M, Comte R, Ruff M, Fenner K, Singer H (2020) Assessing emissions from pharmaceutical manufacturing based on temporal high-resolution mass spectrometry data. Environ Sci Technol. (in press). https://doi.org/10.1021/acs.est.9b07085
51. Ruff M, Mueller MS, Loos M, Singer HP (2015) Quantitative target and systematic nontarget analysis of polar organic micro-pollutants along the river Rhine using high resolution mass-spectrometry – identification of unknown sources and compounds. Water Res 87:145–154
52. Schollee JE, Schymanski EL, Avak SE, Loos M, Hollender J (2015) Prioritis zing unknown transformation products from biologically-treated wastewater using high resolution mass spectrometry, multivariate statistics, and metabolic logic. Anal Chem 87:12121–12129
53. Newton SR, McMahen RL, Sobus JR, Mansouri K, Williams AJ, McEachran AD, Strynar MJ (2018) Suspect screening and non-targeted analysis of drinking water using point-of-use filters. Environ Pollut 234:297–306. https://doi.org/10.1016/j.envpol.2017.11.033
54. Brunner AM, Dingemans MML, Baken KA, van Wezel AP (2019) Prioritising anthropogenic chemicals in drinking water and sources through combined use of mass spectrometry and ToxCast toxicity data. J Hazard Mater 364:332–338. https://doi.org/10.1016/j.jhazmat.2018.10.044
55. Gago-Ferrero P, Krettek A, Fischer S, Wiberg K (2018) Ahrens. Suspect screening and regulatory databases: a powerful combination to identify emerging micropollutants. Environ Sci Technol 52(12):6881–6894. https://doi.org/10.1021/acs.est.7b06598
56. Kiefer K, Müller A, Singer H, Hollender J (2019) New relevant pesticide transformation products in groundwater detected using target and suspect screening for agricultural and urban micropollutants with LC-HRMS. Water Res 165:114972. https://doi.org/10.1016/j.watres.2019.114972
57. Troger R, Kohler SJ, Franke V, Bergstedt O, Wiberg K (2020) Sci Total Environ, 706. http://eawag-bbd.ethz.ch/predict/aboutPPS.html. Accessed 05 Apr 2020

58. Wicker J, Lorsbach T, Gütlein M, Schmid E, Latino D, Kramer S, Fenner K (2016) enviPath – the environmental contaminant biotransformation pathway resource. Nucleic Acids Res 44 (D1):D502–D508
59. Moriya, Y.; Shigemizu, D.; Hattori, M.; Tokimatsu, T.; Kotera, M.; Goto, S.; Kanehisa, M. PathPred: an enzyme-catalyzed metabolic pathway prediction server. Nucleic Acids Res. 2010, 38, W138−W143. http://oasis-lmc.org/products/software/catalogic.aspx. Accessed 05 Apr 2020
60. Arora PK, Bae H (2014) Integration of bioinformatics to biodegradation. Biol Proced 16:8
61. Rucker C, Kummerer K (2012) Modeling and predicting aquatic aerobic biodegradation – a review from a user's perspective. Green Chem 14(4):875–887
62. Ruttkies C, Neumann S, Posch S (2019) Improving MetFrag with statistical learning of fragment annotations. BMC Bioinf 20. https://doi.org/10.1186/s12859-019-2954-7
63. Djoumbou-Feunang Y, Pon A, Karu N, Zheng J, Li C, Arndt D, Gautam M, Allen F, Wishart DS (2019) CFM-ID 3.0: significantly improved ESI-MS/MS prediction and compound identification. Metabolites 9. https://doi.org/10.3390/metabo9040072
64. Dührkop K, Fleischauer M, Ludwig M, Aksenov AA, Melnik AV, Meusel M, Dorrestein PC, Rousu J, Böcker S (2019) SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. Nat Methods 16:299–302. https://doi.org/10.1038/s41592-019-0344-8
65. Schymanski EL, Gerlich M, Ruttkies C, Neumann S (2014) Solving CASMI 2013 with MetFrag, MetFusion and MOLGEN-MS/MS. Mass Spectrom 3(SpecialIssue 2):S0036
66. Schymanski EL, Ruttkies C, Krauss M, Brouard C, Kind T, Duhrkop K, Allen F, Vaniya A, Verdegem D, Bocker S, Rousu J, Shen H, Tsugawa H, Sajed T, Fiehn O, Ghesquiere B, Neumann S (2017) Critical Assessment of Small Molecule Identification 2016: automated methods. J Cheminform 9(1):22
67. Huntscha S, Hofstetter TB, Schymanski EL, Spahr S, Hollender J (2014) Biotransformation of benzotriazoles: Insights from transformation product identification and compound-specific isotope analysis. Environ Sci Technol 48(8):4435–4443
68. Aalizadeh R, Thomaidis NS, Bletsou AA, Gago-Ferrero P (2016) Quantitative structure–retention relationship models to support nontarget high-resolution mass spectrometric screening of emerging contaminants in environmental samples. J Chem Inf Model 56:1384–1398. https://doi.org/10.1021/acs.jcim.5b00752
69. Bade R, Bijlsma L, Miller TH, Barron LP, Sancho JV, Hernández F (2015) Suspect screening of large numbers of emerging contaminants in environmental waters using artificial neural networks for chromatographic retention time prediction and high-resolution mass spectrometry data analysis. Sci Total Environ 538:934–941. https://doi.org/10.1016/j.scitotenv.2015.08.078
70. Ulrich N, Schüürmann G, Brack W (2011) Linear solvation energy relationships as classifiers in non-target analysis—a capillary liquid chromatography approach. J Chromatogr A 1218:8192–8196. https://doi.org/10.1016/j.chroma.2011.09.031
71. Gerlich M, Neumann S (2013) J Mass Spectrom 48:291e298
72. Escher B, Leusch F (2012) Bioanalytical tools in water quality assessment. IWA Publishing, London
73. Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP, Hollender J (2014) Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ Sci Technol 48:2097–2098. https://doi.org/10.1021/es5002105
74. Gago-Ferrero P, Bletsou AA, Damalas DE, Aalizadeh R, Alygizakis NA, Singer HP et al (2020) Wide-scope target screening of>2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes. J Hazard Mater 387:121712. https://doi.org/10.1016/j.jhazmat.2019.121712
75. Wode F, van Baar P, Dünnbier U, Hecht F, Taute T, Jekel M, Reemtsma T (2000) Current and legacy micropollutants on a wastewater infiltration site with a UPLC-high resolution MS target screening method. Water Res 2015(69):274–283

76. D'Andrilli J, Cooper WT, Foreman CM, Marshall AG (2015) An ultrahigh-resolution mass spectrometry index to estimate natural organic matter Lability: FTICRMS organic matter molecular Lability index. Rapid Commun Mass Spectrom 29(24):2385–2401
77. Lavonen EE, Kothawala DN, Tranvik LJ, Gonsior M, Schmitt-Kopplin P, Köhler SJ (2015) Tracking changes in the optical properties and molecular composition of dissolved organic matter during drinking water production. Water Res 85:286–294
78. Sleighter RL, Hatcher PG (2008) Molecular characterisation of dissolved organic matter (DOM) along a river to ocean transect of the lower Chesapeake Bay by ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Mar Chem 110(3–4):140–152
79. Hawkes JA, Dittmar T, Patriarca C, Tranvik L, Bergquist J (2016) Evaluation of the Orbitrap mass spectrometer for the molecular fingerprinting analysis of natural dissolved organic matter. Anal Chem 88:7698–7704
80. Wu Z, Rodgers RP, Marshall AG (2004) Two- and three-dimensional van Krevelen diagrams: a graphical analysis complementary to the Kendrick mass plot for sorting elemental compositions of complex organic mixtures based on ultrahigh-resolution broadband Fourier transform ion cyclotron resonance mass measurements. Anal Chem 76(9):2511–2516
81. Fang Z, He C, Li Y, Chung KH, Xu C, Shi Q (2017) Fractionation and characterisation of dissolved organic matter (DOM) in refinery wastewater by revised phase retention and ion-exchange adsorption solid phase extraction followed by ESI FT-ICR MS. Talanta 162:466–473
82. Cortés-Francisco N, Caixach J (2013) Molecular characterisation of dissolved organic matter through a desalination process by high resolution mass spectrometry. Environ Sci Technol 47 (17):9619–9627
83. Pereira AS, Islam MS, Gamal El-Din M, Martin JW (2013) Ozonation degrades all detectable organic compound classes in Oil Sands process-affected water; an application of high-performance liquid chromatography/Orbitrap mass spectrometry: ozonation degrades all detectable organic compound classes in OSPW. Rapid Commun Mass Spectrom 27 (21):2317–2326
84. Gonsior M, Zwartjes M, Cooper WJ, Song W, Ishida KP, Tseng LY, Jeung MK, Rosso D, Hertkorn N, Schmitt-Kopplin P (2011) Molecular characterisation of effluent organic matter identified by ultrahigh resolution mass spectrometry. Water Res 45(9):2943–2953
85. Mesfioui R, Love NG, Bronk DA, Mulholland MR, Hatcher PG (2012) Reactivity and chemical characterisation of effluent organic nitrogen from wastewater treatment plants determined by Fourier transform ion cyclotron resonance mass spectrometry. Water Res 46 (3):622–634
86. Bade R, Rousis NI, Bijlsma L, Gracia-Lor E, Castiglioni S, Sancho JV, Hernandez F (2015) Screening of pharmaceuticals and illicit drugs in wastewater and surface waters of Spain and Italy by high resolution mass spectrometry using UHPLC-QTOF MS and LCLTQ-Orbitrap MS. Anal Bioanal Chem 407(30):8979–8988
87. Remucal CK, Cory RM, Sander M, McNeill K (2012) Low molecular weight components in an aquatic humic substance as characterized by membrane Dialysis and Orbitrap mass spectrometry. Environ Sci Technol 46(17):9350–9359
88. Kellerman AM, Dittmar T, Kothawala DN, Tranvik LJ (2014) Chemodiversity of dissolved organic matter in lakes driven by climate and hydrology. Nat Commun 5:3804
89. Riedel T, Zark M, Vähätalo AV, Niggemann J, Spencer RGM, Hernes PJ, Dittmar T (2016) Molecular signatures of biogeochemical transformations in dissolved organic matter from ten world rivers. Front Earth Sci 4:85
90. Verkh Y, Rozman M, Petrovic M (2018) A non-targeted high-resolution mass spectrometry data analysis of dissolved organic matter in wastewater treatment. Chemosphere 200:397e404
91. Postigo C, Andersson A, Harir M, Bastviken D, Gonsior M, Schmitt-Kopplin P, Gago-Ferrero P, Ahrens L, Ahrens L, Wiberg K (2021) Unraveling chemodiversity of halogenated disinfection by-products formed during drinking water treatment using targeted and

non-targeted screening tools. J Hazard Mater. (Online) https://www.sciencedirect.com/science/article/pii/S2213343720307867. https://doi.org/10.1016/j.jece.2020.104437
92. Sanchís J, Jaén-Gil A, Gago-Ferrero P, Munthali E, Farré MJ (2020) Characterisation of organic matter by HRMS in surface waters: effects of chlorination on molecular fingerprints and correlation with DBP formation potential. Water Res 176:115743
93. Farré MJ, Jaén-Gil A, Hawkes J, Petrovic M, Catalán N (2019) Orbitrap molecular fingerprint of dissolved organic matter in natural waters and its relationship with NDMA formation potential. Sci Total Environ
94. Yuthawong V, Kasuga I, Kurisu F, Furumai H (2019) Molecular-level changes in dissolved organic matter compositions in Lake Inba water during KMnO4 oxidation: assessment by Orbitrap mass spectrometry. J Water Environ Technol 17:27–39
95. Sörengård M, Ahrens L, Alygizakis N, Jensen PE, Gago-Ferrero P (2021) Non-target and suspect screening strategies for electrodialytical soil remediation evaluation: assessing changes in the molecular fingerprints and per- and polyfluoroalkyl substances (PFASs). J Environ Chem Eng. (Under revision)
96. Zhang H, Zhang Y, Shi Q, Hu J, Chu M, Yu J, Yang M (2012) Study on transformation of natural organic matter in source water during chlorination and its chlorinated products using ultrahigh resolution mass spectrometry. Environ Sci Technol 46(8):4396–4402
97. Minor EC, Steinbring CJ, Longnecker K, Kujawinski EB (2012) Characterisation of dissolved organic matter in Lake Superior and its watershed using ultrahigh resolution mass spectrometry. Org Geochem 43:1–11
98. Lu M, Zhang Z, Qiao W, Wei X, Guan Y, Ma Q, Guan Y (2010) Remediation of petroleum-contaminated soil after composting by sequential treatment with Fenton-like oxidation and biodegradation. Bioresour Technol 101(7):2106–2113
99. Kim S, Kramer RW, Hatcher PG (2003) Graphical method for analysis of ultrahigh- resolution broadband mass spectra of natural organic matter, the Van Krevelen diagram. Anal Chem 75 (20):5336–5344
100. Marshall JW, Schmitt-Kopplin P, Schuetz N, Moritz F, Roullier-Gall C, Uhl J, Colyer A, Jones LL, Rychlik M, Taylor AJ (2018) Monitoring chemical changes during food sterilisation using ultrahigh resolution mass spectrometry. Food Chem 242:316–322

Perspectives on the Feasibility of Using Enzymes for Pharmaceutical Removal in Wastewater

Muhammad Bilal, Hafiz M. N. Iqbal, and Damiá Barceló

Contents

Abstract This particular chapter spotlights the growing environmental concerns and hazardous consequences of numerous organic contaminants so-called emerging

M. Bilal
School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian, China
e-mail: bilaluaf@hotmail.com

H. M. N. Iqbal (✉)
Tecnologico de Monterrey, School of Engineering and Sciences, Monterrey, NL, Mexico
e-mail: hafiz.iqbal@tec.mx

D. Barceló
Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Catalan Institute for Water Research (ICRA), Girona, Spain

College of Environmental and Resources Sciences, Zhejiang A&F University, Hangzhou, China
e-mail: dbcqam@cid.csic.es

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 119–144, DOI 10.1007/698_2020_661,

contaminants (ECs). These ECs are being detected, though in different quantities, in different environmental matrices and wastewater treatment systems. With ever-increasing awareness, people are now more concerned about the wide-spread distribution of pharmaceutically related active compounds in water matrices. In turn, the free flow of ECs in water matrices poses notable adverse effects on human, aquatic animals, and naturally occurring plants, even at very small concentrations. Due to inadequacies and ineffectiveness of, in practice, physical and chemical-based remediation processes, robust treatment approaches, such as microorganisms and their novel enzyme-based degradation/removal of ECs, are of supreme interest. This chapter focuses on various pharmaceutically related ECs and their efficient mitigation from water matrices. Following a brief introduction, the focus is given to two main treatment approaches, i.e., (1) remediation of pharmaceutically active compounds by crude (pristine) and purified enzymes (i.e., lignin peroxidase, manganese peroxidase, soybean peroxidase, horseradish peroxidase, and laccases) and (2) immobilized enzyme-assisted degradation of pharmaceutically active compounds.

Keywords Biocatalysis, Biological risks, Enzymes, Hazardous compounds, Toxicity, Wastewater treatment

1 Introduction

A continuous rise in worldwide population and urbanization and their associated increase in the consumption of pharmaceuticals have redirected the researches' attention to the search for novel materials for rapid and efficient decontamination of both wastewaters and drinking waters. In addition to extensive heavy metal pollution problems, water contamination by pharmaceutical compounds has become a growing worldwide concern [1]. The concentrations of persistent organic pollutants like personal care products, pharmaceuticals, and their metabolites are continuously rising in the natural environment due to human activities [2–5]. The major source of pharmaceutical pollutants is the WWTP effluents. WWTPs are not designed to completely eliminate environmental pollutants, and thus they can percolate through WWTPs and incorporated into the aquatic systems (streams and rivers) [6]. For example, the contents of diclofenac (an anti-inflammatory drug) and carbamazepine reached 0.99 and 0.95 μg/L, respectively, in WWTP effluents [7]. Particularly, a detectable level of diclofenac has been identified in drinking waters, surface waters, and groundwaters in the range of ng/L to μg/L in Sweden, Spain, Switzerland, and the Baltic region [8–10]. Apart from this, other pharmaceuticals including tramadol, carbamazepine, ibuprofen, oxazepam, and naproxen have also recently been detected in drinking water supplies in some countries. These concentrations of pharmaceuticals can induce serious environmental threats such as

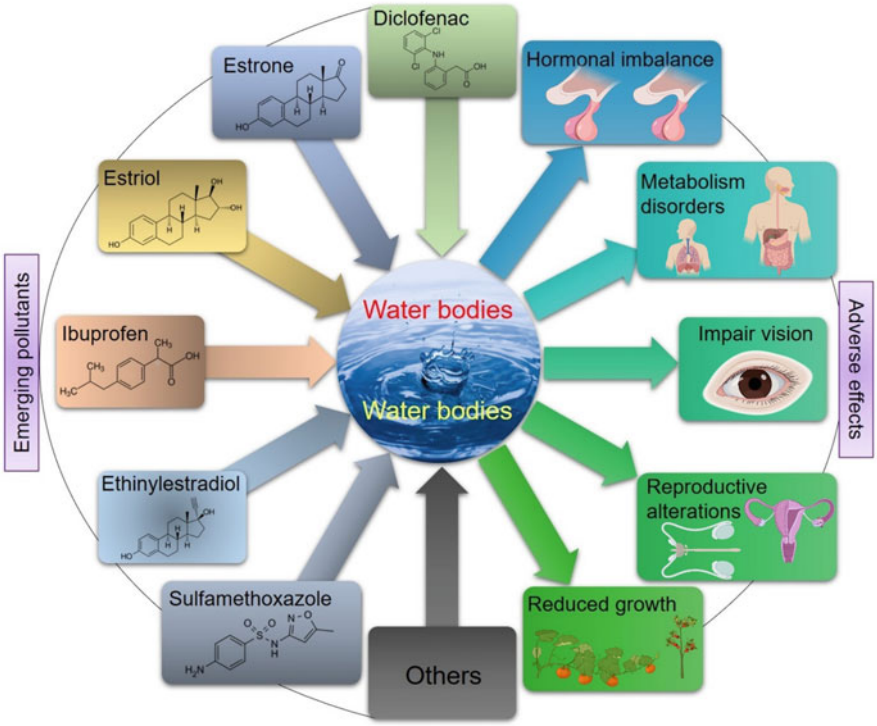


Fig. 1 Notable adverse effects of numerous ECs. Reprinted from Morsi et al. [12] with permission from Elsevier

congenital disorders, physical abnormalities, impairments of the endocrine and reproductive system, and feminization of some fish species [11, 12]. Due to the capability of micropollutants and pharmaceutically active compounds to cause adverse effects to the ecosystem and human health, they have attracted the principal research focus in recent days. Some notable adverse effects of numerous ECs are shown in Fig. 1 [12].

In this avenue, enzymatic treatment of water and wastewater has been advocated as a lucrative alternative due to their high biocatalytic performance and selectivity. Oxidoreductases such as laccase, MnP, LiP, and versatile peroxidase have garnered increasing research interest for potential aptitude to remove a large number of antibiotics and pharmaceutically active residues in wastewater samples.

2 Remediation of Pharmaceutically Active Compounds by Crude/Purified Enzymes

In recent years, enzymes have been extensively utilized for the removal of pharmaceutically active residues and antibiotics into innocuous compounds in wastewater samples. Application of microorganisms or their derived enzymes such as wood-rotting fungi has been recognized as a robust substitute for classical wastewater treatments. These microbes have demonstrated a substantial transformation ability to a vast number of environmental contaminants by secreting a group of ligninolytic enzymes, such as MnP, LiP, VP, and laccase. Peroxidases are characterized as heme and non-heme peroxidases. Peroxidases, in which heme groups serve as an active center, are an important class of oxidoreductases with pronounced ability to oxidize substrates using hydrogen peroxide (Fig. 2) [13]. These enzymes carry out the oxidation-reduction-mediated biotransformation of various classes of hazardous contaminants such as textile dyes, phenols, chlorinated phenols, cresols, dioxins, pesticides, herbicides, and personal care and pharmaceutically active products [13–16]. Among oxidoreductases, peroxidases and laccase are the most widely investigated class of enzymes for bioremediation because of their high capability in the biodegradation and decomposition of diverse organic pollutants. These enzymes form highly reactive free radicals that transform the parent contaminants into smaller compounds that are more decomposable and thus possess negligible toxicity.

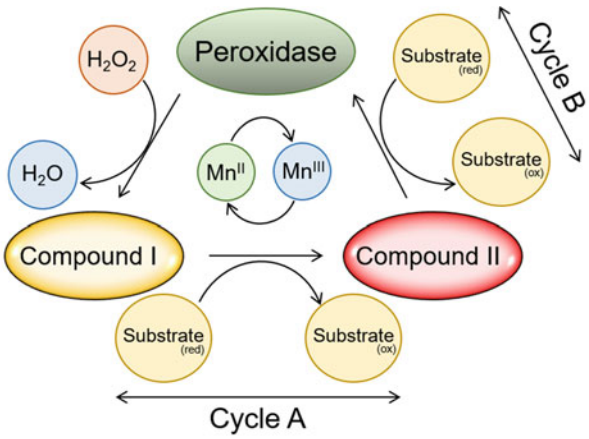


Fig. 2 A simplified schematic illustration of the catalytic cycle of peroxidases. Reprinted from Bilal et al. [13] with permission from Elsevier

2.1 Lignin Peroxidase: Catalytic Features and Pharmaceutical Degradation

LiP (EC 1.11.1.14) is a heme-containing enzyme that can catalyze the degradation of aromatic and halogenated phenolic substances with high redox potential. The range of isoelectric point and molecular weight of LiP has been documented as 3.3 to 4.7 and 38 kDa to 43 kDa, respectively [17]. This monomeric hemoprotein optimally executes its functions at acidic pH (around pH 3.0) in the presence of veratryl alcohol as a natural substrate [18]. As potent oxidants, microbial LiPs have demonstrated the aptitude for oxidative degradation of a wide spectrum of recalcitrant aromatic contaminants, including antibiotics and pharmaceutically active compounds. For instance, crude LiP extracted from the ligninolytic culture of *Phanerochaete chrysosporium* catalyzed the effective degradation of tetracycline and oxytetracycline [19]. Zhang and Geißen [20] markedly removed diclofenac and carbamazepine using crude LiP from *P. chrysosporium*. The high degradation properties of LiP toward persistent aromatic pollutants make it a versatile biocatalyst for diverse biotechnological applications. High redox potentialities of LiP to degrade lignin and other compounds of high redox potential have been ascribed to its exposed tryptophan residue (Trp171), which generates a reactive tryptophanyl radical on the enzyme's surface by relocating electron to the heme molecule. Differences in tryptophan milieu are associated with modifying the enzyme characteristics such as catalytic performance, substrate specificity, and functional stability [21]. The removal efficacy of carbamazepine remained stable in the range of 60–80% for 100 days in a fungal bioreactor that consists of three major biocatalysts including MnP, LiP, and laccase [22].

2.2 Manganese Peroxidase: Catalytic Features and Pharmaceutical Degradation

MnPs are known for their pronounced Mn^{2+}-mediated oxidative capacity for a large number of xenobiotic and aromatic compounds, including various industrial dyes, phenols, and non-phenols [23]. Though MnPs have been inspected for about three decades since their discovery with the fungal LiPs, very limited attention is devoted to MnPs [24, 25]. In recent times, there has been exceptional interest in the use of MnPs owing to their ubiquitous presence in almost all kinds of WRF and some soil-litter decomposers [26, 27]. MnPs originated from WRF are largely associated with the biotransformation of plant biomass and the removal of various refractory pollutants including antibiotics, endocrine disrupters, and pharmaceutically active residues. Moreover, these lignin-degrading enzymes are capable of complete bioconversion of recalcitrant lignin into carbon dioxide and water [27]. Consequently, MnPs have found prominent applications in various biotechnological sectors such as textiles, food, bioremediation, organic synthesis, and paper and pulp

manufacturing [28, 29]. The Mn^{2+}-mediated catalytic mechanism of MnPs in oxidizing various aromatic compounds has been well elucidated. Mn^{2+} binds to MnP in a typical region formed by three conserved acidic amino acids, i.e., Glu35, Glu39, and Asp179, in MnP of *P. chrysosporium* [30]. MnP then oxidizes Mn^{2+} to Mn^{3+}, which subsequently is chelated with organic acids and serves as a diffusible oxidizing agent for a large spectrum of aromatic substances [31–33]. Two isoforms of MnPs, MnP1, and MnP2 and a laccase, Lac1, extracted from *T. polyzona* KU-RNW027 exhibited the potential for removing pharmaceutical compounds without a redox-mediated system. Under the optimized reaction environments of pH 4.5 and 50°C, the catalytic efficiencies of these enzymes led to effective remediation and removal of commonly consumed antibiotics ciprofloxacin, tetracycline, amoxicillin, and doxycycline [34]. Wen et al. [35] reported 84.3% and 72.5% removal of oxytetracycline and tetracycline within 4 h by applying 40 U/L of crude MnP extract from the *P. chrysosporium*. The tetracycline removal efficiency of MnP was markedly enhanced by the addition of H_2O_2 during the degradation reaction.

2.3 Soybean Peroxidase and Chloroperoxidase: Catalytic Features and Pharmaceutical Degradation

As a widely studied enzyme, SBP (EC. 1.11.1.7) is regarded as a lucrative biocatalyst and is present in considerable quantities in the soybean hulls [36, 37]. The SBP-assisted biocatalytic system presents a high biodegradation potential for a range of antibiotics, organic pollutants, and emerging contaminants by generating extremely reactive free radicals. Alneyadi et al. reported that both SBP and chloroperoxidase (CPO)-based treatment catalyzed the degradation of sulforhodamine B with the concomitant formation of at least one different degradation intermediate. Further, SBP-aided reaction results in degradation and detoxification of sulforhodamine B, whereas the CPO treatment does not affect the toxicity removal of this aromatic contaminant (Fig. 3) [38, 39]. In another study, Almaqdi et al. [40] conducted experiments for sulfamethoxazole degradation by a combinatorial approach using SBP enzymatic system as well as an advanced oxidation process (UV + H_2O_2). Initial experiments using the individual SBP + H_2O_2 system had no degradation effect; however, the sulfamethoxazole was profoundly transformed by the inclusion of 1-hydroxybenzotriazole as a redox mediator to the reaction system. Results revealed over 80% of the sulfamethoxazole was removed within an initial 10 min of reaction using a mixture of SBP + H_2O_2 system in the presence of HOBT mediators by producing various intermediates. To evaluate the remediation efficiencies of different peroxidases, a mixture containing 21 diverse arrays of emerging pollutants including antibiotics, textile dyes, analgesics, pesticides, herbicides, hormones, pharmaceutical, and personal care products and nonsteroidal anti-inflammatory drugs was independently exposed to MnP, HRP, SBP, CPO, and lactoperoxidase (LPO). Experimental results showed that all of the tested

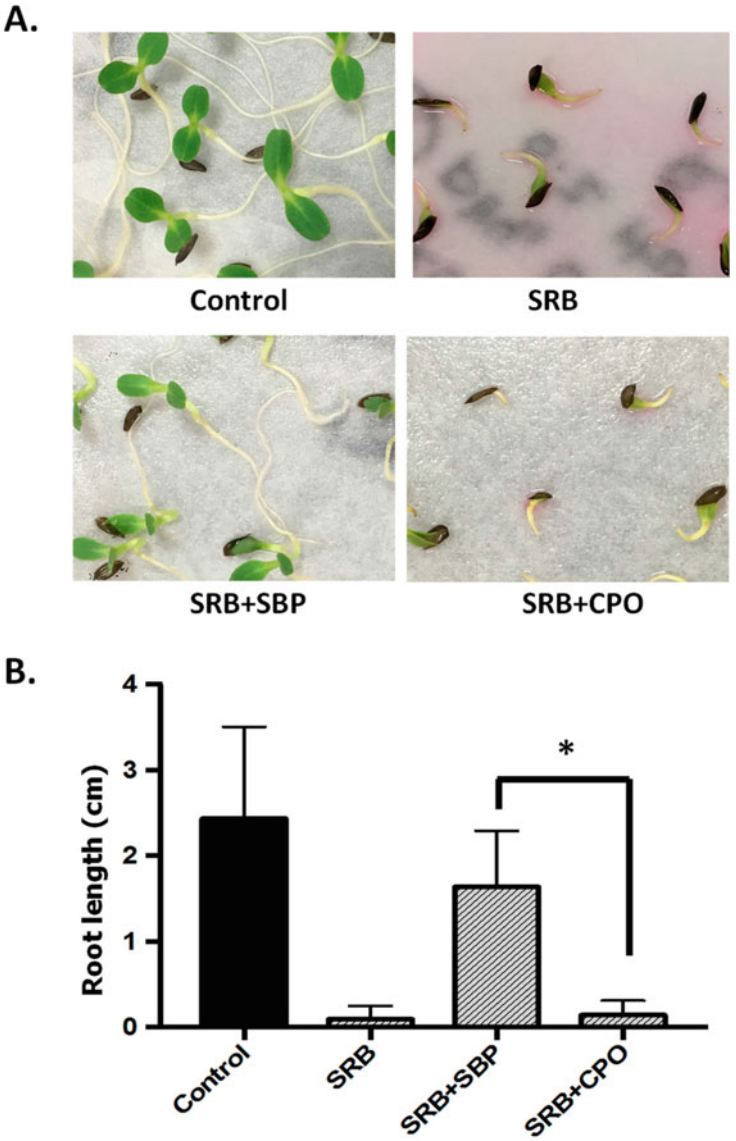


Fig. 3 SRB dye toxicity on *Lactuca sativa* seed. (**a**) A representative sample of *L. sativa* seed treated by SRB dye (10 ppm), SRB dye decolorized by SBP (SRB + SBP) and SRB dye treated by CPO (SRB + CPO); (**b**) root length (cm) after treating the seeds with SRB dye samples. Statistical analysis was performed using unpaired t-test ($n = 80$). The asterisk (*) shows a significant difference ($p < 0.05$). Reprinted from Alneyadi et al. [38], an open-access article distributed under the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). Copyright (2017) the authors. Licensee MDPI, Basel, Switzerland

peroxidases have shown marked ability to decompose meloxicam (a nonsteroidal anti-inflammatory drug), with SBP as a slightly superior catalyst compared with others. Nevertheless, it appears that all of the organic contaminants were not equally bio-transformed and degraded by the catalytic actions of peroxidases. For illustration, SBP was not capable of degrading roxithromycin (a semi-synthetic macrolide antibiotic), and it also found to recalcitrant to MnP and CPO treatments exhibiting only about 25% transformation. On the other hand, HRP and LPO treatment induced efficient degradation with about 95% and 80% degradation, respectively [40].

2.4 *Horseradish Peroxidase: Catalytic Features and Pharmaceutical Degradation*

Plant HRP (EC 1.11.1.7) is a Class III peroxidase that has attracted incredible interest as a widely studied enzyme to oxidizing a variety of phenolic substrates such as alkylphenols, phenols, halophenols, bisphenol A, triclosan, lignosulfonates, and hormones by one-electron transfer [41–43]. The supplementation of H_2O_2 in the HRP-catalyzed reaction results in the biotransformation of non-phenolic compounds (aliphatic amines), organic acids (fulvic acid, humic acid, hydroxycinnamic acid, and perfluorooctanoic acid), polychlorinated biphenyls, persistent organic pollutants, and even recalcitrant water contaminants such as swine manure and azo dyes [44, 45]. In a current study, Yang et al. [46] reported for the first time the efficient removal of six sulfonamides including sulfadiazine, sulfapyridine, sulfathiazole, sulfamerazine, sulfamethoxazole, and sulfamethoxypyridazine from aqueous environment by HRP-mediated catalytic reaction. When spiked together, the concomitant sulfonamides can serve as mediating agents for accelerated removal of sulfamethoxazole. Up to 47% of diclofenac was removed by a native form of HRP [47].

2.5 *Laccases: Catalytic Features and Pharmaceutical Degradation*

As trendy enzymes, laccase is a multi-copper containing blue oxidase enzyme that belongs to oxidoreductases. The induction, formation, and secretion of extracellular laccase varies in the presence of different physical and nutrient growth conditions like pH, temperature, moisture, aeration, nutrients, mediators, and inhibitors. It is revealed that copper plays a role in the induction of laccases by most of the laccase producers [48]. Laccases typically catalyze their substrates (phenolic as well as non-phenolics, i.e., aromatic amines, phenol, or its derivatives) oxidation with a simultaneous four-electron reduction of molecular oxygen to form a corresponding radical, with water as a by-product (Fig. 4) [13]. Though laccases can be extracted

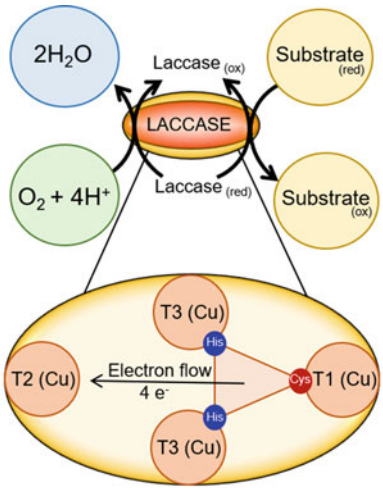


Fig. 4 A simplified schematic illustration of the catalytic cycle of laccase. Reprinted from Bilal et al. [13] with permission from Elsevier

from fungi, bacteria, plants, and insects [49], microbial-derived laccases, in particular WRF, have garnered increasing interest because of broad substrate specificity and high oxidation capability. It drives the oxidative cleavage of a diversity of phenolic compounds, diamines, aromatic amines, and electron-rich substrates accompanied by simultaneous mono-electronic reduction of molecular oxygen to water. Laccases accelerate the conversion of phenoxyl radicals to the ketone, demethoxylation, and cleavage of carbon in phenolic structures. Oxidation and biotransformation of a spectrum of non-phenolic structures of lignin by laccase can also be possible through the inclusion of different natural/synthetic redox mediators including 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), syringaldehyde, acetosyringone, vanillin, and 1-hydroxybenzotriazole (HOBT). During the last few years, the application of microbial laccases has been widely explored for the bioremediation of pharmaceuticals, xenobiotics, organic pollutants, endocrine-disrupting compounds, dyes, polychlorinated biphenyls, organochlorines, polyaromatic hydrocarbons, and 2,4,6-trinitrotoluene [13–15, 50–52].

Pharmaceutical products released openly by pharmaceutical manufacturers or hospital activities have substantial health-related apprehensions. In a very recent study, laccase treatment drives the dehydrochlorination of an insecticide, lindane, to form non-toxic and environmentally friendlier by-products (Fig. 5) [53]. A promising aspect of laccases functions is the cleavage of different substrates without the necessity of external source of Mn^{2+}, H_2O_2, or co-factors that are generally included in the case of other peroxidases such as MnP or LiP. Guardado et al. [54] appraised the degradation capability of a novel *Pycnoporus sanguineus CS43* laccase for decomposing various pharmaceutical micropollutants. Degradation profile indicated a removal efficacy of 40%, 80%, and 100% for ciprofloxacin, amoxicillin, and sulfamethoxazole, respectively, within 3 h of syringaldehyde-mediated catalytic reaction time. The recombinant laccase from *T. versicolor* evinced to be a highly proficient catalyst for the decomposition of pharmaceuticals-based environmental

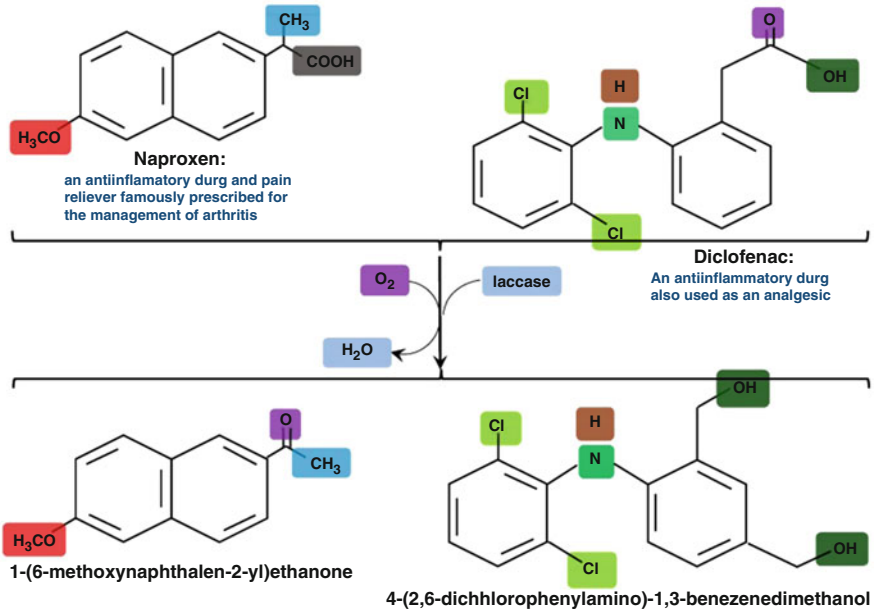


Fig. 5 The oxidative cleavage of some pharmaceuticals by laccase enzyme. Reprinted from Unuofin et al. [53] with permission under Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). Copyright (2019) the authors. Licensee MDPI, Basel, Switzerland

pollutants. After 24 h of catalytic treatment with a recombinant enzyme, the sulfamethoxazole and diclofenac were removed to 51.1% and 46.8%, respectively. A complete degradation efficiency was obtained within 1 h by the supplementation of 1 mM ABTS as a redox mediator [55]. Alharbi et al. [56] investigated the removal of four pharmaceutical compounds, i.e., sulfamethoxazole (SMX), diclofenac (DCF), carbamazepine (CBZ), and trimethoprim (TMP), separately as well as a mixture at various concentrations, by laccase from *T. versicolor*. In contrast to mixtures, the pharmaceuticals tested individually were more efficiently degraded under identical reaction environments. For instance, diclofenac (5 mg/L) was degraded to an undetectable level within 8 h when treated in the individual experiment, whereas the removal time was increased to 24 h following exposure to the catalytic system in the form of a mixture with other pharmaceutical compounds. The comparable tendency was noted with other tested pharmaceuticals, where 82 vs. 34%, 95 vs. 39%, and 56 vs. 49% degradation occurred after 48 h of biocatalytic treatment with carbamazepine, trimethoprim, and sulfamethoxazole ascertained separately or as in mixture forms, respectively. Toxicity analysis revealed the non-toxicity of all the laccase-treated pharmaceutical and effluent samples. Table 1 summarizes recent degradation studies of pharmaceutically active compounds by free laccase enzymes.

Table 1 Removal studies of pharmaceutical compounds by free laccases under various operating conditions

Enzyme source	Pharmaceutical compound	Reaction conditions	Removal (%)	Reference
Aspergillus oryzae	Ciprofloxacin hydrochloride	Enzyme loading of 0.02% (w/v), temperature 60°C, power input 75 W, frequency 22 kHz, and agitation 200 rpm	51	Sutar and Rathod [57]
Aspergillus oryzae	Cetirizine dihydrochloride	Optimal experimental parameters were enzyme loading of 0.02%, temperature 50°C, power input 100 W, frequency 25 kHz, and agitation 200 rpm	91	Sutar and Rathod [58]
Pycnoporus sanguineus CS43	Amoxicillin	Pollutant concentration, 20 mg L^{-1} Redox mediator syringaldehyde Incubation time, 3 h	80	Guardado et al. [54]
Pycnoporus sanguineus CS43	Sulfamethoxazole	Pollutant concentration, 20 mg L^{-1} Redox mediator syringaldehyde Incubation time, 3 h	100	Guardado et al. [54]
Pycnoporus sanguineus CS43	Ciprofloxacin	Pollutant concentration, 20 mg L^{-1} Redox mediator syringaldehyde Incubation time, 3 h	40	Guardado et al. [54]
Trametes versicolor	Doxycycline	Reaction mixture contained 10^{-4} M doxycycline, partially purified laccase (10 nkat mL^{-1}), malonate buffer (50 mM, pH 4.5), and 0.2 mM HBT as a redox mediator	100	Suda et al. [59]
Trametes versicolor	Chlortetracycline	Reaction mixture contained 10^{-4} M chlortetracycline, partially purified laccase (10 nkat mL^{-1}), malonate buffer (50 mM, pH 4.5), and 0.2 mM HBT as a redox mediator	100	Suda et al. [59]
Trametes versicolor	Tetracycline	Reaction mixture contained 10^{-4} M tetracycline, partially purified laccase (10 nkat mL^{-1}), malonate buffer (50 mM, pH 4.5), and 0.2 mM HBT as a redox mediator	100	Suda et al. [59]
Trametes versicolor	Oxytetracycline	Reaction mixture contained 10^{-4} M oxytetracycline, partially purified laccase (10 nkat mL^{-1}), malonate buffer (50 mM, pH 4.5), and 0.2 mM HBT as a redox mediator	100	Suda et al. [59]
Trametes versicolor	Tetracycline	Antibiotic of 100 μg/mL, laccase 17.5 μg/mL, pH 7.0, temperature 20°C, incubation time 18 h	78%	Llorca et al. [60]

3 Enzyme Immobilization, Techniques, and Unique Advantages

Enzymes work perfectly under normal physiological environments, and their functionalities are highly based on their conformations. Harsh and adverse environmental conditions that are usually experienced in effluent streams such as high ionic strength, extreme temperatures, presence of inhibitors, and very low or high pH can affect the conformation of the enzyme, thus resulting in its denaturation [61, 62]. These inadequacies can be overcome by immobilizing enzyme on a solid support. Immobilization is a process in which the enzyme is attached to an insoluble support carrier, where it is held in a proper geometrical conformation resulting in increased stability and reusability of the enzyme [14, 63]. The immobilization process converts the enzyme from its homogenous form to a heterogeneous catalyst (immobilized enzyme) to give an immobilized biocatalyst [64]. The immobilized enzymes can be used for the continuous bioremediation of great volumes of effluent effectively [61].

The immobilization of enzymes to different biocompatible supports can be achieved using three major techniques. The first method is binding to a carrier, and it can be further subdivided into the physical binding, also known as adsorption, and chemical binding via covalent linkages [65]. In adsorption, the catalyst is adsorbed to the outside surface of inert support, which can be a glass, matrix, or alginate beads. This technique is not very efficient, and the coupling between the enzyme and support is feeble to keep the enzyme attached to its place. Covalent binding entails the attachment of the biocatalyst to support by covalent bonds directly or using a cross-linking reagent such as glutaraldehyde, which will be attached to the enzyme at one side and the support from its other side. Covalent binding is more effective and stronger than adsorption. The second method is entrapment or encapsulation in which the enzyme is either trapped in a polymeric matrix network or encapsulated within a solid carrier. The last technique is the development of cross-linking of enzyme aggregates (CLEAs) or crystals (CLECs), which are considered as carrier-free immobilized biocatalytic systems [63, 65]. It is important to mention that the solid support used in immobilization should be inexpensive, ecologically friendly, and non-toxic and does not exhibit any undesirable effect on the biodegraded solution [14].

The use of carrier-immobilized enzymes can address most of the issues that are faced when using an enzyme in the free form. Non-immobilized forms of enzymes cannot be reused and recycled after the treatment and are considered highly expensive. Immobilization overcomes part of these issues as it allows for the repeated usability of enzymes and increases their recycling efficiencies in multiple continuous cycles [13, 66]. Additionally, immobilization allows the long-term and durable stability of enzymes as they become more resistant to degradation and denaturation and stabilized against harsh temperatures, pH, and pressure conditions [13, 67]. Since the enzyme immobilization process offers numerous advantages

compared to the free state of the enzymes, it can be considered as an easy and effective way to boost up the catalytic potentialities of enzymes.

4 Immobilized Peroxidases for Removal of Pharmaceutically Active Compounds

Though the free form of biocatalysts may be efficient in removing pharmaceutically active compounds, their scale-up exploitation is associated with some drawbacks. The soluble nature of free enzymes in the aqueous media renders them challenging to retain in the reactor system. Moreover, instability toward thermal or chemical denaturation diminishes their applicability to establish enzyme-driven processes, which in turn profoundly increase the processing cost. The deployment of carrier-immobilized enzyme enables the easy retrieval of both the products and enzyme molecules facilitating their multiple-time reutilization in consecutive biocatalytic operations, controlled reactions termination, and wider bioreactor configurations. In the last decade, immobilized peroxidases have been effectively used in many studies for the removal of different pharmaceutically active compounds due to their elevated functional stability and repeated usability (Table 2). Touahar et al. [68] developed a versatile and multifunctional combined cross-linked enzyme aggregate (combi-CLEA) to remove an array of different pharmaceuticals. To this end, three oxidative enzymes including versatile peroxidase from *B. adusta*, laccase from *T. versicolor*, and glucose oxidase from *A. niger* were concurrently cross-linked to prepare combi-CLEAs. The as-synthesized combi-CLEAs presented the better capability for resisting denaturing environments such as low pH 3.0 and elevated temperature of 60°C. The immobilized biocatalytic system exhibited high removal efficiency to synthetic wastewater and pharmaceutical cocktail consisting of diazepam, fenofibrate, caffeine, naproxen, acetaminophen, indomethacin, ketoprofen, diclofenac, mefenamic acid, ciprofloxacin, bezafibrate, trimethoprim, and carbamazepine. The MnP-Tween 80 system comprising unsaturated fatty acid has shown the potential to eliminate the antidepressant sertraline and miconazole resulting in 85% and 88% removal of sertraline and miconazole after enzyme exposure to 24 h. The developed enzyme system was also able to remove intermediate metabolites of sertraline such as desmethylsertraline [71].

In a recent report, Pylypchuk et al. [1] engineered novel enzyme-based nanocomposites by adsorbing LiP and HRP onto magnetite nanoparticles followed by sol-gel encapsulation in a silica matrix, and they used these for the removal of common pharmaceutical pollutants in a green process (Fig. 6a). Encapsulation results in an improvement in the thermal stability profile of the biocatalysts. The encapsulated enzyme exhibited notable selectivity in oxidative removal of the organic contaminants carbamazepine, paracetamol, and diclofenac with high activity. Particularly, sol-gel immobilized HRP and LiP-based nanostructured composites showed catalytic activity over 20 successive removal cycles for 20 days at a high

Table 2 Removal studies of pharmaceutical compounds by peroxidases under various operating conditions

Enzyme source	Enzyme name	Immobilization support	Pharmaceutical compound	Reaction conditions	Removal (%)	Reference
Commercial	Horseradish peroxidase and lignin peroxidase	Magnetic sol-gel encapsulated horseradish peroxidase and lignin peroxidase composite	Carbamazepine	Carbamazepine solution (1 mL) was added to 30 mg of sol-gel composites sample under vigorous shaking in the presence of 3 μL of H_2O_2 (3.6%)	68	Pylypchuk et al. [1]
Commercial	Horseradish peroxidase and lignin peroxidase	Magnetic sol-gel encapsulated horseradish peroxidase and lignin peroxidase composite	Diclofenac	Diclofenac solution (1 mL) was added to 30 mg of sol-gel composites sample under vigorous shaking in the presence of 3 μL of H_2O_2 (3.6%)	64	Pylypchuk et al. [1]
Versatile peroxidase from *Bjerkandera adusta*, laccase *Trametes versicolor*, and glucose oxidase *Aspergillus niger*	Laccase, versatile peroxidase, and glucose oxidase	Combined cross-linked enzyme aggregates from laccase, versatile peroxidase, and glucose oxidase	Acetaminophen, naproxen, mefenamic acid, indomethacin, diclofenac, ketoprofen, caffeine, diazepam, ciprofloxacin, trimethoprim, fenofibrate, bezafibrate, and carbamazepine	Treatment was carried out at pH 5.0, 20°C in 125 mL Erlenmeyer flask at 150 rpm for 5 h. Free form of enzymes or combi-CLEA was included to achieve final Lac and Mn-oxidizing activities of 750 U/L and 250 U/L, respectively	>80	Touahar et al. [68]
Caldaromyces fumago	Chloroperoxidase	Chitosan microspheres	Sulfamethoxazole	The removal was determined in a volume of 1 mL containing 20 μM of the substrate, 20 mM	>80	García-Zamora et al. [69]

				of KCl, and 1 macrosphere in a 60 mM phosphate buffer at pH 3.0 under gentle agitation for 10 min		
Caldaromyces fumago	Chloroperoxidase	Chitosan macrospheres	Naproxen	–	>80	García-Zamora et al. [69]
Caldaromyces fumago	Chloroperoxidase	Chitosan macrospheres	Tetracycline	–	>80	García-Zamora et al. [69]
Pichia methanolica	Lignin peroxidase	Fe_3O_4@SiO_2@polydopamine nanoparticles	Tetracycline	1.0 g of immobilized LiP was employed to degrade pollutants with 5 mg/L of original concentration in 200 mL of sodium tartrate buffer for different periods	100	Guo et al. [70]
Pichia methanolica	Lignin peroxidase	Fe_3O_4@SiO_2@polydopamine nanoparticles	Dibutyl phthalate	–	100	Guo et al. [70]
Pichia methanolica	Lignin peroxidase	Fe_3O_4@SiO_2@polydopamine nanoparticles	5-Chlorophenol	–	100	Guo et al. [70]
Pichia methanolica	Lignin peroxidase	Fe_3O_4@SiO_2@polydopamine nanoparticles	Phenol	–	100	Guo et al. [70]
Pichia methanolica	Lignin peroxidase	Fe_3O_4@SiO_2@polydopamine nanoparticles	Phenanthrene	–	79	Guo et al. [70]
Pichia methanolica	Lignin peroxidase	Fe_3O_4@SiO_2@polydopamine nanoparticles	Fluoranthene	–	73	Guo et al. [70]
Pichia methanolica	Lignin peroxidase	Fe_3O_4@SiO_2@polydopamine nanoparticles	Benzo(a)pyrene	–	65	Guo et al. [70]

temperature of 55°C. In contrast to native enzymes, the encapsulated biocatalysts also displayed improved stability in acidic medium. Under the optimal conditions, sol-gel-assisted LiP composites showed 64% and 68% degradation of diclofenac and carbamazepine, respectively. Both diclofenac and carbamazepine were completely degraded at pH 3.0 and 55°C within 3 days by treatment with both immobilized LiP and HRP. After characterizing decomposedproducts of the drug through NMR spectroscopy, a decomposition pathway was proposed by the peroxidase enzymes (Fig. 6b). It was noted that the degradation of diclofenac by oxidases initiates from hydroxylation of its structural molecule. For instance, two major degradation metabolites, viz., 4′-hydroxy diclofenac and 5-hydroxy diclofenac, were detected by laccase-assisted catalytic treatment. Quinones like diclofenac-2,5-iminoquinone might also be generated as diclofenac degradation intermediates, but only 5–10% of the degraded diclofenac was converted into quinones, indicating that a major portion of this drug (90–95%) was probably removed via other oxidation routes [72].

5 Immobilized Laccases for Removal of Pharmaceutically Active Compounds

Immobilized form of laccases from many strains of WRF has been demonstrated for the degradation and removal of various pharmaceutically active compounds in wastewater effluents. Yang et al. [73] exploited an immobilized form of laccase and tested its ability to degrading different antibiotics. The method that they used for the immobilization process is CLEAs as they prepared magnetic-CLEAs for laccase and used it for the biodegradation of antibiotics. Results showed that laccase M-CLEAs were capable of degrading more than 80% of tetracycline within 12 h treatment. Three degradation products of tetracycline were detected using LC-TOF-MS designated as TP 459, TP 431, and TP 396 that have elution time at 2.69, 6.01, and 6.35 min, respectively. In a very recent study, Bilal et al. [13–15] demonstrated the ability of free and immobilized laccase for the degradation of bisphenol A, which is an organic synthetic compound [14]. In their work, laccase was covalently attached to chitosan beads using glutaraldehyde as a coupling agent. The immobilized biocatalyst showed good stability and preserved 71.24% of its initial activity after ten cycles of treatments. Bisphenol A was almost completely degraded (more than 99%) by the immobilized laccase after 150 min. Likewise, there are a plethora of studies that have documented the ability of immobilized biocatalytic systems to degrade different contaminants of emerging concerns efficiently. A summary of recent degradation studies of different pharmaceutical pollutants by immobilized laccase-based biocatalytic systems is summarized in Table 3. Immobilization of biocatalysts on different supports is a promising and ecologically friendly technique for the elimination of different pollutants from wastewater. The role of three natural phenolic substances, including acetosyringone, syringaldehyde, and p-coumaric acid, was evaluated as mediators in eliminating a routinely used

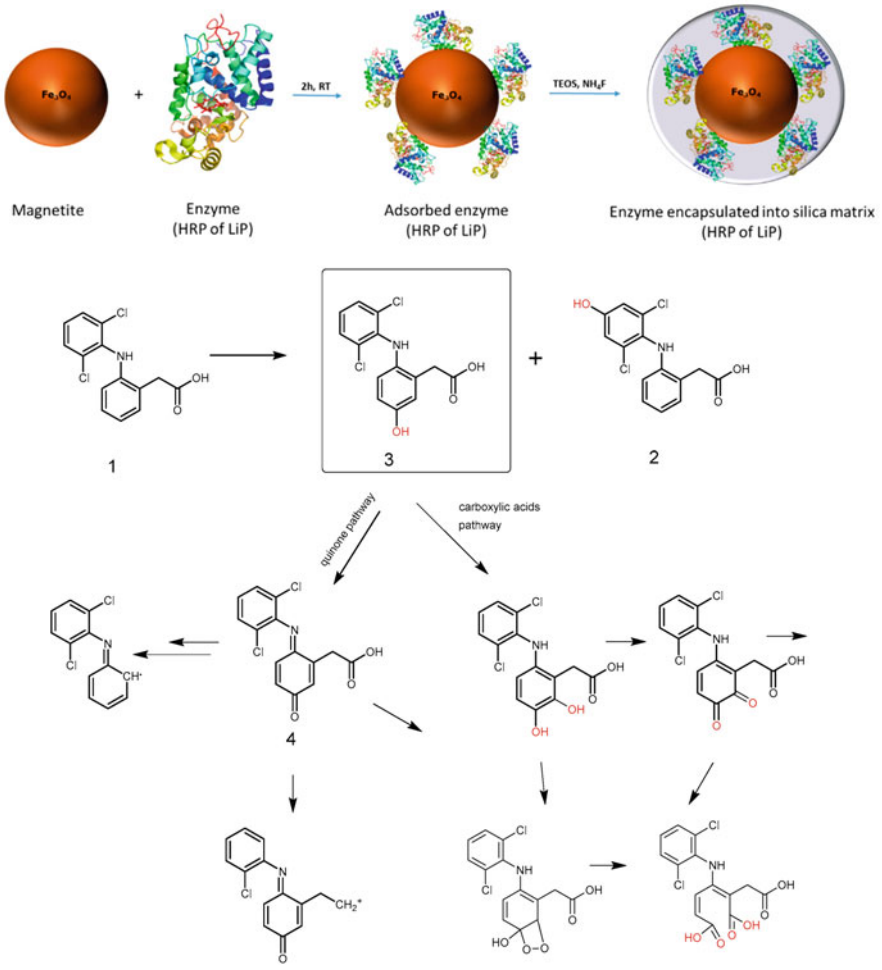


Fig. 6 (**a**) Sol-gel encapsulation of HRP and LiP peroxidases adsorbed onto magnetite nanoparticles. (**b**) Possible pathways in diclofenac enzymatic oxidative decomposition. Reprinted from Pylypchuk et al. [1] an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/)

recalcitrant pharmaceutical carbamazepine by the native as well as insolubilized laccases. Among these mediators, p-coumaric acid facilitated the optimal biocatalytic performance for removing carbamazepine with over 60% elimination efficiency after 96 h of enzymatic treatment [79].

Enzyme immobilization onto porous membranes, so-called the development of enzyme-based membrane reactors, is an attractive method to solve the issue associated with the free enzymes due to the provision of high surface area by membranes for biocatalytic reactions. Noticeable advantages of laccases immobilized on membranes are reusability, durable shelf life, and steadiness against pH and temperature

Table 3 Removal studies of pharmaceutical compounds by immobilized laccases under various operating conditions

Enzyme source	Immobilization support	Pharmaceutical compound	Reaction conditions	Removal (%)	Reference
Trametes versicolor	Bentonite-derived mesoporous materials	Tetracycline	Reaction mixture containing tetracycline solution (10 mg/L) and the immobilized enzyme was placed at 303 K for 2 h in the presence of 1-hydroxybenzotriazole	60	Wen et al. [74]
Phoma sp. UHH 5-1-03	Cross-linked to polyvinylidene fluoride membranes	Acetaminophen	Concentration, 10 μM; 20 U L-1 of immobilized enzyme; incubation at room temperature for 24 h using 180 rpm	>85	Jahangiri et al. [75]
Phoma sp. UHH 5–1-03	Cross-linked to polyvinylidene fluoride membranes	Mefenamic acid	Concentration, 10 μM; 20 U L-1 of immobilized enzyme; incubation at room temperature for 24 h using 180 rpm	>85	Jahangiri et al. [75]
Trametes versicolor	Hybrid bioreactor consisting of a combined cross-linked laccase and tyrosinase aggregates	Acetaminophen, naproxen, mefenamic acid, ibuprofen, ketoprofen, indomethacin, trimethoprim, ciprofloxacin, ofloxacin, caffeine, carbamazepine, bezafibrate, fenofibrate, atenolol	Pharmaceutical solution (10 mg/L) was mixed with the combi-CLEAs to a final activity of 80 U/L of Lac and 50 U/L of Tyr The experiment was continuously operated 5 days at room temperature	100	Ba et al. [76]
Trametes versicolor	Polyacrylonitrile-biochar composite nanofibrous membrane	Chlortetracycline	Chlortetracycline concentration (200 ppb) in water was pumped at three different fluxes (1, 2, and 3 mL/cm^2·h) into the test setup in the dead-end configuration	58.3%, 40.7%, and 22.6% chlortetracycline removal efficiency at flux rates of 1, 2, and 3 mL/h·cm^2	Taheran et al. [77]

Trametes versicolor	Oxygen functionalized nanobiochars	Carbamazepine	50 mg immobilized laccase was dispersed in 20 mL of carbamazepine solution (20 ng/mL) and allowed to react at 25°C and 200 rpm for 24 h	83% and 86% degradation in spiked water and secondary effluent, respectively	Naghdi et al. [78]
Trametes versicolor	Membrane hybrid reactor	Carbamazepine	Carbamazepine solution with an initial concentration of 20 μM was incubated with immobilized laccase after 96 h	60	Ji et al. [79]
Trametes versicolor	Cross-linked enzyme aggregates on amine-functionalized magnetic nanoparticles	Acetaminophen, atenolol, diclofenac, epoxy carbamazepine, and mefenamic acid	Reaction mixture containing 100 μg/L of each of pharmaceuticals was reacted with 1,000 U/L of the immobilized enzyme for 6 h using 0.1 mM ABTS at pH 7.0 and 125 rpm	100	Kumar and Cabana [80]
Aspergillus oryzae	Enzymatic membrane reactor	Diclofenac	Loading rate 480 mg/L d (diclofenac); redox-mediator syringaldehyde (5 mM) The reactor was operated at a flux of 1.1 L/m2 h via a peristaltic pump with an 8 min on and 1 min off cycle	>80	Nguyen et al. [81]

fluctuations [82]. Laccase enzyme produced by *T. versicolor* was subjected to immobilization on an electrospun polyacrylonitrile-biochar composite membrane and applied for the degradation of a commonly used chlortetracycline antibiotic from aqueous solution. The newly developed immobilized biocatalyst catalyzed 58.3%, 40.7%, and 22.6% degradation of chlortetracycline continuously at flux rates of 1, 2, and 3 mL/h·cm^2 [77]. Cross-linking of laccase from *Phoma* sp. UHH 5-1-03 to polyvinylidene fluoride membranes by electron beam irradiation was employed to remove a mixture of pharmaceutical pollutants including indomethacin, naproxen, bezafibrate, acetaminophen, ketoprofen, and mefenamic acid in actual municipal wastewater samples. Degradation profile of the immobilized laccase showed greater removal efficiency (more than 80%) of acetaminophen and mefenamic acid in wastewater in continuously eliminating operational mode.

6 Concluding Remarks and Outlook

In conclusion, based on the above-discussed literature with suitable examples, environmental contamination with a range of anthropogenic pollutants of emerging concern has become a global problem. This chapter is of particular interest, which highlights the significant potential of numerous enzymes in pristine or immobilized forms for efficient removal/degradation of ECs. The growing contamination of water matrices with a controlled or uncontrolled discharge of incompletely or inadequately treated hazardous industrial wastes harshly affects the whole living ecosystem. Considering the above-discussed scenarios, there is a dire need to develop highly efficient bioremediation strategies that are clean, green, sustainable, and environmental-friendly and can replace the in-practice inefficient remediation approaches. Various wastewater treatment technologies using crude (pristine), purified enzymes, and immobilized enzyme systems are discussed with a particular emphasis on the degradation and detoxification of pharmaceutically active compounds. This study emphasized that the enzyme-based biocatalytic treatment processes have gained unprecedented importance in mitigating an array of ECs typically present in wastewater effluents. Despite an effective technology with proven bioremediation potential, enzymes exploitability is currently far from being and has not yet been incorporated in large-scale water treatment systems. Future studies should be attentive to increase the application of enzymes under the actual treatment environments. Moreover, an in-depth understanding of the extensive environmental and commercial influences regarding the practical applicability of enzyme-based biocatalytic agents to achieve efficient remediation of numerous contaminants of emerging concern is critical to attaining the sustainability of immobilized enzyme technology.

Acknowledgments Authors are grateful to their representative universities/institutes for providing literature facilities.

Conflict of Interests Authors declare no conflict of interest in any capacity, including financial and competing.

References

1. Pylypchuk IV, Daniel G, Kessler VG, Seisenbaeva GA (2020) Removal of diclofenac, paracetamol, and carbamazepine from model aqueous solutions by magnetic Sol–Gel encapsulated horseradish peroxidase and lignin peroxidase composites. Nano 10(2):282
2. Ali N, Khan A, Bilal M, Malik S, Badshah S, Iqbal H (2020) Chitosan-based bio-composite modified with Thiocarbamate moiety for decontamination of cations from the aqueous media. Molecules 25(1):226
3. Ali N, Said A, Ali F, Raziq F, Ali Z, Bilal M et al (2020) Photocatalytic degradation of congo red dye from aqueous environment using cobalt ferrite nanostructures: development, characterization, and photocatalytic performance. Water Air Soil Pollut *231*(2):50
4. Ebele AJ, Abdallah MAE, Harrad S (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerg Contam 3(1):1–16
5. Khan A, Ali N, Bilal M, Malik S, Badshah S, Iqbal H (2019) Engineering functionalized chitosan-based sorbent material: characterization and sorption of toxic elements. Appl Sci 9 (23):5138
6. Petrović M, Gonzalez S, Barceló D (2003) Analysis and removal of emerging contaminants in wastewater and drinking water. TrAC Trends Anal Chem 22(10):685–696
7. Tixier C, Singer HP, Oellers S, Müller SR (2003) Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. Environ Sci Technol 37(6):1061–1068
8. Aldekoa J, Medici C, Osorio V, Pérez S, Marcé R, Barceló D, Francés F (2013) Modelling the emerging pollutant diclofenac with the GREAT-ER model: application to the Llobregat River basin. J Hazard Mater 263:207–213
9. Buser HR, Poiger T, Müller MD (1998) Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: rapid photodegradation in a lake. Environ Sci Technol 32 (22):3449–3456
10. Hallgren P, Wallberg P (2015) Background report on pharmaceutical concentrations and effects in the Baltic Sea. In: Policy area hazards of the EU strategy for the Baltic Sea Region. Swedish Environmental Protection Agency, Stockholm
11. Belhaj D, Baccar R, Jaabiri I, Bouzid J, Kallel M, Ayadi H, Zhou JL (2015) Fate of selected estrogenic hormones in an urban sewage treatment plant in Tunisia (North Africa). Sci Total Environ 505:154–160
12. Morsi R, Bilal M, Iqbal HMN, Ashraf SS (2020) Laccases and peroxidases: the smart, greener and futuristic biocatalytic tools to mitigate recalcitrant emerging pollutants. Sci Total Environ:136572
13. Bilal M, Adeel M, Rasheed T, Zhao Y, Iqbal HMN (2019) Emerging contaminants of high concern and their enzyme-assisted biodegradation – a review. Environ Int 124:336–353
14. Bilal M, Jing Z, Zhao Y, Iqbal HM (2019) Immobilization of fungal laccase on glutaraldehyde cross-linked chitosan beads and its bio-catalytic potential to degrade bisphenol A. Biocatal Agric Biotechnol 19:101174
15. Bilal M, Ashraf SS, Barceló D, Iqbal HM (2019) Biocatalytic degradation/redefining "removal" fate of pharmaceutically active compounds and antibiotics in the aquatic environment. Sci Total Environ 691:1190–1211
16. Bilal M, Rasheed T, Iqbal HMN, Yan Y (2018) Peroxidases-assisted removal of environmentally-related hazardous pollutants with reference to the reaction mechanisms of industrial dyes. Sci Total Environ 644:1–13

17. Glumoff T, Harvey P, Molinari S, Goble M, Frank G, Palmer JM et al (1990) Lignin peroxidase from *Phanerochaete chrysosporium* molecular and kinetic characterization of isozymes. Eur J Biochem 187:515–520
18. Furukawa T, Bello FO, Horsfall L (2014) Microbial enzyme systems for lignin degradation and their transcriptional regulation. Front Biol 9:448–471
19. Wen X, Jia Y, Li J (2009) Degradation of tetracycline and oxytetracycline by crude lignin peroxidase prepared from *Phanerochaete chrysosporium*–a white rot fungus. Chemosphere 75 (8):1003–1007
20. Zhang Y, Geißen SU (2010) In vitro degradation of carbamazepine and diclofenac by crude lignin peroxidase. J Hazard Mater 176(1–3):1089–1092
21. Ivancich A, Mazza G, Desbois A (2001) Comparative electron paramagnetic resonance study of radical intermediates in turnip peroxidase isozymes. Biochemistry 40:6860–6866
22. Zhang Y, Geißen SU (2012) Elimination of carbamazepine in a non-sterile fungal bioreactor. Bioresour Technol 112:221–227
23. Heinfling A, Ruiz-Dueñas FJ, Martínez MJ, Bergbauer M, Szewzyk U, Martínez AT (1998) A study on reducing substrates of manganese-oxidizing peroxidases from Pleurotus eryngii and Bjerkandera adusta. FEBS Lett 428(3):141–146
24. Glenn JK, Gold MH (1985) Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete, Phanerochaete chrysosporium. Arch Biochem Biophys 242(2):329–341
25. Paszczyński A, Huynh VB, Crawford R (1985) Enzymatic activities of an extracellular, manganese-dependent peroxidase from Phanerochaete chrysosporium. FEMS Microbiol Lett 29(1–2):37–41
26. Bilal M, Asgher M, Parra-Saldivar R, Hu H, Wang W, Zhang X, Iqbal HM (2017) Immobilized ligninolytic enzymes: an innovative and environmental responsive technology to tackle dye-based industrial pollutants–a review. Sci Total Environ 576:646–659
27. Kellner H, Luis P, Pecyna MJ, Barbi F, Kapturska D, Krüger D et al (2014) Widespread occurrence of expressed fungal secretory peroxidases in forest soils. PLoS One 9(4):e95557
28. Champagne PP, Ramsay JA (2005) Contribution of manganese peroxidase and laccase to dye decoloration by Trametes versicolor. Appl Microbiol Biotechnol 69(3):276
29. Maciel MJM, Ribeiro HCT (2010) Industrial and biotechnological applications of ligninolytic enzymes of the basidiomycota: a review. Electron J Biotechnol 13(6):14–15
30. Sundaramoorthy M, Kishi K, Gold MH, Poulos TL (1994) The crystal structure of manganese peroxidase from Phanerochaete chrysosporium at 2.06-A resolution. J Biol Chem 269 (52):32759–32767
31. Hildén K, Mäkelä MR, Steffen KT, Hofrichter M, Hatakka A, Archer DB, Lundell TK (2014) Biochemical and molecular characterization of an atypical manganese peroxidase of the litter-decomposing fungus Agrocybe praecox. Fungal Genet Biol 72:131–136
32. Li L, Liu B, Yang J, Zhang Q, He C, Jia R (2019) Catalytic properties of a short manganese peroxidase from Irpex lacteus F17 and the role of Glu166 in the Mn2+-independent activity. Int J Biol Macromol 136:859–869
33. Wong DW (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol 157(2):174–209
34. Lueangjaroenkit P, Teerapatsakul C, Sakka K, Sakka M, Kimura T, Kunitake E, Chitradon L (2019) Two manganese peroxidases and a laccase of *Trametes polyzona* KU-RNW027 with novel properties for dye and pharmaceutical product degradation in redox mediator-free system. Mycobiology 47:217–229
35. Wen X, Jia Y, Li J (2010) Enzymatic degradation of tetracycline and oxytetracycline by crude manganese peroxidase prepared from *Phanerochaete chrysosporium*. J Hazard Mater 177 (1–3):924–928
36. Bódalo A, Gomez JL, Gomez E, Bastida J, Maximo MF (2006) Comparison of commercial peroxidases for removing phenol from water solutions. Chemosphere 63(4):626–632

37. Fernandes M, Souza DH, Henriques RO, Alves MV, Skoronski E, Junior AF (2020) Obtaining soybean peroxidase from soybean hulls and its application for detoxification of 2, 4-dichlorophenol contaminated water. J Environ Chem Eng 8:103786
38. Alneyadi A, Shah I, AbuQamar S, Ashraf S (2017) Differential degradation and detoxification of an aromatic pollutant by two different peroxidases. Biomol Ther 7(1):31
39. Alneyadi AH, Ashraf SS (2016) Differential enzymatic degradation of thiazole pollutants by two different peroxidases–a comparative study. Chem Eng J 303:529–538
40. Almaqdi KA, Morsi R, Alhayuti B, Alharthi F, Ashraf SS (2019) LC-MSMS based screening of emerging pollutant degradation by different peroxidases. BMC Biotechnol 19(1):83
41. Arnoldsson K, Andersson PL, Haglund P (2012) Formation of environmentally relevant brominated dioxins from 2,4,6,-tribromophenol via bromoperoxidase-catalyzed dimerization. Environ Sci Technol 46(13):7239–7244
42. Baumer JD, Valério A, de Souza SMGU, Erzinger GS, Furigo Jr A, de Souza AAU (2018) Toxicity of enzymatically decolored textile dyes solution by horseradish peroxidase. J Hazard Mater 360:82–88
43. Zhou H, Yang D, Qiu X, Wu X, Li Y (2013) A novel and efficient polymerization of lignosulfonates by horseradish peroxidase/H2O2 incubation. Appl Microbiol Biotechnol 97 (24):10309–10320
44. Colosi LM, Pinto RA, Huang Q, Weber WJJ (2009) Peroxidase-mediated degradation of perfluorooctanoic acid. Environ Toxicol Chem Int J 28(2):264–271
45. Ling KQ, Li WS, Sayre LM (2008) Oxidations of N-(3-indoleethyl) cyclic aliphatic amines by horseradish peroxidase: the indole ring binds to the enzyme and mediates electron-transfer amine oxidation. J Am Chem Soc 130(3):933–944
46. Yang L, Shi Y, Li J, Fang L, Luan T (2018) Transformation of aqueous sulfonamides under horseradish peroxidase and characterization of sulfur dioxide extrusion products from sulfadiazine. Chemosphere 200:164–172
47. Xu R, Si Y, Li F, Zhang B (2015) Enzymatic removal of paracetamol from aqueous phase: horseradish peroxidase immobilized on nanofibrous membranes. Environ Sci Pollut Res 22 (5):3838–3846
48. Berry TD, Filley TR, Blanchette RA (2014) Oxidative enzymatic response of white-rot fungi to single-walled carbon nanotubes. Environ Pollut 193:197–204
49. Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, Sannia G (2010) Laccases: a never-ending story. Cell Mol Life Sci 67(3):369–385
50. Asgher M, Noreen S, Bilal M (2017) Enhancing catalytic functionality of Trametes versicolor IBL-04 laccase by immobilization on chitosan microspheres. Chem Eng Res Des 119:1–11
51. Asgher M, Noreen S, Bilal M (2017) Enhancement of catalytic, reusability, and long-term stability features of Trametes versicolor IBL-04 laccase immobilized on different polymers. Int J Biol Macromol 95:54–62
52. Moreno AD, Ibarra D, Eugenio ME, Tomás-Pejó E (2019) Laccases as versatile enzymes: from industrial uses to novel applications. J Chem Technol Biotechnol 95:481. https://doi.org/10.1002/jctb.6224
53. Unuofin JO, Okoh AI, Nwodo UU (2019) Aptitude of oxidative enzymes for treatment of wastewater pollutants: a laccase perspective. Molecules (Basel, Switzerland) 24(11):2064
54. Guardado ALP, Belleville MP, Alanis MDJR, Saldivar RP, Sanchez-Marcano J (2019) Effect of redox mediators in pharmaceuticals degradation by laccase: a comparative study. Process Biochem 78:123–131
55. Litwińska K, Bischoff F, Matthes F, Bode R, Rutten T, Kunze G (2019) Characterization of recombinant laccase from Trametes versicolor synthesized by Arxula adeninivorans and its application in the degradation of pharmaceuticals. AMB Express 9(1):102
56. Alharbi SK, Nghiem LD, Van De Merwe JP, Leusch FD, Asif MB, Hai FI, Price WE (2019) Degradation of diclofenac, trimethoprim, carbamazepine, and sulfamethoxazole by laccase from Trametes versicolor: transformation products and toxicity of treated effluent. Biocatal Biotransformation 37(6):399–408

57. Sutar RS, Rathod VK (2015) Ultrasound assisted laccase catalyzed degradation of ciprofloxacin hydrochloride. J Ind Eng Chem 31:276–282
58. Sutar RS, Rathod VK (2015) Ultrasound assisted enzyme catalyzed degradation of cetirizine dihydrochloride. Ultrason Sonochem 24:80–86
59. Suda T, Hata T, Kawai S, Okamura H, Nishida T (2012) Treatment of tetracycline antibiotics by laccase in the presence of 1-hydroxybenzotriazole. Bioresour Technol 103(1):498–501
60. Llorca M, Rodríguez-Mozaz S, Couillerot O, Panigoni K, de Gunzburg J, Bayer S et al (2015) Identification of new transformation products during enzymatic treatment of tetracycline and erythromycin antibiotics at laboratory scale by an on-line turbulent flow liquid-chromatography coupled to a high resolution mass spectrometer LTQ-Orbitrap. Chemosphere 119:90–98
61. Mugdha A, Usha M (2012) Enzymatic treatment of wastewater containing dyestuffs using different delivery systems. Sci Rev Chem Commun 2(1):31–40
62. Yang RL, Zhao XJ, Wu TT, Bilal M, Wang ZY, Luo HZ, Yang WJ (2019) A novel and highly regioselective biocatalytic approach to acetylation of helicid by using whole-cell biocatalysts in organic solvents. Catal Commun 128:105707
63. Asgher M, Shahid M, Kamal S, Iqbal HMN (2014) Recent trends and valorization of immobilization strategies and ligninolytic enzymes by industrial biotechnology. J Mol Catal B Enzym 101:56–66
64. Zdarta J, Meyer AS, Jesionowski T, Pinelo M (2018) Developments in support materials for immobilization of oxidoreductases: a comprehensive review. Adv Colloid Interf Sci 258:1–20
65. Sheldon RA, van Pelt S (2013) Enzyme immobilisation in biocatalysis: why, what and how. Chem Soc Rev 42(15):6223–6235
66. Ren S, Li C, Jiao X, Jia S, Jiang Y, Bilal M, Cui J (2019) Recent progress in multienzymes co-immobilization and multienzyme system applications. Chem Eng J 373:1254–1278
67. Bilal M, Asgher M (2015) Dye decolorization and detoxification potential of Ca-alginate beads immobilized manganese peroxidase. BMC Biotechnol 15(1):111
68. Touahar IE, Haroune L, Ba S, Bellenger JP, Cabana H (2014) Characterization of combined cross-linked enzyme aggregates from laccase, versatile peroxidase and glucose oxidase, and their utilization for the elimination of pharmaceuticals. Sci Total Environ 481:90–99
69. García-Zamora JL, León-Aguirre K, Quiroz-Morales R, Parra-Saldívar R, Gómez-Patiño MB, Arrieta-Baez D et al (2018) Chloroperoxidase-mediated halogenation of selected pharmaceutical micropollutants. Catalysts 8(1):32
70. Guo J, Liu X, Zhang X, Wu J, Chai C, Ma D et al (2019) Immobilized lignin peroxidase on Fe3O4@ SiO2@ polydopamine nanoparticles for degradation of organic pollutants. Int J Biol Macromol 138:433–440
71. Inoue S, Igarashi Y, Yoneda Y, Kawai S, Okamura H, Nishida T (2015) Elimination and detoxification of fungicide miconazole and antidepressant sertraline by manganese peroxidase-dependent lipid peroxidation system. Int Biodeterior Biodegradation 100:79–84
72. Forrez I, Carballa M, Verbeken K, Vanhaecke L, Ternes T, Boon N, Verstraete W (2010) Diclofenac oxidation by biogenic manganese oxides. Environ Sci Technol 44(9):3449–3454
73. Yang J, Lin Y, Yang X, Ng TB, Ye X, Lin J (2017) Degradation of tetracycline by immobilized laccase and the proposed transformation pathway. J Hazard Mater 322:525–531
74. Wen X, Zeng Z, Du C, Huang D, Zeng G, Xiao R et al (2019) Immobilized laccase on bentonite-derived mesoporous materials for removal of tetracycline. Chemosphere 222:865–871
75. Jahangiri E, Thomas I, Schulze A, Seiwert B, Cabana H, Schlosser D (2018) Characterisation of electron beam irradiation-immobilised laccase for application in wastewater treatment. Sci Total Environ 624:309–322
76. Ba S, Haroune L, Soumano L, Bellenger JP, Jones JP, Cabana H (2018) A hybrid bioreactor based on insolubilized tyrosinase and laccase catalysis and microfiltration membrane remove pharmaceuticals from wastewater. Chemosphere 201:749–755

77. Taheran M, Naghdi M, Brar SK, Knystautas EJ, Verma M, Surampalli RY (2017) Degradation of chlortetracycline using immobilized laccase on Polyacrylonitrile-biochar composite nanofibrous membrane. Sci Total Environ 605:315–321
78. Naghdi M, Taheran M, Brar SK, Kermanshahi-pour A, Verma M, Surampalli RY (2017) Immobilized laccase on oxygen functionalized nanobiochars through mineral acids treatment for removal of carbamazepine. Sci Total Environ 584:393–401
79. Ji C, Hou J, Wang K, Zhang Y, Chen V (2016) Biocatalytic degradation of carbamazepine with immobilized laccase-mediator membrane hybrid reactor. J Membr Sci 502:11–20
80. Kumar VV, Cabana H (2016) Towards high potential magnetic biocatalysts for on-demand elimination of pharmaceuticals. Bioresour Technol 200:81–89
81. Nguyen LN, Hai FI, Price WE, Leusch FD, Roddick F, McAdam EJ et al (2014) Continuous biotransformation of bisphenol A and diclofenac by laccase in an enzymatic membrane reactor. Int Biodeterior Biodegradation 95:25–32
82. Wang Q, Cui J, Li G, Zhang J, Huang F, Wei Q (2014) Laccase immobilization by chelated metal ion coordination chemistry. Polymers 6(9):2357–2370

Fungal Reactors: A Solution for the Removal of Pharmaceuticals in Urban and Hospital Wastewater

Josep Anton Mir-Tutusaus and Montserrat Sarrà

Contents

Abstract The pharmaceutical occurrence in surface and ground water bodies is due to low efficiency of the wastewater treatment plants in removing those pollutants. Their biological transformation using white-rot fungi has been proposed due to their unspecific intracellular and extracellular oxidoreductase enzymatic systems. This chapter summarizes and analyzes the studies performed on pharmaceuticals removal from urban and hospital wastewater using fungal reactors operating in batch or continuous mode. Due to low fungal growth rate, all reactors are based on the biomass reuse through the retention through the pellet morphology or membrane, or by immobilization on a support. The treatment of real wastewater in non-sterile conditions requires the assessment of effect of the native microorganism on the fungal treatment. The chapter also offers an insight into fungal enzymatic systems,

J. A. Mir-Tutusaus and M. Sarrà (✉)
Departament d'Enginyeria Química Biològica i Ambiental, Escola d'Enginyeria, Universitat Autònoma de Barcelona, Barcelona, Spain
e-mail: jantonz@gmail.com; Montserrat.Sarra@uab.cat

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 145–162, DOI 10.1007/698_2020_660,

types of reactors, and strategies to reduce bacteria effect and consequently maintaining fungal activity during a continuous treatment during long periods.

Keywords Biomass retention, Immobilization, Non-sterile conditions, Real wastewater

1 Introduction

Current wastewater treatment plants are not designed to remove pharmaceuticals; as a consequence, their effluents are one of the main sources of this pollution in surface and ground water bodies [1]. Although the concentration of those compounds is usually low, ranging from ng/L to μg/L, there is an increasing concern due their high activity even at a very low concentration. Among the many alternatives studied to remove emerging pollutants, biological treatments are preferred because they are regarded as a more environmentally friendly process. Nevertheless, not all biological systems have evidenced the same efficiency in removing pharmaceuticals compounds from wastewater. Among these eco-friendly treatments, the use of white-rot fungi (WRF) is an interesting option because these fungi have demonstrated the capacity to degrade a wide range of recalcitrant organic compounds, including emerging pollutants, due to their unspecific enzymatic system. Despite their high potential, an application at full scale does not exist. One of the drawbacks of the technology is the difficulty in maintaining the fungal activity for a long period of time since bacteria exert competitive pressure in fungal survival. So, the reactor design and the operational strategies are key aspects to consider in achieving a scale-up process to remove emerging pollutants using WRF.

This chapter shows some of the latest research efforts at improving fungal bioreactors for removing pharmaceuticals from urban and hospital wastewater. The first section presents some general aspects of the WRF and their enzymatic systems, focusing on pollutant biodegradation. The second section is devoted to describing the type of reactors using fungi as retained biomass or as immobilized biomass. Finally, the third section reviews treatments of urban and hospital wastewater with fungi while focusing on the removal of pharmaceutically active compounds.

2 White-Rot Fungi

It is not uncommon in nature to find among fungal species the ability to break down cellulose and hemicellulose, polysaccharides that together with lignin form the main components of lignocellulose. This process is part of the primary metabolism and as such, it yields a positive energy gain. However, the ability to depolymerize the third

component, lignin, is much more scarce [2]. Lignin is a complex and heterogeneous polyphenolic polymer, the degradation of which does not result in a net energy gain; rather, it is a step used by few organisms to access polysaccharides inside lignin-carbohydrate complexes and exploit this ecological niche. White-rot fungi (WRF) are a group of fungal species that are able to do just that. In nature they achieve depolymerization of lignin through a mixture of extracellular ligninolytic enzymes and mediators and intracellular monooxygenases. This enzymatic system happens to be very unspecific and has been studied for transforming a wide range of micropollutants [3, 4].

WRF owe their name, *white-rot*, to the whitish aspect that a wood takes after being attacked by these fungi. Most known WRF are basidiomycetes, but also certain ascomycetes are able to produce white rot. Some relevant fungal species that have been studied for their ability to remove pharmaceuticals include *Ganoderma* spp., *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, and *Trametes versicolor* [5].

2.1 Enzymatic System of the Fungi

The enzymatic system of fungi is very complex and diverse. In regard to xenobiotic transformation, it can be classified into extracellular and intracellular. However, metabolites generated by either method may be catabolized extra- and intracellularly, metabolites produced by intracellular enzymes may be excreted, and metabolites produced by extracellular enzymes may be internalized. Additionally, transformation products (TPs) may form conjugates, be further transformed, or result in mineralization. TPs may be excreted at different oxidation stages or be transformed by fungal, non-fungal, or abiotic oxidation (Fig. 1).

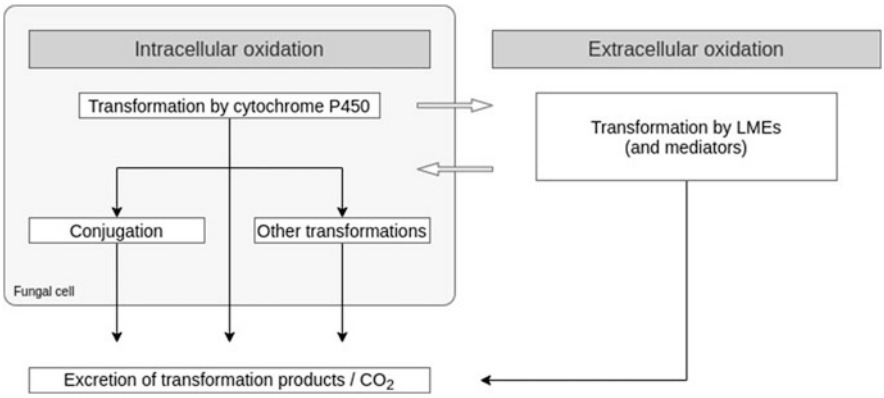


Fig. 1 Simplified schema of micropollutant transformation pathways in white-rot fungi

2.1.1 Extracellular Enzymatic Mechanism

The extracellular enzymatic mechanism of WRF is composed mainly of oxidoreductases called lignin-modifying enzymes (LMEs) and mediators, all of which are mainly active in the acidic pH range. Several LMEs have been described in the literature, four of which are described below; however, they are not the only enzymes involved in lignin transformation [6]:

- Lignin peroxidase (LiP). Monomeric glycosylated hemoproteins with relatively low specificity, although their preferred substrates are non-phenolic compounds [7]. Like most LMEs, LiP is involved in lignin transformation, but it is not essential, as some fungi capable of lignin transformation do not produce this enzyme. Their reaction mechanism is H_2O_2-dependent one-electron oxidation and is usually mediated by redox mediator veratryl alcohol [8, 9].
- Manganese peroxidase (MnP). Heme glycosylated enzyme, its reaction mechanism is H_2O_2-dependent, via one-electron oxidation of Mn^{2+} to Mn^{3+}, which subsequently oxidates the organic substrate [9, 10]. Mn^{3+} can diffuse into the lignified cell wall and attack it from inside, sometimes with the help of some organic acids such as oxalic acid that chelates Mn^{3+} and acts as redox mediator.
- Laccase (Lac). Laccases are multicopper enzymes and are produced by several wood-transforming basidiomycetes and ascomycetes. Their active site contains four copper atoms, and their reaction mechanism involves the reduction of oxygen to water [11, 12]. Small organic compounds, known as mediators, can be oxidized by laccases, and these molecules may further attack other substrates, thus widening the range of compounds laccase can transform.
- Versatile peroxidase (VP). These enzymes, first described in *Pleurotus* and *Bjerkandera* species, are peroxidases with MnP-LiP hybrid properties, capable of transforming typical substrates of both previous peroxidases (Mn^{2+} and veratryl alcohol, respectively) [7, 13]. Thanks to their distinct multiple binding sites, they can also transform aromatic compounds not efficiently attacked by MnP and LiP [14].

Mediators are small, low-molecular-weight redox molecules that are able to diffuse through the lignocellulosic complex. They are especially important because due to the molecular size of LMEs, interactions with lignin are improbable. The mediators, once oxidized by LMEs, remain stable radicals that can oxidize compounds that originally were not substrate of LMEs. Conveniently, these mediators are often produced by fungal metabolism itself, notably 3-hydroxyanthranilic acid, veratryl alcohol, oxalate, malate, and fumarate [15].

2.1.2 Intracellular Enzymatic Mechanism

The main intracellular enzymes involved in xenobiotic transformation are related to the cytochrome P450 superfamily. They are intracellular monooxygenases typically found in mammals that are involved in the metabolism of a wide range of

compounds. The reaction mechanism involves the reduction of O_2 to H_2O, while oxidizing the substrate. Cytochrome P450 can, therefore, transform aromatic or aliphatic structures of many molecules, including organic pollutants.

In fungus, cytochrome P450 has been shown to play a role in the metabolism of anti-inflammatory drugs, lipid regulators and analgesics, among other compounds [16–19].

2.2 *Bio-Oxidation/Redox-Mediated System*

In addition to their extracellular oxidation potential, white-rot fungi show the ability to reduce lignin depolymerization products, such as aromatic aldehydes, acids, and quinones, through different intracellular and membrane-bound systems. So, the simultaneous actions of both systems, extracellular oxidative enzymes and intracellular reduction mechanism, lead to the establishment of redox cycles. One of the functions of these cycles is the production of reactive oxygen species (ROS), i.e., superoxide anion radicals ($O_2\cdot^-$), H_2O_2 and hydroxyl radicals (·OH) depending on the fungus, the depolymerization product, and the oxidizing enzyme [20]. Among the ROS produced by fungi, hydroxyl radical has the highest redox potential, and it can be chemically generated by the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow OH^- + \cdot OH + Fe^{3+}$). But the biological generation of the hydroxyl radical involves lignin-oxidizing enzymes to produce H_2O_2, fungal mycelia, and its metabolic products as metal chelating agents. Therefore, the use of mediators in lignin and/or environmental pollutants degradation by ligninolytic enzymes performs similarly to the advanced oxidation process in the generation of highly reactive radicals. The process applied to evidence the degradation of several selected pharmaceuticals through the quinone redox cycling mechanism can be achieved from molecular oxygen, incubating fungi and chelated ferric ion. The advanced bio-oxidation process can be achieved by incubating the fungi with a lignin-derived quinone (2,6-dimethoxy-1,4-benzoquinone, DBQ) and chelated ferric ion (Fe^{3+}-oxalate) [21]. Under these conditions, DBQ are converted to hydroquinone ($DBQH_2$) by an intracellular quinone reductase; subsequently, extracellular lignin-modifying enzyme (laccase or peroxidase) oxidizes $DBQH_2$ to semiquinone radicals ($DBQ\cdot^-$). Then the Fenton's reagent is formed by these radical's autoxidation catalyzed by Fe^{3+} ($DBQ\cdot^- + Fe^{3+}$-oxalate $\rightarrow$ DBQ + Fe^{2+}-oxalate and Fe^{2+}-oxalate + $O_2 \leftrightarrow Fe^{3+}$-oxalate + $O_2\cdot^-$). Finally, the $O_2\cdot^-$ dismutation produces H_2O_2 precursor of hydroxyl radicals. This process was first evidenced by Gómez-Toribio et al. [20], who established the conditions to enhance the radical production.

The unspecificity, the short reaction time required, and the high oxidation potential of $^{\bullet}OH$ radicals are the main advantages of the process in removing recalcitrant organic pollutants. Despite these advantages, reports of effluent treatment are scarce, even at lab scale, due to quinone depletion probably due to radical attack. Recent studies are focused in using gallic acid as a mediator because it is also a transformation product of the lignin and it can generate semiquinone compounds through the fungal redox cycle [22].

3 Type of Reactors with Fungi

3.1 Retained Fungi

Several strategies exist to retain fungal biomass inside the bioreactor. A prominent one is the auto-immobilization of fungal mass in the form of pellets. This immobilization allows for decoupling the solids retention time and the hydraulic retention time (i.e., better separation of fungal biomass and liquid matrix). In addition to retain the fungus inside the reactor, other advantages can be observed for pelleted biomass versus free hyphae: improved settling, possibility of biomass reuse, low clogging effect, high volumetric productivity, and lower adhesion to parts of the reactor [23–25]. The fact that pellet formation is often separated from the treatment stage is also an advantage. Different approaches for pellet growth can be found in the literature [26–28]. In general, it can be acknowledged that pellet diameter can be controlled by shear stress, often controlled by agitation/aeration speed, and inoculum concentration [29, 30]. However, pelleted biomass comes with some limitations, such as lower nutrient and oxygen transfer inside the pellet, which can result in autolysis and difficulty of maintaining fungal pellets during long-term operations [23, 29, 31].

Some representative reactors with pelleted biomass comprise stirred tanks, fluidized beds, and airlift/bubble columns:

- Stirred tank reactor (STR). It consists of a tank with mechanical agitation that provides both agitation and a mechanism to disperse air, which is usually supplied at the bottom of the reactor. Agitation speed is a main variable in these systems, which controls the diameter of the pellets, the stress of the cells, and the mass and oxygen transfer. Increased shear stress and agitation may lead to the rupture of pellets [32].
- Airlift reactor and bubble column reactor. The airlift reactor incorporates a cylindrical baffle that separates the liquid moving down from the liquid rising. This baffle provides an engineered path that air bubbles follow, therefore ensuring proper aeration and mixing by recirculation. It does not need any mechanical agitation. The bubble diameter and the flow rate of aeration control the diameter and fluffiness of the pellets, the mass, and oxygen transfer. These systems produce less shear stress than their mechanically stirred counterparts and require less energy. Bubble column reactors similarly rely on aeration for oxygen transfer and mixing purposes, but they do not use any baffle; instead, a gas distributor on the bottom ensures (non-uniform) agitation [33].
- Fluidized bed reactor (FBR). The fungal pellets are suspended in the stream of air and influent, both of which flow from the bottom of the reactor. The mixing does not require mechanical agitation and similarly to airflit reactors; the airflow controls pellet diameter and mass/oxygen transfer. Poor fluidization is a problem in fluidized bed reactors using pelleted biomass due to pellet aggregation; partial biomass purging helps in reducing this difficulty. Some improvements in the system involve (1) fluidization by air pulses, which improves fluidization quality, avoids conglomerates of mycelia, and maintains more uniform pellets [29, 34],

and (2) reactor head with a diameter larger than the reactor column, which aids in reducing foam formation, facilitates gas-liquid phase separation, and facilitates pellet formation by allowing pellets to spin before descending through the column [35].

The use of membrane bioreactors (MBRs) constitutes another important retention strategy. MBRs have long been used both in urban and industrial wastewater treatment [36–38], and there has been an increased interest in micropollutant removal on these systems [39]. Fungal MBRs tend to take the form of a bubble reactor coupled with a membrane stage and rely on air flow for mixing [40]. The membrane enables the retention of fungal biomass while allowing the flow of medium, thus allowing for shorter hydraulic retention times than other fungal reactors. Additionally, depending on the membrane type and its pore size, the retention of enzymes and other microorganisms is also possible [3, 41, 42]. Membrane fouling and durability of the membrane are a concern in membrane reactors (especially in fungal MBRs), which require more maintenance and monitoring [43, 44].

3.2 Immobilized Fungi

A lot of diversity can be found in the literature about fungal reactors with immobilized biomass. Some diversity comes from the fact that fungi can be immobilized onto a wide variety of carriers, both inert, stainless steel mesh, polyamide fiber net, fiberglass net, polyurethane foam, polyurethane cubes, foam glass beads, etc. [45, 46], and non-inert, some of which can also be partially used as a carbon source: pine chips, pallet wood, pine bark, hazelnut shell, nutshell, etc. [47, 48].

- Trickling packed bed reactors. Some particularly successful reactors using immobilized fungi are trickling packed-bed reactors. They consist of a packed bed full of packing material that may be already colonized by the fungus or rely on the reactor operation to grow the fungus onto the material. The air is usually pumped from the bottom of the reactor and the influent is fed from the top. Some setups also include a recycling loop to recirculate the effluent [47–49].
- Packed bed reactor. Another alternative is a packed bed reactor. The operation of such reactors may not require aeration (oxygen is supplied dissolved in the influent), and they tend to be operated with an upflow of medium [50, 51].
- Moving packed bed reactor. A moving packed bed reactor is another option for WRF, in which the packing material with the immobilized fungus is placed inside a holed cylinder cage rotating inside the reactor container. The air is pumped from the bottom of the reactor with an air diffuser [45].
- Rotating biological contactors (RBCs). These reactors consist of spaced disks mounted on a rotating shaft, which is half submerged on an open container that contains the influent. The reactor is first operated with growth medium until a

biofilm of the target fungus is grown onto the disks [52]. A r*otating suspension cartridge reactor* is a variation of the RBC that use holed cartridges filled with the fungus immobilized onto packing material [53]. Another reactor inspired by the RBC is the rotating drum reactor, which consists of an inner tube, filled with the immobilized fungus, located in the center of the longitudinal axis of an open polyvinylchloride channel [54]. These types of reactor do not need aeration because the rotating shaft ensures that biomass is intermittently submerged in the liquid and in contact with air.

4 Treatments of UWW and HWW with Fungi

Some prominent wastewater treatments are reviewed in this section. It is significant to note that at the time of writing only a few studies can be found that use real wastewater. And of the ones that do treat real wastewater, only some of them do so mimicking real-world variables: environmentally significant micropollutant concentration and non-sterile conditions. In addition, most studies are run at laboratory scale, being few the experiments found at pilot-scale plants and none, to the best of the authors' knowledge, at full-scale WWTPs.

4.1 Batch System

Most of the white-rot fungal bioreactor batch treatments reviewed in this section used fluidized bed bioreactors. Those studies can be now regarded as a stepping stone needed to demonstrate the feasibility of white-rot fungal reactor treatments of real wastewater, and put some light into the effect of sterility in the removal of pharmaceutical active compounds from that matrix (Table 1).

Cruz-Morató et al. [56] demonstrated for the first time the degradation of pharmaceuticals at pre-existent concentrations in a WRF bioreactor treating real urban wastewater under non-sterile conditions. In subsequent experiments, they demonstrated the possibility of removing a mixture of pharmaceuticals and endocrine disruptor compounds in non-sterile hospital wastewater (HWW), with presumably a higher micropollutant initial concentration [58]. They performed parallel experiments with heat-sterilized and non-sterile hospital wastewater, and both operations reduced the concentration and estrogenic activity of the influent. The non-sterile treatment, however, performed substantially worse with a 53.3% removal of the initial micropollutant load while the sterile operation showed a 83.2% removal. *T. versicolor*, the WRF used in the study, played a major role in the pollutant degradation.

A 10 L FBR was used to remove spiked micropollutants from non-sterile HWW. The setup removed 87% of X-ray contrast agent iopromide and 98.5% antibiotic ofloxacin in sterile HWW, while similar results were obtained for the non-sterile

Table 1 Batch treatments based on white-rot fungi

Source	Reactor	Biomass morphology	Matrix	Spiked Matrix	Sterility	Duration of the treatment (day)	Pharmaceuticals
Gros et al. [55]	Fluidized bed reactor	Pellets	Hospital wastewater	No	No	8	Iopromide; ofloxacin
Gros et al. [55]	Fluidized bed reactor	Pellets	Hospital wastewater	No	Yes	8	Iopromide; ofloxacin
Cruz-Morató et al. [56]	Fluidized bed reactor	Pellets	Urban wastewater	No	No	8	Naproxen; ibuprofen; acetaminophen; salicylic acid; ketoprofen; codeine; erythromycin; metronidazole; ciprofloxacin; azithromycin; cephalexin; propranolol; carbamazepine; 10,11-epoxyCBZ; 2-hydroxyCBZ; acridone; citalopram
Cruz-Morató et al. [56]	Fluidized bed reactor	Pellets	Urban wastewater	No	Yes	8	Naproxen; ibuprofen; acetaminophen; salicylic acid; ketoprofen; codeine; erythromycin; metronidazole; ciprofloxacin; azithromycin; cephalexin; propranolol; carbamazepine; 10,11-epoxyCBZ; 2-hydroxyCBZ; acridone; citalopram
Badia-Fabregat et al. [57]	Fluidized bed reactor	Pellets	Veterinary hospital wastewater	No	No	15	Furosemide; salicylic acid; ketoprofen; ibuprofen; piroxicam; diclofenac; naproxen; indomethacin; metronidazole; ciprofloxacin; diazepam; fluvastatin; gemfibrozil; atorvastatin; clopidogrel; albendazole; ranitidine
Cruz-Morató et al. [58]	Fluidized bed reactor	Pellets	Hospital wastewater	No	No	8	51 PhACs
Cruz-Morató et al. [58]	Fluidized bed reactor	Pellets	Hospital wastewater	No	Yes	8	49 PhACs
Ferrando-Climent et al. [59]	Fluidized bed reactor	Pellets	Hospital wastewater	No	No	8	Ciprofloxacin; tamoxifen; ifosfamide; cyclophosphamide; vincristine; docetaxel; paclitaxel; etoposide; methotrexate; azathioprine
Ferrando-Climent et al. [59]	Fluidized bed reactor	Pellets	Hospital wastewater	No	Yes	8	Ciprofloxacin; tamoxifen; ifosfamide; cyclophosphamide; vincristine; docetaxel; paclitaxel; etoposide; methotrexate; azathioprine

operation: 65.4% and 99%, respectively [55]. A similar pilot-scale reactor was used to test the ability of *T. versicolor* to degrade recalcitrant anticancer drugs from HWW, both raw and sterilized [59]. This paper highlighted the interesting case of ciprofloxacin, which was more efficiently eliminated under non-sterile conditions. This fact was attributed to a synergistic contribution between fungi and wastewater-native bacteria, although no further discussion was offered.

That "synergistic contribution" was evaluated in a different study using DGGE for microbial community assessment [57]. The experiment achieved a longer batch operation, 15 days, using *T. versicolor* in a 1.5 L lab-scale fluidized bed bioreactor treating veterinary hospital wastewater. Under non-sterile conditions, the treatment showed a 66% removal of the micropollutants present at pre-existent concentrations, while no removal was observed in the uninoculated control reactor. It was demonstrated that the presence of WRF was mandatory for degradation to occur, and they hypothesized that the synergistic effect was due to the native wastewater microorganisms, especially bacteria, degrading fungal transformation products and metabolites. However, no direct relationship between detected non-WRF microorganisms and degraded pollutants could be established. Finally, the study also highlighted the necessity of adjusting nutrient addition in non-sterile treatments taking into account the nutrient consumption of native microorganisms.

4.2 Continuous Treatment

It is known that a continuous wastewater treatment is preferred over batch treatment unless the volume treated is very low. As a result, more continuous white-rot fungal treatments can be found in the literature, although one could argue that they could not have existed without prior reactor batch studies, seeing that all of them are more recent (Table 2).

All operations reviewed in this section were conducted in non-sterile conditions and can be classified by the reactors they used: fluidized bed reactors (FBR), rotating biological contactors (RBC), and trickling bed reactors.

The *fluidized bed reactor* operations reviewed in this section all used pelleted biomass. As discussed in Sect. 3, the use of pellets enables the retention of fungal biomass while allowing the flow of liquid. However, attention must be paid to oxygen and mass transfer inside the pellets. The need for optimizing the nutrients addition in non-sterile continuous treatments, both carbon-to-nitrogen ratio and quantity to reduce the cost of operation and the COD of the effluent, was highlighted by Badia-Fabregat et al. [57]. It also identified some WRF-bacteria and WRF-other fungi interactions and described the inactivation of the fungus *T. versicolor*, which could be confirmed with DGGE. The operation lasted 26 days and removed a 44% of overall pharmaceutical load, but the fungus was inactivated during some periods of time. An experiment with a similar FBR, yet with spiked anti-inflammatories (at a much higher concentration than the concentration found in the environment), included the pre-treatment of influent through UV light, coagulation-flocculation

Table 2 Continuous treatments based on white-rot fungi

Source	Reactor	Biomass morphology	Matrix	Spiked matrix	Sterility	Duration of the treatment (day)	HRT	Pharmaceuticals
Kresinová et al. [60]	Trickling bed reactor	Grown in straw	Effluent from secondary treatment	No	No	28	46 h–8 h	Bisphenol A; estrone (E1); 17-α-ethynylestradiol (EE2); triclosan; 4-n-nonylphenol
Zhang and Geißen [61]	Plate bioreactor	Immobilization in polyether foam	Effluent from WWTP	Yes	No	100	36 h	Carbamazepine
Badia-Fabregat et al. [57]	Fluidized bed reactor	Pellets	Veterinary hospital wastewater	No	No	26	3.3 d	Furosemide; salicylic acid; ketoprofen; ibuprofen; piroxicam; diclofenac; naproxen; indomethacin; metronidazole; ciprofloxacin; diazepam; fluvastatin; gemfibrozil; atorvastatin; clopidogrel; albendazole; ranitidine
Mir-Tutusaus et al. [62]	Fluidized bed reactor	Pellets	Hospital wastewater (flocculated)	Yes	No	28	3 d	Ibuprofen; ketoprofen
Mir-Tutusaus et al. [63]	Fluidized bed reactor	Pellets	Hospital wastewater (flocculated)	No	No	56	3 d	Acetaminophen; diclofenac; ibuprofen; ketoprofen; thiabendazole; ciprofloxacin; ronidazole; sulfamethoxazole; trimethoprim; ofloxacin; warfarin; valsartan; atenolol; sotalol; furosemide; hydrochlorothiazide; ranitidine ; loratadine; atorvastatin; gemfibrozil; 10.11-epoxycarbamazepine; 2-hydroxycarbamazepine; acridone; carbamazepine; citalopram; diazepam; norfluoxetine; olanzapine; sertraline; trazodone; venlafaxine; dexamethasone

(continued)

Table 2 (continued)

Source	Reactor	Biomass morphology	Matrix	Spiked matrix	Sterility	Duration of the treatment (day)	HRT	Pharmaceuticals
Cruz del Álamo et al. [52]	Rotating biological contactor	Immobilized on rotating biological contactors	Effluent from primary treatment	No	No	22	24 h	Amoxicillin; metronidazole; sulfamethoxazole; carbamazepine; 4-acetamidoantipyrine; gemfibrozil; hydrochlorothiazide; iohexol
Torán et al. [48]	Fluidized bed reactor	Pellets with wood core	Hospital wastewater (flocculated)	Yes	No	28	3 d	Ibuprofen; ketoprofen; naproxen
Torán et al. [48]	Trickling packed-bed reactor	Immobilized on pallet wood	Hospital wastewater (flocculated)	Yes	No	49	3 d	Ibuprofen; ketoprofen; naproxen
Mir-Tutusaus et al. [29]	Fluidized bed reactor	Pellets	Hospital wastewater (flocculated)	Yes	No	21	3 d	Ibuprofen; ketoprofen; naproxen
Mir-Tutusaus et al. [64]	Fluidized bed reactor	Pellets	Hospital wastewater (flocculated)	No	No	91	3 d	74 pharmaceutically active compounds.
Cruz del Álamo et al. [22]	Rotating biological contactor	Immobilized on rotating biological contactors	Effluent from primary treatment of a pilot WWTP	Yes	No	40	1 d	Antipyrine; clofibric acid; atenolol; caffeine; carbamazepine; diclofenac; gemfibrozil; hydrochlorothiazide; ibuprofen; ranitidine; sulfamethoxazole; sulpiride

and both strategies, and the operation of the same reactor in sequential batch and continuous mode [62]. The study's objective was to decrease the influent microbial concentration in an attempt to increase the operation time. Specifically, the main objective was to increase the time the white-rot fungus was active inside the reactor. The best-performing strategy was the coagulation-flocculation pre-treatment step coupled to a continuous fungal treatment, which led to an operation that lasted 28 days with an average removal of 80% of the spiked concentration. A similar study with spiked anti-inflammatories was conducted by Torán et al. [48] using fungal pellets with a wooden core, so as to avoid the addition of an external carbon and nitrogen source. The operation ensured fungal viability up to day 21, when removal performance degraded.

A 2-L FBR inoculated with pelleted *T. versicolor* was operated for 56 days treating flocculated HWW at environmentally relevant concentration of pharmaceutically active compounds [63]. A DGGE and sequencing approach was used to evaluate the microbial community arisen in inoculated and uninoculated reactors, and it revealed a possible inhibition of *T. versicolor* activity due to *Candida*. Further studies with similar reactors but spiked pharmaceuticals identified an optimal carbon-to-nitrogen ratio of 7.5 in regard to nutrient addition (i.e., glucose and ammonium nitrate) and found that an aeration of 0.8 $L \cdot min^{-1}$ and a pellet diameter of around 2 mm helped in extending the treatment length and fungal activity [29]. A long-term continuous treatment of hospital wastewater was performed using the nutrient addition, aeration, and pellet diameter values found previously [64]. The study removed a 70% of influent pharmaceutical concentration for 91 days and demonstrated the presence during the whole treatment of *T. versicolor* by means of qPCR.

Del Álamo et al. [22] operated a 10 L *rotating biological contactor* with real wastewater (collected after the primary treatment of a pilot scale WWTP) for 40 days. The white-rot fungus *T. versicolor* was grown in the form of biofilm into the disks in the start-up period prior to the wastewater treatment. During the treatment phase, the operation achieved a 40–95% removal of 12 micropollutants spiked at 50 $\mu g \cdot L^{-1}$ each without the need of addition of nutrients (which can be regarded as a mandatory requirement in FBRs). Additionally, RBCs do not typically need an external aeration source, as the aeration is attained through rotation of the reactor itself. However, the study does report the addition of promoters of advanced bio-oxidation: metallic species in the form of Fe^{3+}-oxalate and Mn^{2+}-nitrate and quinone-like compounds such as gallic acid. A similar experiment was conducted with UWW at environmentally relevant micropollutant concentration, without spiking of pharmaceutical compounds [52]. The operation also used promoters of bio-oxidation and lasted 22 days when using the primary effluent of a full-scale WWTP. A 2 L *plate bioreactor* was used for the treatment of the effluent of urban WWTP with spiked carbamazepine [61]. The WRF *P. chrysosporium* was immobilized in a sheet of polyether foam submerged in a recipient with the WWTP effluent, placed in a water bath at 34–37°C. In this case no bio-oxidation promoters were added, but the addition of carbon and nitrogen sources was needed to maintain the removal of the spiked carbamazepine around 60%.

The *trickle bed reactors* reviewed in this section used WRF immobilized or grown on lignocellulosic substrates. The first experiment reviewed is a pilot-scale study that used a reactor of 30 L working volume and treated the secondary effluent of a WWTP [60]. The operation used the fungus *Pleurotus ostreatus* (an edible industrial mushroom that can be cultivated in farms), and it decreased by 76% of the concentration of the endocrine-disrupting compounds (EDCs) analyzed. The operation lasted 28 days, and it demonstrated the possibility of a fungal-based tertiary treatment for the degradation of EDCs. Another study evaluated the ability of *T. versicolor* immobilized in pallet wood of removing wastewater with spiked pharmaceutical compounds in a lab-scale trickle packed-bed reactor [48]. This study was not intended as a tertiary treatment but as an onsite treatment for hospital wastewater in order to reduce pharmaceutical load. The experiment successfully removed pharmaceutical compounds for 49 days in non-sterile flocculated wastewater without the addition of carbon or nitrogen source (as it is needed in FBRs).

5 Conclusions

Biotechnology processes based on fungal systems at industrial scale have been developed along the history, for example, antibiotic production. But, regarding wastewater treatment, although the high degrading capacity of the fungi, particularly to antibiotics and pharmaceutical compounds, treatment processes are still scarce even at lab scale. In general, it seems that bacterial concentration in raw wastewater hindered the robust operation of the batch and continuous treatments reviewed. Nevertheless, the reduction of this microbiological load, either using a pre-treatment (e.g., coagulation-flocculation) or by feeding the reactor a more processed influent (e.g., effluent from primary or secondary treatment), helped maintaining the fungus active for a longer period. The use of WRF immobilized onto lignocellulosic substrates avoided the need of nutrients addition, although systems with this type of biomass tend to need longer start-up periods for biomass growth. So, the technology is mature enough for different pilot-scale reactor setups to flourish soon, and such studies should be encouraged.

References

1. Verlicchi P, Al Aukidy M, Zambello E (2012) Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment – a review. Sci Total Environ 429:123–155. https://doi.org/10.1016/j.scitotenv.2012.04.028
2. Cragg SM, Beckham GT, Bruce NC, Bugg TDH, Distel DL, Dupree P, Etxabe AG, Goodell BS, Jellison J, McGeehan JE, McQueen-Mason SJ, Schnorr K, Walton PH, Watts JEM, Zimmer M (2015) Lignocellulose degradation mechanisms across the tree of life. Curr Opin Chem Biol. https://doi.org/10.1016/j.cbpa.2015.10.018

3. Nguyen LN, Hai FI, Yang S, Kang J, Leusch FDL, Roddick F, Price WE, Nghiem LD (2013) Removal of trace organic contaminants by an MBR comprising a mixed culture of bacteria and white-rot fungi. Bioresour Technol 148:234–241. https://doi.org/10.1016/j.biortech.2013.08.142
4. Tuomela M, Hatakka A (2019) 6.17 – oxidative fungal enzymes for bioremediation. In: Moo-Young M (ed) Comprehensive biotechnology3rd edn. Pergamon, Oxford, pp 224–239. https://doi.org/10.1016/B978-0-444-64046-8.00349-9
5. Schwarze FWMR (2007) Wood decay under the microscope. Fungal Biol Rev. https://doi.org/10.1016/j.fbr.2007.09.001
6. Pointing SB (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57:20–33. https://doi.org/10.1007/s002530100745
7. Martínez AT (2002) Molecular biology and structure-function of lignin-degrading heme peroxidases. Enzym Microb Technol. https://doi.org/10.1016/S0141-0229(01)00521-X
8. Hammel KE (1995) Mechanisms for polycyclic aromatic hydrocarbon degradation by ligninolytic fungi. Environ Health Perspect 105:41–43. https://doi.org/10.2307/3432477
9. Hofrichter M, Ullrich R, Pecyna MJ, Liers C, Lundell T (2010) New and classic families of secreted fungal heme peroxidases. Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-010-2633-0
10. Hofrichter M (2002) Review: lignin conversion by manganese peroxidase (MnP). Enzym Microb Technol 30:454–466. https://doi.org/10.1016/S0141-0229(01)00528-2
11. Gianfreda L, Xu F, Bollag J-M (1999) Laccases: a useful group of oxidoreductive enzymes. Biorem J 3:1–26. https://doi.org/10.1080/10889869991219163
12. Tuor U, Winterhalter K, Fiechter A (1995) Enzymes of white-rot fungi involved in lignin degradation and ecological determinants for wood decay. J Biotechnol. https://doi.org/10.1016/0168-1656(95)00042-O
13. Camarero S, Sarkar S, Ruiz-Dueñas FJ, Martínez MJ, Martínez ÁT (1999) Description of a versatile peroxidase involved in the natural degradation of lignin that has both manganese peroxidase and lignin peroxidase substrate interaction sites. J Biol Chem 274:10324–10330. https://doi.org/10.1074/jbc.274.15.10324
14. Carabajal M, Kellner H, Levin L, Jehmlich N, Hofrichter M, Ullrich R (2013) The secretome of Trametes versicolor grown on tomato juice medium and purification of the secreted oxidoreductases including a versatile peroxidase. J Biotechnol 168:15–23. https://doi.org/10.1016/j.jbiotec.2013.08.007
15. Leonowicz A, Matuszewska A, Luterek J, Ziegenhagen D, Wojtaś-Wasilewska M, Cho N-S, Hofrichter M, Rogalski J (1999) Biodegradation of lignin by white rot fungi. Fungal Genet Biol 27:175–185. https://doi.org/10.1006/fgbi.1999.1150
16. Cerniglia CE (1997) Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J Ind Microbiol Biotechnol. https://doi.org/10.1038/sj.jim.2900459
17. Golan-rozen N, Chefetz B, Ben-Ari J, Geva J, Hadar Y (2011) Transformation of the recalcitrant pharmaceutical compound carbamazepine by pleurotus ostreatus: role of cytochrome P450 monooxygenase Anda manganese peroxidase. Environ Sci Technol 45:6800–6805. https://doi.org/10.1021/es200298t
18. Marco-Urrea E, Pérez-Trujillo M, Cruz-Morató C, Caminal G, Vicent T (2010) White-rot fungus-mediated degradation of the analgesic ketoprofen and identification of intermediates by HPLC-DAD-MS and NMR. Chemosphere 78:474–481. https://doi.org/10.1016/j.chemosphere.2009.10.009
19. Marco-Urrea E, Pérez-Trujillo M, Vicent T, Caminal G (2009) Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by Trametes versicolor. Chemosphere 74:765–772. https://doi.org/10.1016/j.chemosphere.2008.10.040
20. Gómez-Toribio V, García-Martín AB, Martínez MJ, Martínez ÁT, Guillén F (2009) Enhancing the production of hydroxyl radicals by Pleurotus eryngii via quinone redox cycling for pollutant removal. Appl Environ Microbiol 75:3954–3962. https://doi.org/10.1128/AEM.02138-08

21. Marco-Urrea E, Radjenović J, Caminal G, Petrović M, Vicent T, Barceló D (2010) Oxidation of atenolol, propranolol, carbamazepine and clofibric acid by a biological Fenton-like system mediated by the white-rot fungus Trametes versicolor. Water Res 44:521–532. https://doi.org/10.1016/j.watres.2009.09.049
22. Cruz del Álamo A, Pariente MI, Vasiliadou I, Padrino B, Puyol D, Molina R, Martínez F (2018) Removal of pharmaceutical compounds from urban wastewater by an advanced bio-oxidation process based on fungi Trametes versicolor immobilized in a continuous RBC system. Environ Sci Pollut Res 25:34884–34892. https://doi.org/10.1007/s11356-017-1053-4
23. El-Enshasy HA (2007) Filamentous fungal cultures – process characteristics, products, and applications. In: Bioprocessing for value-added products from renewable resources. Elsevier, Amsterdam, pp 225–261. https://doi.org/10.1016/B978-044452114-9/50010-4
24. Rodarte-Morales AI, Feijoo G, Moreira MT, Lema JM (2012) Biotransformation of three pharmaceutical active compounds by the fungus Phanerochaete chrysosporium in a fed batch stirred reactor under air and oxygen supply. Biodegradation 23:145–156. https://doi.org/10.1007/s10532-011-9494-9
25. Xin B, Xia Y, Zhang Y, Aslam H, Liu C, Chen S (2012) A feasible method for growing fungal pellets in a column reactor inoculated with mycelium fragments and their application for dye bioaccumulation from aqueous solution. Bioresour Technol 105:100–105. https://doi.org/10.1016/j.biortech.2011.11.062
26. Borràs E, Blánquez P, Sarrà M, Caminal G, Vicent T (2008) Trametes versicolor pellets production: low-cost medium and scale-up. Biochem Eng J 42:61–66. https://doi.org/10.1016/j.bej.2008.05.014
27. Espinosa-Ortiz EJ, Rene ER, Pakshirajan K, van Hullebusch ED, Lens PNLL (2015) Fungal pelleted reactors in wastewater treatment: applications and perspectives. Chem Eng J 283:553–571. https://doi.org/10.1016/j.cej.2015.07.068
28. Nair RB, Lennartsson PR, Taherzadeh MJ (2016) Mycelial pellet formation by edible ascomycete filamentous fungi, Neurospora intermedia. AMB Express 6:31. https://doi.org/10.1186/s13568-016-0203-2
29. Mir-Tutusaus JAJA, Caminal G, Sarrà M (2018) Influence of process variables in a continuous treatment of non-sterile hospital wastewater by Trametes versicolor and novel method for inoculum production. J Environ Manag 212:415–423. https://doi.org/10.1016/j.jenvman.2018.02.018
30. Sitanggang AB, Wu H-S, Wang SS, Ho Y-C (2010) Effect of pellet size and stimulating factor on the glucosamine production using Aspergillus sp. BCRC 31742. Bioresour Technol 101:3595–3601. https://doi.org/10.1016/j.biortech.2009.12.084
31. Pazouki M, Panda T (2000) Understanding the morphology of fungi. Bioprocess Eng 22:127–143. https://doi.org/10.1007/s004490050022
32. Moreira MT, Feijoo G, Lema JM (2003) Fungal bioreactors: applications to white-rot fungi. Rev Environ Sci Biotechnol 2:247–259. https://doi.org/10.1023/B:RESB.0000040463.80808.dc
33. Kantarci N, Borak F, Ulgen KO (2005) Bubble column reactors. Process Biochem. https://doi.org/10.1016/j.procbio.2004.10.004
34. Moreira MT, Sanromán A, Feijoo G, Lema JM (1996) Control of pellet morphology of filamentous fungi in fluidized bed bioreactors by means of a pulsing flow. Application to Aspergillus Niger and Phanerochaete chrysosporium. Enzym Microb Technol 19:261–266. https://doi.org/10.1016/0141-0229(95)00244-8
35. Blánquez P, Sarrà M, Vicent T (2008) Development of a continuous process to adapt the textile wastewater treatment by fungi to industrial conditions. Process Biochem 43:1–7. https://doi.org/10.1016/j.procbio.2007.10.002
36. Grandclément C, Seyssiecq I, Piram A, Wong-Wah-Chung P, Vanot G, Tiliacos N, Roche N, Doumenq P (2017) From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: a review. Water Res 111:297–317. https://doi.org/10.1016/j.watres.2017.01.005

37. Song W, Xie B, Huang S, Zhao F, Shi X (2020) Aerobic membrane bioreactors for industrial wastewater treatment. In: Current developments in biotechnology and bioengineering. Elsevier, Amsterdam, pp 129–145. https://doi.org/10.1016/b978-0-12-819809-4.00006-1
38. Thomas O, Thomas MF (2017) Urban wastewater. In: UV-visible spectrophotometry of water and wastewater. Elsevier, Amsterdam, pp 281–315. https://doi.org/10.1016/B978-0-444-63897-7.00009-3
39. Goswami L, Vinoth Kumar R, Borah SN, Arul Manikandan N, Pakshirajan K, Pugazhenthi G (2018) Membrane bioreactor and integrated membrane bioreactor systems for micropollutant removal from wastewater: a review. J Water Process Eng. https://doi.org/10.1016/j.jwpe.2018.10.024
40. Yang S, Hai FI, Nghiem LD, Nguyen LN, Roddick F, Price WE (2013) Removal of bisphenol A and diclofenac by a novel fungal membrane bioreactor operated under non-sterile conditions. Int Biodeterior Biodegrad 85:483–490. https://doi.org/10.1016/j.ibiod.2013.03.012
41. Asif MB, Hai FI, Singh L, Price WE, Nghiem LD (2017) Degradation of pharmaceuticals and personal care products by white-rot fungi—a critical review. Curr Pollut Rep 3:88–103. https://doi.org/10.1007/s40726-017-0049-5
42. Nguyen LN, Hai FI, Yang S, Kang J, Leusch FDL, Roddick F, Price WE, Nghiem LD (2014) Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by Trametes versicolor: role of biosorption and biodegradation. Int Biodeterior Biodegrad 88:169–175. https://doi.org/10.1016/j.ibiod.2013.12.017
43. Asif MB, Hai FI, Jegatheesan V, Price WE, Nghiem LD, Yamamoto K (2018) Applications of membrane bioreactors in biotechnology processes. In: Current trends and future developments on (bio-) membranes: membrane processes in the pharmaceutical and biotechnological field. Elsevier, Amsterdam, pp 223–257. https://doi.org/10.1016/B978-0-12-813606-5.00008-7
44. Iorhemen O, Hamza R, Tay J (2016) Membrane bioreactor (MBR) technology for wastewater treatment and reclamation: membrane fouling. Membranes (Basel) 6:33. https://doi.org/10.3390/membranes6020033
45. Bardi A, Yuan Q, Siracusa G, Becarelli S, Di Gregorio S, Tigini V, Levin DB, Petroni G, Munz G (2019) Stability of fungal biomass continuously fed with tannic acid in a non-sterile moving-packed bed reactor. J Environ Manag 247:67–77. https://doi.org/10.1016/j.jenvman.2019.06.036
46. Gao D, Zeng Y, Wen X, Qian Y (2008) Competition strategies for the incubation of white rot fungi under non-sterile conditions. Process Biochem 43:937–944. https://doi.org/10.1016/j.procbio.2008.04.026
47. Ehlers GA, Rose PD (2005) Immobilized white-rot fungal biodegradation of phenol and chlorinated phenol in trickling packed-bed reactors by employing sequencing batch operation. Bioresour Technol 96:1264–1275. https://doi.org/10.1016/j.biortech.2004.10.015
48. Torán J, Blánquez P, Caminal G (2017) Comparison between several reactors with Trametes versicolor immobilized on lignocellulosic support for the continuous treatments of hospital wastewater. Bioresour Technol 243:966–974. https://doi.org/10.1016/j.biortech.2017.07.055
49. Li X, Xu J, de Toledo RA, Shim H, De Toledo RA, Shim H (2015) Enhanced removal of naproxen and carbamazepine from wastewater using a novel countercurrent seepage bioreactor immobilized with Phanerochaete chrysosporium under non-sterile conditions. Bioresour Technol 197:465–474. https://doi.org/10.1016/j.biortech.2015.08.118
50. Basak B, Jeon B-HH, Kurade MB, Saratale GD, Bhunia B, Chatterjee PK, Dey A (2019) Biodegradation of high concentration phenol using sugarcane bagasse immobilized *Candida tropicalis* PHB5 in a packed-bed column reactor. Ecotoxicol Environ Saf 180:317–325. https://doi.org/10.1016/j.ecoenv.2019.05.020
51. Kurade MB, Waghmode TR, Xiong JQ, Govindwar SP, Jeon BH (2019) Decolorization of textile industry effluent using immobilized consortium cells in upflow fixed bed reactor. J Clean Prod 213:884–891. https://doi.org/10.1016/j.jclepro.2018.12.218
52. Cruz del Álamo A, Pariente MI, Martínez F, Molina R, del Álamo AC, Pariente MI, Martínez F, Molina R (2020) Trametes versicolor immobilized on rotating biological contactors as

alternative biological treatment for the removal of emerging concern micropollutants. Water Res 170:115313. https://doi.org/10.1016/j.watres.2019.115313
53. Li X, Xu J, de Toledo RA, Shim H (2016) Enhanced carbamazepine removal by immobilized Phanerochaete chrysosporium in a novel rotating suspension cartridge reactor under non-sterile condition. Int Biodeterior Biodegradation 115:102–109. https://doi.org/10.1016/j.ibiod.2016.08.003
54. Beltrán-Flores E, Torán J, Caminal G, Blánquez P, Sarrà M (2020) The removal of diuron from agricultural wastewaters by Trametes versicolor immobilized on pinewood in simple channel reactors. Sci Total Environ 728:138414. https://doi.org/10.1016/j.scitotenv.2020.138414
55. Gros M, Cruz-Morato C, Marco-Urrea E, Longrée P, Singer H, Sarrà M, Hollender J, Vicent T, Rodriguez-Mozaz S, Barceló D (2014) Biodegradation of the X-ray contrast agent iopromide and the fluoroquinolone antibiotic ofloxacin by the white rot fungus Trametes versicolor in hospital wastewaters and identification of degradation products. Water Res 60:228–241. https://doi.org/10.1016/j.watres.2014.04.042
56. Cruz-Morató C, Ferrando-Climent L, Rodriguez-Mozaz S, Barceló D, Marco-Urrea E, Vicent T, Sarrà M (2013) Degradation of pharmaceuticals in non-sterile urban wastewater by Trametes versicolor in a fluidized bed bioreactor. Water Res 47:5200–5210. https://doi.org/10.1016/j.watres.2013.06.007
57. Badia-Fabregat M, Lucas D, Pereira MA, Alves M, Pennanen T, Fritze H, Rodríguez-Mozaz S, Barceló D, Vicent T, Caminal G (2015) Continuous fungal treatment of non-sterile veterinary hospital effluent: pharmaceuticals removal and microbial community assessment. Appl Microbiol Biotechnol 100:2401–2415. https://doi.org/10.1007/s00253-015-7105-0
58. Cruz-Morató C, Lucas D, Llorca M, Rodriguez-Mozaz S, Gorga M, Petrovic M, Barceló D, Vicent T, Sarrà M, Marco-Urrea E, Petrović M, Barceló D, Vicent T, Sarrà M, Marco-Urrea E, Petrovic M, Barceló D, Vicent T, Sarrà M, Marco-Urrea E (2014) Hospital wastewater treatment by fungal bioreactor: removal efficiency for pharmaceuticals and endocrine disruptor compounds. Sci Total Environ 493:365–376. https://doi.org/10.1016/j.scitotenv.2014.05.117
59. Ferrando-Climent L, Cruz-Morató C, Marco-Urrea E, Vicent T, Sarrà M, Rodriguez-Mozaz S, Barceló D (2015) Non conventional biological treatment based on Trametes versicolor for the elimination of recalcitrant anticancer drugs in hospital wastewater. Chemosphere 136:9–19. https://doi.org/10.1016/j.chemosphere.2015.03.051
60. Kresinová Z, Linhartová L, Filipová A, Ezechiáš M, Mašín P, Cajthaml T, Křesinová Z, Linhartová L, Filipová A, Ezechiáš M, Mašín P, Cajthaml T (2018) Biodegradation of endocrine disruptors in urban wastewater using Pleurotus ostreatus bioreactor. New Biotechnol 43:53–61. https://doi.org/10.1016/j.nbt.2017.05.004
61. Zhang Y, Geißen S-UU (2012) Elimination of carbamazepine in a non-sterile fungal bioreactor. Bioresour Technol 112:221–227. https://doi.org/10.1016/j.biortech.2012.02.073
62. Mir-Tutusaus JA, Sarrà M, Caminal G (2016) Continuous treatment of non-sterile hospital wastewater by Trametes versicolor: how to increase fungal viability by means of operational strategies and pretreatments. J Hazard Mater 318:561–570. https://doi.org/10.1016/j.jhazmat.2016.07.036
63. Mir-Tutusaus JA, Parladé E, Llorca M, Villagrasa M, Barceló D, Rodriguez-Mozaz S, Martinez-Alonso M, Gaju N, Caminal G, Sarrà M (2017) Pharmaceuticals removal and microbial community assessment in a continuous fungal treatment of non-sterile real hospital wastewater after a coagulation-flocculation pretreatment. Water Res 116:65–75. https://doi.org/10.1016/j.watres.2017.03.005
64. Mir-Tutusaus JA, Parladé E, Villagrasa M, Barceló D, Rodríguez-Mozaz S, Martínez-Alonso M, Gaju N, Sarrà M, Caminal G (2019) Long-term continuous treatment of non-sterile real hospital wastewater by Trametes versicolor. J Biol Eng 13:47. https://doi.org/10.1186/s13036-019-0179-y

Novel Constructed Wetland Configurations for the Removal of Pharmaceuticals in Wastewater

Mònica Escolà Casas and Víctor Matamoros

Contents

Abstract Nature-based solutions such as constructed wetlands (CWs) are a cost-effective option to perform wastewater treatment. Multiple studies have already shown that common CW configurations can remove pharmaceutically active compounds (PhACs) from wastewater mainly due to sorption, biodegradation, and photodegradation processes. Even so, recent research has proposed novel CW configurations to improve PhAC removal. This chapter will review existing studies on the three following novel CW approaches: (1) CWs' filling material modification, (2) biodegradation enhancement, and (3) integration of CWs with intensive wastewater treatment technologies. Waste-to-product filling materials such as biochar and cork can be used to enhance CWs' adsorption capacities. On the other side, biodegradation can be improved by incorporating microbial fuel cells, forced aeration, or

M. Escolà Casas (✉) and V. Matamoros
Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain
e-mail: mecgam@cid.csic.es

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 163–190, DOI 10.1007/698_2020_681,

bioaugmentation technologies into CWs. Finally, combinations of CWs with intensive wastewater treatments may offer a wider range of biodegradation pathways for PhACs and a reduction of the resulting transformation products (TPs). Future research on CWs should include the monitoring of TPs, a higher number of PhACs, ecotoxicological effects, and antimicrobial resistance. In addition, one of the main limitations of CW technology is still the high surface area required, which may be solved by future studies combining novel CW technologies.

Keywords Bioaugmentation, Constructed wetlands, Filling material, Forced aeration, Microbial fuel cells, Pharmaceuticals

1 Introduction

Constructed wetlands (CWs) are probably one of the clearest examples of a nature-based solution for wastewater treatment. Wetlands have been involved in the treatment of the wastewater since humans started to discharge sewage into the environment [1]. However, it was not until the early 1950 that artificial wetlands started to be built to treat wastewater [2]. During the last decades, CWs were developed into fully engineered systems, and nowadays they are defined as a sustainable, low-cost, robust, and efficient wastewater treatment technology which, unlike other technologies, provides ecosystem services [1]. Among these ecosystem services, CWs support animal biodiversity, which contributes to dampen the occurrence of zoonosis [3] and therefore can minimize the risk of future disease outbreaks.

CWs have been typically designed to remove biochemical oxygen demand (BOD), nutrients (nitrogen and phosphorus), total suspended solids, and bacteria [4–6]. Nevertheless, different studies have also reported their ability to remove the so-called contaminants of emerging concern (CEC) from urban and domestic wastewaters, which include pharmaceutically active compounds (PhACs) [7–11]. Furthermore, CWs are also capable of removing PhACs from livestock, swine [12], hospital [13], and aquaculture [14] wastewaters. The removal of PhACs in CWs involves different processes such as adsorption to the filling material, biodegradation by the soil's or rhizosphere's microbial community, and plant uptake [11] but also photodegradation, hydrolysis, and volatilization [15, 16]. According to this, the efficiency of CWs to degrade PhACs from wastewater depends on their physicochemical properties, the characteristics of the filling material, the planted species, the microbial populations, the wastewater characteristics, and the environmental conditions [17]. As PhACs belong to different chemical families with very diverse physicochemical properties, the removal mechanisms are complex and almost compound-specific.

The main aim of this chapter is to provide a review of novel CW configurations for the attenuation of PhACs from wastewater. Nevertheless, to understand the

attenuation processes of PhACs in CWs, a general overview on common CW configurations will also be included. Novel configurations will be classified on three strategies: (1) modifying the filling material, (2) enhancing the biodegradation mechanisms, and (3) combining CWs with other wastewater treatment technologies. This chapter will also explain the challenges that novel CW configurations need to overcome regarding the attenuation of PhACs and the derived future research trends. Finally, conclusions about the removal of PhACs in novel CWs will be stated.

2 Common CW Configurations Used for the Removal of PhACs

Typically, CWs can be designed as subsurface flow constructed wetlands (SSFCWs), where the water level is maintained below the surface of the medium placed in the beds [18], or as surface flow constructed wetlands (SFCWs), which are flooded systems that expose the water to the atmosphere (Fig. 1) [2]. Based on water flow direction, SSFCWs are subdivided into horizontal flow (HF) and vertical flow (VF) systems [19] (Fig. 1). In addition, there are hybrid systems, which consist of various types of those CW designs combined according to the wastewater treatment needs (Fig. 1). Despite their design, all CWs work in a similar way: solids are removed by filtration and settling, BOD declines due to consumption of organic compounds by the microbial community, ammonia is oxidized by microorganisms under aerobic conditions, nitrate is reduced under anaerobic conditions, and

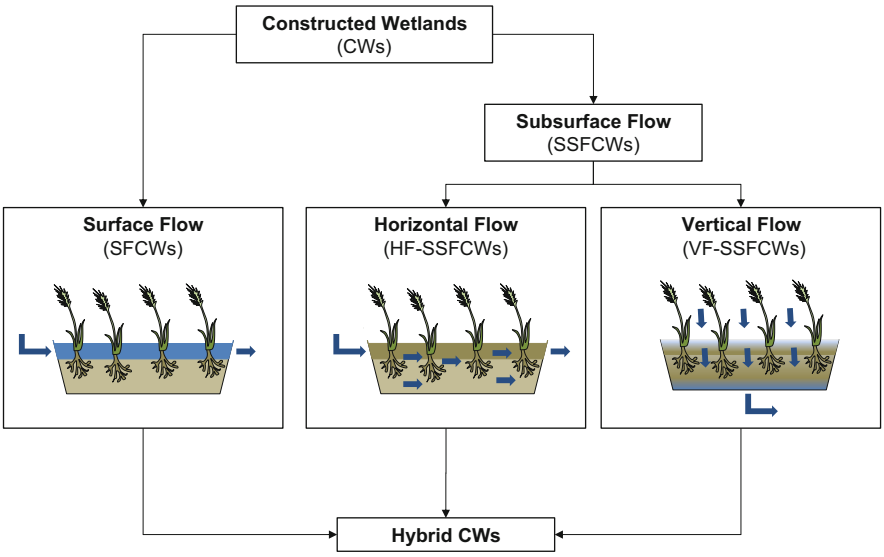


Fig. 1 Scheme of most common CW configurations used for the removal of PhACs

phosphorus may be absorbed by sediments [4]. All these processes are facilitated by plants, which maintain a favorable environment for microbial communities [20]. The removal of these pollutants has been the main focus of research in CW configurations [1], and several thorough reviews about this topic have been already written [4–6].

2.1 Removal of PhACs by SSFCWs

SSFCWs are mainly performing as a secondary treatment with relatively high organic matter loading rates [21]. However, HF-SSFCWs have successfully been used for both secondary and tertiary treatment [6].

HF-SSFCWs can degrade organic matter by sedimentation and filtration as well as by aerobic and anaerobic microbial processes [6]. In such systems, redox potential measurements have confirmed that the environment is anoxic-anaerobic, but aerobic microenvironments may exist around the plant roots, around algae blooms, or near the surface [11]. It has been seen that aerobic conditions lead to more efficient biodegradation pathways of PhACs than anoxic-anaerobic conditions [11]. Therefore, shallower (and thus more aerobic) HF-SSFCWs have shown to be better at degrading most PhACs [22]. However, not all PhACs are degraded under aerobic conditions [11], and the presence of oxic, anoxic, and anaerobic environments offers the possibility to degrade a wider range of PhACs. For example, the biodegradation of diclofenac has been linked to anaerobic conditions or even to the combination of oxic-anoxic conditions [23].

VF-SSFCWs, with unsaturated flow and therefore mainly with an aerobic environment, appeared to consistently perform better in terms of PhAC removal compared to HF-SSFCWs [24, 25].

2.2 Removal of PhACs by SFCWs

Generally, SFCWs are mostly used as a tertiary treatment of wastewater with low organic matter loading rates [21] and can achieve similar CEC removal efficiencies to those obtained by advanced wastewater treatment systems [26, 27].

SFCWs typically consist of a sequence of shallow basins and water control structures that maintain water depth [2]. SCFWs allow water to flow on top of the ground and to be exposed to the atmosphere and direct sunlight, allowing the photodegradation of some PhACs [28]. As the removal of some PhACs depends on sunlight irradiation (photodegradation) and temperature (biodegradation), different removals may be obtained depending on site and season [26]. According to this, an extended retention time of the water (i.e., 1 month) promotes photodegradation and biodegradation reactions so removal efficiencies of PhACs can reach as high values as those reported for HF-SSFCWs and conventional WWTPs [26]. In

particular, SFCWs have shown to have the same capacity to remove antibiotics as conventional activated sludge wastewater systems [29]. In addition, the short exposure (25 days) to low levels of antibiotics (ng/L to μg/L) in SFCWs did not promote the proliferation of antibiotic resistance genes (ARG), which were as abundant as in natural wetlands [29]. However, the increase of ARG after longer exposure periods should be assessed [29].

2.3 Removal of PhACs with Hybrid CW Systems

In the last years, the combined use of the abovementioned CW configurations has been tested for removing PhACs. Depending on the nature of the compounds to be degraded, some CW designs are better suited than others; so the combination of different CW systems can considerably reduce the total discharge of PhACs [30]. For example, three different real-scale hybrid treatment combinations (one 3-pond system and two different pond-SFCW-HF-SSFCW systems) obtained PhAC removals mainly over 70% [31]. These systems had highest removal efficiency in the first treatment step (pond, SFCW, or SSFCW) probably due to dependency on concentration [31]. Another example is a study testing a full-scale hybrid CW, consisting of a VF-SSFCW, a HF-SSFCW, and a SFCW [32]. This hybrid configuration achieved removals over 80% of all PhACs and other CEC, thanks to the diversity of physicochemical conditions occurring in the different CW configurations, which allowed synergies between biotic and abiotic removal mechanisms [32].

3 Novel CW Approaches for Pharmaceutical Removal

Multiple studies are exploring novel CW designs to boost the attenuation of PhACs from wastewater (Fig. 2). These studies follow three strategies: (1) modification of the filling material or support matrix, (2) enhancement of the biodegradation mechanisms, and (3) combination of CWs with other wastewater treatment technologies. The use of these strategies for removing PhACs from wastewater will be the main focus of this chapter.

3.1 Modifying the Filling Material of CWs

The choice of the filling material mainly influences the adsorption capacity of the CWs for removing PhACs, but it can also promote biofilm growth [33], increase residence time, and shape the microbial community, which will ultimately determine the biodegradation of pollutants [34]. The latest years, alternative CW approaches based on the replacement of the filling material (e.g., clays and biochar) as well as

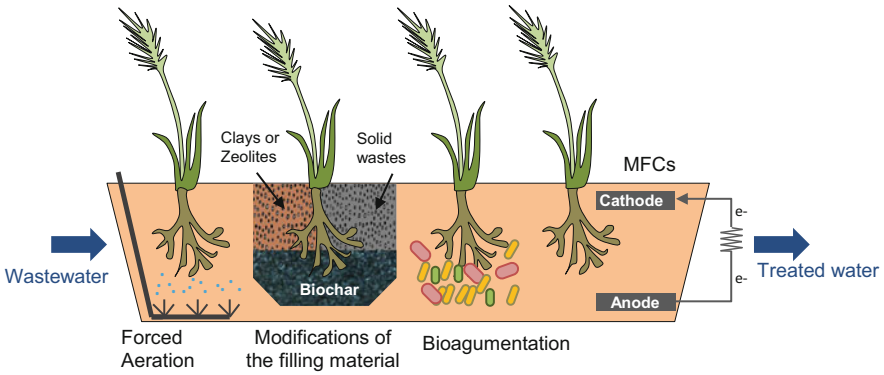


Fig. 2 Overview of novel CW configurations discussed in this chapter. Modifications of the filling material (clays or zeolites, solid wastes, and biochar) are discussed in Sect. 3.1. Strategies for enhancing biodegradation (microbial fuel cells (MFCs), forced aeration, and bioaugmentation) are discussed in Sect. 3.2. A HF-SSFCW has been represented here, but novel CW configurations may be applied to other types of CWs

giving new usages to materials (e.g., waste products) have been studied for the removal of carbon, nitrogen, and phosphorus content. However, these new approaches have also shown to improve the removal of xenobiotic compounds such as PhACs.

3.1.1 Clays and Zeolites

Clays can be used to enhance the removal of PhACs in CWs. Thermally processed clay materials like lightweight expanded clay aggregate (LECA) (300 US dollars per m^3, 0.28 kg/L density[1]) or exfoliated vermiculite (117 US dollars per m^3, 0.060–0.12 kg/L density[2]) have been successfully used for regular wastewater treatment as filters or as filling material in CWs [35]. These materials have also lately been tested for the removal of PhACs, and promising results have been obtained in several studies. Among such materials, the use of LECA has shown excellent results with the attenuation of several PhACs (Table 1) [35, 36, 38, 39, 42]. For example, the removals of ibuprofen and carbamazepine in planted LECA were both over 82% in two separate studies [38, 39] (Table 1). Also, exfoliated vermiculite may have some specific applications in CWs as it has shown higher and faster adsorption capacities per unit mass than LECA, but, in general, its low density makes it less efficient than LECA per unit of volume [35].

With the same composition as clays but with different crystallographic structure, zeolites are another appealing material that can be applied in CWs. Zeolites are

[1]Data for bulk acquisition of Leca® LWA in https://www.specialistaggregates.com/ in May 2020.
[2]Data for bulk acquisition of exfoliated vermiculite in https://www.perlipol.com.pl/ in May 2020.

Table 1 Assessment of different filling materials (LECA, exfoliated vermiculite, zeolites, cork, and biochar) which have been or could be applied in CWs for the attenuation of different PhACs

Compound and concentration tested	Technology	Dimensions	Type of water	Removal efficiency (%)	Hydraulic parameters	Reference
Atenolol 1 mg/L	SSFCW, LECA (unplanted and planted)	Microcosm batch	Real wastewater after secondary treatment	Unplanted: 82% Planted: 92–95%	HRT = 4 days	[36]
Caffeine 3.8–4.7 μg/L	Biochar (unplanted, active biofilm/inactive biofilm/no biofilm)	Laboratory scale	Real autoclaved wastewater	97–99% (range active biofilm/inactive biofilm/no biofilm)	HLR: 50 Lm^2/days	[37]
Carbamazepine 6.9–2.3 μg/L	Biochar (unplanted active biofilm/inactive biofilm/no biofilm)	Laboratory scale	Real autoclaved wastewater	Active biofilm: 99% Inactive biofilm: 73% No biofilm: 99%	HLR: 50 Lm^2/days	[37]
Carbamazepine 58 μg/L	SSFCW, LECA (planted and unplanted)	Laboratory scale	Real wastewater	Planted: 89% Unplanted: 95%	HRT = 3 days	[38]
Carbamazepine 1,000 μg/L	SSFCW, LECA (planted and unplanted, winter and summer)	Microcosm batch	Real wastewater after secondary treatment	Planted: 88–96% Unplanted: 87–86% (winter and summer, respectively)	HRT = 7 days	[39]
Carbamazepine 35 mg/L	Granulated cork sorption	Laboratory-scale batch	Real wastewater after secondary treatment	63%	Contact time = 6 days	[40]
Clofibric acid 1 mg/L	SSFCW, LECA (planted and unplanted, winter and summer)	Microcosm batch	Real wastewater after secondary treatment	Planted: 48–75% Unplanted: 40–43% (winter and summer, respectively)	HRT = 7 days	[39]
Clofibric acid 35 mg/L	Granulated cork sorption	Laboratory-scale batch	Real wastewater after secondary treatment	8%	Contact time = 6 days	[40]

(continued)

Table 1 (continued)

Compound and concentration tested	Technology	Dimensions	Type of water	Removal efficiency (%)	Hydraulic parameters	Reference
Erythromycin 11.6 µg/L	HF-SSFCW (planted zeolite)	Mesocosm scale	Raw wastewater	83%	HLR: 96 Lm^2/days	[41]
Furosemide 1–11 mg/L	Cork and LECA, adsorption (unplanted)	Laboratory scale	Ultra-pure water	LECA: >89%, concentration dependent Cork: 73–77%	HRT = 7 days	[42]
Gemfibrozil 1–30 mg/L	LECA and exfoliated vermiculite adsorption (unplanted)	Laboratory scale	Ultra-pure water	LECA: 95–100% Exfoliated vermiculite: 35–40%	LECA: HRT = 4 days Exfoliated vermiculite: HRT = 2 days	[35]
Ibuprofen 33 µg/L	SSFCW, LECA (planted and unplanted)	Laboratory scale	Real wastewater	Planted: 89% Unplanted: 94%	HRT = 3 days	[38]
Ibuprofen 1 mg/L	SSFCW, LECA (planted and unplanted, winter and summer)	Microcosm batch	Real wastewater after secondary treatment	Planted: 82–96% Unplanted: 73–91% (winter and summer, respectively)	HRT = 7 days	[39]
Ibuprofen 35 mg/L	Granulated cork sorption (unplanted)	Laboratory-scale batch	Real wastewater after secondary treatment	95–100%	Contact time = 6 days	[40]
Lincomycin 46 ng/L	HF-SSFCW (planted zeolite)	Mesocosm scale	Raw wastewater	55%	HLR: 96 Lm^2/days	[41]
Mefenamic acid 1–30 mg/L	LECA and exfoliated vermiculite adsorption (unplanted)	Laboratory scale	Ultra-pure water	LECA: 95–100% Exfoliated vermiculite: 35–40%	LECA: HRT = 4 days Exfoliated vermiculite: HRT = 2 days	[35]

Metoprolol 1.9–3.1 μg/L	Biochar (unplanted active biofilm/inactive biofilm/no biofilm)	Laboratory scale	Real autoclaved wastewater	95–99% (range active biofilm/inactive biofilm/no biofilm)	HLR: 50 Lm^2/days	[37]
Monensin 144 ng/L	HF-SSFCW (planted zeolite)	Mesocosm scale	Raw wastewater	95%	HLR: 96 Lm^2/days	[41]
Naproxen 1–30 mg/L	LECA and exfoliated vermiculite adsorption (unplanted)	Laboratory scale	Ultra-pure water	LECA: 95–100% Exfoliated vermiculite: 80%	LECA: HRT = 4 days Exfoliated vermiculite: HRT = 2 days	[35]
Novobiocin 6 ng/L	HF-SSFCW (planted zeolite)	Mesocosm scale	Raw wastewater	100%	HLR: 96 Lm^2/d	[41]
Ofloxacin 7 ng/L	HF-SSFCW (planted zeolite)	Mesocosm scale	Raw wastewater	100%	HLR: 96 Lm^2/days	[41]
Oxytetracycline 233 ng/L	IVCW (zeolites vs. quartz)	Laboratory scale	Swine wastewater aerobically digested	Planted zeolite: 90% Planted quartz: 70–80%	HRT = 7 days	[43]
Ranitidine 3.2–7.2 μg/L	Biochar (unplanted active biofilm/inactive biofilm/no biofilm)	Laboratory scale	Real autoclaved wastewater	97–99% (range active biofilm/inactive biofilm/no biofilm)	HLR: 50 Lm^2/days	[37]
Sulfadiazine 33 μg/L	SSFCW, LECA (planted and unplanted)	Laboratory scale	Real wastewater	Planted: 67% Unplanted: 94%	HRT = 3 days	[38]
Sulfadiazine 22 ng/L	IVCW (zeolites vs. quartz)	Laboratory scale	Swine wastewater aerobically digested	Planted zeolite: 60–70% Planted quartz: 30–40%	HRT = 7 days	[43]
Sulfamethazine 10 ng/L	IVCW (zeolites vs. quartz)	Laboratory scale	Swine wastewater aerobically digested	Planted zeolite: 60–70% Planted quartz: 50–60%	HRT = 7 days	[43]
Sulfamethazine 8 ng/L	HF-SSFCW (planted zeolite)	Mesocosm scale	Raw wastewater	52%	HLR: 96 Lm^2/days	[41]
Sulfamethoxazole 0.6 ng/L	IVCW (zeolites vs. quartz)	Laboratory scale	Swine wastewater aerobically digested	Planted zeolite: 45–50% Planted quartz: 45–50%	HRT = 7 days	[43]

(continued)

Table 1 (continued)

Compound and concentration tested	Technology	Dimensions	Type of water	Removal efficiency (%)	Hydraulic parameters	Reference
Sulfamerazine 96 ng/L	HF-SSFCW (planted zeolite)	Mesocosm scale	Raw wastewater	100%	HLR: 96 Lm^2/days	[41]
Tetracycline 539 ng/L	IVCW (zeolites vs. quartz)	Laboratory scale	Swine wastewater aerobically digested	Planted zeolite: 80–90% Planted quartz: 70–80%	HRT = 7 days	[43]

HRT hydraulic retention time, *HLR* hydraulic loading rate

natural or artificially produced aluminosilicate minerals which are normally used for their adsorption, ion exchange, molecular sieve, and catalytic properties [44]. In CWs, zeolites alone or applied together with other materials have shown to have a successful removal of nitrogen from domestic, urban, and livestock wastewaters, with a less satisfactory removal of chemical oxygen demand (COD) and phosphorus [45]. Interestingly, the usage of zeolites in CWs has shown to improve the removal efficiency of PhACs such as antibiotics as well as ARGs [41, 43] (Table 1). Although natural zeolites are relatively affordable (1,000 US dollar per ton [46]) and abundant, they are still a limited natural resource and producing them artificially implies energetic costs. This is a disadvantage compared to other available materials that can be cheaply produced like LECA or obtained from waste products (e.g., cork and biochar), but their efficiency in removing antibiotics and ARGs may pay off the costs.

3.1.2 Solid Wastes

The use of solid wastes from agriculture, industry, and construction to fill CWs has been explored as a way to reduce landfill disposal of those materials in the context of circular economy [47]. Among the different tested materials, cork granulates showed the ability to adsorb organic compounds [47]. Despite their selective adsorption potential, cork-filled treatment wetlands appeared to be specifically an appropriate technology to treat nitrate-polluted groundwater [48] and to adsorb some PhACs [40, 42] (Table 1). It was also noted that cork allowed shorter equilibration times and larger removal of PhACs per weight of sorbent than LECA materials [40]. In another study, LECA reached greater removals of furosemide than cork (Table 1), but cork granulates achieved higher adsorption in the first 24 h [42]. Therefore, cork granulates were suggested as preliminary filter to retain most of the PhAC concentration with a low hydraulic retention time (HRT), followed by a LECA filter [42]. In addition, cork sorbents have become a potential low-cost choice (240–260 US dollars per m^3; 0.06–0.22 kg/L density[3]) as they can be obtained from cork industrial wastes [49]. Nevertheless, no pilot or full-scale CW system based on cork material has been built to demonstrate the real effectiveness of this material. Other solid wastes like oyster shells were able to remove 17–60% of antibiotics, but COD and nitrogen removals were not as satisfactory [41]. Similar results have also been reported with other comparable materials (snail shells) [47].

3.1.3 Biochar

Biochar used as filling material in CWs seems to be a promising option to enhance PhAC removal. Biochar is an interesting charcoal-like product obtained when

[3]Data for bulk acquisition of natural cork granulates in https://www.corklink.com/ in May 2020.

biomass (which can be a waste product) is heated at elevated temperatures with little or no available oxygen (pyrolysis). Biochar price varies between 90 and 3,000 US dollars per ton, mostly depending on the country of production [50]. When applied to the soil, biochar enhances soil's fertility and quality by increasing the moisture retention capacity, attracting beneficial fungi and microbes, and retaining nutrients in the soil [51]. Due to this, biochar is used as filling material in CWs, mainly to remove nitrogen [52] but also COD, phosphorus [53], and even phenols and metals [54]. In spite of this, there are currently no available studies on the effect of biochar applied in CWs towards PhAC removal from wastewater. Nonetheless, trials in unplanted biofilters based on biochar suggest that its usage may enhance degradation of PhACs in CWs. Dalahmeh et al. [37] observed that filters based on biochar resulted in the simultaneous biodegradation and adsorption of recalcitrant pharmaceuticals, like carbamazepine (73–99%), and less recalcitrant compounds like metoprolol, caffeine, and ranitidine (Table 1). In the case of the recalcitrant carbamazepine, the obtained removals were achieved mainly by adsorption processes, but some biodegradation still occurred (Table 1). Same authors suggested that as biofilm contributed to biodegradation, biochar with biofilm could extend adsorbent lifetime.

All in all, the usage of different filling materials in CWs seems to improve the removal of PhACs and seems especially useful to remove recalcitrant compounds, which can only be removed by sorption. However, all reported studies (Table 1) were conducted at laboratory, microcosm, or mesocosm scale. Therefore, further studies should move towards testing such materials in pilot- and real-scale CWs, with longer operation times. Future studies need to evaluate the applicability of these materials under real wastewater conditions because clogging, material degradation, and exhaustion of the sorption capacity (the main removal mechanism for some PhACs) may eventually happen.

3.2 Enhancing the Biodegradation Capacity of CWs

Besides modifying the filling material, another approach to enhance the elimination of PhACs from wastewater is to enhance the biodegradation capacity of CWs. This is done by stimulating the microbiological activity through different mechanisms.

3.2.1 Microbial Fuel Cell

A new research line based on the integration of microbial fuel cells (MCFs) (Fig. 3) into CWs (MFC-CWs) has recently been developed (Fig. 2). In a MFC, exoelectrogenic bacteria oxidize organic and inorganic substrates, and electrons are shifted from the anode, via a conductive material and a resistor, to a higher redox electron acceptor, like oxygen, at the cathode [56]. These bioelectrochemical systems and the related microbial electrolysis cell use the electric current to improve pollutant degradation [1].

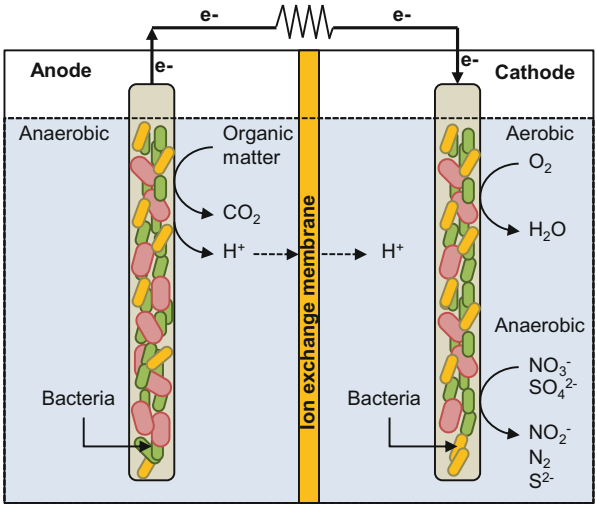


Fig. 3 Scheme of a typical two-chambered MFC separated by an ion exchange membrane. Electrons and protons are generated from organic matter oxidation via anaerobic respiration or fermentation by microorganisms in the anode chamber. Electrons are transferred to the cathode through an external circuit and produce electricity. Protons are transferred to the cathode through the ion exchange membrane. Electrons and protons react with oxygen (aerobic cathode), nitrate, or sulfate (anaerobic cathode). Adapted from [55]

Limited studies have been conducted applying MFC-CWs towards CEC degradation. In a recent study, MFCs have been integrated into an upper-flow CW (MFC-UCW) [57] (Table 2). In that study, at least 60% of 10 mg/L of ibuprofen and bisphenol-A were removed from wastewater with a HRT of 0.7 days [57]. In an even more recent study, MFC-CWs with different configurations were tested for the removal of sulfadiazine, carbamazepine, naproxen, and ibuprofen [60] (Table 2). These studies demonstrated that the operation of the MFC-CW system increased the accumulation of PhACs (sulfadiazine, carbamazepine, naproxen, and ibuprofen) on the electrode layers by electrosorption, which ended up selecting bacterial communities with enhanced xenobiotics metabolism. This resulted in an enhanced removal of PhACs but also with an enriched number of sulfonamide ARGs [60]. Such findings suggested that MFC-CW can be a promising technology for the removal of PhACs, but potential risks due to the accumulation of ARGs should be assessed.

3.2.2 Aerated CWs

Oxygen availability in CWs results in improved biodegradation, reduced clogging, enhanced removal of organic nutrients, and reduced land area requirement [8]. Therefore, another way to enhance biodegradation of PhACs in CWs is the incorporation of forced aeration in their design (Fig. 2). Forced aeration has been implemented in both, HF- and VF-SSFCW to treat wastewater with very high organic loads, which

Table 2 Overview of articles that tested the removal of PhACs from wastewater in CWs with enhanced biodegradation mechanisms (MCFs, forced aeration, and bioaugmentation)

Compound and concentration tested	Technology	Dimensions	Type of water	Removal efficiency (%)	Hydraulic parameters	Reference
Caffeine 64 μg/L	HF-SSFCW with forced aeration VF-SSFCW with forced aeration Two-cell recirculating system	Pilot-scale	Real wastewater after primary treatment (septic tank)	HF-SSFCW with forced aeration: 100% VF-SSFCW with forced aeration: 99% Two-cell reciprocating system: 99% (93–100% with non-aerated VF and HF-SSFCW)	HRT > 3 days	[58]
Caffeine 25 μg/L	SFCW SFCW-stabilization tank	Laboratory-scale	Synthetic wastewater	SFCW: 98% SFCW-stabilization tank: 98.2% Both systems: 13 days aerated +17days non-aerated, removal rate did not vary	HRT = 7 days	[59]
Carbamazepine 4 mg/L	Up-flow CW-MFC with activated carbon	Laboratory-scale	Synthetic wastewater	>99% (both HRTs) Accumulation on the anode	HRT=3 and 1.5 days	[60]
Carbamazepine 3 μg/L	HF-SSFCW with forced aeration VF-SSFCW with forced aeration Two-cell reciprocating system	Pilot-scale	Real wastewater after primary treatment (septic tank)	HF-SSFCW with forced aeration: −5% VF-SSFCW with forced aeration: −4% Two-cell reciprocating system: −5% (−8 to −4% with non-aerated VF-SSFCW and 2% with HF-SSFCW)	HRT > 3 days	[58]
Diclofenac μg/L 5.58 ± 2.95	VF-SSFCW With aeration, planted	Pilot-scale	Real wastewater after primary treatment	58% (same achieved in non-aerated, unsaturated system)	HLR = 0.095 m/days (intermittent loading)	[61]

Diclofenac 6 μg/L	HF-SSFCW with forced aeration VF-SSFCW with forced aeration Two-cell reciprocating system	Pilot-scale	Real wastewater after primary treatment (septic tank)	HF-SSFCW with forced aeration: 34% VF-SSFCW with forced aeration: 74% Two-cell reciprocating system: 75% (53–78% with non-aerated VF-SSFCW and 17% with HF-SSFCW)	HRT > 3 days	[58]
Ibuprofen 50 mg/L	HF-SSFCWs, bioaugmentation in planted system	Laboratory-scale	Synthetic wastewater	75–80% (5% higher than without bioaugmentation) Biodegradation 80 days earlier in bioaugmented CWs	HLR=40 Lm^2/days	[62]
Ibuprofen 10 mg/L	Up-flow CW-MFC with activated carbon	Laboratory-scale	Synthetic wastewater	96% (9% higher than control)	HRT = 0.7 days	[57]
Ibuprofen 4 mg/L	Up-flow CW-MFC with activated carbon	Laboratory-scale	Synthetic wastewater	>99% (both HRTs) Accumulation on anode	HRTs=3 and 1.5 days	[60]
Ibuprofen 100 μg/L	Forced aeration, planted and unplanted CWs	Mesocosm	Synthetic wastewater	65% for unplanted (higher than non-aerated unsaturated systems (45%)) 68–97% for planted (same as non-aerated saturated and unsaturated systems)	HLR=0.34 m/days	[63]
Ibuprofen 44.5 ± 13.0 μg/L	VF-SSFCW With aeration, planted	Pilot-scale	Real wastewater after primary treatment	99% (95% achieved in non-aerated, saturated systems)	HLR of 0.095 m/days (intermittent loading)	[61]
Ibuprofen 24 μg/L	HF-SSFCW with forced aeration VF-SSFCW with forced aeration	Pilot-scale	Real wastewater after primary treatment (septic tank)	HF-SSFCW with forced aeration: 95% VF-SSFCW with forced aeration: 98%	HRT > 3 days	[58]

(continued)

Table 2 (continued)

Compound and concentration tested	Technology	Dimensions	Type of water	Removal efficiency (%)	Hydraulic parameters	Reference
	Two-cell recipro-cating system			Two-cell reciprocating system: 99% (95–98% with non-aerated VF-SSFCWs, 9% with non-aerated HF-SSFCW)		
Metformin 910 ng/L	VF-SSFCW with forced aeration, planted	Pilot-scale	Real wastewater after pri-mary treatment (direct from sewer, after settling)	99% (30–40% non-aerated)	HRT = 1 day	[64]
Naproxen 4 mg/L	Up-flow CW-MFC with activated carbon	Laboratory-scale	Synthetic wastewater	>99% (both HRTs) Accumulation on the anode	HRTs = 3 and 1.5 days	[60]
Naproxen 24 µg/L	HF-SSFCW with forced aeration VF-SSFCW with forced aeration Two-cell recipro-cating system	Pilot-scale	Real wastewater after pri-mary treatment (septic tank)	HF-SSFCW with forced aeration: 84% VF-SSFCW with forced aeration: 94% Two-cell reciprocating system: 99% (89–97% with non-aerated VF-SSFCWs and 23% with HF-SSFCW)	HRT > 3 days	[58]
Sulfadiazine 4 mg/L	Up-flow CW-MFC with activated carbon	Laboratory-scale	Synthetic wastewater	>99% (both HRTs) Accumulation on anode	HRTs=3 and 1.5 days	[60]
Valsartan 2,270 ng/L	VF-SSFCW Forced aeration, planted	Pilot-scale	Real wastewater after pri-mary treatment (direct from sewer, after settling)	99% (30–40% non-aerated)	HRT = 1 day	[64]

HRT hydraulic retention time, *HLR* hydraulic loading rate

are oxygen limited and therefore achieve poor removal of nitrogen [52, 65]. HF-SSFCWs particularly are often oxygen limited due to permanent water clogging of the reed bed [66]. For regular pollutants, aeration can increase the nitrification rate of such CWs, but the economic costs of this might not pay off [66].

Regarding PhACs, it has been observed that aerated saturated CWs have a higher microbial diversity than unsaturated systems [63], which might result in the biodegradation of a wider range of compounds. Forced aeration has only been tested for a few PhACs (Table 2). For ibuprofen, forced aeration achieved 65% removal in unplanted HF-SSFCWs, a 20% higher than that found for equivalent saturated or unsaturated systems; however, aeration had not much influence when comparing equivalent planted systems [63]. Similar results were achieved in planted VF-SSFCW with forced aeration, where ibuprofen and diclofenac removals (99% and 58%, respectively) were not different from VF-SSFCWs operating with non-aerated unsaturated flows [61]. Also, aeration in VF-SSFCWs has been tested during municipal and hospital wastewater treatment [64]. In this study, aeration did not enhance the removal of sotalol, carbamazepine, and diclofenac (less than 50% removal in aerated and non-aerated systems); but it resulted in a complete removal (99%) of valsartan and metformin, which were poorly removed in non-aerated systems (17% and 68%, respectively). Finally, another study tested aerated and non-aerated HF- and VF-SSFCWs and, while there were only small differences in VF-SSFCWs (0–16% greater removals in aerated systems), HF-SSFCW aeration improved the removal efficiencies of ibuprofen, naproxen, and diclofenac from 19 to 95%, from 23 to 84%, and from 17 to 24%, respectively [58]. According to those studies, forced aeration seems to be useful to increase the biodegradation of PhACs in systems with low dissolved oxygen levels such as HF-SSFCWs or unplanted systems. Nonetheless, this is mostly valid for compounds that undergo aerobic degradation or degradation in aerobic-anaerobic environments, and, depending on the circumstances, the installation and maintenance costs might not pay off the increased removals.

3.2.3 Bioaugmentation

Bioaugmentation consists in adding biological material to improve pollutant biodegradation and ecosystem function [67]. In wastewater treatment, this is usually translated into adding the required cultured microorganisms into the media to accelerate the degradation of contaminants (Fig. 2). To do so, microorganisms or uncharacterized microbial consortiums isolated from the same media to be treated are usually preferred, as they may persist longer and perform better than externally obtained microorganisms [67].

Bioaugmentation has shown to be able to treat wastewater from different origins (pharmaceutical factories, pulp mills, and other factories) containing different organic and inorganic chemicals, but in CWs it has mainly been studied to enhance nitrogen and phosphorus removal [68–70]. Regarding organic contaminants, one

study demonstrated that bioaugmentation enhanced atrazine removal in CWs from 74.6 to 87.1% [71]. For PhACs, there is only one available study that tested the addition of ibuprofen-degrading bacteria into CWs [62] (Table 2). In this study, wastewater containing 11–92 μg/L of ibuprofen was incubated with 500 mg/L of ibuprofen to isolate a bacterium living solely on ibuprofen. After that, a biofilm of the selected bacteria (*Sphingobium yanoikuyae*) was artificially grown on gravel, which was later on applied to a HF-SSFCW. The inoculated gravel improved the removal efficiency of ibuprofen in the CW only by 5%. However, the key finding of the study was that the addition of these bacteria reduced with more than 80 days the stabilization time of the CW compared with the non-inoculated CWs [62]. In addition, this study found that plants were important to maintain the ibuprofen-degrading bacteria community because roots provided aerobic environments.

The limitation of this novel approach comes from the fact that inoculated microorganisms might be washed out, predated, or killed by environmental conditions; periodic inoculation must be considered to maintain the biomass, which is translated into costs and complex operation [68]. To avoid continuous inoculation, microorganism immobilization has been pointed as a solution [72]. In this case, CWs are especially well suited for it as bacteria could be immobilized in the filling material.

As it happens with the filling material approaches, most processes to enhance biodegradation have been mainly tested at laboratory scale, and further testing in pilot and real scale should be the next research step. This will be important to evaluate the applicability of these technologies in terms of costs and efficiency. Forced aeration is the only approach that has been tested on pilot-scale systems, and it has shown to enhance PhAC removal. According to the results, forced aeration seems to improve the attenuation of PhACs by 20–60% in comparison to conventional HF-SSFCWs, but limited improvements have been observed in comparison to conventional VF-SSFCWs.

3.3 CWs Combined with Other Wastewater Treatment Technologies

Most of the times, solutions to remove PhACs from wastewater are thought in terms of using one specific type of technology, and therefore limitations have to be accepted. However, the combination of complementary treatment processes could overcome some of those limitations. In this sense, intensive technologies such as membrane bioreactors (MBRs), moving bed biofilm reactors (MBBRs), or advanced oxidation processes (AOPs) combined with CWs should be taken into consideration. The specific combination of these intensive technologies with CWs has barely been explored in terms of PhACs elimination.

MBRs and MBBRs are intensive biological wastewater treatment techniques. The combination of these techniques with CWs is not common, and, to the knowledge of the authors, these combinations have not been tested to remove PhACs. So far, only

common wastewater treatment studies have been conducted by using these combinations. For example, a submerged MBR (SMBR) acting as a secondary treatment step was coupled with an integrated VF-SSFCW which acted as a tertiary treatment step and reached 97.5, 87.5, and 83.5% removals for COD, total nitrogen, and total phosphorus, respectively, with HRTs of 7.5 h in the SMBR and 11.5 h in the VF-SSFCW [73]. A CW combined with a MBBR has also been tested for the removal of COD, total nitrogen, and total phosphorus and achieved removals of 85.9–97.5%, 46.1–84.5%, and 27.9–69.5%, respectively (HRT: 8–15 h) [74]. Even though these combination systems were not tested for PhACs yet, they could be an interesting solution to eliminate PhACs due to their capacity to develop different microbial communities in each of the technologies and therefore diversifying the possible degradation pathways of these compounds. In fact, MBBRs are able to eliminate certain organic micropollutants in several stages of the wastewater treatment [75–77].

The AOPs are techniques aimed at the production of hydroxyl radicals, one of the most powerful oxidant species, capable of oxidizing a range of organic contaminants [78]. Due to the oxidation process, these techniques generate transformation products (TPs) that can be more toxic than the parent compounds [79]. For this reason, CWs could be added after AOPs to eliminate such products that, moreover, could be more biodegradable than the parent compounds.

Among AOPs, photocatalysis is based on aqueous-phase hydroxyl radical chemistry that combines low-energy UV-A light with semiconductors like TiO_2 that act as photocatalysts [80]. As photocatalysis can be conducted with sunlight, it matches the low-cost approach of CWs. In fact, Melián et al. [80] observed that the combination of photocatalysis with CWs was capable of achieving a complete removal of 100 mg/L of phenol and the generated TPs over 1 day. This was also tested by conducting the photocatalysis of several pesticides spiked into ultra-pure water, mixing the photocatalyzed products with wastewater, and applying it to CWs [81]. Like this, complete removals of most compounds and a reduction of at least 78% of toxicity were achieved, achievements that photodegradation or CWs could not accomplish independently [81]. In the same way, mild photocatalysis combined with sludge-based biological treatment achieved complete removal of recalcitrant PhACs such as diclofenac and gemfibrozil [82]. Additionally, it has been shown that natural organic matter released from CW plants allowed a higher photodegradation kinetic rates of recalcitrant compounds like carbamazepine and paraxanthine (ca. 0.001 min^{-1}, both) than organic matter from a WWTP effluent (ca. 0.00016 min^{-1}, both) [83]. Therefore, photocatalysis combined with CWs could be an interesting sustainable and low-cost solution to enhance the removal of PhACs from wastewater.

Besides photocatalysis, ozonation has also been tested in combination with CWs. In ozonation, oxidation occurs via the ozone itself or by the generated hydroxyl radicals. The combination of HF-SSFCW followed by a polishing ozonation step enhanced the removals of PhACs from real WWTP influent during a laboratory-scale experiment, and ibuprofen and naproxen removals increased from 92 to 94% and 83% to 96%, respectively [84]. In this case, the HF-SSFCW conducted a

secondary wastewater treatment, while ozonation was established as a tertiary wastewater treatment. Nonetheless, this CW-ozone combination could be also used as a whole tertiary treatment unit. In this case, ozonation could be used as a first polishing step, and afterwards CWs could biodegrade the remaining PhACs and TPs. In any case, ozonation is an expensive technology, with a potential explosion hazard and toxicity related to its generation [85]. Therefore, before using ozone, other options to eliminate PhACs should be evaluated.

To summarize, the combined use of intensive biological wastewater treatment (MBR and MBBR) or AOPs techniques with CWs is a promising solution to eliminate PhACs. Until now, those combinations have not been yet investigated or only tested at laboratory scale. Particularly, the combination of AOPs with CWs may help to reduce the amount of energy needed to oxidize PhACs as well as to minimize the presence of oxidized TPs generated by the former technology. Furthermore, the water effluent from CWs has a greater ecological richness than that from AOPs, comparable to that found in continental surface waters. Therefore further investigation is needed in this field.

4 Challenges and Future Trends

Despite CWs being a low-cost alternative to conventional WWTPs with a remarkable capacity to remove PhACs, there are still some challenges to be overcome to become a more competitive technology (Fig. 4). Therefore, future research trends should address those challenges that, in several cases, are shared with other wastewater treatment technologies.

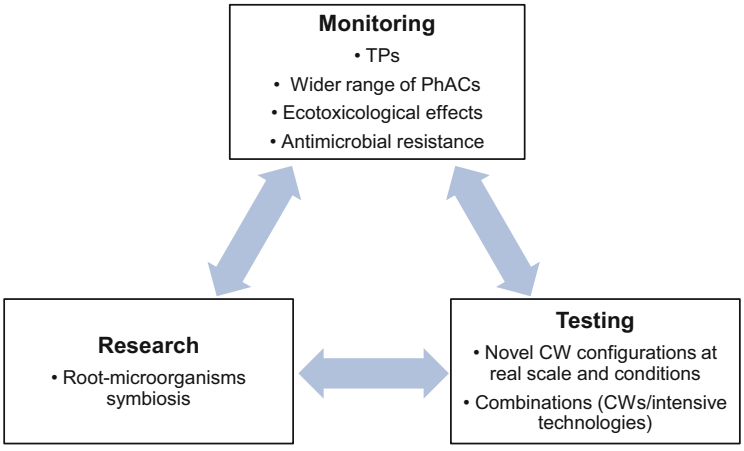


Fig. 4 Overview of future research strategies to overcome the challenges of wastewater treatment with CWs. Monitoring, research, and testing actions also relate to each other

Monitoring: Firstly, most studies about PhAC degradation by CWs or other wastewater treatment technologies have focused on the removal of the parent compounds. However, PhAC degradation may result in TPs which may be more or less toxic than the original compound. As an attempt to solve this, TP formation assessment via toxicity tests has been proposed to complement chemical analysis [86]. On the other side, limited studies in CWs, and only for a few compounds, have demonstrated that several TPs are left after water treatment [8]. Further studies should address the formation and toxicity of TPs [87]. For example, one TP of diclofenac was identified after passing through filtration-UV-chlorination [88], but it was not detected after a CW treatment [89, 90]. Thereafter, the same authors found out that the presence of aquatic plants aided to enhance the removal of this TP from water [91]. Second, PhACs include a large list of compounds, but only a few of them have been studied repeatedly in CWs, hindering a deeper understanding of treatment processes. This phenomenon, known as Matthew Effect, is generally occurring in the study of PhACs in the environment [92]. Taking into account the recent advances in analytical chemistry, future research should analyze a wider range and larger number of compounds. In relation to that, the ecotoxicological effects of PhACs and their TPs should also be monitored and assessed. While conventional toxicological studies of a few compounds can be performed to complement chemical analysis [86, 93], new in silico tools using big data, machine learning, and artificial intelligence seem to be the future way to conduct ecotoxicological assessment studies in CWs [94]. Finally, antimicrobial resistance is a raising threat in global health, and wastewater treatment has been pointed out as one of the main sources of ARGs [95]. Several authors have claimed that CWs are a potential spot for horizontal gene transfer that could diffuse antimicrobial resistant bacteria and ARG to the aquatic environment [96, 97]. This could be particularly significant when bacteria are inoculated into CWs to enhance nutrient and PhAC removal (bioaugmentation). In this case, it would be important to start characterizing the antimicrobial resistance profile of the inoculated bacteria [96]. In contrast, some studies point towards the potential of CWs to attenuate antimicrobial resistance [96, 98]. For example, SFCWs have shown removals of 56–100% for 12 antibiotics without increasing the amount of ARGs [29]. In addition, the usage of zeolites in a SSFCW mesocosm achieved good ARG removal (85% for HLR = 10 cm/days) [41]. Future research should verify and explore the utility of CWs to fight antimicrobial resistance.

Research: An important challenge that CW technology needs to overcome is the high surface area required for wastewater treatment. Besides adsorption, biodegradation is the most important process in CWs, which is remarkably enhanced by the symbiotic interactions between the plants and the microbial community [96]. These interactions are mainly conducted through the release of chemical root exudates as it has been observed in recent publications [99]. Therefore, this interaction is predominantly happening within the root zone (rhizosphere), reducing the effective volume of the CW. By enhancing the symbiotic interactions between plants and the surrounding microbial communities, the surface area/volume demand of the CW could be reduced [100]. This could be performed by the addition of synthetic chemical root exudates to the CWs, as it has already been employed in the enhancement of the

bioremediation of contaminated soils [101, 102]. Therefore, future research trends on CWs will certainly need to focus on the understanding of the role of root exudates on the biodegradation enhancement of PhACs.

Testing: Novel CW configurations have only been tested at laboratory scale. Some novel CW configurations have shown promising results removing PhACs but only on a laboratory scale and under controlled conditions (e.g., temperature and synthetic wastewater). Future research should put efforts into testing these novel configurations at pilot or full scale under real conditions. This will be essential to evaluate the applicability of these technologies in terms of costs and efficiency. On the other hand, nearly all novel CW configurations enhance only one removal mechanism: sorption or biodegradation. This approach is favorable to study and understand the efficiency of the technology, but it limits the type of PhACs that can be removed. As a solution, recent studies have started to test combinations of the available novel CW configurations. For example, a full-scale CW has been constructed using LECA, which enhances sorption and biofilm growth, while it operates with forced aeration, which enhances biodegradation [103]. With such configuration, the system achieved removals over 78% of 10 out of 11 PhACs, with high removal of recalcitrant compounds such as carbamazepine and diclofenac. These types of approaches, as well as the combination of novel CWs with intensive wastewater treatment technologies, should be further studied. Additionally, synergies between different approaches have to be considered. For example, the nature of the filling material of the CW determines the associated microbial communities, but it could also be used to enhance the immobilization of bacteria when applying bioaugmentation.

5 Concluding Remarks

CWs are sustainable, low-cost, robust, and efficient wastewater treatment systems that can remove PhACs at least as effectively as conventional WWTPs. It is known that most PhACs undergo biodegradation under aerobic conditions and only a few of them are degraded under anoxic-anaerobic conditions. Nevertheless, lately, different novel CW configurations have been developed to improve the attenuation of PhACs from wastewater. First, novel filling materials offer enhanced adsorption capacities which are, up to now, the only mechanism to remove recalcitrant compounds. Besides PhACs, zeolites have shown capacity to remove ARGs. Interestingly, novel filling materials can also be part of circular economy, as they can be obtained from waste materials. Second, biodegradation processes can be boosted in CWs. MCF-CWs have shown to increase removal of PhACs, but the accumulation of antibiotics on the anode layer could trigger antimicrobial resistance processes. Forced aeration enhanced the biodegradation rates of PhACs by 20–60% in comparison to HF-SSFCWs, but limited improvements were obtained in VF-SSFCWs. Bioaugmentation for PhACs has only been tested for ibuprofen but has shown to be effective for other types of organic contaminants like atrazine. Also,

bioaugmentation may shorten the CW adaptation phase, starting to degrade PhACs earlier. Third, the combination of CWs with intensive wastewater treatment technologies such as MBBR and MBR may offer a broader range of biodegradation pathways, while CWs placed after AOPs may allow the biodegradation of generated TPs. Finally, future research should assess TPs, expand the number of studied PhACs, assess ecotoxicity, evaluate antimicrobial resistance processes, investigate root-microorganism symbiosis, and test additional CW combinations to improve CW wastewater treatment technology.

Acknowledgments The authors gratefully acknowledge the financial support of the Spanish Ministry of Science, Innovation and Universities under Project CTM2017-91355-EXP and Generalitat de Catalunya for the Beatriu de Pinós grant-programme.

References

1. Carvalho PN, Arias CA, Brix H (2017) Constructed wetlands for water treatment: new developments. Water. https://doi.org/10.3390/w9060397
2. Vymazal J (2010) Constructed wetlands for wastewater treatment. Water. https://doi.org/10.3390/w2030530
3. Ostfeld RS (2009) Biodiversity loss and the rise of zoonotic pathogens. Clin Microbiol Infect. https://doi.org/10.1111/j.1469-0691.2008.02691.x
4. Fitch MW (2014) Constructed wetlands. In: Comprehensive water quality and purification. https://doi.org/10.1016/B978-0-12-382182-9.00053-0
5. Scholz M, Lee B (2005) Constructed wetlands: a review. Int J Environ Stud 62:421–447. https://doi.org/10.1080/00207230500119783
6. Vymazal J, Kröpfelová L (2009) Removal of organics in constructed wetlands with horizontal sub-surface flow: a review of the field experience. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2008.08.032
7. Hijosa-Valsero M, Matamoros V, Sidrach-Cardona R et al (2010) Comprehensive assessment of the design configuration of constructed wetlands for the removal of pharmaceuticals and personal care products from urban wastewaters. Water Res:44. https://doi.org/10.1016/j.watres.2010.04.022
8. Ilyas H, van Hullebusch ED (2020) Performance comparison of different types of constructed wetlands for the removal of pharmaceuticals and their transformation products: a review. Environ Sci Pollut Res Int. https://doi.org/10.1007/s11356-020-08165-w
9. Li Y, Zhu G, Ng WJ, Tan SK (2014) A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: design, performance and mechanism. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2013.09.018
10. Matamoros V, Bayona JM (2013) Removal of pharmaceutical compounds from wastewater and surface water by natural treatments. Compr Anal Chem 62:409–433. https://doi.org/10.1016/B978-0-444-62657-8.00012-4
11. Verlicchi P, Zambello E (2014) How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2013.10.085
12. Liu L, Hong LY, Wang Z et al (2014) Behavior of tetracycline and sulfamethazine with corresponding resistance genes from swine wastewater in pilot-scale constructed wetlands. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2014.06.015

13. Vo HNP, Koottatep T, Chapagain SK et al (2019) Removal and monitoring acetaminophen-contaminated hospital wastewater by vertical flow constructed wetland and peroxidase enzymes. J Environ Manage. https://doi.org/10.1016/j.jenvman.2019.109526
14. Bôto M, Almeida CMR, Mucha AP (2016) Potential of constructed wetlands for removal of antibiotics from saline aquaculture effluents. Water. https://doi.org/10.3390/w8100465
15. Loffler D, Rombke J, Meller M, Ternes TA (2005) Environmental fate of pharmaceuticals in water/sediment systems. Environ Sci Technol 39:5209–5218. https://doi.org/10.1021/es0484146
16. Verlicchi P, Al Aukidy M, Jelic A et al (2014) Comparison of measured and predicted concentrations of selected pharmaceuticals in wastewater and surface water: a case study of a catchment area in the Po Valley (Italy). Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2013.10.026
17. Dordio AV, Carvalho AJP (2013) Organic xenobiotics removal in constructed wetlands, with emphasis on the importance of the support matrix. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2013.03.008
18. Brix H (1994) Use of constructed wetlands in water pollution control: historical development, present status, and future perspectives. In: Water science and technology
19. Ávila C, Matamoros V, Reyes-Contreras C et al (2014) Attenuation of emerging organic contaminants in a hybrid constructed wetland system under different hydraulic loading rates and their associated toxicological effects in wastewater. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2013.10.065
20. Brix H (2003) Plants used in constructed wetlands and their functions. Proceedings of 1st international seminar on the use of aquatic macrophytes for wastewater treatment in constructed wetlands
21. Kadlec RH, Wallace S (2008) Treatment wetlands. CRC Press, Boca Raton
22. Matamoros V, Bayona JM (2006) Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. Environ Sci Technol. https://doi.org/10.1021/es0607741
23. Ávila C, Pedescoll A, Matamoros V et al (2010) Capacity of a horizontal subsurface flow constructed wetland system for the removal of emerging pollutants: an injection experiment. Chemosphere. https://doi.org/10.1016/j.chemosphere.2010.08.006
24. Matamoros V, Arias C, Brix H, Bayona JM (2009) Preliminary screening of small-scale domestic wastewater treatment systems for removal of pharmaceutical and personal care products. Water Res 43. https://doi.org/10.1016/j.watres.2008.10.005
25. Matamoros V, Arias C, Brix H, Bayona JM (2007) Removal of pharmaceuticals and personal care products (PPCPs) from urban wastewater in a pilot vertical flow constructed wetland and a sand filter. Environ Sci Technol. https://doi.org/10.1021/es071594+
26. Matamoros V, García J, Bayona JM (2008) Organic micropollutant removal in a full-scale surface flow constructed wetland fed with secondary effluent. Water Res:42. https://doi.org/10.1016/j.watres.2007.08.016
27. Matamoros V, Salvadó V (2012) Evaluation of the seasonal performance of a water reclamation pond-constructed wetland system for removing emerging contaminants. Chemosphere. https://doi.org/10.1016/j.chemosphere.2011.09.020
28. Matamoros V, Duhec A, Albaigés J, Bayona JM (2009) Photodegradation of carbamazepine, ibuprofen, ketoprofen and 17α-ethinylestradiol in fresh and seawater. Water Air Soil Pollut. https://doi.org/10.1007/s11270-008-9765-1
29. Berglund B, Khan GA, Weisner SEB et al (2014) Efficient removal of antibiotics in surface-flow constructed wetlands, with no observed impact on antibiotic resistance genes. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2013.12.128
30. Rühmland S, Wick A, Ternes TA, Barjenbruch M (2015) Fate of pharmaceuticals in a subsurface flow constructed wetland and two ponds. Ecol Eng. https://doi.org/10.1016/j.ecoleng.2015.01.036

31. Hijosa-Valsero M, Matamoros V, Martín-Villacorta J et al (2010) Assessment of full-scale natural systems for the removal of PPCPs from wastewater in small communities. Water Res 44. https://doi.org/10.1016/j.watres.2009.10.032
32. Ávila C, Bayona JM, Martín I et al (2015) Emerging organic contaminant removal in a full-scale hybrid constructed wetland system for wastewater treatment and reuse. Ecol Eng. https://doi.org/10.1016/j.ecoleng.2014.07.056
33. Hadjiev D, Dimitrov D, Martinov M, Sire O (2007) Enhancement of the biofilm formation on polymeric supports by surface conditioning. Enzyme Microb Technol. https://doi.org/10.1016/j.enzmictec.2006.06.022
34. Zhang L, Lyu T, Ramírez Vargas CA et al (2018) New insights into the effects of support matrix on the removal of organic micro-pollutants and the microbial community in constructed wetlands. Environ Pollut. https://doi.org/10.1016/j.envpol.2018.05.028
35. Dordio AV, Miranda S, Prates Ramalho JP, Carvalho AJP (2017) Mechanisms of removal of three widespread pharmaceuticals by two clay materials. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2016.05.091
36. Dordio A, Pinto J, Dias CB et al (2009) Atenolol removal in microcosm constructed wetlands. Int J Environ Anal Chem. https://doi.org/10.1080/03067310902962502
37. Dalahmeh S, Ahrens L, Gros M et al (2018) Potential of biochar filters for onsite sewage treatment: adsorption and biological degradation of pharmaceuticals in laboratory filters with active, inactive and no biofilm. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2017.08.178
38. Özengin N, Elmaci A (2016) Removal of pharmaceutical products in a constructed wetland. Iran J Biotechnol. https://doi.org/10.15171/ijb.1223
39. Dordio A, Carvalho AJP, Teixeira DM et al (2010) Removal of pharmaceuticals in microcosm constructed wetlands using Typha spp. and LECA. Bioresour Technol. https://doi.org/10.1016/j.biortech.2009.09.001
40. Dordio AV, Gonçalves P, Texeira D et al (2011) Pharmaceuticals sorption behaviour in granulated cork for the selection of a support matrix for a constructed wetlands system. Int J Environ Anal Chem. https://doi.org/10.1080/03067319.2010.510605
41. Chen J, Wei XD, Liu YS et al (2016) Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: optimization of wetland substrates and hydraulic loading. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2016.04.176
42. Machado AI, Dordio A, Fragoso R et al (2017) Furosemide removal in constructed wetlands: comparative efficiency of LECA and Cork granulates as support matrix. J Environ Manage. https://doi.org/10.1016/j.jenvman.2017.08.002
43. Du L, Zhao Y, Wang C et al (2020) Removal performance of antibiotics and antibiotic resistance genes in swine wastewater by integrated vertical-flow constructed wetlands with zeolite substrate. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.137765
44. USGS (2020) Zeolites statistics and information. Zeolites Stat Inf.: https://www.usgs.gov/centers/nmic/zeolites-statistics-and-information. Accessed May 2020
45. Wang H, Xu J, Sheng L (2020) Purification mechanism of sewage from constructed wetlands with zeolite substrates: a review. J Clean Prod. https://doi.org/10.1016/j.jclepro.2020.120760
46. CM Zeolite (2013) Zeolite CM discussion note: Castle Mountain Zeolite or polyacrylamide as water saver crystals. http://www.cmzeolites.com.au/_literature_149098/Zeolite_Compared_to_PAM_as_a_Water_Saver_Cystal
47. Mateus DMR, Pinho HJO (2018) Screening of solid waste as filler material for constructed wetlands. Paper presented in IOP Conference Series: Earth and Environmental Science
48. Aguilar L, Gallegos Á, Arias CA et al (2019) Microbial nitrate removal efficiency in groundwater polluted from agricultural activities with hybrid cork treatment wetlands. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2018.10.426
49. Mateus DMR, Pinho HJO (2020) Evaluation of solid waste stratified mixtures as constructed wetland fillers under different operation modes. J Clean Prod. https://doi.org/10.1016/j.jclepro.2020.119986

50. Maroušek J, Strunecký O, Stehel V (2019) Biochar farming: defining economically perspective applications. Clean Techn Environ Policy 21:1389. https://doi.org/10.1007/s10098-019-01728-7
51. Lehmann J (2007) Bio-energy in the black. Front Ecol Environ. https://doi.org/10.1890/1540-9295(2007)5[381:BITB]2.0.CO;2
52. Zhou X, Jia L, Liang C et al (2018) Simultaneous enhancement of nitrogen removal and nitrous oxide reduction by a saturated biochar-based intermittent aeration vertical flow constructed wetland: effects of influent strength. Chem Eng J. https://doi.org/10.1016/j.cej.2017.11.066
53. Gupta P, Ann TW, Lee SM (2016) Use of biochar to enhance constructed wetland performance in wastewater reclamation. Environ Eng Res. https://doi.org/10.4491/eer.2015.067
54. Abedi T, Mojiri A (2019) Constructed wetland modified by biochar/zeolite addition for enhanced wastewater treatment. Environ Technol Innov. https://doi.org/10.1016/j.eti.2019.100472
55. Clark DP, Pazdernik NJ (2016) Chapter 12 – environmental biotechnology. In: Clark DP, Pazdernik NJBT-B, Second E (eds). Academic Cell, Boston, pp 393–418
56. Corbella C, Puigagut J (2015) Microbial fuel cells implemented in constructed wetlands: fundamentals, current research and future perspectives. Contrib Sci. https://doi.org/10.2436/20.7010.01.220
57. Li H, Zhang S, Yang XL et al (2019) Enhanced degradation of bisphenol A and ibuprofen by an up-flow microbial fuel cell-coupled constructed wetland and analysis of bacterial community structure. Chemosphere. https://doi.org/10.1016/j.chemosphere.2018.11.022
58. Kahl S, Nivala J, van Afferden M et al (2017) Effect of design and operational conditions on the performance of subsurface flow treatment wetlands: emerging organic contaminants as indicators. Water Res:125. https://doi.org/10.1016/j.watres.2017.09.004
59. Li J, Zhou Q, Campos LC (2017) Removal of selected emerging PPCP compounds using greater duckweed (*Spirodela polyrhiza*) based lab-scale free water constructed wetland. Water Res. https://doi.org/10.1016/j.watres.2017.09.002
60. Li H, Cai Y, Gu Z et al (2020) Accumulation of sulfonamide resistance genes and bacterial community function prediction in microbial fuel cell-constructed wetland treating pharmaceutical wastewater. Chemosphere. https://doi.org/10.1016/j.chemosphere.2020.126014
61. Ávila C, Nivala J, Olsson L et al (2014) Emerging organic contaminants in vertical subsurface flow constructed wetlands: influence of media size, loading frequency and use of active aeration. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2014.06.128
62. Balciunas EM, Kappelmeyer U, Harms H, Heipieper HJ (2020) Increasing ibuprofen degradation in constructed wetlands by bioaugmentation with gravel containing biofilms of an ibuprofen-degrading Sphingobium yanoikuyae. Eng Life Sci. https://doi.org/10.1002/elsc.201900097
63. Zhang L, Lyu T, Zhang Y et al (2018) Impacts of design configuration and plants on the functionality of the microbial community of mesocosm-scale constructed wetlands treating ibuprofen. Water Res:131. https://doi.org/10.1016/j.watres.2017.12.050
64. Auvinen H, Havran I, Hubau L et al (2017) Removal of pharmaceuticals by a pilot aerated sub-surface flow constructed wetland treating municipal and hospital wastewater. Ecol Eng. https://doi.org/10.1016/j.ecoleng.2016.12.031
65. Uggetti E, Hughes-Riley T, Morris RH et al (2016) Intermittent aeration to improve wastewater treatment efficiency in pilot-scale constructed wetland. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2016.03.195
66. Rous V, Vymazal J, Hnátková T (2019) Treatment wetlands aeration efficiency: a review. Ecol Eng. https://doi.org/10.1016/j.ecoleng.2019.06.006
67. Free A, McDonald MA, Pagaling E (2018) Diversity-function relationships in natural, applied, and engineered microbial ecosystems. Adv Appl Microbiol. https://doi.org/10.1016/bs.aambs.2018.07.002

68. Meng P, Pei H, Hu W et al (2014) How to increase microbial degradation in constructed wetlands: influencing factors and improvement measures. Bioresour Technol. https://doi.org/10.1016/j.biortech.2014.01.095
69. Shao Y, Pei H, Hu W et al (2014) Bioaugmentation in lab scale constructed wetland microcosms for treating polluted river water and domestic wastewater in northern China. Int Biodeter Biodegr. https://doi.org/10.1016/j.ibiod.2014.05.027
70. Zhao X, Yang J, Bai S et al (2016) Microbial population dynamics in response to bioaugmentation in a constructed wetland system under 10°C. Bioresour Technol. https://doi.org/10.1016/j.biortech.2016.01.043
71. Zhao X, Bai S, Li C et al (2019) Bioaugmentation of atrazine removal in constructed wetland: performance, microbial dynamics, and environmental impacts. Bioresour Technol. https://doi.org/10.1016/j.biortech.2019.121618
72. Suzana Cudia SM, Claudia M, Fiúza LMCG et al (2013) Immobilization of microbial cells: a promising tool for treatment of toxic pollutants in industrial wastewater. Afr J Biotechnol. https://doi.org/10.5897/ajb12.2677
73. Xiao ER, Liang W, He F et al (2010) Performance of the combined SMBR-IVCW system for wastewater treatment. Desalination. https://doi.org/10.1016/j.desal.2008.11.041
74. Lai C, Sun Y, Guo Y et al (2020) A novel integrated bio-reactor of moving bed and constructed wetland (MBCW) for domestic wastewater treatment and its microbial community diversity. Environ Technol. https://doi.org/10.1080/09593330.2019.1709904
75. Escolà Casas M, Chhetri RK, Ooi G et al (2015) Biodegradation of pharmaceuticals in hospital wastewater by staged moving bed biofilm reactors (MBBR). Water Res:83. https://doi.org/10.1016/j.watres.2015.06.042
76. Torresi E (2017) Removal of micropollutants in moving bed biofilm reactors (MBBRs). Dissertation, Danish Technical University
77. Torresi E, Escolà Casas M, Polesel F et al (2017) Impact of external carbon dose on the removal of micropollutants using methanol and ethanol in post-denitrifying moving bed biofilm reactors. Water Res:108. https://doi.org/10.1016/j.watres.2016.10.068
78. Liu R, Zhao Y, Doherty L et al (2015) A review of incorporation of constructed wetland with other treatment processes. Chem Eng J. https://doi.org/10.1016/j.cej.2015.05.023
79. Reungoat J, Escher BI, Macova M et al (2012) Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. Water Res:46. https://doi.org/10.1016/j.watres.2011.11.064
80. Melián JAH, Araña J, Ortega JA et al (2008) Comparative study of phenolics degradation between biological and photocatalytic systems. J Sol Energy Eng. https://doi.org/10.1115/1.2969800
81. Araña J, Garriga i Cabo C, Fernández Rodríguez C et al (2008) Combining TiO2-photocatalysis and wetland reactors for the efficient treatment of pesticides. Chemosphere. https://doi.org/10.1016/j.chemosphere.2007.10.008
82. de Wilt HA (2018) Pharmaceutical removal: synergy between biological and chemical processes for wastewater treatment. Dissertation, Wageningen University
83. Lee E, Shon HK, Cho J (2014) Role of wetland organic matters as photosensitizer for degradation of micropollutants and metabolites. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2014.05.001
84. Lancheros JC, Madera-Parra CA, Caselles-Osorio A et al (2019) Ibuprofen and naproxen removal from domestic wastewater using a horizontal subsurface flow constructed wetland coupled to ozonation. Ecol Eng. https://doi.org/10.1016/j.ecoleng.2019.05.007
85. Ozonetech (2020) Ozone safety. https://www.ozonetech.com/ozone-safety. Accessed May 2020
86. Escher BI, Fenner K (2011) Recent advances in environmental risk assessment of transformation products. Environ Sci Technol. https://doi.org/10.1021/es1030799
87. Yin L, Wang B, Yuan H et al (2017) Pay special attention to the transformation products of PPCPs in environment. Emerg Contam. https://doi.org/10.1016/j.emcon.2017.04.001

88. Matamoros V, Salvadó V (2013) Evaluation of a coagulation/flocculation-lamellar clarifier and filtration-UV-chlorination reactor for removing emerging contaminants at full-scale wastewater treatment plants in Spain. J Environ Manage 117:96–102. https://doi.org/10.1016/j.jenvman.2012.12.021
89. Matamoros V (2010) Attenuation of emerging contaminants by wetland systems. Cases from Catalonia and Denmark. http://www.phytosudoe.eu/wp-content/uploads/2018/10/10oct_2_Matamoros_emerging-contaminants.pdf
90. Matamoros V, Sala L, Salvadó V (2012) Evaluation of a biologically-based filtration water reclamation plant for removing emerging contaminants: a pilot plant study. Bioresour Technol. https://doi.org/10.1016/j.biortech.2011.11.036
91. Matamoros V, Nguyen LX, Arias CA et al (2012) Evaluation of aquatic plants for removing polar microcontaminants: a microcosm experiment. Chemosphere. https://doi.org/10.1016/j.chemosphere.2012.04.004
92. Daughton CG (2014) The Matthew effect and widely prescribed pharmaceuticals lacking environmental monitoring: case study of an exposure-assessment vulnerability. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2013.06.111
93. Han GH, Hur HG, Kim SD (2006) Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: occurrence and toxicity to *Daphnia magna*. Environ Toxicol Chem 25:265–271. https://doi.org/10.1897/05-193r.1
94. Kar S, Sanderson H, Roy K et al (2020) Ecotoxicological assessment of pharmaceuticals and personal care products using predictive toxicology approaches. Green Chem. https://doi.org/10.1039/C9GC03265G
95. Pazda M, Kumirska J, Stepnowski P, Mulkiewicz E (2019) Antibiotic resistance genes identified in wastewater treatment plant systems – a review. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2019.134023
96. Riva V, Riva F, Vergani L et al (2020) Microbial assisted phytodepuration for water reclamation: environmental benefits and threats. Chemosphere. https://doi.org/10.1016/j.chemosphere.2019.124843
97. Zhang S, Lu YX, Zhang JJ et al (2020) Constructed wetland revealed efficient sulfamethoxazole removal but enhanced the spread of antibiotic resistance genes. Molecules. https://doi.org/10.3390/molecules25040834
98. Chen QL, Cui HL, Su JQ et al (2019) Antibiotic Resistomes in plant microbiomes. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2019.02.010
99. de la Paz A, Salinas N, Matamoros V (2019) Unravelling the role of vegetation in the attenuation of contaminants of emerging concern from wetland systems: preliminary results from column studies. Water Res 166. https://doi.org/10.1016/j.watres.2019.115031
100. SIMAGUA Project (2019) CTM2017-91355-EXP. https://simaguasite123me/. Accessed May 2020
101. Lu H, Sun J, Zhu L (2017) The role of artificial root exudate components in facilitating the degradation of pyrene in soil. Sci Rep. https://doi.org/10.1038/s41598-017-07413-3
102. Urbaniak M, Mierzejewska E, Tankiewicz M (2019) The stimulating role of syringic acid, a plant secondary metabolite, in the microbial degradation of structurally-related herbicide. MCPA PeerJ. https://doi.org/10.7717/peerj.6745
103. Auvinen H, Gebhardt W, Linnemann V et al (2017) Laboratory- and full-scale studies on the removal of pharmaceuticals in an aerated constructed wetland: effects of aeration and hydraulic retention time on the removal efficiency and assessment of the aquatic risk. Water Sci Technol. https://doi.org/10.2166/wst.2017.328

Microalgae-Based Processes for the Removal of Pharmaceuticals in Wastewater

Paqui Blánquez, Andrea Hom-Díaz, Teresa Vicent, and Benoit Guieysse

Contents

Abstract Microalgae-based technologies deliver economic and effective treatment for municipal and industrial effluents while also providing capacity for nutrient recovery and the development of algae-derived products. In order to assess the potential of microalgae-based technologies for the removal of pharmaceuticals (PhACs) found in wastewater effluents, this chapter aims to (1) introduce microalgae and their ecological significance; (2) describe the principle of pollutant removal in

P. Blánquez (✉), A. Hom-Díaz, and T. Vicent
Chemical, Biological and Environmental Engineering Department, Escola d'Enginyeria, Universitat Autònoma de Barcelona, Barcelona, Spain
e-mail: paqui.blanquez@uab.cat; andreahomdiaz@gmail.com; teresa.vicent@uab.cat

B. Guieysse
School of Engineering and Advanced Technology, Massey University, Palmerston North, New Zealand
e-mail: B.J.Guieysse@massey.ac.nz

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 191–222, DOI 10.1007/698_2020_682,

microalgae-based systems; (3) review the general mechanisms of pharmaceutical removal occurring during wastewater treatment and discuss their potential significance in microalgae-based systems; (4) describe the main photobioreactors used for microalgae-based wastewater treatment; and (5) summarize recent progress on microalgae-based PhACs removal with particular focus on efficiency and mechanisms at pilot and full-scale systems. Overall, the data available demonstrate microalgae based-systems providing capacities for PhACs removal via biodegradation, biosorption and bioaccumulation that are similar or higher than the capacities commonly reported during conventional biological treatment. In addition, microalgae-based wastewater system also provides the capacity for pollutant photodegradation and volatilization. Full-scale demonstration during long-term treatment remains needed.

Keywords Microalgae, Pharmaceuticals (PhACs), Photobioreactors, Wastewater treatment

1 An Introduction to Microalgae and Microalgae-Based Wastewater Treatment

1.1 Microalgae Biochemistry and Ecological Significance

Microalgae represent a phylogenetically diverse group of unicellular eukaryotes that can be found grouped into chains. These microorganisms are found in almost any environment (e.g. lakes, rivers, oceans, soil), including in extreme habitats (e.g. ice, hypersaline environments), and can develop symbiotic relationships with other organisms (e.g. with fungi in lichen). Prokaryotic cyanobacteria have similar abilities and functions, and consequently, eukaryotic and prokaryotic microbial phototrophs will henceforth be commonly referred to as microalgae for simplicity.

Microalgae carry out the photosynthetic reduction of carbon dioxide (CO_2) into biomass using sunlight as energy source. Briefly, light-dependent reactions photolyse water into O_2 and convert sunlight energy onto energy carrier ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate). Light-independent reactions then use these ATP and NAPDH to reduce CO_2 into 3-carbon sugars that are then further processed into cellular components. The conversion of sunlight energy into chemical energy is typically carried out by chlorophyll, but other pigments can be involved. Beside carbon fixation, microalgae must also access the minerals needed to build cell components and enzymes, especially nitrogen (N) and phosphorus (P). N is found in amino acids (the component of proteins) and nucleotides (the component of nucleic acids) and can represent up to 10% of the total dry cell weight. N is generally obtained from ammonium (NH_4^+) or nitrate (NO_3^-), and the latter must reduce the former before incorporation

into amino acids. P is typically found in phospholipids, nucleotides and ATP and represents approximately 1% of the total dry cell weight, although various microalgae can intracellularly accumulate P as polyphosphate up to 3–4% of the cell weight [1]. Interestingly, nutrient starvation (especially N) can trigger lipid accumulation in microalgae cells, with interesting application for bioenergy generation.

Microalgae have critical global ecological functions as primary producer (converting carbon dioxide into organic matter) and oxygen producer and for nitrogen fixation. Microalgae can however also generate ecological issues when they have been introduced in new environments, or when nutrient pollution (N and/or P) cause excessive algae growth leading to the eutrophication of water bodies. As anthropogenic pollution causes massive eutrophication issues worldwide, microalgae may also contribute to global anthropogenic N_2O emissions [2] because these microorganisms can synthetize this potent greenhouse gas, including during wastewater treatment [3]. Fortunately, the ability of microalgae to effectively scavenge N and P from aquatic bodies also provides means of water treatment and nutrient recycle [1], as further discussed below.

1.2 Microalgae Biotechnology

Industrial applications of microalgae currently focus on food production using a few species (e.g. *Chlorella vulgaris* and *Arthrospira platensis*) cultivated in raceway ponds, but there is considerable interest in also using microalgae to produce high-value bioactive compounds such as pharmaceuticals, nutraceuticals or cosmetics. In addition, and while microalgae cultivation for bioenergy (e.g. biodiesel) generation has generated considerable interest in the past, this application is nowadays considered to be neither economic nor sustainable, and research in this area is now focusing on combining microalgae-based wastewater treatment with bioenergy generation.

Microalgae are commonly found (at least periodically) in maturation and stabilization ponds during wastewater treatment (WWT), but their growth in these systems is opportunistic (and often problematic) rather than engineered. In contrast, the high rate algal ponds (HRAPs) developed by Oswald and co-workers in the 1960s were specifically designed and operated to enhance microalgae activity by optimizing light supply to these microorganisms (see Sect. 3). These systems rely on the exchange of materials between microalgae and heterotrophic microorganisms to remove pollutants: the microalgae carry out the photosynthetic fixation of CO_2 into biomass, thereby releasing O_2 from the photolytic oxidation of water. This O_2 is then used as a final electron acceptor by heterotrophs converting organic pollutants into biomass and CO_2, the latter becoming available as a carbon source for microalgae photosynthetic growth (Fig. 1). This in situ biological oxygen supply can significantly reduce energy consumption in comparison to mechanically aerated treatment alternatives (Box 1). Many microalgae can also carry out heterotrophic growth at

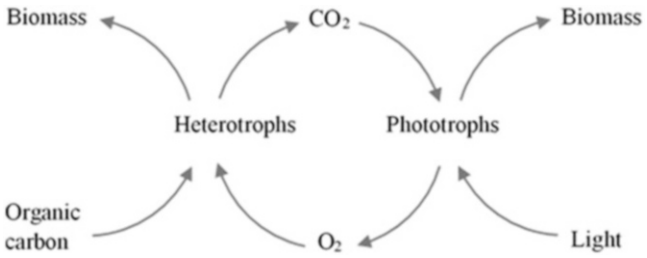


Fig. 1 Principle of organic pollutant removal in HRAP (adapted from [4])

night and even during daytime and therefore provide additional capacity (to strict heterotrophs) for removing organic pollutants.

In addition to providing O_2, photosynthesis occurring during microalgae-based WWT produces additional biomass, which improves N and P removal by assimilation. This additional biomass must however be harvested to achieve wastewater discharge compliance, and sludge management can represent up to 50% of WWT costs. Sludge can be anaerobically digested, and the additional algal biomass means more biogas is produced per unit of wastewater treated than during 'conventional' treatment [5]. However, the algal biomass can be hard to digest anaerobically, and digestion releases N and P into a stream (digestate) that must be further managed. The digestate and/or undigested biosolids may be used as fertilizer [5], but this practice is practically limited by logistic issues (fertilizers are only needed in certain places at certain times) and the risks of pollutant spreading (e.g. pathogens or heavy metals) onto agricultural products (this also limits the use of raw biomass as animal feed). Sludge management is therefore especially critical in the context of hazardous pollutants such as pharmaceuticals because sorption onto the sludge can cause the potential release (or transfer) of these pollutants during sludge disposal. There is currently considerable interest in developing biorefineries based on algae-based WWT, but major risks remain when the end products are intended for animal or human consumption, and these processes are difficult to control considering the variability of wastewater.

Box 1 Microalgae-Based Wastewater Treatment: A Case Study

Posadas et al. [6] sized a HRAP for the treatment of secondary domestic wastewater with a daily average flow rate of 920 m^3/d representative of 2000 people equivalents (PE). The treatment system required to achieve year-round bCOD (biodegradable chemical oxygen demand) and TN (total nitrogen) compliance under temperate climate was estimated to 12.9 m^2 per PE, for an energy requirement for pond-mixing of 0.039 kWh per m^3 of wastewater treated. In comparison, treating the same effluent with an activated sludge process would only require around 0.04 m^2 per PE, but at an energy

(continued)

Box 1 (continued)
input of 0.23 kWh/m^3 for combined bCOD/TN removal [7]. As can be seen, biological oxygen supply can significantly reduce energy consumption compared to mechanically aerated treatment alternatives, but the relatively low photosynthetic efficiency of microalgae means large areas are required to supply enough light to the microalgae. This large land requirement can be limiting but land value generally appreciates over time, and the large treatment volume thus provided buffers flow variations. Critically, Posadas et al. [6] also showed that sizing an HRAP for N removals de facto provides enough oxygenation capacity for bCOD removal, even without prior primary treatment (preliminary treatment is still needed). Altogether, Posadas et al.'s [6] analysis demonstrated HRAPs are best used when combined bCOD and TN is needed at relatively small scale. This does not, however, exclude their integration within existing treatment systems to, as examples, provide nutrient removal following secondary treatment [8], centrate treatment [9] or upgrade biogas [10].

2 Pharmaceutical Removal Under Controlled Conditions During Simulated Microalgae-Based Wastewater Treatment

2.1 General Mechanisms of Pharmaceutical Removal During Wastewater Treatment

The mechanisms driving pharmaceuticals (PhACs) removal during conventional biological WWT are well-established and include hydrolysis, volatilization, biodegradation, biosorption and bioaccumulation. During advanced WWT, the mechanisms targeted include sorption (e.g. onto activated carbon) and chemical oxidation with radicals generated chemically or photochemically. A distinction should be made between 'destructive' mechanisms that (should) convert pollutants into harmless substances (e.g. hydrolysis, biodegradation and chemical oxidation) and mechanisms that only transfer these pollutants into another medium (e.g. a sorbent that must be regenerated or safely disposed of). Destructive mechanisms may however lead to the formation toxic and/or recalcitrant products: this must be assessed and avoided.

The field efficiency of the mechanisms listed above depends on the chemistry of pollutants (some being intrinsically more biodegradable or reactive than others), their concentrations (the rate of removal generally increases with concentration), wastewater composition and environmental conditions (e.g. temperature). Removal is thus especially influenced by the presence of substances that can either enhance (e.g. radical precursors or co-substrates for co-metabolism) or reduce (e.g. substances competing for radicals or sorption sites) removal. The presence of

interfering substances is especially challenging because emerging pollutants such as PhACs are typically found at very low concentrations in wastewater, meaning they are easily 'outcompeted' by other substances.

2.2 Relevance of Individual Mechanisms to Microalgae-Based Wastewater Treatment

To establish the potential significance of a removal mechanism, pollutant removal must often be assessed under specific conditions that 'isolate' this mechanism against a negative control where no significant removal occurs. In the context of microalgae-based WWT, hydrolytic removal is therefore typically assessed in darkness in clean water (this test is often the negative control) and/or chemically sterilized wastewater. Photodegradation is tested under light irradiation in either clean (sometimes buffered) water, defined media (where known interfering substances are added at known concentrations) or sterilized wastewater (that can also be spiked with interfering substances). Biodegradation can be tested under darkness (under artificial aeration, if needed) or under light illumination (using UV filters and/or controls to avoid/assess photodegradation) in defined media or wastewater inoculated with active microalgae or microalgae consortium. Biosorption and bioaccumulation can be tested with dead or live biomass (generally under darkness). It can be difficult to distinguish between mechanisms (e.g. biosorption from bioaccumulation), so several controls and advanced analytics will be needed. Various levels of reactants and/or combinations of conditions should also be tested (e.g. incubation times, light intensity, biomass concentration), especially when kinetics are quantified, and experiments must be replicated under controlled conditions to demonstrate statistical significance. The need to conduct many experiments under rigorously controlled conditions generally means that relevance must be sacrificed for data quality (e.g. the pollutants are often spiked at relatively high concentrations, and microcosms are incubated under constant artificial light, temperature, etc.). The results can therefore statistically demonstrate the significance of a mechanism under the conditions tested, but they may not evidence that the mechanism investigated is also significant under real-life conditions (e.g. low pollutant concentration, variable conditions, etc.). The following sections describe and demonstrated mechanisms and discuss their full-scale potential during microalgae-based treatment. Please refer to the reviews by Wang et al. [11], Leng et al. [12], [13] and Norvill et al. [14] for more examples and in-depth discussion.

Biodegradation by heterotrophs is a major mechanism of PhACs removal during conventional biological WWT and is generally best carried out under aerobic conditions. This potential is also established for microalgae and microalgal consortia for a broad range of PhACs. For example, Peng et al. [15] studied the biotransformation of progesterone and norgestrel by the microalgae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. Using controls, these authors showed that hydrolysis and

volatilization were negligible and that biosorption to dead algae cells or bioaccumulation to live cells had little impact under the conditions studied. Instead, they reported pollutant-dependent and species-dependent biodegradation and, based on the transformation products detected, showed this biodegradation likely involved hydroxylation, reduction and oxidation as main pathways.

While many PhACs can inhibit microalgae at very high concentrations found in certain effluents (ppm), toxicity will typically not prevent biodegradation at the concentration found in domestic wastewater (ppt). Instead, low pollutant concentrations may limit biodegradation when the degrading microorganisms do not 'recognize' a pollutant as a potential substrate below a certain threshold. For this reason, co-metabolism is suspected to be the main mechanism for trace pollutant biodegradation during biological WWT.

Biodegradation by microalgae and other microorganisms generally involves a series of sequential steps catalysed by enzymatic systems such as cytochrome P450 [15, 16]. As most pharmaceuticals are relatively soluble in comparison to persistent pollutants such as dioxins and PCBs, lack of catabolic ability and/or low bioavailability is unlikely to prevent their biodegradation. Each reaction step also typically yields products that are more soluble and readily biodegradable, meaning there is little risk of metabolite accumulation. A high biodiversity is generally seen as beneficial for the removal of hazardous pollutants as it increases the chance that the required metabolic abilities are available and as it can provide alternative pathways via synergetic interactions (although competition also occurs). In this regard, microalgae-based WWT systems harbour microalgae as well as diverse heterotrophic communities that are also likely to possess a high metabolic versatility [6]. These systems can also harbour ammonia oxidizers with well-established pollutants' co-metabolic abilities.

Biodegradation efficiency typically increases with the mean cell retention time (MCRT) during conventional biological WWT. A high MCRT is practically achieved by recycling (e.g. activated sludge process), retaining (e.g. membrane bioreactors) or attaching (e.g. trickling biofilter) cells during conventional WWT. However, cells are typically not recycled, retained or attached in photobioreactors, although these configurations have been successfully tested [17–19]. Instead, the MCRT is increased via operation at high average hydraulic retention times (HRTs) as the HRT equals the MCRT when cells are not retained or recycled. Hence, biodegradation should also be considered as the major removal mechanism during full-scale microalgae-based WWT, and the microalgae may either biodegrade the pollutants or support other biodegrading species. Finally, HRAP broth conditions can vary from supersaturated in O_2 during daytime (when photosynthetic O_2 output $>$ O_2 demand) to anaerobic at night (if atmospheric O_2 transfer $<$ demand). This variability may enhance biodiversity and enable anaerobic-aerobic degradation pathways [20].

Adsorption onto bacterial and microalgal biomass is a well-established passive mechanism for the removal of relatively hydrophobic PhACs. Biosorption efficiency is highly dependent on pollutant structure (hydrophobicity and functional groups) and sorbent properties (i.e. the hydrophobicity and functional groups found on algal

cell walls and extracellular substances). For example, Santaeufemia et al. [21] reported oxytetracycline maximum sorption capacities of 29.18 mg/d and 4.54 mg/g for live and dead biomass of the microalga *Phaeodactylum tricornutum*, respectively, while de Godos et al. [22] evidenced tetracycline sorption onto biomass dominated by *Chlorella vulgaris*. At full scale, the significance of this mechanism will also depend on interfering substances competing for binding sites and environmental conditions impacting sorption equilibrium and/or kinetics (e.g. temperature, water hardness, pH). A high biomass productivity is also critical, meaning photosynthetic growth should provide enhanced capabilities for pollutant removal via adsorption.

Bioaccumulation involves the active (energy-demanding) transfer and storage of pollutants inside the cells. Bioaccumulation efficiency is highly dependent on pollutant structure and algae biology. For example, Stravs et al. [16] studied the bioaccumulation of 15 fungicides and 9 PhACs (in various mixtures) in the cyanobacteria *Microcystis aeruginosa* and *Synechococcus* sp. as well as the green alga *Chlamydomonas reinhardtii*. These authors reported bioaccumulation factors up to 1,000 and above and species-dependant correlations between the hydrophobicity of a pollutant and its bioconcentration factor. As for biosorption, enhanced biomass productivity can increase the significance of bioaccumulation during microalgae-based WWT.

Photodegradation is a well-established removal mechanism for many PhACs in aquatic environments [23] and during certain advanced oxidation processes (AOPs). This mechanism can involve direct photolysis, when the pollutant absorbs light energy (generally UV-B or low UV-A) and is then converted into a new product, and photooxidation when light irradiation causes the formation of radicals that then oxidize the pollutants (these radicals can be formed from the irradiations of photosensitizers, or they can be released by photosynthesizing microalgae). The potential of these mechanisms is well demonstrated in laboratory studies: for example, Bai and Acharya [24] evaluated the removal of seven endocrine-disrupting chemicals (EDCs) in wastewater (WW) inoculated with the green microalga *Nannochloris* sp. Using various inoculums, these authors demonstrated triclosan was removed by photolysis, even in the presence of the microalgae, but this mechanism has little impact on the other pollutants tested. Efficiency is therefore dependent on pollutant structure (e.g. ability to absorb UV radiation) and broth characteristics (e.g. presence of photosensitizers or competing compounds). Given that photobioreactors (PBs) are specifically designed to optimize light supply (see Sect. 3), PhACs photodegradation may be significant during microalgae-based WWT (unlike during conventional WWT). It is however difficult to assess the full-scale potential of photodegradation because the microalgae can ‘shade’ pollutants (or photosensitizers) from direct light exposure. This effect may however be counteracted by mixing, enabling frequent (but brief) exposure to high-light intensities at or near the reactor surface. Finally, photodegradation can be incomplete but photodegradation products can be subsequently biodegraded [25].

Hydrolysis is generally not seen as a main route of PhACs removal during WWT [26], although certain compounds such as β-lactam antibiotics can hydrolyse rapidly

in microalgae microcosms [27]. In these cases, hydrolysis may initiate biodegradation. To our knowledge, this mechanism is often not tested under the combination of high temperature and high pH that can occur during microalgae-based WWT, meaning it may be underestimated during laboratory studies. Hydrolysis can be enzymatically catalysed, but this mechanism should then be considered as biodegradation.

Volatilization of pollutants has been evidenced during laboratory assays, but only in the case of volatile substances with high Henry's law constant (H) values. For example, Matamoros et al. [28] reported the volatilization of non-ionized pollutants with H values of 0.1234–0.323 Pa m^3 mol^{-1} (4-octylphenol, galaxolide and tributyl phosphate) in aerated 2.5 L microcosms containing green microalgae, whereas ionized (e.g. ibuprofen) and low H pollutants (e.g. caffeine) were not removed by this mechanism. The high surface-to-volume ratio of HRAPs may support significant volatilization for high H-values of PhACs, but low concentration will strongly limit mass transfer from the aqueous broth to the atmospheric phase.

To conclude, microalgae-based WWT design and operation enables the development of diverse populations of phototrophic and heterotrophic microorganisms, many of which harbour heterotrophic biodegradation capacities. Microalgae activity may also support bacterial biodegradation via synergistic associations, and phototrophic biomass production likely benefits PhACs removal via biosorption and bioaccumulation (following biomass harvesting and disposal). Microalgae-based WWT systems are also specifically designed to optimize light supply to microalgae, which may enable PhACs photolysis and photodegradation and, in specific cases, pollutant volatilization from open ponds. Photosynthetic activity also drives daily variations in temperature, pH and dissolved oxygen (DO) concentrations that may impact the kinetics of removal mechanisms, including hydrolysis. High HRT operation will finally improve the quantitative significance of all removal mechanisms considered.

Overall, the design and operation of microalgae-based WWT appears to support the removal potential of all known mechanisms. This is especially important given that the efficiency of individual mechanism depends on pollutant structure, meaning microalgae-based WWT should be able to remove a broad range of contaminants (from volatile to biodegradable and hydrophobic). Different removal mechanisms may compete for pollutant removal and thus provide redundancy, and several mechanisms may even be required for complete treatment: for example, bioaccumulation may be required before cells can initiate biodegradation, and biosorption/bioaccumulation may act as a buffer to maintain efficiency under rapidly changing influent pollutant concentration or when the main removal process ceases. This effect was demonstrated by Hom-Diaz et al. [29] who showed the removal of the antibiotic ciprofloxacin was driven by photodegradation during daytime and biosorption at night. Similar results were reported by Norvill et al. [30] for tetracycline. Table 1 provides a summary of the removal mechanisms and their potential significance.

Table 1 Potential significance of main pharmaceutical removal mechanisms in HRAPs (see [14] for further discussion and original references; volatilization is not considered as broadly significant and is therefore not included)

Mechanism	Critical parameters	Relevance to HRAP
Pharmaceuticals are mainly removed via biodegradation and biosorption during conventional WWT. Biodegradation is generally more efficient for soluble pollutants, while biosorption is more efficient for hydrophobic pollutants, but many other factors must be taken into consideration	High biodiversity and aerobic conditions are generally beneficial[a] A high MCRT can be achieved by recycling, retaining, or attaching cells, or by using a large reactor volume (high HRT) A high MCRT increases biodiversity, which increases biodegradation potential	Microalgae can biodegrade numerous pharmaceuticals and indirectly support other degrading species The variability of HRAP broth conditions may enable new biodegradation pathways[b] HRAP are generally operated at high MCRT (=HRT) of 7–10 days, which is comparable to most WWT processes[c]
Sorption onto activated carbon is commonly used for hazardous pollutant removal. Alternative materials can be used, including active biomass, but costs and other issues have hitherto prevented commercialization	Biosorption and bioaccumulation efficiency depends on pollutant structure, cell biology and biochemistry. At constant biosorption affinity/bioaccumulation ratio, biomass productivity determines the amount of pollutant removed when biomass is harvested	Microalgae can enhance pharmaceutical removal via biosorption and bioaccumulation. Algae activity also boosts the overall biomass productivity
Photolysis and photodegradation (direct or indirect) are powerful mechanisms of pollutant removal in many aquatic environments	Removal kinetics and efficiency are highly dependent on wastewater chemistry (interfering substance or sensitizers), light attenuation and individual pollutant chemistry	High HRT operation and mixing increase the probability of pollutant exposure to UV and/or radicals. Algal biomass causes rapid light attenuation, but algal activity may enhance photodegradation via the production of radicals and photosensitizers and/or by increasing pH, DO and temperature during daytime
Hydrolysis enables the breakdown of large biopolymers prior to their biodegradation	Hydrolysis is highly dependent on wastewater composition, pollutant chemistry and HRT	Hydrolysis is only significant for a few pharmaceuticals. The combination of high HRT operation, high pH and high temperature may increase hydrolysis

[a]Reduced pollutants, such as pentachlorophenol, are often best biodegraded anaerobically by serving as electron acceptor. Anaerobic degradation is however often incomplete and must be completed by aerobic treatment

[b]Facultative ponds are stratified (from anaerobic in the sedimentation zone to aerobic near the surface) and other stabilization ponds can be anaerobic or aerobic. Most 'intensive' WWT processes are operated aerobically, but certain activated sludge process configuration supports cell recycle between anaerobic, anoxic and aerobic zones. Biofilm bioreactors are generally operated aerobically but anaerobic micro-zones can be found in deep biofilm (or biogranules)

[c]Higher MCRT are used in membrane and biofilm bioreactors targeting hazardous pollutants

3 Algae Reactor Design for Wastewater Treatment

The geometry and operation of photobioreactors (PBs) determine how light is supplied to the microalgae and, therefore, how efficient the microalgae use this light during photosynthesis. The light source and its intensity are critical factors for microalgal growth, and light must often be 'diluted' throughout the entire culture, while excessive shading should be reduced by mixing. Several external factors affect microalgae activity during cultivation outdoors, such as day/night cycles and seasonal or geographic changes in climatic conditions. The pH of the culture medium affects the biochemical reactions taking place and is often optimal around pH 7–9. During daytime, pH can increase if the photosynthetic uptake of CO_2 exceeds its replenishment rate via atmospheric diffusion and microbial respiration, and in these cases, the pH also decreases overnight as photosynthesis stops [31].

3.1 Photobioreactor Design

Works on large-scale algal cultures began in the United States in 1948–1950, and the first significant outdoor pilot plant studies on the production of *Chlorella* were carried out in 1951 (Massachusetts, USA). It was not until the 1950s that commercial farming started in Germany. The potential of microalgae to provide oxygen and capabilities for biomass valorization during wastewater treatment was also demonstrated in the USA in the 1960s. Microalgae-based WWT indeed provides additional capacities for nutrient and organic matter removal at a much lower energy requirement compared to conventional activated sludge systems relying on mechanical aeration [32, 33]. Microalgae-based WWT has therefore been demonstrated to deliver economic and efficient treatment [34, 35], and, from the perspective of algae cultivation, domestic wastewater streams are readily available and cost-effective substrates for microalgal biomass production [36–39]. Many bioreactors can be used for the dual purpose of removing nutrients from wastewaters and growing biomass, and several reviews have discussed these aspects in detail [4, 40–44]. Algae cultivation systems are commonly classified as open and closed systems as further discussed below.

3.1.1 Closed Systems

One of the first closed photobioreactors was built by Gudin [45] as a pilot plant with a total working volume of 6.5 m^3. This reactor was operated for several years to cultivate *Porphyridium cruentum* (productivity: 20–25 g/ (m^2·d)) for the production of an exocellular sulphated polysaccharide used as a thickening agent. Many designs have been tested at the laboratory or pilot scale since this pioneer work (Fig. 2), including vertical reactors, flat plate, plastic bags, green wall panel and annular and

Fig. 2 Microalgal closed photoreactors. Top: vertical column; Bottom left: flat panel; Bottom right: horizontal tubular

tubular reactors. The use of closed photobioreactors prevents direct evaporation, minimizes contamination risks and CO_2 losses and potentially enables high light utilization efficiencies boosting biomass productivity; all these attributes also lead to more reproducible conditions. The disadvantages of closed PBs include high construction costs, biomass fouling, sedimentation, leakage [46] and difficulties in economically controlling temperature during outdoor cultivation. In addition, the scale-up of closed systems can be limited by high respiration losses at high cell density operation [47]. Consequently, closed PBs are currently best used for the cultivation of pure strains and high-value algal products [48], and their design seeks to optimize light supply, mixing and mass transfer according to the unique physiological and growth characteristics of the strain cultivated.

In order to reduce equipment costs, transparent bags (i.e. plastic) have been located on rigid frames [49]. Tubular systems are widely used and made of transparent pipes with small internal diameters (the working volume can be increased by extending the tube length). The main disadvantage of tubular PBs is the high mixing energy consumption in long continuous tubes [44]. Vertical column PBs are made of vertical tubing equipped with a gas sparging system installed at the bottom of the

reactor as agitation system. Bubble column and airlift reactors are examples of vertical column PBs [44]. The configuration of column PBs is simple as these reactors contain no moving parts. Hence, microalgae only experience mild shear stresses. This configuration also support efficient gas-liquid mass transfer, and bubble behaviour plays an important role in CO_2 transfer in the microalgae solution, which in turn affects microalgal growth and CO_2 uptake efficiency. Flat panel PBs are simple and advantageous in terms of biomass production and energy consumption as large illumination surface areas can improve light supply. Mixing is achieved using rising air bubbles.

3.1.2 Open Systems

Open ponds are the most common platform used for large-scale outdoor microalgae cultivation due to their relatively low capital and operational costs. They are also the most suitable for WWT, where they are usually known as high rate algal ponds (HRAPs). In these systems, microalgae are cultivated in 0.2–0.5 m deep open channels stirred with a paddle wheel, which is economic and easy to build and operate. On the downside, open ponds are prone to contamination, due to constant exposure to atmospheric pollutants, and prone to low productivity resulting from poor mixing, low light penetration and difficult control. Evaporation losses can also be considerable.

Microalgae can be found in three main types of ponds used for wastewater treatment: facultative ponds, maturation ponds and HRAPs (Fig. 3). Facultative

Fig. 3 Microalgal open system: high rate algal pond

and maturation ponds are often collectively referred to as waste stabilization ponds (WSP) or WWT lagoons and are widely used by farms and small communities [50–52]. While facultative ponds are 1–2 m deep and receive raw WW or anaerobic waste effluent, maturation ponds are 1–1.5 m deep and receive secondary effluents from conventional WWT: maturation ponds are therefore used for tertiary WWT with the main objective of pathogen removal [14]. A major disadvantage of WWT ponds is the relatively large land requirement compared with electromechanical treatment systems [5]. HRAPs retain most of the advantages of conventional ponds (simplicity and economy) but overcome many of their drawbacks (poor and highly variable effluent quality, limited nutrient removal). Critically, HRAPs also recovers wastewater nutrients as a harvestable algal/bacterial biomass that can be used as fertilizer or biofuel [5]. The effluent can be partially recycled in order to compensate evaporation losses [53]. HRAPs require low power input and are relatively easy to maintain [47]. They can be constructed of concrete, glass fibre or membrane [54] and can receive raw wastewater, primary effluent or effluent from a previous waste treatment pond [14]. A HRAP combined with gravity settling pretreatment (to remove suspended solids) and followed by secondary clarification (to remove algal/bacterial biomass) would fit within the footprint of an existing two-pond oxidation pond system [5]. HRAPs are operated under long HRT (8–14 d) to enable sufficient phototroph growth to provide oxygen for aerobic biodegradation. Daily variations in pH and dissolved oxygen (DO) occur due to photosynthetic activity, and pond temperature has a direct influence on cell density [55]. Pond performance and ecology (e.g. algal/bacterial dominance) can also be affected by environmental conditions.

HRAPs have now also been studied for emerging contaminant (EC) removal [55, 56]. Although single-species microalgae cultures have been applied for nutrient and EC removal [15, 57–59], it is difficult to maintain a microalgal monoculture in open processes. Accordingly, several studies have reported the advantages of co-cultivated photosynthetic microorganisms during WWT. Koreivienė et al. [60] thus reported high nutrient removal efficiencies from municipal WW using a non-native consortium composed by *Chlorella* sp. and *Scenedesmus* sp. Renuka et al. [61] described the potential of using a consortium of non-native and native species to obtain a self-flocculating culture in the treatment of a primary-treated sewage effluent. Most of the consortia reported involve *Chlorella* and *Scenedesmus* species [60–63] (Box 2).

Box 2 Full-Scale Wastewater Treatment Plant: A Case Study

An important example of full-scale wastewater treatment plant is the All-Gas project in the El Torno treatment plant in Chiclana, supported by the European Union. It was initiated to demonstrate the sustainable large-scale production of biofuels based on the low-cost cultivation of microalgae. It is the largest site in the world for the production of biofuel from algae harvested using wastewater. Pretreated wastewater is transformed into an algae biofuel which provides four times the distance of conventional biofuels such as sugar ethanol or palm oil

(continued)

Box 2 (continued)
diesel per L. The complete process chain is designed for a cultivation area of up to 10 ha, making wastewater treatment energy self-sufficient, and recycling the nitrogen and phosphorus from wastewater into microalgal biomass. The full process chain at demonstration scale has been implemented, consisting of:

- A cultivation area composed of four raceway ponds with a surface area of 200 m^2 each in which an average of 100 ton/ha-year biomass can be produced (Fig. 4).
- A separation and thickening system based on dissolved air flotation (3 units). At this stage, the algae broth is concentrated 100 times at very low energy requirement.
- A 750 m^3 anaerobic digester in which more than 200 L of biomethane per kg of added volatile solid can be produced.
- The demo operation showed that each hectare of algae culture has a wastewater treatment capacity of around 1,000 m^3/d, producing biomethane above 13,000 kg CH4/year – enough to fuel 20 cars (at 4.5 kg/100 km and 15,000 km/year). This represents four times more than what is achieved with conventional biofuels, such as bioethanol from sugarcane or biodiesel from palm oil, both yielding about 5,000 L/ha/year.

All-Gas project is an example of circular economy, since a waste is converted sustainably into raw materials with added value, providing an innovative and environment-friendly process.

Fig. 4 Raceway ponds of All-Gas project in the El Torno treatment plant in Chiclana, Spain (EU FP7 All-Gas project). Source: AQUALIA

4 Removal of Pharmaceutical Compounds from Wastewaters in Microalgae Reactors

The mechanisms for pollutant removal involved during conventional or advanced treatments cannot be directly extrapolated to algae-based wastewater (WW) treatment because algae photosynthesis causes specific environmental and ecological conditions to occur [14]. Unfortunately, PhACs removal has not yet been extensively studied during microalgae-based WWT, and Table 2 summarizes published results on the elimination of PhACs in algae photobioreactors in synthetic WW and real WW. Some of these studies are also discussed in recently published reviews on organic micropollutant removal [13, 44, 72, 73].

4.1 Pharmaceutical Removal in Synthetic WW

The first specific study on PhACs removal during microalgal WWT treatment was published by de Godos et al. [22] who reported the removal of the antibiotic tetracycline from synthetic WW using a pilot-scale HRAP (14 L). Tetracycline was added after 46 days of operation and reached a stable value of 0.79 ± 0.02 mg/L. Active removal was confirmed with an efficiency increasing from 60 ± 1% on day 61 to 69 ± 1% on day 76. The main mechanisms involved were photodegradation and biosorption.

Another antibiotic was selected by Hom-Diaz et al. [29] who evaluated the removal of ciprofloxacin (CPX) in indoor and outdoor HRAPs. These microalgae systems were fed with primary wastewater effluent. When spiked at 2 mg/L, CPX was efficiently removed from photobioreactors continuously operated under various conditions of artificial illumination and hydraulic residence times (7–3 d). These findings were confirmed in a 1,000 L pilot HRAP operated outdoors (Fig. 5) and laboratory batch assays performed using HRAP microcosms incubated under the same outdoor conditions. Spiked experiments were conducted by stopping the wastewater influent supply during 24 h and immediately adding CPX to an initial concentration of 2 mg/L. HRAP was also monitored throughout the day to assess photodegradation and sorption. CPX removal occurred immediately after spiking in all assays, independent of the time of the day. There was a pollutant removal of 15% during daytime and 18–22% at night time. The final CPX removal percentage was 38% after 24 h. Photodegradation also took place during continuous CPX removal, and it was not strongly influenced by the suspended biomass concentration. Hom-Diaz et al. [29] concluded that the main CPX removal mechanisms were sorption and photodegradation, although biodegradation may also occur.

Some ECs can be transformed into metabolites that are even more detrimental to human health and natural ecosystems than their parent compounds. Ismail et al. [64] therefore studied the degradation of a mixture of the analgesics ketoprofen, paracetamol and aspirin by focusing on toxicity reduction and the elimination of the

Table 2 Studies of PhACs removal in algae photobioreactors treating synthetic or real WW

Reactor type	PhAC	WW	Operational description	Removal (%)	References
HRAP (14 L)	Tetracycline 0.79 ± 0.02 mg/L	Synthetic	Addition at the steady state	60–69 ± 1%	de Godos et al., [22]
HRAP (1,000 L) (Fig. 5)	Ciprofloxacin 2 mg/L	Urban WW primary influent	Batch experiments a) Sunrise spike (24 h) b) Sunset spike (night) c) Sunset spike (24 h)	a) 15% during light hours, up to 23% at the end of the experiment b) 18% c) 22% during night hours, up to 38% at the end of the experiment	Hom-Diaz et al., [29]
Stirred tank (5 L)	Ketoprofen 0.5 mM, paracetamol 0.25–0.5 mM, aspirin 0.5 mM	Synthetic	HRT 3d–4d	95% removal (HRT 4d)	Ismail et al., [64]
Tubular periphyton PB (30 L)	Carbamazepine ibuprofen, hydrochlorothiazide, gemfibrozil 10 μg/L each one	Synthetic	HRT 2d–4d	Carbamazepine 6.45%, ibuprofen 48.7%, hydrochlorothiazide 26.2%, gemfibrozil 20.6%	Kang et al., [65]
Semiopen multitubular PB (1,200 L) (Fig. 6)	17β-Estradiol (2 mg/L)	Toilet WW	Batch operation	Night 81% Day 77–55%	Parladé et al., [66]
HRAP (470 L) (Fig. 7)	26 emerging contaminants 0–24 μg/L	Urban WW primary influent	HRT 4d-8d	84–59% (warm and cold season, respectively) Ibuprofen, ketoprofen, naproxen, diclofenac, carbamazepine: average 40% and 62% at 4d and 8d, respectively	Matamoros et al., [56]
Open PB (650 L)	52 PhACs 0.16–2.9 μg/L	Urban WW and CO_2	Repeated (7 d)	a) >90% for atenolol, bisoprolol, metoprolol, clarithromycin, bupropion, atracurium, diltiazem and terbutaline b) 50–90% for 14 PhACs c) Very low or nonquantifiable for 18 PhACs,	Gentili and Fick, [67]

(continued)

Table 2 (continued)

Reactor type	PhAC	WW	Operational description	Removal (%)	References
				11% Carbamazepine	
Semiopen multitubular PB (1,200 L) (Fig. 6)	16 PhACs anti-inflammatories 50–59 μg/L, antibiotics 65–5,662 ng/L	Toilet WW	HRT 8d-12d	Anti-inflammatories >98% Antibiotics >48%	Hom-Diaz et al., [68]
HRAP (9,600 L)	64 PhACs average concentration 223 mg/L	Urban WW	HRT 6d	Analgesics and anti-inflammatories >85% Diclofenac 55–71% Antibiotics 63% Other 77%	Villar-Navarro et al., [69]
Semiopen multitubular PB (8,500 L) (Fig. 8)	PhACs and other micropollutants The highest for carbamazepine 510 ng/L	Agricultural *	HRT 16 d	Ibuprofen 50% Diclofenac 61% Carbamazepine <40%	García-Galán et al. [70]
HRAP (470 L) (Fig. 7)	12 PhACs and 26 TPs 166–23,811 ng/L	Urban WW	HRT 4.5 d	Acetaminophen 100% Ibuprofen 79% Sulfamethoxazole 80% Diclofenac 53% Carbamazepine 0%	García-Galán et al. [71]

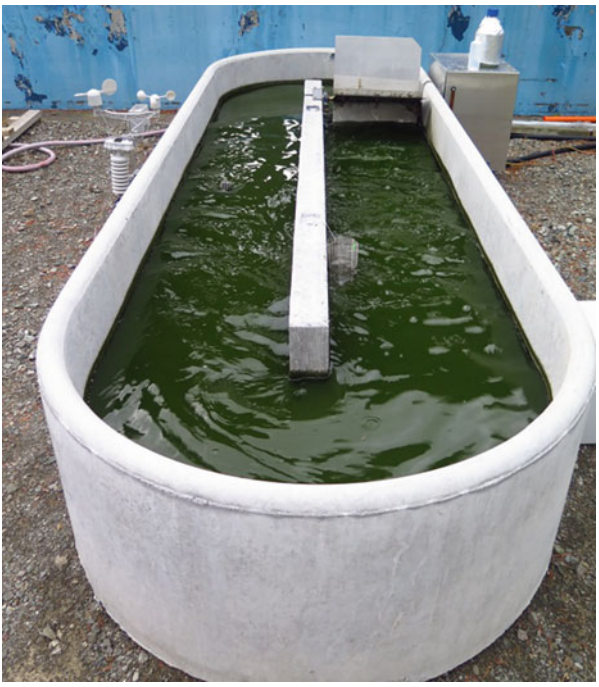


Fig. 5 Concrete 1,000 L HRAP located in Palmerston North, New Zealand. Courtesy of Dr. Andrea Hom-Diaz

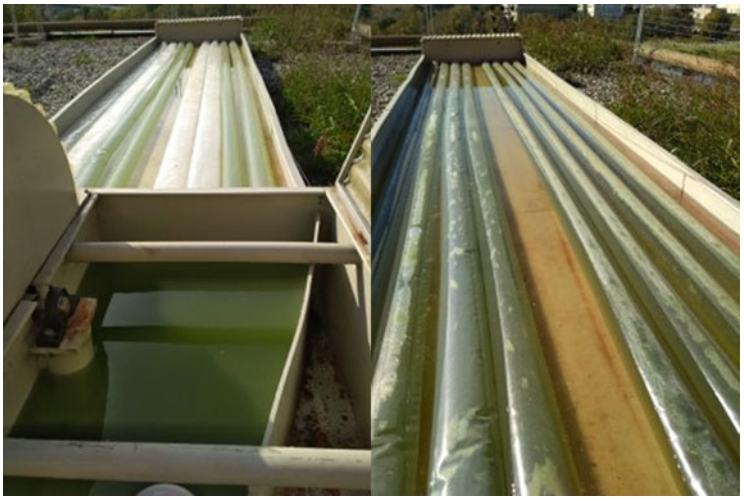


Fig. 6 1,200 L semiopen multitubular PB located on the roof of the Chemical, Biological and Environmental Engineering Department at the Universitat Autònoma de Barcelona, Spain. Courtesy of Dr. Andrea Hom-Diaz

Fig. 7 470 L HRAPs located outdoors at the laboratory of the GEMMA research group (Universitat Politècnica de Catalunya – BarcelonaTech, Spain). Courtesy of Dr. Ivet Ferrer

Fig. 8 Full-scale semi-closed tubular PBR located in Agropolis, Barcelona, Spain. Courtesy of Dr. Ivet Ferrer

metabolites formed. An artificial microalgal-bacterial consortium was used in a stirred-tank photobioreactor. The toxic paracetamol metabolite p-aminophenol was formed at a HRT of 3 days, and increasing the HRT to 4 days enhanced

bioremediation efficiency. After acclimatization under continuous illumination, both the analgesics and p-aminophenol were removed by 95%.

Kang et al. [65] employed periphyton to treat a synthetic wastewater spiked with the pharmaceuticals and personal care products (PPCPs) carbamazepine (CBZ), ibuprofen (IBU), hydrochlorothiazide (HCT) and gemfibrozil (GEM) as well as the industrial chemical bisphenol A (BPA). The photobioreactor was made of acrylic glass and divided into five channels. The reactors were illuminated using LED lamps, and four treatments with different illuminances (7,000–5,000 lx) and HRT (4-2d) were carried out. After 10 d of pollutant-free operation, standard solutions of the PPCPs were added to the storage tanks resulting in a concentration of 10 μg/L for each compound. CBZ and GEM were poorly removed (6.45–20.6%), HCT and IBU were moderately removed (26.2–48.7%), and BPA was effectively removed (72–86.4%). High HRT operation enhanced nutrient and PPCP removal, but change in illumination period had contrasting effects on the nutrients and PPCP removals, suggesting that dark/light illumination cycles supported the development of microbial communities with high PPCP removal capacity. These results agree with the findings by Hom-Diaz et al. [68] who reported that there is no correlation between the removal of PhACs and biomass concentration in the photobioreactor. Kang et al. [65] thus suggested that changes of dark/light illumination periods could be a strategy to improve micropollutant removal in periphyton photobioreactors.

Few publications have studied the evolution of bacteria and microalgae populations and their influence on pollutant removal in photobioreactors. Kang et al. [65] identified algae belonging to 4 phyla and 11 genera in their study. The cyanophyta *Phormidium* accounted for over 94% of the total algae DNA extracted. As *Phormidium* was therefore likely the dominant algal genus in all treatments, the differences in nutrient and PPCP removal efficiencies observed should be more related to the bacterial composition. Kang et al. [65] reported that a high bacterial diversity favoured nutrient removal but that a lower bacterial diversity was associated with better PPCP removal.

The hormone 17β-estradiol (E2) is a widespread emerging contaminant (EC) detected in urban and industrial WW [74]. Due to their environmental fate and toxicological effects, the EU commission has proposed to list E2, diclofenac and 17-α-ethinylestradiol (EE2) as priority substances. Although the concentrations currently detected in WW are in the range of ng to μg/L, E2 and EE2 can induce adverse ecological effects and can act as endocrine disruptors [75]. High E2 removal has been achieved in microalgal monocultures in spiked experiments [59, 76]. Parladé et al. [66] later aimed to assess how E2 was removed and how microbial community changed during 1 year of photobioreactor operation for treating toilet wastewater. Briefly, the system was designed as a 1,200 L semiopen multitubular PR (Fig. 6). Two distribution chambers were placed at each end of the tubes to transfer and distribute the culture evenly between the tubes. The PB was operated at HRTs of 8 and 12 days. E2 spiking experiments were conducted during steady-state operation corresponding to different seasons of the year.

Complete E2 removal was achieved during the first assay (8d HRT) and 81% of the removal took place during the night. Two assays conducted at 12 d HRT showed

lower final removal (77% and 55%), but the behaviour was similar as rapid removal took place during the first 10–13 h followed by a slower removal rate. Complete removal of the hormone could thus be attained under favourable seasonal conditions at temperatures between 10 and 25°C, and more than 50% of the pollutant was still removed in 24 h at low temperature and under low solar irradiation. Estrone (E1) has been reported as an E2 biodegradation product from the microalgae *Chlorella vulgaris* [77], although it can also be formed during E2 oxidation processes. In the study of Parladé et al. [66], E1 was formed at the same rate as E2 disappeared, and a rapid conversion occurred at the beginning of the experiment. The transformation rate decreased after 5 h, and E1 formation was still taking place at the end of the experiment since E2 was not completely removed. E1 was formed in the presence of active algal biomass but was not detected in the killed controls, under either light or dark conditions, demonstrating that E1 was a product from biodegradation. Sorption ranged from 8% to 50%, suggesting that E2 sorption varied with biomass composition. As earlier reported by Hom-Diaz et al. [59], algal biomass composition has a direct effect on the adsorption of pollutants. Parladé et al. [66] concluded that both adsorption and biodegradation were fast both in light and in darkness.

Parladé et al. [66] also evaluated the effect of bioaugmentation with a pure *Scenedesmus* culture on E2 removal. Eukaryotic diversity was assessed using denaturing gradient gel electrophoresis, and samples were taken immediately before and after 24 h of each E2 spike. No changes occurred after E2 addition, suggesting that the oestrogenic compound did not have any effect on populations. The analysis revealed the presence of five different phyla within the Eukarya domain and two unclassified sequences. Briefly, the PB microalgae flora was dominated by *Chlorella* species which remained present at all stages, followed by *Pseudospongiococcum* in less abundance. Other abundant phylotypes, which were only present episodically, included protozoa, rotifer or parasitic chytrid species that fed on the algae. It is likely that changes in the microbial community were partially responsible for the differences in E2 removal reported. Bioaugmentation with *Scenedesmus* could not outmatch the native PB community in terms of biomass yield. The PB community was composed of transient species that appeared and disappeared over time and stable microalgae like *Chlorella*, which remained unaffected by seasonal variations. Parladé et al. [66] concluded that a high biodiversity with some well-established species was preferable to a high biomass concentration for E2 removal.

In addition to supporting satisfactory WWT efficiency, the biomass accumulated during microalgae-based WWT can be valorized as, for example, animal feed, soil conditioners, plant fertilizers or biofuel [72]. Ismail et al. [64] extensively analysed the biomass harvested and reported total crude protein, carbohydrate and lipid levels of 50%, 16% and 7%, respectively. According to Brown et al. [78], the levels of protein, carbohydrate and lipid can range from 6% to52%, 5% to 23% and 7% to 23%, respectively, in microalgal biomass. The high level of crude protein reported by Ismail et al. [64] is likely a consequence of the high nitrogen supply. Moreover, the total protein concentration of the harvested biomass was rich in essential (65.5%), nonessential (29%) and conditional amino acids (5.5%), which suggests a ‘high-quality protein-rich’ biomass suitable for use as animal feed. Interestingly,

the harvested biomass contained a good blend of medium and long-chain fatty acids that make it a good biodiesel-producing candidate. The water-extractable fraction of the biomass possessed a high phenolic content and antioxidant capacity. Ismail et al. [64] concluded that microalgae-based WWT could provide an integrated eco-friendly and cost-efficient strategy for remediating pharmaceutical wastewater.

4.2 Pharmaceutical Removal in Real WW

Matamoros et al. [56] studied the effect of HRT and meteorological conditions on the removal efficiencies of 26 ECs (pharmaceuticals, fragrances, fire retardants and pesticides, among others) in two HRAP pilot plants fed with urban wastewater. The pilot plant was located outdoors at the laboratory of the GEMMA research group (Universitat Politècnica de Catalunya – BarcelonaTech, Spain). Urban wastewater was pumped from a municipal sewer to a homogenization tank. From there, the wastewater was screened and conveyed to two identical parallel lines, each equipped with a primary settler, a pilot high-rate algal pond and a secondary clarifier for biomass separation (Fig. 7). The experimental HRAPs were made of PVC raceway ponds equipped with a paddle wheel for stirring the mixed liquor. Each HRAP had a nominal volume of 0.47 m^3 at a water depth of 0.3 m and a surface area of 1.54 m^2.

The concentration of ECs in the HRAP influent ranged from undetected to 24 μg/L. Caffeine, acetaminophen and ibuprofen were usually detected at >9 μg/L in the influent, and these pollutants were efficiently removed (>90%). Ketoprofen and naproxen were removed by 60–90%, diclofenac and diazinon were removed by 40–60%, while carbamazepine was poorly removed by <40%. The results showed removal efficiencies varied greatly with pollutant chemistry. The most abundant pollutants in the biomass were the most hydrophobic ones, such as musk fragrances, but adsorption did not seem to be a quantitatively significant removal mechanism for any PhACs. No significant differences in removal performance were observed at the two HRTs tested during the warm season, but significant differences were found during the cold season for those compounds previously known to be affected by biodegradation (i.e. caffeine, ibuprofen and naproxen), photodegradation (i.e. ketoprofen and triclosan) and sorption or volatilization (i.e. musk fragrances). Most of the compounds were nevertheless removed at 4 d HRT in both seasons. In contrast to other authors, Matamoros et al. [56] reported that the effect of seasonality on pollutant removal performance was low or null (i.e. for caffeine, acetaminophen and ibuprofen) for the most abundant compounds. To assess the potential for detoxification, Matamoros et al. [56] computed the hazard quotients (HQs) based on the chemical composition of water samples and predicted non-effect concentrations (PNECs) for different aquatic organisms. Acetaminophen, ibuprofen and oxybenzone exhibited high HQs in influent wastewater samples (HQ > 1), mainly due to their high concentrations. After treatment in the HRAP system, all the studied ECs had an HQ < 1. Matamoros et al. [56] thus concluded that HRAPs were at least

as efficient as conventional activated sludge, constructed wetlands and waste stabilization ponds for EC removal and detoxification.

While algae can be simultaneously used for WWT treatment, CO_2 abatement and bioenergy production, few studies have considered the potential use of cultivating algae to simultaneously treat flue gases and municipal wastewater containing pharmaceuticals. Gentili and Fick [67] investigated the removal of pharmaceuticals in urban WW in a 650 L open outdoor photobioreactor supplied with flue gas. The photobioreactor was constructed following the open-pond principle, where water flow was generated by a mechanical device. It was supported by a metal frame and constructed of thin fibreglass to allow light penetration from the top (as in a traditional open pond), sides and bottom. The reactor also had an empty space in its centre to improve illumination. Municipal wastewater was treated during 1 week in several batches. Flue gas (9% CO_2) from a combined heat and power plant incinerating municipal and industrial solid wastes (Umeå Energi, Umeå, Sweden) was bubbled through the algal culture at a flow rate of approximately 3 L/min. The pH in all batches was 8.3 ± 0.9. Bubbling was stopped at night to prevent medium acidification in the absence of light. Experiments were conducted from April to May 2012 to study the performance of the algae under different environmental conditions of light and temperature (10–32°C). The concentrations of the 52 pharmaceuticals detected ranged from 0.16 to 2.9 μg/L. Removal efficiencies were very high (>90%) for atenolol, bisoprolol, metoprolol, clarithromycin, bupropion, atracurium, diltiazem and terbutaline, moderate (50–90%) for 14 PhACs and very low or nonquantifiable for 18 PhACs. These results agreed with past studies on different biological WW treatment processes [79, 80]. Many pharmaceuticals were metabolized and excreted as glucuronides or other conjugated metabolites that can be converted back to their parent compounds by enzymatic processes. Deconjugation can indeed occur in sewage treatment processes and fungal WW treatment, showing negative removal efficiencies for macrolide antibiotics, carbamazepine and other pharmaceuticals. Several pharmaceuticals were degraded more efficiently in the algal photobioreactor than in the influent treated without microalgae. On average, only 8 pharmaceuticals were not removed nor had negative removal rates in the presence of microalgae compared to 20 without microalgae. The reduction in pharmaceuticals content was positively correlated with light intensity inside the culture, dissolved oxygen concentration and the reduction in nitrogen content. Some authors have suggested that photolysis is a critical removal pathway for several micropollutants including pharmaceuticals [56, 81]. However, and although Gentili and Fick [67] recognized the importance of this mechanism in the elimination of PhACs, the removal of PhACs was lower in a control PB free-of-algae than in the inoculated system.

Hom-Diaz et al. [68] studied the removal of PhACs in an outdoor pilot tubular PB treating toilet WW at HRTs of 8 days and 12 days (Period I and Period II, respectively). The experimental set-up was previously described (Fig. 6). Toilet WW was collected from the toilet drain of the Chemical, Biological and Environmental Engineering Department (Universitat Autònoma de Barcelona) and settled. The supernatant was conducted to a second settler from which the WW was pumped

into the microalgal PB. The HRT of the settlers (also used as homogenization tanks) was 48 h. The PB treated 150 L/day (8 d HRT) in Period I and 100 L/day (12 d HRT) in Period II. The PBR performance was monitored from September to December 2015. Nutrient removal efficiency was very high but slightly different for the two periods. No large differences in the influent PhAC levels were observed between October and December. Five anti-inflammatory compounds (acetaminophen, ibuprofen, naproxen, salicylic acid and ketoprofen) were detected: acetaminophen (paracetamol) was found at the highest concentrations (50.2–58.7 μg/L) in both sampling periods and was removed by >98%. Ibuprofen, the second most abundant compound detected in the influent WW during both operating periods (39.0–52.8 μg/L), was also removed by >98%, evidencing again a high biodegradability noted in past studies. The antibiotics ciprofloxacin and ofloxacin were detected at concentrations of 2,629–294 ng/L and 5,662–65 ng/L, respectively. Azithromycin was only detected in Period I (385 ng/L), and erythromycin was only detected in Period II (661 ng/L). Antibiotic removal efficiencies varied between periods, but remained >48%. Higher removal efficiencies were reported during Period II despite the lower temperature and light irradiation. Hom-Diaz et al. [68] stated this could be a consequence of the HRT increase from 8 to 12 days, in agreement with the study by Matamoros et al. [56]. Hom-Diaz et al. [68] also studied the harvesting of the algal biomass since its separation from the treated effluent is essential for discharge compliance and biomass valorization.

Villar-Navarro et al. [69] studied the efficacy of PhACs removal from urban wastewater by comparing a conventional treatment using activated sludge against a nonconventional treatment scheme involving HRAPs. This research was performed at the WWTP of Cadiz (Spain). The nonconventional treatment line was a pilot-scale installation within the WWTP (Fig. 4) consisting of three upflow anaerobic sludge blanket (UASB) reactors followed by six HRAPs. These were open shallow ponds (9.6 m^3 each) equipped with a paddle wheel. After grit and grease removal, the WWTP influent was diverted from the conventional to the nonconventional line. The wastewater was either treated through the UASB reactors before entering the HRAPs (February to April 2015) or directly directed onto the HRAPs (May to July 2015). The HRT in the HRAPs was between 72 and 216 h (144 h average). The HRAPs removed 74% and 92% of dissolved nitrogen and phosphorous respectively, while the conventional treatment only satisfactorily removed phosphorus, probably due to insufficient HRT in the biological reactor. Sixty-four out of 81 target PhACs were detected in the WW influent, at an average total concentration of 223 mg/L. Analgesics and anti-inflammatories were the predominant group in the influent (>100 mg/L) and were removed above 85% during both conventional and nonconventional treatments. The average removal efficiency of diclofenac in the conventional line was 19%, where it was never exceeding 30%. In comparison, this pollutant was removed between 55 and 71% following HRAP treatment. These results agreed with past demonstrations of the influence of light on diclofenac removal. Removal from the HRAP was also higher for antibiotics (63%) and other PhACs (77%). The average removal efficiencies were however similar (94% vs. 92%) for both treatment lines when comparing total PhACs concentrations.

The research by García-Galán et al. [70] focused on the treatment of agricultural runoff water for biomass production. Agricultural runoff can contain a wide variety of hazardous contaminants including pesticides, heavy metals and numerous veterinary PhACs (mainly antibiotics and their metabolites). This study was performed over two different periods (December and April) in batch and continuous (16 d HRT) modes. The PB was a full-scale (8.5 m^3) semi-closed hybrid tubular horizontal photobioreactor (HTH-PB, Fig. 8), similar to the reactor used by Hom-Diaz et al. [68], located at the Universitat Politècnica de Catalunya facilities (Barcelona, Spain). The feed for the HTH-PB was pumped from a drainage channel that runs through different cropped fields in the area, and it also received the discharge from an urban WWTP.

PhACs removal was evaluated during four consecutive days at the end of the continuous experiment. Carbamazepine was detected at the highest concentration in the influent (average 510 ng/L) followed by diclofenac (average 150 ng/L). The poor removal efficiency obtained for carbamazepine (<40%) agrees with prior results in microalgae systems [56, 67]. The best removal efficiency was achieved with diclofenac (61%). Diclofenac removal rates can range from 0% to 90% during municipal wastewater treatment, but Villar-Navarro et al. [69] concluded diclofenac removal was higher in algae systems (55–71%) than during conventional treatment with activated sludge. These results agree with findings from Zhang et al. [82] who reported that the removal of diclofenac can be mostly attributed to photodegradation, while carbamazepine could not be photodegraded, biodegraded or adsorbed. The removal of ibuprofen achieved by García-Galán et al. [70] was low when compared to removals obtained in HRAP studies [56, 69] and in a similar tubular PB [68].

García-Galán et al. [71] recently published a new research related to PhACs removal in microalgal systems using the set-up described by Matamoros et al. [56]. Two HRAP systems (Fig. 7) were tested with and without primary treatment during the beginning of summer (high solar irradiation). The aim was to assess the effect of primary treatment on the removal of 12 PhACs and 26 of their main metabolites in urban wastewater. Each HRAP had a volume of 470 L and was operated continuously at 4.5 d HRT. The concentration of the 12 PhACs and their transformation products detected (15) before and after primary treatment was not statistically different. The analgesic ibuprofen (IBU) was detected at the highest concentrations (330–23,811 ng/L). Diclofenac (DCF) and acetylsalicylic acid (AcSAc) were detected in all influent samples at 271–2126 ng/L and 166–5,428 ng/L, respectively. Acetaminophen (ACM) was detected up to 7,800 ng/L, and the antibiotic sulfamethoxazole (SMX) was detected in all influent samples at 70–900 ng/L. This compound is considered ubiquitous in natural systems and has been identified as the main responsible for the increase of sulfonamide resistance genes in WWTPs [83]. The antidepressant venlafaxine (VFX) and the antiepileptic carbamazepine (CBZ) were present in all the influent samples at average concentrations ranging between 664 and 702 ng/L and at concentrations <33 ng/L, respectively. The very low concentration of CBZ detected is not surprising since CBZ can be found in conjugate form in wastewater and therefore its concentration is often underestimated. Some PhACs metabolites were already

detected in the influents, including metabolites of IBU, CBZ and SMX, as reported by Jelic et al. [84] who noted degradation may occur in sewer networks. The results showed pretreatment had no impact on PhACs removal from the HRAPs. SMX and metronidazole (MTZ) removal averaged 80% and > 89% respectively, but these pollutants were not detected in biomass algae, which suggests that sorption did not cause their removal. García-Galán et al. [71] therefore concluded that biodegradation should be considered as the main elimination route of the antibiotics during microalgae treatment. High removals were also obtained for ACM (100%) and IBU (79%), in agreement with past studies early cited. DCF was removed around 53%, which is much lower than reported by Matamoros et al. [56] using the same experimental HRAPs during the warm/summer season. DCF was however detected at quite high concentrations in biomass samples, so bioadsorption/bioaccumulation could partly account for its elimination. CBZ was not removed, confirming the low biodegradability of this drug, although other authors obtained removals of 23–60% in HRAPs [56, 69]. García-Galán et al. [71] concluded that HRAPs could be operated without primary treatment and provided high removal efficiencies for a broad range of PhACs, including metabolites and transformation products. Bioadsorption/bioaccumulation to microalgal biomass was needed to remove non-biodegradable compounds such as venlafaxine.

5 Concluding Remarks

Overall, nearly all studies conducted at laboratory to full scale and using synthetic media or real wastewater converge in demonstrating that microalgae-based wastewater technologies support efficient removal of pharmaceutical compounds during WWT, with the added advantage of removing more nutrients, producing potentially valuable biomass and reducing energy demand in comparison to mechanically aerated treatment systems. Biodegradation is often suspected as the main removal mechanism, but biosorption/bioaccumulation and photodegradation can also be important. Because pollutant removal by specific removal mechanisms is highly dependent on pollutant chemistry, the broader range of pollutant-removing mechanism potentially taking place during microalgae-based WWT should provide more efficient and resilient pharmaceutical removal.

The HRT is a key design parameter for the removal of microcontaminants during microalgae-based WW treatment (as in the absence of cell retention or recycling, the HRT is also the mean cell residence time). The relative long HRTs used during microalgal-based treatment systems should therefore favour all removal mechanisms, including especially photodegradation and hydrolysis. Experimental data indeed confirm high HRT operation is generally beneficial to pollutant removal.

References

1. Shilton AN, Powell N, Guieysse B (2012) Plant based phosphorus recovery from wastewater via algae and macrophytes. Curr Opin Biotechnol 23(6):884–889. https://doi.org/10.1016/j.copbio.2012.07.002
2. Plouviez M, Shilton A, Packer MA, Guieysse B (2019) Nitrous oxide emissions from microalgae: potential pathways and significance. J Appl Phycol 31:1–8. https://doi.org/10.1007/s10811-018-1531-1
3. Plouviez M, Chambonniere P, Shilton A, Packer M, Guieysse B (2019) Nitrous oxide (N2O) emissions during real domestic wastewater treatment in an outdoor pilot-scale high rate algae pond. Algal Res 44. https://doi.org/10.1016/j.algal.2019.101670
4. Muñoz R, Guieysse B (2006) Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res 40:2799–2815. https://doi.org/10.1016/j.watres.2006.06.011
5. Craggs R, Sutherland D, Campbell H (2012) Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. J Appl Phycol 24:329–337. https://doi.org/10.1007/s10811-012-9810-8
6. Posadas E, Alcántara C, García-Encina PA, Gouveia L, Guieysse B, Norvill Z, Acién FG, Markou G, Congestri R, Koreiviene J, Muñoz R (2017) Microalgae-Based Biofuels and Bioproducts. 3 - Microalgae cultivation in wastewater, 67–91. From Feedstock Cultivation to End-products. Woodhead Publishing Series in Energy. https://doi.org/10.1016/B978-0-08-101023-5.00003-0
7. Metcalf and Eddy, Inc (2011) In: Franklin LM, David Stensel H (eds) Wastewater engineering. Treatment and reuse, 4th edn. George Tchobanoglous
8. Arbib Z, Ruiz J, Álvarez-Díaz P, Garrido-Pérez C, Barragán J, Perales JA (2013) Effect of pH control by means of flue gas addition on three different photo-bioreactors treating urban wastewater in long-term operation. Ecol Eng 57:226–235. https://doi.org/10.1016/j.ecoleng.2013.04.040
9. Ledda C, Romero Villegas GI, Adani F, Acién Fernández FG, Molina Grima E (2015) Utilization of centrate from wastewater treatment for the outdoor production of *Nannochloropsis gaditana* biomass at pilot scale. Algal Res 12:17–25. https://doi.org/10.1016/j.algal.2015.08.002
10. Bahr M, Díaz I, Domínguez A, González-Sánchez A, Muñoz R (2014) Microalgal-biotechnology as a platform for an integral biogas upgrading and nutrient removal from anaerobic effluents. Environ Sci Technol 48:573–581. https://doi.org/10.1021/es403596m
11. Wang Y, Liu J, Kang D, Wu C, Wu Y (2017) Removal of pharmaceuticals and personal care products from wastewater using algae-based technologies: a review. Rev Environ Sci Biotechnol 16:717–735. https://doi.org/10.1007/s11157-017-9446-x
12. Leng L, Wei L, Xiong Q, Xu S, Li W, Lv S, Lu Q, Wan L, Wen Z, Zhou W (2020) Use of microalgae based technology for the removal of antibiotics from wastewater: a review. Chemosphere 238:124680. https://doi.org/10.1016/j.chemosphere.2019.124680
13. Xiong J-Q, Kurade MB, Jeon B-H (2018) Can microalgae remove pharmaceutical contaminants from water? Curr Opin Biotechnol 36(1):30–44. https://doi.org/10.1016/j.tibtech.2017.09.003
14. Norvill ZN, Shilton A, Guieysse B (2016) Emerging contaminant degradation and removal in algal wastewater treatment ponds: identifying the research gaps. J Hazard Mater 313:291–309. https://doi.org/10.1016/j.hazmat.2016.03.085
15. Peng F-Q, Ying G-G, Yang B, Liu S, Lai H-J, Liu Y-S, Chen Z-F, Zhou G-J (2014) Biotransformation of progesterone and norgestrel by two freshwater microalgae (Scenedesmus obliquus and Chlorella pyrenoidosa): transformation kinetics and products identification. Chemosphere 95:581–588. https://doi.org/10.1016/j.chemosphere.2013.10.013
16. Stravs MA, Pomati F, Hollender J (2017) Exploring micropollutant biotransformation in three freshwater phytoplankton species. Environ Sci Process Impacts 19(6):822–832. https://doi.org/10.1039/c7em00100b

17. Ferrando L, Matamoros V (2020) Attenuation of nitrates, antibiotics and pesticides from groundwater using immobilised microalgae-based systems. Sci Total Environ 703 (10):134740. https://doi.org/10.1016/j.scitotenv.2019.134740
18. Muñoz R, Köllnera C, Guieysse B (2009) Biofilm photobioreactors for the treatment of industrial wastewaters. J Hazard Mater 161(1):29–34. https://doi.org/10.1016/j.jhazmat.2008.03.018
19. Park JBK, Craggs RJ, Shilton AN (2011) Recycling algae to improve species control and harvest efficiency from a high rate algal pond. Water Res 45:6637–6649. https://doi.org/10.1016/j.watres.2011.09.042
20. Ronen Z, Abeliovich A (2000) Anaerobic-aerobic process for microbial degradation of tetrabromobisphenol A. Appl Environ Microbiol 66:2372–2377. https://doi.org/10.1128/AEM.66.6.2372-2377.2000
21. Santaeufemia S, Torres E, Mera R, Abalde J (2016) Bioremediation of oxytetracycline in seawater by living and dead biomass of the microalga Phaeodactylum tricornutum. J Hazard Mat 320:315–332. https://doi.org/10.1016/j.jhazmat.2016.08.042
22. de Godos I, Muñoz R, Guieysse B (2012) Tetracycline removal during wastewater treatment in high-rate algal ponds. J Hazard Mater 229-230:446–449. https://doi.org/10.1016/j.jhazmat.2012.05.106
23. Challis JK, Hanson ML, Friesen KJ, Wong CS (2014) A critical assessment of the photodegradation of pharmaceuticals in aquatic environments: defining our current understanding and identifying knowledge gaps. Environ Sci Process Impacts 16(4):672–696. https://doi.org/10.1039/c3em00615h
24. Bai X, Acharya K (2019) Removal of seven endocrine disrupting chemicals (EDCs) from municipal wastewater effluents by a freshwater green alga. Environ Pollut 247:534–540. https://doi.org/10.1016/j.envpol.2019.01.075
25. Poirier-Larabie S, Segura PA, Gagnona C (2016) Degradation of the pharmaceuticals diclofenac and sulfamethoxazole and their transformation products under controlled environmental conditions. Sci Total Environ 557–558:257–267. https://doi.org/10.1016/j.scitotenv.2016.03.057
26. Monteiro SC, Boxal ABA (2010) Occurrence and fate of human pharmaceuticals in the environment in: reviews of environmental contamination and toxicology. In: Reviews of Environmental Contamination and Toxicology, vol 202. Springer, New York. https://doi.org/10.1007/978-1-4419-1157-5_2
27. Längin A, Alexy R, König A, Kümmerer K (2009) Deactivation and transformation products in biodegradability testing of ß-lactams amoxicillin and piperacillin. Chemosphere 75 (3):347–354. https://doi.org/10.1016/j.chemosphere.2008.12.032
28. Matamoros V, Uggetti E, Garcia J, Bayona JM (2016) Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: a laboratory scale study. J Hazard Mater 301:197–205. https://doi.org/10.1016/j.jhazmat.2015.08.050
29. Hom-Diaz A, Norvill ZN, Blanquez P, Vicent T, Guieysse B (2017) Ciprofloxacin removal during secondary domestic wastewater treatment in high rate algal ponds. Chemosphere 180:33–41. https://doi.org/10.1016/j.chemosphere.2017.03.125
30. Norvill ZN, Toledo-Cervantes A, Blanco S, Shilton A, Guieysse B, Muñoz R (2017) Photodegradation and sorption govern tetracycline removal during wastewater treatment in algal ponds. Bioresour Technol 232:35–43. https://doi.org/10.1016/j.biortech.2017.02.011
31. Sutherland DL, Howard-Williams C, Turnbull MH, Paul A, Craggs RJ (2015) Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 184:222–229. https://doi.org/10.1016/j.biortech.2014.10.074
32. Park JBK, Craggs RJ, Shilton AN (2011) Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 102:35–42. https://doi.org/10.1016/j.biortech.2010.06.158
33. Romero-Villegas GI, Fiamengo M, Acién-Fernández FG, Molina-Grima E (2018) Utilization of centrate for the outdoor production of marine microalgae at the pilot-scale in raceway

photobioreactors. J Environ Manag 228:506–516. https://doi.org/10.1016/j.jenvman.2018.08.020
34. de la Noüe J, Laliberté G, Proulx D (1992) Algae and waste water. J Appl Phycol 4:247–254. https://doi.org/10.1007/BF02161210
35. Oswald WJ (1988) Micro-algae and wastewater treatment. In: Borowitzka MA (ed) Microalgal biotechnology. Cambridge University Press, Cambridge, pp 691–707
36. Batstone DJ, Hülsen T, Mehta CM, Keller J (2015) Platforms for energy and nutrient recovery from domestic wastewater: a review. Chemosphere 140:2–11. https://doi.org/10.1016/j.chemosphere.2014.10.021
37. Kong QX, Li L, Martinez B, Chen P, Ruan R (2010) Culture of microalgae *Chlamydomonas reinhardtii* in wastewater for biomass feedstock production. Appl Biochem Biotechnol 160:9–18. https://doi.org/10.1007/s12010-009-8670-4
38. Seco A, Aparicio S, González-Camejo J, Jiménez-Benítez A, Mateo O, Mora JF, Noriega-Hevia G, Sanchis-Perucho P, Serna-García R, Zamorano-López N, Giménez JB, Ruiz-Martinez A, Aguado D, Barat R, Borrás L, Bouzas A, Martí N, Pachés M, Ribes J, Robles A, Ruano MV, Serralta J, Ferrer J (2018) Resource recovery from sulphate rich sewage through an innovative anaerobic-based water resource recovery facility (WRRF). Water Sci Technol 78 (9):1925–1936. https://doi.org/10.2166/wst.2018.492
39. Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, Wang Y, Ruan R (2010) Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. Appl Biochem Biotechnol 162:1174–1186. https://doi.org/10.1007/s12010-009-8866-7
40. Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: a review of enclosed system designs and performances. Biotechnol Prog 22:1490–1506. https://doi.org/10.1021/bp060065r
41. Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001
42. Rawat I, Kumar R, Mutanda T, Bux F (2011) Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl Energy 88:3411–3424. https://doi.org/10.1016/j.apenergy.2010.11.025
43. Shen Y, Yuan W, Pei ZJ, Wu Q, Mao E (2009) Microalgae mass production methods. Trans ASABE 52:1275–1287
44. Vo HNP, Ngo HH, Guo W, Minh T, Nguyen H, Liu Y, Liu Y, Nguyen DD, Chang SW (2019) A critical review on designs and applications of microalgae-based photobioreactors for pollutants treatment. Sci Total Environ 651(1):1549–1568. http://dx.doi.org/10.1016 /j.scitotenv.2018.09.282
45. Gudin C (1976) Method of growing plant cells. US Patent 3,955,317
46. Huang Q, Jiang F, Wang L, Yang C (2017) Design of photobioreactors for mass cultivation of photosynthetic organisms. Engineering 3(3):318–329. https://doi.org/10.1016/J.ENG.2017.03.020
47. Ugwu CU, Aoyagi H, Uchiyama H (2008) Photobioreactors for mass cultivation of algae. Bioresour Technol 99:4021–4028. https://doi.org/10.1016/j.biortech.2007.01.046
48. Chen CY, Yeh KL, Aisyah R, Lee DJ, Chang JS (2011) Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol 102:71–81. https://doi.org/10.1016/j.biortech.2010.06.159
49. Tredici MR, Rodolf L (2004) Reactor for industrial culture of photosynthetic microorganisms 2004/074423 A2
50. Crites RW, Middlebrooks EJ, Reed SC (2006) Natural systems for wastewater treatment methods, vol 96. https://doi.org/10.1016/S0026-0576(98)80320-6
51. Heaven S, Lock AC, Pak LN, Rspaev MK (2003) Waste stabilisation ponds in extreme continental climates: a comparison of design methods from the USA, Canada, northern Europe and the former Soviet Union. Water Sci Technol 48:25–33. https://doi.org/10.2166/wst.2003.0078

52. Shilton A, Walmsley N (2005) Introduction to pond treatment technology. In: Shilton A (ed) Pond treatment technology. IWA Publishing, London, p 479
53. Handler RM, Canter CE, Kalnes TN, Lupton FS, Kholiqov O, Shonnard DR, Blowers P (2012) Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: analysis of the prior literature and investigation of wide variance in predicted impacts. Algal Res 1:83–92. https://doi.org/10.1016/j.algal.2012.02.003
54. Brennan L, Owende P (2010) Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 14:557–577. https://doi.org/10.1016/j.rser.2009.10.009
55. García J, Green BF, Lundquist T, Mujeriego R, Hernández-Mariné M, Oswald WJ (2006) Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. Bioresour Technol 97:1709–1715. https://doi.org/10.1016/j.biortech.2005.07.019
56. Matamoros V, Gutierrez R, Ferrer I, García J, Bayona JM (2015) Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study. J Hazard Mater 288:34–42. https://doi.org/10.1016/j.jhazmat.2015.02.002
57. Escapa C, Coimbra RN, Paniagua S, García AI, Otero M (2015) Nutrients and pharmaceuticals removal from wastewater by culture and harvesting of Chlorella sorokiniana. Bioresour Technol 185:276–284. https://doi.org/10.1016/j.biortech.2015.03.004
58. Escapa C, Coimbra RN, Paniagua S, García AI, Otero M (2016) Comparative assessment of diclofenac removal from water by different microalgae strains. Algal Res 18:127–134. https://doi.org/10.1016/j.algal.2016.06.008
59. Hom-Diaz A, Llorca M, Rodríguez-Mozaz S, Vicent T, Barceló D, Blánquez P (2015) Microalgae cultivation on wastewater digestate: b-estradiol and 17a ethynylestradiol degradation and transformation products identification. J Environ Manag 155:106–113. https://doi.org/10.1016/j.jenvman.2015.03.003
60. Koreivienė J, Valčiukas R, Karosienė J, Baltrėnas P (2014) Testing of Chlorella/Scenedesmus microalgae consortia for remediation of wastewater, CO 2 mitigation and algae biomass feasibility for lipid production. J Environ Eng Landsc Manag 22:105–114. https://doi.org/10.3846/16486897.2013.911182
61. Renuka N, Sood A, Ratha SK, Prasanna R, Ahluwalia AS (2013) Evaluation of microalgal consortia for treatment of primary treated sewage effluent and biomass production. J Appl Phycol 25:1529–1537. https://doi.org/10.1007/s10811-013-9982-x
62. Qin L, Wang Z, Sun Y, Shu Q, Feng P, Zhu L, Xu J, Yuan Z (2016) Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. Environ Sci Pollut Res 23:8379–8387. https://doi.org/10.1007/s11356-015-6004-3
63. Su Y, Mennerich A, Urban B (2012) Coupled nutrient removal and biomass production with mixed algal culture: impact of biotic and abiotic factors. Bioresour Technol 118:469–476. https://doi.org/10.1016/j.biortech.2012.05.093
64. Ismail MM, Essam TM, Ragab YM, El-Sayed AE-kB, Mourad FE (2017) Remediation of a mixture of analgesics in a stirred-tank photobioreactor using microalgal-bacterial consortium coupled with attempt to valorise the harvested biomass. Bioresour Technol 232:364–371. https://doi.org/10.1016/j.biortech.2017.02.062
65. Kang D, Zhao Q, Wu Y, Wu C, Xiang W (2018) Removal of nutrients and pharmaceuticals and personal care products from wastewater using periphyton photobioreactors. Bioresour Technol 248:113–119. https://doi.org/10.1016/j.biortech.2017.06.153
66. Parladé E, Hom-Diaz A, Blánquez P, Martínez-Alonso M, Vicent T, Gaju N (2018) Effect of cultivation conditions on ß-estradiol removal in laboratory and pilot-plant photobioreactors by an algal-bacterial consortium treating urban wastewater. Water Res 137:86–96. https://doi.org/10.1016/j.watres.2018.02.060
67. Gentili FG, Fick J (2017) Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants. J Appl Phycol 29:255–262. https://doi.org/10.1007/s10811-016-0950-0

68. Hom-Diaz A, Jaén-Gil A, Bello-Laserna I, Rodríguez-Mozaz S, Vicent T, Barceló D, Blánquez P (2017) Performance of a microalgal photobioreactor treating toilet wastewater: pharmaceutically active compound removal and biomass harvesting. Sci Total Environ 592:1–11. https://doi.org/10.1016/j.scitotenv.2017.02.224
69. Villar-Navarro E, Baena-Nogueras RM, Paniw M, Perales JA (2018) Removal of pharmaceuticals in urban wastewater: high rate algae pond (HRAP) based technologies as an alternative to activated sludge based processes. Wat Res 139:19–29. https://doi.org/10.1016/j.watres.2018.03.072
70. García-Galán MJ, Gutiérrez R, Uggetti E, Matamoros V, García J, Ferrer I (2018) Use of full-scale hybrid horizontal tubular photobioreactors to process agricultural runoff. Biosyst Eng 166:138–149. https://doi.org/10.1016/j.biosystemseng.2017.11.016
71. García-Galán MJ, Arashiroa L, Santos LHMLM, Insa S, Rodríguez-Mozaz S, Barceló D, Ferrer I, Garfí M (2020) Fate of priority pharmaceuticals and their main metabolites and transformation products in microalgae-based wastewater treatment systems. J Hazard Mater 390:121771. https://doi.org/10.1016/j.jhazmat.2019.121771
72. Garrido-Cardenas JA, Manzano-Agugliaro F, Acien-Fernandez FG, Molina-Grima E (2018) Microalgae research worldwide. Algal Res 35:50–60. https://doi.org/10.1016/j.algal.2018.08.005
73. Tolboom SN, Carrillo-Nieves D, Rostro-Alanis M, Quiroz R, Barceló D, Iqbal HMN, Parra-Saldivar R (2019) Algal-based removal strategies for hazardous contaminants from the environment – a review. Sci Total Environ 665:358–366. https://doi.org/10.1016/j.scitotenv.2019.02.129
74. Santos LHMLM, Araújo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MCBSM (2010) Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J Hazard Mater 175:45–95. https://doi.org/10.1016/j.jhazmat.2009.10.100
75. Jobling S, Nolam M, Tyler CR, Brighty G, Sumpter JP (1998) Widespread sexual disruption in wild fish. Environ Sci Technol 32:2498–2506. https://doi.org/10.1021/es9710870
76. Shi W, Wang L, Rousseau DPL, Lens PNL (2010) Removal of estrone, 17a ethinylestradiol, and 17b-estradiol in algae and duckweed-based wastewater treatment systems. Environ Sci Pollut Res Int 17:824–833. https://doi.org/10.1007/s11356-010-0301-7
77. Lai KM, Scrimshaw MD, Lester JN (2002) Biotransformation and bioconcentration of steroid estrogens by *Chlorella vulgaris*. Appl Environ Microbiol 68:859–864. https://doi.org/10.1128/AEM.68.2.859
78. Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture. Aquaculture 151:315–331. https://doi.org/10.1016/S0044-8486(96)01501-3
79. Gros M, Petrović M, Ginebreda A, Barceló D (2010) Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ Int 36:15–26. https://doi.org/10.1016/j.envint.2009.09.002
80. Mir-Tutusaus JA, Parladé E, Villagrasa M, Barceló D, Rodriguez-Mozaz S, Marinez-Alonso M, Gaju N, Sarrà M, Caminal G (2019) Long-term continuous treatment of non sterile real hospital wastewater by Trametes versicolor. J. Biol Eng 13:47. https://doi.org/10.1186/s13036-019-0179-y
81. de Wilt A, Butkovskyi A, Tuantet K, Hernandez Leal L, Fernandes TV, Langenhoff A, Zeeman G (2016) Micropollutant removal in an algal treatment system fed with source separated wastewater streams. J Hazard Mater 304:84–92. https://doi.org/10.1016/j.jhazmat.2015.10.033
82. Zhang N, Liu G, Liu H, Wang Y, He Z, Wang G (2011) Diclofenac photodegradation under simulated sunlight: effect of different forms of nitrogen and kinetics. J Hazard Mater 192 (1):411–418. https://doi.org/10.1016/j.jhazmat.2011.05.038
83. Guo X, Pang W, Dou C, Yin D (2017) Sulfamethoxazole and COD increase abundance of sulfonamide resistance genes and change bacterial community structures within sequencing batch reactors. Chemosphere 175:21–27. https://doi.org/10.1016/j.chemosphere.2017.01.134
84. Jelic A, Rodriguez-Mozaz S, Barceló D, Gutierrez O (2015) Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Water Res 68:98–108. https://doi.org/10.1016/j.watres.2014.09.033

Removal of Pharmaceuticals from Wastewater by Membrane Bioreactors: Factors, Mechanisms, and Perspectives

Faisal I. Hai and William E. Price

Contents

Abstract This chapter reviews the performance of membrane bioreactors (MBR) for the removal of pharmaceuticals from wastewater. Many factors affect pharmaceuticals removal by MBR. The factors include physicochemical characteristics of the pharmaceuticals and MBR operational parameters. The presence of a membrane allows various adsorption mechanisms for pharmaceuticals. Conceptually MBRs can deliver better removal of pharmaceuticals than conventional activated sludge (CAS) processes because of operation under longer solids retention times (SRTs), higher mixed liquor suspended solids (MLSS) concentrations, and complete retention of suspended solids. However, the available reports from lab and full-scale plants are sometimes conflicting. Further work is required to improve our understanding of the overall fate and degradation pathways of pharmaceuticals in MBRs.

Keywords Membrane bioreactor, Pharmaceuticals, Biodegradation, Adsorption, Sludge retention time

F. I. Hai (✉) and W. E. Price
Strategic Water Infrastructure Laboratory, The University of Wollongong, Wollongong, NSW, Australia
e-mail: faisal@uow.edu.au; wprice@uow.edu.au

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 223–238, DOI 10.1007/698_2020_676,

1 Introduction

The conventional wastewater treatment plants were not designed to remove emerging contaminants such as pharmaceuticals. The ineffective removal of pharmaceuticals from wastewater is a significant concern because pharmaceuticals are biologically active and can present detrimental effects to the aquatic environment. The effects include acute and chronic toxicity to aquatic organisms, accumulation in the ecosystem, and various adverse effects on human health [1]. Membrane bioreactors (MBR) are an advancement over the conventional activated sludge processes. It combines biodegradation and membrane separation in a single step. Details on the basic principle, design, and operation of MBRs are available elsewhere [2]. This chapter provides a brief overview of the factors and mechanisms of pharmaceuticals removal by MBR.

2 Pharmaceuticals Removal by MBR

Compared to the conventional activated sludge (CAS) processes, MBRs can operate under longer solids retention times (SRTs), higher mixed liquor suspended solids (MLSS) concentration, and complete retention of suspended solids. Hai et al. [3] noted that MBRs may offer additional removal mechanisms for pharmaceuticals as follows: (1) the membrane is an effective barrier to solids, onto which pharmaceuticals may adsorb [4–6]; (2) pharmaceuticals may also adsorb on membrane surface [7]; and (3) the longer SRT in MBR may enhance pharmaceuticals biodegradation.

Compared to CAS, better and/or more stable removal by MBR has been reported for pharmaceuticals which have moderate to high biodegradability. On the other hand, similar to CAS, low and unstable removal performance by MBR has been observed for biologically persistent hydrophilic pharmaceuticals [8–10]. In some cases, similar removal efficiency has been observed for CAS and MBR. Examples of such pharmaceuticals include naproxen, acetaminophen, ibuprofen, and paroxetine [11, 12]. Some pharmaceuticals, namely, carbamazepine and hydrochlorothiazide have been widely reported to be poorly removed by both CAS and MBR [13, 14]. Thus based on the extent of their removal, pharmaceuticals can be categorized into (1) better removal by MBR (e.g., ketoprofen, mefenamic acid, and naproxen); (2) easy removal by both CAS and MBR (e.g. ibuprofen); and (3) poor removal by either of them (e.g., carbamazepine, clofibric acid, dichlorprop, and diclofenac) [3].

Gonzalez et al. [15] observed similar high removal (over 95%) of linear alkylbenzene sulfonates compounds by CAS and MBR processes. However, MBR performance was more stable and independent of the influent concentrations. Furthermore, MBR effluent quality in terms of chemical oxygen demand (COD), NH_4^+ concentration, and total suspended solids (TSS) was consistently better. Radjenovic et al. [13] reported better removal of the pharmaceutical compounds investigated, for

example, bezafibrate, diclofenac, ketoprofen, gemfibrozil, pravastatin, ranitidine, and ofloxacin. Better or more uniform removal of pharmaceutical compounds by MBR may be due to the smaller sludge flocs which improve mass transfer, and thus removal efficiency increases [13]. Gobel et al. [16] reported 20% better removal of sulfamethoxazole by MBR. Compared to unstable removal of trimethoprim, clarithromycin, and dehydro-erythromycin by CAS, MBR achieved up to 90% removal of these pharmaceuticals at SRTs over 60 days [16]. Radjenovic et al. [13] reported better removal by MBR of a range of pharmaceuticals including β-blockers (atenolol and metoprolol), lipid regulators, and cholesterol-lowering medicine (gemfibrozil, bezafibrate, clofibric acid, and pravastatin), antibiotics (ofloxacin and erythromycin), anti-ulcer agent (ranitidine), and some analgesics and anti-inflammatory drugs (propyphenazone, mefenamic acid, and diclofenac). In a pilot scale study by Radjenovic et al. [14] compounds such as mefenamic acid, diclofenac, indomethacin, pravastatin, propyphenazone, and gemfibrozil were poorly removed by CAS. However, they were better removed by MBR.

Post-treatment by ultrafiltration following CAS treatment was reported to increase antibiotics removal by around 30% [6]. This improvement was attributed to adsorption of the antibiotics on suspended biomass and on the biomass attached to membrane surface. Boonyaroj et al. [4] reported a similar observation. Urase et al. [17] note that in MBRs additional removal of hydrophobic pharmaceuticals may occur due to the extra retention of the pharmaceuticals on suspended solids deposited on the membrane. Li et al. [18] reported that dosing of powdered activated carbon to MBR can lead to significant additional removal of pharmaceuticals. However, for hydrophilic pharmaceuticals, such additional removal by MBR may not occur [19].

It appears that MBR performs better than CAS for pharmaceuticals with moderate biodegradability, while the removal performance of CAS and MBR may be similar for easily biodegradable and resistant compounds. For example, in batch experiments with MBR and CAS sludge, similar kinetic biodegradation constant (k_{biol}) of ≤ 0.1 L/(g_{SS}. d) for diclofenac was observed by Joss et al. [20]. Clara et al. [21] reported on the importance of SRT on pharmaceuticals removal by both CAS and MBR. They noted that when operated at comparable SRT, no significant differences in the treatment efficiency between CAS and MBR may be observed. It is, however, noteworthy that pharmaceuticals removal by MBR is more stable (Figs. 1 and 2) [22]. While MBR may not lead to higher removal efficiencies, it shows a more consistent performance and stable response to fluctuating pharmaceuticals load in wastewater. Additionally, MBR installations are more compact and offer more flexibility to fine-tune biological performance.

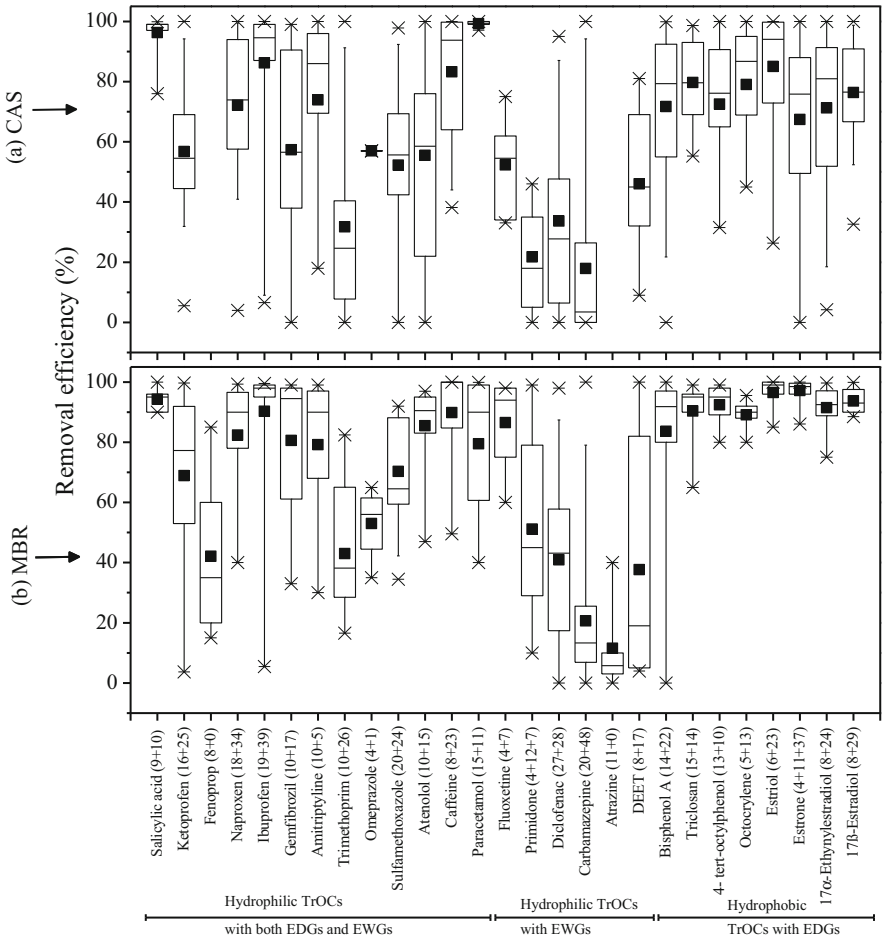


Fig. 1 Variations in the removal of emerging contaminants including pharmaceuticals by (**a**) CAS and (**b**) MBR. Box-and-whisker plots show information about the interquartile range, median (horizontal line in the box), min and max (whiskers), and average (block square in the box). Numbers in parenthesis on the X-axis represent the no. of data points (MBR + CAS). Adapted by permission from Asif, Ansari, Chen, Nghiem, Price, and Hai [22]

3 Factors and Mechanisms Affecting Pharmaceuticals Removal by MBR

The mechanisms of pharmaceuticals removal do not follow a general rule since their relative contribution depends on the physicochemical properties of the pharmaceuticals, the origin and composition of the wastewater, and the operating parameters of the wastewater treatment facility [11].

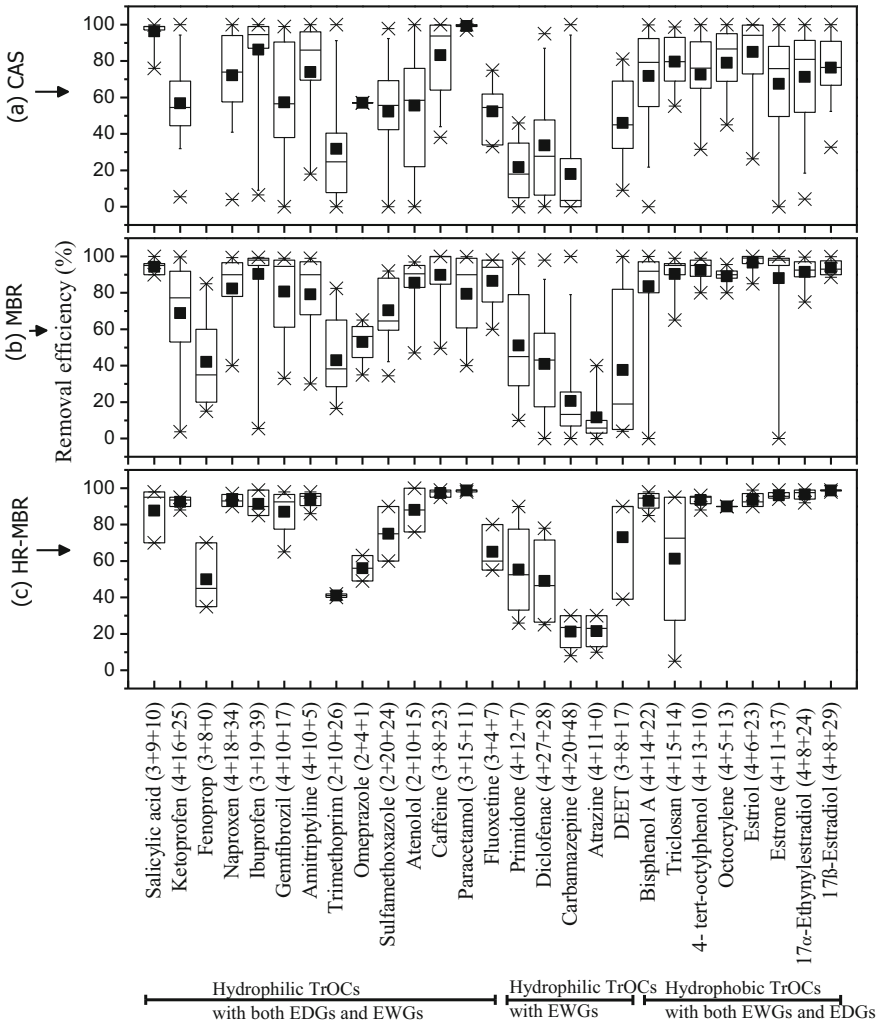


Fig. 2 Box-and-whisker plot showing the biodegradation of emerging trace organic contaminants (TrOCs) including pharmaceuticals in (**a**) CAS, (**b**) conventional MBR, and (**c**) high retention MBR. This chapter discusses the case of conventional MBR. For discussion on high retention MBR, which is an emerging format, see the Ref. [3]. Figure reproduced with permission from Asif, Ansari, Chen, Nghiem, Price, and Hai [22]

3.1 Characteristics of the Pharmaceuticals

Removal of pharmaceuticals even from the same therapeutic group may be different [23]. Indeed Tadkaew et al. [10] observed no general trend for any of the six therapeutic classes of pharmaceuticals investigated: their removal efficiencies by MBR treatment varied widely (10–98%) even within the same class of compounds.

Thus it may not be possible to predict removal based on the therapeutic group of pharmaceutical. This is because compounds within the same pharmaceutical group can possess significantly different chemical composition. For example, among the antibiotics, while the macrolides have one ring with side chains or sugars, every sulfonamide has two relatively small rings connected by a sulfur atom and C–N bonds. Thus sulfonamides are more polar and hydrophilic in nature compared to the macrolides (log $K_{ow} < 1$ and log $K_{ow} > 3$, respectively). This has important implications for their removal by MBRs [6]. On the other hand, Onesios et al. [23] observed antiseptic removals of greater than 70% in majority of the studies reviewed in their article.

Four broad mechanisms govern pharmaceuticals removal from aqueous phase: photolysis, volatilization, sorption, and biodegradation. The relative contribution of these mechanisms depends on the physicochemical properties of the compounds and the operating condition of MBR. Because of the high MLSS concentration in the bioreactor, removal by photolysis may be limited. If sufficiently volatile, pharmaceuticals can be transferred from the aqueous phase to the gas phase. Similarly, if they are sufficiently hydrophobic, they can partition to the solid phase.

Log D or the effective octanol-water partitioning coefficient can be used to denote the hydrophobicity of pharmaceuticals. This can be calculated as log $D_{(pH)} = \log K_{ow} - \log (1+10^{(pH\text{-}pKa)})$. In general, when log D of a pharmaceutical is about 3 or higher, sorption to biosolids can be a major removal mechanism. In addition to hydrophobic interactions, a number of other reactions can influence the partition of the organic compound between the solid and the liquid phase. These include complex formations with metal ions, ion exchange, and hydrogen bonding [24]. The hydrophobic and non-hydrophobic interactions can occur simultaneously. For example, fluoroquinolone antibiotics are very hydrophilic, but they adsorb onto sludge significantly via electrostatic interactions [25]. Stevens-Garmon et al. [26] reported that the positively charged pharmaceutically active compounds they studied had the highest sorption potential (log $K_d = 2.8$–3.8), as compared to the neutral and negatively charged chemicals.

For compounds with low biosorption, removal mainly occurs by biodegradation [27]. Suarez et al. [28] proposed that compounds could be classified according to their k_{biol} into very highly ($k_{biol} > 5$ L/(g $_{SS}$. d)), highly ($1 < k_{biol} < 5$ L/(g $_{SS}$. d)), moderately ($0.5 < k_{biol} < 1$ L/(g $_{SS}$. d)), and hardly ($k_{biol} < 0.5$ L/(g $_{SS}$. d)) biodegradable. Available reports show the relationship between the molecular structure and/or functional moieties of pharmaceutical and their biodegradability during MBR treatment [10, 19, 29]. For example, compounds with complex structure (e.g., alkyl chain branch) [30] and toxic groups (e.g., halogens groups [31]) show higher resistance to complete biodegradation. Tadkaew et al. [10] systematically studied the influence of electron withdrawing groups (EWGs) and electron donating groups (EDGs) on pharmaceuticals removal: hydrophilic and moderately hydrophobic (log $D < 3.2$) compounds possessing strong electron withdrawing functional groups showed removal efficiency of less than 20%. By contrast, high removal efficiencies were observed with most compounds bearing electron donating functional groups such as hydroxyl and primary amine groups.

3.2 Operating Parameters

3.2.1 Mixed Liquor Suspended Solids Properties

When biosorption is significant, higher MLSS concentration can result in better removal of pharmaceuticals from the aqueous phase [32]. On the other hand, a minimum level of MLSS must be maintained to sustain biodegradation. For example, Li et al. [33] found that the biodegradation of hormones depended significantly on the level of mixed liquor volatile suspended solids (MLVSS) concentration which varied between the low levels of 0.435 and 1.75 g/L. In a study by Shariati et al. [34], the removal of acetaminophen improved from 20 to 40% when the MLSS concentration increased from 2 to 15 g/L. This performance improvement can be attributed to biodegradation improvement as acetaminophen is a hydrophilic compound. Better acclimatization of the microorganisms to the influent can improve biodegradation. For example, naproxen removal performance of a pilot plant increased from 27 to 99% during the first 300 days, possibly due to acclimation of bacteria to this pharmaceutical [35].

3.2.2 Solids Retention Time (SRT)

With increasing SRT, the microbial population in an MBR may become more diversified (e.g., slow-growing bacteria eventually reaching effective population numbers). The microorganisms may also diversify their metabolic activity in response to the lower food to microorganism (F/M) ratio in MBR under long SRT [36].

A survey conducted in some full-scale CAS plants revealed better removal of gemfibrozil and diclofenac under SRTs over 30 days than below 15 days [37]. Similarly Kimura et al. [38] reported better removal of ketoprofen and diclofenac by MBR at an SRT of 65 days compared to that at an SRT of 15 days. Tambosi et al. [39] also observed roxithromycin removal to increase when SRT was doubled from the initial SRT of 15 days.

The minimum SRT required for effective removal depends on the compound. Caffeine and oxybenzone are well removed from the aqueous phase by MBR. They showed a critical SRT of less than 5 d [40]. On the other hand, compounds categorized as showing poor removal (e.g., galaxolide and tris(2-chloroethyl) phosphate) were reported to require an SRT greater than 15 d. For compounds such as macrolide antibiotics azithromycin, erythromycin, and clarithromycin, a clear increase in removal was observed when SRT exceeded 60 days, whereas, in the same study, higher reduction of roxithromycin was observed already at a SRT of 33 days [16].

Figure 3 indicates that there is no universal relationship between SRT and pharmaceuticals removal by MBR. Nevertheless, the removal of a few pharmaceuticals such as diclofenac, sulfamethoxazole, and mefenamic acid could improve

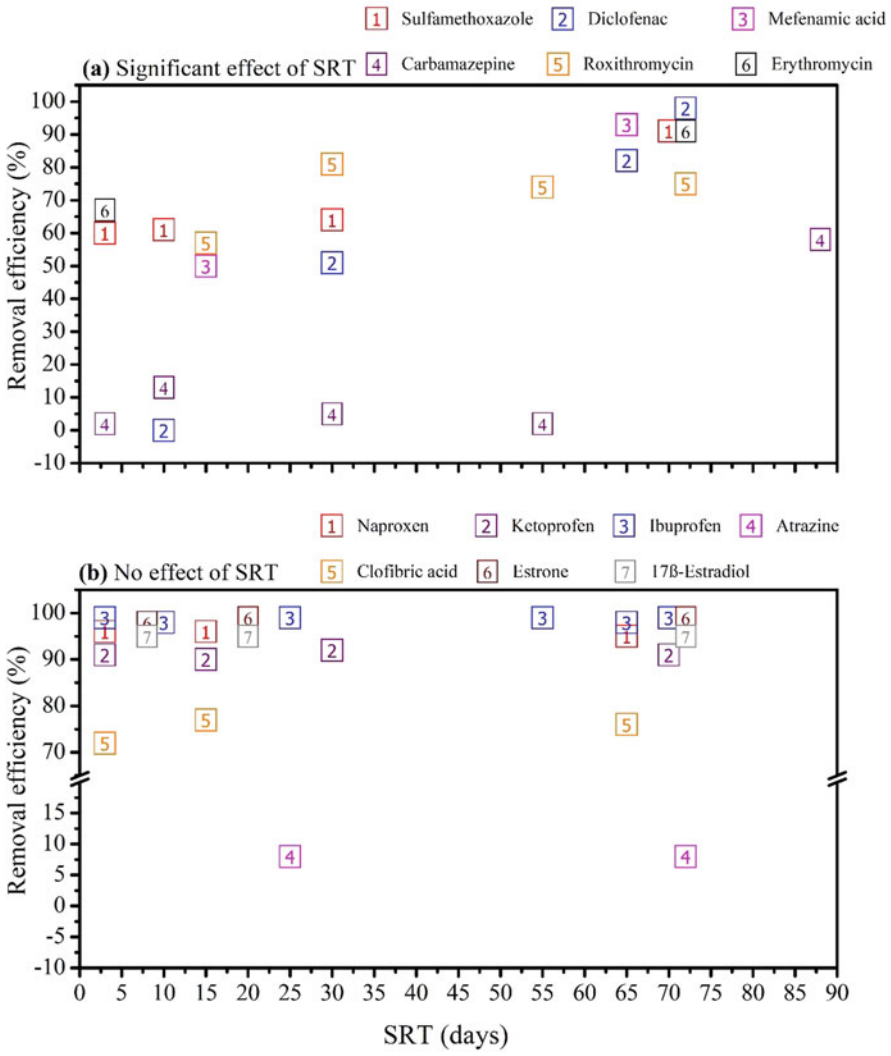


Fig. 3 Effect of SRT on the aqueous phase removal of the selected compounds by MBRs. Reproduced with permission from Asif, Ansari, Chen, Nghiem, Price, and Hai [22]

significantly with the increase in SRT. On the other hand, SRT did not influence the removal of easily biodegradable as well as resistant hydrophilic compounds [22]. Notably, in MBRs, SRT is independent of HRT. Therefore most MBR studies report no obvious effect of HRT on pharmaceuticals [3]. For example, no discernible influence of HRT (3.9–8 h) on BPA removal was observed in an MBR [41].

3.2.3 Pharmaceutical Loading

The concentration of pharmaceuticals in wastewater is usually in microgram to nanogram per liter level. At such low concentrations, biodegradation of pharmaceuticals can happen only if a primary substrate is available for the corresponding bacteria to grow on. Thus cometabolism may be the main removal mechanism of pharmaceuticals, meaning that in presence of other substrate, bacteria break down the pharmaceuticals but do not use them as carbon source. For instance, in batch biodegradation tests, bezafibrate, naproxen, and ibuprofen were degraded only via cometabolism [42]. On the other hand, in a lab-scale sequencing batch reactor study, sulfamethoxazole was biodegraded in presence of acetate, but there was an indication that the microorganisms used the degraded antibiotic as a nitrogen source [43].

3.2.4 Mixed Liquor pH

Wastewater pH can influence pharmaceuticals removal by influencing the physiology of the microorganisms (i.e., optimum pH for enzymatic activity) and the solubility of pharmaceuticals in wastewater [11]. Depending on the pH, pharmaceuticals can exist in different protonation states. This can affect the hydrophobicity (log *D*) at different pH values [11, 44]. For example, during sludge dewatering and conditioning with lime, the pH is increased to over 9. This can cause desorption of pharmaceuticals. On the other hand, compared to neutral pH, at an acidic pH, Urase et al. [17] observed improved removal of several pharmaceuticals, such as ibuprofen, fenoprofen, naproxen, and diclofenac. The removal of the neutral compounds remained unchanged. Despite the possibility of enhanced adsorption of certain ionizable pharmaceuticals on sludge at acidic pH, it may have detrimental impact on microbial groups that are involved in removal of TOC, TN, and/or total phosphorous (TP) removal [45, 46].

3.2.5 Mixed Liquor Temperature

Temperature fluctuation can cause deterioration of treated water quality and disrupt the treatment system [11, 47]. This is because temperature conditions significantly affect microbial growth and activity as well as solubility and other physicochemical properties of organics [11]. Relatively high effluent concentrations of certain pharmaceuticals during low winter temperature are observed [48, 49]. On the other hand, in lab-scale MBR experiments, Hai et al. [50] observed that compared to the operation in the temperature range of 10–35°C, operation at 10 and 45°C led to significantly reduced removal efficiency of the hydrophilic pharmaceuticals investigated.

3.2.6 Mixed Liquor Dissolved Oxygen Concentration

Biodegradation of organics can occur under aerobic (molecular oxygen available), denitrifying (no molecular oxygen available, nitrate available), or anaerobic (neither molecular oxygen nor nitrate available) conditions. Different removal efficiencies of pharmaceuticals have been observed for anaerobic, anoxic, and aerobic conditions [51]. For example, when DO level was high, Thompson et al. [52] observed biodegradation to be responsible for the majority of triclosan removal, while sorption became the dominant removal mechanism when the DO level was low. Suarez et al. [28] classified pharmaceuticals as follows: (1) highly biodegradable under aerobic and anoxic conditions, e.g., ibuprofen, fluoxetine; (2) highly biodegradable under aerobic conditions but persistent in the anoxic reactor, e.g., diclofenac, naproxen, roxithromycin, and erythromycin; (3) moderately biodegradable under aerobic and anoxic conditions, e.g., citalopram; and (4) resistant to biological transformation, for example, sulfamethoxazole, trimethoprim, carbamazepine, and diazepam.

A few studies have recommended combining aerobic/anoxic/anaerobic treatment sequences. Zwiener and Frimmel [53] observed better degradation of diclofenac in an anoxic biofilm reactor. Park et al. [54] reported high removal of carbamazepine removal by a constructed wetland under anoxic conditions. In a study by Suarez et al. [35], several pharmaceuticals such as fluoxetine, trimethoprim, and erythromycin showed significant biodegradation during anoxic treatment, while the remaining pharmaceuticals were only removed during the aerobic treatment [35].

In addition to the studies on the effect of anoxic/aerobic operation regimes on pharmaceuticals removal by MBR, some studies have highlighted the relationship between achievement of nitrification and/or denitrification and pharmaceuticals removal. There is circumstantial evidence that micropollutant biodegradation can be cometabolically mediated under operating conditions that allow for enrichment of nitrifiers [55, 56].

4 Bioaugmented MBR for Pharmaceutical Removal

Bioaugmentation can be implemented by adding special microbes which either use micropollutant as the main carbon source or can degrade micropollutants due to "cometabolism." Only a few bioaugmentation studies involving MBR are available. Pure culture of white-rot fungi (WRF) can degrade micropollutants effectively in absence of contamination from other microbes [57]. WRF secrete enzymes such as laccase and different kinds of peroxidases [58–62]. However, in non-sterile environment, which is common for wastewater conditions, the performance of WRF usually diminishes over time. Yang et al. [63] tested a fungal MBR. The MBR was first inoculated with the WRF *Trametes versicolor*, but over time a mixed bacteria-fungi community developed within the bioreactor. A synthetic wastewater comprising bisphenol A and diclofenac was treated by the MBR. It is noteworthy that

diclofenac is recalcitrant to bacterial degradation. In batch pure cultures, the fungus degraded both micropollutants effectively. However, during MBR operation, diclofenac removal was not as effective, possibly because of continuous loss of the extracellular enzymes with treated effluent and contamination by bacteria. Nevertheless at a micropollutant loading of around 400 μg/L d, steady removal of bisphenol A (80–90%) and diclofenac (~55%) was observed. Nguyen, Hai, Yang, Kang, Leusch, Roddick, Price, and Nghiem [64] investigated the performance of a fungal MBR for the removal of 30 micropollutants from synthetic wastewater. When compared to a conventional bacterial MBR, the bioaugmented MBR showed 30–55% better removal for some micropollutants, namely, pentachlorophenol, fenoprop, naproxen, clofibric acid, ketoprofen, and diclofenac. Importantly, contamination by bacteria significantly reduces the performance of fungal MBR during operation over long term [65–70]. Such contamination can (1) reduce the growth and enzyme secretion by WRF and (2) consume/destabilize the produced enzymes [63, 71, 72]. Bacteria are fast-growing prokaryotes. Thus they can outperform WRF and dominate in fungal MBR [72, 73]. Some strategies to mitigate bacterial contamination have been reviewed by Asif, Hai, Singh, Price, and Nghiem [74]. The strategies include biomass replacement, immobilized fungal growth, influent pretreatment, and use of micro-screen for fungal retention within the bioreactor, while allowing passage of bacteria. An alternative MBR is enzymatic membrane bioreactor (EMBR) which combines an enzymatic bioreactor with a membrane which can retain the enzyme [75–78].

5 Conclusion and Perspective

This chapter critically discusses the potential advantages of the MBR process over conventional CAS process for the biodegradation and removal of pharmaceuticals. MBR offers some advantages for a range of pharmaceuticals because of its operation under longer SRT, higher biomass concentrations, and complete retention of suspended solids. Additionally, pharmaceuticals are adsorbed either by the membrane directly or onto the retained solids. The longer SRT in MBR may enhance biodegradation of pharmaceuticals. Nevertheless, in reality, performance of MBR over CAS depends on the operating conditions and the particular pharmaceutical being investigated. Additionally, there are a number of probable reasons why laboratory-scale studies do not exactly match removals reported from their full-scale counterparts. Such reasons include the use of arbitrarily chosen pharmaceuticals concentrations that are much higher than those encountered in wastewater and in the environment.

References

1. Deblonde T, Cossu-Leguille C, Hartemann P (2011) Emerging pollutants in wastewater: a review of the literature. Int J Hyg Environ Health 214:442–448
2. Hai FI, Yamamoto K, Lee C-H (2019) Membrane biological reactors: theory, modeling, design, management and applications to wastewater reuse, 2nd edn. IWA Publishing, London, ISBN electronic: 9781780409177. https://doi.org/10.2166/9781780409177
3. Hai FI, Nghiem LD, Khan SJ, Asif MB, Price WE, Yamamoto K (2019) Removal of emerging trace organic contaminants (TrOC) by MBR. In: Hai FI, Yamamoto K, Lee C-H (eds) Membrane biological reactors: theory, modeling, design, management and applications to wastewater reuse. IWA Publishing, London, pp 413–468. https://doi.org/10.2166/9781780409177_0413
4. Boonyaroj V, Chiemchaisri C, Chiemchaisri W, Theepharaksapan S, Yamamoto K (2012) Toxic organic micro-pollutants removal mechanisms in long-term operated membrane bioreactor treating municipal solid waste leachate. Bioresour Technol 113:174–180
5. Johnson AC, Sumpter JP (2001) Removal of endocrine-disrupting chemicals in activated sludge treatment works. Environ Sci Technol 35:4697–4703
6. Sahar E, Messalem R, Cikurel H, Aharoni A, Brenner A, Godehardt M, Jekel M, Ernst M (2011) Fate of antibiotics in activated sludge followed by ultrafiltration (CAS-UF) and in a membrane bioreactor (MBR). Water Res 45:4827–4836
7. Chang S, Waite TD, Schäfer AI, Fane AG (2003) Adsorption of the endocrine-active compound estrone on microfiltration hollow fiber membranes. Environ Sci Technol 37:3158–3163
8. Clara M, Strenn B, Gans O, Martinez E, Kreuzinger N, Kroiss H (2005) Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. Water Res 39:4797–4807
9. Joss A, Keller E, Alder AC, Göbel A, McArdell CS, Ternes T, Siegrist H (2005) Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Res 39:3139–3152
10. Tadkaew N, Hai FI, McDonald JA, Khan SJ, Nghiem LD (2011) Removal of trace organics by MBR treatment: the role of molecular properties. Water Res 45:2439–2451
11. Cirja M, Ivashechkin P, Schäffer A, Corvini P (2008) Factors affecting the removal of organic micropollutants from wastewater in conventional treatment plants (CTP) and membrane bioreactors (MBR). Rev Environ Sci Biotechnol 7:61–78
12. Oulton RL, Kohn T, Cwiertny DM (2010) Pharmaceuticals and personal care products in effluent matrices: a survey of transformation and removal during wastewater treatment and implications for wastewater management. J Environ Monit 12:1956–1978
13. Radjenovic J, Petrovic M, Barceló D (2007) Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. Anal Bioanal Chem 387:1365–1377
14. Radjenovic J, Petroviic M, Barcelo 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 Res 43:831–841
15. Gonzalez S, Petrovic M, Barcelo D (2007) Removal of a broad range of surfactants from municipal wastewater – comparison between membrane bioreactor and conventional activated sludge treatment. Chemosphere 67:335–343
16. Gobel A, McArdell CS, Joss A, Siegrist H, Giger W (2007) Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci Total Environ 372:361–371
17. Urase T, Kagawa C, Kikuta T (2005) Factors affecting removal of pharmaceutical substances and estrogens in membrane separation bioreactors. Desalination 178:107–113
18. Li X, Hai FI, Nghiem LD (2011) Simultaneous activated carbon adsorption within a membrane bioreactor for an enhanced micropollutant removal. Bioresour Technol 102:5319–5324
19. Reemtsma T, Zywicki B, Stueber M, Kloepfer A, Jekel M (2002) Removal of sulfur-organic polar micropollutants in a membrane bioreactor treating industrial wastewater. Environ Sci Technol 36:1102–1106

20. Joss A, Zabczynski S, Gobel A, Hoffmann B, Loffler D, McArdell CS, Ternes TA, Thomsen A, Siegrist H (2006) Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. Water Res 40:1686–1696
21. Clara M, Kreuzinger N, Strenn B, Gans O, Kroiss H (2005) The solids retention time – a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res 39:97–106
22. Asif MB, Ansari AJ, Chen S-S, Nghiem LD, Price WE, Hai FI (2018) Understanding the mechanisms of trace organic contaminant removal by high retention membrane bioreactors: a critical review. Environ Sci Pollut Res 26:34085–34100
23. Onesios K, Yu J, Bouwer E (2009) Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation 20:441–466
24. Boxall ABA, Blackwell P, Cavallo R, Kay P, Tolls J (2002) The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicol Lett 131:19–28
25. Göbel A, McArdell CS, Joss A, Siegrist H, Giger W (2007) Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci Total Environ 372:361–371
26. Stevens-Garmon J, Drewes JE, Khan SJ, McDonald JA, Dickenson ERV (2011) Sorption of emerging trace organic compounds onto wastewater sludge solids. Water Res 45:3417–3426
27. Joss A, Keller E, Alder AC, Gobel A, McArdell CS, Ternes T, Siegrist H (2005) Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Res 39:3139–3152
28. Suarez S, Lema JM, Omil F (2010) Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. Water Res 44:3214–3224
29. Kümmerer K, Al-Ahmad A (1997) Biodegradability of the anti-tumour agents 5-fluorouracil, cytarabine, and gemcitabine: impact of the chemical structure and synergistic toxicity with hospital effluent. Acta Hydrochim Hydrobiol 25:166–172
30. Corvini P, Schäffer A, Schlosser D (2006) Microbial degradation of nonylphenol and other alkylphenols – our evolving view. Appl Microbiol Biotechnol 72:223–243
31. Hai FI, Tadkaew N, McDonald JA, Khan SJ, Nghiem LD (2011) Is halogen content the most important factor in the removal of halogenated trace organics by MBR treatment? Bioresour Technol 102:6299–6303
32. Auriol M, Filali-Meknassi Y, Tyagi RD, Adams CD, Surampalli RY (2006) Endocrine disrupting compounds removal from wastewater, a new challenge. Process Biochem 41:525–539
33. Li F, Yuasa A, Obara A, Mathews AP (2005) Aerobic batch degradation of 17-β estradiol (E2) by activated sludge: effects of spiking E2 concentrations, MLVSS and temperatures. Water Res 39:2065–2075
34. Shariati FP, Mehrnia MR, Salmasi BM, Heran M, Wisniewski C, Sarrafzadeh MH (2010) Membrane bioreactor for treatment of pharmaceutical wastewater containing acetaminophen. Desalination 250:798–800
35. Suarez S, Reif R, Lema JM, Omil F (2012) Mass balance of pharmaceutical and personal care products in a pilot-scale single-sludge system: influence of T, SRT and recirculation ratio. Chemosphere 89:164–171
36. Ternes TA, Joss A, Siegrist H (2004) Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environ Sci Technol 38:392A–399A
37. Lishman L, Smyth SA, Sarafin K, Kleywegt S, Toito J, Peart T, Lee B, Servos M, Beland M, Seto P (2006) Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Sci Total Environ 367:544–558
38. Kimura K, Hara H, Watanabe Y (2007) Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors. Environ Sci Technol 41:3708–3714

39. Tambosi JL, de Sena RF, Favier M, Gebhardt W, Jose HJ, Schroder HF, Moreira RdFPM (2010) Removal of pharmaceutical compounds in membrane bioreactors (MBR) applying submerged membranes. Desalination 261:148–156
40. Oppenheimer J, Stephenson R, Burbano A, Liu L (2007) Characterizing the passage of personal care products through wastewater treatment processes. Water Environ Res 79:2564–2577
41. Chen J, Huang X, Lee D (2008) Bisphenol A removal by a membrane bioreactor. Process Biochem 43:451–456
42. Quintana JB, Weiss S, Reemtsma T (2005) Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. Water Res 39:2654–2664
43. Drillia P, Dokianakis SN, Fountoulakis MS, Kornaros M, Stamatelatou K, Lyberatos G (2005) On the occasional biodegradation of pharmaceuticals in the activated sludge process: the example of the antibiotic sulfamethoxazole. J Hazard Mater 122:259–265
44. Tadkaew N, Sivakumar M, Khan SJ, McDonald JA, Nghiem LD (2010) Effect of mixed liquor pH on the removal of trace organic contaminants in a membrane bioreactor. Bioresour Technol 101:1494–1500
45. Zhang T, Liu Y, Fang HHP (2005) Effect of pH change on the performance and microbial community of enhanced biological phosphate removal process. Biotechnol Bioeng 92:173–182
46. Baldwin DD, Campbell CE (2001) Short-term effects of low pH on the microfauna of an activated sludge wastewater treatment system. Water Qual Res J Can 36:519–535
47. Morgan-Sagastume F, Allen DG (2003) Effects of temperature transient conditions on aerobic biological treatment of wastewater. Water Res 37:3590–3601
48. Sui Q, Huang J, Deng S, Chen W, Yu G (2011) Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ Sci Tech 45:3341–3348
49. Vieno NM, Tuhkanen T, Kronberg L (2005) Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environ Sci Technol 39:8220–8226
50. Hai FI, Tessmer K, Nguyen LN, Kang J, Price WE, Nghiem LD (2011) Removal of micropollutants by membrane bioreactor under temperature variation. J Membr Sci 383:144–151
51. Joss A, Andersen H, Ternes T, Richle PR, Siegrist H (2004) Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. Environ Sci Technol 38:3047–3055
52. Thompson A, Griffin P, Stuetz R, Cartmell E (2005) The fate and removal of triclosan during wastewater treatment. Water Environ Res 77:63–67
53. Zwiener C, Frimmel FH (2003) Short-term tests with a pilot sewage plant and biofilm reactors for the biological degradation of the pharmaceutical compounds clofibric acid, ibuprofen, and diclofenac. Sci Total Environ 309:201–211
54. Park N, Vanderford BJ, Snyder SA, Sarp S, Kim SD, Cho J (2009) Effective controls of micropollutants included in wastewater effluent using constructed wetlands under anoxic condition. Ecol Eng 35:418–423
55. Yi T, Harper Jr WF (2007) The link between nitrification and biotransformation of 17-α-ethinylestradiol. Environ Sci Tech 41:4311–4316
56. Vader JS, van Ginkel CG, Sperling FMGM, de Jong J, de Boer W, de Graaf JS, van der Most M, Stokman PGW (2000) Degradation of ethinyl estradiol by nitrifying activated sludge. Chemosphere 41:1239–1243
57. Yang S, Hai FI, Nghiem LD, Roddick F, Price WE, Moreira MT, Magram SF (2013) Understanding the factors controlling the removal of trace organic contaminants by white-rot fungi and their lignin modifying enzymes: a critical review. Bioresour Technol 141:97–108

58. Marco-Urrea E, Pérez-Trujillo M, Blánquez P, Vicent T, Caminal G (2010) Biodegradation of the analgesic naproxen by Trametes versicolor and identification of intermediates using HPLC-DAD-MS and NMR. Bioresour Technol 101:2159–2166
59. Kang A-R, Choi H-T, Song H-G (2008) Optimization of bisphenol A biodegradation by Trametes versicolor. Korean J Microbiol 44:37–42
60. Marco-Urrea E, Gabarrell X, Sarrà M, Caminal G, Vicent T, Reddy CA (2006) Novel aerobic perchloroethylene degradation by the white-rot fungus Trametes versicolor. Environ Sci Technol 40:7796–7802
61. Tran NH, Urase T, Kusakabe O (2010) Biodegradation characteristics of pharmaceutical substances by whole fungal culture Trametes versicolor and its laccase. J Water Environ Technol 8:125–140
62. Nguyen LN, Hai FI, Yang S, Kang J, Leusch FDL, Roddick F, Price WE, Nghiem LD (2014) Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by Trametes versicolor: role of biosorption and biodegradation. Int Biodeter Biodegr 88:169–175
63. Yang S, Hai FI, Nghiem LD, Nguyen LN, Roddick F, Price WE (2013) Removal of bisphenol A and diclofenac by a novel fungal membrane bioreactor operated under non-sterile conditions. Int Biodeterior Biodegradation 85:483–490
64. Nguyen LN, Hai FI, Yang S, Kang J, Leusch FD, Roddick F, Price WE, Nghiem LD (2013) Removal of trace organic contaminants by an MBR comprising a mixed culture of bacteria and white-rot fungi. Bioresour Technol 148:234–241
65. Jelic A, Cruz-Morató C, Marco-Urrea E, Sarrà M, Perez S, Vicent T, Petrović M, Barcelo D (2012) Degradation of carbamazepine by Trametes versicolor in an air pulsed fluidized bed bioreactor and identification of intermediates. Water Res 46:955–964
66. Zhang Y, Geißen S-U (2012) Elimination of carbamazepine in a non-sterile fungal bioreactor. Bioresour Technol 112:221–227
67. Ferrando-Climent L, Cruz-Morató C, Marco-Urrea E, Vicent T, Sarrà M, Rodriguez-Mozaz S, Barceló D (2015) Non conventional biological treatment based on Trametes versicolor for the elimination of recalcitrant anticancer drugs in hospital wastewater. Chemosphere 136:9–19
68. Cruz-Morató C, Ferrando-Climent L, Rodriguez-Mozaz S, Barceló D, Marco-Urrea E, Vicent T, Sarrà M (2013) Degradation of pharmaceuticals in non-sterile urban wastewater by Trametes versicolor in a fluidized bed bioreactor. Water Res 47:5200–5210
69. Cruz-Morató C, Lucas D, Llorca M, Rodriguez-Mozaz S, Gorga M, Petrovic M, Barceló D, Vicent T, Sarrà M, Marco-Urrea E (2014) Hospital wastewater treatment by fungal bioreactor: removal efficiency for pharmaceuticals and endocrine disruptor compounds. Sci Total Environ 493:365–376
70. Badia-Fabregat M, Lucas D, Tuomivirta T, Fritze H, Pennanen T, Rodríguez-Mozaz S, Barceló D, Caminal G, Vicent T (2017) Study of the effect of the bacterial and fungal communities present in real wastewater effluents on the performance of fungal treatments. Sci Total Environ 579:366–377
71. Espinosa-Ortiz EJ, Rene ER, Pakshirajan K, van Hullebusch ED, Lens PN (2016) Fungal pelleted reactors in wastewater treatment: applications and perspectives. Chem Eng J 283:553–571
72. Libra JA, Borchert M, Banit S (2003) Competition strategies for the decolorization of a textile-reactive dye with the white-rot fungi Trametes versicolor under non-sterile conditions. Biotechnol Bioeng 82:736–744
73. Hai FI, Yamamoto K, Nakajima F, Fukushi K (2009) Factors governing performance of continuous fungal reactor during non-sterile operation – the case of a membrane bioreactor treating textile wastewater. Chemosphere 74:810–817
74. Asif MB, Hai FI, Singh L, Price WE, Nghiem LD (2017) Degradation of pharmaceuticals and personal care products by white-rot fungi – a critical review. Curr Pollut Rep 3:88–103

75. Nguyen LN, Hai FI, Dosseto A, Richardson C, Price WE, Nghiem LD (2016) Continuous adsorption and biotransformation of micropollutants by granular activated carbon-bound laccase in a packed-bed enzyme reactor. Bioresour Technol 210:108–116
76. Nguyen LN, Hai FI, Price WE, Kang J, Leusch FD, Roddick F, van de Merwe JP, Magram SF, Nghiem LD (2015) Degradation of a broad spectrum of trace organic contaminants by an enzymatic membrane reactor: complementary role of membrane retention and enzymatic degradation. Int Biodeter Biodegr 99:115–122
77. Lloret L, Eibes G, Feijoo G, Moreira MT, Lema JM (2013) Application of response surface methodology to study the removal of estrogens in a laccase-mediated continuous membrane reactor. Biocatal Biotransformation 31:197–207
78. Nguyen LN, Hai FI, Price WE, Leusch FD, Roddick F, McAdam EJ, Magram SF, Nghiem LD (2014) Continuous biotransformation of bisphenol A and diclofenac by laccase in an enzymatic membrane reactor. Int Biodeter Biodegr 95:25–32

Adsorptive Removal of Pharmaceutically Active Compounds from Wastewater

Frederik Zietzschmann

Contents

Abstract This chapter provides some fundamental aspects on adsorption of pharmaceutically active compounds in wastewater treatment scenarios, with a strong focus on actual practicality and the most recent scientific output. It discusses the key properties of adsorbing substances and adsorbent materials impacting the associated removals. The text demonstrates how adverse competitive effects by dissolved organic matter substantially impact adsorption of targeted compounds. This chapter further highlights possibilities for efficiently exploiting the available capacities of powdered and granular adsorbents. It introduces several practical and easily applicable means for prediction of the occurring adsorptive processes. In addition, the text

F. Zietzschmann (✉)
Berliner Wasserbetriebe, Berlin, Germany

Delft University of Technology, Delft, The Netherlands

Technische Universität Berlin, Berlin, Germany
e-mail: frederik.zietzschmann@bwb.de

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 239–268, DOI 10.1007/698_2020_687,

provides some insights into available adsorbents and new material developments, comparing their advantages, downsides, and overall potentials for successful application. The last section gives a short overview on the associated environmental impacts and costs of adsorptive stages when installed on wastewater treatment plants. The conclusion summarizes the main findings and relates them to open research questions and additional future needs beyond strictly scientific disciplines.

Keywords Activated carbon, Adsorbent, Adsorption, Advanced wastewater treatment, Dissolved organic matter, Pharmaceutically active compound

1 Introduction

To address the problem of pharmaceutically active compounds (PhACs) in the water cycle (e.g., [1, 2]), authorities are exploring managerial and regulative measures [3, 4], ultimately implying advanced technological solutions [5, 6]. A major goal of all approaches is to diminish PhAC emissions into the aquatic environment from point sources – of which wastewater treatment plants (WWTPs) are by far the most relevant [7]. In comparison to PhAC treatment in drinking water works, removing PhACs in WWTPs protects the entire water cycle (rivers, lakes, groundwaters) and thus also drinking water resources [8].

The variety of available techniques in advanced wastewater treatment for PhAC removal includes, among others, improved biological stages, oxidative-reductive approaches, membrane processes, and *adsorption*. When compared to the other techniques, adsorption stands out due to a multitude of features leading to several advantages:

- *Broad effect*, since many adsorbent materials are highly heterogeneous and can adsorb compounds with largely varying chemical properties
- *True removal of contaminants*, since contaminant molecules attach onto the adsorbent and therefore literally exit the aqueous phase (in contrast to chemical transformation in oxidative-reductive processes and brine production in membrane processes)
- *Low-tech and robust*, since reactors must primarily assure good contact between water and adsorbent (no temperature or chemical reaction control, etc.)
- *Renewable resources-based*, since manifold organic carbon-based raw materials are suitable as adsorbents (e.g., plant detritus)
- *Cheap*, since adsorbents can be produced from otherwise unused materials (e.g., organic waste)
- *Safe*, since many adsorbent materials are not harmful for the environment or for humans

However – despite these advantages – adsorption of PhACs in wastewaters or WWTP effluents involves several peculiarities and difficulties. In addition, the substantiality of the above-listed advantages strongly depends on the adsorbent material considered and the application settings (water type, PhAC, etc.). Acknowledging these specificities of adsorption in wastewater treatment for PhAC removal, this chapter gives an overview on current knowledge, applications, and assessment tools.

2 Challenges of PhAC Adsorption in Wastewaters

2.1 Properties of PhACs and Adsorbents

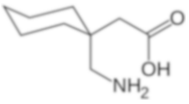
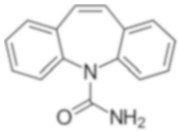
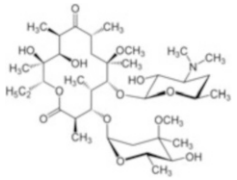
2.1.1 PhACs

Refractory PhACs that remain in the liquid phase during wastewater treatment are often relatively polar, hydrophilic molecules [1, 2, 9]. This is undesirable because many adsorbent materials such as activated carbon generally target compounds with overall low polarity and low hydrophilicity (e.g., [10]). Accordingly, efficient adsorptive removal of polar/hydrophilic compounds often requires comparatively high adsorbent doses [11–13]. Increased hydrophilicity or decreased hydrophobicity of a PhAC, as indicated by low distribution coefficients logD, can lead to dramatically reduced loadings on activated carbons; polarizability similarly can affect adsorptive PhAC removals [14]. Poor removability generally occurs for extremely polar compounds such as charged ions like gabapentin (cf. Table 1) which typically occurs as a zwitterion in aqueous phases (e.g., [15]).

However, hydrophobic or aromatic systems within PhAC molecules can overcompensate adverse charge effects; such PhACs are still removed very well – as in the case of diclofenac or bezafibrate [16, 17]. Moreover, certain charge effects can even increase PhAC removal, for example, in the case of metoprolol [18]. This substance carries a positive charge around typical real water pHs (6–8), allowing it to interact very well with negatively charged adsorbent surfaces as those of most activated carbons [19]. The overall affinity of aromatic structures toward many adsorbents is underlined by the typically high adsorptive removals of PhACs carrying aromatic electron systems, like carbamazepine (cf. Table 1).

In addition to hydrophobicity/charge/aromaticity, molecular size of target PhACs can strongly affect their removal by different adsorbents. Many materials used for aqueous adsorption treatment are extremely porous (e.g., [20]), and targeted compounds must be able to enter the pore structure. Otherwise, they cannot adequately access adsorption sites and would suffer from size-exclusion effects [21–23]. An example for a comparatively large PhAC is clarithromycin with a molecular weight of 748 g/mol, shown in Table 1, right. Besides the large molecular size of clarithromycin, its low aromaticity, high polarizability, and proton-donating groups

Table 1 Examples for typical PhACs found in wastewaters and WWTP effluents, with names, medical application, and some major molecular properties [25]

Name	Gabapentin	Carbamazepine	Clarithromycin
Medical application	Analgesic, antiepileptic, veterinary drug	Anticonvulsant/ antiepileptic	Antibiotic
Molecular structure			
Molar mass (g/mol)	171.2	236.3	748.0
Solubility (mg/L)	4,490	17.7	0.342
Log D at pH 7	−1.1	2.45	1.25
Charge at pH 7	+-(zwitterion)	Neutral	+
Aromatic rings	0	2	0

are good examples for manifold molecular properties of PhACs, ultimately determining a PhAC's overall adsorbability by intricate interactions.

Despite adverse effects of very large molecular sizes, PhACs which are *too small* in relation to the average pore size of an adsorbent may not be removed well, either. For example, very small water contaminants like nitrobenzene adsorb very poorly onto activated carbons with a relatively large average pore size and otherwise extremely high specific internal surfaces [11, 19]. This may appear unexpected but probably results from poor overall interactions with the adsorbent surface inside pores that are too large. Despite such potentially detrimental effects, PhACs of low molecular size have the advantage of rapid diffusion within an adsorbent's porous system, allowing them to reach adsorption sites rapidly [24].

2.1.2 Adsorbents

The pore size of adsorbents logically influences the involved transport phenomena as well [24, 26, 27]. Large average pore sizes of adsorbents allow for rapid establishment of near-equilibrium conditions, whereas this takes longer for adsorbents of low average pore size [19]. Similarly, the particle size of adsorbents strongly influences the velocity of the overall process. Small particle sizes help in reducing the travel distances of PhACs within adsorbent particles and often lead to substantially improved performance [28]. Since intraparticle diffusion is generally the slowest mass transfer step in adsorptive systems, super-fine adsorbents can improve the overall process dramatically [29]. However, reducing the average particle size of

adsorbents can also have detrimental effects on adsorbent capacities [30] and particle removal (cf. Sect. 4.1.1).

As indicated, the surface chemistry of adsorbents plays a crucial role in removing PhACs from aqueous solutions. In particular, charged PhACs can be repulsed from identical charges on adsorbent surfaces, or attracted by opposite charges [20]. In addition, a large variety of charge interactions, like dispersive/van der Waals forces, H-bonds, electron donor-acceptor interactions, and electrostatic attraction/repulsion, largely impact the adsorption of PhACs [31]. It is noteworthy that the surface chemistry of adsorbents strongly depends on, and can be adjusted by, the respective production approach [32]. An often unnoticed effect of carbonaceous adsorbents is their ability to induce redox reactions of PhACs, depending on the chemical surface composition of the adsorbent [33].

2.2 Competitive Adsorption Phenomena

2.2.1 Concentration Aspects

Most organic contaminants in raw wastewater normally occur in concentrations in the low to medium µg/L range. This concentration range is a combined result of (1) use and discharge of PhACs by polluters (households, industry) and (2) dilution and degradation/transformation processes in sewers. In WWTP effluents, the concentrations are lower, usually in the range of ng/L or µg/L [34]. Thus, the concentrations of PhACs are some 1,000 to >100,000 times lower than those of bulk dissolved organic matter (DOM), whose concentration usually ranges within 10–30 mg/L in WWTP effluents (~5–15 mg/L dissolved organic carbon, DOC). Since both PhACs and DOM are attracted toward adsorbent surfaces, they compete for the available adsorption sites, with substantial adverse effects on PhAC removal. The vast concentration difference of PhACs and DOM makes competitive adsorption phenomena appear quite unsurprising. The severity of competitive adsorption is related to DOM concentrations of treated waters in most cases [35], which makes wastewaters and WWTP effluents particularly unfavorable, due to overall very high DOM concentrations [36].

An example for adverse competitive DOM impacts on PhAC adsorption is shown in Fig. 1 via isotherms of carbamazepine adsorbing onto activated carbon from different waters. For pure water, the carbamazepine loading amounts to ~22 µg/mg at a liquid-phase concentration of 1 µg/L. At the same liquid-phase concentration, the loading declines to ~2.6 µg/mg in WWTP effluent at an initial carbamazepine concentration $c_0 = 58$ µg/L and ~0.27 µg/mg at $c_0 = 2.5$ µg/L, respectively. Given that typical WWTP effluents contain carbamazepine concentrations around 1–3 µg/L, loadings are thus ~100-fold decreased compared to pure water. Note that in real waters like the WWTP effluent shown in Fig. 1, the PhAC initial concentration has a direct impact on the position of the isotherm [37]. At increased initial PhAC concentrations, more PhAC molecules are present and can compete against DOM,

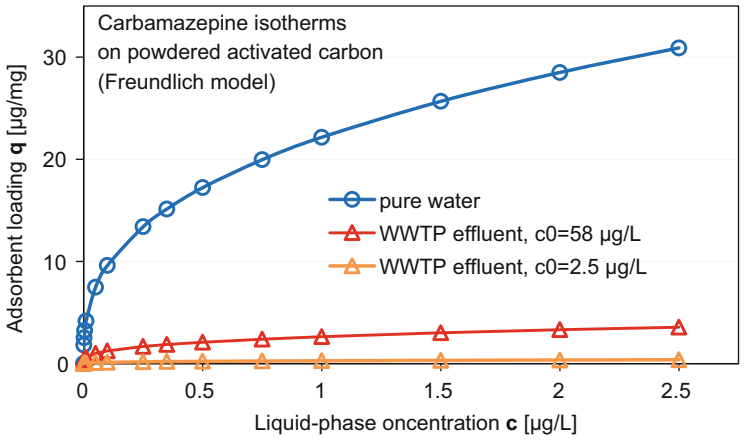


Fig. 1 Isotherms of carbamazepine (loadings q vs liquid-phase concentrations c after 48 h contact time, modeled as pseudo single-solute Freundlich isotherms based on real data) on powdered activated carbon (*Norit SAE Super*) in pure water and WWTP effluent (Berlin, Germany, DOC = 10–12 mg/L) at two different initial concentrations (c_0), depicted concentrations limited to ranges typically found in WWTP effluents for carbamazepine; unpublished data by the author

leading to overall higher loadings. In contrast, isotherms in pure water – without competing DOM – are mostly independent of the initial PhAC concentration.

2.2.2 Properties of Competitive DOM

DOM encompasses ubiquitously present background organic water constituents, originating from (1) microbes/plants/fungi/animals, (2) various anthropogenic applications, and (3) physical/chemical/biological transformation/degradation of substances from the former two groups. For these origins, DOM can have vastly variable molecular compositions, and the diversity of DOM constituents in a single water sample is enormous. The adsorption of DOM and therefore the competitiveness of DOM against PhAC adsorption relate to the large variability of DOM constituents [36–40].

To understand the underlying physical-chemical phenomena of DOM competitive adsorption, model substances can be helpful [19, 31, 41–46]. Nevertheless, despite the important fundamental insights of such studies, the actual mechanisms of competitive DOM adsorption in real waters remain partly unknown: The major complexity of DOM cannot be comprehensively simulated by model compounds. The only way to obtain manageable analytical information on DOM is to use "overall" characteristics from sum parameters like DOC or optical properties (e.g., ultraviolet/visual (UV-vis) light absorption). More advanced analytical techniques provide vastly more information but simultaneously lead to increased complexity of the data that we have to understand (e.g., [47, 48]).

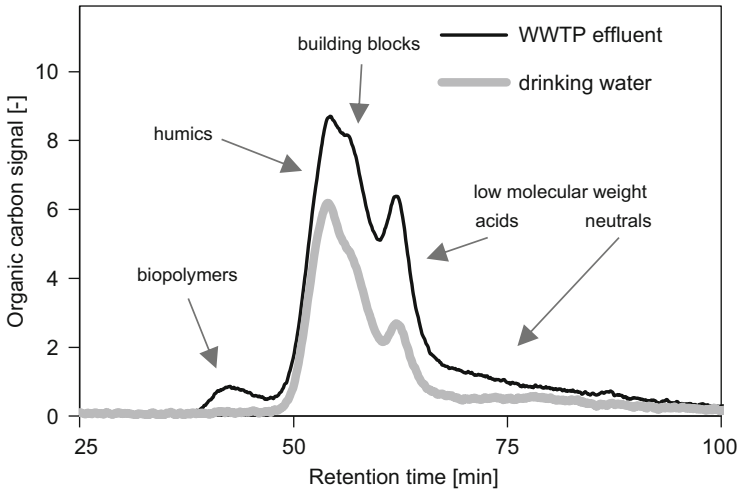


Fig. 2 LC-OCD chromatograms for Berlin (Germany) WWTP effluent (DOC = 10.9 mg/L) and drinking water (DOC = 5.1 mg/L) with indicators for typical DOM fractions according to [57]; unpublished data by the author

Advanced DOM characterization by liquid size-exclusion chromatography with online organic carbon and UV_{254} detection (LC-OCD-UV or LC-OCD) appears to provide a balance of increased analytical information and data manageability with respect to competitive adsorption. LC-OCD-UV demonstrates that different DOM fractions impose variable competitive stress on PhACs [49–53]. In particular, low molecular weight (LMW) constituents of DOM exert strong adverse impacts on PhAC adsorption [54–56]. These LMW DOM compounds have molecular weights of ~100–1,000 g/mol, thus in the range of most PhACs. Obviously, PhACs and LMW DOM target similar adsorption sites on many adsorbent materials and therefore compete with each other, leading to negative outcomes on PhAC removal. Exemplary LC-OCD chromatograms for Berlin WWTP effluent and drinking water (for comparison) are given in Fig. 2, with indicators for the typical DOM fractions encountered in real waters. LC-OCD bases upon size-exclusion chromatography which is why the largest DOM fractions (biopolymers/polysaccharides) elute the earliest and the smallest fractions (LMW acids and neutrals) elute the latest. Regarding competitive adsorption, the LMW acids and neutrals are the fractions that cause the strongest adverse effects against PhAC adsorption [52, 54, 55]. Relative to the total DOM, WWTP effluent contains higher amounts of these LMW compounds than drinking water (cf. Fig. 2).

2.2.3 Practical Implications

For the large majority of adsorbents, competitive adsorption is the most important phenomenon when considering PhAC removal in real waters, particularly in

wastewaters. Obviously, this has substantial impacts on operation of adsorptive treatment stages in practice, e.g., in reactor dimensioning, or experimental tests for performance assessments. The following important aspects have to be considered for correct practical application, particularly for carbonaceous adsorbents as the most widely used material:

(a) The position of PhAC adsorption isotherms in typical loading-vs-concentration diagrams moves upward with increasing initial concentration of the PhAC (Fig. 1). This is a direct result of the increased amount of PhAC able to compete with DOM for adsorption sites [37]. Therefore, the adsorbent capacity for a PhAC is linearly proportional to the PhAC initial concentration in a given real water [58].
(b) This leads to constant relative PhAC removals for fixed doses of powdered adsorbents in a given real water, independent of the PhAC initial concentration [35, 58–61].
(c) In granular adsorbent applications, adsorbent capacities are linearly correlated to PhAC initial concentrations, meaning that relative breakthrough curves (depicted over specific throughputs, e.g., bed volumes) are independent of the PhAC initial concentration [52, 62, 63].
(d) The linear proportionality between adsorbent capacities and initial PhAC concentration in real waters is sometimes *falsely* described as "linear isotherm." This becomes obvious when considering the ideal adsorbed solution theory (IAST) equivalent background compound (EBC) model for variable initial PhAC concentration at constant EBC concentrations [36, 37, 58].
(e) Besides impacts on adsorption isotherms, competitive DOM constituents hamper the mass transfer/kinetics, resulting in increased contact times in real waters compared to pure/synthetic waters [51]. The slower mass transfer occurs in powdered and granular applications and has substantial effects on reactor design, residence times, and invest as well as operational costs.
(f) Competitive adsorption affects weak adsorbates more severely than strong adsorbates, and operators/practitioners should meticulously check if adsorptive removals of weak adsorbates are sufficient [15, 28, 64].
(g) Different types of wastewaters induce varying adverse effects on PhAC adsorption due to the variable competitiveness of different DOM fractions; WWTP effluents are generally preferable as compared to raw wastewaters or influents [40].
(h) The importance of the above-listed aspects for PhAC adsorption from real waters can strongly depend on the type of adsorbent. Non-carbonaceous adsorbents may behave differently to a large extent. For example, zeolites usually suffer far less from competitive adsorption but usually are only effective for specific PhACs [65].

2.3 *Inorganic Water Composition*

The pH and salt composition of a solution can have strong effects on adsorptive mechanisms. It is normally not feasible to adjust these parameters in practical applications; but knowing their influences can help in understanding the occurring phenomena. This can lead to improved adsorbent choices, e.g., by increasing the point of zero charge (pH_{PZC}) aiming at predominantly protonated adsorbent surfaces, alleviating adsorption of negatively charged PhACs [19, 31]. Since pH or ionic composition normally does not vary strongly for most wastewaters, potential favorable or adverse effects are usually relatively stable for a given treatment location/wastewater. However, some WWTPs are partially or strongly influenced by dilution, intermittent industrial discharges, or other factors inducing much variation to the treated waters. In such cases, variable inorganic wastewater composition should be considered regarding potential effects on PhAC adsorption.

3 Adsorbent Materials

The variety of adsorbent materials for PhAC removal is very large. A rough overview on research output related to various adsorbent materials for wastewater purification is given in Fig. 3, comparing yearly scientific publications, as obtained by searching for a fixed set of terms relating to PhACs, wastewater, and adsorption, combined with different material classifications. The search for "activated carbon"

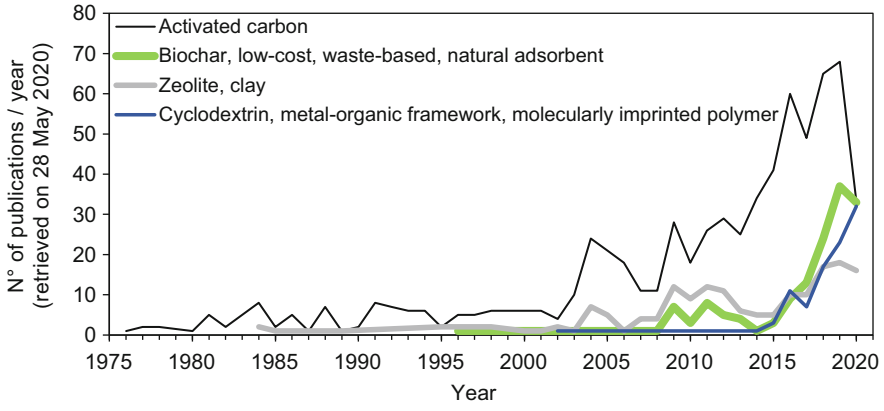


Fig. 3 Number of publications per year from a Scopus® literature search on 28 May 2020 using the command "[XYZ] AND wastewater AND adsorption AND (pharmaceutical OR antibiotic OR (organic AND (pollutant OR contaminant OR compound OR substance))) AND NOT (desalination OR (heavy metal) OR (ion exchange) OR salt OR inorganic)" in the search fields "article title" and "keyword," with *[XYZ]* referring to the search items in the legend above [66]

clearly returns the highest numbers of hits each year, but publications on new adsorbents are certainly on the rise during the last 5 years or so.

3.1 Carbon-Based Materials

3.1.1 Activated Carbon

Activated carbon (AC) is by far the most commonly used adsorbent for the removal of organic contaminants (including PhACs) from impacted waters. Its ratio of performance to costs is still unrivalled, and besides water purification, AC has additional applications in hundreds of industries. Thus, the overall availability is vast, resulting in a rapidly growing global market projected to pass 3.3×10^9 kg in 2021 [67]. Theoretically, this amount would allow for the treatment of ~1.7×10^{14} L of water at a typical AC dose of 20 mg/L, thus enough for treating 50 L of water per person per day year-round at a world population of 9×10^9. With manifold different production techniques [20, 68], the product diversity is enormous.

This diversity results in strongly variable PhAC removals, as demonstrated in Fig. 4 which compares the removals of 2 PhACs and a metabolite, as achieved with 18 different powdered activated carbons (PACs). Almost all PACs demonstrate the highest removal for carbamazepine, followed by 4-formylaminoantipyrine and gabapentin. The results in Fig. 4 further underline that there are large differences between the performances achieved with the various products tested here. For example, product "B" reaches >95% carbamazepine, ~70% 4-formylaminoantipyrine, and ~25% gabapentin removal, respectively. In contrast, product "K" achieves <5%, 5–10%, and ~10%, respectively. These data highlight

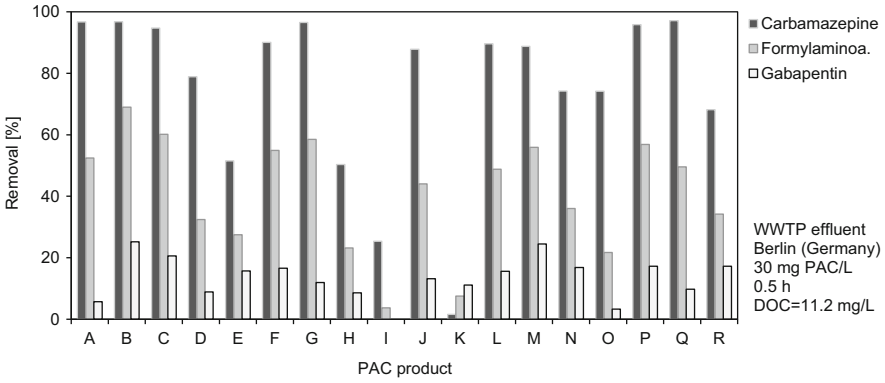


Fig. 4 Removal of carbamazepine, 4-formylaminoantipyrine (metabolite of analgesic metamizole), and gabapentin by 18 different PACs from WWTP effluent (Berlin, Germany) at 30 mg PAC/L and 30 min contact time, initial DOC = 11.2 mg/L; unpublished data by the author

the importance to assess the respective scenario prior to application, especially regarding targeted PhACs and available products.

3.1.2 Low-Cost Carbonaceous Adsorbents

An extensive amount of studies examines low-cost adsorbents for PhAC removal; several reviews provide detailed overviews on the tested materials [69–71] and production techniques [72, 73]. Despite the plenty of studies on alternative adsorbents, two fundamental shortcomings often jeopardize their practical applicability: (1) use of de-ionized/pure/synthetic water instead of real (waste)water and (2) unrealistically high PhAC concentrations in mg/L range (instead of ng/L or μg/L range) – leading to substantially overestimated potentials (cf. Sect. 2.2.1). Under more realistic conditions, low-cost adsorbents may only reach similar performance as activated carbons of moderate quality [73, 74]. Nonetheless, from environmental and economical perspectives, less efficient low-cost adsorbents like biochar can still be a better choice than activated carbons due to their reduced global warming and/or health effects [75] and reduced costs [76].

3.1.3 Special Carbonaceous Materials

Special carbonaceous adsorbent materials cover a niche for applications where the required amounts are low or where the costs play a subordinate role. In wastewater treatment both conditions are seldomly met, and such adsorbents are normally not applied unless production techniques can be transferred to a larger scale and thus be reduced in costs. The materials often have special properties, e.g., they are partly magnetic, allowing for alleviated removal from treated waters and reuse [77], or highly activated with specific internal surfaces up to $>3{,}000\ m^2/g$ [78], or contain doping elements such as nitrogen leading to modified internal surface chemistries [79].

3.2 Other Materials

3.2.1 Zeolites and Clays

Clays are silicate-based inorganic minerals of highly variable composition, and zeolites are a subgroup of silicates, with aluminum replacing silicon in defined positions within the mineral framework. The overall costs are usually cheap as compared to commercially available high-grade activated carbons. Clay/zeolitic minerals have defined symmetrical pores, resulting in highly specific adsorptive behavior. For example, the cage-like pore structure of zeolites allows for specific removal of substances that fit into the cages, reducing the proneness to competitive

adsorption [65]. However, zeolites may be less effective when a multitude of PhACs shall be removed simultaneously [59]. For some PhACs like the antibiotic sulfamethoxazole, zeolites can reach relatively good removals from real WWTP effluent in the range 43% to 76% with 10 mg/L dosages [80], thus comparable to removals achieved with ACs [12]. A general peculiarity of adsorption onto zeolites can be S-shaped isotherms because of increased adsorbate-adsorbate interactions at high concentrations/loadings [81]. Despite these examples for ongoing research activity, there is still a lack of widespread insights into PhAC adsorption onto clay-based materials under realistic conditions [82].

3.2.2 Oligo- and Polysaccharides, Metal-Organic Frameworks, and Molecularly Imprinted Polymers

Cyclodextrins are natural circular oligosaccharides whose application as adsorbents for PhAC removal from contaminated waters is relatively new [83, 84]. The application on practical scales is still challenging since cyclodextrins swell upon contact with water, making controlled usage delicate. β-Cyclodextrins appear to be less prone to DOM adsorption competition than ACs but also demonstrate less overall adsorption capacity; doped onto cellulose microcrystals, they achieved some promising granular applications on the lab scale [18]. Other saccharide-based adsorbents include chitosans [85] and various chitosan-containing composite materials. Metal-organic frameworks (MOFs) are a new class of highly tailored synthetic material combinations of inorganic building blocks and organic linkers. Their application for (waste)water purification appears promising [86] but is strongly limited due to the elaborate synthesis of MOFs. Similarly, molecularly imprinted polymers are a new class of potential adsorbents [87], but selectivity for certain PhACs and elaborate production are bottlenecks for wider application.

3.3 Comparison

Despite the above-given examples for new developments, the choice of adsorbent base material is still relatively straightforward, since carbonaceous adsorbents are clearly the most widespread. A fundamental factor is the related costs, which range from 1 €/kg to 5 €/kg for bulk activated carbons, e.g., 1.60 €/kg for GAC and 1.80 €/kg for PAC [88]. Most "high-end" adsorbent materials cannot easily compete with such prices. Furthermore, the capacities for production need to be in the range of several tons per day for supplying a *single* large-scale WWTP (e.g., 250,000 m^3/day $\times$ 20 g/m^3 = 5 t/day).

In addition, the choice strongly depends on the respective goal. In most cases, broad effects are sought with the aim of removing a multitude of PhACs, some of which might not even be in the scope of analytical monitoring. This aspect, again, favors carbonaceous adsorbents over more specialized materials like zeolites or

cyclodextrins. Due to their highly defined internal structure, especially zeolites have strong affinities for very specific PhACs, e.g., regarding size or charge, while blocking out non-suitable PhACs. Logically, a wide range of PhACs with largely variable structural/chemical properties suffers from such exclusive material characteristics [59].

4 Practical Aspects

4.1 Powdered Applications

Powdered adsorbents such as powdered activated carbon (PAC) are fine particles in the low to medium µm range. They are mixed to the treated water either in dry form or as concentrated slurries, at variable locations within WWTPs. After the desired contact time, powdered adsorbent and water are separated by flocculation, sedimentation, filtration, centrifugation, or combinations thereof.

4.1.1 Process Options

The primary goal in powdered adsorption process optimization is to exploit the available adsorbent capacity to the fullest extent possible while minimizing the required contact time. To achieve these goals, contact between adsorbents and treated water should be intensified where possible. However, competitive adsorption by DOM impedes rapid exploitation of adsorbent capacities and decelerates mass transfer (cf. Sect. 2.2.3). Therefore, several means for improved/prolonged adsorbent-water contact have been developed, resulting in different options for powdered adsorbent processes:

(a) *Direct dosage into existing biological stages* on WWTP, with continuous integration of the powdered adsorbent into sludge flocs, subsequent separation of the adsorbent with the sludge, and recirculation of the sludge-adsorbent slurry into the biology influent [88, 89]. Realization of this design is relatively straightforward since WWTPs have to be modified only marginally (installation of adsorbent dosage). However, increased DOM concentrations and competitiveness in early WWTP stages can reduce the overall efficiency to a considerable extent [40].

(b) *Direct dosage into advanced wastewater treatment stages* like clarifiers or filtration. Separation of the powdered adsorbent is usually achieved by coagulation-sedimentation and/or rapid/deep-bed filtration. If existing filtration stages are used for adsorbent separation, the filtration regimes (i.e., backwash intervals) are usually affected due to the increased loads to the filter material [90, 91]. Even distribution of the powdered adsorbent over the filter bed can substantially increase the performance [92].

(c) *Implementation of separate advanced slurry contactors/reactors,* followed by separation by sedimentation/filtration/centrifugation with recirculation of the adsorbent slurry for repeated usage of the adsorbent and increasing adsorbent concentrations in the system. This setup requires extensive additional modifications to wastewater treatment trains but normally rewards the efforts with superior adsorbent exploitation [15, 93–95].

Independent of the kind of powdered adsorbent process, separation of the adsorbent from the slurry is always necessary to avoid discharge of loaded adsorbents into receiving waters [96]. This is particularly important for very fine adsorbent materials like super-fine PAC and therefore demands for well-adjusted coagulation [97]. Nonetheless, super-fine PAC can have substantial improvements regarding adsorption capacities and required contact times as compared to conventional PAC [98], resulting in reduced slurry adsorber sizes and shorter contact times [29]. Additional improvements regarding adsorbent-water separation can be achieved by membrane filtration which usually allows for complete retention of adsorbent particles [99].

A general drawback of PAC as compared to GAC is that PAC normally cannot be reactivated equally well because of the following factors: (1) the overall burn-off during thermal reactivation is higher for PAC, (2) used PAC is usually mixed with various wastewater sludge components which cause interferences, and (3) the adsorptive qualities of PAC decrease more severely at the relatively high temperatures needed [100]. However, examples exist of pilot-scale thermal reactivation and vacuum regeneration [100, 101]. For adsorbents like zeolites, which can withstand strongly oxidizing agents, regeneration by in situ oxidation, e.g., using ozone, of adsorbed PhACs appears to be an interesting option [102].

4.1.2 Performance Prediction

Predicting the adsorptive performance is important for efficient operation and reduced costs; however, various factors influence the process efficiency. For example, variable incoming flows can increase/reduce contact times of adsorbent and water, and variable water composition, e.g., regarding competitive DOM concentrations, can influence PhAC removal. In addition, different grades of delivered adsorbent batches can induce variations of the overall adsorptive removal as well. Last but not least, it is important to realize that the relationship between PhAC removal and adsorbent dosage is strongly nonlinear in most cases and therefore the effects of variable dosages are not always easy to estimate [103].

Theoretical predictions of adsorption performance involve various concepts, e.g., quantitative structure-activity relationships (QSARs) relating adsorbent, PhAC, and water characteristics to performance indicators like PhAC removal [14, 104]. An important shortcoming of such semi-descriptive methods for performance predictions is that they often lack the integration of water quality parameters. Thus, they do not account for competitive adsorption, and the associated models usually have to be empirically corrected for influences of DOM [105]. This can be overcome by

initializing competitive adsorption models in an integrated fashion, additionally using water quality characteristics such as the concentration of LMW DOM [36].

Despite the usefulness of the above-given examples of performance prediction for powdered adsorbents in lab-based studies, these approaches are seldom useful on truly practical scales. The problem is that they require a relatively large amount of analytical information on the adsorbent, the PhAC, and the water composition – all of which are not always easily available, particularly not on operating full-scale plants. Therefore, strictly operational performance assessments, which should be available without substantial loss of time, have to be simplified. However, direct online measurements of PhAC removals are not possible with the currently available analytical techniques, since they require trained personnel on advanced analytical equipment and sophisticated sample preparation (high-performance liquid chromatography-mass spectrometry).

Therefore, several approaches exist to project adsorptive PhAC removals by using surrogate parameters' adsorptive removal, as was initially conceived for oxidative stages in a similar way [106]. The AC-induced removals of PhACs, either as individual PhAC or averaged over several PhACs, can be correlated to easily measurable sum parameters, e.g., spectrometric or DOC. For example, ultraviolet absorption at 254 nm (UV_{254}) allows for relatively precise performance assessments, mostly independent of:

- PAC product and concentration [11, 107]
- PhAC initial concentration [36]
- WWTP effluent [13, 36]
- PAC recirculation stage [108]
- Coagulation, if adequately corrected for [109]

An example for such a correlation between the removal of the lipid-lowering agent bezafibrate and the removal of UV_{254} is given in Fig. 5, showing a mostly linear relation of the two parameters. Note that in Fig. 5, the linearity covers the entire range of the y-axis, which is a result of the quite similar PAC doses of 20 mg/L and 30 mg/L used here. In cases of higher variability of the PAC doses, such correlations are normally L-shaped since the PhAC removals approach 100% while the UV_{254} removals continuously increase [11]. This concept is also practically applicable, and PAC dosages can be empirically adjusted toward the desired performances using online measurements on pilot or full scales [110, 111].

4.2 Granular Applications

Granular adsorbent particles typically lie in the range of 0.5 mm to 3 mm and are applied as loose bulk fillings in fixed-bed filters. The treated water passes through the filter bed, coming into contact with the adsorbent particles and thus promoting adsorption of PhACs. In contrast to powdered adsorbent applications, granular applications contain a large excess adsorbent during the initial phase of operation.

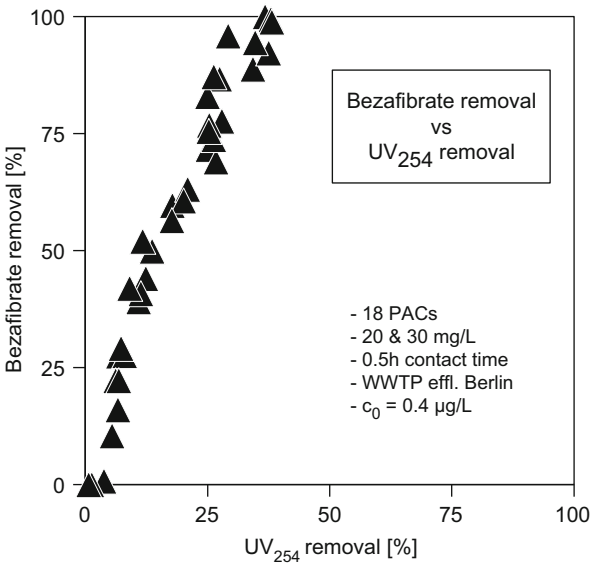


Fig. 5 Removals of the lipid-lowering agent bezafibrate vs removals of UV_{254} for various PAC products, two PAC doses, 0.5 h contact time in Berlin WWTP effluent; unpublished data by the author

During continuous operation, more and more volume of water is treated, and the ratio of adsorbent mass to treated water approaches values similar to powdered applications. Similar to "classical" granular filter materials, granular adsorbent media have to be backwashed regularly, especially when treating turbid waters – like wastewaters.

4.2.1 Operation Fundamentals

Mass transfer resistances, particularly within adsorbent pores of larger granular media, are the reason that not all PhAC molecules can enter the same adsorbent grains at the same time. Some PhAC molecules travel farer down the filter bed and, at a certain point, reach the filter effluent – without having been adsorbed. This marks the beginning of breakthrough, and PhAC effluent concentrations continuously rise thereafter, resulting in the typical occurrence of breakthrough curves (BTCs). Examples for BTCs, depicted as the dimensionless relative effluent concentration (effluent concentration divided by influent concentration, c/c_0) over the specific throughput in bed volumes fed to the filter, are shown in Fig. 6. Note that the only difference between the exemplary BTCs in Fig. 6 originates from differently fast film and/or internal mass transfer rates; the overall adsorption capacities are the same in all three cases.

Ideally, BTCs are very steep, indicating fast external and internal mass transfer and near-complete exploitation of the adsorber capacity (cf. black line in Fig. 6). Such BTCs can be expected in well-designed adsorbers without competitive effects by DOM. More realistically, BTCs in strongly competitive waters like WWTP

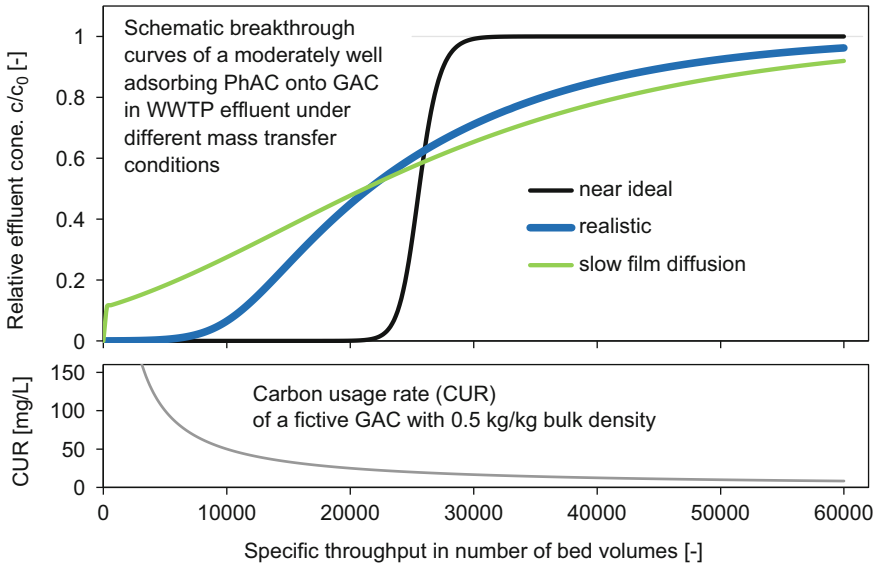


Fig. 6 *Top:* Examples for BTCs of a fictive moderately well-adsorbing PhAC onto GAC from WWTP effluent in three scenarios with variable film and internal mass transfer resistances; thin gray line indicates $c/c_0 = 1$; modeled as pseudo single-solute BTCs using the FAST 2.1 software [116]. *Bottom:* Carbon usage rate (CUR) corresponding to the specific throughput in number of bed volumes for a GAC with bulk density 0.5 kg/L

effluents are far from ideal, usually with early increases of c/c_0 and overall flat appearance (cf. blue line in Fig. 6), indicating slow usage of the available adsorption capacity [52, 62]. This shape of BTC is predominantly caused by pore-blocking DOM, which hampers PhAC mass transfer toward and within the adsorbent pores [103]. Increased water-adsorbent contact times, usually expressed via the empty bed contact time (EBCT), can help in improving the BTC shape [112]. However, this effect cannot be arbitrarily exploited and levels off at a certain filter height/EBCT [55, 113]. Moreover, practical considerations limit the EBCT since it directly relates to the adsorbent amount and thus, the adsorber dimensioning, which logically should be kept reasonably small for economic reasons.

Unfortunately, flat and stretched appearance of BTCs in strongly competitive waters can be profoundly aggravated if the advective mass transfer toward the adsorbent grains is inefficient (cf. green line in Fig. 6), characterized by almost immediate breakthrough. Such BTCs indicate poorly conceived adsorbers, either characterized by very low Reynolds numbers $\ll 1$ or grains being too big (e.g., ~2.5 mm) [114]. In addition to DOM interference by pore blocking, strong direct site competition by DOM can also occur, particularly for weakly adsorbing PhACs. In such cases, competing DOM can displace previously adsorbed PhAC, and this can lead to temporary concentration overshoots with $c/c_0 > 1$ [115].

The so-called carbon usage rate (CUR, Fig. 6 bottom) serves as a measure for the efficiency of granular adsorber operation. Ideally, CURs at specific levels of

breakthrough are as low as possible. For example, at a minimum removal of $\geq$70% ($c/c_0 \leq 0.3$), the throughput on the blue BTC is maximally ~16,000 bed volumes, corresponding to a CUR of ~31 mg/L. With the dimension of mass per volume (e.g., mg/L), CURs are comparable to dosages of powdered adsorbents. Also, the CUR is helpful since many granular adsorbents are sold by mass (not volume) and have different bulk densities. Note that the definition of the CUR as a "rate" is very disadvantageous, since the term "rate" usually relates to time-dependent quantities like speeds or reaction rates. A more suitable expression would simply be *carbon usage*.

4.2.2 Process Options

Process options for granular adsorbers, and combinations thereof, are various, e.g., upward or downward flow fixed-bed systems [117] and filter connections in series or in parallel for profound performance improvements [88, 118, 119]. More advanced options include continuously backwashed/moving-bed or fluidized-bed adsorbers with advantages due to uninterrupted operation [120]. Continuously backwashed filters with conventional media like sand can often be easily upgraded to adsorbers [121]. Such modifications are particularly attractive for small- and medium-sized WWTPs, as they are feasible by simple adjustments and an exchange of the filter media. Setups with fluidized beds, held in place with filtration membranes, allow for usage of micro-grain granular adsorbents with superb kinetic properties [64]. A relatively widespread application of granular adsorbers is as polishing step for the effluents of oxidative stages, to reduce potential harmful toxic effects from by-products [122].

Despite the above-given examples for new approaches to granular adsorption, most setups are still relatively "classic" fixed-bed filters with granular activated carbon. Due to the vast knowledge on rapid filtration, granular adsorbers are relatively straightforward to operate, and to date, manifold examples of successful application exist [113]. Granular adsorbers are only slightly different in their operation than typical rapid filters, e.g., filter velocities are lower and, therefore, contact times are longer. Very generally, the treated water passes through a bed of 1–2.5 m height, at velocities of, e.g., 5–12 m/h, resulting in EBCTs of 5–30 min. Shorter EBCTs cause rapid and inefficient PhAC breakthrough curves, whereas longer EBCTs are normally not feasible as of space constraints (cf. Sect. 4.2.1). Also, the advantageous effects of increasing EBCTs for reducing adverse kinetic impacts by DOM tend to flatten at a certain point [52].

Due to the comparatively slow continuous usage of adsorbent in granular applications, operation times often exceed several months or years. Therefore, substantial additional biological effects occur, increasing the overall removal of various PhACs beyond what could be expected of solely adsorptive removals [117]. The long operational times of granular adsorbers also require regular backwashing due to particulate deposits within the filter media. Since wastewaters are comparatively turbid waters, the overall frequency of backwashes in treating such waters is high,

e.g., >1/day, thus much higher than in, e.g., drinking water treatment. The high turbidity is also the reason why granular adsorption is normally limited to WWTP effluents, thus wastewaters whose loads of suspended solids do not require interrupting filter operation too frequently for backwashing [123].

For many granular adsorbents, regeneration and reactivation are attractive options to increase the operation times of filter media and therefore raise the overall efficiency. Considering that reactivated GAC products cost about 1/5 of virgin GAC, the reuse of spent filter materials can also result in profound economic benefits [113]. Of course, adsorbent reuse is also more environmentally friendly, given that many adsorbents are resource-consuming during their production [88]. There are manifold possibilities for adsorbent regeneration and reactivation, ranging from thermal [124] to chemical, microbial, and vacuum processes [101].

4.2.3 Performance Prediction

The interactions in granular adsorbers between the adsorbent internal (specific internal surface, pore volume distribution) and external properties (particle size), water composition (PhACs, DOM), and operational parameters (EBCT, flow conditions, long operation times) make performance prediction generally more delicate than in powdered applications. In addition, regular backwash can partly disturb the filter media filling and lead to stratification [125]. A very straightforward tool for robust and online performance prediction is via the use of easily measurable surrogate parameters which allow for inferring PhAC removals, e.g., UV_{254} or fluorescence [126].

An elaborate but mostly accurate method for adsorber performance prediction involves rapid small-scale column tests (RSSCTs), miniature duplicates of large-scale filters with identical flow conditions [115]. RSSCTs allow for shortening filter run times and decreasing necessary volumes up to several orders of magnitude. Despite their overall successful application for WWTP effluent scenarios [62], caution must be kept regarding blockage of the filter bed and associated operational difficulties [127]. Using DOC concentrations and different RSSCTs allows for the determination of fouling parameters to account for pore-blocking effects [128].

A general finding from various RSSCTs (and larger-scale tests) is that BTCs on GAC are mostly independent of the PhAC influent concentration in a given water [62, 129]. This translates into a linear relationship between the GAC capacity and the influent concentration [62]. Furthermore, the analytical information on LMW DOM (cf. Fig. 2) proves useful for the alignment of BTCs in different types of waters [62]. Granular adsorber performance can further be predicted via deterministic models, however requiring manifold simplifications as in Fig. 6 [116], or BTC data for prior fitting [55]. More complex models include, thus, direct adsorption site competitive effects by DOM [130] and pore-blocking effects [131], yet difficulties remain regarding initialization. Regression-based models like quantitative structure-activity relationships (QSARs) use adsorbent or PhAC properties, among others, and BTC data to obtain best-fit parameters [132, 133].

4.3 Applicability, Costs, and Environmental Impacts

The decision if adsorption is a feasible option for PhAC removal in a specific wastewater treatment scenario depends on various factors. Profound practical experience has been gained in full-scale advanced wastewater treatment using adsorption (and oxidative techniques) during the last years in a variety of countries, e.g., Switzerland [134] and Germany [135]. The available resources of information from various case studies and fully operational plants contain different treatment designs/approaches, sizes, and locations, among others. In addition, many full-scale research activities are still ongoing, providing more details on special adsorption stages designs or adsorbent materials (e.g., [119]). Despite such plenty of knowledge, decision-makers need to assess each case individually for monetary, societal, and environmental benefits and expenditures.

The costs for advanced wastewater treatment cover relatively wide ranges, depending on the targeted PhACs' removability/adsorbability, the respective WWTP size, construction and operational design, and applied adsorbents and chemicals, among other factors. For German adsorptive treatment stages using activated carbon, numbers of 0.05 €/m^3 up to 0.30 €/m^3 in WWTP effluent are reported, translating to 2 €/a/inhabitant up to 12 €/a/inhabitant [88]. Note, however, that such cost calculations can lose their validity rapidly due to increasing capital costs as a consequence of augmented demand, inflation, and other factors.

Since adsorbent production and operation of treatment stages both consume resources and energy, potential adverse environmental impacts have to be crucially assessed. For example, the energy demand for the production of virgin activated carbon is comparatively high and becomes more feasible if reuse/reactivation of spent adsorbent is considered, too [136]. Reactivation is normally only available for GAC, not for PAC. However, PAC increases the heating value of the accruing sludge, thus partly balancing the drawback of not being reactivatable. Moreover, the source material for activated carbon production can increase the cumulated energy consumption of advanced wastewater treatment stages by >100%, e.g., when switching from coconut husk to lignite. Similarly, the global warming potential profoundly depends on source materials, possibility for reactivation, adsorbent usage, as well as the location of treatment [88].

Examples for the cumulated energy consumption and global warming potential, including all indirect effects (e.g., production), for different advanced wastewater treatment scenarios with activated carbon, are given in Fig. 7. The error margins outline the high variability as induced by different material sources for the produced activated carbon: renewable (left, coconut husk) versus fossil (right, lignite). Note that the PAC and GAC scenarios were chosen for similar expectable PhAC removals [88] – and differ substantially considering the GAC-related CURs corresponding to the shown bed volumes of throughput (cf. Fig. 6). The reason for this higher GAC consumption as compared to PAC is adverse kinetic DOM impacts, resulting in unfavorable BTC shape. Remarkably, the ecologic benefits from reactivation,

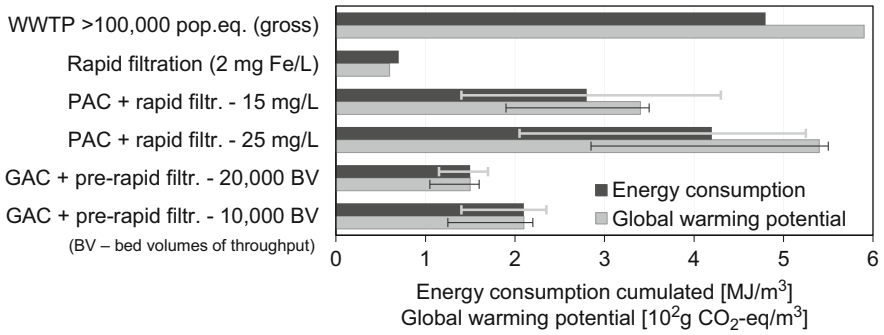


Fig. 7 Energy consumption (cumulated) and global warming potential per m^3 WWTP effluent for two PAC (dosages) and two GAC (throughput) treatment scenarios, with error bars indicating min/max values (see text for more information) and average values for typical large WWTPs (without advanced treatment) and rapid filtration for comparison (data adapted from [88])

incorporated within the GAC data in Fig. 7, clearly outweigh the drawback from unfavorable BTCs.

5 Conclusion and Outlook

Adsorption is an increasingly viable option for the removal of pharmaceutically active compounds from wastewaters, with several countries installing full-scale capacities in various locations. The insights gained on efficient adsorbent materials, installation, operation, performance prediction, and adsorptive mechanisms are substantial. The variety of process designs comprises simple upgrades of existing plants, e.g., dosage of powdered adsorbents into biological basins, as well as more sophisticated installations, like reaction chambers with sludge recirculation. Similarly, granular adsorbents can serve as exchange material for media in existing rapid filters – or as filling of newly conceived downstream adsorbers. Future research needs to optimize processes regarding high and continuous removal of pharmaceutically active compounds, with a particular focus on understanding and alleviating adverse competitive effects of dissolved organic matter.

Besides profound developments in adsorber design and operation, demands for improved adsorbent materials push manufacturers toward increasing efficiency and reducing environmental impacts. Carbonaceous adsorbents are still by far the most widespread, but various new structures such as zeolites, cyclodextrins, and metal-organic frameworks create growing interests – at least from a scientific perspective. The competitiveness of these new adsorbents will depend on the achievability of broad removal of pharmaceuticals, large production volumes, aggressive prices, toxicological harmlessness, and low impacts during production.

Last but not least, improvements of water quality by advanced treatments should not be pitted against external environmental costs. To avoid impacts from adsorbent

manufacture and its excessive usage, we must exploit additional means, too. Among these are overthinking exaggerated pharmaceutical consumption, e.g., in livestock, designing degradable pharmaceuticals, and exploiting renewable resources for adsorbent and energy production.

References

1. Reemtsma T, Weiss S, Mueller J, Petrovic M, Gonzalez S, Barcelo D et al (2006) Polar pollutants entry into the water cycle by municipal wastewater: a European perspective. Environ Sci Technol 40(17):5451–5458
2. Schulze S, Zahn D, Montes R, Rodil R, Quintana JB, Knepper TP et al (2019) Occurrence of emerging persistent and mobile organic contaminants in European water samples. Water Res 153:80–90
3. Swiss Confederation (2016) Verordnung des UVEK zur Überprüfung des Reinigungseffekts von Massnahmen zur elimination von organischen Spurenstoffen bei Abwasserreinigungsanlagen (ordinance of the UVEK on the review of purification effects by measures for the elimiation of organic micro-pollutants in wastewater treatment plants). Document number 814.201.231. https://www.admin.ch/opc/de/classified-compilation/20160123/index.html
4. European Commission (2018) Watch list of substances for Union-wide monitoring in the field of water policy. Document number 32018D0840. http://data.europa.eu/eli/dec_impl/2018/840/oj
5. Blair B, Nikolaus A, Hedman C, Klaper R, Grundl T (2015) Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. Chemosphere 134:395–401
6. Eggen RIL, Hollender J, Joss A, Scharer M, Stamm C (2014) Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. Environ Sci Technol 48(14):7683–7689
7. Ruff M, Mueller MS, Loos M, Singer HP (2015) Quantitative target and systematic non-target analysis of polar organic micro-pollutants along the river Rhine using high-resolution mass-spectrometry – identification of unknown sources and compounds. Water Res 87:145–154
8. McCance W, Jones OAH, Edwards M, Surapaneni A, Chadalavada S, Currell M (2018) Contaminants of emerging concern as novel groundwater tracers for delineating wastewater impacts in urban and peri-urban areas. Water Res 146:118–133
9. Reemtsma T, Berger U, Arp HPH, Gallard H, Knepper TP, Neumann M et al (2016) Mind the gap: persistent and Mobile organic compounds water contaminants that slip through. Environ Sci Technol 50(19):10308–10315
10. Worch E (2012) Adsorption Technology in Water Treatment – fundamentals, processes, and modeling. De Gruyter, Berlin
11. Zietzschmann F, Altmann J, Ruhl AS, Dünnbier U, Dommisch I, Sperlich A et al (2014) Estimating organic micro-pollutant removal potential of activated carbons using UV absorption and carbon characteristics. Water Res 56:48–55
12. Mailler R, Gasperi J, Coquet Y, Deshayes S, Zedek S, Cren-Olive C et al (2015) Study of a large scale powdered activated carbon pilot: removals of a wide range of emerging and priority micropollutants from wastewater treatment plant effluents. Water Res 72:315–330
13. Altmann J, Ruhl AS, Zietzschmann F, Jekel M (2014) Direct comparison of ozonation and adsorption onto powdered activated carbon for micropollutant removal in advanced wastewater treatment. Water Res 55:185–193

14. de Ridder DJ, Villacorte L, Verliefde AR, Verberk JQ, Heijman SG, Amy GL, van Dijk JC (2010) Modeling equilibrium adsorption of organic micropollutants onto activated carbon. Water Res 44(10):3077–3086
15. Zietzschmann F, Dittmar S, Splettstößer L, Hunsicker J, Dittmann D, Meinel F et al (2019) Fast empirical lab method for performance projections of large-scale powdered activated carbon re-circulation plants. Chemosphere 215:563–573
16. Wang J, Wang S (2016) Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: a review. J Environ Manag 182:620–640
17. Radovic LR, Silva IF, Ume JI, Menéndez JA, Leon CALY, Scaroni AW (1997) An experimental and theoretical study of the adsorption of aromatics possessing electron-withdrawing and electron-donating functional groups by chemically modified activated carbons. Carbon 35 (9):1339–1348
18. Ling Y, Alzate-Sánchez DM, Klemes MJ, Dichtel WR, Helbling DE (2020) Evaluating the effects of water matrix constituents on micropollutant removal by activated carbon and β-cyclodextrin polymer adsorbents. Water Res 173:115551
19. Dittmar S, Zietzschmann F, Mai M, Worch E, Jekel M, Ruhl AS (2018) Simulating effluent organic matter competition in micropollutant adsorption onto activated carbon using a surrogate competitor. Environ Sci Technol 52(14):7859–7866
20. Bandosz TJ, Ania CO, Dfaz JAMN, Beguin F, Choma J, Ingaki M et al (2006) Activated carbon surfaces in environmental remediation. Elsevier, Amsterdam
21. Kilduff JE, Karanfil T, Chin YP, Weber WJ (1996) Adsorption of natural organic polyelectrolytes by activated carbon: a size-exclusion chromatography study. Environ Sci Technol 30(4):1336–1343
22. Ding L, Snoeyink VL, Marinas BJ, Yue ZR, Economy J (2008) Effects of powdered activated carbon pore size distribution on the competitive adsorption of aqueous atrazine and natural organic matter. Environ Sci Technol 42(4):1227–1231
23. Ji LL, Liu FL, Xu ZY, Zheng SR, Zhu DQ (2010) Adsorption of pharmaceutical antibiotics on template-synthesized ordered micro- and mesoporous carbons. Environ Sci Technol 44 (8):3116–3122
24. Piai L, Dykstra JE, Adishakti MG, Blokland M, Langenhoff AAM, van der Wal A (2019) Diffusion of hydrophilic organic micropollutants in granular activated carbon with different pore sizes. Water Res 162:518–527
25. U.S. National Library of Medicine (2020) ChemIDplus advanced. https://chem.nlm.nih.gov/chemidplus/. Accessed 28 May 2020
26. Aschermann G, Schröder C, Zietzschmann F, Jekel M (2019) Organic micropollutant desorption in various water matrices – activated carbon pore characteristics determine the reversibility of adsorption. Chemosphere 237:124415
27. Pelekani C, Snoeyink VL (1999) Competitive adsorption in natural water: role of activated carbon pore size. Water Res 33(5):1209–1219
28. Nowotny N, Epp B, von Sonntag C, Fahlenkamp H (2007) Quantification and modeling of the elimination behavior of ecologically problematic wastewater micropollutants by adsorption on powdered and granulated activated carbon. Environ Sci Technol 41(6):2050–2055
29. Bonvin F, Jost L, Randin L, Bonvin E, Kohn T (2016) Super-fine powdered activated carbon (SPAC) for efficient removal of micropollutants from wastewater treatment plant effluent. Water Res 90:90–99
30. Takaesu H, Matsui Y, Nishimura Y, Matsushita T, Shirasaki N (2019) Micro-milling superfine powdered activated carbon decreases adsorption capacity by introducing oxygen/hydrogen-containing functional groups on carbon surface from water. Water Res 155:66–75
31. Kah M, Sigmund G, Xiao F, Hofmann T (2017) Sorption of ionizable and ionic organic compounds to biochar, activated carbon and other carbonaceous materials. Water Res 124:673–692

32. Salame II, Bandosz TJ (2001) Surface chemistry of activated carbons: combining the results of temperature-programmed desorption, boehm, and potentiometric titrations. J Colloid Interf Sci 240(1):252–258
33. Nielsen L, Biggs MJ, Skinner W, Bandosz TJ (2014) The effects of activated carbon surface features on the reactive adsorption of carbamazepine and sulfamethoxazole. Carbon 80:419–432
34. Tran NH, Reinhard M, Gin KY-H (2018) Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. Water Res 133:182–207
35. Graham MR, Summers RS, Simpson MR, MacLeod BW (2000) Modeling equilibrium adsorption of 2-methylisoborneol and geosmin in natural waters. Water Res 34(8):2291–2300
36. Zietzschmann F, Aschermann G, Jekel M (2016) Comparing and modeling organic micro-pollutant adsorption onto powdered activated carbon in different drinking waters and WWTP effluents. Water Res 102:190–201
37. Najm IN, Snoeyink VL, Richard Y (1991) Effect of initial concentration of a Soc in natural-water on its adsorption by activated carbon. J Am Water Works Ass 83(8):57–63
38. Summers RS, Haist B, Koehler J, Ritz J, Zimmer G, Sontheimer H (1989) The influence of background organic-matter on Gac adsorption. J Am Water Works Ass 81(5):66–74
39. Rabolt B (1988) Untersuchungen zur konkurrierenden adsorption von Mikroverunreinigungen und natürlichen organischen Wasserinhaltsstoffen. Doctoral Dissertation, Technische Universität Dresden, Dresden
40. Streicher J, Ruhl AS, Gnirss R, Jekel M (2016) Where to dose powdered activated carbon in a wastewater treatment plant for organic micro-pollutant removal. Chemosphere 156:88–94
41. Kilduff JE, Karanfil T, Weber WJ (1996) Competitive interactions among components of humic acids in granular activated carbon adsorption systems: effects of solution chemistry. Environ Sci Technol 30(4):1344–1351
42. Li QL, Snoeyink VL, Mariaas BJ, Campos C (2003) Elucidating competitive adsorption mechanisms of atrazine and NOM using model compounds. Water Res 37(4):773–784
43. Pignatello JJ, Kwon S, Lu YF (2006) Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): attenuation of surface activity by humic and fulvic acids. Environ Sci Technol 40(24):7757–7763
44. Yu ZR, Peldszus S, Huck PM (2008) Adsorption characteristics of selected pharmaceuticals and an endocrine disrupting compound – naproxen, carbamazepine and nonylphenol – on activated carbon. Water Res 42(12):2873–2882
45. Nam SW, Choi DJ, Kim SK, Her N, Zoh KD (2014) Adsorption characteristics of selected hydrophilic and hydrophobic micropollutants in water using activated carbon. J Hazard Mater 270:144–152
46. Dittmar S, Ruhl AS, Jekel M (2017) Untersuchung von Modellstoffen zur simulation der Adsorptionskonkurrenz in gereinigten Abwässern (Model substances for simulating competitive adsorption in treated wastewaters). Vom Wasser 115(2):45–47. In German
47. Hertkorn N, Frommberger M, Witt M, Koch BP, Schmitt-Kopplin P, Perdue EM (2008) Natural organic matter and the event horizon of mass spectrometry. Anal Chem 80 (23):8908–8919
48. Matilainen A, Gjessing ET, Lahtinen T, Hed L, Bhatnagar A, Sillanpaa M (2011) An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. Chemosphere 83(11):1431–1442
49. Zietzschmann F, Mitchell R-L, Jekel M (2015) Impacts of ozonation on the competition between organic micro-pollutants and effluent organic matter in adsorption onto powdered activated carbon. Water Res 84:153–160
50. Aschermann G, Neubert L, Zietzschmann F, Jekel M (2019) Impact of different DOM size fractions on the desorption of organic micropollutants from activated carbon. Water Res 161:161–170

51. Li QL, Snoeyink VL, Marinas BJ, Campos C (2003) Pore blockage effect of NOM on atrazine adsorption kinetics of PAC: the roles of PAC pore size distribution and NOM molecular weight. Water Res 37(20):4863–4872
52. Kennedy AM, Summers RS (2015) Effect of DOM size on organic micropollutant adsorption by GAC. Environ Sci Technol 49(11):6617–6624
53. Wang Q, Zietzschmann F, Yu J, Hofman R, An W, Yang M, Rietveld LC (2020) Projecting competition between 2-methylisoborneol and natural organic matter in adsorption onto activated carbon from ozonated source waters. Water Res 173:115574
54. Zietzschmann F, Worch E, Altmann J, Ruhl AS, Sperlich A, Meinel F, Jekel M (2014) Impact of EfOM size on competition in activated carbon adsorption of organic micro-pollutants from treated wastewater. Water Res 65:297–306
55. Zietzschmann F, Müller J, Sperlich A, Ruhl AS, Meinel F, Altmann J, Jekel M (2014) Rapid small-scale column testing of granular activated carbon for organic micro-pollutant removal in treated domestic wastewater. Water Sci Technol 70(7):1271–1278
56. Al Mardini F, Legube B (2010) Effect of the adsorbate (Bromacil) equilibrium concentration in water on its adsorption on powdered activated carbon. Part 3: competition with natural organic matter. J Hazard Mater 182(1–3):10–17
57. Huber SA, Balz A, Abert M, Pronk W (2011) Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography – organic carbon detection – organic nitrogen detection (LC-OCD-OND). Water Res 45(2):879–885
58. Knappe DRU, Matsui Y, Snoeyink VL, Roche P, Prados MJ, Bourbigot MM (1998) Predicting the capacity of powdered activated carbon for trace organic compounds in natural waters. Environ Sci Technol 32(11):1694–1698
59. Rossner A, Snyder SA, Knappe DRU (2009) Removal of emerging contaminants of concern by alternative adsorbents. Water Res 43(15):3787–3796
60. Kovalova L, Knappe DRU, Lehnberg K, Kazner C, Hollender J (2013) Removal of highly polar micropollutants from wastewater by powdered activated carbon. Environ Sci Pollut R 20 (6):3607–3615
61. Matsui Y, Fukuda Y, Inoue T, Matsushita T (2003) Effect of natural organic matter on powdered activated carbon adsorption of trace contaminants: characteristics and mechanism of competitive adsorption. Water Res 37(18):4413–4424
62. Zietzschmann F, Stützer C, Jekel M (2016) Granular activated carbon adsorption of organic micro-pollutants in drinking water and treated wastewater – aligning breakthrough curves and capacities. Water Res 92:180–187
63. Corwin CJ, Summers RS (2011) Adsorption and desorption of trace organic contaminants from granular activated carbon adsorbers after intermittent loading and throughout backwash cycles. Water Res 45(2):417–426
64. Mailler R, Gasperi J, Coquet Y, Bulete A, Vulliet E, Deshayes S et al (2016) Removal of a wide range of emerging pollutants from wastewater treatment plant discharges by micro-grain activated carbon in fluidized bed as tertiary treatment at large pilot scale. Sci Total Environ 542:983–996
65. Jiang N, Shang R, Heijman SGJ, Rietveld LC (2018) High-silica zeolites for adsorption of organic micro-pollutants in water treatment: a review. Water Res 144:145–161
66. Elsevier (2020) Scopus document search. https://www.scopus.com. Accessed 28 May 2020
67. Statista (2020) Market volume of activated carbon worldwide in 2016 and 2021. Statista GmbH. https://www.statista.com/statistics/963555/global-market-volume-activated-carbon/. Accessed 16 May 2020
68. Danish M, Ahmad T (2018) A review on utilization of wood biomass as a sustainable precursor for activated carbon production and application. Renew Sust Energ Rev 87:1–21
69. Silva CP, Jaria G, Otero M, Esteves VI, Calisto V (2018) Waste-based alternative adsorbents for the remediation of pharmaceutical contaminated waters: has a step forward already been taken? Bioresour Technol 250:888–901

70. Quesada HB, Baptista ATA, Cusioli LF, Seibert D, de Oliveira BC, Bergamasco R (2019) Surface water pollution by pharmaceuticals and an alternative of removal by low-cost adsorbents: a review. Chemosphere 222:766–780
71. Tran HN, Nguyen HC, Woo SH, Nguyen TV, Vigneswaran S, Hosseini-Bandegharaei A et al (2019) Removal of various contaminants from water by renewable lignocellulose-derived biosorbents: a comprehensive and critical review. Crit Rev Environ Sci Technol 49 (23):2155–2219
72. Tan XF, Liu SB, Liu YG, Gu YL, Zeng GM, Hua XJ et al (2017) Biochar as potential sustainable precursors for activated carbon production: multiple applications in environmental protection and energy storage. Bioresour Technol 227:359–372
73. Benstoem F, Becker G, Firk J, Kaless M, Wuest D, Pinnekamp J, Kruse A (2018) Elimination of micropollutants by activated carbon produced from fibers taken from wastewater screenings using hydrothermal carbonization. J Environ Manag 211:278–286
74. Shimabuku KK, Kearns JP, Martinez JE, Mahoney RB, Moreno-Vasquez L, Summers RS (2016) Biochar sorbents for sulfamethoxazole removal from surface water, stormwater, and wastewater effluent. Water Res 96:236–245
75. Thompson KA, Shimabuku KK, Kearns JP, Knappe DRU, Summers RS, Cook SM (2016) Environmental comparison of biochar and activated carbon for tertiary wastewater treatment. Environ Sci Technol 50(20):11253–11262
76. Kearns JP, Wellborn LS, Summers RS, Knappe DRU (2014) 2,4-D adsorption to biochars: effect of preparation conditions on equilibrium adsorption capacity and comparison with commercial activated carbon literature data. Water Res 62:20–28
77. Rocha LS, Pereira D, Sousa É, Otero M, Esteves VI, Calisto V (2020) Recent advances on the development and application of magnetic activated carbon and char for the removal of pharmaceutical compounds from waters: a review. Sci Total Environ 718:137272
78. Li SJ, Han KH, Li JX, Li M, Lu CM (2017) Preparation and characterization of super activated carbon produced from gulfweed by KOH activation. Microporous Mesoporous Mater 243:291–300
79. Schneidermann C, Jackel N, Oswald S, Giebeler L, Presser V, Borchardt L (2017) Solvent-free Mechanochemical synthesis of nitrogen-doped Nanoporous carbon for electrochemical energy storage. ChemSusChem 10(11):2416–2424
80. de Sousa DNR, Insa S, Mozeto AA, Petrovic M, Chaves TF, Fadini PS (2018) Equilibrium and kinetic studies of the adsorption of antibiotics from aqueous solutions onto powdered zeolites. Chemosphere 205:137–146
81. Jiang N, Erdős M, Moultos OA, Shang R, Vlugt TJH, Heijman SGJ, Rietveld LC (2020) The adsorption mechanisms of organic micropollutants on high-silica zeolites causing S-shaped adsorption isotherms: an experimental and Monte Carlo simulation study. Chem Eng J 389:123968
82. Thiebault T (2020) Raw and modified clays and clay minerals for the removal of pharmaceutical products from aqueous solutions: state of the art and future perspectives. Crit Rev Environ Sci Technol 50(14):1451–1514
83. Morin-Crini N, Crini G (2013) Environmental applications of water-insoluble beta-cyclodextrin-epichlorohydrin polymers. Prog Polym Sci 38(2):344–368
84. Sikder MT, Rahman MM, Jakariya M, Hosokawa T, Kurasaki M, Saito T (2019) Remediation of water pollution with native cyclodextrins and modified cyclodextrins: a comparative overview and perspectives. Chem Eng J 355:920–941
85. Zhang X, Bai RB (2003) Mechanisms and kinetics of humic acid adsorption onto chitosan-coated granules. J Colloid Interface Sci 264(1):30–38
86. Tian C, Zhao J, Ou X, Wan J, Cai Y, Lin Z et al (2018) Enhanced adsorption of p-Arsanilic acid from water by amine-modified UiO-67 as examined using extended X-ray absorption fine structure, X-ray photoelectron spectroscopy, and density functional theory calculations. Environ Sci Technol 52(6):3466–3475

87. Meng Z, Chen W, Mulchandani A (2005) Removal of estrogenic pollutants from contaminated water using molecularly imprinted polymers. Environ Sci Technol 39(22):8958–8962
88. Metzger S, Alt K, Benstoem F, Biebersdorf N, Boehler M, Bornemann C et al (2019) Aktivkohleeinsatz auf kommunalen Kläranlagen zur Spurenstoffentfernung (Activated carbon application on municipal wastewater treatment plants for trace compound removal). Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e.V. (DWA), Hennef (Germany). (In German)
89. Boehler M, Zwickenpflug B, Hollender J, Ternes T, Joss A, Siegrist H (2012) Removal of micropollutants in municipal wastewater treatment plants by powder-activated carbon. Water Sci Technol 66(10):2115–2121
90. Altmann J, Sperlich A, Jekel M (2015) Integrating organic micropollutant removal into tertiary filtration: combining PAC adsorption with advanced phosphorus removal. Water Res 84:58–65
91. Loewenberg J, Zenker A, Krahnstover T, Boehler M, Baggenstos M, Koch G, Wintgens T (2016) Upgrade of deep bed filtration with activated carbon dosage for compact micropollutant removal from wastewater in technical scale. Water Res 94:246–256
92. Altmann J, Ruhl AS, Sauter D, Pohl J, Jekel M (2015) How to dose powdered activated carbon in deep bed filtration for efficient micropollutant removal. Water Res 78:9–17
93. Meinel F, Zietzschmann F, Ruhl AS, Sperlich A, Jekel M (2016) The benefits of powdered activated carbon recirculation for micropollutant removal in advanced wastewater treatment. Water Res 91:97–103
94. Nicolet L, Rott U (1999) Recirculation of powdered activated carbon for the adsorption of dyes in municipal wastewater treatment plants. Water Sci Technol 40(1):191–198
95. Karelid V, Larsson G, Bjorlenius B (2017) Effects of recirculation in a three-tank pilot-scale system for pharmaceutical removal with powdered activated carbon. J Environ Manag 193:163–171
96. Krahnstöver T, Wintgens T (2018) Separating powdered activated carbon (PAC) from wastewater – technical process options and assessment of removal efficiency. J Environ Chem Eng 6(5):5744–5762
97. Nakazawa Y, Matsui Y, Hanamura Y, Shinno K, Shirasaki N, Matsushita T (2018) Minimizing residual black particles in sand filtrate when applying super-fine powdered activated carbon: coagulants and coagulation conditions. Water Res 147:311–320
98. Ando N, Matsui Y, Kurotobi R, Nakano Y, Matsushita T, Ohno K (2010) Comparison of natural organic matter adsorption capacities of super-powdered activated carbon and powdered activated carbon. Water Res 44(14):4127–4136
99. Loewenberg J, Zenker A, Baggenstos M, Koch G, Kazner C, Wintgens T (2014) Comparison of two PAC/UF processes for the removal of micropollutants from wastewater treatment plant effluent: process performance and removal efficiency. Water Res 56:26–36
100. Hernandez LA, Harriott P (1976) Regeneration of powdered active carbon in fluidized bed. Environ Sci Technol 10(5):454–456
101. Salvador F, Martin-Sanchez N, Sanchez-Hernandez R, Sanchez-Montero MJ, Izquierdo C (2015) Regeneration of carbonaceous adsorbents. Part II: chemical, microbiological and vacuum regeneration. Microporous Mesoporous Mater 202:277–296
102. Reungoat J, Pic JS, Manéro MH, Debellefontaine H (2007) Adsorption of nitrobenzene from water onto high silica zeolites and regeneration by ozone. Sep Sci Technol 42(7):1447–1463
103. Quinlivan PA, Li L, Knappe DRU (2005) Effects of activated carbon characteristics on the simultaneous adsorption of aqueous organic micropollutants and natural organic matter. Water Res 39(8):1663–1673
104. Li L, Quinlivan PA, Knappe DRU (2005) Predicting adsorption isotherms for aqueous organic micropollutants from activated carbon and pollutant properties. Environ Sci Technol 39 (9):3393–3400
105. Worch E (2010) Competitive adsorption of micropollutants and NOM onto activated carbon: comparison of different model approaches. J Water Supply Res Technol 59(5):285–297

106. Bahr C, Schumacher J, Ernst M, Luck F, Heinzmann B, Jekel M (2007) SUVA as control parameter for the effective ozonation of organic pollutants in secondary effluent. Water Sci Technol 55(12):267–274
107. Ziska AD, Park M, Anumol T, Snyder SA (2016) Predicting trace organic compound attenuation with spectroscopic parameters in powdered activated carbon processes. Chemosphere 156:163–171
108. Zietzschmann F, Altmann J, Hannemann C, Jekel M (2015) Lab-testing, predicting, and modeling multi-stage activated carbon adsorption of organic micro-pollutants from treated wastewater. Water Res 83:52–60
109. Altmann J, Zietzschmann F, Geiling EL, Ruhl AS, Sperlich A, Jekel M (2015) Impacts of coagulation on the adsorption of organic micropollutants onto powdered activated carbon in treated domestic wastewater. Chemosphere 125:198–204
110. Rößler A, Metzger S (2016) Application of SAC(254) measurement for the assessment of micropollutant removal in the adsorptive treatment stage of a municipal wastewater treatment plant. Water Pract Technol 11(2):503–515
111. Altmann J, Massa L, Sperlich A, Gnirss R, Jekel M (2016) UV254 absorbance as real-time monitoring and control parameter for micropollutant removal in advanced wastewater treatment with powdered activated carbon. Water Res 94:240–245
112. Kennedy AM, Reinert AM, Knappe DRU, Ferrer I, Summers RS (2015) Full- and pilot-scale GAC adsorption of organic micropollutants. Water Res 68:238–248
113. Benstoem F, Nahrstedt A, Boehler M, Knopp G, Montag D, Siegrist H, Pinnekamp J (2017) Performance of granular activated carbon to remove micropollutants from municipal wastewater-A meta-analysis of pilot- and large-scale studies. Chemosphere 185:105–118
114. Meinel F, Ruhl AS, Sperlich A, Zietzschmann E, Jekel M (2015) Pilot-scale investigation of micropollutant removal with granular and powdered activated carbon. Water Air Soil Pollut 226(1)
115. Crittenden JC, Reddy PS, Arora H, Trynoski J, Hand DW, Perram DL, Summers RS (1991) Predicting Gac performance with rapid small-scale column tests. J Am Water Works Ass 83 (1):77–87
116. Schimmelpfennig S, Sperlich A (2011) FAST – fixed-bed adsorption simulation tool 2.1beta. Berlin, Germany. http://www.fast-software.de/
117. Altmann J, Rehfeld D, Träder K, Sperlich A, Jekel M (2016) Combination of granular activated carbon adsorption and deep-bed filtration as a single advanced wastewater treatment step for organic micropollutant and phosphorus removal. Water Res 92:131–139
118. Crittenden JC, Trussell RR, Hand DW, Howe KJ, Tchobanoglous G (2012) MWH's water treatment – principles and design, 3rd edn. Wiley, Hoboken (acid-free paper)
119. Nahrstedt A, Rohn A, Alt K, Wu X, Schlösser F, Schröder K-H (2016) Mikroschadstoffelimination mittels granulierter Aktivkohle im Ablauf der Kläranlage Gütersloh-Putzhagen – Kurzbericht (micropollutant elimination by granular activated carbon in the effluent of WWTP Gütersloh-Putzhagen – short report). IWW Rheinisch-Westfälisches Institut für Wasserforschung gemeinnützige GmbH. In German
120. Wunderlin P, Meier A, Grelot J (2017) Elimination von Mikroverunreinigungen auf ARA: Aktueller Stand der Verfahren und künftige Entwicklungen. Aqua Gas 11:60–70. In German
121. Hertel M, Maurer P, Steinmetz H (2014) Auswahl und Überprüfung granulierter Aktivkohlen (GAK) für den Einsatz in kontinuierlich gespülten Filtern (selection and screening of granular activated carbons for application in continuously backwashed filters). Wasser Abfall 16 (11):40–45. In German
122. Bourgin M, Beck B, Boehler M, Borowska E, Fleiner J, Salhi E et al (2018) Evaluation of a full-scale wastewater, treatment plant upgraded with ozonation and biological post-treatments: abatement of micropollutants, formation of transformation products and oxidation by-products. Water Res 129:486–498
123. Bornemann C, Hachenberg M, Kazner C, Herr J, Jagemann P, Lyko S et al (2012) Ertüchtigung kommunaler Kläranlagen, insbesondere kommunaler

Flockungsfiltrationsanlagen durch den Einsatz von Aktivkohle – MICROFlock; Abschlussbericht (upgrading municipal wastewater treatment plants, especially flocculation filters, by activated carbon; final report). RWTH Aachen. In German
124. Salvador F, Martin-Sanchez N, Sanchez-Hernandez R, Sanchez-Montero MJ, Izquierdo C (2015) Regeneration of carbonaceous adsorbents. Part I: thermal regeneration. Microporous Mesoporous Mater 202:259–276
125. Ruhl AS, Zietzschmann F, Altmann J, Meinel F, Sperlich A, Jekel M (2015) Stratification of granular activated carbon filters for advanced wastewater treatment. Water Air Soil Pollut 226 (11)
126. Anumol T, Sgroi M, Park M, Roccaro P, Snyder SA (2015) Predicting trace organic compound breakthrough in granular activated carbon using fluorescence and UV absorbance as surrogates. Water Res 76:76–87
127. Freihardt J, Jekel M, Ruhl AS (2017) Comparing test methods for granular activated carbon for organic micropollutant elimination. J Environ Chem Eng 5(3):2542–2551
128. Kennedy AM, Reinert AM, Knappe DRU, Summers RS (2017) Prediction of full-scale GAC adsorption of organic micropollutants. Environ Eng Sci 34(7):496–507
129. Summers RS, Kim SM, Shimabuku K, Chae SH, Corwin CJ (2013) Granular activated carbon adsorption of MIB in the presence of dissolved organic matter. Water Res 47(10):3507–3513
130. Worch E (2008) Fixed-bed adsorption in drinking water treatment: a critical review on models and parameter estimation. J Water Supply Res Technol 57(3):171–183
131. Schideman LC, Marinas BJ, Snoeyink VL, Campos C (2006) Three-component competitive adsorption model for fixed-bed and moving-bed granular activated carbon adsorbers. Part I. Model development. Environ Sci Technol 40(21):6805–6811
132. de Ridder DJ, McConville M, Verliefde ARD, van der Aa LTJ, Heijman SGJ, Verberk JQJC et al (2009) Development of a predictive model to determine micropollutant removal using granular activated carbon. Drink Water Eng Sci 2(2):57–62
133. Liang CH, Chiang PC, Chang EE (2007) Modeling the behaviors of adsorption and biodegradation in biological activated carbon filters. Water Res 41(15):3241–3250
134. VSA (2020) VSA-plattform "Verfahrenstechnik Mikroverunreinigungen". Verband Schweizer Abwasser- und Gewässerschutzfachleute. https://www.micropoll.ch. Accessed 30 May 2020
135. KomS (2020) Micropollutants Competence Centre BW. Deutsche Vereinigung für Wasserwirtschaft, Abwasser und Abfall e. V. (DWA). https://koms-bw.de/en. Accessed 30 May 2020
136. Mousel D, Palmowski L, Pinnekamp J (2017) Energy demand for elimination of organic micropollutants in municipal wastewater treatment plants. Sci Total Environ 575:1139–1149

Removal of Pharmaceutically Active Compounds (PhACs) in Wastewater by Ozone and Advanced Oxidation Processes

Javier Marugán, Jorge Rodríguez-Chueca, Santiago Esplugas, Carme Sans, and Sixto Malato

Contents

J. Marugán (✉)
Department of Chemical and Environmental Technology, ESCET, Universidad Rey Juan Carlos, Madrid, Spain
e-mail: javier.marugan@urjc.es

J. Rodríguez-Chueca
Department of Chemical and Environmental Engineering Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid, Madrid, Spain
e-mail: jorge.rodriguez.chueca@upm.es

S. Esplugas and C. Sans
Department of Chemical Engineering and Analytical Chemistry, Faculty of Chemistry, Universitat de Barcelona, Barcelona, Spain
e-mail: santi.esplugas@ub.edu; carmesans@ub.edu

S. Malato
Plataforma Solar de Almería (CIEMAT), Carretera de Senés, Almería, Spain
e-mail: smalato@psa.es

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 269–298, DOI 10.1007/698_2020_664,

Abstract During the last decades, many water treatment processes have been proposed to deal with the increasing water quality requirements demanded for the urban wastewater effluents (UWWE). Among them, the so-called Advanced Oxidation Processes (AOPs) include a wide range of technologies based on the generation of very reactive and non-selective radical species with a strong oxidation potential that can readily remove not only PhACs but also other organic micropollutants. Together with ozone-based processes, these technologies constitute promising alternatives to be used as final barrier to remove these contaminants before the discharge of the effluents to the environment. This chapter focuses on the processes with higher potential to be implemented at large scale for the removal of PhACs in urban wastewater treatment plants: ozone-based, Fenton, and UV/H_2O_2 processes. Along the chapter, the fundamentals of these processes are introduced, including some of the more relevant modifications from the basic processes, and the more recent studies about their application for the removal of PhACs from UWWE at pilot and full scale are reviewed. From a scientific and technical point of view, the results from these researches confirm the successful removal of a wide range of PhACs and other micropollutants, although the absolute efficiency depends on the water matrix and the specific substances monitored along the treatment. As final remarks, other aspects are also included, such as the need to control the potential formation of by-products during the process and the required optimization of the processes to become competitive in economic terms.

Keywords Fenton, Hydrogen peroxide, Hydroxyl radicals, Ozone, UV

1 Introduction

The available technologies to remove contaminants in aqueous solution are very diverse. Physical treatments just concentrate the contaminants, whereas chemical and biochemical processes degrade these compounds, mainly oxidizing the contaminants. Glaze et al. [1] defined "the Advanced Oxidation Processes (AOPs) as those involved in the generation of hydroxyl radicals in sufficient quantity to affect water purification." Glaze referred to water treatments involving UV/H_2O_2/O_3 used in "light conditions," that is, room temperature and atmospheric pressure. Later on, the definition of AOPs was extended to the role of other radicals generated, as hydroperoxyl, persulfate, or peroxymonosulfate [2–4], and other treatments like Fenton, TiO_2 photocatalysis, electrochemistry, ultrasounds, non-thermal plasma, and wet oxidation were included in AOPs (Fig. 1). The distinction of applications at light conditions is nowadays questionable. The name AOPs is also doubtful because some of the named processes are used in the reduction instead of the oxidation attack to the contaminants. Nowadays, the applications to the AOPs have been extended from water to wastewaters, air, and soil, mainly at laboratory

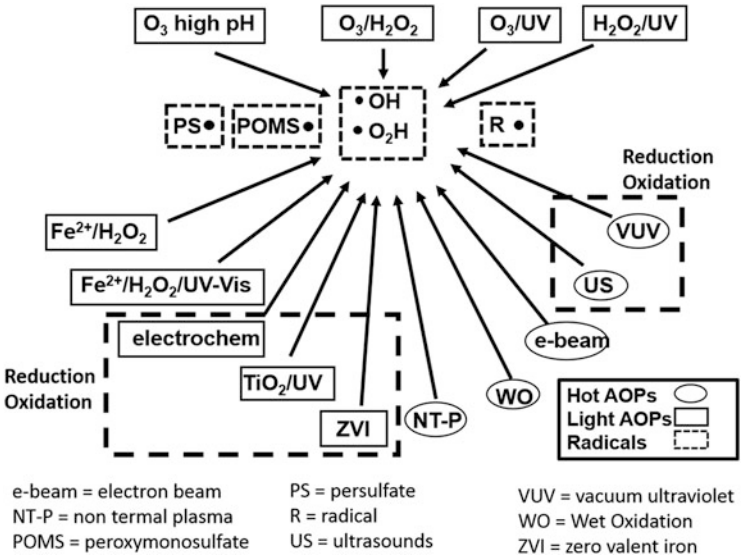


Fig. 1 Advanced oxidation processes

and pilot scales. Only ozone-based AOPs, Fenton, and UV/H_2O_2 are implemented at industrial scale for water and wastewater treatment.

AOPs involve chemical reactions promoting the degradation of the contaminants, and it is more a transformation of compounds than a simple fading. In addition, it is important to consider the possibility to generate by-products more harmful than the parent compounds. In consequence, biodegradability and toxicology tests of treated waters turn as important as the chemical analysis of the contaminant. The water matrix (pure, groundwater, wastewater) also influences in the efficiency and the removal rate of these contaminants [5], mainly because the oxidant is also consumed by other compounds in the water matrix (organic matter, ions, particulate matter). For most wastewaters, contaminant concentration and effluent toxicity decrease, and biodegradability increases as AOP treatment is extended. Because of this and the cost of chemicals and energy involved in AOP treatments, the combination of these technologies with other less costly treatments as biodegradation, adsorption, and membrane separation has been extensively studied [6].

Nowadays, there is major concern about the presence of PhACs in wastewater treatment plant effluents. These compounds are continuously released to the environment, and, in many cases, natural degradation is not enough to remove them efficiently. Consequently, PhACs remain in natural waters affecting the water quality and human health. Many reviews on the applications of AOPs for the treatment of water and wastewater containing PhACs compounds can be found in the literature [7–10]. Today it is possible to find extensive information on removal of PhACs in wastewater by AOPs, mainly at lab and pilot plant scale. Accordingly, this section aims to present a general vision of the removal of PhACs in wastewater by

AOPs. Specific care is devoted to the AOPs implemented at industrial scale, that is, ozone-based AOPs, Fenton, and UV/H_2O_2 processes.

2 Ozone-Based Processes

After more than 100 years of experience on industrial-scale applications of ozone, nowadays ozonation is already a chemical treatment process extensively applied on municipal and industrial wastewater disinfection and oxidation of recalcitrant organic microcontaminants (drugs, pesticides, phenolic compounds, etc.) and inorganic chemicals (iron, manganese, sulfites, etc.).

Among current environmental concerns, the accumulation of microcontaminants and particularly PhACs in natural waters has triggered numerous studies on ozonation and related technologies, like ozone/hydrogen (known as peroxone process) or catalytic ozonation of wastewaters. The effectiveness of ozone-based processes on the removal of PhACs strongly depends on chemical and engineering parameters, like ozone generation and transfer from the gas phase to the wastewater, PhACs reactivity with ozone, and, particularly, composition of the water matrix. This section summarizes the current knowledge on PhACs remediation in wastewaters by ozone-based processes.

2.1 Fundamentals

Ozone (O_3) is a pale blue gas with a robust oxidizing capacity ($E_0 = 2.07$ eV), slightly soluble in water. It easily decomposes to oxygen atoms and molecules; that is why in water treatment, ozone must be generated "in situ" [11]. This gas with high oxidation potential is produced by submitting oxygen molecules to high electrical voltages. There are different methods for producing ozone being the corona discharge one of the most used by far. This one consists of passing oxygen between two electrodes separated by a dielectric material and connected to a source of high voltage producing a corona discharge. The oxygen in the discharge gap is converted to ozone.

Once ozone is produced, it is bubbled into a reactor containing the solution to be treated. The ozone transfer efficiency from gas to liquid phase is fundamental to achieve optimal reaction kinetics rates between ozone and dissolved compounds. This transfer is mainly controlled by physical parameters, such as temperature, gas flow rate, bubble size, ozone partial pressure, and reactor geometry, and also chemical factors like pH, ionic strength, composition of aqueous solutions, etc. [12]. Therefore, it is important to distinguish between applied ozone dose and transferred ozone dose (TOD), which is defined by Eq. (1) and represents the accumulated amount of ozone that is transferred to the water sample per unit of volume and time:

$$TOD = \int_0^t \frac{F_{gas}}{V_{liq}} \cdot \left([O_3]_{gas,in} - [O_3]_{gas,out} \right) \cdot dt \quad (1)$$

Ozone in the liquid phase can react with organic compounds in two ways, direct or indirect. These two reaction pathways lead to different oxidation products, and they are controlled by different reaction kinetics. Direct ozone reactions with organic water constituents (comprising PhACs) are quite selective mechanisms including cycloaddition, electrophilic substitution, and electron transfer reactions. Thus, molecular ozone preferably reacts with organic matter with double bonds, activated aromatic groups, or amines moieties [13, 14]. The indirect reaction pathway involves hydroxyl radicals (•OH), mainly generated by radical chain decomposition of ozone initiated by hydroxide ions (OH^-) in water. Reaction between O_3 and OH^- is very slow ($k = 70\ M^{-1}\ s^{-1}$); therefore indirect ozone reactions through hydroxyl radicals only contribute to pollutants removal at neutral or alkaline pH conditions. Hydroxyl radicals are extremely reactive transient species, more reactive, and significantly less selective than molecular ozone. Hydroxyl radicals' reactivity involves three main mechanisms: addition reactions, hydrogen abstraction reactions, and electron transfer reactions, being addition reactions to double bonds (like C=C, C=N, S=O) and aromatic rings the most common and fast [14].

Other •OH generation mechanism is ozone reactions with the dissolved organic matter (DOM) contained in wastewater matrices, particularly with DOM aromatic functional groups. The electron-rich aromatic components of DOM can undergo electron transfer with O_3 generating the intermediate ozonide anion ($O_3^{\bullet-}$) which can give rise to •OH generation. Similarly, phenolic moieties generated by hydroxylation of aromatic DOM moieties can also lead to •OH production by electron transfer with O_3. However, DOM present in water also acts as radical chain inhibitor, scavenging •OH or consuming O_3 without further •OH or chain carrier production [15]. Even with this dual behavior of DOM, organic matter content in wastewaters dramatically increases ozone consumption for pharmaceuticals depletion.

Moreover, some inorganic anions present in the wastewater can interfere in the ozonation process. Carbonate and bicarbonate anions are well-known •OH scavengers and radical chain inhibitors, since they contribute to the termination of the ozone decomposition mechanism [16]. Other important species is nitrite, contributing to ozone depletion of 3.4 g O_3 per g NO_2-N. Finally, organic particulate matter content of water consumes both O_3 and •OH, increasing overall ozone requirements.

Considering all the issues exposed, ozonation performance for the removal of PhACs in wastewaters hardly depends on the quantity and type of organic matter and inorganic constituents of the water matrix.

2.1.1 Ozonation Kinetics

There are many experimental shreds of evidence, at laboratory, pilot plant, and industrial scale, demonstrating the effectiveness of ozonation for the oxidation of

Table 1 Micropollutants classification and some PhACs with their second-order reaction rate constant with ozone and hydroxyl radical (adapted from [17])

Group of MPs	Reactivity	k_{O3} [M^{-1} s^{-1}]	$k_{\bullet OH}$ [M^{-1} s^{-1}]
I	*High with O_3 and $^{\bullet}OH$*	*$>1 \times 10^5$*	*$>5 \times 10^9$*
Triclosan	Antimicrobials	3.8×10^7	9.6×10^9
Diclofenac	NSAID	1.0×10^6	7.5×10^9
Sulfamethoxazole	Antibiotics	5.7×10^5	5.5×10^9
Carbamazepine	Anticonvulsant	3.0×10^5	8.8×10^9
Trimethoprim	Antibiotic	2.7×10^5	6.9×10^9
Naproxen	NSAID	2.0×10^5	9.6×10^9
II	*Moderate with O_3 and high with $^{\bullet}OH$*	*$10^{-1} \times 10^5$*	*$>5 \times 10^9$*
Gemfibrozil	Lipid regulator	5.0×10^4	10×10^9
Atenolol	B-blocker	1.7×10^3	8.0×10^9
III	*Low with O_3 and high with $^{\bullet}OH$*	*<10*	*$>5 \times 10^9$*
Ibuprofen	NSAID	9.6	7.4×10^9
Phenytoin	Antiepileptic	<10	6.3×10^9
Primidone	Anticonvulsant	<10	6.7×10^9
IV	*Low with O_3 and moderate with $^{\bullet}OH$*	*<10*	*1×10^9–5×10^9*
Meprobamate	Antianxiety drug	<1	3.7×10^9
V	*Low with both O_3 and $^{\bullet}OH$*	*<10*	*$<1 \times 10^9$*

PhACs in wastewaters. Since common municipal wastewater pH is neutral or slightly alkaline, ozonation efficiency directly depends on the second-order rate constants of reactions between the pharmaceutical (PhAC) and both ozone and hydroxyl radicals, as well as the ozone and •OH exposures ($\int[O_3]dt$ and $\int[\bullet OH]dt$) as described by Eq. (2):

$$-\ln\left(\frac{[\mathrm{PhAC}]}{[\mathrm{PhAC}]_0}\right) = k_{O3} \cdot \int [O_3]dt + k_{\bullet OH} \cdot \int [^{\bullet}OH]dt \tag{2}$$

Most organic micropollutants, including PhACs, present high reactivity with •OH, with second-order rate constants higher than 10^9 M^{-1} s^{-1}. However, kinetics in direct ozone reactions largely depends on the structure of the target chemicals due to the specific reaction pathways of molecular ozone. Consequently, the second-order rate constants for ozone oxidation of organic compounds vary more than eight orders of magnitude, between 0.1 M^{-1} s^{-1} and 10^7 M^{-1} s^{-1}. Lee and coworkers classified micropollutants depending on their reactivity with ozone and •OH [17]. Classification goes for group I, including the most reactive compounds with both oxidants down to group V, containing the most resistant to ozonation treatment. The second-order kinetic constant of most common PhACs, both with ozone and •OH, is already available in the literature. Table 1 includes micropollutants classification and some examples of PhACs, together with their second-order reaction rate constant with ozone and hydroxyl radical.

The acid-base dissociation constant (pK_a) of a PhAC is also a key physicochemical parameter influencing ozonation performance. As the majority of drugs are weak acids and/or bases, the knowledge of their dissociation constant helps in understanding the ionic form a molecule will take across a range of pH values. This is particularly important since the reactivity of ozone with some organic dissociating compounds extraordinarily changes with pH by several orders of magnitude [13].

2.1.2 Peroxone

The promotion of in situ generation of •OH by combining ozone with hydrogen peroxide (H_2O_2) is known as peroxone process. The generation mechanism is based on the favored reaction of ozone with HO_2^-, the equilibrium deprotonated form of H_2O_2 [18]. On the other hand, H_2O_2 reaction with ozone is very slow ($k < 0.01\ M^{-1}\ s^{-1}$), while its reactivity with •OH is substantially faster ($k = 2.7 \times 10^7\ M^{-1}\ s^{-1}$). Consequently, increasing H_2O_2 concentration may result in an overall process efficiency decline, due to the reduction of the available amount of •OH in the reaction media [18].

There are many experimental pieces of evidence on the benefits of peroxone in terms of PhACs abatement and bromates production reduction, compared with single ozonation, for drinking water applications [19]. However, this enhancement in micropollutants abatement is not evident for urban wastewaters presenting a higher pollution load, at the current industrial ozone doses applied [20].

2.1.3 Catalytic Ozonation

Ozone combined with homogeneous and heterogeneous catalysts can also enhance •OH production. Homogeneous process, using transition metals in solution, and heterogeneous catalysts, like metal oxides, supported metals, and even minerals or metallic residues, have been widely studied demonstrating the benefits associated to the potential generation of •OH [21, 22].

However, several problems arise related to the use of catalysts that makes industrial application quite difficult. Thus, the homogeneous process requires metals removal from water effluents, increasing treatment costs. Heterogeneous systems deal with problems like catalyst fouling and deactivation, metals leakage into water media, and synthesis and/or preparation costs. All these drawbacks, together with the objective to improve the overall efficiency, are the reasons that, nowadays, catalytic ozonation is one of the main research topics in water treatment.

2.2 Removal of PhACs from Wastewaters by Ozonation

Although PhACs were not within the 45 substances/groups of substances included in the EU priority substances list (established in Directive 2013/39/EU), 5 out of 15 contaminants of emerging concern (CECs) in the latest watch list are antibiotics (Decision 2018/810/EU). Moreover, the Swiss Federal Office for the Environment (FOEN) established a list of 12 indicator substances to be removed by at least 80% in the upgraded wastewater treatment plants, among them are 11 PhACs (amisulpride, carbamazepine, citalopram, clarithromycin, diclofenac, metoprolol, hydrochlorothiazide, venlafaxine, candesartan, irbesartan, and mecoprop).

Although ozonation efficiency directly depends on the second-order rate constants of PhACs with ozone and •OH, urban wastewater effluents (UWWE) composition, mainly its effluent organic matter (EfOM) content, is the main parameter controlling overall process efficiency. This is the reason why ozone dosage for UWWE is commonly referred as specific ozone dose, that is, mg of ozone supplied per mg of dissolved organic carbon [mg O_3 mg C^{-1}]. Under the current applied specific ozone doses between 0.4 and 0.6 mg O_3 mg C^{-1}, there has been reported acceptable (50–80%) to good (>80%) efficiencies removal for PhACs belonging to groups I and II (i.e., triclosan, naproxen, trimethoprim, ciprofloxacin, clarithromycin, diclofenac, erythromycin, metoprolol, sulfamethoxazole, among others). PhACs with rate constants with ozone lower than 10 M^{-1} s^{-1} (from group III to V) are considered ozone-resistant compounds (like meprobamate, X-ray contrast agent iopromide, etc.). They are partially or poorly abated when applying ozone doses up to about 1.0 mg O_3 mg C^{-1} [23, 24]. Higher ozone doses would be necessary to improve their depletion, increasing ozone treatment cost. Figure 2 shows some PhACs removal obtained from concentrated municipal wastewaters at different applied specific ozone dose [25].

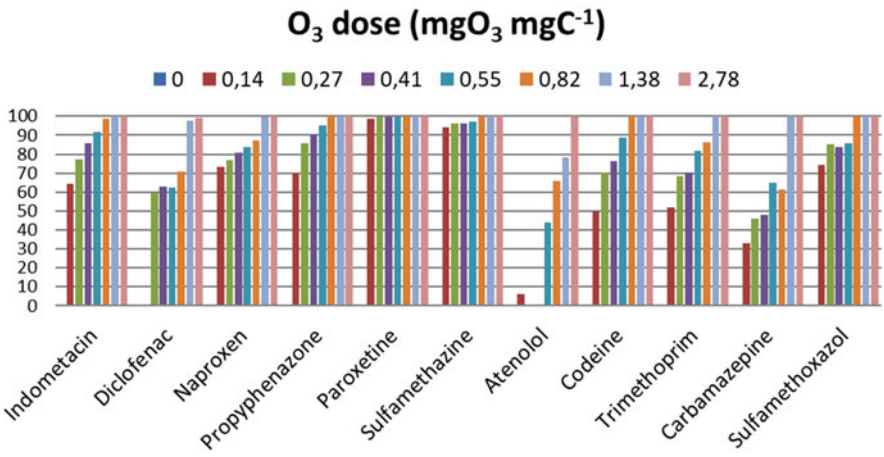


Fig. 2 PhACs removal at different ozone doses [25]

In UWWE ozonation, there is a competition for ozone between the PhACs and the wastewater constituents, that is, EfOM and some inorganic ions. These compounds rapidly consume transferred ozone in a process called primary ozonation or instantaneous ozone demand (IOD) stage. Wastewater organic and inorganic constituents also strongly scavenge the hydroxyl radicals formed through ozone decomposition reactions, resulting in poor availability of this oxidant in the reaction medium. After IOD completion, a secondary ozonation stage characterized by a slower ozone consumption and significantly lower •OH scavenging rate starts. However, UWWE ozonation typically employs ozone dosages below IOD fulfillment [26]; thus a satisfactory removal of recalcitrant PhACs is not accomplished. Application of peroxone (O_3/H_2O_2) process for the intensification of •OH oxidation neither appears to contribute significantly to recalcitrant PhACs removal in wastewaters, probably because the ozone decomposition process, at the current applied doses, is strongly controlled by reactions with EfOM [27].

As every year water resources become scarcer, wastewater reclamation and reuse gains prominence as an alternative to increasing water supplies in many areas of the world. Therefore, obtaining high-quality reclaimed water is already imperative. Ozonation is a water treatment technology with a proven capability to remove microcontaminants, including PhACs, from UWWE. However, efforts to further remove both highly reactive and also ozone-resistant micropollutants during ozonation and ozone-based processes are still necessary, especially for water entering the reuse cycle either in potable (drinkable) form or in non-potable (not drinkable) form.

2.2.1 Control of Ozonation By-products

Ozonation rarely leads to UWWE mineralization but to the so-called transformation products (TPs). A combination of advanced analytical techniques and the knowledge of mechanisms of ozone and •OH reactions with PhACs allows the elucidation of many of these products. Although the existence or not of the functional pharmacophores in the TPs structures can be an indicator of their potential hazard, their potential risks to humans and the environment associated to their presence in water matrices, particularly for water reuse, should be assessed. Protocols comprising a wide range of specific and unspecific toxicity bioassays have been developed over the last years, as well as computer-aided methods based on the existing knowledge for a number of model compounds [28].

The generation of bromate, an oxidation by-product from bromide, also needs to be carefully evaluated. Bromate is a highly carcinogenic compound, and its generation can be significant in the ozonation of industrial wastewaters and sewer systems with saline intrusion. Even though the process is still not fully understood due to its highly complicated chemistry, efficient strategies for its mitigation during wastewater ozonation are nowadays fairly well-known, such as low ozone dosage and the use of the peroxone process [29].

The generation of other oxidation by-products from water pollutants and EfOM transformations during ozonation can also bring some toxicity concerns – like

N-nitrosodimethylamine (NDMA) that originated, for example, from the oxidation of sulfonamide moiety of some antibiotics—and others, like aldehydes, ketones, and carboxylic acids, although in general less toxic, that are formed in significant concentrations. These compounds contribute to the increase of the biodegradability of ozonized effluents, which may trigger some technical and additional environmental problems, like formation of toxic disinfection by-products, bacterial growth in distribution systems, or eutrophication in receiving water bodies [30]. In order to mitigate problems with oxidation products, ozonation is integrated with post-treatments with other wastewater technologies, such as granular activated carbon and biofiltration strategies.

2.2.2 Municipal Wastewater Ozonation at Industrial Scale

UWWE have been proven as the main source of PhACs in natural water compartments. Protection of sensitive waters and drinking water resources together with load reduction for downstream water use has become a priority for some countries, like Switzerland. Moreover, the reclamation and reuse of municipal wastewater is a well-established practice in many areas of the world with water scarcity, like California and the southeast of Australia, and these activities will continue to increase.

High-quality effluents and water supplies can be obtained by further treatment of UWWE. Ozone is nowadays recognized as one of the best chemical oxidants for reducing the amount of micropollutants discharged with UWWE. This strategy is the one adopted by Switzerland and it is currently under development. By 2040, circa 100 wastewater treatment plants (out of a total of 650) will be upgraded with a tertiary treatment either with ozonation or powdered activated carbon [31]. In other European countries such as France, the Netherlands, or Germany, similar practices are currently under evaluation.

Ozonation is also one of the short- or medium-term future prospect technology in wastewater reclamation for water reuse. This technology can be employed after activated sludge when reuse is intended for agricultural and municipal applications or as part of a multibarrier advanced treatment in the case of indirect and direct potable reuse. Many cities have integrated reuse plans into their water management strategies, for example, to irrigate parks, playgrounds, and golf courses and for other non-potable uses such as toilet flushing, fire protection, street cleaning, and fountains. For example, Los Angeles has set the goal of reusing 40% of its municipal wastewaters within 20 years. St. Petersburg in Florida completely reuses all of its wastewater through a specific distribution system, at a cost about one-third less than the drinking-quality water. Many studies are being conducted on the feasibility of reclaiming wastewater to drinking quality. Although implementation results are still scarce, areas facing limited water availabilities and high costs of developing new supplies are considering reclaimed water as a potential source of potable water in the near future.

3 Fenton and Fenton-Like Processes

3.1 Fundamentals

Fenton reaction was described for the first time by Henry John Horstman Fenton, who discovered that H_2O_2 could be activated in the presence of ferrous ions to oxidize tartaric acid [32]. The mechanism of Fenton oxidation is quite complex [33]. Briefly, it relies on the reaction between ferrous iron (as catalyst) and H_2O_2 (as oxidant) generating •OH, as stated in the simplified sequence of reactions 3 and 4. The regeneration of ferrous iron from ferric iron by reaction 4 is the rate-limiting step in the catalytic iron cycle, if iron is added in small amounts. The values of the kinetic constants have been reported by Sychev and Isaak [34]. It has only been in 1968 that it has been first suggested as means for wastewater treatment by Bishop et al. [35]. In the presence of H_2O_2 and at pH < 3, the reaction system is autocatalytic, because Fe (III) reacts with H_2O_2 giving Fe(II), which is generated at a slow rate:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-, k = 53 - 76\ M^{-1}s^{-1} \quad (3)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ + HO_2^{\bullet}, k = 1 - 2 \times 10^{-2}\ M^{-1}s^{-1} \quad (4)$$

Many Fenton-based processes have risen in the last years, suggesting the future intensification of the use of the classical Fenton process coupled to radiation or electrochemistry, and/or involving heterogeneous catalysts. The optimization of the catalyst and oxidant doses makes the process capable of treating complex water matrices. It has long been demonstrated that the increase of H_2O_2 concentration results in a higher generation of •OH, which in turn leads to the increase of the degradation rate. Nevertheless, the use of excessive oxidant concentration is not encouraged either, since massive amount of H_2O_2 can induce antagonistic reactions (i.e., reaction of the oxidant with the produced hydroxyl radicals) and thus the formation of radicals that are less reactive than the hydroxyl radicals.

ZVI-based in situ chemical oxidation has been proposed as a potential alternative for the traditional Fenton reaction because ZVI would leach dissolved iron species regularly and activate molecular oxygen. ZVI can be used to oxidize organic and inorganic compounds in the presence of oxygen. The important oxidants are hydroxyl radicals, ferryl ions, and superoxide radicals [36]. In the oxidation of ZVI in aerobic water, hydrogen peroxide is produced as an intermediate product (reaction 5). Further reaction for the formation of H_2O_2 is the oxidation of ferrous iron by O_2 (reaction 6). H_2O_2 can react with ferrous iron by Fenton (reaction 3). Acidic conditions promote the production of highly reactive •OH (reaction 7), whereas neutral and basic conditions lead to the formation of oxidizing species such as ferryl iron (reaction 8). Moreover, fast recycling of ferric iron into ferrous species at the metal surface can take place (reaction 9). It has been also recently proposed [37] an analogous process using persulfate (reaction 10) for PhACs removal. Acidic conditions will be not discussed here, as an application for

UWWE containing PhACs would not be a consistent application, as stated in the following paragraphs.

$$Fe^0 + 2H_2O + O_2 \rightarrow Fe^{2+} + H_2O_2 + 2OH^- \quad (5)$$

$$Fe^{2+} + O_2^{\bullet -} + 2H^+ \rightarrow Fe^{3+} + H_2O_2 \quad (6)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (7)$$

$$Fe^{2+} + H_2O_2 \rightarrow FeO^{2+} + H_2O \quad (8)$$

$$2Fe^{3+} + Fe^0 \rightarrow 3Fe^{2+} \quad (9)$$

$$Fe^0 + S_2O_8^{2-} \rightarrow Fe^{2+} + 2SO_4^{2-} \quad (10)$$

Chelating agents are substances with several coordinating sites and can form bonds with metal ions. They can be used at pH higher than 3 to form complexes with Fe(III)/Fe(II), and keep it soluble, thus enhancing the production of oxidative species by the reaction of H_2O_2 with Fe(III)/Fe(II). The criteria to successfully achieve such goal have been recently defined by Clarizia et al. [38]: lowering costs, minimal increase of total organic carbon (TOC), low toxicity, and high biodegradability. Different chelating agents have been tested (most common have been humic acids, fulvic acids, citric acid, oxalic acid, aminopolycarboxylic acids).

3.2 *Removal of PhACs from Wastewaters by Fenton and Fenton-Like Processes*

Treating UWWE resulting in rapid and complete removal of pharmaceutically active compounds (PhACs) should take also into account that the process should be mild (to remove microcontaminants from huge streams of water not large quantities of catalyst, oxidant, or energy, keeping the environmental advantage of PhACs removal) and competitive with best available technologies (BAT) as ozonation and adsorption on active carbon (PAC and GAC) [24]. The subsequent use or reuse of water should be taken into account, for large quantities of iron could impede the reuse in irrigation of crops, the main application of treated water. Recommended maximum concentration in irrigation water is Fe <5.0 mg/L, as it is not toxic to plants in aerated soils [39]. However, FAO established the limit in Fe from 5 mg/L for long-term use until 20 mg/L for short-term use (periods of up to 20 years on fine-textured neutral or alkaline soil) [40]. Wastewater reuse for irrigation in agriculture is by far the most established end use in low-income countries as well as in arid and semi-arid ones [41]. Another important factor to take into account is the legislation that establishes environmental limits of iron emissions. Specifically, in Andalucia (Spain, Decree 109/2015), a limit of 3.3 and 2.2 mg/L is established for wastewater

discharge in coastal or surface waters, respectively. High conductivity would not be also recommendable for reuse for irrigation.

BAT already applied at real scale in a few countries for PhACs removal in UWWE have been reported to range in 0.04–0.10 €/m^3 investment costs for ~50,000 population equivalent (PE) and 0.03–0.09 €/m^3 operation costs. Therefore, for mid-scale plants (~50,000 PE), the costs range from 0.10 to 0.15 €/m^3. Capitalized investment-related and operation cost yield to the annual costs for the given life cycle are stated in Rizzo et al. [24], decreasing further with the increasing plant size. Therefore, Fenton for PhACs removal in UWWE should be applied at low iron concentration (in the range of a few mg/L) and minimizing the use of additional chemicals to improve the efficiency, as it would dramatically increase costs up to the available BAT.

Because of the low solubility product of ferric iron hydroxide ($K_S(Fe(OH)_3) \approx 10^{-37}$), precipitation starts at pH 2.5–3.5 depending on the iron concentration and the temperature. The precipitation process starts with the formation of dimers and oligomers, which at continuation gradually polymerize further and lose water until forming finally insoluble iron hydroxides (e.g., goethite or hematite). At pH above 4, the formation and precipitation of ferric oxyhydroxides inhibit both the production of •OH radicals and the regeneration of Fe(II) [42]. The precipitation and aging processes are also temperature-dependent, and more and faster precipitation takes place at higher temperatures [43]. The precipitate is difficult to re-dissolve through acidification and insoluble above pH 1–1.5 [44]. Therefore, conventional Fenton should be applied at acidic pH.

However, urban wastewater effluents containing PhACs also contain inorganic carbon as carbonates, mainly as hydrogen carbonate (HCO_3^-), in the range of hundreds of mg/L. Roughly, HCO_3^- 250 mg/L needs around 200 mg/L H_2SO_4 for being balanced and 50 mg/L H_2SO_4 for attaining pH < 3. Therefore, 250 kg H_2SO_4 are needed per 1,000 m^3 of urban wastewater effluent. Before water disposal or reuse, pH 7–8 should be recovered, using at least NaOH 40 mg/L, or other neutralizing agents. The salinity of effluent would increase >0.5 mS/cm, leading also to considerable loads of iron-containing sludge that will require further management. Applying conventional Fenton to urban wastewater effluents would dramatically increase effluent salinity, environmental impact, and treatment costs. Therefore, from the point of view of the authors, it could be disregarded for PhACs removal.

It has also been demonstrated that by adding iron at different steps (i.e., sequential iron dosage), it is possible to operate Fenton at initial neutral pH but substantially increasing iron dosing and decreasing the reaction rate compared to Fenton at acidic pH [45]. Using Fenton at neutral pH is not viable without adding large quantities of iron and long treatment times, as Fe(II) 30 mg/L and 90 min or 72 min of reaction for eliminating 51 or 32 microcontaminants (ranging from tens of nanogram per liter to micrograms per liter) detected in urban wastewater effluents from two different treatment plants [46]. Similar results were obtained by Soriano-Molina et al. [47] as only 35% of PhACs and other microcontaminants were removed from UWWE with $FeSO_4$ (Fe 20 mg/L) at circumneutral pH. Increasing iron concentration >5 mg/

L would impede proper reuse of treated water in many crops, as stated earlier. It can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum and may result in unsightly deposits on plants, equipment, and buildings [48]. But when using Fe <5.0 mg/L, Fenton could not remove almost any PhACs [49].

Another important factor that strongly influences Fenton performance concerning the removal of PhACs is the complex chemical composition of organic matter present (OM) in UWWE. OM components react readily with hydroxyl radicals, thus reducing the radical concentration and the direct attack of HO• toward the PhACs. Under the inherent wastewater pH, Fe(III) can be complexed by DOM resulting in the formation of stable and soluble complexes that can participate in further reactions [50]. Humic substances are the largest fraction of dissolved organic matter in natural water, and an important fraction of UWWE, as they result from microbiological and chemical transformation of organic debris. They contain carboxylic acids, phenolic compounds, alcoholic quinine, and amino and amido groups which enable them to support ion exchange and redox processes and to form complexes. They can also facilitate the reduction of Fe(III) to Fe(II) due to the presence of quinone moiety [51].

The occurrence of inorganic anions (i.e., chlorides, sulfates, carbonates) in wastewater can influence the degradation rate of PhACs during the Fenton treatment by consuming HO•. They can also complex the iron species ($FeCl_2^+$, $FeCl_2^+$, and $Fe(SO_4)^{2-}$) interfering with the reduction of Fe(III) to Fe(II) that is required to sustain Fenton mechanism. The inorganic anions scavenge the hydroxyl radicals to generate inorganic radicals ($Cl^{\bullet}$, $SO_4^{-\bullet}$, and $CO_3^{\bullet-}$) which, in turn, react with the organic contaminants, albeit at a slower rate [5]. $SO_4^{-\bullet}$ have recently gained great attention due to the high redox potential [52], reacting preferentially through an electron transfer mechanism. Phosphate is a specific case as it can precipitate the iron catalyst as iron(III) phosphate in a wide range of pH.

In conclusion, conventional Fenton or Fenton at circumneutral pH seems to be not suitable for PhACs removal in UWWE. Other available processes based on electrochemical processes [53] would be also disregarded due to a lack of efficiency (and therefore high costs) with usual UWWE due to the low salt content (0.9–2.2 mS/cm conductivity). Therefore, other research line has been explored and are now hot topics in Fenton and Fenton-like processes: zero-valent iron (ZVI) and application of chelating agents to form complexes with Fe and permit Fenton reaction at high pH.

The use of waste-metallic iron shavings instead of commercial ZVI powders showed promising results for the elimination of PhACs [54] but always after activation by immersion in acid solution to remove the surface layer of the oxidized iron (Fig. 3).

It has been demonstrated the negligible effect of the air/ZVI system for the treatment of the wastewater, unlike the promising results reported in the degradation of model pollutants. It is clear the significant role of both ZVI and H_2O_2 together in the reaction system. The formation of iron oxides supported on the metallic iron

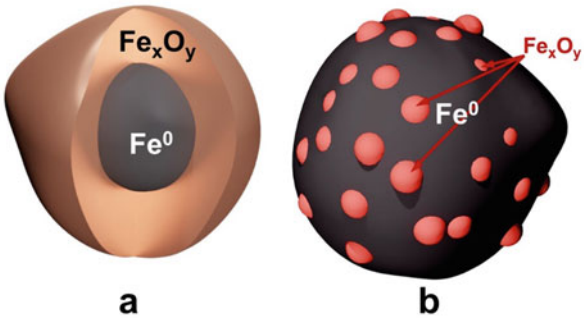


Fig. 3 Waste-metallic iron with Fe^0 inside (**a**). Formation of iron oxides supported on the metallic Fe^0 surface at circumneutral pH (**b**)

surface (Fig. 3b) is plausible at circumneutral pH, which would also reduce the presence of the iron species in the treated aqueous solution [55].

Many chelating agents presented important drawbacks to be applied for PhACs removal in UWWE. Carboxylic acids (citric acid, oxalic acid) add an important extra dissolved organic carbon and reduced final pH; humic substances resulted in low degradation rates due to the quite low amount (few mg/L) that can be found in UWWE. Therefore, it is required extracting and concentrating actions from water which is not easy and does not fulfill the "lowest-cost" principle. Aminopolycarboxylic acids (APCAs) act similarly to polycarboxylic acids, and they are used widely in domestic products, industrial applications, and soil remediation. APCAs contain several carboxylate groups linked to one or more nitrogen atoms and can chelate metal ions by forming one or more heteroatomic rings around the ions [56].

EDTA, as one of the most common APCAs, has been used widely. However, the concern about the potential of toxicity and low biodegradability makes it a persistent organic pollutant and limits its applicability as iron chelate [57] for treatment of UWWE by Fenton processes. EDDS (ethylenediaminedisuccinic acid) is a structural isomer of EDTA and is reported to have metal-complexing properties similar to EDTA [58]. Complete and rapid biodegradation of [S,S]-EDDS (SS-ethylenediaminedisuccinic acid) has been described in many environmental matrices as water [59] and soil [60], being easily biodegraded by activated sludge [61]. Therefore, [S,S]-EDDS is safe when used as Fe-chelating agent during Fenton treatment of UWWE. Currently, 80% of fertilizers used in agriculture are synthetic iron chelates. In addition, the effect of the sub-products generated by photodecomposition has been previously investigated resulting non-toxic for crops [62]. Therefore, its use as a possible iron chelate for wastewater treatment and further reuse in agriculture seems to be a plausible option.

The removal of PhACs in the EDDS-driven Fenton reaction was successful at pH between 8 and 9 [63]. Therefore, that of EDDS application may be limited by its rapid reaction with •OH that involves the decomposition of EDDS and the decrease in efficiency of Fenton process. It is important to highlight that the treatment of several grams of PhACs per cubic meter of UWWE should be achieved in just a few

minutes to be an alternative to the previously mentioned BAT. In conclusion, it would be possible to apply EDDS-iron complex only in these circumstances.

4 UV/Peroxide Processes

4.1 Fundamentals

The system UV/hydrogen peroxide (UV/H_2O_2) is one of the most extensively studied AOPs for the removal of water pollutants, both chemical and biological [64, 65]. In this case, the generation of the •OH comes from the photolysis of the hydrogen peroxide, specifically through the cleavage of the peroxide bond (O-O). Baxendale and Wilson [66] reported the formation of two hydroxyl radicals per absorbed photon with a quantum yield close to 1.0 at 254 nm according to reaction 11:

$$H_2O_2 \xrightarrow{h\nu} 2\,^{\bullet}OH,\ \phi_{254nm} = 1.0\ \text{mol Einstein}^{-1} \tag{11}$$

The photolysis of H_2O_2 is controlled by certain parameters such as H_2O_2 concentration, UV-C dose, UV wavelength, pH, and alkalinity of water. The optimal control of these parameters allows maximizing the efficiency of the treatment. Obviously, the increase of the UV dose leads to an increase in the efficiency of the generation of •OH. In contrast, the concentration of H_2O_2 is a critical parameter. Since the molar absorption coefficient of H_2O_2 is low ($\varepsilon_{254nm} = 18.6\ M^{-1}\ cm^{-1}$), a high concentration is required to absorb a significant fraction of UV in the range of 200–300 nm (the maximum absorbance of hydrogen peroxide is around 220 nm). However, excessive dosage can cause scavenging of •OH according to the reactions (12)–(14):

$$^{\bullet}OH + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \tag{12}$$

$$HO_2^{\bullet} + H_2O_2 \rightarrow {}^{\bullet}OH + H_2O + O_2 \tag{13}$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \tag{14}$$

Besides, the activation of H_2O_2 is affected by the UV transmittance of the medium, or in other words, the effectiveness of UV/H_2O_2 process is susceptible to water matrix. Alkaline pH favored the process, as the molar absorption coefficient of the HO_2^- conjugate base is much higher ($\varepsilon_{254nm} = 240\ M^{-1}\ cm^{-1}$), improving significantly the efficiency of the photon absorption step. However, water alkalinity strongly affects the yield of the process, as carbonates and bicarbonates present act as •OH scavengers (reactions 15 and 16), reducing the net availability of •OH for the degradation of pollutants:

$$^{\bullet}OH + CO_3^{2-} \rightarrow OH^- + CO_3^{\bullet -} \; k = 2 \times 10^8 \; M^{-1} \; s^{-1} \quad (15)$$

$$^{\bullet}OH + HCO_3^- \rightarrow OH^- + HCO_3^{\bullet} \; k = 1.5 \times 10^7 \; M^{-1} \; s^{-1} \quad (16)$$

Among the advantages of the UV/H_2O_2 processes for the removal of chemical pollutants, it can be mentioned that their degradation might occur also via direct photolysis and the possibility of carrying out the simultaneous disinfection of water by the damages produced by the UV-C on the DNA of microorganisms [67]. In addition, the design of the treatment facilities is simple [68], and existing facilities can be adapted [69], taking advantage of the high degree of maturity and widely full-scale application of UV disinfection processes. Moreover, UV/H_2O_2 minimizes the formation of by-products, as trihalomethanes (THMs) and haloacetic acids (HAAs) generated as a consequence of reaction of chlorine used as oxidant with natural organic matter (NOM) [70]. Finally, UV/H_2O_2 is an AOP with a relative low cost if it is compared with other advanced treatments. This low cost gives it great versatility, being useful as a single or complementary treatment to other treatments (coupled with filtration processes, adsorption, etc.) [70–72]. The cost of the UV/H_2O_2 process is associated with the intensity required from the UV lamps and their low energy efficiency [69]. The introduction of UV-LED lamps promises to reduce the associated costs, although now they are not competitive because of the associated cooling costs [73].

As main limitations of the process, it can be mentioned the strong sensitivity of the efficiency to water turbidity, reducing UV transmittance, and water matrix effects, especially radical scavenging substances. For this reason, the application of this technology is especially of interest as a tail-end technology treating the final effluent before discharge. It is also worth noting that H_2O_2 is a powerful oxidizing substance, so its handling and storage require some specific precautions.

Recently, the scientific community has focused their interest in the application of a modification of UV/H_2O_2 system, based on substituting the H_2O_2 by persulfate salts as sodium or potassium persulfate and potassium peroxymonosulfate [74]. The substantial difference with UV/H_2O_2 system is the additional generation of sulfate radicals, with a comparable redox potential to hydroxyl radicals (2.5–3.1 V vs. 1.8–2.7 V for $SO_4^{\bullet -}$ and •OH, respectively) [75]. The reactions 17 and 18 show the generation of hydroxyl and/or sulfate radicals by cleavage of the O-O bond of persulfate (PS) and peroxymonosulfate (PMS) anion [76]:

$$S_2O_8^{2-} \xrightarrow{h\nu} 2SO_4^{\bullet -}, \; \phi_{254nm} = 1.4 \text{ mol/Einstein} \quad (17)$$

$$HSO_5^-/SO_5^{2-} \xrightarrow{h\nu} SO_4^{\bullet -} + {}^{\bullet}OH, \phi_{254nm} = 1.04 \text{ mol/Einstein} \quad (18)$$

These treatment systems are as simple to apply as UV/H_2O_2, with the only difference that persulfate salts are solids and hydrogen peroxide is liquid. In both

cases, the oxidizing character of both substances forces precautions for storage and handling.

4.2 Removal of PhACs from Wastewaters by UV/H_2O_2

As it has been previously mentioned, the UV/H_2O_2 process is a promising technology with a high level of maturity thanks to the extensive bench-scale studies carried out during the last decades. However, the study of the process continues nowadays due to the current challenges and associated human risks, such as the formation of by-products [77], the presence of substances of emerging concern [78], and antibiotic resistance genes [79].

The high level of bench-scale tests of the UV/H_2O_2 system has helped through the scaling up of pilot- and even full-scale application, as a feasible technology for the removal of PhACs. Along this section, it will be shown the state of the art about pilot- and full-scale application of UV/H_2O_2 system for the removal of PhACs and other micropollutants or emerging concern. Table 2 summarizes recent reports about the application of UV/H_2O_2, UV/PS, and UV/PMS processes at pilot scale, whereas Table 3 summarizes some recent studies at full scale. As it can be observed, there is an important variety of working conditions in the presented researches, such as different water matrices, working pH, UV dose, concentration of hydrogen peroxide, flow rates, kind of reactor, or even the studied PhACs, making difficult the direct comparison of the results. Nevertheless, there is a common trend, that is, the use of UV/H_2O_2 system as a final step in a tertiary treatment in a wastewater treatment plant, in some cases treating directly the effluent after the secondary treatment and in other cases treating the effluent after some previous stages as microfiltration, ultrafiltration, reverse osmosis, adsorption, etc. Besides, there is a common pattern in most of the cases, which is the comparison of the process with other AOPs, including UV/Cl_2 system.

Miklos et al. [68] reported the viability of applying UV-C/H_2O_2 as a tertiary treatment for municipal wastewater, studying the removal efficiency of this technology over 15 different organic pollutants occurring at trace level in UWWE. The study included the optimization of working conditions at bench scale and the scaling up at pilot scale. Using a dose of 10 mg/L of hydrogen peroxide, these authors reported the UV-C/H_2O_2 system as a successful option to remove water PhACs. Between the most important remarks, they pointed out the important variations of •OH exposure as a consequence of the fluctuations on nitrite concentration and the scavenging effect of substances in water. A common feature in most of the studies is the significant differences in the removal efficiency depending on the specific substance, as a direct consequence of their chemical structure and properties. For instance, Miklos et al. [68] reported the total removal of diclofenac (DCF) but a null efficiency over tris(2-carboxyethyl)phosphine (TCEP).

On the other hand, Zhang et al. [70] reported the comparison between UV/H_2O_2, UV/free chlorine, and UV/chloramines in the removal of 0.5 log units of 1,4-dioxane

Table 2 Summary of recent pilot-scale UV/H_2O_2 studies for the removal of PhACs and other micropollutants in different water matrices

Reference	Location	Water matrix	[Oxidant]	pH	Operation mode	Reactor characteristics	UV dose	Flow rate (m^3/h)	Reactor volume (L)	Pollutants	Yield (%)
Sarathy et al. [80]	Canada	Surface water	H_2O_2 = 10 mg/L	N.S.	Continuous	Trojan UVSwift 4L12 Trojan UVPhox 8AL20	N.S.	MP reactor: 22–65 LP reactor: 11–22	N.S.	Natural organic matter	> 58%
Sichel et al. [81]	Germany	Tap water	H_2O_2 = 5 mg/L	7	Continuous	WTL 40, 80, and 200, Siemens AG	N.S.	0.25	N.S.	BTA, TTA, DEA, CBZ, SMX, DCF, IPM	50–100%
Lester et al. [82]	Boulder (USA)	Secondary effluent wastewater	H_2O_2 = 10 mg/L	N.S.	Continuous	Trojan 100 W LP Trojan 400 W MP	750 mJ/cm^2 1,500 mJ/cm^2	0.11–0.23	N.S.	SCL, LMT, GMB, CLAR, ATN, 2,4-D, CBZ, MTP, TMP, VEN, DPD	750 mJ/cm^2: 18–54% 1,500 mJ/cm^2: 37–71%
Chu et al. [83]	Singapore	Fresh water	H_2O_2 = 0.5–10 mg/L	6.3	Continuous	Trojan UVSwift 4 MP UV lamps; each of 3 kW	N.S.	40–60	N.S.	BPA, E2, PFOA, PFOS, NDMA, DCF	45–100%, except for PFOS and PFOA, 5–20%
Miralles-Cuevas et al. [84]	Almería (Spain)	Simulated fresh water	H_2O_2 = 10, 25, and 50 mg/L PS = 10, 25, and 50 mg/L	N.S.	Continuous	3 UV-C lamps, 230 W	115 W/m^2 (254 nm)	1.2	5.21	AP, CFN, CBZ, CPX, SMX	65–99%

(continued)

Table 2 (continued)

Reference	Location	Water matrix	[Oxidant]	pH	Operation mode	Reactor characteristics	UV dose	Flow rate (m^3/h)	Reactor volume (L)	Pollutants	Yield (%)
Sarasidis et al. [85]	Thessaloniki (Greece)	Tap water	H_2O_2 = 10–80 mg/L	7.8	Continuous	PURO 4S, 4 UV-C lamps	N.S.	0.027	15	DCF	100%
Krystynik et al. [86]	Czech Republic	Groundwater	H_2O_2 = 2 mM/h	7.62	Continuous	TUV UVC TL-D 36 W G13 Philips	N.S.	1.27	42.4	TCE, PCE	100%
Miklos et al. [68]	Munich (Germany)	Secondary effluent wastewater	H_2O_2 = 10 mg/L	N.S.	Continuous	WEDECO LBX 90e WEDECO LBX 10	800 mJ/cm^2 (254 nm)	LBX 90e: 35 LBX 10: 11	LBX 90e: 45 LBX 10: 13	DCF, IOP, SMX, CLI, TRA, BZN, VEN, SOT, PRI, CBZ, GBP, TCEP	44–99.6% 1–90% 1–81%
Baresel et al. [87]	Stockholm (Sweden)	Wastewater	H_2O_2 = 10–50 mg/L	6.8–7	Continuous	Van Remmen UV Technology	3,000–10,000 mJ/m^2 (254 nm)	1–2	50	Sum of pharmaceuticals	50–80%
Rodríguez-Chueca et al. [74]	Madrid (Spain)	Secondary effluent wastewater	H_2O_2; PS and PMS = 0.01–5 mM	7.23	Continuous	Philips TUV PL-L 95 W/4 P HO 1CT/25	5.7–57 J/L	0.06–0.6	0.47	DCF, IBP, SMX, CBZ, ATN, SMZ, ATZ, TCS, CFN, SCL	23–100%
Wünsch et al. [88]	Germany	Rhine river	H_2O_2 = 4 mg/L	8.1 ± 0.1	Continuous	N.S.	6,000 mJ/m^2 (254 nm)	N.S.	N.S.	EDTA, ACE, IPA, IME, MET, BTZ, IPR	10–95%
Zhang et al. [70]	Fountain Valley (USA)	Permeate reverse osmosis	H_2O_2 = 3.1–6.2 mg/L	5.7 and 7	Continuous	Trojan 257 W LPHO	≈1,000 mJ/cm^2 (254 nm)	1.33	9.7	1,4-Dioxane	65–78%

N.S., Not specified

Table 3 Summary of recent full-scale UV/H_2O_2 studies for the removal of PhACs and other micropollutants in different water matrices

Reference	Location	Water matrix	pH	Operation mode	Reactor characteristics	[Oxidant]	UV dose	Flow rate (m^3/h)	Reactor volume (L)	Pollutants	Yield (%)
Wang et al. [89]	Cornwall (Canada)	St. Lawrence River	6.5, 7.5, and 8.5	Continuous	Trojan UVSwift 8 L24	H_2O_2 = 1, 2.9, 4.8 mg/L	2,000 ± 150 mJ cm^{-2} (200–400 nm)	360	720	Geosmin, MIB, CFN	93% for geosmin; 83% for MIB; 77% for CFN
Rodríguez-Chueca et al. [69]	Toledo (Spain)	Secondary effluent wastewater	7.17 ± 0.04	Continuous	WEDECO LBX400e	H_2O_2; PS and PMS =0.05–0.5 mM	42–170 J/L	28, 75, 114	140	LDC, TRA, ODT, VLX, NVNF, CBZ, OCBZ, MIR, GBP, METO, SFX, BTR, TT, DEET, TBY, TCEP, TCPP, TPP, HHCB, HHCB-I, HHCB-II, AHTN, ATII, OTNE	Average removal of 48% with PMS; 55% with H_2O_2
Wetterau et al. [72]	California (USA)	Permeate reverse osmosis	5.6	Continuous	Trojan 30AL50 UVPhox Trojan 72AL75 UVPhox	H_2O_2 = 1–4 mg/L	N.S.	568–947	N.S.	1,4-Dioxane	1.2 log
Rodríguez-Chueca et al. [90]	Toledo (Spain)	Secondary effluent wastewater	7.17 ± 0.04	Continuous	WEDECO LBX 400e	H_2O_2; PS and PMS	42–170 J/L	28, 75, 114	140	Antibiotics (AB): AZT, CLAR,	AB: average removal

(continued)

Table 3 (continued)

Reference	Location	Water matrix	pH	Operation mode	Reactor characteristics	[Oxidant]	UV dose	Flow rate (m^3/h)	Reactor volume (L)	Pollutants	Yield (%)
						=0.05–0.5 mM				CPFX, OFX, SMX, SFD, SFP, TMP, MTZ, CLI Antibiotic resistance genes (ARG): intI1, sul1, sul2, blaOXT, blaTEM, qnrS	of 29% with PMS; 36% with H_2O_2 ARG: average removal of 0.31 log with PMS; 0.55 log with H_2O_2
Wang et al. [71]	Mississauga (Canada)	UF effluent	6.5 and 8	Continuous	Trojan UVSwift ECT16L30	H_2O_2 = 5–10 mg/L	16 medium-pressure lamps (12.3 kW). Three UV ballast power levels (50, 70, and 100%)	625	316	SCL, CFN, DBP (THMs, HAA, and AOX)	At pH 6.5, 0.3–1 log; at pH 8, 0.2–0.8 log

N.S., Not specified

(1,4-D) to regenerate wastewater, as well as the assessment of the generation of disinfection by-products (DBPs). Their main conclusion is that the UV/free chlorine system is the most efficient at pH 5.7, while at pH 7, because of the scavenging caused by the OCl^-, the most efficient treatment was UV/H_2O_2. In any case, the UV/free chlorine system achieved a cheaper cost than UV/H_2O_2 and UV/chloramines treatments, and the generation of DBPs was similar in all the cases but promoting the generation of different substances. Other authors, as Baresel et al. [87], also reported a good efficiency of UV/H_2O_2 in removing micropollutants but confirm that they are still not economically competitive regarding other technologies such as ozonation or activated carbon.

Miralles-Cuevas et al. [84] compared from the economical point of view the application of UV/H_2O_2 using UV-C and solar radiation. They concluded that the main contribution to the operating costs comes from reagents and electricity from lamps operation. However, according to their results, the main difference between using solar and UV systems on the removal of PhACs is the optimal concentration of the oxidant. Rodríguez-Chueca et al. [74] compared the efficiency of UV/H_2O_2 with modification treatments using PS and PMS as oxidants with very low residence times. In general, the use of PMS and PS as oxidants led to higher removal yield than using hydrogen peroxide. Both persulfate anions allowed to reach the total removal of some PhACs under the most demanding operating conditions. Just the removal of sucralose (SCL) was more effective by using H_2O_2.

Other authors, such as Sarasidis et al. [85], studied the efficiency of UV/H_2O_2 to regenerate powdered activated carbon (PAC) used on the removal of PhACs. With promising results on the removal of DCF, the authors proposed a further investigation on the mechanisms of regeneration of PAC because it involves adsorption, desorption, and oxidation. Some authors reported the use of UV/H_2O_2 on the treatment of fresh surface water, as it is the case of Wünsch et al. [88], studying the impact of a UV/H_2O_2 before soil aquifer treatment (SAT). They assessed the efficiency on the abatement of different PhACs and other micropollutants present in Rhine river water, using a pilot plant consisting of a UV-C reactor and two parallel soil columns. These coupled systems enhanced the removal of micropollutants by an additive effect of the unit processes. However, in almost all cases, the UV/H_2O_2 system is responsible for the abatement of the studied micropollutants, being only the metformin primarily abated by SAT.

The disadvantages reported by some authors in terms of efficiency and cost of UV/H_2O_2 in comparison with other AOPs have slowed down the implementation of technology on a full scale. Despite this, there are reports about the application of UV/H_2O_2 at full-scale, in some cases as research studies, but in others in real water facilities using this technology in the removal of PhACs and other micropollutants (Table 3). For instance, Wang et al. [71] compared the efficiency of UV/H_2O_2 and UV/Cl_2 on the removal of micropollutants in water adapting the tertiary treatment of the Lorne Park Water Treatment Plant in Canada. Both AOPs were applied over the permeate of the filtration step. The conclusions obtained are quite similar to the ones showed by Zhang et al. [70] at pilot scale. At pH 6.5, the generation of •OH was almost twice by UV/Cl_2 than UV/H_2O_2, but at pH 8.0, •OH formation was

negligible during UV/Cl_2 system. Similar conclusions comparing UV/Cl_2 and UV/H_2O_2 were reached by other authors, such as Wang et al. [89] applying the technologies in the Cornwall Water Purification Plant (Canada) and Wetterau et al. [72] in WRD Leo J. Vander Lans Advanced Water Treatment Facility in California (USA). In this last case, Wetterau et al. [72] pointed that the same level of 1,4-dioxane reduction can be achieved with UV/Cl_2 at roughly one-third the dose as UV/H_2O_2, reducing the use of reagents or the size of the UV reactor.

Finally, Rodríguez-Chueca et al. [69] modified the tertiary treatment of municipal wastewater treatment plant in Toledo (Spain) to test different UV-driven AOPs in the existing UV-C equipment. Among them, UV/H_2O_2, UV/PMS, and UV/PS were tested using different flow rates, or in other words different UV-C doses and UV-C contact time. In general, photolysis of H_2O_2 and PMS led to similar results considering the average removal of the sum of the studied micropollutants, with a slightly higher performance using H_2O_2 (55% with H_2O_2 vs. 48% with PMS). These results, together with the lower cost of H_2O_2, made UV/H_2O_2 more attractive than the use of PMS for the removal of micropollutants. In a similar study, Rodríguez-Chueca et al. [90] analyzed the removal of antibiotics and antibiotic resistance genes from the effluents. In general, the addition of the oxidants improved significantly the efficiency of the removal of the antibiotics in comparison with the UV alone. However, the removal of antibiotic resistance genes was maximum when only UV is dosed, without oxidants. This fact suggested a compromising situation because the highest antibiotics removal corresponded to the lowest removal of genes. The reason is that the generation of hydroxyl and sulfate radicals, triggered by light absorption of the oxidants, actually reduces the availability of photons for the direct damages on the DNA. Consequently, although in theory both processes can take place simultaneously, to achieve a high efficiency in the removal on the PhACs and the antibiotic resistance genes, much higher UV doses have to be used.

5 Concluding Remarks

In this chapter the fundamentals of several advanced oxidation processes for the removal of PhACs in urban wastewater effluents have been summarized, remarking that the most suitable oxidation technique would depend mainly on the water matrix and the final use of the effluent.

Nowadays, O_3, O_3/H_2O_2, and UV/H_2O_2 treatments constitute good options to succeed in the removal of PhACs as tertiary treatments in municipal wastewaters. Modified processes such as catalytic ozonation or the use of alternative peroxides are under study.

Applying conventional Fenton at acidic pH to urban wastewater effluents would dramatically increase effluent salinity, environmental impact, and treatment costs disregarding it as an option for PhACs removal from this type of effluents. Many iron-chelating agents (carboxylic acids, humic substances, EDTA) to operate Fenton-like processes at circumneutral pH presented important drawbacks to be

applied for PhACs removal in UWWE. EDDS appears as a possible iron chelate for UWWE treatment, and further reuse in agriculture seems to be a plausible option if the treatment would be completed in just a few minutes. Modifications of Fenton and Fenton-like processes at circumneutral pH are under study in many research labs, but they are still far away from their application.

Independently on the oxidation treatment used to remove PhACs, it should be considered the potential formation of by-products during the oxidative treatment.

Treating urban wastewater effluents for removal of PhACs should be mild, minimizing the use of additional chemicals, and economically competitive with best available technologies as ozonation and adsorption on active carbon, reported to range between 0.10 and 0.15 €/m^3.

References

1. Glaze WH, Kang JW, Chapin DH (1987) The chemistry of water treatment processes involving ozone, hydrogen peroxide and ultraviolet radiation. Ozone Sci Eng 9:335–352. https://doi.org/10.1080/01919518708552148
2. Andreozzi R, Caprio V, Insola A, Marotta R (1999) Advanced oxidation processes (AOP) for water purification and recovery. Catal Today 53:51–59. https://doi.org/10.1016/S0920-5861(99)00102-9
3. Parsons S (2004) Advanced oxidation processes for water and wastewater treatment. IWA Publishing, London. https://doi.org/10.2166/9781780403076
4. Stefan MI (2017) Advanced oxidation processes for water treatment: fundamentals and applications, vol 16. IWA Publishing, London, p 9781780407197. https://doi.org/10.2166/9781780407197
5. Lado Ribeiro AR, Moreira NFF, Li Puma G, Silva AMT (2019) Impact of water matrix on the removal of micropollutants by advanced oxidation technologies. Chem Eng J 363:155–173. https://doi.org/10.1016/j.cej.2019.01.080
6. Ortiz I, Mosquera A, Lema J, Esplugas S (2015) Advanced technologies for water treatment and reuse. AICHE J 61:3146–3158. https://doi.org/10.1002/aic.15013
7. Ahmed MB, Zhou JL, Ngo HH, Guoa W, Thomaidis NS, Xuca J (2017) Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: a critical review. J Hazard Mater 323:274–298. https://doi.org/10.1016/j.jhazmat.2016.04.045
8. Kanakaraju D, Glass BD, Oelgemoller M (2018) Advanced oxidation process-mediated removal of pharmaceuticals from water: a review. J Environ Manag 219:189–207. https://doi.org/10.1016/j.jenvman.2018.04.103
9. Wang J, Wang S (2016) Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: a review. J Environ Manag 182:620–640. https://doi.org/10.1016/j.jenvman.2016.07.049
10. Yang Y, Ok YS, Kim K, Kwon EE, Tsang YF (2017) Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: a review. Sci Total Environ 596–597:303–320. https://doi.org/10.1016/j.scitotenv.2017.04.102
11. Langlais B, Reckhow DA, Brink DR (1991) Ozone in water treatment. Application and engineering. Lewis Publishers, Chelsea
12. Tiwari G, Bose P (2007) Determination of ozone mass transfer coefficient in a tall continuous flow counter-current bubble contactor. Chem Eng J 132(1–3):215–225. https://doi.org/10.1016/j.cej.2006.12.025

13. Beltran FJ (2004) Ozone reaction kinetics for water and wastewater systems. CRC Press, Boca Raton. https://doi.org/10.1201/9780203509173
14. von Sonntag C, von Gunten U (2012) Chemistry of ozone in water and wastewater treatment: from basic principles to applications. IWA Publishing, London. https://doi.org/10.2166/9781780400839
15. Pocostales JP, Sein MM, Knolle W, von Sonntag C, Schmidt TC (2010) Degradation of ozone-refractory organic phosphates in wastewater by ozone and ozone/hydrogen peroxide (peroxone): the role of ozone consumption by dissolved organic matter. Environ Sci Technol 44:8248–8253. https://doi.org/10.1021/es1018288
16. Acero JL, von Gunten U (2000) Influence of carbonate on the ozone/hydrogen peroxide based advanced oxidation process for drinking water treatment. Ozone Sci Eng 22:305–328. https://doi.org/10.1080/01919510008547213
17. Lee Y, Gerrity D, Lee M, Bogeat AE, Salhi E, Gamage S, Trenholm RA, Wert EC, Snyder SA, von Gunten U (2013) Prediction of micropollutant elimination during ozonation of municipal wastewater effluents: use of kinetic and water specific information. Environ Sci Technol 47:5872–5881. https://doi.org/10.1021/es400781r
18. Merényi G, Lind J, Naumov S, von Sonntag C (2010) Reaction of ozone with hydrogen peroxide (peroxone process): a revision of current mechanistic concepts based on thermokinetic and quantum-chemical considerations. Environ Sci Technol 44:3505–3507. https://doi.org/10.1021/es100277d
19. von Gunten U, Oliveras Y (1997) Kinetics of the reaction between hydrogen peroxide and hypobromous acid: implication on water treatment and natural systems. Water Res 31:900–906. https://doi.org/10.1016/S0043-1354(96)00368-5
20. Pisarenko AN, Stanford BD, Yan D, Gerrity D, Snyder SA (2012) Effects of ozone and ozone/peroxide on trace organic contaminants and NDMA in drinking water and water reuse applications. Water Res 46:316–326. https://doi.org/10.1016/j.watres.2011.10.021
21. Malvestiti JA, Cruz-Alcalde A, López-Vinent N, Dantas RF, Sans C (2019) Catalytic ozonation by metal ions for municipal wastewater disinfection and simultaneous micropollutants removal. Appl Catal B Environ 259:118104. https://doi.org/10.1016/j.apcatb.2019.118104
22. Nawrocki J, Kasprzyk-Hordern B (2010) The efficiency and mechanisms of catalytic ozonation. Appl Catal B Environ 99:27–42. https://doi.org/10.1016/j.apcatb.2010.06.033
23. Bourgin M, Beck B, Boehler M, Borowska E, Fleiner J, Salhi E, Teichler R, von Gunten U, Siegrist H, Mcardell CS (2018) Evaluation of a full-scale wastewater treatment plant upgraded with ozonation and biological post-treatments: abatement of micropollutants, formation of transformation products and oxidation by-products. Water Res 129:486–498. https://doi.org/10.1016/j.watres.2017.10.036
24. Rizzo L, Malato S, Antakyali D, Beretsou VG, Maja BD, Gernjak W, Heath E, Ivancev-Tumbas I, Karaolia P, Lado AR, Mascolo G, Mcardell CS, Schaar H, Silva AMT, Fatta-Kassinos D (2019) Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. Sci Total Environ 655:986–1008. https://doi.org/10.1016/j.scitotenv.2018.11.265
25. 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/H_2O_2 and ozone. J Hazard Mat 263:268–274. https://doi.org/10.1016/j.jhazmat.2013.05.030
26. Cruz-Alcalde A, Esplugas S, Sans C (2019) Abatement of ozone-recalcitrant micropollutants during municipal wastewater ozonation: kinetic modelling and surrogate-based control strategies. Chem Eng J 360:1092–1100. https://doi.org/10.1016/j.cej.2018.10.206
27. Cruz-Alcalde A, Esplugas S, Sans C (2020) Continuous H_2O_2 addition in peroxone process: performance improvement and modelling in wastewater effluents. J Hazard Mater 387:121993. https://doi.org/10.1016/j.jhazmat.2019.121993
28. Lee M, Blum LC, Schmid E, Fenner K, von Gunten U (2017) A computer-based prediction platform for the reaction of ozone with organic compounds in aqueous solution: kinetics and mechanisms. Environ Sci Process Impacts 19:465–476. https://doi.org/10.1039/c6em00584e

29. Chon K, Salhi E, von Gunten U (2015) Combination of UV absorbance and electron donating capacity to assess degradation of micropollutants and formation of bromate during ozonation of wastewater effluents. Water Res 81:388–397. https://doi.org/10.1016/j.watres.2015.05.039
30. Cruz-Alcalde A, Esplugas S, Sans C (2020) New insights on the fate of EfOM during ozone application for effective abatement of micropollutants in wastewater effluents. Sep Pur Tech 237:116468. https://doi.org/10.1016/j.seppur.2019.116468
31. Federal Office for the Environment (FOEN), Switzerland. https://www.bafu.admin.ch/bafu/en/home.html
32. Fenton HJH (1894) Oxidation of tartaric acid in presence of iron. J Chem Soc 65:899–910. https://doi.org/10.1039/CT8946500899
33. Pignatello JJ, Oliveros E, MacKay A (2006) Advanced oxidation processes for organic contaminant destruction based on the Fenton reaction and related chemistry. Crit Rev Environ Sci Technol 36:1–84. https://doi.org/10.1080/10643380500326564
34. Sychev AY, Isaak VG (1995) Iron compounds and the mechanisms of the homogeneous catalysis of the activation of O_2 and H_2O_2 and of the oxidation of organic substrates. Russ Chem Rev 64:1105–1129. https://doi.org/10.1070/RC1995v064n12ABEH000195
35. Bishop DF, Stern G, Fleischmann M, Marshall LS (1968) Hydrogen peroxide catalytic oxidation of refractory organics in municipal wastewaters. Ind Eng Chem Process Des Dev 7:110–117. https://doi.org/10.1021/i260025a022
36. Stieber M, Putschew A, Jekel M (2011) Treatment of pharmaceuticals and diagnostic agents using zero-valent iron - kinetic studies and assessment of transformation products assay. Environ Sci Technol 45:4944–4950. https://doi.org/10.1021/es200034j
37. Wu J, Wang B, Cagnetta G, Huang J, Wang Y, Deng S, Yua G (2020) Nanoscale zero valent iron-activated persulfate coupled with Fenton oxidation process for typical pharmaceuticals and personal care products degradation. Sep Pur Technol 239:116534. https://doi.org/10.1016/j.seppur.2020.116534
38. Clarizia L, Russo D, Di Somma I, Marotta R, Andreozzi R (2017) Homogeneous photo-Fenton processes at near neutral pH: a review. Appl Catal B Environ 209:358–371. https://doi.org/10.1016/j.apcatb.2017.03.011
39. Rowe DR, Abdel-Magid IM (1995) Handbook of wastewater reclamation and reuse. CRC Press, p 550
40. FAO (2003) Users manual for irrigation with treated wastewater. FAO regional Office of the near East, Cairo, Egypt. http://www.fao.org/tempref/GI/Reserved/FTP_FaoRne/morelinks/Publications/English/Usersmanual-en.pdf
41. Drechsel P, Mara DD, Bartone C, Scheierling SM (2010) Improving wastewater use in agriculture: an emerging priority. World Bank policy research working paper no. 5412, 111 p. https://doi.org/10.1596/1813-9450-5412
42. Bigda RJ (1995) Consider Fenton's chemistry for wastewater treatment. Chem Eng Prog 91:62–66
43. Krýsová H, Jirkovský J, Krýsa J, Mailhot G, Bolte M (2003) Comparative kinetic study of atrazine photodegradation in aqueous $Fe(ClO_4)_3$ solutions and TiO_2 suspensions. Appl Catal B Environ 40:1–12. https://doi.org/10.1016/S0926-3373(01)00324-1
44. Sulzberger B, Laubscher H, Karametaxas G (1994) Photoredox reactions at the surface of iron (III)(hydr-)oxides. In: Helz GR, Zepp RG, Crosby DG (eds) Aquatic and surface photochemistry. Lewis Publishers, Boca Raton, pp 53–74. https://doi.org/10.1201/9781351069847-4
45. Carra I, Malato S, Jiménez M, Maldonado MI, Sánchez Pérez JA (2014) Microcontaminant removal by solar photo-Fenton at natural pH run with sequential and continuous iron additions. Chem Eng J 235:132–140. https://doi.org/10.1016/j.cej.2013.09.029
46. Estrada-Arriaga EB, Cortés-Muñoz JE, González-Herrera A, Calderón-Mólgora CG, Rivera-Huerta ML, Ramírez-Camperos E, Montellano-Palacios L, Gelover-Santiago SL, Pérez-Castrejón S, Cardoso-Vigueros L, Martín-Domínguez A, García-Sánchez L (2016) Assessment of full-scale biological nutrient removal systems upgraded with physico-chemical processes for

the removal of emerging pollutants present in wastewaters from Mexico. Sci Total Environ 571:1172–1182. https://doi.org/10.1016/j.scitotenv.2016.07.118
47. Soriano-Molina P, Miralles-Cuevas S, Esteban García B, Plaza-Bolaños P, Sánchez Pérez JA (2020) Two strategies of solar photo-Fenton at neutral pH for the simultaneous disinfection and removal of contaminants of emerging concern. Comparative assessment in raceway pond reactors. Catal Today. https://doi.org/10.1016/j.cattod.2019.11.028
48. FAO (1985) Ayers RS, Westcot DW (eds) Water quality for agriculture. Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/3/t0234e/t0234e00.htm
49. De la Cruz N, Gimenez J, Esplugas S, Grandjean D, de Alencastro LF, Pulgarin C (2012) Degradation of 32 emergent contaminants by UV and neutral photo-Fenton in domestic wastewater effluent previously treated by activated sludge. Water Res 46:1947–1957. https://doi.org/10.1016/j.watres.2012.01.014
50. Fukushima M, Tatsumi K (2001) Degradation pathways of pentachlorophenol by photo-Fenton systems in the presence of iron(III), humic acids and hydrogen peroxide. Environ Sci Technol 35:1771–1778. https://doi.org/10.1021/es001088j
51. Lipczynska-Kochany E, Kocjany J (2008) Effect of humic substances on the Fenton treatment of wastewater at acidic and neutral pH. Chemosphere 73:745–750. https://doi.org/10.1016/j.chemosphere.2008.06.028
52. Lian L, Yao B, Hou S, Fang J, Yan S, Song W (2017) Kinetic study of hydroxyl and sulfate radical-mediated oxidation of pharmaceuticals in wastewater effluents. Environ Sci Technol 51:2954–2962. https://doi.org/10.1021/acs.est.6b05536
53. Sirés I, Brillas E (2012) Remediation of water pollution caused by pharmaceutical residues based on electrochemical separation and degradation technologies: a review. Environ Int 40:212–229. https://doi.org/10.1016/j.envint.2011.07.012
54. Mackulak T, Nagyová K, Faberová M, Grabic R, Koba O, Gál M, Birosová L (2015) Utilization of Fenton-like reaction for antibiotics and resistant bacteria elimination in different parts of WWTP. Environ Toxicol Pharmacol 40:492–497. https://doi.org/10.1016/j.etap.2015.07.002
55. Segura Y, Martínez F, Melero JA (2013) Effective pharmaceutical wastewater degradation by Fenton oxidation with zero-valent iron. Appl Catal B Environ 136–137:64–69. https://doi.org/10.1016/j.apcatb.2013.01.036
56. Zhang Y, Zhou M (2019) A critical review of the application of chelating agents to enable Fenton and Fenton-like reactions at high pH values. J Hazard Mater 362:436–450. https://doi.org/10.1016/j.jhazmat.2018.09.035
57. Schmidt CK, Fleig M, Sacher F, Brauch HJ (2004) Occurrence of aminopolycarboxylates in the aquatic environment of Germany. Environ Pollut 13:107–124. https://doi.org/10.1016/j.envpol.2004.01.013
58. Egli T (2001) Biodegradation of metal-complexing aminopolycarboxylic acids. J Biosci Bioeng 92:89–97. https://doi.org/10.1016/S1389-1723(01)80207-3
59. Jaworskal JS, Schowanek D, Feijtel TCJ (1999) Environmental risk assessment for trisodium [s, s]-ethylene diamine disuccinate, a biodegradable chelator used in detergent applications. Chemosphere 38(15):3591–3625. https://doi.org/10.1016/S0045-6535(98)00573-6
60. Tandy S, Ammann A, Schulin R, Nowack B (2006) Biodegradation and speciation of residual SS-ethylenediaminedisuccinic acid (EDDS) in soil solution left after soil washing. Environ Pollut 142:191–199. https://doi.org/10.1016/j.envpol.2005.10.013
61. Vandevivere PC, Saveyn H, Verstraete W, Feijtel TCJ, Schowanek DR (2001) Biodegradation of metal-[S,S]-EDDS complexes. Environ Sci Technol 2001(35):1765–1770. https://doi.org/10.1021/es0001153
62. Hernández-Apaolaza L, Lucena JJ (2011) Influence of irradiation time and solution concentration on the photochemical degradation of EDDHA/Fe^{3+}: effect of its photodecomposition products on soybean growth. J Sci Food Agric 91:2024–2030. https://doi.org/10.1002/jsfa.4414
63. Huang W, Brigante M, Wu F, Mousty C, Hanna K, Mailhot G (2013) Assessment of the Fe (III)–EDDS complex in Fenton-like processes: from the radical formation to the degradation of bisphenol A. Environ Sci Technol 47:1952–1959. https://doi.org/10.1021/es304502y

64. Rodríguez-Chueca J, Ormad MP, Mosteo R, Ovelleiro JL (2015) Kinetic modeling of *Escherichia coli* and *Enterococcus* sp. inactivation in wastewater treatment by photo-Fenton and H_2O_2/UV–vis processes. Chem Eng Sci 138:730–740. https://doi.org/10.1016/j.ces.2015.08.051
65. Wang WL, Wu QY, Huang N, Xu ZB, Lee MY, Hu HY (2018) Potential risks from UV/H_2O_2 oxidation and UV photocatalysis: a review of toxic, assimilable, and sensory-unpleasant transformation products. Water Res 141:109–125. https://doi.org/10.1016/j.watres.2018.05.005
66. Baxendale JH, Wilson JA (1957) Photolysis of hydrogen peroxide at high light intensities. Trans Faraday Soc 53:344–356. https://doi.org/10.1039/TF9575300344
67. Pablos C, Marugán J, van Grieken R, Serrano E (2013) Emerging micropollutant oxidation during disinfection processes using UV-C, UV-C/H_2O_2, UV-A/TiO_2 and UV-A/TiO_2/H_2O_2. Water Res 47:1237–1245. https://doi.org/10.1016/j.watres.2012.11.041
68. Miklos DB, Hartl R, Michel P, Linden KG, Drewes JE, Hübner W (2018) UV/H_2O_2 process stability and pilot-scale validation for trace organic chemical removal from wastewater treatment plant effluents. Water Res 136:169–179. https://doi.org/10.1016/j.watres.2018.02.044
69. Rodríguez-Chueca J, Laski E, García-Cañibano C, Martín de Vidales MJ, Encinas Á, Kuch B, Marugán J (2018) Micropollutants removal by full-scale UV-C/sulfate radical based advanced oxidation processes. Sci Total Environ 630:1216–1225. https://doi.org/10.1016/j.scitotenv.2018.02.279
70. Zhang Z, Chuang YH, Szczuka A, Ishida KP, Roback S, Plumlee MH, Mitch WA (2019) Pilot-scale evaluation of oxidant speciation, 1,4-dioxane degradation and disinfection byproduct formation during UV/hydrogen peroxide, UV/free chlorine and UV/chloramines advanced oxidation process treatment for potable reuse. Water Res 164:114939. https://doi.org/10.1016/j.watres.2019.114939
71. Wang C, Moore N, Bircher K, Andrews S, Hofmann R (2019) Full-scale comparison of UV/H_2O_2 and UV/Cl_2 advanced oxidation: the degradation of micropollutant surrogates and the formation of disinfection byproducts. Water Res 161:448–458. https://doi.org/10.1016/j.watres.2019.06.033
72. Wetterau G, Bruce Chalmers R, Schulz C (2018) Full-scale testing of UV/chlorine for potable reuse. IUVA News 20(3):11–15. https://iuvanews.com/stories/pdf/IUVANews_2018_Quarter3_links-Wetterau.pdf
73. Matafonova G, Batoev V (2018) Recent advances in application of UV light-emitting diodes for degrading organic pollutants in water through advanced oxidation processes: a review. Water Res 132:177–189. https://doi.org/10.1016/j.watres.2017.12.079
74. Rodríguez-Chueca J, García-Cañibano C, Lepistö RJ, Encinas Á, Pellinen J, Marugán J (2019) Intensification of UV-C tertiary treatment: disinfection and removal of micropollutants by sulfate radical based advanced oxidation processes. J Hazard Mater 372:94–102. https://doi.org/10.1016/j.jhazmat.2018.04.044
75. Neta P, Huie RE (1985) Free-radical chemistry of sulfite. Environ Health Perspect 64:209–217. https://doi.org/10.1289/ehp.8564209
76. Herrmann H (2007) On the photolysis of simple anions and neutral molecules as sources of O^-/OH, SO_x^- and Cl in aqueous solution. Phys Chem Chem Phys 9:3935–3964. https://doi.org/10.1039/B618565G
77. Cai L, Li L, Yu S, Guo J, Kuppers S, Dong L (2019) Formation of odorous by-products during chlorination of major amino acids in east Taihu Lake: impacts of UV, UV/PS and UV/H_2O_2 pre-treatments. Water Res 162:427–436. https://doi.org/10.1016/j.watres.2019.07.010
78. Qiu W, Zheng M, Sun J, Tian Y, Fang M, Zheng Y, Zhang T, Zheng C (2019) Photolysis of enrofloxacin, pefloxacin and sulfaquinoxaline in aqueous solution by UV/H_2O_2, UV/Fe(II), and UV/H_2O_2/Fe(II) and the toxicity of the final reaction solutions on zebrafish embryos. Sci Total Environ 651(1):1457–1468. https://doi.org/10.1016/j.scitotenv.2018.09.315
79. Yoon Y, Chung HJ, Di Wen DY, Dodd MC, Hur HG, Lee Y (2017) Inactivation efficiency of plasmid-encoded antibiotic resistance genes during water treatment with chlorine, UV, and UV/H_2O_2. Water Res 123:783–793. https://doi.org/10.1016/j.watres.2017.06.056

80. Sarathy SR, Stefan MI, Royce A, Mohseni M (2011) Pilot-scale UV/H_2O_2 advanced oxidation process for surface water treatment and downstream biological treatment: effects on natural organic matter characteristics and DBP formation potential. Environ Technol 32 (15):1709–1718. https://doi.org/10.1080/09593330.2011.553843
81. Sichel C, Garcia C, Andre K (2011) Feasibility studies: UV/chlorine advanced oxidation treatment for the removal of emerging contaminants. Water Res 45(19):6371–6380. https://doi.org/10.1016/j.watres.2011.09.025
82. Lester Y, Ferrer I, Thurman EM, Linden KG (2014) Demonstrating sucralose as a monitor of full-scale UV/AOP treatment of trace organic compounds. J Hazard Mater 280:104–110. https://doi.org/10.1016/j.jhazmat.2014.07.009
83. Chu X, Xiao Y, Hu J, Quek E, Xie R, Pang T, Xing Y (2016) Pilot-scale UV/H_2O_2 study for emerging organic contaminants decomposition. Rev Environ Health 31(1):71–74. https://doi.org/10.1515/reveh-2016-0008
84. Miralles-Cuevas S, Darowna D, Wanag A, Mozia S, Malato S, Oller I (2017) Comparison of UV/H_2O_2, UV/$S_2O_8^{2-}$, solar/Fe(II)/H_2O_2 and solar/Fe(II)/$S_2O_8^{2-}$ at pilot plant scale for the elimination of micro-contaminants in natural water: an economic assessment. Chem Eng J 310:514–524. https://doi.org/10.1016/j.cej.2016.06.121
85. Sarasidis VC, Plakas KV, Karabelas AJ (2017) Novel water-purification hybrid processes involving in-situ regenerated activated carbon, membrane separation and advanced oxidation. Chem Eng J 328:1153–1163. https://doi.org/10.1016/j.cej.2017.07.084
86. Krystynik P, Masin P, Kluson P (2018) Pilot scale application of UV-C/H_2O_2 for removal of chlorinated ethenes from contaminated groundwater. J Water Supply Res T AQUA 67 (4):414–422. https://doi.org/10.2166/aqua.2018.144
87. Baresel C, Harding M, Junestedt C (2019) Removal of pharmaceutical residues from municipal wastewater using UV/H_2O_2. IVL Swedish Environmental Research Institute 2019. Report number B 2354. https://sjostad.ivl.se/download/18.2299af4c16c6c7485d05de/1567162616009/B2354%20UV-H2O2%20rapport.pdf
88. Wünsch R, Plattner J, Cayon D, Eugster F, Gebhardt J, Wülser R, von Guten U, Wintgens T (2019) Surface water treatment by UV/H_2O_2 with subsequent soil aquifer treatment: impact on micropollutants, dissolved organic matter and biological activity. Environ Sci Water Res Technol 5:1709–1722. https://doi.org/10.1039/C9EW00547A
89. Wang D, Bolton JR, Andrews SA, Hofmann R (2015) UV/chlorine control of drinking water taste and odour at pilot and full-scale. Chemosphere 136:239–244. https://doi.org/10.1016/j.chemosphere.2015.05.049
90. Rodríguez-Chueca J, Varella Della Giustina S, Rocha J, Fernandes T, Pablos C, Encinas A, Barceló D, Rodríguez-Mozaz S, Manaia CM, Marugán J (2019) Assessment of full-scale tertiary wastewater treatment by UV-C based-AOPs: removal or persistence of antibiotics and antibiotic resistance genes? Sci Total Environ 652:1051–1061. https://doi.org/10.1016/j.scitotenv.2018.10.223

Removal and Degradation of Pharmaceutically Active Compounds (PhACs) in Wastewaters by Solar Advanced Oxidation Processes

Sixto Malato, Jaime Giménez, Isabel Oller, Ana Agüera, and José Antonio Sánchez Pérez

Contents

S. Malato (✉)
Plataforma Solar de Almería (CIEMAT), Almería, Spain
e-mail: Sixto.malato@psa.es

J. Giménez
Departamento de Ingeniería Química, Facultad de Química, Universitat de Barcelona, Barcelona, Spain
e-mail: j.gimenez.fa@ub.edu

I. Oller
Plataforma Solar de Almería (CIEMAT), Almería, Spain
e-mail: Isabel.oller@psa.es

A. Agüera
Solar Energy Research Centre (CIESOL), Joint Centre University of Almeria-CIEMAT, Almeria, Spain
e-mail: aaguera@ual.es

J. A. Sánchez Pérez
Solar Energy Research Centre (CIESOL), Joint Centre University of Almeria-CIEMAT, Almeria, Spain

Department of Chemical Engineering, University of Almería, Almeria, Spain
e-mail: jsanchez@ual.es

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 299–326, DOI 10.1007/698_2020_688,

Abstract Most advanced oxidation processes (AOPs) are based on combination of oxidants, catalysts and radiation. Main disadvantages of these processes are their high energy consumption and operation costs. The use of solar radiation could be a way to overcome these drawbacks, but only two processes fit with this approach: heterogeneous photocatalysis with semiconductors and homogeneous photocatalysis through photo-Fenton-like processes. Solar AOPs (SAOPs) present special interest for the degradation of pharmaceutically active compounds (PhACs) as they can be easily adapted to comply with fundamental principles of green chemistry. This chapter states the main concepts of (1) solar semiconductor photocatalysis including its management with specific solar photoreactors; (2) solar photo-Fenton processes application, with special emphasis in circumneutral pH operation, for PhAC elimination; (3) optimized treatment validation under actual conditions with sensitive and selective analytical approaches; and (4) finally photoreactor modelling as a key tool to integrate the effect of temperature, PhAC concentration and radiation absorption in kinetics for a proper prediction of treated volume of water per unit of photoreactor surface and time.

Keywords Kinetic modelling, Photocatalysis, Photo-Fenton, Photoreactor, Solar treatment

1 Introduction

Most advanced oxidation processes (AOPs) are based on combination of oxidants (O_3/H_2O_2), oxidant and catalysts (Fe^{2+}/H_2O_2), oxidant and radiation (UV/H_2O_2) or radiation and catalysts (UV/TiO_2), although there are AOPs that combine all of the above with other processes [1]. One of the main disadvantages of these processes is their high energy consumption, mainly electrical, which makes them economically demanding. One of the strategies to reduce costs is their combination with other technologies, or the use of renewable energies, as solar radiation. In addition, advances in photoreactors, modelling, modes of operation and control strategies can reduce costs [2]. SAOPs are "near ambient temperature and pressure water treatment processes driven by solar energy which involve the generation of hydroxyl radicals in sufficient quantity to effective water purification", using an adaption of the pioneer definition made by [3].

Nowadays, there are two processes that can be carried out with solar energy: heterogeneous photocatalysis with semiconductors and homogeneous photocatalysis

through photo-Fenton-like processes. Both are catalytic, which represents a certain advantage by compensating the consumption of reagents as in other AOPs. In the case of heterogeneous photocatalysis, TiO_2 is, currently, the most effective catalyst, as is discussed in Sect. 2 of this chapter. In photo-Fenton-like processes, iron no longer acts based on its aquacomplexes, and H_2O_2 is even replaced by other oxidants [4], as is discussed in Sect. 3. In this sense, literature on SAOPs and their applications have experienced an exponential growth in recent years and today are remarkably active and productive research fields within the scope of environmental chemistry and engineering. Evidence of this productivity includes the large number of related research works published recently. For example, in between 2010 and 2019, a search of SAOPs topic on the Scopus database returned more than 13,500 entries. These numbers have been continuously increasing since 1996 (26 entries) up to >2,500 (2019). This demonstrates the worldwide interest in this research field with multiple applications including PhAC treatment in water.

SAOP technology and equipment have much in common with solar thermal applications. Consequently, reactors and other components of the photocatalytic plants initially followed the design of solar thermal collectors, such as parabolic trough collectors or non-concentrating collectors [5]. However, from the first developments, their non-feasibility was shown [6] due to the following issues: (1) the fluid must be exposed to solar UV radiation, so the absorber must be transparent, (2) high temperature (>50°C) must be avoided, and (3) SAOPs must capture only short wavelength radiation (UVA and part of visible radiation) to promote photoreactions. Note that the concentration factor, RC, is the ratio between the area of the collector and the absorber. Therefore, in SAOPs, $RC = 1$ is required. Finally, such collectors must be simple and inexpensive and work with little pressure drop. In this way, the use of tubular absorbers (photoreactors) has clear advantages, and they are available in different sizes and materials, being the most obvious choice in systems that work under pressure conditions.

SAOPs present special interest for degradation of PhACs in secondary effluents from municipal wastewater (WW) treatment plants (MWWTPs) as they can be easily adapted to comply with many of the 12 fundamental principles of green chemistry. Anastas and Eghbali [7] reviewed 10 years ago those principles in the work entitled "design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances". This definition and the concept of green chemistry were first formulated at the beginning of the 1990s [8]. SAOPs cover 9 of 12 fundamental principles of green chemistry: (1) prevent waste, (2) generate substances with little or no toxicity to human health and the environment, (3) do not use auxiliary substances (e.g. solvents, separation agents, etc.), (4) minimize energy requirements, (5) do not use derivatization steps, (6) are catalytic, (7) use chemicals that are recovered at the end of their function (catalysts) and they do not persist in the environment (or break down into innocuous degradation products as H_2O_2), (8) could be controlled by real-time monitoring and, finally, (9) are safe (near ambient temperature and pressure, not hazardous chemicals are used).

Since the pioneering work on SAOP cost comparison published by Bauer et al. [9], the studies related to SAOP costs are normally based on data obtained at

laboratory or pilot plant scale. Although the final goal is always the possible industrial implementation, little information is found about the cost involved at such a scale. There is a need for unification of cost assessment criteria that would help in the comparison of results obtained by different research groups as well as cost scaling-up [10]. Photo-Fenton system seems to be the one that provides the best reaction rates when treating WW containing organic contaminants, thus requiring photoreactors with lower volume and lower economic investments. Although the best performance of solar photo-Fenton process compared to solar heterogeneous photocatalysis might not be a universal rule, a huge body of published works clearly point towards the existence of faster reaction rates for the photo-Fenton system [2].

Muñoz et al. [11] compared solar photo-Fenton and heterogeneous photocatalytic processes by using Life Cycle Assessment (LCA) showing less environmental impact from the former. LCA is thus increasingly important to understand the environmental impact of implementing any SAOP and for comparing alternative SAOPs with different processes [12]. Indeed, LCA applied to SAOP in combination with other technologies could substantially help in taking decisions for the efficient removal of PhACs [13]. The first step in a LCA is the definition of the main goal (for instance, environmental impact related to each m^3 of treated secondary effluents from MWWTPs). The inventory analysis step involves data collection to quantify system inputs and outputs, that is, evaluation of raw materials, energy consumption and pollutant production. The proposed methodologies have been demonstrated to be valid tools for the analysis of cost and environmental consequences of SAOPs. This would also help to inform future policy development on PhACs and their elimination, which is currently in its infancy.

The efficiency of SAOPs for PhAC removal is deeply related to ambient conditions, as it depends on solar irradiance and operation temperature. Therefore, it is highly influenced by MWWTP location. In spite of their importance, the combined effect of these variables on SAOPs has not been explored yet though lately some interesting studies have been reported [14]. The feasibility of any SAOPs should be based on two different scaling-up strategies for each location: one based on the average ambient conditions and the other on the most unfavourable ambient conditions, always monthly based. Different photoreactor sizes, treatment costs and LCA would be obtained, being a not obvious decision.

Green chemistry constitutes a good framework for SAOPs, but the relative "greenness" cannot be evaluated solely based on compliance with these principles, due to their qualitative nature. LCA can be a better tool for environmental assessment. Results and conclusions obtained by means of streamlined LCA studies based exclusively on laboratory-derived data do not match correct conclusions. Detailed LCA studies applied to SAOPs require at least demonstration projects in order to supply enough quality data. Environmental impact of SAOPs increases in locations where less solar radiation is available, due to the progressive increase in solar collector area and water flow requirements. Therefore, the differences in environmental impact among the different SAOPs and with regard to non-solar AOP or conventional technology prevent from speaking about SAOPs as a group of "green" technologies for PhAC elimination, unless the discussion is on particular options for treating particular WWs.

2 Solar Semiconductor Photocatalysis

Photocatalysis is based on the use of semiconductors able to promote electrons from the valence band to the conduction band, by absorbing radiation of adequate wavelength and with the sufficient energy to overcome the semiconductor bandgap.

The beginning of modern photocatalysis could be the work of Fujishima and Honda [15], although titanium dioxide was used as an electrode and not as suspended particles. The objective of the work was to obtain hydrogen from water, which was the initial objective of photocatalysis with titanium dioxide during the 1970s and until the mid-1980s [16, 17]. Since then, the number of works on the subject has grown continuously. According to the Web of Science, introducing the keywords photocatalysis-TiO_2 and during the last 40 years (1980–2019), 27,011 works on the subject have been published. Using the keywords photocatalysis-TiO_2-drugs, the number of references is 1,265, and, delimiting with photocatalysis-TiO_2-solar-drugs, they are 301. All this indicates the impact of the topic focused on PhAC elimination. In addition to the papers, numerous books have been published, as an example the books of Ollis and Al-Ekabi [18] and Marcí and Palmisano [19] could be cited, both show the evolution of photocatalysis, and the last one is very recent, which indicates the topicality of the subject. Reviews also continue to appear allowing for an update and a retrospective view of the state of the art [20–22].

Titanium dioxide is one of the most used semiconductors in photocatalysis because it is cheap, stable and easy to obtain. Other semiconductors, such as zinc oxide, iron oxide, etc., were also tested, but they gave worse results and presented stability problems. A handicap of water photocatalysis for hydrogen production was the low yield. It was tried to solve the problem by doping the catalyst with different elements, mainly noble metals, favouring the charge separation and avoiding the electron/hole pair recombination.

At the end of the 1970s, photocatalysis began to be used for the treatment of inorganic contaminants [23], taking advantage of the electrons generated to reduce cations. The electrons in the conduction band can produce the photo-reduction (Eq. 1) of the compound (C) or the generation of radicals from oxygen (Eq. 2). The next step, almost immediately, was its use in the treatment of organic pollutants, taking advantage of the generation of hydroxyl radicals with a high oxidation potential [24, 25]. The holes generated in the valence band are responsible for the production of hydroxyl radicals (Eq. 3) or for the direct photo-oxidation (Eq. 4) of some compounds (C).

$$C + e^- \rightarrow C^{\cdot -} \tag{1}$$

$$O_2 + e^- \rightarrow O_2^{\cdot -} \tag{2}$$

$$H_2O + h^+ \rightarrow HO^\bullet + H^+ \tag{3}$$

$$C + h^+ \rightarrow C^{\bullet +} \quad (4)$$

Solar radiation was also early used in the elimination of contaminants by photocatalysis [26–29]. In the late 1990s and early this century, titanium dioxide was specifically applied for the abatement of different types of PhACs [30, 31], such as antibiotics (sulfamethoxazole, trimethoprim, etc.), β-blockers (propranolol, metoprolol, etc.), nonsteroidal anti-inflammatory PhACs-NSAIDs (diclofenac, naproxen, ibuprofen, etc.), analgesics (paracetamol, etc.), etc. The percentage of elimination depends on the type of contaminant and its functional groups. Likewise, attacks on the molecule occur primarily by hydroxyl radicals but also by other types of radicals and agents. In addition, it is interesting to determine and to eliminate the reaction intermediates formed since these may be more dangerous than the initial contaminant.

The elimination of PhACs has been studied by using different types of photoreactors with different geometries: annular, flat, cylindrical, tubular, etc. Different types of lamps have been employed, such as Xe lamps, used in solar simulators, since they have an emission spectrum very similar to solar in the ultraviolet range. BLB lamps, Hg UV lamps and other types of radiation have also been utilized. Natural sunlight as radiation source was also used in the photocatalytic degradation of PhACs with different types of reactors [32, 33]. Those that offered better yields are compound parabolic collectors (CPCs; see Figs. 1 and 2), since they present better sunlight capture taking profit of both direct and diffuse radiation. The percentage of pollutant removal depends a lot on the particular operating conditions: type of pollutant, initial concentration, TiO_2 concentration, reactor geometry, etc. In this regard, it should be noted that, as the catalyst concentration increases, the reaction rate increases but a value is reached (it can range between 0.2 and 1 g/L of TiO_2) from which the reaction rate does not increase any more since opacity and light-scattering phenomena occur.

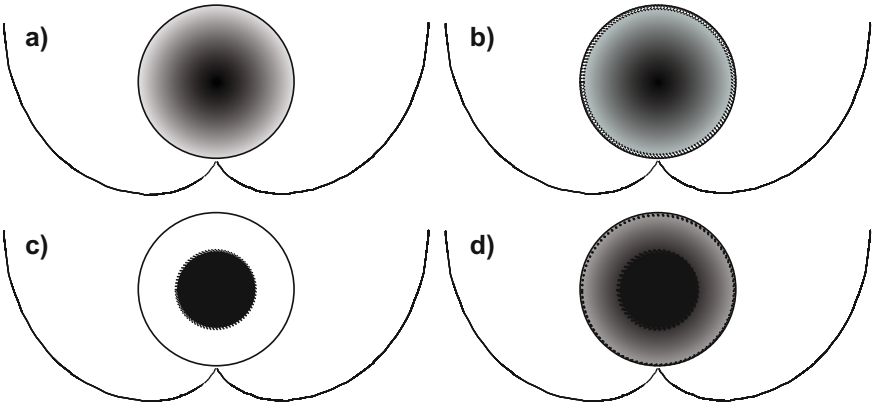


Fig. 1 View of different catalyst configurations in CPCs: (**a**) slurry, (**b**) supported on absorber wall, (**c**) supported on an inner tube, (**d**) supported on absorber wall and on an inner tube

Fig. 2 View of CPCs (**a**) and basic schematic concept of RPR (**b**)

The use of artificial light usually makes the process faster, but the electricity consumption makes treatment more expensive. From this point of view, solar reactors can make the process competitive since the cost of energy is zero, but obviously not the cost of fixed assets. Efficiency and costs can be calculated in different ways. One of them is the electric energy per order (E_{EO}, the electric energy in kWh required to degrade a contaminant C by one order of magnitude) or the electric energy per mass (E_{EM}, the electric energy in kWh required to bring about the degradation of a unit mass), both proposed by Prof. Bolton [34]. It is also possible to estimate kW/ppm, Euros/ppm, kW/L, Euro/L, etc. [12].

Titanium dioxide absorbs radiation in the UV band, which represents a very small percentage of the solar radiation that reaches the earth. In order to broaden the absorption spectrum, the catalyst has been doped with different elements (noble metals, B, N, etc.), although the improvement has not been very substantial in the majority of cases. On the other hand, the fact of working with doped catalysts makes the process more expensive and can generate problems of stability and loss of activity along treatment time.

The catalyst used as particles in suspension presents the problem of its separation from the reaction medium, although in some cases and depending on the particle size, it is possible to separate it by sedimentation. To avoid this problem, attempts have been made of working with the catalyst (TiO_2) supported on the reactor walls, deposited on balls with which the reactor was filled or on materials, such as graphene or other carbon products. This avoids the problem of sedimentation, but presents two additional problems: on the one hand, a worse use of radiation with respect to the catalyst in suspension and, on the other hand, the stability of the supported catalysts can be lower due to poor adhesion of TiO_2 on the support. Figure 1 shows different possible configurations of supported catalysts in CPCs (see also Fig. 2 for a general view) in comparison with a slurry, where radiation field is conceptually shown (as darker, less photons would be available).

The time required for the degradation of PhACs is dependent on the water characteristics, and it is often established through studies using the WW of interest.

The main causes why PhAC degradation rate diminishes through matrix effects are [35] (1) scavenging of hydroxyl radicals by bicarbonate, chloride, sulphate and other anions, producing lower oxidant potential radicals; (2) screening effect, when matrix components have light absorption at same wavelengths than catalyst; (3) turbidity, which avoids light transmission through the water; and (4) adsorption onto catalyst surface of some organic and inorganic species (e.g. NOM or phosphate).

A key issue for scaling-up is the modelling of photocatalytic processes with titania in suspension. It is a complex matter, since the reaction rate depends not only on conventional kinetic parameters but also on the radiation absorbed in the medium. Therefore, when modelling the system, the following parameters must be considered for semiconductor photocatalysis (more details could be found in Sect. 5, including modelling of homogeneous photocatalysis):

- Reaction medium: pH, concentration of reactants, adsorption of reactants on catalyst surface, intermediate concentration, final concentration of products, colour, temperature. In this way, it should be noted that actual WW treatment with TiO_2 could present problems because of the presence of organic matter, particles, etc., which represents a competition for light absorption and light-scattering problems, too.
- Catalyst: type of semiconductor-catalyst used, catalyst concentration, particles diameter, specific area, structure.
- Reactor: geometry (tubular, annular, cylindrical, etc.), dimensions (volume, diameter, height, etc.), radiation concentrators (geometry, location), location with respect to light and flow model.
- Fluid dynamics: feed flow rate, O_2 feed flow, mixing.
- Operation (batch, continuous and recirculation): volume, total experimental time, time and volume of samples, feed flow rate, air feed flow, recirculation flow.
- Radiation: power of radiation source, spectrum, radiation reaching the reactor, radiation entering the reactor, radiation absorbed, use and type of filters. Since light plays a fundamental role in the reaction rate, the influence of radiation on the kinetic equation must be introduced. Different models have been used to estimate the absorbed radiation, from analytical models to probabilistic models [36–38]. Radiation models will be widely explained in Sect. 5 of this chapter.

3 Degradation of PhACs by Solar Photo-Fenton Process

Among those AOPs successfully applied for the remediation of different types of WW, Fenton and photo-Fenton processes have shown to be highly efficient for the elimination and degradation of a wide range of contaminants and bio-recalcitrant materials [39]. Both have shown great potential to be commercialized and finally applied at industrial scale [40].

Fenton process is extensively covered in another chapter of this book, but briefly the mechanism is based in H_2O_2 decomposition by iron (II) at acidic pH [39]. In this

mechanism, the regeneration of iron (II) from iron (III) is considered the limiting step in the catalytic cycle, even more when the iron is present at low concentrations (Eqs. 5 and 6). In this sense, Fenton's reactions are clearly accelerated in the presence of light, due to the catalytic regeneration of iron (II). This process is known as photo-Fenton, occurring at wavelengths lower than 580 nm and so opening the possibility to be powered by solar energy as renewable irradiation source [1]. Precisely, a large amount of studies have been published on the use of solar UV radiation in the photo-Fenton process bringing out the potential on the reduction of electric energy consumption and increasing its environmental sustainability [2]. Another advantage of using solar radiation is that certain complexes which intervene in Fenton's reaction absorb UV light and part of the visible light of the solar spectrum, increasing the generation of hydroxyl radicals and so the efficiency of the process [41]. Iron (III) complexes suffer a ligand to metal charge transfer, provoking the dissociation in iron (II) and the oxidized ligand (Eq. 7) [39].

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + HO^{\bullet} \quad \left(K\tilde{7}0\ M/s\right) \tag{5}$$

$$Fe^{+3} + H_2O_2 \rightarrow Fe^{+2} + HO_2^{\bullet} + H^+ \quad \left(K\tilde{1} - 2 \times 10^{-2}\ M/s\right) \tag{6}$$

$$Fe^{+3}(L)_n + h\upsilon \rightarrow Fe^{+2}(L)_{n-1} + L_{ox}^{\bullet} \tag{7}$$

In this context, it is important to stress that the optimum pH to operate photo-Fenton process is 2.8, as, under this condition, precipitation of iron is avoided and the maximum generation of active species of iron is also assured ($FeOH^+$ and $Fe(OH)^{2+}$). At pH higher than 3, the reactivity of the system starts to decrease, and at values higher than 4, dissolved iron starts to precipitate as ferric hydroxide [42]. This issue can be clearly considered as one of the main challenges investigated by the scientific community in the last years, with the main objective of successfully applying the highly powerful photo-Fenton process for the elimination of PhACs and other microcontaminants. PhACs and their metabolites, detergents, personal care products, flame retardants, industrial additives, steroids and hormones are present at extremely low concentrations in, for instance, superficial waters, non-treated urban WWs and effluents of MWWTPs (between 50 and 150 μg/L) [43–45]. Special awareness is placed on PhACs as they have been discovered in almost all environmental water matrices including surface water (lakes, rivers, streams, estuaries and seawater), groundwater, WWTP effluents and influents and sludge. Even, they have been broadly detected in polar regions [46].

This global challenge must face important modifications in existing WWTP infrastructures, in which the integration of photo-Fenton as a technical and economical feasible process has become an important research focus from the green, environmental and sustainable point of view [47–49]. One of the main requirements in such investigation is the importance of performing photo-Fenton tertiary treatment at neutral pH, as addition of reagents for adjusting this parameter in urban WWs would provoke different economic and environmental disadvantages: increase in

effluent salinity, environmental impact and increasing treatment operating costs (including the generation of sludge) [50].

As it has been widely studied, the application of photo-Fenton process at neutral pH is considered a promising alternative, thanks to the use of iron complexing agents for maintaining iron in solution, as well as the possibility of being powered by solar irradiation as a renewable, abundant and pollution-free energy source [42, 51]. Nevertheless and from the operational point of view, it is always necessary to avoid the addition of high concentrations of such complexing agents, as an implicit increase in total organic carbon will occur, with the consequent reduction in the efficiency of the treatment as well as an increase in operating costs. In general, iron complexing agents commonly used are macromolecules with carboxylic and/or amino groups which absorb light in the UV-visible range suffering also self-photocatalytic degradation: oxalate [52], ferrioxalate [53], EDTA [50, 54], EDDS [55, 56], NTA [57], citrate [58–60] and humic acids [61]. Low biodegradability and toxicity of EDTA and the reduced pH range in which citric acid or oxalate could be used, as well as the high cost of EDDS, have pushed recent studies to evaluate the feasibility of using natural waters (containing polyphenolic compounds) [62] and agro-industrial residues [63] as complexing agents. Though their efficiency has been proved, it was demonstrated that the iron complexes generated are not stable enough showing activity only for a few minutes at the beginning of the process and so attaining low degradation efficiencies at circumneutral pH.

Nowadays, [S,S]-ethylenediamine-N,N′-disuccinic acid (EDDS) is one of the chelating agents most used in the application of solar photo-Fenton tertiary treatments to maintain iron in solution. In recent years, EDDS has gained the attention of researchers as a stable iron complexing agent, and Fe^{3+}-EDDS complex has been widely studied since the pioneering work of Gilles Mailhot group in France [54, 64]. In addition, EDDS is highly biodegradable [65], fulfilling the principle of environmental sustainability. As already mentioned, different parameters can influence the chelate-modified photo-Fenton process and the contaminants removing, including the type and load of chelating agents and reagents (iron, hydrogen peroxide, etc.), pH, applied energy, type of organic pollutant, water matrices, etc. [66, 67].

Looking for low-cost and more competitive commercial applications, the use of solar energy is being widely studied to perform photo-Fenton tertiary treatment for the elimination of PhACs. Thus, in locations with water scarcity problems and abundant solar radiation, the development of solar technologies for WW reusing looks as a plausible alternative water resource [14].

Solar photoreactors mostly tested and reported in literature for PhAC degradation in different water matrices are:

- Solar simulators at laboratory scale. In the last years, neutral pH is stated, thanks to chelating agents and different water matrices evaluated. However, many studies still deal with distilled water or natural water in which target PhACs are spiked and even low pHs applied (closer to optimal photo-Fenton pH). In addition, extremely high concentrations of PhACs are sometimes considered far

from those actually found in WWs [4, 68, 69]. Traditionally, best-operating conditions for solar photo-Fenton process performance are evaluated at laboratory scale in solar simulators (Xe lamps). Trovó et al. [60] studied the elimination of the antibiotic amoxicillin by solar photo-Fenton by using this methodology. In the last years, the degradation of PhAC contained in simulated and actual effluents at more realistic concentrations (even near neutral pH) is also tackled under solar simulation devices at laboratory scale [70–72].

- Compound parabolic collectors (CPCs). CPCs are static collectors with surface following an involute around a cylindrical reactor tube (made of borosilicate glass) placed in the axis of an aluminium reflector. CPCs can make efficient use of both direct and diffuse solar radiation, without the need for solar tracking. Most of literature on solar photo-Fenton applications, at pilot plant scale, has reported the use of CPC for microcontaminant removal, including PhACs [56, 73]. When industrial WWs (with high organic content) have to be treated by photo-Fenton, high concentrations of oxidant species (hydroxyl radicals and others) are required. Besides, many WWs must be confined without contact with the environment for a safe operation. In this sense, CPC photoreactors (Fig. 2a) have been demonstrated to be highly efficient for solar irradiation capture with adequate absorber tube diameter and iron concentration ([74]) and so must be selected as the best option. Nevertheless, increasingly studies are focused on synthetic or actual WW spiked with PhACs or even monitoring actually present PhACs by advanced analytical tools such as liquid chromatography coupled to mass spectrometry [75, 76]. The advanced analytical techniques are a very powerful tool to follow the degradation of PhACs, allowing detection and monitoring of the reaction intermediates produced.
- Raceway pond reactors (RPR). One of the main weaknesses of CPC photoreactors lays on their manufacture and amortization costs. But, when the objective is the elimination of PhACs present at concentrations at least thousand times lower than other organics in industrial WW, the process needs less concentration of hydroxyl radicals and, consequently, less iron concentration and solar irradiance density. In such a case, the possibility of using open photoreactors, as RPRs, is promising, because they are less efficient in photon capture but much more simple to manufacture and with lower costs (per surface unit). RPRs have been widely applied for microalgal mass culture [77, 78]. They consist of extensive reactors with channels in a closed loop, provided with a paddle wheel connected to an engine to recirculate the water (Fig. 2b). They can be designed and operated at different light path lengths (liquid depth can be varied), higher than CPC, therefore permitting lower iron concentration. Worse solar irradiation capture could be compensated by higher surface due to lower costs. Treating non-toxic superficial waters, non-treated urban WWs or effluents of MWWTPs permits to operate open photoreactors. Few published works have already demonstrated the efficiency of solar photo-Fenton at neutral pH in RPRs for the elimination of PhACs contained in different actual effluents of MWWTPs [79].

Combining technologies have also been demonstrated to be promising, though further research is still lacking in this topic. For instance, combination of Fenton with reverse osmosis reported removal rate up to 99% of amoxicillin according to Zhang et al. [57]. Nanofiltration membrane system followed by a mild solar photo-Fenton tertiary treatment for the elimination of PhAC has been also successfully reported by Miralles-Cuevas et al. [58, 59, 80]. Such published works showed how the preconcentration of microcontaminants (including PhACs) as well as the reduction of the total volume of water to be treated resulted in a significant reduction of reagent consumption and surface area of solar photoreactor, compared to the application of solar photo-Fenton alone. From the work published by Miralles-Cuevas et al., it was also concluded that the combination of NF membranes with solar photo-Fenton process improved total costs, though operating costs at neutral pH when using EDDS were higher than conventional photo-Fenton at pH 3, mainly due to the high cost of this chelating agent [81]. In addition, it has been confirmed that several transformation products could be more resistant than their original parent compounds. Therefore, the existence of transformation products must be essentially investigated after the treatment in order to evaluate the effectiveness of the solar photo-Fenton process [82–84].

Another important challenge lies on the proper selection of the technology to be applied for the elimination of PhACs. In this regard, it is always crucial to assess how environmentally effective is the proposed treatment in reducing ecotoxicity impact of such microcontaminants and if, by doing so, potential ecotoxicity could increase elsewhere or even cause other environmental impacts. This aspect is tackled by performing Life Cycle and Risk Assessments [13, 85–87].

4 Application of Advanced Analytical Techniques for Assessing the Efficiency of AOPs

Nowadays, it is well known that the application of conventional analytical techniques is insufficient for assessing the efficiency of AOPs in removing PhACs in WW. Dedicated methodologies, focused on the identification and monitoring of organic microcontaminants (OMCs), are required to provide a comprehensive evaluation of the treatment efficiency.

In any case, evaluating the behaviour of “all contaminants” present in WW is a difficult or rather impossible task, given the complexity of the samples; the low concentration at which these compounds are present, compared to other components of the matrix; and the large number of potential contaminants. Thus, most reported degradation studies focus on a limited group of compounds.

Test protocols generally include adding the selected OMCs at high concentrations in simulated or actual WW and their monitoring by high-performance liquid chromatography (HPLC) with classical detectors (UV or DAD) during the treatments. These tests are very useful as preliminary studies and can be used for the initial

optimization of treatment parameters (type and concentration of reagents, treatment times, etc.) or even for comparison purposes, to determine the most suitable treatment for a certain application. As an example, Klamerth et al. [88] selected 15 OMCs as model compounds to optimize a modified photo-Fenton treatment at neutral pH by using EDDS. Selected compounds included PhACs representing various therapeutic classes, such as antibiotics (flumequine, ofloxacin, sulfamethoxazole), analgesic and antipyretic drugs (acetaminophen, ibuprofen, antipyrine), nonsteroidal anti-inflammatory drugs (ketorolac, diclofenac), antiepileptic drugs (carbamazepine), hormones (progesterone), psychoactive drugs (caffeine) and also personal care products (triclosan) and pesticides (atrazine, isoproturon, hydroxybiphenyl). All of them provide a reliable response in a conventional UPLC-UV/DAD system, with limits of detection and quantification from 1.5 to 10 μg/L. MWWTP effluents were spiked at two concentration levels (100 and 15 μg/L), and the concentration profile of each compound was monitored by recording the UV signal for each compound at the wavelength of maximum absorption. Experiments with the highest concentration level were monitored by direct injection of samples into the UPLC-UV/DAD system, thus providing a simple, inexpensive and rapid evaluation of results. When the lowest concentration level was selected, preconcentration of the samples (50-fold) was required to obtain a useful OMC transformation profile along the treatment.

Despite the interest of this approach, validation of the optimized treatments under actual concentration levels and operation conditions is required, since, in general, degradation percentages depend on the type and concentration of compounds present in WW. To provide a comprehensive assessment of a treatment process in real WW, the use of more sensitive and selective analytical approaches is required. Gas chromatography (GC) and HPLC coupled to mass spectrometry (MS) represent the best choice. HPLC-MS is mostly applied in PhAC determination due to its versatility and wide scope of analysis, extended to thermolabile, highly polar or with limited volatility compounds. GC-MS is reserved only for volatile and non-polar compounds, in order to avoid the application of derivatization processes, required to improve detectability of more polar and less volatile compounds [89]. Derivatization is a time-consuming technique, prone to analyte losses and non-recommended in multiresidue analysis. Therefore, HPLC or, more recently, ultra-high-performance liquid chromatography (UHPLC), which provides higher resolution and shorter analysis times, has become the preferred advanced technique for multiclass PhAC analysis.

Another advantage associated to the use of LC-based methods is the possibility of using direct sample injection. Direct injection (DI) technique is becoming a popular option in the analysis of environmental matrices due to its inherent advantages, such as (1) reduction of solvent consumption, in line with fundamental principles of green chemistry, (2) analysis time reduction and (3) lower cost, compared to the use of conventional extraction procedures, such as liquid-liquid extraction (LLE) or solid-phase extraction (SPE). This technique also increases sample throughput, eliminates analyte losses associated with the extraction procedures and provides adequate reproducibility and low sample contamination. Campos-Mañas et al. [90] developed

a DI-based analytical method to be applied in the analysis of 115 OMCs (including PhACs) in WW effluents. Sample pre-treatment was reduced to acetonitrile addition and filtration of the mixture previous to HPLC-MS analysis. 92% of the studied compounds showed limits of quantification $\leq$100 ng/L and mean recoveries in the range of 70–120%, with RSD values $\leq$20%. Up to 67 compounds could be determined in actual WW effluent samples at concentrations ranging from 10 ng/L to 26 μg/L, thus demonstrating the applicability of the method. This strategy was also successfully applied to validate, in realistic and variable conditions, a solar photo-Fenton process at neutral pH with Fe^{3+}-EDDS [79]. The treatment was applied to five WW effluents of diverse origin and composition. The removal of more than 80% of total load of PhACs and other OMCs (from 18 to 45 compounds) could be monitored, and the efficiency of the photo-Fenton process to treat effluents of very different physicochemical composition was demonstrated.

Although DI has indisputable advantages and allows a rapid and inexpensive monitoring of PhACs along the treatments, an enrichment step may still be required to evaluate the reaction kinetics at very low concentrations, mainly at the end of treatment, when most compounds have reached maximum degradation. In this case, SPE is the recommended sample preparation technique, due to its ease of use, low solvent consumption and great availability of sorbent materials, which facilitate the adaptation of the technique to multiple applications. In multiresidue analysis, polymeric sorbents, such as Isolute ENV+, Strata-X or more frequently Oasis HLB material, represent the best choice and provide high capacity and retention of a broad class of compounds in a wide range of polarities. Other alternatives, such as mixed-mode polymeric sorbents, which improve selectivity and retention of ionic compounds, or highly specific molecularly imprinted polymers (MIP), have also been applied to improve the selectivity of the methods, but their use is restricted to a limited number of selected compounds.

A typical multiclass SPE procedure involves a previous filtration of the sample by a 0.7 μm glass fibre filter, to remove suspended solids, and a pH adjustment, typically at pH 7–8 as a compromise solution, although some more acidic PhACs, such as some antibiotics, may require lower pH for proper recovery [91]. Final elution of retained compounds is usually accomplished with methanol.

After pre-treatment step, identification and quantification of pharmaceuticals in WW samples is usually performed by MS techniques. Modern MS systems using hybrid triple quadrupole (QqQ-MS) or quadrupole linear ion trap (QqLIT-MS) analysers allow very low detection limits to be reached when operating in MRM (multiple reaction monitoring) mode, and they represent, without any doubt, the best choice for target quantitative analysis. Despite the excellent performance characteristics of these systems, matrix effects involving ion suppression or enhancement are frequent, contributing to a significant degradation of the analytical accuracy and reproducibility. Electrospray ionization sources, which are routinely used in WW analysis, due to its suitability for polar and ionic compounds determination, are particularly prone to matrix effects. To avoid or minimize matrix effects, several strategies have been proposed, such as improvement on clean-up protocols or HPLC separations, matrix dilution, use of standard addition method, external matrix

matched standards, analogue-structure internal standards or isotopically labeled standards [92]. The latter represent the more robust approach, but the cost and availability of the standards limit their extensive use.

Although most degradation studies focus on the quantification and monitoring of selected compounds originally present in raw WW, one aspect that is becoming increasingly relevant concerns the identification of transformation products (TPs), generated during the treatments. It is well known that the absence of parent contaminants does not guarantee the good quality of treated water, and WW treatments must consider the formation of TPs to minimize the environmental impact of the effluents. However, the identification and structure proposal of a priori unknown compounds entail great difficulty, and in many cases, only a tentative proposal can be accepted, as the analytical reference standards of such TPs are not always available for confirmation [93]. The analytical techniques proposed for quantitative analysis of target compounds are inefficient in TP identification, and the application of high-resolution mass spectrometry (HR-MS) is required due to its high confirmatory capabilities, derived from the high resolving power and the mass accuracy in MS and MS/MS modes. Time-of-flight (TOF) and Orbitrap analysers, or more frequently their hybrid configurations (QTOF, QOrbitrap), represent the best choice for this type of analysis. MS/MS fragmentation data is used for structure elucidation, as spectral databases are not useful in most cases. Previous knowledge of the most common transformations taking place in the different treatments, the structural similarity with the precursor and the information on the elemental composition of the TPs candidates, provided by the mass spectrometer, are essential in the proposal of their structures.

As a first approach, assays for the identification of TPs must be carried out individually for each compound, in distilled water and at relatively high concentrations of the selected contaminant, in order to obtain detectable concentrations of TPs without applying any preconcentration step, thus avoiding losses of the more polar TPs. Samples are then analysed by HR-MS. The use of suspect screening strategies has been recently proposed to facilitate the TP detection and identification [94]. Suspect analysis consists of the creation of searching lists containing the monoisotopic masses of tentative TPs, collected from literature or by using computational (in silico) prediction tools, such as PathPred [95] or EAWAG-BBD pathway prediction system [96]. The exact masses of the predicted TPs are extracted from the chromatograms, and a series of confirmation criteria, based on the plausibility of the retention time, isotopic pattern and observed fragmentation pattern, are applied to confirm the proposed structure. Jaén-Gil et al. [97] have successfully applied this strategy to the identification of the TPs of metoprolol and its metabolite metoprolol acid in UV/H_2O_2-treated WWs, identifying 24 TPs with potential ecotoxicological implications. Once the TPs are identified and the transformation pathway is proposed, the formation of the reported TP can be investigated under more realistic conditions.

As a final remark, it can be said that the monitoring of PhACs, other OMCs and their TPs during WW treatments is essential to evaluate their efficiency and guarantee the quality and safety of treated water. There are several analytical strategies available, and their selection will be based on the objective of the analysis. Figure 3 summarizes the most frequently used analytical procedures and techniques.

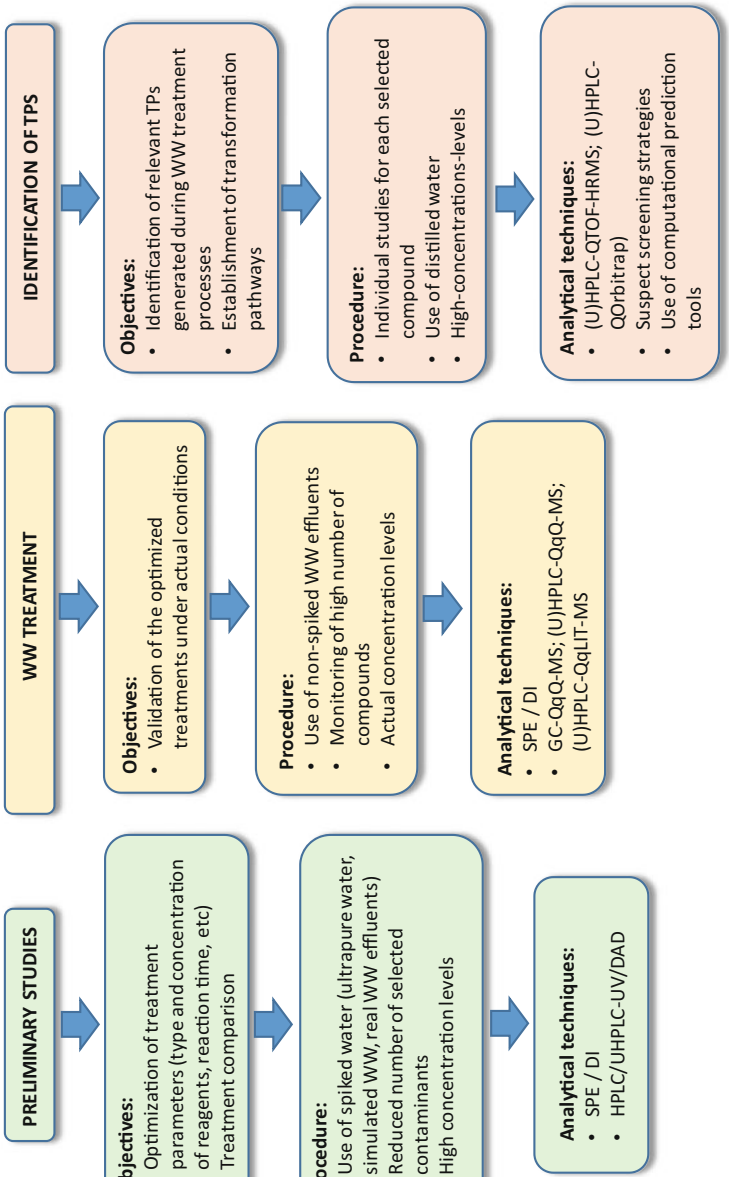


Fig. 3 Flow diagram of procedures and analytical strategies applied in the degradation studies of PACs in WW

5 Modelling of Solar Photoreactors

Reactor modelling starts with accurate kinetic models. In addition to temperature and reactant concentrations, light distribution inside the reactor and photon absorption phenomena play an essential role on the modelling solar photoreactors. Difficulty is higher when considering the myriad of reactions taking place simultaneously in radical chain mechanisms as happens in AOPs. In this regard, different approaches have been considered from empirical to mechanistic models. The former approach uses a mathematical function to fit the response of the main variables to the factors causing changes in the system. The latter uses rate law equations and kinetic parameters. Among SAOPs for PhAC removal in complex media, such as MWWTP secondary effluents, homogeneous photo-Fenton process has several advantages with regard to heterogeneous TiO_2 photocatalysis. Photo-Fenton process yields higher reaction rates, and fouling on catalyst surface is not a problem. Conversely, it is strongly dependent on pH. Most of literature about modelling of solar photoreactors deals with photo-Fenton, at both acidic and neutral pH.

As for the empirical approach, response surface methodology and neural network models provide valuable information on PhAC degradation in a selected experimental framework. Schenone et al. [98] applied a three-level factorial experimental design to model the photo-Fenton degradation of the herbicide 2,4-dichlorophenoxyacetic acid at circumneutral pH with the ferrioxalate complex. Rivas et al. [99] used a Langmuir-Hinshelwood-type model to predict apparent first-order rate constant for the removal of the neonicotinoid pesticide acetamiprid as well as H_2O_2 consumption, by solar photo-Fenton at acidic pH in a raceway pond reactor. Giannakis et al. [100] developed second-degree models for the removal of the PhAC venlafaxine by photo-Fenton by using the response surface methodology (3 pH-specific central composite designs). The models were used as input for the desirability functions to obtain the optimal regions to operate the photo-Fenton process.

These empirical models provide mathematical expressions useful for reactor design in the experimental range, but their main drawback is that they do not provide information about the reaction mechanism. In addition, they cannot be applied globally and consequently extrapolation is not possible.

To overcome these limitations, attention is paid to mechanistic models. To this end, the knowledge of photoreactors' solar field is crucial. To determine the radiation field, the radiative transfer equation (RTE) must be solved. It describes the radiation intensity at any position along a ray pathway through the reaction bulk. In homogeneous systems (without scattering), the photon emission by the absorbing species can be neglected, and the RTE is defined by Eq. (8) [101], where I_λ is the spectral specific intensity; Ω and s denote the direction and the distance of photon transport, respectively; and κ_λ is the volumetric absorption coefficient.

$$\frac{dI_\lambda(s,\Omega,t)}{ds} = -\kappa_\lambda(s,t)\, I_\lambda(s,\Omega,t) \tag{8}$$

In a homogeneous medium, the change in the radiation intensity along the ray trajectory is only due to the absorption process in the reaction bulk. This variation can be expressed by an equation that involves the volumetric absorption coefficient. This coefficient is function of the wavelength and variables such as temperature and composition. The absorption coefficient could be determined accurately by studying the photon absorption by atoms or molecules exposed to a radiation field. Macroscopically, the volumetric absorption coefficient is linearly dependent on the concentration of absorbing species. In Eq. (9) [101] i denotes each absorbing species, $\kappa_{T,\lambda}$ is the volumetric absorption coefficient, $\alpha_{\lambda,i}$ is the molar absorptivity, and C_i stands for the molar concentration of the absorbing species.

$$\kappa_{\lambda,i} = \alpha_{\lambda,i} C_i \tag{9}$$

The local volumetric rate of photon absorption (LVRPA) represents the amount of photons absorbed per unit of time and reaction volume. It depends on the photon source, the concentration of absorbing species, the optical properties of the system and the reactor geometry. Although it is defined for monochromatic radiation, it can be extended to polychromatic sources by integrating over the wavelength range. When working under solar UV radiation, LVRPA values corresponding to the direct and diffuse radiation are calculated in a different way. In homogeneous systems, in which radiation can be modelled with one spatial coordinate, the monochromatic LVRPA corresponding to the absorption of direct radiation can be calculated by Eq. (10) [102], where $q_{w,\lambda}$ is the discretized incident radiation, κ_λ is the volumetric absorption coefficient of the absorbing species and $\kappa_{T,\lambda}$ is the volumetric absorption coefficient of the medium.

$$e^a_\lambda(x,t) = q_{w,\lambda}\kappa_\lambda(t)\exp\left[-\kappa_{T,\lambda}(t)x\right] \tag{10}$$

The concept of diffuse radiation absorption is more complex. Brandi et al. [103] applied the hypothesis of azimuthal symmetry (rays propagate in one spatial coordinate (x) and one angular coordinate (θ)) to model the absorption of diffuse radiation in a simple lab-scale photoreactor. In this case, RTE for homogeneous systems can be defined by Eq. (11) [103]. Integrating Eq. (11) with the boundary conditions corresponding to photons moving both forward and backward, the diffuse LVRPA can be calculated by Eq. (12):

$$\cos\theta \frac{dI_{\lambda,\Omega}(x,t)}{dx} + \kappa_\lambda(x,t) I_{\lambda,\Omega}(x,t) = 0 \tag{11}$$

$$e_{\lambda}^{a}(x,t) = 2\Pi I_{\lambda}^{0}\kappa_{\lambda}(t)\int_{-\frac{\Pi}{2}}^{\frac{\Pi}{2}} \exp\left[-\frac{\kappa_{\lambda}(t)}{cos\theta}x\right] \text{sen}\theta d\theta \tag{12}$$

$$e^{a}(x,t) = \int_{\lambda\,\min}^{\lambda\,\max} e_{\lambda}^{a}(x,t)d\lambda \tag{13}$$

$$\langle e^{a}(x,t)\rangle_{V_R} = \frac{1}{L_R}\int_{0}^{L_R} e^{a}(x,t)dx \tag{14}$$

As mentioned above, LVRPA is defined for monochromatic radiation. For solar radiation (polychromatic radiation), LVRPA can be computed by Eq. (13). Furthermore, in perfectly mixed reactors with constant cross-sectional under photolimitation conditions (the reaction rate linearly depends on the photon absorption), LVRPA can be averaged across the reactor volume giving the VRPA, Eq. (14) [103], where L_R and V_R stand for reactor length and reactor volume, respectively.

For reported kinetic models for PhAC removal by photo-Fenton process at acidic pH taking into account the VRPA, Andreozzi et al. [104] studied the oxidation of an aromatic heterocyclic compound, benzothiazole, taking into account 21 reactions. The model successfully predicted the influence of H_2O_2, Fe^{3+} and sulphate concentrations on the system reactivity. Conte et al. [105] proposed a kinetic model of 15 reactions for the degradation of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) including the effects of iron concentration, H_2O_2:2,4-D initial concentration ratio, temperature and radiation level. Cabrera Reina et al. [106] proposed a simplified kinetic model of nine reactions for the mineralization of the PhAC paracetamol in raceway pond reactors, and the effect of the VRPA and the liquid depth were included. With the same PhAC, Audino et al. [107] developed a kinetic model in an annular photoreactor computing the VRPA. These studies were carried out to remove organic contaminants in the concentration range of mg/L. Nonetheless, most of PhACs appear in WW at concentrations in the μg/L range. In this regard, a mechanistic model consisting of a set of only eight reactions was proposed for the removal of 100 μg/L of acetamiprid in synthetic MWWTP secondary effluent, taking into account the effect of temperature and photon absorption. The model was validated in raceway pond reactors of 5 and 15 cm of liquid depth (120 and 360 L capacity) under natural sunlight [108]. The model predicted that reaction rate linearly increases with the VRPA up to VRPA values of 866 μEinstein/m^3/s and at higher values the process became photosaturated.

Regarding photo-Fenton process operated at neutral pH, chelating agents are used to keep iron in solution as mentioned before. For instance, Conte et al. [109] studied the influence of different inorganic anions on ferrioxalate complex photo-activity and proposed a kinetic model for the removal of 2,4-dichlorophenoxyacetic acid at circumneutral pH, including the hydroxyl radical scavenging effect of anions. In the case of using EDDS as iron complexing agent, the higher absorptivity of Fe^{3+}-EDDS in comparison with Fe^{3+} makes photosaturation of the reaction rate to take place at VRPA values higher than 1,547 μEinstein/m^3/s pointing out the relevant role of light absorbing species in the photo-Fenton kinetics. In particular, a new mechanistic

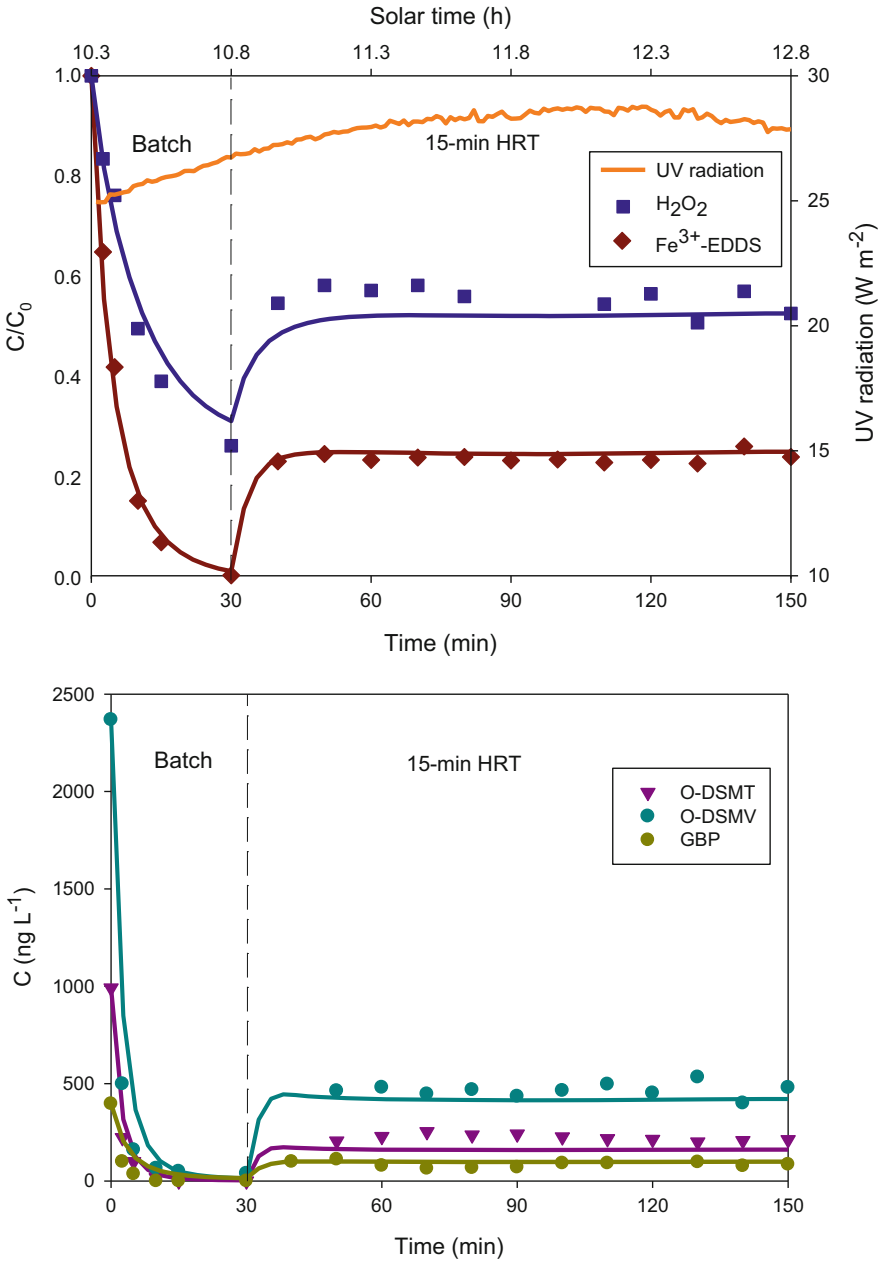


Fig. 4 Model predictions of the performance of a 5-cm-deep RPR operated in continuous flow mode at 15 min HRT to treat MWWTP secondary effluent containing PhACs (O-desmethyltramadol (O-DSMT), O-desmethylvenlafaxine (O-DSMV), gabapentin (GBP)) by solar photo-Fenton with 0.1 mM Fe^{3+}-EDDS and 0.88 mM H_2O_2. The reactor started in batch mode for 30 min and then continuous flow lasted for 120 min. Solid lines represent model predictions and points, experimental data

model of 18 reactions has been reported for Fe^{3+}-EDDS system, following the removal of the 3 most abundant PhACs detected in 5 different actual secondary MWWTP effluents (gabapentin, *O*-desmethyltramadol and *O*-desmethylvenlafaxine). The model constitutes a tool for the design of continuous raceway pond reactors (RPRs) for PhAC removal by solar photo-Fenton process [110]. The treatment capacity of the RPR in terms of volume of water treated per unit of reactor surface and time was successfully predicted as a function of the liquid depth and the hydraulic residence time (HRT), taking into account the availability of solar UV radiation. Figure 4 shows the model predictions compared with the experimental data during the treatment of an actual MWWTP secondary effluent in a 5-cm-deep RPR at pilot plant scale. For instance, the reactor's surface required to treat 100 m^3/day can be calculated as follows: at 15 min of HRT, the treatment capacity would be 600 L/m^2/day for 6 h of continuous flow operation per day. The ratio 100/0.6 gives a reactor surface of 167 m^2. The RPR volume for 5 cm of liquid depth is 8.35 m^3.

6 Concluding Remarks

Application of SAOPs to PhAC elimination in MWWTP secondary effluents must procure beneficial impact on the environment, public health and a greener economy. However, some barriers still need to be overcome, such as the design of new photoreactors specifically for the degradation of contaminants present in the range of micro-gramme per litre; optimization of operating conditions, in particular, solar radiation-catalyst interaction; and long-term reliability of solar operation. Industrial and market acceptance of SAOPs will increase considerably if some of these issues are correctly addressed in the near future. Total costs of best available technologies (BAT), already applied at actual scale in a few countries for PhAC elimination from treated MWWTP secondary effluents, have been informed to range from 0.10 to 0.15 €/m^3 for ~50,000 population equivalent. Capitalized investment-related and operation costs, for the given life cycle, are stated by Rizzo et al. [48], decreasing further with increasing plant size. Therefore, development of SAOPs for PhAC removal should consider these figures to gain a market niche.

Elimination of PhACs in large water volumes needs mild operating conditions (low catalyst and oxidant concentrations). Therefore, environmental impact evaluation (by Life Cycle Assessment) is always crucial for a proper selection of the technology to be applied. For that, validation of the optimized treatments at real conditions using advanced analytical approaches based on mass spectrometry and confident (reproducible) kinetic models are needed.

Acknowledgements I. Oller and S. Malato wish to thank the Spanish Ministry of Economy and Competitiveness for funding under the ECOSAFEFARMING Project (International Joint Programming Actions, reference: PCIN-2017-005) and 2016 Water and FACCE JPIs Joint Call. J. Giménez wish to thank the Spanish Ministry of Economy and Competitiveness (project CTQ2017-86466-R,

MINECO/FEDER, UE) and AGAUR-Generalitat de Catalunya (project 2017SGR-131) for funds received. A. Agüera and J.A. Sánchez Pérez wish to thank contribution of the LIFE ULISES project funded by the European Union under the LIFE Financial Programme Grant Agreement No. LIFE18 ENV/ES/000165.

References

1. Gogate PR, Pandit AB (2004) A review of imperative technologies for WW treatment I: oxidation technologies at ambient conditions. Adv Environ Res 8:501–551. https://doi.org/10.1016/S1093-0191(03)00032-7
2. Malato S, Fernández-Ibáñez P, Maldonado MI, Blanco J, Gernjak W (2009) Decontamination and disinfection of water by solar photocatalysis: recent overview and trends. Catal Today 147:1–59. https://doi.org/10.1016/j.cattod.2009.06.018
3. Glaze W, Kang JW, Chapin DH (1987) The chemistry of water treatment processes involving ozone, hydrogen peroxide and ultraviolet radiation. Ozone Sci Eng 9:335–352. https://doi.org/10.1080/01919518708552148
4. Ahmed MM, Chiron S (2014) Solar photo-Fenton like using persulphate for carbamazepine removal from domestic WW. Water Res 48:229–236. https://doi.org/10.1016/j.watres.2013.09.033
5. Dillert R, Cassano AE, Goslich R, Bahnemann D (1999) Large scale studies in solar catalytic WW treatment. Catal Today 54:267–282
6. Malato S, Blanco J, Vidal A, Richter C (2002) Photocatalysis with solar energy at a pilot-plant scale: an overview. Appl Catal B Environ 37:1–15
7. Anastas P, Eghbali N (2010) Green chemistry: principles and practice. Chem Soc Rev 39:301–312. https://doi.org/10.1039/b918763b
8. Collins TJ (1997) In green chemistry, Macmillan encyclopedia of chemistry, vol 2. Simon and Schuster, Macmillan, New York, pp 691–697
9. Bauer R, Waldner G, Fallmann H, Hager S, Klare M, Krutzler T (1999) The photo-Fenton reaction and the TiO2/UV process for waste water treatment-novel developments. Catal Today 53:131–144
10. Giménez J, Esplugas S, Malato S, Peral J (2019) Economic assessment and possible industrial application of a (photo) catalytic process: a case study. In: Marci G, Palmisano L (eds) Heterogeneous photocatalysis: relationships with heterogeneous catalysis and perspectives. Elsevier, Amsterdam, pp 235–267
11. Muñoz I, Peral J, Ayllón JA, Malato S, Passarinho P, Domènech X (2006) Life cycle assessment of a coupled solar photocatalytic-biological process for WW treatment. Water Res 40:3533–3540
12. Giménez J, Bayarri B, González O, Malato S, Peral J, Esplugas S (2015) Advanced oxidation processes at laboratory scale: environmental and economic impacts. ACS Sustain Chem Eng 3:3188–3196. https://doi.org/10.1021/acssuschemeng.5b00778
13. Gallego-Schmid A, Tarpani RRZ, Miralles-Cuevas S, Cabrera-Reina A, Malato S, Azapagic A (2020) Environmental assessment of solar photo-Fenton processes in combination with nanofiltration for the removal of micro-contaminants from real. Sci Total Environ 650:2210–2220. https://doi.org/10.1016/j.scitotenv.2018.09.361
14. Cabrera Reina A, Miralles-Cuevas S, Cornejo L, Pomares L, Polo J, Oller I, Malato S (2020) The influence of location on solar photo-Fenton: process performance, photoreactor scaling-up and treatment cost. Renew Energy 145:1890–1900
15. Fujishima A, Honda K (1972) Electrochemical photolysis of water at a semiconductor electrode. Nature 238:37–38

16. Borgarello E, Kiwi J, Pelizzetti E, Visca M, Grätzel M (1981) Photochemical cleavage of water by photocatalysis. Nature 289(5794):158–160
17. Herrmann JM, Disdier J, Mozzanega MN, Pichat P (1979) Heterogeneous photocatalysis: in situ photoconductivity study of TiO2 during oxidation of isobutane into acetone. J Catal 60 (3):369–377
18. Ollis DF, Al-Ekabi H (eds) (1993) Photocatalytic purification and treatment of water and air. Elsevier, New York. https://doi.org/10.1016/j.apcatb.2012.05.036
19. Marcí G, Palmisano L (eds) (2019) Heterogeneous photocatalysis. Relationships with homogeneous catalysis and perspectives. Elsevier, Amsterdam
20. Awfa D, Ateia M, Johnson MS, Yoshimura C (2018) Photodegradation of pharmaceuticals and personal care products in water treatment using carbonaceous-TiO2 composites: a critical review of recent literature. Water Res 142:26–45. https://doi.org/10.1016/j.watres.2018.05.036
21. Fagan R, McCormack DE, Dionysiou DD, Pillai SC (2016) A review of solar and visible light active TiO2 photocatalysis for treating bacteria, cyanotoxins and contaminants of emerging concern. Mater Sci Semicond Process 42:2–14. https://doi.org/10.1016/j.mssp.2015.07.052
22. Kumar A, Khan M, He J, Lo IMC (2020) Recent developments and challenges in practical application of visible-light-driven TiO2-based heterojunctions for PPCP degradation: a critical review. Water Res 170:115356. https://doi.org/10.1016/j.watres.2019.115356
23. Frank SN, Bard AJ (1977) Heterogeneous Photocatalytic oxidation of cyanide and sulfite in aqueous solutions at semiconductor powders. J Phys Chem 81(15):1484–1488
24. Fujihira M, Satoh Y, Osa T (1981) Heterogeneous photocatalytic oxidation of aromatic compounds on TiO2. Nature 293(5829):206–208
25. Ollis DF, Pelizzetti E, Serpone N (1991) Photocatalyzed destruction of water contaminants. Environ Sci Technol 25(9):1522–1529
26. Curcó D, Giménez J, Marco P (1996) Photocatalytic degradation of phenol: comparison between pilot plant scale and laboratory results. Sol Energy 56(5):387–400
27. Malato S, Blanco J, Richter C, Curcó D, Giménez J (1997) Low-concentrating CPC collectors for photocatalytic water detoxification. Comparison with a medium concentrating solar collector. Water Sci Technol 35(4):157–164
28. Matthews RW, McEvoy SR (1992) Destruction of phenol in water with sun, sand, and photocatalysis. Sol Energy 49(6):507–513
29. Pelizzetti E, Carlin V, Minero C, Pramauro E, Vincenti M (1992) Degradation pathways of atrazine under solar light and in the presence of TiO2 colloidal particles. Sci Total Environ 123–124:161–169
30. Calza P, Sakkas VA, Medana C, Baiocchi C, Dimou A, Pelizzetti E, Albanis T (2006) Photocatalytic degradation study of diclofenac over aqueous TiO2 suspensions. Appl Catal B Environ 67:197–205. https://doi.org/10.1016/j.apcatb.2006.04.021
31. Molinari R, Pirillo F, Loddo V, Palmisano L (2006) Heterogeneous photocatalytic degradation of pharmaceuticals in water by using polycrystalline TiO2 and a nanofiltration membrane reactor. Catal Today 118:205–213. https://doi.org/10.1016/j.cattod.2005.11.091
32. Méndez-Arriaga F, Maldonado MI, Giménez J, Esplugas S, Malato S (2009) Abatement of ibuprofen by solar photocatalysis process: enhancement and scale up. Catal Today 144:112–116. https://doi.org/10.1016/j.cattod.2009.01.028
33. Pérez-Estrada L, Malato S, Agüera A, Fernández-Alba AR (2007) Degradation of dipyrone and its main intermediates by solar AOPs identification of intermediate products and toxicity assessment. Catal Today 129:207–214. https://doi.org/10.1016/j.cattod.2007.08.008
34. Bolton JR, Bircher KG, Tumas W, Tolman CA (2001) Figures-of-merit for the technical development and application of advanced oxidation technologies for both electric- and solar-driven systems (IUPAC technical report). Pure Appl Chem 73(4):627–637
35. Lado Ribeiro AR, Moreira NFF, Li Puma G, Silva AMT (2019) Impact of water matrix on the removal of micropollutants by advanced oxidation technologies. Chem Eng J 363:155–173. https://doi.org/10.1016/j.cej.2019.01.080

36. Acosta-Herazo R, Monterroza-Romero J, Mueses MA, Machuca-Martinez F, Li Puma G (2016) Coupling the six flux absorption-scattering model to the Henyey-Greenstein scattering phase function: evaluation and optimization of radiation absorption in solar heterogeneous photoreactors. Chem Eng J 302:86–96. https://doi.org/10.1016/j.cej.2016.04.127
37. Cassano AE, Alfano OM (2000) Reaction engineering of suspended solid heterogeneous photocatalytic reactors. Catal Today 58(2–3):167–197. https://doi.org/10.1016/S0920-5861(00)00251-0
38. Curcó D, Giménez J, Addardak A, Cervera-March S, Esplugas S (2002) Effects of radiation absorption and catalyst concentration on the photocatalytic degradation of pollutants. Catal Today 76:177–188. https://doi.org/10.1016/S0920-5861(02)00217-1
39. Pignatello JJ, Oliveros E, MacKay A (2006) Advanced oxidation processes for organic contaminant destruction based on the Fenton reaction and related chemistry. Crit Rev Environ Sci Technol 36:1–84. https://doi.org/10.1080/10643380500326564
40. Gupta VK, Ali I, Saleh TA, Nayak A, Agarwal S (2012) Chemical treatment technologies for waste-water recycling-an overview. RSC Adv 2:6380–6388. https://doi.org/10.1039/C2RA20340E
41. Oppenländer T (2003) Photochemical purification of water and air: advanced oxidation processes (AOPs)-principles, reaction mechanisms, reactor concepts. Wiley, Weinheim. https://doi.org/10.1002/9783527610884
42. Clarizia L, Russo D, di Somma I, Marotta R, Andreozzi R (2017) Homogeneous photo-Fenton processes at near neutral pH: a review. Appl Catal B Environ 209:358–371. https://doi.org/10.1016/j.apcatb.2017.03.011
43. Bui XT, Vo TPT, Ngo HH, Guo WS, Nguyen TT (2016) Multicriteria assessment of advanced treatment technologies for micropollutants removal at large-scale applications. Sci Total Environ 563-564:1050–1067. https://doi.org/10.1016/j.scitotenv.2016.04.191
44. Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S, Wang XC (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during WW treatment. Sci Total Environ 473:619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065
45. Pal A, Gin KY-H, Lin AY-C, Reinhard M (2010) Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. Sci Total Environ 408:6062–6069. https://doi.org/10.1016/j.scitotenv.2010.09.026
46. Patel M, Kumar R, Kishor K, Mlsna T, Pittman CUJ, Mohan D (2019) Pharmaceuticals of Emerging Concern in aquatic systems: chemistry, occurrence, effects, and removal methods. Chem Rev 119:3510–3673. https://doi.org/10.1021/acs.chemrev.8b00299
47. Alharbi SK, Price WE (2017) Degradation and fate of pharmaceutically active contaminants by advanced oxidation processes. Curr Pollut Rep 3:268–280. https://doi.org/10.1007/s40726-017-0072-6
48. Rizzo L, Malato S, Antakyali D, Beretsou VG, Đolić MB, Gernjak W, Heath E, Ivancev-Tumbas I, Karaolia P, Lado Ribeiro AR, Mascolo G, McArdell CS, Schaar H, Silva AMT, Fatta-Kassinos D (2019) Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban WW. Sci Total Environ 655:986–1008. https://doi.org/10.1016/j.scitotenv.2018.11.265
49. Rodríguez-Narváez O, Rodríguez OM, Peralta-Hernández JM, Goonetilleke A, Bandala E (2017) Treatment technologies for emerging contaminants in water: a review. Chem Eng J 323:361–380. https://doi.org/10.1016/j.cej.2017.04.106
50. De Luca A, Dantas RF, Esplugas S (2014) Assessment of iron chelates efficiency for photo-Fenton at neutral pH. Water Res 61:232–242. https://doi.org/10.1016/j.watres.2014.05.033
51. Zhan Y, Zhou M (2019) A critical review of the application of chelating agents to enable Fenton and Fenton-like reactions at high pH values. J Hazard Mater 362:436–450. https://doi.org/10.1016/j.jhazmat.2018.09.035

52. Klamerth N, Malato S, Maldonado MI, Agüera A, Fernández-Alba AR (2011) Modified photo-Fenton for degradation of emerging contaminants in municipal WW effluents. Catal Today 161:241–246. https://doi.org/10.1021/es903455p
53. Durán A, Montegudo JM, San Martín I (2018) Operation costs of the solar photo-catalytic degradation of pharmaceuticals in Water: a mini-review. Chemosphere 211:482–488. https://doi.org/10.3390/nano9091194
54. Huang W, Brigante M, Wu F, Hanna K, Mailhot G (2012) Development of a new homogenous photo-Fenton process using Fe(III)-EDDS complexes. J Photochem Photobiol A Chem 239:17–23. https://doi.org/10.1021/es304502y
55. De la Obra I, Ponce-Robles L, Miralles-Cuevas S, Oller I, Malato S, Sánchez Pérez JA (2017) Microcontaminant removal in secondary effluents by solar photo-Fenton at circumneutral pH in raceway pond reactors. Catal Today 287:10–14. https://doi.org/10.1016/j.cattod.2016.12.028
56. Klamerth N, Malato S, Agüera A, Fernández-Alba A (2013) Photo-Fenton and modified photo-Fenton at neutral pH for the treatment of emerging contaminants in WW treatment plant effluents: a comparison. Water Res 47:833–840. https://doi.org/10.1016/j.watres.2012.11.008
57. Zhang G, Ji S, Xi B (2006) Feasibility study of treatment of amoxicillin wastewater with a combination of extraction, Fenton oxidation and reverse osmosis. Desalination 196(1–3):32–42. https://doi.org/10.1016/j.desal.2005.11.018
58. Miralles-Cuevas S, Oller I, Sánchez-Pérez JA, Malato S (2014b) Removal of pharmaceuticals from MWTP effluent by nanofiltration and solar photo-Fenton using two different iron complexes at neutral pH. Water Res 64:23–31. https://doi.org/10.1016/j.watres.2014.06.032
59. Miralles-Cuevas S, Oller I, Ruiz Aguirre A, Sánchez Pérez JA, Malato Rodríguez S (2014a) Removal of pharmaceuticals at microg L-1 by combined nanofiltration and mild solar photo-Fenton. Chem Eng J 239:68–74. https://doi.org/10.1016/j.cej.2013.10.047
60. Trovó AG, Pupo Nogueira RF, Agüera A, Fernandez-Alba AR, Malato S (2011) Degradation of the antibiotic amoxicillin by photo-Fenton process – chemical and toxicological assessment. Water Res 45:1394–1402. https://doi.org/10.1016/j.watres.2010.10.029
61. Ballesteros SG, Costante M, Vicente R, Mora M, Amat AM, Arques A, Einschlag FSG (2017) Humic-like substances from urban waste as auxiliaries for photo-Fenton treatment: a fluorescence EEM-PARAFAC study. Photochem Photobiol Sci 16:38–45. https://doi.org/10.1039/C6PP00236F
62. Papoutsakis S, Pulgarin C, Oller I, Sánchez-Moreno R, Malato S (2016) Enhancement of the Fenton and photo-Fenton processes by components found in WW from the industrial processing of natural products: the possibilities of cork boiling WW reuse. Chem Eng J 304:890–896. https://doi.org/10.1016/j.cej.2016.07.021
63. Ruíz-Delgado A, Roccamante MA, Oller I, Agüera A, Malato S (2019) Natural chelating agents from olive mill WW to enable photo-Fenton-like reactions at natural pH. Catal Today 328:281–285. https://doi.org/10.1016/j.cattod.2018.10.051
64. Wu Y, Brigante M, Dong M, De Sainte-Claire P, Mailhot G (2014) Toward a better understanding of Fe(III)–EDDS photochemistry: theoretical stability calculation and experimental investigation of 4-tert-butylphenol degradation. J Phys Chem A 118:396–403. https://doi.org/10.1021/jp409043e
65. Vandevivere PC, Saveyn H, Verstraete W, Feijtel TCJ, Schowanek DR (2001) Biodegradation of metal-[S,S]-EDDS. Complexes. Environ Sci Technol 35:1765–1770. https://doi.org/10.1021/es0001153
66. Gutierrez-Mata AG, Velazquez-Martínez S, Álvarez-Gallegos A, Ahmadi M, Hernández-Pérez JA, Ghanbari F, Silva-Martínez S (2017) Recent overview of solar Photocatalysis and solar photo-Fenton processes for WW treatment. Hindawi Int J Photoenergy:27. https://doi.org/10.1155/2017/8528063
67. Souza BM, Marinho BA, Moreira FC, Dezotti MW, Boaventura RAR, Vilar VJP (2017) Photo-Fenton oxidation of 3-amino-5-methylisoxazole: a by-product from biological

breakdown of some pharmaceutical compounds. Environ Sci Pollut Res 24:6195–6204. https://doi.org/10.1007/s11356-015-5690-1
68. Ahmed MM, Brienza M, Goetz V, Chiron S (2014) Solar photo-Fenton using peroxymonosulfate for organic micropollutants removal from domestic WW: comparison with heterogeneous TiO2 photocatalysis. Chemosphere 117:252–256. https://doi.org/10.1016/j.chemosphere.2014.07.046
69. Mirzaei A, Chen Z, Haghighat F, Yerushalmi L (2017) Removal of pharmaceuticals from water by homo/heterogonous Fenton-type processes- a review. Chemosphere 174:665–688. https://doi.org/10.1016/j.chemosphere.2017.02.019
70. Costa EP, Roccamante M, Amorim CC, Oller I, Sánchez Pérez JA, Malato S (2020) New trend on open solar photoreactors to treat micropollutants by photo-Fenton at circumneutral pH: increasing optical pathway. Chem Eng J 385:123982. https://doi.org/10.1016/j.cej.2019.123982
71. Cuervo Lumbaque E, Araújo DS, Klein TM, Lopes-Tiburtius ER, Argüello J, Sirtori C (2019) Solar photo-Fenton-like process at neutral pH: Fe(III)-EDDS complex formation and optimization of experimental conditions for degradation of pharmaceuticals. Catal Today 328:259–266. https://doi.org/10.1016/j.cattod.2019.01.006
72. Koltsakidou A, Antonopoulou M, Sykiotou M, Evgenidou E, Konstantinou I, Lambropoulou D (2017) Photo-Fenton and Fenton-like processes for the treatment of the antineoplastic drug 5-fluorouracil under simulated solar radiation. Environ Sci Pollut Res 24:4791–4800. https://doi.org/10.1007/s11356-016-8138-3
73. Alalm MG, Tawfik A, Ookawara S (2015) Degradation of four pharmaceuticals by solar photo-Fenton process: kinetics and costs estimation. J Environ Chem Eng 3:46–51. https://doi.org/10.1016/j.jece.2014.12.009
74. Malato S, Blanco J, Maldonado MI, Fernandez-Ibañez P, Alarcon D, Collares M, Farinha J, Correia J (2004) Engineering of solar photocatalytic collectors. Sol Energy 77:513–524
75. Hinojosa Guerra MM, Oller Alberola I, Malato Rodriguez S, Agüera López A, Acevedo Merino A, Quiroga Alonso JM (2019) Oxidation mechanisms of amoxicillin and paracetamol in the photo-Fenton solar process. Water Res 156:232–240. https://doi.org/10.1016/j.watres.2019.02.055
76. Ioannou-Ttofa L, Raj S, Prakash H, Fatta-Kassinos D (2019) Solar photo-Fenton oxidation for the removal of ampicillin, total cultivable and resistant E. coli and ecotoxicity from secondary-treated WW effluents. Chem Eng J 355:91–102. https://doi.org/10.1016/j.cej.2018.08.057
77. Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306
78. Davis R, Aden A, Pienkos PT (2011) Techno-economic analysis of autotrophic microalgae for fuel production. Appl Energy 88:3524–3531
79. Soriano-Molina P, Plaza-Bolaños P, Lorenzo A, Agüera A, García-Sánchez JL, Malato S, Sánchez Pérez JA (2019) Assessment of solar raceway pond reactors for removal of contaminants of emerging concern by photo-Fenton at circumneutral pH from very different municipal WW effluents. Chem Eng J 366:141–149. https://doi.org/10.1016/j.cej.2019.02.074
80. Miralles-Cuevas S, Oller I, Agüera A, Ponce-Robles L, Sánchez Pérez JA, Malato S (2015) Removal of microcontaminants from MWTP effluents by combination of membrane technologies and solar photo-Fenton at neutral pHs. Catal Today 252:78–83. https://doi.org/10.1016/j.cattod.2014.11.015
81. Miralles-Cuevas S, Oller I, Agüera A, Sánchez Pérez JA, Malato S (2017) Strategies for reducing cost by using solar photo-Fenton treatment combined with nanofiltration to remove microcontaminants in real municipal effluents: Toxicity and economic assessment. Chem Eng J 318:161–170. https://doi.org/10.1016/j.cej.2016.06.031
82. Della-Flora A, Wilde ML, Pinto IDF, Lima ÉC, Sirtori C (2020) Degradation of the anticancer drug flutamide by solar photo-Fenton treatment at near-neutral pH: identification of transformation products and in silico (Q)SAR risk assessment. Environ Res 183:109223. https://doi.org/10.1016/j.envres.2020.109223
83. De Melo Santos MM, da Silva TD, de Lucena ALA, Napoleão DC, Duarte MMMB (2020) Degradation of Ketoprofen, Tenoxicam, and meloxicam drugs by photo-assisted peroxidation

and photo-Fenton processes: identification of intermediates and toxicity study. Water Air Soil Pollut 231:35. https://doi.org/10.1007/s11270-020-4401-9
84. Majumder A, Gupta B, Gupta AK (2019) Pharmaceutically active compounds in aqueous environment: a status, toxicity and insights of remediation. Environ Res 176:108542. https://doi.org/10.1016/j.envres.2019.108542
85. Ioannou-Ttofa L, Foteinis S, Chatzisymeon E, Michael-Kordatou I, Fatta-Kassinos D (2017) Life cycle assessment of solar-driven oxidation as a polishing step of secondary-treated urban effluents. J Chem Technol Biotechnol 92:1315–1327. https://doi.org/10.1002/jctb.5126
86. Salazar LM, Grisales CM, Garcia DP (2019) How does intensification influence the operational and environmental performance of photo-Fenton processes at acidic and circumneutral pH. Environ Sci Pollut Res 26:4367–4380. https://doi.org/10.1007/s11356-018-2388-1
87. Tarpani RRZ, Azapagic A (2018) Life cycle environmental impacts of advanced WW treatment techniques for removal of pharmaceuticals and personal care products (PPCPs). J Environ Manag 215:258–272. https://doi.org/10.1016/j.jenvman.2018.03.047
88. Klamerth N, Malato S, Agüera A, Fernández-Alba AR, Mailhot G (2012) Treatment of municipal WW treatment plant effluents with modified photo-Fenton as a tertiary treatment for the degradation of micro pollutants and disinfection. Environ Sci Technol 46:2885–2892. https://doi.org/10.1021/es204112d
89. Rosal R, Rodríguez A, Perdigón-Melón JA, Petre A, García-Calvo E, Gómez MJ, Agüera A, Fernández-Alba AR (2010) Occurrence of emerging pollutants in urban WW and their removal through biological treatment followed by ozonation. Water Res 44:578–588. https://doi.org/10.1016/j.watres.2009.07.004
90. Campos-Mañas MC, Plaza-Bolaños P, Sánchez-Pérez JA, Malato S, Agüera A (2017) Fast determination of pesticides and other contaminants of emerging concern in treated WW using direct injection coupled to highly sensitive ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 1507:84–94. https://doi.org/10.1016/j.chroma.2017.05.053
91. Maia AS, Paíga P, Delerue-Matos C, Castro PML, Tiritan ME (2020) Quantification of fluoroquinolones in WWs by liquid chromatography-tandem mass spectrometry. Environ Pollut 259:113927. https://doi.org/10.1016/j.envpol.2020.113927
92. González-Antuña A, Domínguez-Romero JC, García-Reyes JF, Rodríguez-González P, Centineoc G, García Alonso JI, Molina-Díaz A (2013) Overcoming matrix effects in electrospray: quantitation of β–agonists in complex matrices by isotope dilution liquid chromatography–mass spectrometry using singly 13C-labeled analogues. J Chromatogr A 1288:40–47. https://doi.org/10.1016/j.chroma.2013.02.074
93. Schymanski EL, Jeon J, Gulde R, Fenner K, Ruff M, Singer HP, Hollender J (2014) Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ Sci Technol 48:2097–2098. https://doi.org/10.1021/es5002105
94. Bletsou AA, Jeon J, Hollender J, Archontaki E, Thomaidis NS (2015) Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment. TrAC Trends Anal Chem 66:32e44. https://doi.org/10.1016/j.trac.2014.11.009
95. Kyoto University (2018) Pathpred: pathway prediction server [WWW document]. https://www.genome.jp/tools/pathpred
96. EAWAG (2018) EAWAG-BBD pathway prediction system [WWW document]. http://eawag-bbd.ethz.ch/predict
97. Jaén-Gil A, Buttiglieri G, Benito A, Gonzalez-Olmos R, Barceló D, Rodríguez-Mozaz S (2019) Metoprolol and metoprolol acid degradation in UV/H2O2 treated WWs: an integrated screening approach for the identification of hazardous transformation products. J Hazard Mater 380:120851. https://doi.org/10.1016/j.jhazmat.2019.120851
98. Schenone AV, Conte LO, Botta MA, Alfano OM (2015) Modeling and optimization of photo-Fenton degradation of 2,4-D using ferrioxalate complex and response surface methodology (RSM). J Environ Manag 155:177–183. https://doi.org/10.1016/j.jenvman.2015.03.028
99. Rivas G, Carra I, García Sánchez JL, Casas López JL, Malato S, Sánchez Pérez JA (2015) Modelling of the operation of raceway pond reactors for micropollutant removal by solar

photo-Fenton as a function of photon absorption. Appl Catal B Environ 178:210–217. https://doi.org/10.1016/j.apcatb.2014.09.015
100. Giannakis S, Hendaoui I, Rtimi S, Fürbringer JM, Pulgarin C (2017) Modeling and treatment optimization of pharmaceutically active compounds by the photo-Fenton process: the case of the antidepressant venlafaxine. J Environ Chem Eng 5:818–828. https://doi.org/10.1016/j.jece.2016.12.050
101. Cassano AE, Martin CA, Brandi RJ, Alfano OM (1995) Photoreactor analysis and design: fundamentals and applications. Ind Eng Chem Res 34:2155–2201. https://doi.org/10.1021/ie00046a001
102. Conte LO, Schenone AV, Alfano OM (2016) Photo-Fenton degradation of the herbicide 2,4-D in aqueous medium at pH conditions close to neutrality. J Environ Manag 170:60–69. https://doi.org/10.1016/j.jenvman.2016.01.002
103. Brandi RJ, Citroni MA, Alfano OM, Cassano AE (2003) Absolute quantum yields in photocatalytic slurry reactors. Chem Eng Sci 58:979–985. https://doi.org/10.1016/S0009-2509(02)00638-3
104. Andreozzi R, D'Apuzzo A, Marotta R (2000) A kinetic model for the degradation of benzothiazole by Fe3+-photo-assisted Fenton process in a completely mixed batch reactor. J Hazard Mater 80:241–257. https://doi.org/10.1016/S0304-3894(00)00308-3
105. Conte LO, Farias J, Albizzati ED, Alfano OM (2012) Photo-Fenton degradation of the herbicide 2,4-dichlorophenoxyacetic acid in laboratory and solar pilot-plant reactors. Ind Eng Chem Res 51:4181–4191. https://doi.org/10.1021/ie2023228
106. Cabrera Reina A, Santos-Juanes L, García Sánchez JL, Casas López JL, Maldonado Rubio MI, Li Puma G, Sánchez Pérez JA (2015) Modelling the photo-Fenton oxidation of the pharmaceutical paracetamol in water including the effect of photon absorption (VRPA). Appl Catal B Environ 166–167:295–301. https://doi.org/10.1016/j.apcatb.2014.11.023
107. Audino F, Conte LO, Schenone AV, Pérez-Moya M, Graells M, Alfano OM (2018) A kinetic study for the Fenton and photo-Fenton paracetamol degradation in an annular photoreactor. Environ Sci Pollut Res 26(5):4312–4323. https://doi.org/10.1007/s11356-018-3098-4
108. Sánchez Pérez JA, Soriano-Molina P, Rivas G, García Sánchez JL, Casas López JL, Fernández SJM (2017) Effect of temperature and photon absorption on the kinetics of micropollutant removal by solar photo-Fenton in raceway pond reactors. Chem Eng J 310:464–472. https://doi.org/10.1016/j.cej.2016.06.055
109. Conte LO, Schenone AV, Giménez BN, Alfano OM (2019) Photo-Fenton degradation of a herbicide (2,4-D) in groundwater for conditions of natural pH and presence of inorganic anions. J Hazard Mater 372:113–120. https://doi.org/10.1016/j.jhazmat.2018.04.013
110. Soriano Molina P, García Sánchez JL, Malato S, Plaza-Bolaños P, Agüera A, Sánchez Pérez JA (2019) On the design and operation of solar photo-Fenton open reactors for the removal of contaminants of emerging concern from WWTP effluents at neutral pH. Appl Catal B Environ 256:117801. https://doi.org/10.1016/j.apcatb.2019.117801

Advanced Electrochemical Processes for the Elimination of Pharmaceutical Compounds in Contaminated Waters

Jelena Radjenovic and Luis Baptista-Pires

Contents

Abstract Electrochemical systems have often been investigated for the removal and degradation of pharmaceuticals in water, mainly focusing on their electrooxidation kinetics and transformation pathways and sometimes relying on sophisticated high-resolution mass spectrometry (HRMS) analysis. Most of these studies are based on ideal experimental designs of high electrolyte conductivity and high contaminant

The title of the original version of this chapter was corrected from "Chemical Processes for the Elimination of Pharmaceutical Compounds in Contaminated Waters" to "Advanced Electrochemical Processes for the Elimination of Pharmaceutical Compounds in Contaminated Waters". A correction to this chapter can be found at https://doi.org/10.1007/698_2021_757

J. Radjenovic (✉)
Catalan Institute for Water Research (ICRA), Girona, Spain

Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain
e-mail: jradjenovic@icra.cat

L. Baptista-Pires
Catalan Institute for Water Research (ICRA), Girona, Spain

University of Girona, Girona, Spain
e-mail: l.baptistapires@gmail.com

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 327–348. DOI 10.1007/698_2020_689,

Published online: 13 October 2020

concentration, resulting in outputs that can hardly be extrapolated to a real case scenario. This chapter discusses a limited number of studies in which the performance of electrochemical systems in removing and degrading pharmaceutical residues was evaluated using real waste streams. The main mechanisms of electrochemical oxidation of pharmaceuticals are explained, with a particular emphasis on the recently reported participation of strong oxidant species, sulfate radicals, in electrooxidation of organic contaminants. Finally, as electrode and reactor design evolves toward three-dimensional geometries with reduced mass transfer limitations, significant attention has been paid to the progress made using flow-through, porous electrode materials for the electrooxidation of pharmaceuticals. Novel, electrocatalytically active, stable membrane and foam/sponge-type electrodes are thoroughly discussed as such materials represent an essential enabler of a wider-scale implementation of electrochemical treatment systems and their integration into water and wastewater treatment infrastructure.

Keywords Boron-doped diamond (BDD), Dimensionally stable anode (DSA), Electrochemical water treatment, PhACs, Reactive electrochemical membrane (REM)

1 Introduction

The ability of electrolysis to remove persistent organic and inorganic pollutants from water has triggered an enormous amount of research toward the end of the twentieth century. This research was intensified by the invention of a new material, boron-doped diamond (BDD) anode in 1987 by Pleskov [1], which was later patented for organic pollutant removal from water by Carey et al. [2]. Compared with other commercial electrode materials based on mixed metal oxide (MMO) coatings, BDD has demonstrated superior performance in terms of its oxidizing power and capacity to form strong oxidizing species, hydroxyl radicals ($HO^{\bullet}$) (Table 1). BDD electrodes also display excellent electrochemical and corrosion stability, longer service life, and stability under polarity reversal, which is crucial for field applications of electrochemical treatment to prevent scaling and fouling at the cathode [5]. Nevertheless,

Table 1 Onset potentials for oxygen evolution reaction (E_{OER}) expressed versus standard hydrogen electrode (SHE) and characteristics of typical anode materials

Electrode	E_{OER}, V/SHE	Conditions	Ref.
Ti/RuO_2	1.5	0.5 M H_2SO_4	[3]
Ti/IrO_2	1.5	0.5 M H_2SO_4	[3]
Ti/SnO_2	1.9	0.05 M H_2SO_4	[3]
Ti/PbO_2	1.9	0.05 M H_2SO_4	[3]
BDD	2.3	0.5 M H_2SO_4	[3]
Ti_4O_7	2.7–3.0[a]	3.7 M H_2SO_4	[4]

[a]No generic OER as oxygen evolution strongly depends on the pore size distribution of Ti_4O_7, where decrease in pore size shifts the OER to more positive values

BDD suffer from two major limitations: (1) synthesis based on chemical vapor deposition (CVD) or high-pressure high-temperature (HPHT) process keeps the price of BDD electrodes at ~6,000 € m^{-2} [6] and makes it difficult to upscale the technology due to low production rates, and (2) anodic polarization of BDD electrode results in termination with various functional groups (e.g., hydroxyl, carbonyl, carboxyl) and leads to a dramatic increase in surface and charge transfer resistance, thus increasing the electrode potentials (i.e., cell voltage) and consequently the energy consumption. Furthermore, BDD electrodes can be produced only in plate (Si, Nb) and mesh (Nb) geometries. It is difficult to produce them in three-dimensional (3D) forms that would offer high specific surface area and thus minimized mass transfer limitations and lower energy consumption for the degradation of trace organic pollutants.

As an alternative to pricey BDD anodes, MMO-type electrodes based on the oxides of ruthenium (RuO_2), iridium (IrO_2), lead (PbO_2), and tin (SnO_2) coated on titanium have been intensively investigated for the removal of organic contaminants from water. In particular, SnO_2-based electrodes, often doped with antimony (Sb_2O_5) to increase the coating conductivity, have drawn significant attention due to their large overpotential for oxygen evolution reaction (OER) and thus capability to efficiently form $HO^{\bullet}$ (Table 1) [7]. The commercial fabrication of Ti/SnO_2-Sb_2O_5-coated titanium electrodes is mostly based on thermal decomposition process and has been used to produce mesh and plate-type geometries. However, Ti/SnO_2-Sb_2O_5 anodes are characterized by the short service life and low corrosion stability, which can be somewhat improved by the interlayer insertion (e.g., IrO_2), addition of polymers, and/or modification of Ti substrate, for example, by the insertion of a TiO_2 nanotube array (NTA) layer [8]. Mesh-type geometries still have limited specific surface area for the degradation of pollutants, although significant improvements in the electrical efficiency in low-conductivity electrolytes can be achieved by using solid polymer electrolytes (e.g., proton exchange membranes) compressed between the mesh anode and cathode [9].

More recently, Magnéli phase materials based on sub-stoichiometric titanium oxide, Ti_4O_7, have gained attention due to their high electric conductivity, comparable to that of graphite, high specific surface area and high stability toward corrosion [10]. The lab-scale fabrication costs were determined to be significantly lower compared with BDD, although the cost and feasibility of a large-scale production of Ti_4O_7 anodes is yet to be determined given that its manufacture involves combustion under H_2 atmosphere at temperatures above 1,000°C [11]. The onset potentials for OER at Ti_4O_7 anodes were reported to vary between 2.7 and 3.0 V/SHE depending on the anode porosity, with smaller pore size yielding higher onset for OER (Table 1) [4].

2 Mechanisms of Electrochemical Oxidation of Pharmaceutical Residues in Water

Electrochemical degradation of trace pollutants is rarely achieved by direct electron transfer (DET), except in the cases when contaminants are resistant to $HO^{\bullet}$ and other oxidant species produced at the anode, and/or when the sorption of contaminant onto the electrode surface is favored. For example, in electrochemical oxidation of reverse osmosis concentrate at Ti/$RuIrO_2$ anode, antidepressant drug sertraline was the only compound among 28 pharmaceuticals and pesticides analyzed that was removed by direct electrolysis at low applied current densities, likely due to its high logK_{OW} (5.3) and thus better adsorption onto the anode surface [12]. To achieve degradation of organic contaminants, electrochemical water treatment is normally conducted at high current densities at which the contribution of DET is significantly lowered. Under these conditions, electrochemical oxidation of pharmaceutical residues and other trace organic pollutants is mediated by the strong oxidant species formed during electrolysis of water such as $HO^{\bullet}$ (Eq. 1), H_2O_2 (Eq. 2) and other reactive oxygen species, as well as Cl_2/HClO/OCl^- (Eqs. 3 and 4), sulfate radical ($SO_4^{\bullet -}$), and persulfate, in the presence of Cl^- and SO_4^{2-} (Eqs. 5–8) [13]:

$$H_2O \rightarrow HO^{\bullet} + H^+ + e^- \quad (1)$$

$$2H_2O \rightarrow H_2O_2 + 2H^+ + 2e^- \quad (2)$$

$$2Cl^- \rightarrow Cl_2 + 2e^- \quad (3)$$

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- \quad (4)$$

$$SO_4^{2-} \rightarrow SO_4^{\bullet -} + e^- \quad (5)$$

$$HSO_4^- + HO^{\bullet} \rightarrow SO_4^{\bullet -} + H_2O \quad (6)$$

$$H_2SO_4 + HO^{\bullet} \rightarrow SO_4^{\bullet -} + H_3O^+ \quad (7)$$

$$SO_4^{\bullet -} + SO_4^{\bullet -} \rightarrow S_2O_8^{2-} \quad (8)$$

Electrochemical generation of reactive species such as $HO^{\bullet}$ is affected by the properties of the electrode material, i.e., OER overpotential [13]. The amount of $HO^{\bullet}$ generated in the system will determine not only the oxidation efficiency of organic contaminants but also the impact of inorganic and organic matrix on the treatment outcome. For example, electrooxidation of highly saline reverse osmosis brine stream at MMO anodes results in the increase in toxicity to *Vibrio fischeri* due to the formation of chlorinated and brominated organic byproducts [12]. In the case of BDD anode, no such increase in toxicity was noted, in spite of the observed increase in concentrations of adsorbable organic halogen (AOX), suggesting that organochlorine and organobromine compounds formed were likely comprised of lower molecular weight compounds, as a consequence of more of $HO^{\bullet}$ produced at the anode and thus enhanced bond breakage [14]. Another interesting feature of BDD

anodes is their ability to form sulfate radical ($SO_4^{\bullet-}$) directly from the electrolysis of sulfate [15]. Although sulfate-based solutions have long been used as inert electrolytes, several studies conducted by Farhat et al. [15] demonstrated the participation of anodically formed $SO_4^{\bullet-}$ in electrooxidation of persistent pharmaceuticals such as carbamazepine, iodinated contrast media (ICM) diatrizoate and iopromide, and lower production of chlorinated organic byproducts when SO_4^{2-} and Cl^- are present simultaneously [16, 17]. Furthermore, BDD/SO_4^{2-} system exhibits oxidative capacitance during an intermittent power supply operation, with prolonged oxidation of diatrizoate observed in the power-off mode [18]. Application of 100 A m^{-2} in cycles of 5 min on/15 min off enabled a saving in the electrical energy consumption by ~35% compared to constant current experiment. Nevertheless, the exact mechanism behind this prolonged oxidation could not be determined and was hypothesized to involve radical chain propagators of $SO_4^{\bullet-}$ formed during the oxidation [19, 20]. Since then, research on electrochemically formed sulfate-based oxidants has intensified, given that in the currently applied oxidation methods based on $SO_4^{\bullet-}$, their formation from persulfate or peroxymonosulfate needs to be induced by not so practical methods based on UV, transition metals, heat, or high pH. Recent studies assigned the formation of $SO_4^{\bullet-}$ to heat activation of anodically formed persulfate that occurs at higher cell potentials due to ohmic heating [21, 22]. Some controversial studies hypothesized a non-radical electrochemical activation of the $S_2O_8^{2-}$ formed by anodic oxidation of sulfate [23, 24]. For example, Song et al. [24] hypothesized the formation of a transition state structure between persulfate and Ti/Pt anode, which enhanced the removal of carbamazepine, sulfamethoxazole, propranolol, and benzoic acid. Similar non-radical persulfate activation at the BDD anode was suggested to enhance the removal of antibiotic ciprofloxacin [23]. However, these observations were based on the decreased but not completely halted oxidation of pollutants in the presence of typical $HO^{\bullet}$ and $SO_4^{\bullet-}$ radical scavengers, *tert*-butanol, and methanol. Although these saturated alcohols are efficient quenchers of dissolved oxidant species, they do not react readily with electrode surfaces and alternative probes, for surface-bound, anodically produced radical oxidants may be better suited, such as allyl alcohols [25]. In a recent study by Cai et al. [26], direct electrogeneration of $SO_4^{\bullet-}$ from SO_4^{2-} at BDD anode was demonstrated using in situ electron paramagnetic resonance spectrometry. The authors observed continued degradation of di-n-butyl phthalate (DnBP), a typical endocrine disruptor, in the power-off phase during an intermittent power supply, which was assigned to the accumulation of $S_2O_8^{2-}$ and its reactivation to $SO_4^{\bullet-}$ via specific degradation intermediates. Similar to the study of Farhat et al. [18], electrical energy consumption was reduced by over 30% compared with continuous power supply operation. Continued generation of $SO_4^{\bullet-}$ was demonstrated by the spin trapping in the absence of applied potential [26] and was observed to lead to continued pollutant oxidation both inside the electrochemical system and in the sample vial upon sampling in the absence of quenchers [18]. Yet, oxidative capacitance and sustained oxidation of organic pollutants in the power-off phase of the BDD/SO_4^{2-} system need to be further investigated to elucidate the underlying propagation mechanisms [26].

The impact of $SO_4^{\bullet-}$, Cl_2/HOCl, $Cl_2^{\bullet-}$ and other oxidants is highly dependent on the nature and reactivity of a given pharmaceutically active pollutant. For instance, reaction rate constants of aromatic compounds with $Cl_2^{\bullet-}$ can vary from $k < 10^5$–$10^7\ M^{-1}\ s^{-1}$ for compounds containing substituents with negative inductive effect to $k = 10^7$–$10^9\ M^{-1}\ s^{-1}$ for compounds containing substituents with positive inductive effect [27]. In addition, electrooxidation of trace organic contaminants will be impacted by the organic and inorganic matrix of the contaminated water. For example, elevated concentrations of HCO_3^- can scavenge reactive species like chlorine radicals and reduce the transformation rates of electron-poor contaminants such as β-blockers [25]. Nevertheless, Cl^- present in virtually any water and wastewater will undoubtedly pose limitations to electrochemical treatment due to the formation of chlorinated organic byproducts from the trace organic pollutants and organic matrix, leading to an increase in the toxicity of the treated water [12, 28, 29]. Electrochlorination of the bulk organic matter yields a range of toxic low-molecular-weight byproducts such as trihalomethanes (THMs) and haloacetic acids (HAAs) [30–32]. Also, at BDD-, Ti_4O_7-, and Ti/SnO_2-type anodes with higher oxidizing power, Cl^- is easily oxidized to toxic chlorate and perchlorate that pose significant challenges to the discharge of the treated effluent [33–35]. Application of an electrochemical system as a single treatment step may be compromised depending on the characteristics of the organic and inorganic matrix of the contaminated water. In this case, downstream treatment options should be considered to reduce the effluent toxicity, such as granular activated carbon and/or reductive posttreatment.

3 Limitations of State of the Art in Electrochemical Water Treatment

Research on electrochemical oxidation for the removal of pharmaceuticals and other pollutants has been mainly performed using clean supporting electrolytes and often unrealistic conditions of both high contaminant concentration (i.e., mg L^{-1} range) and high electrolyte conductivity. This makes it very difficult to extrapolate the obtained results to a real case scenario in terms of the required energy consumption and reported transformation kinetics and pathways of a given compound. More importantly, one of the main bottlenecks of electrochemical treatment systems – generation of toxic chlorinated byproducts, both organic and inorganic – has not been sufficiently addressed as most researchers either used sulfate-, nitrate-, and perchlorate-based electrolytes, or they did not consider that the observed disappearance of trace pharmaceuticals in NaCl supporting electrolyte is achieved by electrochlorination. Another limitation in the field of electrochemical water treatment is dependence of the figures of merit on the experimental design, as it is difficult to compare batch versus continuous mode or batch beaker test versus a batch recirculation reactor. There is a great disparity in the way the obtained results

are reported and expressed, with most authors opting for using the observed pseudo-first-order removal rate constants (k_{OBS}, h^{-1}) of target pollutants, in some cases normalized to the electrode surface area, and current efficiencies (CE, in %) calculated using Eq. (9):

$$CE = 100 \times nFV \frac{(C_0 - C_t)}{It} \tag{9}$$

where n is the number of electrons required for complete oxidation of a specific pollutant (i.e., to CO_2, H_2O, etc.), F is the Faraday constant (96,487 C mol^{-1}), V the electrolyte volume (L), C_0 and C_t are concentrations of compound measured at time $t = 0$ and time t (in mol L^{-1}), I is the applied current (A), and t is the duration of electrolysis (s). Yet, for processes targeting removal of pharmaceuticals and other trace pollutants, electric energy per order (E_{EO}) kWh m^{-3}) is a more appropriate figure of merit that expresses energy required to reduce the concentration of a contaminant by one order of magnitude in a unit volume of water (Eqs. 10 and 11) [13]:

$$E_{EO} = \frac{Pt}{V \log \frac{C_0}{C_t}} \text{ for batch processes} \tag{10}$$

$$E_{EO} = \frac{P}{q \log \frac{C_0}{C_t}} \text{ for continuous processes} \tag{11}$$

where P is the rated power of the system (W), V is volume of water (L) treated in time t (h) in a batch system, q is the flow rate applied in a continuous system (L h^{-1}), and C_0 and C_t are initial and final concentration of the target pollutant where C_t is an order of magnitude lower than C_0 (i.e., $C_0 = 10_{\times} C_t$ and thus $\log \frac{C_0}{C_t} = 1$).

4 Electrochemical Treatment for the Removal of Pharmaceutical Residues from Real Contaminated Water

The first study on electrochemical transformation of a pharmaceutical compound was published in 1964 by Merkle and Discher [36]. They investigated electrolysis of an antipsychotic drug chlorpromazine at Pt electrode as a means of quantitative analysis. Although the potential of this technology for the degradation of organic compounds was known for a long time, it was not until the 2000s when reporting on the occurrence of pharmaceuticals in sewage, surface, and groundwater started to intensify that electrochemical water treatment gained attention. Given that oxidative degradation typically offers more complete degradation of organic pollutants,

electrochemical oxidation has been in the focus of the scientific community much more than the cathodic electrolysis of contaminants.

The number of studies where electrochemical removal of pharmaceuticals was evaluated using real contaminated waste streams is very limited. In electrooxidation using Ti/$RuIrO_2$ anode of reverse osmosis (RO) brine amended with pharmaceuticals at low μg L^{-1} concentrations, most of the target pharmaceuticals completely disappeared when applying 461.5 Ah m^{-3} (cell energy consumption of 1.87 kWh m^{-3}) in continuous mode and somewhat lower charge of 437.9 Ah m^{-3} in batch mode (1.77 kWh m^{-3}), due to the higher accumulation of bulk chlorine species and thus faster transformation of target pollutants [12]. Antibiotics trimethoprim, enrofloxacin, norfloxacin, sulfadiazine and roxithromycin, ß-blockers metoprolol and tramadol, anticonvulsant carbamazepine, nonsteroidal anti-inflammatory drugs (NSAIDs) gemfibrozil and diclofenac, and others were rapidly removed at currents ≥150 A m^{-2}. Pharmaceuticals more recalcitrant to oxidation were NSAID ibuprofen, anti-epileptic drug phenytoin, and ICM iopromide, for which continuous mode achieved less than 70% removal, whereas in batch mode their removal was more complete. In electrooxidation of RO brine using BDD anode, all target pharmaceuticals disappeared after 2 h of electrolysis at 100 A m^{-2} (i.e., applied charge of 700 Ah m^{-3}), except for ibuprofen which was removed up to 55% [32]. The energy consumption for the removal of pharmaceuticals was not reported but at lower current density (50 A m^{-2}) 6.4 kWh m^{-3} was needed for complete removal of chemical oxygen demand (COD). Nevertheless, disappearance of target pollutants does not imply that the treatment was successful. The observed effluent toxicity to *Vibrio fischeri* was increased up to 37-fold to 53-fold in both continuous and batch mode electrooxidation of RO brine at Ti/$RuIrO_2$ anode, suggesting electrochlorination as the dominant oxidation mechanism [12]. Electrochlorination of organic pollutants in the presence of high chloride concentration was demonstrated in a subsequent study focused on identification of transformation products of ß-blocker metoprolol formed using Ti/$RuIrO_2$ and Ti/SnO_2-Sb_2O_5 anode [29]. Metoprolol and its degradation products were transformed to chloro, chloro-bromo, and bromo derivatives at both anodes in the presence of 1.5 g L^{-1} of Cl^- and 1.5 mg L^{-1} of Br^- in RO concentrate (Fig. 1). Metoprolol and its derivatives were transformed faster at Ti/SnO_2-Sb_2O_5 anode, likely due to its capacity to form $HO^•$ that participate in the oxidation besides active chlorine species (Cl_2/HOCl/OCl^-).

In another study, lower residual chlorine concentration was measured in electrooxidation of wastewater effluent spiked with 50 μM of carbamazepine than in the same effluent without the added pharmaceutical suggesting its reaction via electrochlorination mechanism, which was also confirmed by the increase in toxicity [37]. Also, in electrooxidation of hospital wastewater effluent using BDD anode, the monitored pharmaceuticals were removed faster than expected from their $HO^•$ radical rate constants due to their reaction with chlorine species [38]. Hydroxy-ibuprofen and hydroxy-diclofenac were more persistent than their parent compounds, indicating the need for including major human metabolites of pharmaceuticals in the evaluation of the treatment technology.

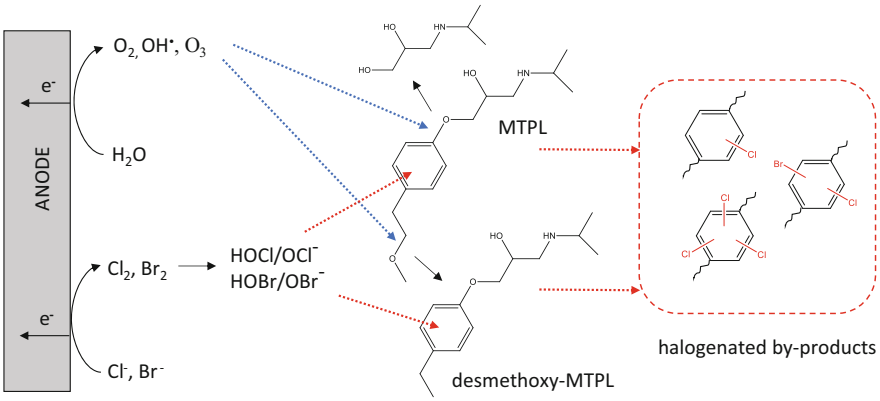


Fig. 1 Schematic representation of the competition between electrochlorination and electrobromination and oxidation by $HO^{\bullet}$ and other reactive oxygen species formed at the anode, during electrooxidation of metoprolol in reverse osmosis brine using BDD anode. Adapted from [29]

Very similar results in terms of electrochlorination of trace organic contaminants were obtained in the treatment of secondary domestic sewage effluent that contained significantly lower Cl^{-} concentration than typically encountered in RO brines. In electrooxidation at BDD anode, most of the identified degradation products of pharmaceuticals carbamazepine, diclofenac, trimethoprim, and venlafaxine were chlorinated [39]. In electrooxidation of pharmaceuticals spiked in secondary municipal wastewater effluent at low μg L^{-1} concentrations, most of the pharmaceuticals were removed after 2 h of electrolysis at 196 A m^{-2} using BDD anode (energy consumption of anodic compartment of 0.64–0.85 kWh m^{-3}) at both acidic and neutral pH, with the exception of ibuprofen and ICM diatrizoate and iopromide [34]. The molecular structures of these compounds are characterized by the presence of electrophilic halogen groups with a negative inductive effect at the aromatic ring (e.g., ICM) or by the absence of nucleophilic substituents with an activating effect on the aromatic ring (e.g., ibuprofen), which diminishes the reactivity of these compounds toward $HO^{\bullet}$, chlorine, and other oxidant species. Ibuprofen was also reported to be the most recalcitrant pharmaceutical residue by Urtiaga et al., with 70% removed after 1 h of treatment at 100 A m^{-2} using BDD anode [40]. They applied ultrafiltration (UF) and reverse osmosis (RO) to treat secondary wastewater effluent, whereas electrooxidation of RO concentrate reduced the total load of pharmaceutical residues from 149 μg L^{-1} to less than 10 μg L^{-1}. The extent of electrochlorination can be decreased by applying lower current densities [12, 41]. In electrooxidation of hospital wastewater at BDD anode, application of 15.6 A m^{-2} yielded low amounts of residual chlorine (0.6 mg L^{-1}) and complete removal of bezafibrate, gemfibrozil, indomethacin, and sulfamethoxazole after 40 min of electrolysis, resulting in the energy consumption of 0.055 kWh m^{-3}. Electrochemically treated effluent was successfully subjected to biological posttreatment with activated sludge.

BDD has often been the anode material of choice for the degradation of pharmaceuticals and other trace pollutants in water. Yet, variations in the structure of BDD electrodes may have a significant impact on their performance and oxidizing capacity. Ultrananocrystalline (UNCD) diamond films with smaller grain sizes are more active and have a much higher surface to volume ratio than their microcrystalline counterparts [42]. Given that in most studies BDD electrodes are commercially purchased, characteristics of the BDD coating and parameters applied during its synthesis are often not included or considered. For example, optimum boron-to-carbon concentration ($C_{B\text{-}C}$) in the gas phase applied in hot filament (HF) CVD technique was determined to be 10,000 ppm, and further increase in $C_{B\text{-}C}$ led to a decrease in the removal efficiencies of carbamazepine, diclofenac, trimethoprim, and venlafaxine [39]. In this study, venlafaxine removal was decreased from 60% to 10% after 240 min of electrolysis at 500 A m^{-2}, when $C_{B\text{-}C}$ was increased from 10,000 to 15,000 ppm. Also, the use of BDD layer grown on structured Si substrate (i.e., Si substrate subjected to wet etching to increase its roughness) yielded nearly 100% of removal efficiency for all target pharmaceuticals, implying the determining impact of the surface morphology of the Si substrate on the performance of anodic oxidation process. Furthermore, the performance of BDD anodes is dependent on the nature of the surface termination groups [13, 43]. BDD anodes produced using CVD method are H-terminated with reduced surface functional groups (i.e., $=CH_2$, $\equiv C–H$), and anodic polarization, typically in concentrated sulfuric acid, generates oxygenated-functional groups such as $\equiv$C-OH, =C=O, and -COOH that are more hydrophilic, yield better anode wettability and act as electroactive sites for the generation of $HO^{\bullet}$ and other oxidants [42]. Electrooxidation of ICM diatrizoate at as-received Nb/BDD anode yielded k_{OBS} of 0.003 h^{-1} in $NaNO_3$ and Na_2SO_4 electrolyte of the same conductivity (i.e., 5.8 mS cm^{-1}), whereas conditioning of the anode increased the k_{OBS} to 0.16 h^{-1} (in $NaNO_3$) and 1.06 h^{-1} (in Na_2SO_4) electrolyte, due to the enabled formation of sulfate radicals ($SO_4^{\bullet -}$) [44].

To obtain a more complete oxidative degradation of pharmaceuticals, some studies used both anodic and cathodic half-cell of an electrochemical unit [45, 46]. For example, reductive deiodination of ICM iopromide at nano Pd-doped graphite felt cathode was combined with oxidative degradation at BDD anode to avoid the formation of persistent iodinated intermediates, typically formed due to the preferential attack of $HO^{\bullet}$ at the alkyl side chains [46]. Cathodic transformation of pharmaceuticals and other trace pollutants is a less attractive process due to the necessity to use noble metal catalyst such as Pd and Pt to achieve enough efficiency of reductive transformation of pollutants. The cathodic half-cell of an electrochemical system has typically been used to produce hydrogen peroxide from the reduction of oxygen, according to Eq. (12):

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \tag{12}$$

H_2O_2 is a weak oxidant with $E^{\circ}(H_2O_2/H_2O) = 1.763$ V/SHE (standard hydrogen electrode) in acidic solution and is activated to $HO^{\bullet}$ by the addition of catalytic amounts of ferrous ion, Fe^{2+} (Eq. 13), in a process known as electro-Fenton [47]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \tag{13}$$

This enables a more complete oxidative degradation of trace organic pollutants that react with the $HO^{\bullet}$ formed in the bulk liquid, in addition to the anodically formed $HO^{\bullet}$ that remain in a proximity of the anode surface. Furthermore, electro-Fenton can be further enhanced by the addition of UV light in a so-called photoelectro-Fenton and solar photoelectro-Fenton processes. The performance of these systems in removing pharmaceutical residues from water has been extensively reviewed in literature [47, 48]. However, great majority of the research on electro-Fenton and photoelectro-Fenton processes has been performed using single component solutions, using high initial contaminant concentration (e.g., in mg L^{-1} range) and high conductivity electrolytes. Very few studies have investigated the process performance when dealing with real contaminated water. For example, electrochemical Fenton-based treatment was successfully applied for the removal of tetracaine, anesthetic drug, in synthetic and urban wastewater [49]. In another study, electro-Fenton was applied to remove NSAIDs diclofenac, naproxen, and ibuprofen from the sewage effluent [50]. In these and other studies, real wastewater had to be amended with 0.3–0.5 mM Fe^{2+} and acidified to pH 2.8–3 to enable the Fenton's reaction. The requirement for the addition of iron and acid and the need to neutralize the treated effluent represent major limitations for the upscale of Fenton-based electrochemical processes.

Due to their exceptional modularity, electrochemical systems can be easily combined with other physicochemical water treatment processes. For example, Zhi et al. [51] combined ozonation and Ti_4O_7 porous anode typically considered as a reactive electrochemical membrane (REM), to achieve a more complete removal of tetracycline, propranolol, ciprofloxacin, and sulfamethoxazole. The energy consumption of the O_3-REM-coupled process was 101.5 kWh kg_{TOC}^{-1}, significantly lower than those of O_3 (241.2 kWh kg_{TOC}^{-1}) and Ti_4O_7 REM (121.5 kWh kg_{TOC}^{-1}), for achieving similar TOC removal (around 77%). In another study, 3D electrochemical system with a packed bed of granular activated carbon (GAC) was combined with ozonation for the treatment of pharmaceutical wastewater [52]. Addition of ozone and electric field enabled in situ regeneration of GAC, enhanced TOC removal, and caused a significant decrease in toxicity to *Vibrio fischeri*.

5 Three-Dimensional, Flow-Through Electrochemical Systems and Development of Electrochemical Membranes

Poor current efficiency and consequently high energy consumption for the removal of pharmaceuticals and other trace organic pollutants have been a major impediment for the upscaling and application of electrochemical systems in water and wastewater treatment. Conventional 2D plate-type electrochemical reactors are characterized by high diffusion limitations and reactions with the electrogenerated $HO^{\bullet}$ limited to a thin boundary layer (~100 μm) at the anode surface. These diffusion limitations can be significantly reduced using flow-through, 3D systems, and porous electrodes that offer higher specific surface area and reduced mass transfer limitations for electrolytic degradation of trace contaminants [13, 43]. However, prerequisite for such systems is the existence of electrocatalytically active, stable porous 3D anode materials. Currently, BDD- and MMO-coated anodes cannot be developed in 3D geometries. Some researchers used carbon-based anodes coated with active catalysts, given that typical carbon-based materials have low electrocatalytic activity for both oxidative and reductive transformation of pollutants. For example, Zhai et al. used MnO catalyst confined in graphite felt for electro-assisted catalytic wet air oxidation of ibuprofen, tetracycline, and other pollutants [53]. Bubbling of oxygen at the bottom of the reactor and polarization of the anode at low potential of 1.0 V/SHE enabled chemisorption of O_2 on MnO, activation of the chemisorbed oxygen species via electrooxidation, and oxidation of organic pollutants by the activated chemisorbed oxygen species. The stability of Mn and other metal catalysts on carbon electrodes may be an issue when dealing with real waste streams due to their high reactivity and potential for leaching.

In an effort to reduce the energy consumption of electrochemical systems, researchers have proposed different porous electrode materials for advection-enhanced mass transfer of trace organic pollutants, based on carbon nanotubes (CNTs), Ti_4O_7, and graphene, among others (Table 2) [60]. Due to the ease and simplicity of producing porous thin films by filtration of CNTs-based ink, CNT-based conductive membranes have been widely investigated for filtration, adsorption, and electrochemical degradation of pharmaceuticals and other organic pollutants [61–63]. Liu et al. applied a multi-walled CNTs (MWCNTs)-based filter for adsorption and electrochemical degradation of tetracycline [54]. They obtained efficient removal of tetracycline with an oxidative flux of 0.025 mol h^{-1} m^{-2} at total cell potential of 2.5 V when using MWCNT anode and Ti cathode, whereas oxidative flux was calculated according to Eq. (14):

$$\text{Electrooxidation flux} = \frac{\left[C\text{in} - C\text{out}\left(\frac{\text{mol}}{\text{L}}\right)\right] * \text{flow rate}\left(\frac{L}{h}\right)}{\text{Effective filter area (m2)}} \tag{14}$$

Table 2 Overview of the studies on electrochemical removal of pharmaceuticals using porous, flow-through electrode materials

Reactor configuration		Operational conditions						
Anode	Cathode	Supporting electrolyte	Flux ($L\ m^{-2}\ h^{-1}$)	E_{WE} (V/SHE)	Target pharmaceutical, initial concentration (μM)	% removal, oxidation flux ($\mu mol\ h^{-1}\ m^{-2}$)	Energy consumption	Ref.
CNT (WE)	CNT	10 mM Na_2SO_4, –	52	0.7	Tetracycline, 200 μM	>99%, 3.80	0.7 kWh kg_{COD}^{-1}	[54]
Carboxilated CNTs (WE)	Ti	10 mM NaCl, pH 6	70.6	2.2	Ibuprofen, 96 μM	79–82%, 1.11	n.r.	[55]
MWCNTs PTFE (WE)	SS	20 mM NaCl, pH 3	176.5	3.2	Amoxicillin, 13 μM Sulfamethoxazole, 20 μM Ciprofloxacin, 15 μM	Amox. 98%; 0.07 Sulfameth. 90%; 0.10 Ciproflox. 75%, 0.06	n.r.	[56]
Graphene nanoplateles/CNT (WE)	Ti	10 mM Na_2SO_4, –	52.9	1.0	Tetracycline, 100 μM	88%, 1.66	n.r.	[57]
Pt	Graphene e-Fenton PTFE	100 mM Na_2SO_4, pH 5.9	20.8	−0.4	Florfenicol, 2.7 μM	90%, 0.11	111 ± 5.5 kWh kg^{-1}	[58]
BDD	Pd-Cu/Ti_4O_7 (WE)	100 mM KH_2PO_4, pH 4.7	300	−1.14	Sulfamethoxazole, 100 μM	96.1%, 0.32	0.33 ± 0.04 kWh m^{-3}	[59]

CNT carbon nanotube membrane electrode, *SS* stainless steel, *BDD* boron-doped diamond, *WE* working electrode

Oxidative flux was increased 2.3-fold when Ti cathode was replaced with MWCNT cathode, which also resulted in a decrease in total cell potential to 1 V due to a higher cathode surface area and thus larger distribution of current, resulting in lower cathodic current density. When the electrochemical filter was applied on drinking water and wastewater effluent samples spiked with tetracycline (0.2 mM), the target antibiotic was completely removed within short residence time (<2 s) and had an oxidative flux of 0.015 and 0.022 mol h^{-1} m^{-2}, respectively (energy consumption of 0.084 kWh m^{-3}). Similar MWCNT-based anode filter was employed for the removal of sulfamethoxazole, ciprofloxacin, and amoxicillin [56]. Although the target antibiotics were adsorbed initially to some extent, breakthrough and increase in effluent concentrations were observed already after the first 5–10 min of continuous operation. Nevertheless, application of anode potentials above 2 V vs Ag/AgCl yielded a steady 70–80% removal of target antibiotics, and the major degradation pathways involved hydroxylation, cleavage of sulfonamide bond and oxidation of aniline moiety (in sulfamethoxazole), oxidation of quinolone ring and the reaction at the piperazinyl ring (in ciprofloxacin), and fracture of the β-lactam ring and oxidation of sulfur atom (in amoxicillin). Besides pharmaceutical and other trace organic pollutant removal (e.g., pesticides, dyes), CNT-based porous electrochemical filters have also been explored for a variety of applications ranging from anti-fouling to viral and bacterial inactivation [64–66]. To enhance the CNT membrane performance and increase its electrocatalytic activity, several authors used atomic doping (boron and nitrogen), nanoparticle functionalization (addition of iron to induce Fenton reactions), and polymeric coatings (e.g., addition of polyaniline to increase conductivity and enhance anodic stability) [67, 68]. For example, electroactive membrane based on MWCNTs and MWCNTs doped with nano-zerovalent iron (nZVI) was applied for sorption and electrochemical oxidation of metoprolol at very low applied potentials, i.e., 0.75 and 1.2 V/SHE, achieving 55% and 97% removal, respectively [69]. nZVI was considered to enhance the electroactive membrane performance through Fenton-type reactions and liberation of Fe^{2+}, and main degradation products identified (e.g., α-hydroxy and α-keto metoprolol, O-desmethyl metoprolol, metoprolol-carboxylic acid) suggested $HO^{\bullet}$-induced transformation. Addition of iron functionalities was also employed to enable the electro-Fenton in a flow-through system using a porous cathodic membrane based on reduced graphene oxide (RGO) [58]. The authors reported 60% mineralization efficiency of antibiotic florfenicol RGO membrane functionalized by -$COOFe^{2+}$ groups and cathodically polarized to −0.6 V/SHE and no mineralization when using RGO membrane without the added iron functionalities. This was enabled by the simultaneous reduction of supplied O_2 to H_2O_2 and its activation to $HO^{\bullet}$ by the -$COOFe^{2+}$ groups introduced into the RGO layer. Despite the efforts made to stabilize CNT-based electrodes, their mechanical stability and electrode degradation remain a handicap for application of this material in electrochemical water treatment. In addition, CNTs are characterized by excessive cost, toxicity, as well as several intrinsic limitations, i.e., low $HO^{\bullet}$ production efficiency; low onset potential for OER (approximately 1 V vs SHE), and consequently low current

efficiency for the degradation of pollutants; and continuous degradation of the anode membrane/electrode at anodic potentials higher than 4 V/SHE [70–72].

In the last 5 years, Magnéli phase Ti_4O_7 anodes have gained significant attention as a promising porous electrode material with high conductivity (1,000 mS cm^{-1}), high corrosion resistance (both to acids and bases), and higher stability under anodic polarization compared with CNTs electrodes [11, 72]. Porous Ti_4O_7 also have a large specific surface area (2.9×10^6 m^{-1}) relative to BDD electrodes (1.0×10^3 m^{-1}), although the electroactive surface area is typically significantly lower than the total surface area due to the poor accessibility of the electrolyte to a large portion of the electrode [6]. All these properties, along with the high capacity for generation of $HO^{\bullet}$, have been exploited for electrooxidation of a range of trace organic pollutants, including antibiotics and other pharmaceuticals [59, 60]. The manufacturing procedure of Ti_4O_7 enables the production of flow-through materials with controlled porosity. The general fabrication method consists of sintering a porous TiO_2 material (tubes, plates, rods, or pellets), followed by a second thermal process where temperatures are increased to 1,000°C under hydrogen atmosphere [11]. Chaplin et al. first applied Magnéli phase-based reactive electrochemical membranes for the degradation of oxalic and terephthalic acid by the electrogenerated $HO^{\bullet}$ [11]. Trellu et al. applied a cylindrical porous anode based on a mixture of Ti_4O_7 and Ti_5O_9 Magnéli phases in cross-flow filtration mode, with the stainless steel rod cathode placed in the center, for the degradation of refractory organic pollutants [73]. Acetaminophen (0.1–2.3 mM) in 50 mM Na_2SO_4 electrolyte was mineralized with 47% current efficiency at 150 A m^{-2}. The authors emphasized the role of both $HO^{\bullet}$ and DET in degrading this pharmaceutical, given that some of the intermediates formed by $HO^{\bullet}$ attack (e.g., 1,4-benzoquinone, oxalic acid) are susceptible only to direct electrolysis. In addition, the authors observed anode fouling at higher organic flux due to the polymerization of aromatic compounds. Magnéli phase materials are also a suitable substrate for the incorporation of catalytic materials or nanoparticles in order to further increase the HO• production or the reductive capacity of REM (e.g., by the addition of Pd). For example, bismuth-doped tin oxide has been incorporated in a Ti_4O_7 substrate by simple electrodeposition and subsequent mild thermal oxidation in water, resulting in higher HO• production compared to the undoped electrode [72]. Also, electrochemical reduction of sulfamethoxazole was improved from 3.8% for the Ti_4O_7 REM to 96.1% for the Pd-Cu-doped REM at −1.14 V/SHE (energy consumption 0.33 kWh m^{-3}) [59]. The reduction was found to proceed through N-O bond scission at the isoxazole ring. When Ti_4O_7 REM was applied as an anode, 95.7% of sulfamethoxazole was removed at 2.03 V/SHE, with an energy consumption of 0.27 kW hm^{-3}, but also enabled mineralization, on the contrary to the reductive transformation. Ti_4O_7 electrodes seem to be a very promising material for the removal of pharmaceuticals and other trace organic pollutants from water. Nevertheless, their performance is yet to be evaluated in environmentally relevant conditions of typical conductivity of wastewater (1.5–3 mS cm^{-1}), groundwater (0.5–2 mS cm^{-1}) and other contaminated water matrices, and also for low ng – μg L^{-1} concentrations of pharmaceuticals. Ti_4O_7 anodes suffer from similar limitations as other anode materials in terms

of the formation of toxic chlorinated byproducts in the presence of Cl^-, i.e., organochlorine compounds and inorganics ClO_3^- and ClO_4^- [35]. Also, more research is needed about the stability of Ti_4O_7 REMs during long-term operation, given that some authors reported irreversible passivation during anodic polarization [74], while other observed restoration of the anode electrocatalytic activity after cathodic polarization [75].

6 Conclusions and Outlook

In order to decrease the energy consumption of electrochemical systems applied for the removal of pharmaceuticals and other persistent trace contaminants, research on novel porous, 3D anode materials with high electrocatalytic activity and stability is essential. Nevertheless, it is crucial to conduct the experiments under environmentally relevant conditions in terms of electrolyte conductivity and composition and contaminant concentration. If a new electrode material is developed, the authors should report its electrochemical stability, for example, by performing accelerated service life tests. Special attention should be paid to the potential of anode materials to generate chlorine and thus induce the formation of chlorinated byproducts, as this is one of the main bottlenecks of electrochemical water treatment. Wider-scale application of electrochemical water treatment systems can only be enabled by addressing their limitations and searching for solutions to minimize the energy consumption and formation of organic and inorganic halogenated byproducts.

Several porous electrode materials have been recently proposed as means to reduce the energy consumption of electrochemical process. Among these, CNT- and Magnéli-based electrochemical membranes are attractive materials due to their high electrical conductivity, specific surface area, and possibility of functionalization. These two materials open the way to pore control and subsequently production of flow-through electrodes with confined active areas in the nanometer to micrometer range. Their production is relatively simple and based on the use of ink solutions (i.e., CNTs) or combustion under reductive atmosphere and sintering (i.e., Magnéli). However, low E_{EO} values and high removal rates for the degradation of trace pollutants are typically achieved when surface functionalization is introduced, e.g., through doping of Sb, Sn, Bi, or Fe_2O_3. This introduces a risk of leaching of toxic metals that needs to be carefully evaluated during electrode operation, particularly at high anode potentials typically required for the degradation of more persistent pollutants. In addition, both CNT and Magnéli phase REMs are yet to be evaluated for low-conductivity electrolyte solutions and low initial contaminant concentration.

More recently, graphene-based membranes were proposed to precisely control the molecular size of elements passed to the permeate side by regulating the interlayer distance between graphene 2D sheets [76]. The ability of graphene membranes to be functionalized (by π-π stacking and oxygen functional groups) is similar to CNTs, although graphene-based materials have more standardized

properties and homogeneity in their production in terms of final size, thickness, and electrical properties [61]. In their pioneering study, Zhou et al. demonstrated that it was possible to precisely control water permeation and flux of graphene-based membranes by applying current, from ultrafast permeation to complete blocking [77]. This was achieved by the application of current to the conductive filaments in the graphene oxide membrane that leads to ionization of the water molecules inside the graphene capillaries and impedes water transport. Graphene-based membranes have been rarely adopted for electrochemical removal of trace organic pollutants [57, 58, 78]. Nevertheless, RGO is a low-cost material that offers interesting opportunities for producing 3D macroscale foams with controlled pore structure, size, and degree of RGO reduction by simple methodologies such as hydrothermal or 3D printing [79, 80]. During the synthesis of RGO materials, their electrical, mechanical, and physicochemical properties (e.g., hydrophobicity) can be tailored for a specific water treatment application. In addition, graphene offers an excellent matrix for functionalization and introduction of other 2D materials (e.g., MXenes, hexagonal boron nitride – hBN) and thus further fine-tuning of their electrocatalytic performance. These and other novel materials offer emerging opportunities for electrochemical systems and different ways to architecture a water treatment system, by designing reactors with directional flows depending on the nanostructures and catalytic improvements made to enhanced surface exposure.

References

1. Pelskov YV, Sakharova AY, Krotova MD, Bouilov LL, Spitsyn BV (1987) Photoelectrochemical properties of semiconductor diamond. J Electroanal Chem Interfacial Electrochem 228:19–27
2. Carey JJ, Christ Jr CS, Lowery SN (1995) Electrolysis of liquid wastes using a doped diamond anode to oxidize solutes. US Patent 5, 247 (Ed.)
3. Panizza M, Cerisola G (2009) Direct and mediated anodic oxidation of organic pollutants. Chem Rev 109:6541–6569
4. Kolbrecka K, Przyluski J (1994) Sub-stoichiometric titanium oxides as ceramic electrodes for oxygen evolution—structural aspects of the voltammetric behaviour of TinO2n−1. Electrochim Acta 39:1591–1595
5. Vinokur N, Miller B, Avyigal Y, Kalish R (1996) Electrochemical behavior of boron-doped diamond electrodes. J Electrochem Soc 143:L238–L240
6. Chaplin BP (2019) The Prospect of electrochemical technologies advancing worldwide water treatment. Acc Chem Res 52:596–604
7. Comninellis C, Chen G (2010) Electrochemistry for the environment. Springer, Berlin
8. Yang Y, Kao LC, Liu Y, Sun K, Yu H, Guo J, Liou SY, Hoffmann MR (2018) Cobalt-doped black TiO_2 nanotube array as a stable anode for oxygen evolution and electrochemical wastewater treatment. ACS Catal 8(5):4278–4287
9. Oriol R, Clematis D, Brillas E, Cortina JL, Panizza M, Sirés I (2019) Groundwater treatment using a solid polymer electrolyte cell with mesh electrodes. ChemElectroChem 6(4):1235–1243
10. Bunce NJ, Bejan D (2014) Pollutants in water – electrochemical remediation using Ebonex electrodes. In: Kreysa G, Ota K-i, Savinell RF (eds) Encyclopedia of applied electrochemistry. Springer, New York, pp 1629–1633

11. Nayak S, Chaplin BP (2018) Fabrication and characterization of porous, conductive, monolithic Ti4O7 electrodes. Electrochim Acta 263:299–310
12. Radjenovic J, Bagastyo A, Rozendal RA, Mu Y, Keller J, Rabaey K (2011) Electrochemical oxidation of trace organic contaminants in reverse osmosis concentrate using RuO2/IrO2-coated titanium anodes. Water Res 45:1579–1586
13. Radjenovic J, Sedlak DL (2015) Challenges and opportunities for electrochemical processes as next-generation technologies for the treatment of contaminated water. Environ Sci Technol 49:11292–11302
14. Bagastyo AY, Batstone DJ, Kristiana I, Escher BI, Joll C, Radjenovic J (2014) Electrochemical treatment of reverse osmosis concentrate on boron-doped electrodes in undivided and divided cell configurations. J Hazard Mater 279:111–116
15. Farhat A, Keller J, Tait S, Radjenovic J (2015) Removal of persistent organic contaminants by electrochemically activated sulfate. Environ Sci Technol 49(24):14326–14333
16. Farhat A, Keller J, Tait S, Radjenovic J (2017) Assessment of the impact of chloride on the formation of chlorinated by-products in the presence and absence of electrochemically activated sulfate. Chem Eng J 330:1265–1271
17. Radjenovic J, Petrovic M (2017) Removal of sulfamethoxazole by electrochemically activated sulfate: implications of chloride addition. J Hazard Mater 333:242–249
18. Farhat A, Keller J, Tait S, Radjenovic J (2018) Oxidative capacitance of sulfate-based boron-doped diamond electrochemical system. Electrochem Commun 89:14–18
19. Rashid R, Mark F, Schuchmann HP, von Sonntag C (1991) SO·−4-induced oxidation of 1,3,6-trimethyluracil and 1,3,5-trimethyluracil (1,3-dimethylthymine) by potassium peroxodisulphate in aqueous solution: an interesting contrast. Int J Radiat Biol 59:1081–1100
20. Schuchmann H-P, Deeble DJ, Olbrich G, Von Sonntag C (1987) The SO− ·4-induced chain reaction of 1,3-dimethyluracil with Peroxodisulphate. Int J Radiation Biol Related Stud Phys Chem Med 51:441–453
21. Cai J, Zhou M, Liu Y, Savall A, Groenen Serrano K (2018) Indirect electrochemical oxidation of 2,4-dichlorophenoxyacetic acid using electrochemically-generated persulfate. Chemosphere 204:163–169
22. Shin Y-U, Yoo H-Y, Ahn Y-Y, Kim MS, Lee K, Yu S, Lee C, Cho K, Kim H-i, Lee J (2019) Electrochemical oxidation of organics in sulfate solutions on boron-doped diamond electrode: multiple pathways for sulfate radical generation. Appl Catal B Environ 254:156–165
23. Matzek LW, Tipton MJ, Farmer AT, Steen AD, Carter KE (2018) Understanding electrochemically activated Persulfate and its application to ciprofloxacin abatement. Environ Sci Technol 52:5875–5883
24. Song H, Yan L, Ma J, Jiang J, Cai G, Zhang W, Zhang Z, Zhang J, Yang T (2017) Nonradical oxidation from electrochemical activation of peroxydisulfate at Ti/Pt anode: efficiency, mechanism and influencing factors. Water Res 116:182–193
25. Barazesh JM, Prasse C, Sedlak DL (2016) Electrochemical transformation of trace organic contaminants in the presence of halide and carbonate ions. Environ Sci Technol 50:10143–10152
26. Cai J, Niu T, Shi P, Zhao G (2019) Boron-doped diamond for hydroxyl radical and sulfate radical anion Electrogeneration, transformation, and voltage-free sustainable oxidation. Small 15:1900153
27. Zhang K, Parker KM (2018) Halogen radical oxidants in natural and engineered aquatic systems. Environ Sci Technol 52:9579–9594
28. Jasper JT, Yang Y, Hoffmann MR (2017) Toxic byproduct formation during electrochemical treatment of latrine wastewater. Environ Sci Technol 51:7111–7119
29. Radjenovic J, Escher BI, Rabaey K (2011) Electrochemical degradation of the β-blocker metoprolol by Ti/Ru0.7Ir0.3O2 and Ti/SnO2-Sb electrodes. Water Res 45:3205–3214
30. Anglada Á, Urtiaga A, Ortiz I, Mantzavinos D, Diamadopoulos E (2011) Boron-doped diamond anodic treatment of landfill leachate: evaluation of operating variables and formation of oxidation by-products. Water Res 45:828–838

31. Bagastyo AY, Radjenovic J, Mu Y, Rozendal RA, Batstone DJ, Rabaey K (2011) Electrochemical oxidation of reverse osmosis concentrate on mixed metal oxide (MMO) titanium coated electrodes. Water Res 45:4951–4959
32. Pérez G, Fernández-Alba AR, Urtiaga AM, Ortiz I (2010) Electro-oxidation of reverse osmosis concentrates generated in tertiary water treatment. Water Res 44:2763–2772
33. Bagastyo AY, Batstone DJ, Kristiana I, Gernjak W, Joll C, Radjenovic J (2012) Electrochemical oxidation of reverse osmosis concentrate on boron-doped diamond anodes at circumneutral and acidic pH. Water Res 46:6104–6112
34. Garcia-Segura S, Keller J, Brillas E, Radjenovic J (2015) Removal of organic contaminants from secondary effluent by anodic oxidation with a boron-doped diamond anode as tertiary treatment. J Hazard Mater 283:551–557
35. Wang L, Lu J, Li L, Wang Y, Huang Q (2020) Effects of chloride on electrochemical degradation of perfluorooctanesulfonate by Magnéli phase Ti4O7 and boron doped diamond anodes. Water Res 170:115254
36. Merkle FH, Discher CA (1964) Electrochemical oxidation of chlorpromazine hydrochloride. J Pharm Sci 53:620–623
37. García-Espinoza JD, Mijaylova-Nacheva P, Avilés-Flores M (2018) Electrochemical carbamazepine degradation: effect of the generated active chlorine, transformation pathways and toxicity. Chemosphere 192:142–151
38. Ouarda Y, Bouchard F, Azaïs A, Vaudreuil M-A, Drogui P, Dayal Tyagi R, Sauvé S, Buelna G, Dubé R (2019) Electrochemical treatment of real hospital wastewaters and monitoring of pharmaceutical residues by using surrogate models. J Environ Chem Eng 7:103332
39. Mordačíková E, Vojs M, Grabicová K, Marton M, Michniak P, Řeháček V, Bořík A, Grabic R, Bruncko J, Mackuľak T, Vojs Staňová A (2020) Influence of boron doped diamond electrodes properties on the elimination of selected pharmaceuticals from wastewater. J Electroanal Chem 862:114007
40. Urtiaga AM, Pérez G, Ibáñez R, Ortiz I (2013) Removal of pharmaceuticals from a WWTP secondary effluent by ultrafiltration/reverse osmosis followed by electrochemical oxidation of the RO concentrate. Desalination 331:26–34
41. Rodríguez-Nava O, Ramírez-Saad H, Loera O, González I (2016) Evaluation of the simultaneous removal of recalcitrant drugs (bezafibrate, gemfibrozil, indomethacin and sulfamethoxazole) and biodegradable organic matter from synthetic wastewater by electro-oxidation coupled with a biological system. Environ Technol 37:2964–2974
42. Yang N, Yu S, Macpherson JV, Einaga Y, Zhao H, Zhao G, Swain GM, Jiang X (2019) Conductive diamond: synthesis, properties, and electrochemical applications. Chem Soc Rev 48:157–204
43. Chaplin BP (2014) Critical review of electrochemical advanced oxidation processes for water treatment applications. Environ Sci Process Impacts 16:1182–1203
44. Radjenovic J, Petrovic M (2016) Sulfate-mediated electrooxidation of X-ray contrast media on boron-doped diamond anode. Water Res 94:128–135
45. Mu Y, Radjenovic J, Shen J, Rozendal RA, Rabaey K, Keller J (2010) Dehalogenation of Iodinated X-ray contrast media in a bioelectrochemical system. Environ Sci Technol 45:782–788
46. Radjenovic J, Flexer V, Donose BC, Sedlak DL, Keller J (2013) Removal of the X-ray contrast media diatrizoate by electrochemical reduction and oxidation. Environ Sci Technol 47:13686–13694
47. Oturan N, Oturan MA (2018) Chapter 8 – electro-fenton process: background, new developments, and applications. In: Martínez-Huitle CA, Rodrigo MA, Scialdone O (eds) Electrochemical water and wastewater treatment. Butterworth-Heinemann, pp 193–221
48. Brillas E (2014) A review on the degradation of organic pollutants in waters by UV photoelectro-Fenton and solar photoelectro-Fenton. J Braz Chem Soc 25:393–417

49. Ridruejo C, Centellas F, Cabot PL, Sirés I, Brillas E (2018) Electrochemical Fenton-based treatment of tetracaine in synthetic and urban wastewater using active and non-active anodes. Water Res 128:71–81
50. Villanueva-Rodríguez M, Bello-Mendoza R, Hernández-Ramírez A, Ruiz-Ruiz EJ (2019) Degradation of anti-inflammatory drugs in municipal wastewater by heterogeneous photocatalysis and electro-Fenton process. Environ Technol 40:2436–2445
51. Zhi D, Wang J, Zhou Y, Luo Z, Sun Y, Wan Z, Luo L, Tsang DCW, Dionysiou DD (2020) Development of ozonation and reactive electrochemical membrane coupled process: enhanced tetracycline mineralization and toxicity reduction. Chem Eng J 383:123149
52. Zhan J, Li Z, Yu G, Pan X, Wang J, Zhu W, Han X, Wang Y (2019) Enhanced treatment of pharmaceutical wastewater by combining three-dimensional electrochemical process with ozonation to in situ regenerate granular activated carbon particle electrodes. Sep Purif Technol 208:12–18
53. Zhai L-F, Duan M-F, Qiao M-X, Sun M, Wang S (2019) Electro-assisted catalytic wet air oxidation of organic pollutants on a MnO@C/GF anode under room condition. Appl Catal B Environ 256:117822
54. Liu Y, Liu H, Zhou Z, Wang T, Ong CN, Vecitis CD (2015) Degradation of the common aqueous antibiotic tetracycline using a carbon nanotube electrochemical filter. Environ Sci Technol 49:7974–7980
55. Bakr AR, Rahaman MS (2016) Electrochemical efficacy of a carboxylated multiwalled carbon nanotube filter for the removal of ibuprofen from aqueous solutions under acidic conditions. Chemosphere 153:508–520
56. Tan T-Y, Zeng Z-T, Zeng G-M, Gong J-L, Xiao R, Zhang P, Song B, Tang W-W, Ren X-Y (2020) Electrochemically enhanced simultaneous degradation of sulfamethoxazole, ciprofloxacin and amoxicillin from aqueous solution by multi-walled carbon nanotube filter. Sep Purif Technol 235:116167
57. Liu Y, Dustin Lee JH, Xia Q, Ma Y, Yu Y, Lanry Yung LY, Xie J, Ong CN, Vecitis CD, Zhou Z (2014) A graphene-based electrochemical filter for water purification. J Mater Chem A 2:16554–16562
58. Jiang W-L, Xia X, Han J-L, Ding Y-C, Haider MR, Wang A-J (2018) Graphene modified electro-Fenton catalytic membrane for in situ degradation of antibiotic Florfenicol. Environ Sci Technol 52:9972–9982
59. Misal SN, Lin M-H, Mehraeen S, Chaplin BP (2020) Modeling electrochemical oxidation and reduction of sulfamethoxazole using electrocatalytic reactive electrochemical membranes. J Hazard Mater 384:121420
60. Trellu C, Chaplin BP, Coetsier C, Esmilaire R, Cerneaux S, Causserand C, Cretin M (2018) Electro-oxidation of organic pollutants by reactive electrochemical membranes. Chemosphere 208:159–175
61. Ando T (2009) The electronic properties of graphene and carbon nanotubes. NPG Asia Mater 1:17–21
62. Sadia AJ, Zhi Z (2016) Electrochemical carbon nanotube filters for water and wastewater treatment. Nanotechnol Rev 5:41–50
63. Vecitis CD, Gao G, Liu H (2011) Electrochemical carbon nanotube filter for adsorption, desorption, and oxidation of aqueous dyes and anions. J Phys Chem C 115:3621–3629
64. Ajmani GS, Goodwin D, Marsh K, Fairbrother DH, Schwab KJ, Jacangelo JG, Huang H (2012) Modification of low pressure membranes with carbon nanotube layers for fouling control. Water Res 46:5645–5654
65. de Lannoy C-F, Jassby D, Gloe K, Gordon AD, Wiesner MR (2013) Aquatic biofouling prevention by electrically charged Nanocomposite polymer thin film membranes. Environ Sci Technol 47:2760–2768
66. Vecitis CD, Schnoor MH, Rahaman MS, Schiffman JD, Elimelech M (2011) Electrochemical multiwalled carbon nanotube filter for viral and bacterial removal and inactivation. Environ Sci Technol 45:3672–3679

67. Gao G, Vecitis CD (2011) Electrochemical carbon nanotube filter oxidative performance as a function of surface chemistry. Environ Sci Technol 45:9726–9734
68. Gao G, Vecitis CD (2012) Doped carbon nanotube networks for electrochemical filtration of aqueous phenol: electrolyte precipitation and phenol polymerization. ACS Appl Mater Interfaces 4:1478–1489
69. Yanez H, Wang Z, Lege S, Obst M, Roehler S, Burkhardt CJ, Zwiener C (2017) Application and characterization of electroactive membranes based on carbon nanotubes and zerovalent iron nanoparticles. Water Res 108:78–85
70. Duan W, Ronen A, Walker S, Jassby D (2016) Polyaniline-coated carbon nanotube ultrafiltration membranes: enhanced anodic stability for in situ cleaning and electro-oxidation processes. ACS Appl Mater Interfaces 8:22574–22584
71. Gao G, Zhang Q, Hao Z, Vecitis CD (2015) Carbon nanotube membrane stack for flow-through sequential regenerative electro-Fenton. Environ Sci Technol 49:2375–2383
72. Gayen P, Chen C, Abiade JT, Chaplin BP (2018) Electrochemical oxidation of atrazine and Clothianidin on Bi-doped SnO2–TinO2n–1 electrocatalytic reactive electrochemical membranes. Environ Sci Technol 52:12675–12684
73. Trellu C, Coetsier C, Rouch J-C, Esmilaire R, Rivallin M, Cretin M, Causserand C (2018) Mineralization of organic pollutants by anodic oxidation using reactive electrochemical membrane synthesized from carbothermal reduction of TiO2. Water Res 131:310–319
74. Waraksa CC, Chen G, Macdonald DD, Mallouk TE (2003) EIS studies of porous oxygen electrodes with discrete particles II. Transmission line modeling. J Electrochem Soc 150:E429–E437
75. El-Sherif S, Bejan D, Bunce NJ (2010) Electrochemical oxidation of sulfide ion in synthetic sour brines using periodic polarity reversal at Ebonex® electrodes. Can J Chem 88:928–936
76. Mi B (2014) Graphene oxide membranes for ionic and molecular sieving. Science 343:740–742
77. Zhou KG, Vasu KS, Cherian CT, Neek-Amal M, Zhang JC, Ghorbanfekr-Kalashami H, Huang K, Marshall OP, Kravets VG, Abraham J, Su Y, Grigorenko AN, Pratt A, Geim AK, Peeters FM, Novoselov KS, Nair RR (2018) Electrically controlled water permeation through graphene oxide membranes. Nature 559:236–240
78. Nidheesh PV (2017) Graphene-based materials supported advanced oxidation processes for water and wastewater treatment: a review. Environ Sci Pollut Res 24:27047–27069
79. Guo H, Lv R, Bai S (2019) Recent advances on 3D printing graphene-based composites. Nano Mater Sci 1:101–115
80. Yousefi N, Lu X, Elimelech M, Tufenkji N (2019) Environmental performance of graphene-based 3D macrostructures. Nat Nanotechnol 14:107–119

Sonochemical Advanced Oxidation Processes for the Removal of Pharmaceuticals in Wastewater Effluents

Efraím A. Serna-Galvis, Judy Lee, Félix Hernández, Ana María Botero-Coy, and Ricardo A. Torres-Palma

Contents

E. A. Serna-Galvis and R. A. Torres-Palma (✉)
Grupo de Investigación en Remediación Ambiental y Biocatálisis (GIRAB), Instituto de Química, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia UdeA, Medellín, Colombia
e-mail: efrain.serna@udea.edu.co; ricardo.torres@udea.edu.co

J. Lee
Department of Chemical and Process Engineering, University of Surrey, Guildford, Surrey, UK
e-mail: j.y.lee@surrey.ac.uk

F. Hernández and A. M. Botero-Coy
Research Institute for Pesticides and Water (IUPA), University Jaume I (UJI), Castellón, Spain
e-mail: hernandf@uji.es; botero@uji.es

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 349–382, DOI 10.1007/698_2020_665,

Abstract Pharmaceuticals in wastewater effluents represent a current environmental concern, which demands application of effective processes. In such regard, sonochemical advanced oxidation processes emerge as an attractive alternative, as illustrated herein. This chapter begins presenting some fundamental aspects of sonochemical treatments (e.g. effects of frequency, power and nature of pharmaceutical pollutants). In second place, the transformations of pharmaceuticals are described, considering topics such as the used analytical techniques and structural modifications of pollutants by chemical effects of ultrasound. Then, treatment of diverse wastewater containing pharmaceuticals are shown, paying special attention to degradations in complex matrices, reactors configurations and combination of ultrasound with membrane filtration processes. Final part is dedicated to highlight the key points presented along the chapter.

Keywords Advanced oxidation process, Environmental remediation, Pharmaceuticals degradation, Ultrasound, Wastewater treatment

1 Fundamental Aspects

1.1 Ultrasound as an Advanced Oxidation Process and Environmental Problem of Pharmaceuticals

Ultrasound comprises mechanical waves having frequencies from 20 to 10,000 kHz (20 kHz to 10 MHz). These waves are utilized in areas such as biomedical diagnostics, material synthesis and environmental chemistry/engineering, among other fields [1]. Herein, we will focus on environmental applications, specifically on the use of ultrasonic waves for degrading organic pharmaceuticals.

Pharmaceuticals are considered contaminants of emerging concern (CECs) because these substances are continuously introduced into the environment at very low concentrations (e.g. ng L^{-1} to μg L^{-1}); they may affect water quality, natural water supplies and ecosystems equilibrium [2]. Additionally, the regulation by governments about pharmaceuticals input into environment is still incipient. Thus, the environmental discharge of pharmaceutical must be limited, and ultrasound-based techniques can be utilized for such purpose.

The generation of radicals in ultrasound systems involves transient acoustic cavitation phenomenon. This consists of formation of bubbles (also called cavities, which contain oxygen gas and/or water vapour) in the liquid; due to the pass of ultrasound waves (expansion and contraction cycles), the cavities grow up until a critical size, and then, they violently collapse [3, 4]. During the collapse temperature and pressure within the cavity is enormous (e.g. 5,000 K and 1,000 atm); these critical conditions lead to rupture of water vapour and oxygen to form radicals

(Eqs. 1–4). Additionally, cavitation can also produce the emission of light (called sonoluminescence) [5, 6].

$$H_2O+))) \rightarrow H\bullet + HO\bullet \quad (1)$$

$$O_2+))) \rightarrow 2\,O\bullet \quad (2)$$

$$H_2O + O\bullet \rightarrow 2\,HO\bullet \quad (3)$$

$$O_2 + H\bullet \rightarrow O\bullet + HO\bullet \quad (4)$$

The formation of radicals by ultrasound can be demonstrated be means of spin trapping and electron spin resonance (ESR) technique [4]. Besides, the formation/accumulation of hydrogen peroxide (coming from recombination of radicals, Eq. 5) is an indirect way to prove the radical generation in an ultrasonic system [7, 8].

$$HO\bullet \rightarrow H_2O_2 \quad (5)$$

Ultrasound is considered an advanced oxidation process (AOP) because this can be used for degrading organic pollutants in aqueous matrices by action of the generated radicals [9]. In the aqueous systems submitted to ultrasound, it has been figured out three degradation zones: (1) interior of cavitation bubble, (2) interface of bubble-liquid and (3) bulk of liquid. Volatile compounds are pyrolysed inside bubble, whereas hydrophobic non-volatile and hydrophilic substances are mainly destroyed at the interface and liquid-bulk zones, respectively [3].

It must be mentioned that the efficiency of sonochemical degradation is influenced by operational parameters, chemical nature of pollutant, matrix components and reactor configuration. All these aspects are analysed and discussed in the following sections.

1.2 *Effect of Frequency and Power on Pharmaceuticals Degradation by Ultrasound*

Physical effects dominate at lower frequencies (20–100 kHz), chemical effects are more significant at frequencies in the range 100–1,000 kHz (1,000 kHz = 1 MHz), and at higher frequencies (>1 MHz) acoustic streaming effect are dominant [10].

For degradation of organic pollutants, frequencies among 20 kHz and 1.2 MHz are commonly used (Table 1). Pharmaceuticals such as cephalexin, diclofenac, estrogenic hormones and ofloxacin have been treated at low frequencies (20–100 kHz) and at very high powers or power densities. Likewise, high frequencies (among 100 kHz and 1.2 MHz) have been widely utilized for degrading acetaminophen, ampicillin, atenolol, levodopa, losartan and triclosan; nevertheless, lower powers (or power densities) than the required at frequencies below 100 kHz can be used. Table 1 shows that if frequencies are varied in the range 100 kHz to

Table 1 Pharmaceuticals degradation at different frequencies

Compound	Frequency (kHz)	Removal	Time (min)	Power conditions	Reference
Cephalexin	24	70% COD	150	Electric 200 W (acoustic 17.3 W)	[13]
Diclofenac	20	~50% Degradation	60	Electric: 100 W L^{-1}	[14]
Oestrogens mixture	20	~80–90% Degradation	25	Electric: 320 W (2,100 W L^{-1})	[15]
Mix of amoxicillin, carbamazepine and diclofenac	20	~20–50% Degradation	60	Electric: 100 W L^{-1}	[16]
Ofloxacin	20	31% Degradation	240	Electric: 640 W L^{-1}	[17]
Atenolol	200	[a]k: 0.01649	–	Electric: 50 W	[18]
	350	[a]k: 0.04476	–		
	620	[a]k: 0.03717	–		
	1,000	[a]k: 0.03720	–		
Acetaminophen	574	95% Degradation	240	Acoustic: 32 W	[19]
	860	92%	240		
Triclosan	574	90%	15	Acoustic: 140 W L^{-1}	[20]
	856	75%	15		
	1,134	72% Degradation	15		
Ampicillin	375	39% Degradation	30	Acoustic: 24.4 W	[8]
	990	26% Degradation	30		
Losartan	375	70% Degradation	30	Acoustic: 88 W L^{-1}	[12]
	575	54% Degradation	30		
	990	15% Degradation	30		

[a]Pseudo-first-order degradation rate constant (k, min^{-1})

1.2 MHz, the highest degradations are observed between 200 and 400 kHz, due to in such range occurs the highest production of radical species [11], which favours the pharmaceuticals degradation [8, 12].

In general, at a fixed power (or power density), the increasing of frequencies results in higher nucleation and production of bubbles that are comparatively smaller in size, for example, at 20 kHz, ~20,000 implosions per second could occur, whereas at 300 kHz, ~100,000 cavitation events per second are possible. This enhances the production of radicals and leads to a higher degradation of pharmaceuticals

[1, 7]. However, it should be indicated that an upper limit exists for the applied frequency above which the active cavitation for pollutants degradation diminishes. Then, if the bubbles have not enough size, the extent of collapse is low and fewer radicals are formed [1].

On the other hand, in the sonochemical systems, a transduction phenomenon (i.e. the transformation of electrical power into acoustic power) is involved. During such transformation, not all electrical power is converted into acoustic power. Then, the actual acoustic power should be determined to establish the sonochemical efficiency of the transducers [21, 22]. The actual acoustic power determination is typically done by calorimetric methods. The sonochemical energy can be assimilated to the calorimetric energy due to the rising up of the temperature in a fixed volume of water. This is represented by the Eq. 6, where *C*p is the specific heat capacity of the solvent (in J kg^{-1} K^{-1}), *m* is the mass of solvent (in kg) and $\Delta T/\Delta t$ means the temperature difference (in K) after a specific sonication time (in s) [21]. Therefore, the efficiency of the energy transduction is calculated as the ratio between the actual acoustic power and the electric power supplied to the system.

$$\text{Acoustic power} = m\ C\text{p}\ (\Delta T/\Delta t) \tag{6}$$

It is well-known that the increasing in the electric power (and consequently in the acoustic power) augments the degradation of pharmaceuticals [1, 18, 19, 22, 23]. A higher power delivers an increasing in the number of cavitation bubbles and the magnitude of the collapse, which lead to a higher production of radicals (and consequently a higher accumulation of H_2O_2, Fig. 1a), favouring the pollutants degradation [24–26], as exemplified in Fig. 1b. However, it should be indicated that excessive powers induce a decrease in the number of bubbles per volume, which is attributed to an increase in bubble coalescence, degassing and liquid agitation. Also, it can be remarked that there is a lower limit or threshold of power. Below such lower limit, the sound field is very small to induce nucleation or bubble growth; consequently, bubbles readily succumb to surface tension effects and dissolve, resulting in a limited cavitation activity [1].

It is important to indicate that although power and frequency are the primary physical parameters that influence sonochemical process, the elimination of pharmaceutical by ultrasound can also be modified by other operational parameters such as aqueous sample temperature, dissolved gas and operation mode (i.e. pulsed vs. continuous waves). Such topics are not considered in this chapter; however valuable information about them is available for reader in the references [1, 23, 27–30].

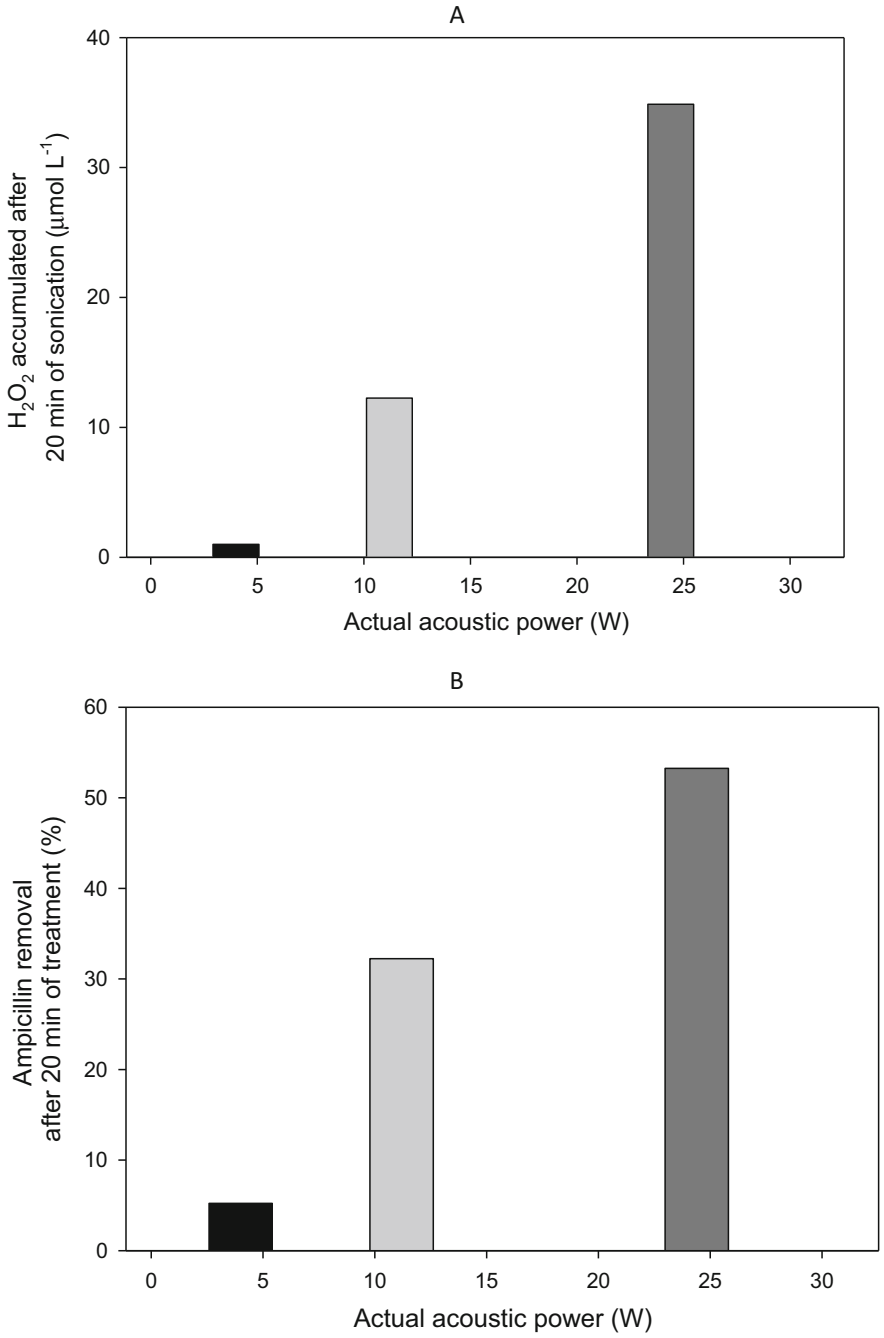


Fig. 1 Effect of power on the sonochemical system. (**a**) Accumulation of hydrogen peroxide (indicator of hydroxyl radical generation) as a function of the acoustic power. Experimental

1.3 *Effect of Pharmaceutical Nature on the Sonochemical Degradation*

Most of organic pharmaceuticals are non-volatile substances. Thereby, degradation of such pollutants in the sonochemical system mainly occurs at the interfacial zone and/or in the solution bulk. Some works have studied the correlation between sonochemical degradation rate for pollutants and physico-chemical parameters (e.g. molar mass, Henry's law constant, second-order reaction constant with hydroxyl radical), indicating that only one property cannot be utilized to predict the degradation of any compound by ultrasound. However, simple correlations may be useful when the considered compounds have similar/related structure [28, 31].

For example, a research about the sonochemical degradation of some antibiotics showed a good correlation between the initial degradation rate (Rd) and the octanol-water partition coefficient (Log *P*). The pharmaceuticals having higher Log *P* values were faster sonodegraded (Table 2). The Log *P* is a parameter related to hydrophobicity. The hydrophobic compounds tend to accumulate at the cavitation bubble interface, where they can have a high interaction with hydroxyl radicals and are prone to a faster degradation by ultrasound action [32].

The closeness of some pharmaceuticals to cavitation bubbles can be indirectly inferred by using interferent substances such as isopropanol (a volatile scavenger of radicals) and ferrous ion (a promoter of radicals in the solution bulk through Fenton reaction, Eq. 7) [12, 28]. Then, substances that due to their hydrophobic nature are placed close to the cavitation bubble will experiment a lower inhibition degradation degree in presence of the radical scavenger than the more hydrophilic ones. On the contrary, in the presence of the radical promoter, the hydrophobic pharmaceuticals are less accelerated than the hydrophilic compounds [12, 32, 33].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO\bullet \tag{7}$$

On the other hand, many pharmaceuticals have acid or basic functional groups on their structure. Hence, structural variations can be promoted by pH changes. This markedly influences the sonochemical degradation, due to modification of the hydrophilic/hydrophobic nature of the pollutants [15, 16, 23, 26]. In the case of molecules having acid moieties such as carboxylic acids and phenols (e.g. acetaminophen, dicloxacillin and naproxen), their degradations are more favoured at pH values below their pK_a of such functional groups, where predominates their non-charged form (Table 3). Meanwhile, pollutants with basic moieties such as amines (e.g. fluoxetine) have higher degradations at pH values above their

Fig. 1 (continued) conditions, 250 mL of distilled water; $pH_{initial}$, 5.6, 20°C, sonication at 375 kHz by using a Meinhardt reactor. (**b**) Removal of ampicillin at diverse acoustic powers. Experimental conditions, 250 mL of aqueous solution of ampicillin at 30 μmol L^{-1}; $pH_{initial}$, 6.5, 20°C, sonication at 375 kHz by using a Meinhardt reactor. Figure was built with own data of the authors

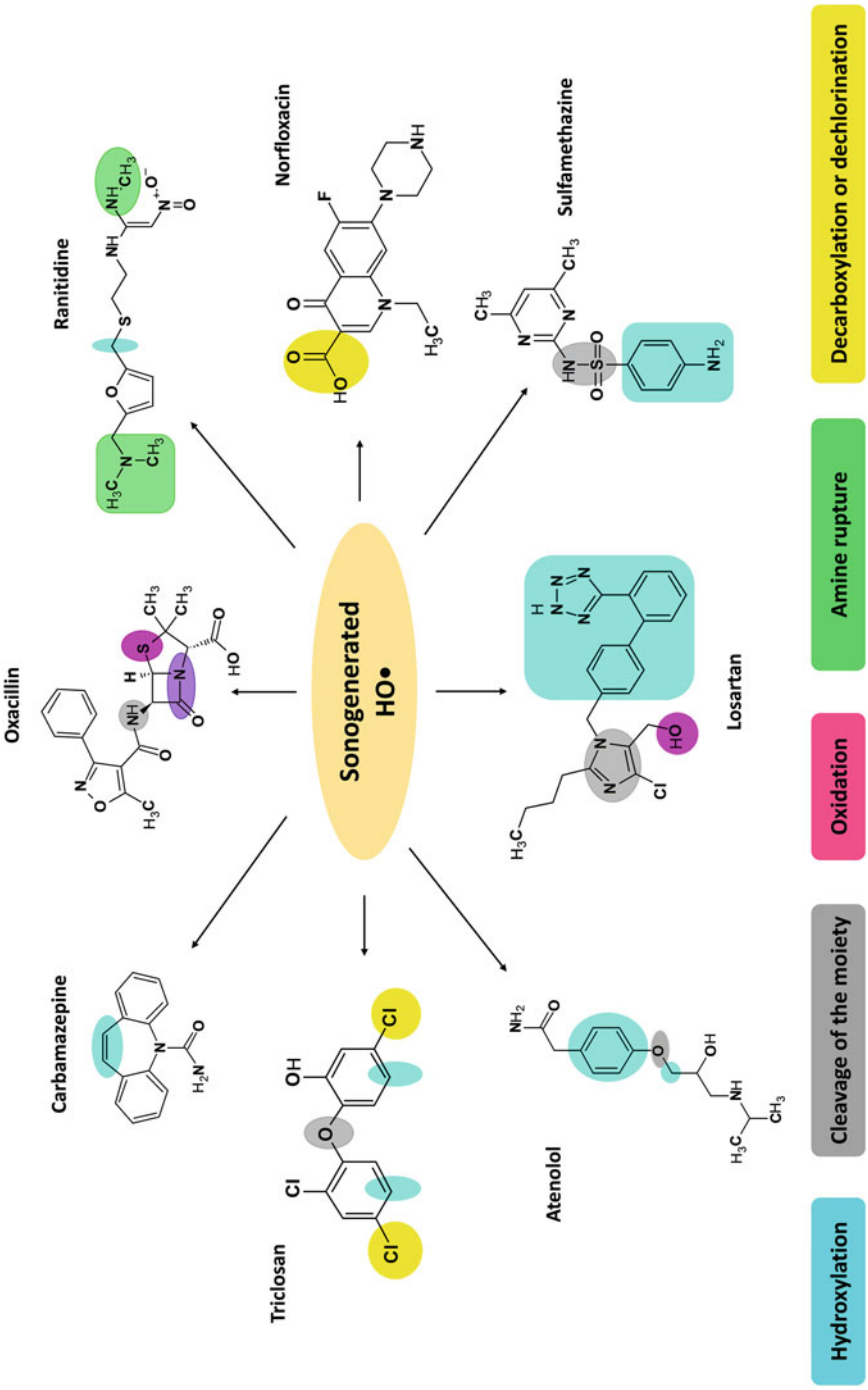


Fig. 2 Some examples of moieties on pharmaceuticals initially attacked by sonogenerated hydroxyl radical during degradation in pure water. Figure was made by the authors. In the case of sulfamethazine (antibiotic), the primary transformations lead to products of the aniline moiety hydroxylation, plus a breakdown of

the sulphonamide group (i.e. the S–N bond) [24]. Besides, the treatment of triclosan (antifungal) by sonochemistry generates compounds coming from hydroxylation, dechlorination and ether cleavage [20]. It can be highlighted that the hydroxyl radical (which has an electron-deficient nature) initially attacks the electron-rich regions (e.g. C=C double bonds, aromatic rings, thioethers, amines and ethers) or unstable moieties (e.g. highly strained rings as β-lactams) on the pharmaceuticals (Fig. 2). Moreover, the hydroxyl radical can also act through a hydrogen abstraction mechanism, which promotes decarboxylation and/or fragmentations

Table 2 Relationship between sonodegradation rate (Rd) of some pharmaceuticals and octanol-water partition coefficient (Log *P*)[a]

Pharmaceutical	Rd (μmol L^{-1})	Log *P*
Cefadroxil	0.4150	−0.40
Ciprofloxacin	0.5600	0.28
Cephalexin	0.7300	0.65
Cloxacillin	0.8600	2.48

[a]Table made by the authors based on Serna-Galvis et al. [32]

Table 3 Effect of pH on sonodegradation of pharmaceuticals

Pharmaceuticals	Acid/base groups	pH values tested	Best degradation at pH	References
Acetaminophen	Phenol (pK_a: 9.5)	3.0, 5.6, 9.5, 11.0 and 12.0	3.0 and 5.6	[26]
Dicloxacillin	Carboxylic acid (pK_a: 2.8)	3.0, 5.7 and 9.0	3.0	[25]
Oestrogens mixture	Phenols ($pK_a > 10$)	3.0, 7.0 and 9.0	3.0	[15]
Ibuprofen	Carboxylic acid (pK_a: 4.9)	3.0, 5.0 and 11.0	3.0	[34]
Naproxen	Carboxylic acid (pK_a: 4.2)	3.0, 6.0 and 10.5	3.0	[35]
Fluoxetine	Amine (pK_a: 10.05)	6.2 and 11.0	11.0	[36]
Cephalexin	Carboxylic acid (pK_a: 3.3–5.2) and amine (pK_a: 7.3)	4.5, 6.5, 7.5, 8.5 and 10	6.5	[13]
Sulfadiazine	Sulphonamide (pK_a: 2.1) and aniline (pK_a: 6.28)	3.0, 5.5, 7.0, 9.0 and 11.0	5.5	[37]

pK_a, where predominates their non-charged species. In the case of pharmaceuticals having both acid and basic moieties, which are able to form zwitterions (e.g. cephalexin and sulfadiazine), their degradations are more favoured at pH values where the zwitterion predominates (i.e. a pH between the pK_a of both groups). This is because such species has an equilibrium with its corresponding neutral non-charged structure (Eq. 8). Overall, the non-charged structures of molecules are more hydrophobic than their corresponding ionic forms (i.e. cation, anion or zwitterion). Therefore, the non-charged forms are closer to the cavitation bubble, which favours their degradation by action of the sonogenerated radicals.

$$^{+}H_3N - R - COO^{-} \leftrightarrow H_2N - R - COOH \tag{8}$$

2 Chemical Transformations of Pharmaceuticals upon Sonochemical Action

2.1 Analytical Techniques to Follow Pharmaceuticals Degradation and Transformation During Sonochemical Treatment

Studies on sonochemical treatments require the measurement of the concentrations of the target compounds under different treatment conditions and along the time. To this aim, it is necessary to apply reliable and sensitive analytical techniques in order to follow the concentrations changes of the parent compound during the treatment, as well as to evaluate the transformations suffered along the process. Ideally, the transformation products (TPs) formed should be elucidated and, if possible, the changes in their concentrations monitored. This implies the use of quantitative techniques for detection and identification of the compounds, particularly when experiments are performed in complex matrix samples, and for the determination of low analyte concentrations. In addition, powerful techniques able to give information about the chemical structures of the compounds and about potential modifications produced in the process, i.e., elucidation techniques, are also needed.

In this field, the importance of chromatographic techniques hyphenated to mass spectrometry is unquestionable. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with triple quadrupole (QqQ), or related analysers, such as QqQ-linear ion trap, e.g., QTRAP, is the technique of choice for quantitative analysis, due to the medium-high polarity of most pharmaceuticals and TPs, while liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) is typically used to identify/elucidate the TPs formed during the treatments [23, 38–42].

2.1.1 Quantitative Analysis

Quantitative analysis of pharmaceuticals in aqueous media, both in environmental studies and in laboratory degradation experiments, is typically performed by LC-MS/MS. This is the technique of choice by several reasons: excellent sensitivity and selectivity, robustness, wide linear dynamic range and fully compatible with aqueous samples without the need to make solvent exchange. There are hundreds of papers reporting the use of LC-MS/MS in the field of pharmaceutical analysis, and a detailed review is out of the scope of this chapter. Instead, some references can be given for those readers interested in this topic [12, 38, 50, 41, 43–49].

Although the advantages of LC-MS/MS are obvious in comparison with LC-based methods using conventional detectors, the latter are still applied in many laboratory experiments that are performed under controlled conditions using clean water (e.g. distilled or Milli-Q) or synthetic water of known composition. In these experiments, “clean” samples are spiked at known concentrations with the

pharmaceuticals under study, and spiking levels use to be at nearly, or above, the ppm levels. Under this "ideal" situation, conventional techniques, such as LC-UV, LC-FD or LC-DAD, can be used to follow the evolution of the concentrations, because there are not sensitivity constrains and/or the need of confirmation of the identity of the compound, as occurs in real-world complex water samples. Thus, a number of publications dealing with pharmaceutical analysis and/or sonochemical treatments report data produced by techniques other than LC-MS/MS [12, 49, 51]. However, when the experiments are performed with real-world (waste) water samples, it is necessary to apply more sensitive techniques, able to determine concentration levels at the ppb (μg L^{-1}) and ppt (ng L^{-1}) levels similar to those found in the samples. The situation can be in part alleviated if the samples are spiked at sufficiently high levels to facilitate monitoring the evolution of the concentrations along the treatment. However, the most critical issue is the correct identification and interferences removal when dealing with complex samples, e.g. wastewater. It is in these cases when the potential of LC-MS/MS is actually exploited due to its excellent performance for quantification. Besides, the acquisition of, at least, two selected reaction monitoring (SRM) transitions and the evaluation of ion ratios and chromatographic retention time allow the reliable identification of the compounds [43, 47]. An illustrative example is the elimination of 15 relevant pharmaceuticals in hospital wastewater after combination of a biological system and a sono-photo-chemical process, which was monitored by LC-MS/MS QqQ [52]. Similarly, LC-MS/MS analysis was used to assess the degradation by sonochemical advanced oxidation processes of 17 contaminants of emerging concern, selected on the basis of their occurrence in urban wastewaters [53].

Although LC-MS/MS is widely accepted for pharmaceutical analysis, there is an issue of major concern when applied to complex-matrix samples. This is matrix effects, which may dramatically affect the ionization of the analytes and consequently the quantification. Matrix effects, if no properly corrected or eliminated, may lead to notable errors in quantification, by default (ionization suppression) or by excess (ionization enhancement). The use of isotope-labelled internal standards (ILIS) is among the best solutions for matrix effects correction, although the own analyte-ILIS should be used for an efficient correction. This implies the acquisition of a notable number of ILIS in multi-residue multiclass methods where many pharmaceuticals are determined. The high cost and low availability of ILIS for some compounds is a limitation to apply this approach, which however is the recommended when only a few pharmaceuticals are under study [43].

Another interesting topic that has arisen in the last few years is the possibility of direct injection of samples in LC-MS/MS methods. Traditionally, a pre-concentration step has been applied for pharmaceuticals determination, typically based on solid-phase extraction (SPE) using different cartridges [38, 44, 46, 49]. However, recent advances in MS instrumentation, particularly in terms of sensitivity improvement, have made possible the direct injection of samples, reaching concentrations at the sub-ppb levels without any type of sample treatment. This is highly convenient, as it saves time and costs, and reduces the possibility of analytical errors associated with sample manipulation. With the LC-MS/MS

new-generation instrumentation, it is possible to avoid the SPE step [43, 54]. We will see in the near future an increase in applications of LC-MS/MS in the pharmaceuticals field making use of direct injection of samples.

We cannot end this section without emphasizing the importance of the quality control of the analyses. There is not discussion about the need to validate the analytical methods applied. However, even if a previous validation is made, it is necessary to apply strict rules for quality control in every batch of samples analysed. The fact that a method works well in a given sample (e.g. analysed by quintuplicate, with satisfactory recovery and precision) does not mean that it will give satisfactory results when applied to other samples, even if they are from the same type than the sample used for validation. Environmental water and wastewater samples, among others, are very different in nature and may have quite different composition and very distinct matrix effects. Therefore, it is necessary to ensure the quality of analysis in real-world samples. One of the best ways to guarantee the reliability of an analytical method is to analyse a notable number of quality control (QC) samples together with the samples under study. QCs are prepared by spiking "real-world blank" samples at known analyte concentrations. QC recoveries are used to assess the quality of data and must be typically between 60 and 140% to be acceptable. QC analysis is not only used to evaluate the recoveries, but the ion intensity ratios used for identification of the compounds [43]. On some occasions, it is hard to find true blank samples for preparation of QCs, due to the presence of the studied compound in all the samples analysed (e.g. some pharmaceuticals in wastewater). This fact makes problematic the recoveries calculation for QCs at low analyte concentration.

2.1.2 Techniques Used for Identification/Elucidation of Transformation Products (TPs)

In the most comprehensive studies, not only the evolution of the pharmaceutical concentration is pursued but also the potential transformation/degradation of the compound. While LC-MS/MS is highly appropriate for quantitative analysis, it is however limited for elucidation purposes. Here, HRMS is the preferred technique due to the useful information contained in accurate-mass full-spectrum acquisition data provided [55–57]. Unfortunately, other common elucidation techniques, such as NMR, IR or X-ray, have little application in degradation experiments because of their limited sensitivity and the low amount of the compound to be elucidated, which is present at very low concentrations in the aqueous media used in the degradation experiments. Gas chromatography-based techniques are rarely used because most of the species are in aqueous phase and contain polar groups [23]. So, LC-HRMS (with analysers such as (Q)Orbitrap or (Q)TOF) is the most applied. In general, the data obtained by this technique is the only information available [39, 40, 42, 58, 59]. This fact, added to the unknown nature of the TPs, makes the elucidation process very complicated [41, 60].

In most cases, only a tentative identification is possible. However, if such identification is well founded and supported by accurate-mass data, with a deep

knowledge of fragmentation rules, the information obtained and the chemical structure proposed for the TPs can be sufficient for the purpose of the work. Thus, the primary transformations of six antibiotics during the sonochemical treatment were studied through establishing the initial degradation products by LC-QTOF MS [32]. The final acquisition of the reference standard is the last step to confirm the identity of the TP, which is a hard task when many TPs are involved due to the low availability of reference standards. Under this situation, the only possibility for full confirmation of the identity is the synthesis of the candidate compound.

HRMS allows the application of different workflows for efficient TPs identification: target analysis for already known TPs with standards available; suspect screening for potential TPs reported in literature and/or predicted by in silico models; and non-target analysis for discovering unknown, non-reported nor previously identified TPs. Searching in environmental samples or in laboratory experiments for target TPs, with reference standards available, is not a big problem, as the accurate-mass information together with the chromatographic retention time and experimental fragments resulting from the injection of the reference standard communicates high confidence in the identification. Suspect screening approaches make use of lists of tentative compounds. The use of a purpose-built database containing TPs reported in the literature (e.g. including elemental composition as well as information about the product ions reported) is a good approach that facilitates the identification of TPs. This strategy allowed the tentative identification by LC-QTOF MS of 12 TPs from eight pharmaceuticals (gemfibrozil, nimesulide, furosemide, paracetamol, propranolol, dipyrone, fluoxetine and diazepam) after application of the Fenton process [61].

The information to create a list of suspect compounds can be collected from software prediction tools and/or databases containing a broad number of compounds to be likely detected [62]. In some cases, several lists (from literature, in-house library, and created by the software used for compound prediction) can be combined for a more comprehensive searching [59]. The prediction of TPs is usually performed in silico using different tools, such as the Eawag-Pathway (enviPath) prediction system (formerly UM-PPS; http://eawag-bbd.ethz.ch/). After TP prediction, suspect screening of expected exact masses of TPs is conducted in HRMS data, usually comparing versus a control sample. The plausibility of retention time, isotopic pattern and ionization mode can be used to reduce the candidate peaks. Finally, MS/MS acquisition can also be performed, which allows matching the chemical structure of the suspected TP to the observed fragmentation.

Another interesting approach to investigate the presence of TPs relies on the assumption that many of them maintain similarity in their chemical structure with the parent compound and therefore present several common fragment ions [55, 60]. Searching for common fragments is a powerful strategy, able to detect and tentatively identify TPs/metabolites in the samples, although it is time-consuming and requires a notable knowledge of mass fragmentation rules. This approach has been applied to the identification of valsartan and losartan TPs produced by application of sonochemical systems or other advanced oxidation processes [12, 52, 63].

2.2 *Transformations of Pharmaceuticals Promoted by Sonochemical Action*

The use of the above detailed techniques for identification of transformation products has shown that the sonochemical treatment initially induce modifications such as hydroxylations, oxidations, fragmentations, decarboxylations, dealkylations or dehalogenations of the pharmaceuticals. For example, oxacillin (antibiotic) has as primary transformation pathways: oxidation of the thioether group, opening of its β-lactam and fragmentation of the central amide, all by attacks of the sonogenerated hydroxyl radical [64]. In the case of norfloxacin (antibiotic), a decarboxylation has been reported, while for cephalexin (antibiotic), the opening of its β-lactam ring is an initial degradation pathway [32]. Acetaminophen (analgesic) experiments hydroxylations initially [65]. Also, atenolol (beta-blocker) exhibits hydroxylation of its aromatic ring and propoxyl group combined to a rupture of the central ether [18].

It is reported that losartan (antihypertensive) experiments cleavage of its imidazole ring, hydroxylations of the biphenyl-tetrazole nucleus and alkyl moiety, plus oxidation of its alcohol group [12]. In turn, carbamazepine (psychiatric pharmaceutical) is initially transformed through hydroxylations and epoxidation at the double bond in the middle of its structure, followed by fragmentations of the primary products [22]. Meanwhile, the ranitidine (antiulcer) is degraded by the HO• attacks to its N,N-dimethylamine, N-methylamine and α-carbons to the sulphur [66].

In general, the pharmaceuticals degradation by action of ultrasound can be schematized as a sequence of three phases (Fig. 3): phase (1) formation of primary intermediates from parent pollutants; phase (2) rupture of the primary intermediates to generate shorter-chain or ring-opened structures and phase (3) transformation of substances from phase 2 into small molecules such as aliphatic carboxylic acids [23]. Although the sonochemical process is able to induce severe transformations on

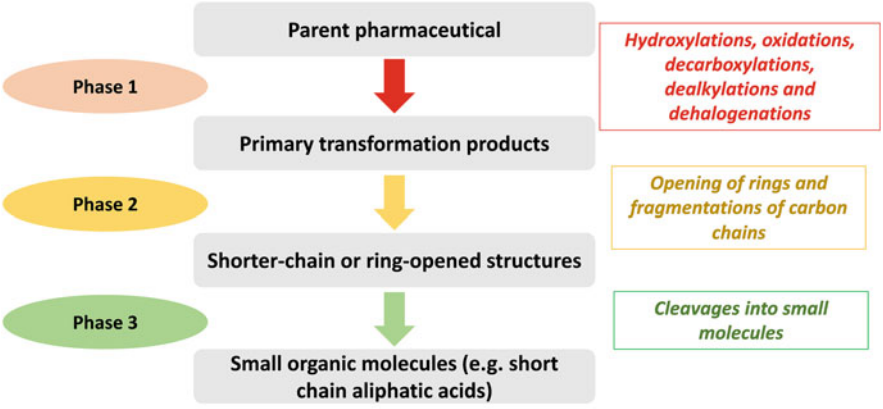


Fig. 3 Schematic sequence of phases during pharmaceuticals degradation by sonochemistry. Figure was made by the authors

pharmaceuticals, it is recognized that this system has a moderate mineralizing ability [7, 8, 23, 64]. This is because most products formed in the phase 3 (e.g. short chain carboxylic acids) have a very hydrophilic character, which limits their interaction with the sonogenerated hydroxyl radical. However, the mineralization degree can be improved by adding to the sonochemical system some reagent (e.g. ferrous ions, persulfate anion, ozone or hydrogen peroxide) that promotes generation of extra radical able to reach the hydrophilic products in the solution bulk [23].

Despite the moderate mineralizing ability of sonochemistry acting alone, during the treatment of antibiotics, this process is able to generate by-products without antimicrobial activity due to the sonogenerated hydroxyl radical attacks the moieties responsible for such activity [32, 64]. Furthermore, in many cases the sonodegradation of pharmaceuticals produces water having smaller molecules more biodegradable and/or less toxic than the parent pollutants [23]. These aspects indicate positive changes exerted by the sonochemical treatment of water polluted with pharmaceuticals.

3 Treatment of Wastewater Polluted with Pharmaceuticals by Ultrasound

3.1 Removal of Pharmaceuticals in Industrial, Municipal and Hospital Wastewaters Effluents

Ultrasound is a versatile advanced oxidation process, which has been applied to treat pharmaceuticals within diverse industrial, municipal and hospital wastewater (Table 4). For instance, degradation of oxacillin or dicloxacillin (antibiotics) in simulated effluents of pharmaceutical industries (the pharmaceuticals plus excipients such as mannitol, calcium carbonate or glucose) has been evaluated [25, 64]. These works show that the elimination of the pollutants or its antimicrobial activity is not affected by the presence of the other ingredients, even when the excipients are much more concentrated than the target pharmaceutical. The selective treatment of these pharmaceuticals is explained considering that due to the hydrophilic nature of the excipients, they are far away of the cavitation bubbles and had no interfering effect on the degradation of the oxacillin or dicloxacillin, which are hydrophobic antibiotics.

The treatment by ultrasound of a mixture of diclofenac (anti-inflammatory), amoxicillin (antibiotic) and carbamazepine (anticonvulsant) spiked on an effluent of urban wastewater treatment plant increased biodegradability and decreased the toxicity of treated samples. Thus, after sonication process application, the treated water is compatible with a biological process or can be discharged into natural aqueous media [16]. Also, the efficiency of ultrasound to reduce hormones oestrogen (E1) and 17-beta-oestradiol (E2) from municipal wastewater has been tested, reporting that after 45 min the process removed 85 and 96% of E1 and E2, respectively [67].

Table 4 Treatment of pharmaceuticals in industrial, municipal and hospital wastewaters by ultrasound

Pharmaceuticals	Wastewater[a]	Ultrasound conditions	References
Dicloxacillin	Simulated PWW (dicloxacillin and glucose)	Frequency: 600 kHz Electric power: 60 W Volume: 300 mL	[25]
Oxacillin	Simulated PWW (oxacillin, mannitol and calcium carbonate)	Frequency: 275 kHz Electric power: 60 W Volume: 250 mL	[64]
Mixture of diclofenac, amoxicillin and carbamazepine	Pharmaceuticals were spiked to an EWWTP	Frequency: 20 kHz Electric power density: 100 W L^{-1} Volume: 200 mL	[16]
Oestrogen (E1) and 17-beta-oestradiol (E2)	Municipal WW	Frequency: 30–60 kHz Electric power density: 70–110 W Volume: 1.5 L	[67]
5-Fluorouracil, ibuprofen, clonidine, estriol, nifedipine and lovastatin	The pharmaceuticals were individually added to an EWWTP	Frequency: 205 kHz Acoustic power density: 45 W L^{-1} Volume: 300 mL	[27]
Seventeen pharmaceuticals Bogotá	Actual EWWTP	Frequency: 375 kHz Acoustic power density: 88 W L^{-1} Volume: 300 mL	[53]
Losartan and valsartan	Pharmaceuticals individually spiked to an EWWTP	Frequency: 375 kHz, Acoustic power density: 88 W L^{-1}, Volume: 300 mL	[12]
Trimethoprim	EWWTP	Frequency: 20 kHz Acoustic power density: 36 W L^{-1} Volume: 200 mL	[68]
Cloxacillin and cefadroxil	Simulated HWW	Frequency: 375 kHz Acoustic power density: 88 W L^{-1} Volume: 300 mL	[32]
Losartan and valsartan	Simulated hospital wastewater	Frequency: 375 kHz Acoustic power density: 88 W L^{-1} Volume: 300 mL	[12]
Fifteen pharmaceuticals	HWW prior treated by an aerobic biological process	Frequency: 375 kHz Acoustic power density: 88 W L^{-1} Volume: 350 mL	[52]

(continued)

Table 4 (continued)

Pharmaceuticals	Wastewater[a]	Ultrasound conditions	References
Amantadine	PWW	*Sono-Fenton:* Frequency: 25 kHz Electric power: 200 W Volume: 50 mL Fe (II): 0.1 mol L^{-1} and H_2O_2: 2.0 mol L^{-1}	[69]
Ciprofloxacin	Pharmaceutical spiked to an EWWTP	*Sono-Fenton:* Frequency: 580 kHz Actual power: 30.6 W Volume: 350 mL Ratio H_2O_2/Fe (II): 6 and H_2O_2: 14.2– 31.8 mmol L^{-1}	[70]
Ampicillin and nafcillin	Simulated EWWTP	*Sono-photo-Fenton:* Frequency: 375 kHz Acoustic power density: 24.4 W L^{-1} Volume: 250 mL Fe (II): 90 μmol L^{-1} UVC lamp: 4 W	[33]
Tinidazole	Simulated PWW and actual PWW	*Sono-peroxide:* Frequency: 120 kHz Electric power: 750 W Volume: 100 mL H_2O_2: 333 mmol L^{-2}	[71]
Mixture of paracetamol, chloramphenicol and diclofenac	Simulated PWW	*Sono-photolysis:* Frequency: 20 kHz Electric power: 80 W Volume: 7.0 L *(Pilot reactor)* H_2O_2: 900 mg L^{-1} UVC lamp: 13 W	[72]
Diclofenac	Pharmaceutical spiked on an actual EWWTP	*Sono-ozonolysis:* Frequency: 20 kHz Electric power density: 400 W L^{-1} Volume: 150 mL Ozone: 31 g h^{-1}	[73]
Diclofenac, sulfamethoxazole and carbamazepine	Mixture of pharmaceutical	*Sono-ozonolysis:* Frequency: 20 kHz Electric power density: 370 W L^{-1} Volume: 200 mL Ozone: 1.3–3.3 g h^{-1}	[74]

[a]*WW* raw wastewater, *EWWTP* effluent of municipal wastewater treatment plant and *PWW* pharmaceutical wastewater

Xiao et al. investigated the individual degradation by sonochemistry of fluorouracil (5-FU), ibuprofen (IBU), clonidine (CLND), estriol (ESTO), nifedipine (NIFE) and lovastatin (LOVS) in a wastewater effluent. Because of the higher concentration of the effluent components, the matrix slowed degradation of the pharmaceuticals compared to elimination in deionized water. Degradations of 5-FU and LOVS were inhibited by the wastewater components in a greater extent than the other pharmaceuticals. The elimination trends indicated that the pharmaceuticals reacting primarily on the bubble surface were slightly affected by the matrix components, whereas substances reacting mainly in the bulk were strongly affected by the effluent. Indeed, some organic matter of the effluent are able to migrate to the cavitation bubble surfaces and compete for the sonogenerated hydroxyl radicals [27].

Losartan and valsartan are other pharmaceuticals treated by ultrasound in municipal wastewater plant effluents. For these compounds, degradations in the effluents were very similar to the obtained in distilled water, indicating that the matrix components had a very low effect on the removal of losartan and valsartan by ultrasound. This was associated with the high hydrophobic character of such pharmaceuticals [12].

In addition to matrices spiked with the pollutants, treatment of actual wastewater, containing pharmaceuticals at realistic μg L^{-1} levels, has been considered. In fact, the degradation of 17 pharmaceuticals (diclofenac, carbamazepine, venlafaxine, ciprofloxacin, norfloxacin, valsartan, losartan, irbesartan, sulfamethoxazole, clarithromycin, azithromycin, erythromycin, metronidazole, trimethoprim and clindamycin) in an effluent of municipal wastewater plant (which had only primary processes) has been reported. The ultrasound action increased the concentrations of four pollutants (diclofenac, sulfamethoxazole, ciprofloxacin and norfloxacin), whereas for the rest of pharmaceuticals, the concentration decreased with the treatment. Such results are rationalized based on both physical and chemical effects of ultrasound. Owing to the effluent contained suspended solids, some pollutants sorbed on such solids increased their concentrations by the physical action of ultrasound (i.e. turbulence and high shearing in the liquid medium by cavitation phenomena), which induces a size reduction of solids and releasing of adsorbed pharmaceuticals. Meanwhile, the removal of the pollutants was associated with the chemical effects of the process (i.e. degrading action of hydroxyl radical, [53]).

As well as effluents from pharmaceutical industry and municipal treatment plants, hospital wastewaters loaded with pharmaceuticals have been also processed by sonication. Indeed, synthetic hospital wastewater contaminated with cloxacillin, cefadroxil, losartan or valsartan has been submitted to ultrasound action. The elimination of cloxacillin, losartan or valsartan in the hospital effluent is very efficient. In contrast, cephalexin (which is very hydrophilic) removal is affected by the matrix components [12, 32].

Other example of elimination of pharmaceutical in wastewater is the sonication of a real effluent of hospital prior treated by an aerobic biological process. In this case the removal of 15 pollutants (acetaminophen, diclofenac, carbamazepine, venlafaxine, loratadine, ciprofloxacin, norfloxacin, valsartan, irbesartan,

sulfamethoxazole, trimethoprim, clarithromycin, azithromycin, erythromycin and clindamycin) was considered. Ultrasound application led to a decreasing of the concentrations of pharmaceuticals except for acetaminophen and valsartan, suggesting that such compounds were liberated from active sludge flocs proceeding from the bio-treatment. When the adsorbed pharmaceuticals are released, they also are susceptible to the chemical effects of ultrasound. The sonogenerated hydroxyl radicals are the main responsible of the elimination of pollutants, leading to ~59% of pondered removal of pharmaceuticals after 90 min of treatment [52].

Although sonication alone has shown a high ability to degrade pharmaceuticals in complex matrices, as an alternative to improve the pollutants degradation and/or reduce the treatment times, the ultrasound action has been complemented by addition of reagents to generate hybrid advanced oxidation processes. Such is the situation of amantadine (antiviral and antiparkinsonian) in a wastewater of the pharmaceutical industry, which was degraded by sono-Fenton (by adding Fe (II) and H_2O_2 at the water sample). The hybrid process lead to a synergistic pharmaceuticals degradation and a significant reduction of wastewater toxicity, thanks the extra formation of radicals by means of the Fenton reaction (Eq. 7) [69]. On the other hand, the feasibility of ciprofloxacin (antibiotic) degradation in a real matrix from a municipal wastewater treatment plant by sono-Fenton was informed. The addition of H_2O_2 and Fe (II) to the sonication system improved both the pharmaceutical elimination and mineralization [70].

Sono-photo-Fenton, which is formed by addition of Fe (II) and UV light to the sonochemical system, has been applied for degrading ampicillin and nafcillin (antibiotics) in a simulated effluent of wastewater treatment plant. This system induced removals of the antibiotics $\geq$90% at 60 min of treatment [33]. Likewise, the simultaneous degradation of several pharmaceuticals in hospital wastewater previously bio-treated or in actual effluents of wastewater treatment plant is significantly increased by the utilization of sono-photo-Fenton. Such system may produce additional radical species at the solution bulk through interaction among iron, hydrogen peroxide and UV light (Eqs. 7 and 9) [52, 53]. Also, it should be mentioned that ultrasound plus hydrogen peroxide, persulfate anion and iron swarf is another hybrid process able to generate extra amounts of radicals. This system has successfully applied to reduce the chemical oxygen demand of pharmaceutical effluents [75].

$$Fe^{3+} + H_2O + \text{UV light} \rightarrow Fe^{2+} + HO\bullet + H^+ \quad (9)$$

The addition of hydrogen peroxide solely is another way to intensify sonochemical degrading action (i.e. sono-peroxide process, Eqs. 10–11), specially at low frequencies. This procedure has been reported for tinidazole (antiparasitic), which was treated in both synthetic and actual pharmaceutical industrial effluents, achieving at 150 min of treatment, 75 and 68%, respectively [71]. Furthermore, low frequency ultrasound (30 kHz) intensified by H_2O_2 and activated carbon (AC) has been applied to pharmaceutical wastewater, decreasing ~79% of chemical oxygen demand of such matrix [76].

$$H_2O_2 +))) \rightarrow 2HO\bullet \quad (10)$$

$$H\bullet + H_2O_2 \rightarrow HO\bullet + H_2O \quad (11)$$

The merging of low frequency ultrasound (20 kHz), hydrogen peroxide and UVC light generates a sono-photolytic system. This kind of process has been utilized for treating paracetamol, chloramphenicol and diclofenac in a simulated pharmaceutical wastewater. The sono-photolytic system exhibited a synergistic effect for the mineralization of this wastewater, achieving 91% of total organic carbon removal after 120 min of treatment, due to the high ability of such system to produce hydroxyl radicals (Eqs. 10–12) [72].

$$H_2O_2 + UVC \rightarrow 2HO\bullet \quad (12)$$

Aside from addition of hydrogen peroxide, activated carbon, iron and light, another possibility is the simultaneous action of low frequency ultrasound and ozone (sono-ozonolysis), which has also shown to be useful for degrading pharmaceuticals in complex matrices. This system is able to improve the mass transfer of ozone to the aqueous medium and the collapsing bubbles yield additional free radicals. For example, the treatment of a mixture of diclofenac, sulfamethoxazole and carbamazepine (simulating a pharmaceutical wastewater) by sono-ozonolysis resulted in an enhanced removal of these pharmaceuticals [74]. Similarly, the removal of organic carbon content from a wastewater treatment plant effluent polluted with diclofenac was improved by the join action of ultrasound and ozone [73].

It must be mentioned that other cases related with the degradation of pharmaceuticals in wastewater by ultrasound alone, intensified or combined with other process, can be found in the following references [77–85]. Besides, from a critical point of view, it should be remarked that the most of works on elimination of pharmaceuticals pollutants in wastewaters lack toxicity or biodegradability analyses to evidence the positive impact of treatments. Moreover, few works on hybrid/intensified processes report or discuss the synergy; thus it is not clear the actual effects of addition of reagents or light to ultrasound. Finally, despite varied application of the ultrasound-based processes to very complex water matrices, the volumes of treatment are typically low (<1.0 L). Therefore, information about performance of these processes at large-scale is still scarce.

3.2 *Ultrasound Generators and Engineering Aspects for the Wastewater Treatments by Ultrasound*

Presented in this section is information on different types of ultrasound generators available and the engineering aspects associated with treating wastewater by ultrasound from lab scale to pilot scale.

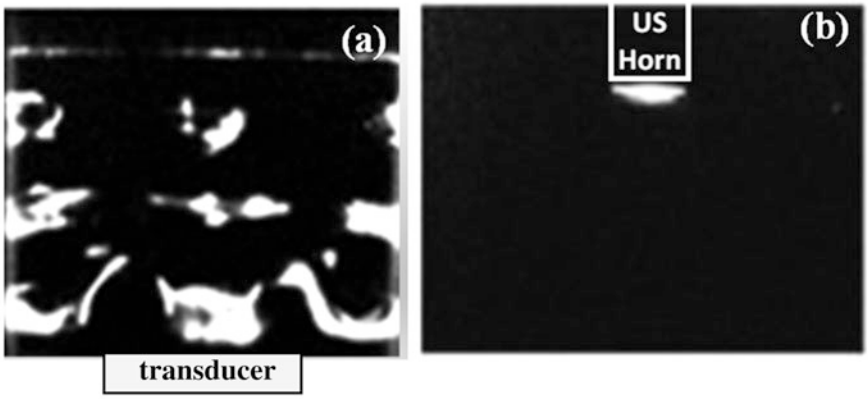


Fig. 4 Sonoluminescence activity (white part) generated by (**a**) 98 kHz plate transducer and (**b**) 20 kHz ultrasonic horn. Reprinted with permission from reference [86]

3.2.1 Type of Ultrasound Generators

Ultrasound is usually generated by a piezoelectric ceramic transducer with a specific resonance frequency that can range from 20 kHz up to 10 MHz and is powered by an amplifier. The transducers are usually glue to a vibrating plate or bottom of an ultrasonic bath with localized cavitation activity (revealed through sonoluminescence) at the pressure antinodal planes perpendicular to the direction of the ultrasound waves (Fig. 4a). Also, power ultrasonic horns have been designed to allow the transduction at a tip, which can vary in diameter from 2 mm up to 45 mm. This leads to high intense cavitation and fluid mixing mainly localized near the horn tip (Fig. 4b) [86]. These ultrasonic horn and bath are usually at the lower end of the ultrasound frequencies (20–100 kHz). For frequencies between 100 kHz and 2 MHz, a specialized reactor setup is usually required coupled with a high-power amplifier. For laboratory scale, one ultrasonic horn or a single plate transducer are generally used for volumes ranging from 1 mL to 1 L and in a batch mode. Meanwhile, for large-scale processing, multiple horns or plate transducers are employed and regularly involve a continuous flow system that requires several passes.

3.2.2 Laboratory-Scale Sonication

As above presented, ultrasound has shown to be effective for degrading pharmaceuticals in diverse water matrices. In this sense, to determine the engineering aspects in terms of the applied frequency, power density and geometry is critical. What is even more important is the spatial distribution of the cavitation activity within the reactor,

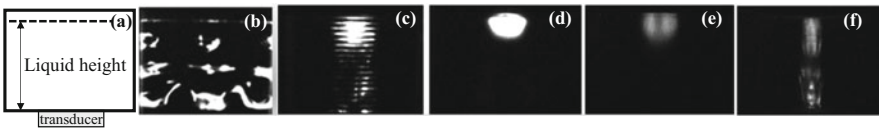


Fig. 5 Spatial distribution of sonoluminescence (SL) intensity for different frequencies: (**a**) configuration of the sonication cell, (**b**) 37 kHz, (**c**) 168 kHz, (**d**) 448 kHz, (**e**) 726 and (**f**) 448 kHz with a surface stabilizer. All images were taken with an exposure time of 30 s and amplifier of 20 W. Reprinted with permission from references [86, 88]

which determines the efficiency of the system and is affected by frequency, power and solution conditions [87].

Figure 5 shows the variation in the spatial distribution of sonoluminescence (SL) from cavitating bubbles generated by different ultrasound frequencies. An optimum frequency for high sonochemical yield has been reported to be related to bubbles characteristics (e.g. population and collapse intensity) with increasing frequency [88]. However, attenuation and spatial distribution of ultrasound within the reactor could also contribute to the optimum frequency for sonochemical yield, and it could be altered by reactor design as illustrated by Fig. 5f, where the spatial distribution is enhanced when the reflectivity of the waves is increased by stabilizing the liquid surface. This would explain the different optimum frequencies being reported between 200 kHz and 800 kHz [1, 89, 90]. Therefore, in addition to the sonochemical yield, it is important to evaluate the spatial distribution of cavitation activity for a given setup.

Laboratory-scale systems are limited from 10 to 1,000 mL, with the cavitation mainly located above the transducer. Often, different sonochemical reactor geometries are used, which includes immersing small vials containing the wastewater solution. For a cylindrical cell with a diameter matching that of the transducer diameters, the entire liquid above the transducer are filled with cavitation bubbles. However, for the rectangular reactor where the width of the reactor extends beyond the transducer diameter, there are regions near the wall that do not have active cavitation taking place. This would thus decrease the overall efficiency of the system [91]. When all the cavitation is concentrated within the vial, the power densities for the different systems would therefore vary and consequently modify the overall pollutants degradation efficiencies [92].

3.2.3 Industrial-Scale Sonication

Full-scale development of ultrasound reactors for wastewater treatment in industry is still incipient. There are a few reports on in-house built pilot-scale studies involving multiple transducers attached to the side of a reactor with volumes ranging from 6 L up to 91 L [93–95]. Figure 6 shows some large-scale ultrasound reactors reported in the literature. The highest ultrasonic reactor was 91 L reported by Gole et al. [95] for the degradation of organic pollutants (per- and poly-fluorinated alkyl sulfonate

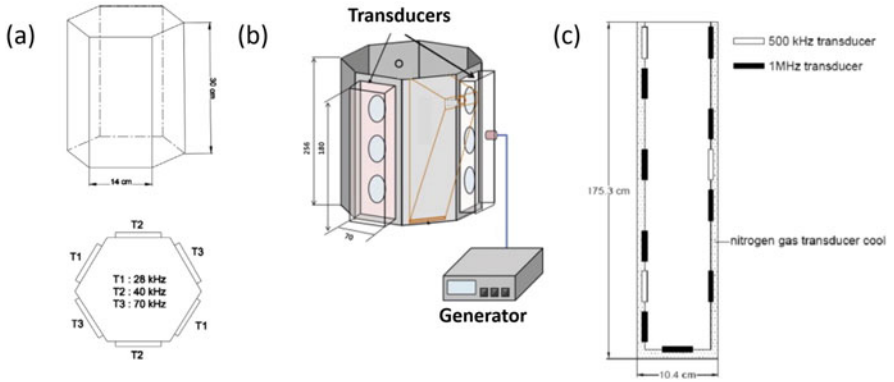


Fig. 6 Large-scale ultrasound reactors reported in the literature. (**a**) 15 L triple frequency reactor (adapted with permission from [94]), (**b**) 6 L reactor with four 332 kHz (adapted with permission from [93]) and (**c**) 91 L multifrequency reactor (adapted with permission from [95])

substances (PFAS)). The reactor was fabricated by PCT System Inc. (Fremont, CA, USA), and it has dimensions of 20.9 × 26.0 × 175.3 cm with 12 transducers, three of these transducers were 500 kHz, and nine were 1 MHz transducers, positioned as indicated in Fig. 6c. It was concluded that the ultrasound treatment was effective at reducing the fluorocarbon substances and it led to a moderate removal of total organic carbon with both single and dual frequency modes [95]. In turn, it has been reported the use of a 140 L ultrasonic reactor equipped with 24 transducers at 350 kHz, each capable of generating free radicals at a rate of 5.2 μmol L^{-1} min^{-1} [96]. However, results on the performance of sonication treatment for specific organic pollutants were no reported (only global parameters).

In the large-scale ultrasound reactor studies, no justification for the chosen design, frequencies and powers were given, and there was a lack of focus on optimizing the conditions controlling cavitation activity and distribution. Upscaling of reactors can potentially be influenced by many parameters that affect the efficiency of sonolysis. This is because changes in liquid volume, liquid height, vessel geometry and solution properties will have significant effect on the ultrasound wave field inside the vessel [97]. As a result, the cavitation activity, which is depended on ultrasound wave field, is affected, and this in turn will affect the efficiency of the sonochemistry. To scale-up ultrasound reactors, there are many optimization factors that can be explored such as sound field distribution, bubbling gas in-line, pH changes and changing the external temperature and pressure. Therefore, there is a need for much more studies on the incremental upscaling ultrasound reactors, especially at high frequencies.

On the other hand, as outlined above, large-scale ultrasound reactors usually require multiple transducers that would need multiple amplifiers to drive each transducer and often cooling is required due to the heating of the transducers. These additional costs associated with the upscaling further hinder the development of pilot-scale ultrasound reactors. An alternative that seems promising to face such

concerns associated with large-scale ultrasound applications is the combination with membrane filtration techniques, allowing to concentrate pollutants and significantly reduce the volumes to be treated, which could be processed by using small ultrasound reactors. This topic is developed in the next section.

3.3 Combinations of Ultrasound with Membrane Filtration Techniques as a Strategy for Overcoming the Issues Related to Large-Scale Application

Membrane filtration is a separation technique that is based on either size or charge, or both depending on the type of membrane material and the compound to be separated. Nanofiltration (NF) and reverse osmosis (RO) have shown high rejection (>85%) of pharmaceuticals from wastewater [98–100]. However, as a separation technology, the process concentrates the pharmaceutical wastes and needs to be combined with other treatment that is capable of destroying the pollutant.

Although ultrasound has shown to be effective for degrading pharmaceutical pollutants, most of the literature on the combination of ultrasound and membrane filtration focuses on the physical impact of ultrasound on enhancing the permeate flux, reduce fouling and membrane surface cleaning [101–103]. A unique hybrid ultrasound-activated carbon-ultrafiltration membrane (USAMe) process to treat three active pharmaceutical ingredients (API), diclofenac, carbamazepine and amoxicillin has been reported [104]. The USAMe composed of glass tubing, encasing a single hollow fibre membrane with powdered activated carbon deposited within the fibre, that is immersed in an ultrasonic bath with a frequency of either 35 kHz or 135 kHz. It was reported that the presence of ultrasound increased the percentage removal of the pharmaceuticals during the treatment. Additionally, 35 kHz was found to be more effective than 135 kHz at sustaining a high removal rate over the duration of the experiment, which led to the conclusion that the main mechanism of ultrasound is to enhance the adsorption of the API onto the activated carbon rather than sonochemically degrading the API [104].

Caretti et al. had used a pilot plant-scale ultrasound reactor to treat a wastewater effluent prior to ultrafiltration (UF) treatment compared to the use of ultrasound to treat the permeate from the UF [96]. It was found that pre-treatment of the effluent prior to UF was more effective (the efficiency of the pilot-scale ultrasound reactor was not reported). However, the configurations proposed in this last work consider an ultrasound reactor at large-scale, which has the limitations previously indicated. Therefore, a new strategy should be considered. In the future, different membrane filtration technologies may be used to concentrate the waste stream (target organic pollutants as pharmaceuticals) and reduce to a more manageable volume to be treated by ultrasonication at small-scale, enhancing the efficiency of pollution elimination and the extraction of clean water permeate that can be recycled.

4 Concluding Remarks

From information presented in this chapter, it can be highlighted the following aspects:

- Elimination of pharmaceuticals by ultrasound is strongly dependent of operational parameters such as frequency and power (or density power).
- The hydrophobic/hydrophilic nature of pharmaceutical pollutants determines their degradation rates by sonochemistry. Furthermore, when the molecules have acid/base functional groups, the hydrophobic/hydrophilic character may be modified through variation of the solution pH.
- The sonogenerated hydroxyl radicals are able to induce primary modifications such as hydroxylations, oxidations, fragmentations, decarboxylations, dealkylations, or dehalogenations to the pharmaceuticals. Meanwhile, the intensive action of radicals leads to transformation of these pollutants into small non-toxic or biocompatible substance; even some degree of pharmaceuticals mineralization is achieved. However, it should be indicated that the most studies about the pharmaceuticals transformations by ultrasound are carried out in very simple water matrices (distilled water, deionized water or milli-Q water). Hence, some future researches on the influence on matrix components on transformations could be developed.
- Ultrasound is able to degrade pharmaceuticals in diverse wastewater; even selective elimination is possible in some cases. The sonochemical action can be enhanced by adding reagents (e.g. ferrous ions) or light. Nevertheless, further investigations should include more information on toxicity and biodegradability of treated wastewater, in addition to synergy calculations to evidence the actual effects of addition of reagents or light to ultrasound.
- Applications at pilot and full scales of ultrasound for wastewater treatment are still incipient. Additionally, it is recognized that the main drawbacks of sonochemical processes for application at large-scale are the high energy costs and the requirements of multiple of amplifiers and cooling systems to avoid the transducers heating. An option to overcome such concerns could be the combination of ultrasound with membrane filtration techniques to concentrate pollutants and significantly reduce the wastewater to volumes which can be treated by using the small available ultrasound reactors.

Acknowledgements The authors thank the financial support provided by the Royal Society (UK) through the project "Sound" methods of remediating emerging contaminants in hospital wastewater (RA4056, ICA\R1\191053) and the financing from COLCIENCIAS through the project No. 111577757323 (Convocatoria 777 de 2017). R.A. Torres-Palma and E.A. Serna-Galvis thank Universidad de Antioquia UdeA for the support provided to their research group through "Programa de Sostenibilidad".

References

1. Wood RJ, Lee J, Bussemaker MJ (2017) A parametric review of sonochemistry: control and augmentation of sonochemical activity in aqueous solutions. Ultrason Sonochem 38:351–370. https://doi.org/10.1016/j.ultsonch.2017.03.030
2. Rivera-Utrilla J, Sánchez-Polo M, Ferro-García MÁ et al (2013) Pharmaceuticals as emerging contaminants and their removal from water. A review. Chemosphere 93:1268–1287. https://doi.org/10.1016/j.chemosphere.2013.07.059
3. Adewuyi YG (2001) Sonochemistry: environmental science and engineering applications. Ind Eng Chem Res 40:4681–4715. https://doi.org/10.1021/ie010096l
4. Riesz P, Berdahl D, Christman CL (1985) Free radical generation by ultrasound in aqueous and nonaqueous solutions. Environ Health Perspect 64:233–252. https://doi.org/10.2307/3430013
5. McNamara WB, Didenko YT, Suslick KS (1999) Sonoluminescence temperatures during multi-bubble cavitation. Nature 401:772–775. https://doi.org/10.1038/44536
6. Beckett MA, Hua I (2001) Impact of ultrasonic frequency on aqueous sonoluminescence and sonochemistry. J Phys Chem A 105:3796–3802. https://doi.org/10.1021/jp003226x
7. Tran N, Drogui P, Brar SK (2015) Sonochemical techniques to degrade pharmaceutical organic pollutants. Environ Chem Lett 13:251–268. https://doi.org/10.1007/s10311-015-0512-8
8. Montoya-Rodríguez DM, Ferraro F, Serna-Galvis EA, Torres-Palma RA (2020) Degradation of the emerging concern pollutant ampicillin in aqueous media by sonochemical advanced oxidation processes – parameters effect, removal of antimicrobial activity and pollutant treatment in hydrolyzed urine. J Environ Manage 261:110224. https://doi.org/10.1016/j.jenvman.2020.110224
9. Mahamuni NN, Adewuyi YG (2010) Advanced oxidation processes (AOPs) involving ultrasound for waste water treatment: a review with emphasis on cost estimation. Ultrason Sonochem 17:990–1003. https://doi.org/10.1016/j.ultsonch.2009.09.005
10. Castelo-Grande T, Augusto PA, Barbosa D (2020) Chapter 14 ultrasound-assisted remediation methods. In: The handbook of environmental remediation: classic and modern techniques. The Royal Society of Chemistry, Cambridge, pp 379–409
11. Kang JW, Hung HM, Lin A, Hoffmann MR (1999) Sonolytic destruction of methyl tert-butyl ether by ultrasonic irradiation: the role of O3, H2O2, frequency, and power density. Environ Sci Technol 33:3199–3205. https://doi.org/10.1021/es9810383
12. Serna-Galvis EA, Isaza-Pineda L, Moncayo-Lasso A et al (2019) Comparative degradation of two highly consumed antihypertensives in water by sonochemical process. Determination of the reaction zone, primary degradation products and theoretical calculations on the oxidative process. Ultrason Sonochem 58:104635. https://doi.org/10.1016/j.ultsonch.2019.104635
13. Guo W, Wang H, Shi Y, Zhang G (2010) Sonochemical degradation of the antibiotic cephalexin in aqueous solution. Water SA 36:651–654. https://doi.org/10.4314/wsa.v36i5.61998
14. Naddeo V, Belgiorno V, Kassinos D et al (2010) Ultrasonic degradation, mineralization and detoxification of diclofenac in water: optimization of operating parameters. Ultrason Sonochem 17:179–185. https://doi.org/10.1016/j.ultsonch.2009.04.003
15. Fu H, Suri RPS, Chimchirian RF et al (2007) Ultrasound-induced destruction of low levels of estrogen hormones in aqueous solutions. Environ Sci Technol 41:5869–5874. https://doi.org/10.1021/es0703372
16. Naddeo V, Meriç S, Kassinos D et al (2009) Fate of pharmaceuticals in contaminated urban wastewater effluent under ultrasonic irradiation. Water Res 43:4019–4027. https://doi.org/10.1016/j.watres.2009.05.027
17. Hapeshi E, Achilleos A, Papaioannou A et al (2010) Sonochemical degradation of ofloxacin in aqueous solutions. Water Sci Technol 61:3141–3146. https://doi.org/10.2166/wst.2010.921

18. Nejumal KK, Manoj PR, Aravind UK, Aravindakumar CT (2014) Sonochemical degradation of a pharmaceutical waste, atenolol, in aqueous medium. Environ Sci Pollut Res 21:4297–4308. https://doi.org/10.1007/s11356-013-2301-x
19. Quesada-Pañate I, Julcour Lebigue C, Jáuregui-Haza U-J et al (2009) Sonolysis of levodopa and paracetamol in aqueous solutions. Ultrason Sonochem 16:610–616. https://doi.org/10.1016/j.ultsonch.2008.11.008
20. Vega LP, Soltan J, Peñuela GA (2019) Sonochemical degradation of triclosan in water in a multifrequency reactor. Environ Sci Pollut Res 26:4450–4461. https://doi.org/10.1007/s11356-018-1281-2
21. Gogate PR, Shirgaonkar IZ, Sivakumar M et al (2001) Cavitation reactors: efficiency assessment using a model reaction. AICHE J 47:2526–2538. https://doi.org/10.1002/aic.690471115
22. Tran N, Drogui P, Zaviska F, Brar SK (2013) Sonochemical degradation of the persistent pharmaceutical carbamazepine. J Environ Manage 131:25–32. https://doi.org/10.1016/j.jenvman.2013.09.027
23. Rayaroth MP, Aravind UK, Aravindakumar CT (2016) Degradation of pharmaceuticals by ultrasound-based advanced oxidation process. Environ Chem Lett 14:259–290. https://doi.org/10.1007/s10311-016-0568-0
24. Gao YQ, Gao NY, Deng Y et al (2013) Factors affecting sonolytic degradation of sulfamethazine in water. Ultrason Sonochem 20:1401–1407. https://doi.org/10.1016/j.ultsonch.2013.04.007
25. Villegas-Guzman P, Silva-Agredo J, Giraldo-Aguirre ALAL et al (2015) Enhancement and inhibition effects of water matrices during the sonochemical degradation of the antibiotic dicloxacillin. Ultrason Sonochem 22:211–219. https://doi.org/10.1016/j.ultsonch.2014.07.006
26. Villaroel E, Silva-Agredo J, Petrier C et al (2014) Ultrasonic degradation of acetaminophen in water: effect of sonochemical parameters and water matrix. Ultrason Sonochem 21:1763–1769
27. Xiao R, Wei Z, Chen D, Weavers LK (2014) Kinetics and mechanism of sonochemical degradation of pharmaceuticals in municipal wastewater. Environ Sci Technol 48:9675–9683. https://doi.org/10.1021/es5016197
28. Xiao R, Diaz-Rivera D, Weavers LK (2013) Factors influencing pharmaceutical and personal care product degradation in aqueous solution using pulsed wave ultrasound. Ind Eng Chem Res 52:2824–2831. https://doi.org/10.1021/ie303052a
29. Xiao R, Diaz-Rivera D, He Z, Weavers LK (2013) Using pulsed wave ultrasound to evaluate the suitability of hydroxyl radical scavengers in sonochemical systems. Ultrason Sonochem 20:990–996. https://doi.org/10.1016/j.ultsonch.2012.11.012
30. Torres-Palma RA, Serna-Galvis EA (2018) Chapter 7 – sonolysis. In: Ameta SC, Ameta RBT (eds) Advanced oxidation processes for waste water treatment. Academic Press, Cambridge, pp 177–213
31. Suri RPS, Singh TS, Abburi S (2010) Influence of alkalinity and salinity on the sonochemical degradation of estrogen hormones in aqueous solution. Environ Sci Technol 44:1373–1379. https://doi.org/10.1021/es9024595
32. Serna-Galvis EA, Montoya-Rodríguez DM, Isaza-Pineda L et al (2018) Sonochemical degradation of antibiotics from representative classes-considerations on structural effects, initial transformation products, antimicrobial activity and matrix. Ultrason Sonochem 50:157–165. https://doi.org/10.1016/j.ultsonch.2018.09.012
33. Montoya-Rodríguez DM, Ávila-Torres Y, Serna-Galvis EA, Torres-Palma RA (2020) Data on treatment of nafcillin and ampicillin antibiotics in water by sonochemistry. Data Br 29:105361. https://doi.org/10.1016/j.dib.2020.105361
34. Méndez-Arriaga F, Torres-Palma R, Pétrier C et al (2008) Ultrasonic treatment of water contaminated with ibuprofen. Water Res 42:4243–4248
35. Im J-K, Heo J, Boateng LK et al (2013) Ultrasonic degradation of acetaminophen and naproxen in the presence of single-walled carbon nanotubes. J Hazard Mater 254–255:284–292. https://doi.org/10.1016/j.jhazmat.2013.04.001

36. Serna-Galvis EA, Silva-Agredo J, Giraldo-Aguirre AL, Torres-Palma RA (2015) Sonochemical degradation of the pharmaceutical fluoxetine: Effect of parameters, organic and inorganic additives and combination with a biological system. Sci Total Environ 524–525:354–360. https://doi.org/10.1016/j.scitotenv.2015.04.053
37. Lastre-Acosta AM, Cruz-González G, Nuevas-Paz L et al (2015) Ultrasonic degradation of sulfadiazine in aqueous solutions. Environ Sci Pollut Res 22:918–925. https://doi.org/10.1007/s11356-014-2766-2
38. Carmona E, Picó Y (2018) The use of chromatographic methods coupled to mass spectrometry for the study of emerging pollutants in the environment. Crit Rev Anal Chem 48:305–316. https://doi.org/10.1080/10408347.2018.1430555
39. Hernández F, Ibáñez M, Bade R et al (2014) Investigation of pharmaceuticals and illicit drugs in waters by liquid chromatography-high-resolution mass spectrometry. TrAC Trends Anal Chem 63:140–157. https://doi.org/10.1016/j.trac.2014.08.003
40. Kosjek T, Heath E (2008) Applications of mass spectrometry to identifying pharmaceutical transformation products in water treatment. TrAC Trends Anal Chem 27:807–820. https://doi.org/10.1016/j.trac.2008.08.014
41. Hernández F, Bakker J, Bijlsma L et al (2019) The role of analytical chemistry in exposure science: focus on the aquatic environment. Chemosphere 222:564–583. https://doi.org/10.1016/j.chemosphere.2019.01.118
42. Ferrando-Climent L, Gonzalez-Olmos R, Anfruns A et al (2017) Elimination study of the chemotherapy drug tamoxifen by different advanced oxidation processes: transformation products and toxicity assessment. Chemosphere 168:284–292. https://doi.org/10.1016/j.chemosphere.2016.10.057
43. Botero-Coy AM, Martínez-Pachón D, Boix C et al (2018) An investigation into the occurrence and removal of pharmaceuticals in Colombian wastewater. Sci Total Environ 642:842–853. https://doi.org/10.1016/j.scitotenv.2018.06.088
44. Bussy U, Li K, Li W (2016) Application of liquid chromatography-tandem mass spectrometry in quantitative bioanalyses of organic molecules in aquatic environment and organisms. Environ Sci Pollut Res 23:9459–9479. https://doi.org/10.1007/s11356-016-6433-7
45. Gracia-Lor E, Sancho JV, Hernández F (2011) Multi-class determination of around 50 pharmaceuticals, including 26 antibiotics, in environmental and wastewater samples by ultra-high performance liquid chromatography–tandem mass spectrometry. J Chromatogr A 1218:2264–2275. https://doi.org/10.1016/j.chroma.2011.02.026
46. Gusmaroli L, Insa S, Petrovic M (2018) Development of an online SPE-UHPLC-MS/MS method for the multiresidue analysis of the 17 compounds from the EU "Watch list". Anal Bioanal Chem 410:4165–4176. https://doi.org/10.1007/s00216-018-1069-8
47. Hernández F, Castiglioni S, Covaci A et al (2018) Mass spectrometric strategies for the investigation of biomarkers of illicit drug use in wastewater. Mass Spectrom Rev 37:258–280. https://doi.org/10.1002/mas.21525
48. Ibáñez M, Gracia-Lor E, Bijlsma L et al (2013) Removal of emerging contaminants in sewage water subjected to advanced oxidation with ozone. J Hazard Mater 260:389–398. https://doi.org/10.1016/j.jhazmat.2013.05.023
49. Kim C, Ryu H-D, Chung EG et al (2018) A review of analytical procedures for the simultaneous determination of medically important veterinary antibiotics in environmental water: sample preparation, liquid chromatography, and mass spectrometry. J Environ Manage 217:629–645. https://doi.org/10.1016/j.jenvman.2018.04.006
50. Rodriguez-Mozaz S, Vaz-Moreira I, Varela Della Giustina S et al (2020) Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment. Environ Int 140:105733. https://doi.org/10.1016/j.envint.2020.105733
51. Michael SG, Michael-Kordatou I, Nahim-Granados S et al (2020) Investigating the impact of UV-C/H2O2 and sunlight/H2O2 on the removal of antibiotics, antibiotic resistance determinants and toxicity present in urban wastewater. Chem Eng J 388:124383. https://doi.org/10.1016/j.cej.2020.124383

52. Serna-Galvis EA, Silva-Agredo J, Botero-Coy AM et al (2019) Effective elimination of fifteen relevant pharmaceuticals in hospital wastewater from Colombia by combination of a biological system with a sonochemical process. Sci Total Environ 670:623–632. https://doi.org/10.1016/j.scitotenv.2019.03.153
53. Serna-Galvis EA, Botero-Coy AM, Martínez-Pachón D et al (2019) Degradation of seventeen contaminants of emerging concern in municipal wastewater effluents by sonochemical advanced oxidation processes. Water Res 154:349–360. https://doi.org/10.1016/j.watres.2019.01.045
54. Boix C, Ibáñez M, Sancho JV et al (2015) Fast determination of 40 drugs in water using large volume direct injection liquid chromatography–tandem mass spectrometry. Talanta 131:719–727. https://doi.org/10.1016/j.talanta.2014.08.005
55. Kern S, Fenner K, Singer HP et al (2009) Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. Environ Sci Technol 43:7039–7046. https://doi.org/10.1021/es901979h
56. Nurmi J, Pellinen J, Rantalainen A-L (2012) Critical evaluation of screening techniques for emerging environmental contaminants based on accurate mass measurements with time-of-flight mass spectrometry. J Mass Spectrom 47:303–312. https://doi.org/10.1002/jms.2964
57. Schollée JE, Schymanski EL, Avak SE et al (2015) Prioritizing unknown transformation products from biologically-treated wastewater using high-resolution mass spectrometry, multivariate statistics, and metabolic logic. Anal Chem 87:12121–12129. https://doi.org/10.1021/acs.analchem.5b02905
58. Boix C, Ibáñez M, Bagnati R et al (2016) High resolution mass spectrometry to investigate omeprazole and venlafaxine metabolites in wastewater. J Hazard Mater 302:332–340. https://doi.org/10.1016/j.jhazmat.2015.09.059
59. Jaén-Gil A, Buttiglieri G, Benito A et al (2019) Metoprolol and metoprolol acid degradation in UV/H2O2 treated wastewaters: an integrated screening approach for the identification of hazardous transformation products. J Hazard Mater 380:120851. https://doi.org/10.1016/j.jhazmat.2019.120851
60. Ibáñez M, Borova V, Boix C et al (2017) UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. J Hazard Mater 323:26–35. https://doi.org/10.1016/j.jhazmat.2016.03.078
61. Cuervo Lumbaque E, Cardoso RM, Dallegrave A et al (2018) Pharmaceutical removal from different water matrixes by Fenton process at near-neutral pH: Doehlert design and transformation products identification by UHPLC-QTOF MS using a purpose-built database. J Environ Chem Eng 6:3951–3961. https://doi.org/10.1016/j.jece.2018.05.051
62. Bletsou AA, Jeon J, Hollender J et al (2015) Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment. TrAC Trends Anal Chem 66:32–44. https://doi.org/10.1016/j.trac.2014.11.009
63. Martínez-Pachón D, Ibáñez M, Hernández F et al (2018) Photo-electro-Fenton process applied to the degradation of valsartan: effect of parameters, identification of degradation routes and mineralization in combination with a biological system. J Environ Chem Eng 6:7302–7311. https://doi.org/10.1016/j.jece.2018.11.015
64. Serna-Galvis EA, Silva-Agredo J, Giraldo-Aguirre AL et al (2016) High frequency ultrasound as a selective advanced oxidation process to remove penicillinic antibiotics and eliminate its antimicrobial activity from water. Ultrason Sonochem 31:276–283. https://doi.org/10.1016/j.ultsonch.2016.01.007
65. Jagannathan M, Grieser F, Ashokkumar M (2013) Sonophotocatalytic degradation of paracetamol using TiO2 and Fe3+. Sep Purif Technol 103:114–118. https://doi.org/10.1016/j.seppur.2012.10.003
66. Elias MT, Chandran J, Aravind UK, Aravindakumar CT (2019) Oxidative degradation of ranitidine by UV and ultrasound: identification of transformation products using LC-Q-ToF-MS. Environ Chem 16:41–54

67. Roudbari A, Rezakazemi M (2018) Hormones removal from municipal wastewater using ultrasound. AMB Express 8:1–8. https://doi.org/10.1186/s13568-018-0621-4
68. Arvaniti OS, Frontistis Z, Nika MC et al (2020) Sonochemical degradation of trimethoprim in water matrices: effect of operating conditions, identification of transformation products and toxicity assessment. Ultrason Sonochem 67:105139. https://doi.org/10.1016/j.ultsonch.2020.105139
69. Zeng P, Du J, Song Y et al (2015) Efficiency comparison for treatment of amantadine pharmaceutical wastewater by Fenton, ultrasonic, and Fenton/ultrasonic processes. Environ Earth Sci 73:4979–4987. https://doi.org/10.1007/s12665-015-4204-2
70. González Labrada K, Alcorta Cuello DR, Saborit Sánchez I et al (2019) Optimization of ciprofloxacin degradation in wastewater by homogeneous sono-Fenton process at high frequency. J Environ Sci Heal A Tox Hazard Subst Environ Eng 53:1139–1148. https://doi.org/10.1080/10934529.2018.1530177
71. Rahmani H, Gholami M, Mahvi AH et al (2014) Tinidazole removal from aqueous solution by sonolysis in the presence of hydrogen peroxide. Bull Environ Contam Toxicol 92:341–346. https://doi.org/10.1007/s00128-013-1193-2
72. Ghafoori S, Mowla A, Jahani R et al (2015) Sonophotolytic degradation of synthetic pharmaceutical wastewater: statistical experimental design and modeling. J Environ Manage 150:128–137. https://doi.org/10.1016/j.jenvman.2014.11.011
73. Naddeo V, Ricco D, Scannapieco D, Belgiorno V (2012) Degradation of antibiotics in wastewater during sonolysis, ozonation, and their simultaneous application: operating conditions effects and processes evaluation. Int J Photoenegy 2012. 624270, 7 p. https://doi.org/10.1155/2012/624270
74. Naddeo V, Uyguner-Demirel CS, Prado M et al (2015) Enhanced ozonation of selected pharmaceutical compounds by sonolysis. Environ Technol 36:1876–1883. https://doi.org/10.1080/09593330.2015.1014864
75. Nachiappan S, Muthukumar K (2013) Treatment of pharmaceutical effluent by ultrasound coupled with dual oxidant system. Environ Technol 34:209–217. https://doi.org/10.1080/09593330.2012.689367
76. Sivakumar R, Muthukumar K (2011) Sonochemical degradation of pharmaceutical wastewater. Clean (Weinh) 39:136–141. https://doi.org/10.1002/clen.200900289
77. Adityosulindro S, Barthe L, González-Labrada K et al (2017) Sonolysis and sono-Fenton oxidation for removal of ibuprofen in (waste)water. Ultrason Sonochem 39:889–896
78. Mowla A, Mehrvar M, Dhib R (2014) Combination of sonophotolysis and aerobic activated sludge processes for treatment of synthetic pharmaceutical wastewater. Chem Eng J 255:411–423. https://doi.org/10.1016/j.cej.2014.06.064
79. Brüggemann H, Köser H, Meyer E, Nguyen T-H (2003) Sonolytic debromination of ambroxol process wastewater. Water Res 37:674–680. https://doi.org/10.1016/S0043-1354(02)00363-9
80. Pulicharla R, Brar SK, Rouissi T et al (2017) Degradation of chlortetracycline in wastewater sludge by ultrasonication, Fenton oxidation, and ferro-sonication. Ultrason Sonochem 34:332–342. https://doi.org/10.1016/j.ultsonch.2016.05.042
81. Monteagudo JM, Durán A, San Martín I (2014) Mineralization of wastewater from the pharmaceutical industry containing chloride ions by UV photolysis of H2O2/Fe(II) and ultrasonic irradiation. J Environ Manage 141:61–69. https://doi.org/10.1016/j.jenvman.2014.03.020
82. Naddeo V, Landi M, Scannapieco D, Belgiorno V (2013) Sonochemical degradation of twenty-three emerging contaminants in urban wastewater. Desalin Water Treat 51:6601–6608. https://doi.org/10.1080/19443994.2013.769696
83. Yang B, Zuo J, Li P et al (2016) Effective ultrasound electrochemical degradation of biological toxicity and refractory cephalosporin pharmaceutical wastewater. Chem Eng J 287:30–37. https://doi.org/10.1016/j.cej.2015.11.033

84. Chandak S, Ghosh PK, Gogate PR (2020) Treatment of real pharmaceutical wastewater using different processes based on ultrasound in combination with oxidants. Process Saf Environ Prot 137:149–157. https://doi.org/10.1016/j.psep.2020.02.025
85. Papoutsakis S, Afshari Z, Malato S, Pulgarin C (2015) Elimination of the iodinated contrast agent iohexol in water, wastewater and urine matrices by application of photo-Fenton and ultrasound advanced oxidation processes. J Environ Chem Eng 3:2002–2009. https://doi.org/10.1016/j.jece.2015.07.002
86. Ashokkumar M, Lee J, Iida Y et al (2009) The detection and control of stable and transient acoustic cavitation bubbles. Phys Chem Chem Phys 11:10118–10121. https://doi.org/10.1039/B915715H
87. Lee J, Yasui K, Tuziuti T et al (2008) Spatial distribution enhancement of sonoluminescence activity by altering sonication and solution conditions. J Phys Chem B 112:15333–15341. https://doi.org/10.1021/jp8060224
88. Lee J, Ashokkumar M, Yasui K et al (2011) Development and optimization of acoustic bubble structures at high frequencies. Ultrason Sonochem 18:92–98. https://doi.org/10.1016/j.ultsonch.2010.03.004
89. Kanthale P, Ashokkumar M, Grieser F (2008) Sonoluminescence, sonochemistry (H2O2 yield) and bubble dynamics: frequency and power effects. Ultrason Sonochem 15:143–150. https://doi.org/10.1016/j.ultsonch.2007.03.003
90. Lesko T, Colussi AJ, Hoffmann MR (2006) Sonochemical decomposition of phenol: evidence for a synergistic effect of ozone and ultrasound for the elimination of total organic carbon from water. Environ Sci Technol 40:6818–6823. https://doi.org/10.1021/es052558i
91. Ashokkumar M, Lee J, Iida Y et al (2010) Spatial distribution of acoustic cavitation bubbles at different ultrasound frequencies. ChemPhysChem 11:1680–1684. https://doi.org/10.1002/cphc.200901037
92. Nalesso S (2019) Antisolvent and reactive crystallisation under variations of ultrasound parameters: effects and comparisons. University of Surrey, Guildford
93. Cui M, Jang M, Ibrahim S et al (2014) Arsenite removal using a pilot system of ultrasound and ultraviolet followed by microfiltration. Ultrason Sonochem 21:1527–1534. https://doi.org/10.1016/j.ultsonch.2014.01.001
94. Manickam S, Zainal Abidin N, Parthasarathy S et al (2014) Role of H2O2 in the fluctuating patterns of COD (chemical oxygen demand) during the treatment of palm oil mill effluent (POME) using pilot scale triple frequency ultrasound cavitation reactor. Ultrason Sonochem 21:1519–1526. https://doi.org/10.1016/j.ultsonch.2014.01.002
95. Gole VL, Fishgold A, Sierra-Alvarez R et al (2018) Treatment of perfluorooctane sulfonic acid (PFOS) using a large-scale sonochemical reactor. Sep Purif Technol 194:104–110. https://doi.org/10.1016/j.seppur.2017.11.009
96. Caretti C, Coppini E, Fatarella E, Lubello C (2011) Membrane filtration and sonication for industrial wastewater reuse. Water Sci Technol 64:2500–2507. https://doi.org/10.2166/wst.2011.800
97. Lee J (2016) Importance of sonication and solution conditions on the acoustic cavitation activity BT – handbook of ultrasonics and sonochemistry. Springer, Singapore, pp 137–175
98. Kimura K, Amy G, Drewes JE et al (2003) Rejection of organic micropollutants (disinfection by-products, endocrine disrupting compounds, and pharmaceutically active compounds) by NF/RO membranes. J Membr Sci 227:113–121. https://doi.org/10.1016/j.memsci.2003.09.005
99. Amy G, Kim T-U, Yoon J et al (2005) Removal of micropollutants by NF/RO membranes. Water Supply 5:25–33. https://doi.org/10.2166/ws.2005.0035
100. Xu P, Drewes JE, Bellona C et al (2005) Rejection of emerging organic micropollutants in nanofiltration–reverse osmosis membrane applications. Water Environ Res 77:40–48. https://doi.org/10.2175/106143005X41609

101. Muthukumaran S, Kentish SE, Stevens GW, Ashokkumar M (2006) Application of ultrasound in membrane separation processes: a review. Rev Chem Eng 22:155–194. https://doi.org/10.1515/REVCE.2006.22.3.155
102. Naddeo V, Borea L, Belgiorno V (2015) Sonochemical control of fouling formation in membrane ultrafiltration of wastewater: effect of ultrasonic frequency. J Water Process Eng 8:e92–e97. https://doi.org/10.1016/j.jwpe.2014.12.005
103. Reuter F, Lauterborn S, Mettin R, Lauterborn W (2017) Membrane cleaning with ultrasonically driven bubbles. Ultrason Sonochem 37:542–560. https://doi.org/10.1016/j.ultsonch.2016.12.012
104. Secondes MFN, Naddeo V, Belgiorno V, Ballesteros F (2014) Removal of emerging contaminants by simultaneous application of membrane ultrafiltration, activated carbon adsorption, and ultrasound irradiation. J Hazard Mater 264:342–349. https://doi.org/10.1016/j.jhazmat.2013.11.039

Correction to: Advanced Electrochemical Processes for the Elimination of Pharmaceutical Compounds in Contaminated Waters

Jelena Radjenovic and Luis Baptista-Pires

Correction to: Chapter "Advanced Electrochemical Processes for the Elimination of Pharmaceutical Compounds in Contaminated Waters" in: Jelena Radjenovic and Luis Baptista-Pires, Hdb Env Chem, https://doi.org/10.1007/698_2020_689

This chapter was inadvertently published with an incorrect title "Chemical Processes for the Elimination of Pharmaceutical Compounds in Contaminated Waters". The title has now been updated correctly to read as "Advanced Electrochemical Processes for the Elimination of Pharmaceutical Compounds in Contaminated Waters".

The updated online version of this chapter can be found at https://doi.org/10.1007/698_2020_689

Sara Rodriguez-Mozaz, Paqui Blánquez Cano, and Montserrat Sarrà Adroguer (eds.), *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment*, Hdb Env Chem (2021) 108: 383–384, https://doi.org/10.1007/698_2021_757,