Universidade do Minho
Escola de Engenharia

Ana Elisa Marques do Lago

Design and tailoring of sorbents coupled with biodegradors for emerging pollutants abatement in aqueous phase

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Design and tailoring of sorbents coupled with biodegradors for emerging pollutants abatement in aqueous phase

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Work developed under supervision of
Professor Doctor Maria Teresa Tavares
Doctor Bruna Andreia Silva

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"It is good to have an end to journey towards; but it is the journey that matters, in the end."

Ursula K. Le Guin

STATEMENT OF INTEGRITY

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RESUMO

Desenho e especificação de sorventes acoplados a biodegradadores para a redução de poluentes emergentes em fase aquosa

A poluição da água provocada por contaminantes emergentes tem-se tornado um problema global crescente devido ao tratamento ineficaz destes poluentes nas estações de tratamento de águas residuais. Em particular, herbicidas e fármacos têm sido recorrentemente detetados em diferentes matrizes aquosas, tornando-se uma ameaça à biodiversidade aquática e terrestre e à saúde humana. É urgente o desenvolvimento de técnicas acessíveis e eficazes para reduzir a sua entrada no meio ambiente.

Esta tese explora a utilização de resíduos orgânicos e inorgânicos como sorventes alternativos para a fixação de herbicidas e de fármacos a partir de matrizes aquosas. Também investiga a funcionalidade de um resíduo florestal como suporte para a formação de biofilmes bacterianos, visando a simulação de biobarreiras sustentáveis em reatores de leito fixo para melhorar o processo de reabilitação. Sepiolite, cortiça e casca de pinheiro foram os materiais selecionados e utilizados como barreiras sustentáveis para processar soluções com atrazina em reatores de coluna de fluxo contínuo. O potencial de reciclagem dos sorventes também foi avaliado através de ciclos consecutivos de adsorção-dessorção. A sepiolite revelou ser o melhor material em termos de capacidade de adsorção e estabilidade após dois ciclos de regeneração, usando um agente lixiviante com baixo impacto ambiental. Em seguida, foi avaliado o processamento de uma solução composta por atrazina, fluoxetina e carbamazepina usando casca de pinheiro natural, bem como modificada quimicamente e termicamente. A casca de pinheiro modificada com HNO_3 revelou ser o melhor biossorvente em termos de captação dos sorbatos. A viabilidade económica dos processos foi avaliada, revelando-se a modificação de superfície com H_2O_2 como o procedimento mais caro. A casca de pinheiro foi também regenerada, utilizando-se um eluente otimizado e reutilizado pelo menos duas vezes, sem que o sorvente perdesse a sua capacidade de adsorção, isto em sistemas fechados. A adsorção dos poluentes pela casca de pinheiro natural também foi investigada num reator do tipo "air-lift", permitindo confirmar a eficácia do sistema para aplicações em maior escala. Por fim, foram concebidas biobarreiras sustentáveis de biofilmes bacterianos (*Streptococcus equisimilis* ou *Pseudomonas putida*) suportados em casca de pinheiro para a retenção de atrazina, em reatores de leito fixo. O sistema com melhor desempenho foi o do biofilme suportado com *S. equisimilis*, atingindo 90,42 % de remoção do poluente, processo atribuído à adsorção pelo biofilme e pelo suporte, bem como à degradação pelas bactérias.

Palavras-chave: Adsorção, biobarreiras sustentáveis, biofilmes, contaminantes emergentes, valorização de resíduos

ABSTRACT

Design and tailoring of sorbents coupled with biodegradors for emerging pollutants abatement in aqueous phase

Environmental water pollution caused by emerging contaminants has become a growing problem worldwide due to their ineffective treatment in wastewater treatment plants. Particularly, herbicides and pharmaceuticals have been recurrently detected in different water matrices, becoming a threat to aquatic and terrestrial biodiversity and to human health. The development of affordable and more effective techniques to reduce their input in the environment is urgent.

This thesis explores the use of organic and inorganic wastes as alternative sorbents for the fixation of herbicides and pharmaceuticals from water matrices. It also investigates the performance of a forest residue as a support for the formation of bacterial biofilms to simulate sustainable biobarriers in packed bed reactors to increase the scope of the whole rehabilitation process. Sepiolite, cork and pine bark were selected and used to act as sustainable barriers to rehabilitate water contaminated with atrazine in continuous column reactors. The recycling potential of sorbents was also assessed by consecutive cycles of adsorption-desorption processes. Sepiolite was revealed as the best material in terms of adsorption capacity and stability after two regeneration cycles, depending on a leaching agent with small environmental impact. The processing of a multi solute solution with atrazine, fluoxetine and carbamazepine was assessed using raw, chemically and thermally modified pine. Pine modified with HNO_3 revealed to be the best biosorbent in terms of overall sorbate entrapment capacity. The economic feasibility of biosorbents was evaluated, reveling that the surface modification with H_2O_2 is the most expensive procedure. Pine was also regenerated, with an optimized extraction eluent, and reused as biosorbent at least twice without losing its adsorption efficiency, in batch mode. Pollutants adsorption by raw pine was also investigated in an air-lift type reactor, confirming the effectiveness of the system for up-scale purposes. Lastly, sustainable biobarriers were designed with bacterial biofilms (*Streptococcus equisimilis* or *Pseudomonas putida*) supported on pine to retain atrazine in packed bed reactors. The best performing system was the one with *S. equisimilis* biofilm, reaching 90.42 % of removal, attributed to adsorption by the biofilm and by the support, as well as to the degradation by bacteria.

Keywords: Adsorption, sustainable biobarriers, biofilms, emerging contaminants, waste valorization

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LIST OF ABBREVIATIONS AND ACRONYMS

AC	activated carbon
ATR-FTIR	attenuated total reflection Fourier transform infrared
ATZ	atrazine
BAF	biological aerated filter
BASR	biofilm airlift suspended reactor
BCBR	biological contact biofilm reactor
BCOR	bio-contact oxidation reactor
BHI	brain heart infusion
BOD	biochemical oxygen demand
BTF	biological trickling filter
CBE	circular bioeconomy
COD	chemical oxygen demand
CRB	carbamazepine
DEK	diethylketone
EC	emerging contaminants
EDS	energy-dispersive X-ray spectroscopy
EPS	extracellular polymeric substances
FBR	fluidized bed reactor
FBSBR	fixed bed sequence batch reactor
FLX	fluoxetine
GAC	granular activated carbon
HTC	hydrothermal carbonization
IFAS	integrated fixed-film activated sludge reactor
LB	luria–bertani
MBBR	moving bed biofilm reactor
MBR	membrane biofilm reactor
MEK	methylethylketone
OD_{620}	optical density at 620 nm
PBBR	packed bed biofilm reactor
PFO	pseudo-first order

pH_{zpc}	pH of zero point of charge
PRB	permeable reactive barriers
PRBB	permeable reactive biobarriers
PSO	pseudo-second order
QS	quorum sensing
ROS	reactive oxygen species
SBBR	sequencing batch biofilm reactor
S_{BET}	surface area
SD	standard deviation
SEM	scanning electron microscopy
SVI	sludge volume index
Sips	three-parameter Langmuir–Freundlich
TEA	terminal electron acceptors
TN	total nitrogen
TP	total phosphorus
UASB	up-flow anaerobic sludge blanket
UHPLC-DAD	ultra-high performance liquid chromatography with diode array detector
VOC	volatile organic compounds
WTP	water treatment plants
WWTP	wastewater treatment plants

SCIENTIFIC OUTPUT

According to the 2nd paragraph of the article 8 of the Portuguese Decree-Law no. 388/70, the scientific outputs of this thesis are listed below.

The results presented in this thesis have been partially published elsewhere.

Papers accepted in peer-reviewed journal articles:

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Silva, B., Rocha, V., **Lago, A**., Costa, F., Tavares, T. Rehabilitation of a complex industrial wastewater containing heavy metals and organic solvents using low cost permeable bio-barriers – from lab-scale to pilot-scale. Separation and Purification Technology, 263 (118381), **2021**

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Costa, F., **Lago, A.**, Barros, Ó., Rocha, V., Vipotnik, Z., Silva, B., Tavares, T., **2020**. Chapter 14 - Retention of organic micro-pollutants by sorption processes, in: Varjani, S., Pandey, A., Tyagi, R.D., Ngo, H.H., Larroche, C.B.T.-C.D. in B. and B. (Eds.), . Elsevier, pp. 331–362.

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Lago, A., Rocha, V., Costa, F., Silva, B., Tavares, T. Rehabilitation of water contaminated with an organic solvent by an aerobic bacterium: tolerance and bioremoval assays**.** WWEM-19 - 5th International Congress on Water, Waste and Energy Management. Paris, France, July 22-24, **2019**

Oral communications in conferences:

Lago, A., Silva, B., Tavares, T. Biowaste valorization for emerging pollutants abatement in aqueous phase. EWaS5- 5th Efficient Water Systems International Conference. Naples, Italy, July 12-15, **2022**

Silva, B., **Lago, A.**, Tavares, T. Modified natural materials as alternative low cost adsorbents for fluoxetine retention from wastewater. EWaS5- 5th Efficient Water Systems International Conference. Naples, Italy, July 12-15, **2022**

Silva, B., Rocha, V., **Lago, A.**, Tavares, T. Valorization of spent coffee grounds as biosorbent for the retention of fluoxetine from water – a cost-effective alternative to activated carbon. 13th International Chemical and Biological Engineering Conference). Aveiro, Portugal, October 2-4, **2018**

CHAPTER I

MOTIVATION AND OUTLINE

This chapter presents the background information supporting this work, as well as its objectives. The outline of the thesis is also presented.

1.1 CONTEXT AND MOTIVATION

Different classes of pollutants as herbicides and pharmaceuticals have been identified in the aquatic environment and classified as emerging contaminants (EC). The release of these non-regulated trace micro pollutants over time from different sources [1,2], adding to the lack of a rigorous legislation and routine monitoring, have made this subject an issue of concern [3]. Their occurrence in the environment makes them partially accountable for the instability and fragility installed in the ecosystems [4] and for their detrimental impact on public health [5,6]. Despite the advances in treatment technologies, the actual process design of existing wastewater treatment plants (WWTP) does not allow the total removal of these micro contaminants [7,8]. The ubiquity of these molecules in the environment underpins the need of a better understanding of their occurrence and the development of disruptive and more efficient processes to remove them [1]. From this point of view the search for greener, environment friendlier and less expensive remediation approaches to prevent water contamination is becoming more and more important.

Adsorption is an attractive physicochemical process used for the entrapment of a wide range of pollutants from aqueous systems [9–12] due to its simplicity and ease of operation over other technologies as precipitation followed by coagulation, membrane filtration and photochemical degradation [13]. It is in fact a valuable pretreatment, able to concentrate pollutants like the emerging ones. Many of these systems are based on the usage of activated carbon (AC) [9]. On the other hand, biosorption entails the usage of biosorbents, sorbents of biological origin, for the removal of contaminants and proved to be useful in the treatment of secondary or tertiary effluents [14,15]. All over the years several adsorption reactor configurations have been developed. The most popular type of flow reactors is fixed bed columns [16–19]. The air-lift type reactor is being also introduced as a potential design, to be used in adsorption processes, once adsorbents are forced to move by gas flow, leading to a more effective contact with the sorbate [20].

Microbial bioremediation of troublesome contaminants in specific ecosystems is increasingly seen as being both cost-effective and reliable [14,21–23]. Common methods for the *in situ* biological protection of ecosystems and for treatment of contaminated sites use permeable reactive biobarriers (PRBB) with engineered structures of enhanced microbial biofilm activity [11]. Biofilm structures usually have high biological activity and sorption area, they provide physical support and protection for microbes, and may enhance the detoxification of micropollutants [15]. The three-dimensional structure offer additional protection from toxic substances and physical perturbation to microbial communities [15]. Some biofilms

are known to entrap organic pollutants [22] and do play a significant role in the fate and transportation of pollutants in aquatic environments [24].

The synergistic effect between sorption and biodegradation has been studied in recent years. Wen *et al.* [25] evaluated vermiculite as a support for a bacterial biofilm of *Arthrobacter* sp. in the degradation of di-(2-ethylhexyl) phthalate and they conclude that the two processes, sorption and degradation, enhanced the overall pollutant removal efficiency. The use of sorption/degradation performed by supported biofilms structures for the removal of pollutants is thus an opportunity that needs to be further explored and improved, especially for high-risk contaminants such as the emerging ones in water.

In this context, the aim of this thesis is the development of a rehabilitation technology applicable to water contaminated with specific EC as herbicides and pharmaceutical drugs. The main purpose is to explore cost-effective and eco-friendly alternatives to conventional treatments used in WWTP to remove target EC in simulated liquid solutions, using sorption and degradation by bacterial processes. Special attention will be paid to the use of low-cost and sustainable materials by recovering organic and inorganic wastes to function as sorbents or as supports for biofilm formation. Firstly, sorption studies will be explored in continuous fixed-bed experiments using those materials as permeable reactive barriers (PRB) and then, the effectiveness of pollutants removal is going to be studied under dynamic flow conditions in air-lift type loop reactor. Lastly, in order to increase the scope of the whole rehabilitation process, sorbents and bacterial biofilms will be designed and tested as biobarriers in a packed bed biofilm reactor (PBBR).

1.2 RESEARCH AIMS

The proposed methodology consists of the systematic evaluation of different unmodified and modified waste materials as sorbents to be used in pollutants fixation, but also to function as supports for the formation of bacterial biofilms in order to enhance the EC elimination process, with the rehabilitation of contaminated water as the final goal.

Within the context described in the previous section, the expected outcome of this PhD research plan is the definition and development of an economically viable and environment friendly system, able to remove atrazine (ATZ), fluoxetine (FLX) and carbamazepine (CRB), selected to work as a first approach to the EC issue, and to be applied as a refining treatment in WWTP, water treatment plants (WTP) or in related industries.

To achieve the main goal of this PhD project, some partial objectives were established:

- Optimization of the analytical protocols for the quantification of EC by ultra-high performance liquid chromatography with diode array detector (UHPLC-DAD);
- Selection of efficient sustainable materials that can be used for both adsorption/biosorption and biofilms supporting;
- Optimization of the selected materials by surface pre-treatments (chemical/ thermal procedures);
- Evaluation of the cost involved in the production of unmodified and modified sorbents;
- Evaluation of the adsorption capacity of those materials in batch mode and in continuous / discontinuous reactors;
- Optimization of the operational parameters for columns and air-lift assays;
- Modelling of the adsorption equilibria and kinetics aiming industrial scaled-up applications and mechanisms definition; establishment of breakthrough thresholds;
- Evaluation of different bacteria able to eliminate partially or completely the target molecules (toxicological and bioremoval assays) at batch mode;
- Assessment of the best operation conditions that maximize the bacterial biofilm formation and attachment to the biocarrier, enabling the entrapment and/or biodegradation of the target EC in columns assays (in PBBR);
- Study of the pollutants desorption from sorbents by different solvents extraction;
- Assessment of the recyclability of sorbents based on adsorption and desorption cycles in batch and columns assays;
- Application of the optimized overall system as a bioreactor for wastewater rehabilitation at lab scale.

1.3 OUTLINE OF THE THESIS

The research activities resulting in this doctoral thesis were developed at Centre of Biological Engineering, University of Minho (Braga, Portugal) under the supervision of Professor Teresa Tavares and Doctor Bruna Silva. To address the above-mentioned objectives, the thesis was structured in six chapters, three of them describing experimental data (from Chapter III to Chapter V):

CHAPTER I presents the motivation, the research goals and the outline of the thesis.

CHAPTER II comprises a comprehensive review of water problematic regarding EC presence, particularly ATZ, FLX and CRB. Treatment technologies, especially using sorption and biological processes are discussed in detail. Additionally, a state of the art comprehending the use of sustainable bacterial biofilm carriers and their use in environment friendly treatment systems is reviewed.

CHAPTER III focus on the exploitation of waste materials (sepiolite) and biomaterials (cork and pine bark) to be used as sorbents to rehabilitate water contaminated with ATZ. ATZ adsorption by those materials and desorption by eluents were firstly assessed in batch assays. Sorbents were employed as PRB in continuous experiments with reusage cycles for up-scale applications.

CHAPTER IV evaluates the sorption capacity and the economic feasibility of raw, chemically and thermally modified pine to treat multi-solute aqueous solutions with ATZ, FLX and CRB. The recyclability of raw pine was evaluated by adsorption-desorption cycles in batch mode using the selected desorption eluent. The effectiveness of pollutants biosorption by raw pine was shown in an air-lift type reactor.

CHAPTER V assesses the synergy between pine and bacterial biofilms (*Streptococcus equisimilis* or *Pseudomonas putida*) in biobarriers for the elimination of ATZ in PBBR. Bacterial adhesion onto the biocarrier was firstly optimized and ATZ toxicity to both bacteria was established. Experiments using the designed biobarriers resulted in a beneficial effect towards the removal of the herbicide.

CHAPTER VI offers the major conclusions, emphasizing the contribution of the study for the development of a sustainable treatment technology for contaminated water, alongside future perspectives on further developments on the topic.

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CHAPTER II

GENERAL INTRODUCTION

This chapter provides a short survey of the topics regarding the broad areas of this thesis: the environmental problematic of EC and the rehabilitation techniques for their removal. Firstly, the presence of pesticides and pharmaceuticals in water matrices is assessed with special emphasis to ATZ, FLX and CRB. Then, the available technologies for their elimination are considered. Sorption processes using sustainable and low-cost materials are the focus and are discussed in detail, as well as their combination with bacterial biofilms for remediation improvement. Tolerance and biodegradation mechanisms of ATZ by bacteria are also contemplated. In the last part, a comprehensive review comparing the use of sustainable and commercial biofilm carriers in biological wastewater rehabilitation is assessed.

This chapter is based on the following publications:

Costa, F.; **Lago, A**.; Rocha, V.; Barros, Ó.; Costa, L.; Vipotnik, Z.; Silva, B.; Tavares, T. A Review on Biological Processes for Pharmaceuticals Wastes Abatement—A Growing Threat to Modern Society. *Environ. Sci. Technol.* **2019**, *53*, 7185–7202, doi:10.1021/acs.est.8b06977.

Costa, F., **Lago, A.**, Barros, Ó., Rocha, V., Vipotnik, Z., Silva, B., Tavares, T., **2020**. Chapter 14 - Retention of organic micro-pollutants by sorption processes, in: Varjani, S., Pandey, A., Tyagi, R.D., Ngo, H.H., Larroche, C.B.T.-C.D. in B. and B. (Eds.), . Elsevier, pp. 331–362. https://doi.org/https://doi.org/10.1016/B978-0-12-819594-9.00014-0

Lago, A.; Rocha, V.; Barros, Ó., Silva, B., Tavares, T. Bacterial biofilm attachment to sustainable carriers as a clean-up strategy for wastewater treatment: a review **(under submission)**

2.1 TARGET EMERGING CONTAMINANTS

A large range of compounds are used daily by society in growing amounts for different human and industrial purposes. Their detection in environmental matrices is pushing the research community to understand their environmental occurrence, fate, behavior, and potential for unintended effects. Some contaminants are reported to have carcinogenic and/or mutagenic properties and are barely removed by degradation processes [1]. This class of pollutant includes the synthetic or naturally occurring substances (mostly organic), designed as EC.

The term "emerging" is used not only to describe new compounds recently discovered, developed, and consumed, but is also applied to substances already present in the environment, though only recently recognized as contaminants [2]. Although EC are often present at trace levels, their presence in the environment is an ever-increasing issue of concern because they may induce known or suspected instability and fragility in ecosystems and may have a detrimental impact on aquatic wildlife and public health [3,4]. Depending on the persisting and recalcitrant characteristics of EC, bioaccumulation in exposed biota through food chains may occur [5].

These molecules have mainly anthropogenic origin and may endanger the water cycle through a multitude of direct and indirect pathways. They can be discharged through industrial, municipal, hospital, or laboratory effluents [6], runoffs from farming practices, manure application and livestock activities [2,7], leachates from subsurface storage of waste [8], sewage sludge land application [9], on-site (septic) waste treatment systems [6,10], and exchanges through groundwater/surface water [2], among other possibilities. However, the discharge of WWTP effluents with inefficient treatment is the main reason for the presence of these micro-pollutants in freshwater resources [11,12]. Activated sludge and trickling filters are the most extensively used processes in conventional treatments, even though they are not designed to degrade and/or remove these emerging pollutants in particular, resulting in their reintroduction into rivers and/or aquifers [13]. EC in waterbodies are then dispersed to other places, including aquifers, estuaries, and marine systems [6] leading to their amplified presence in nature (**Figure 1**).

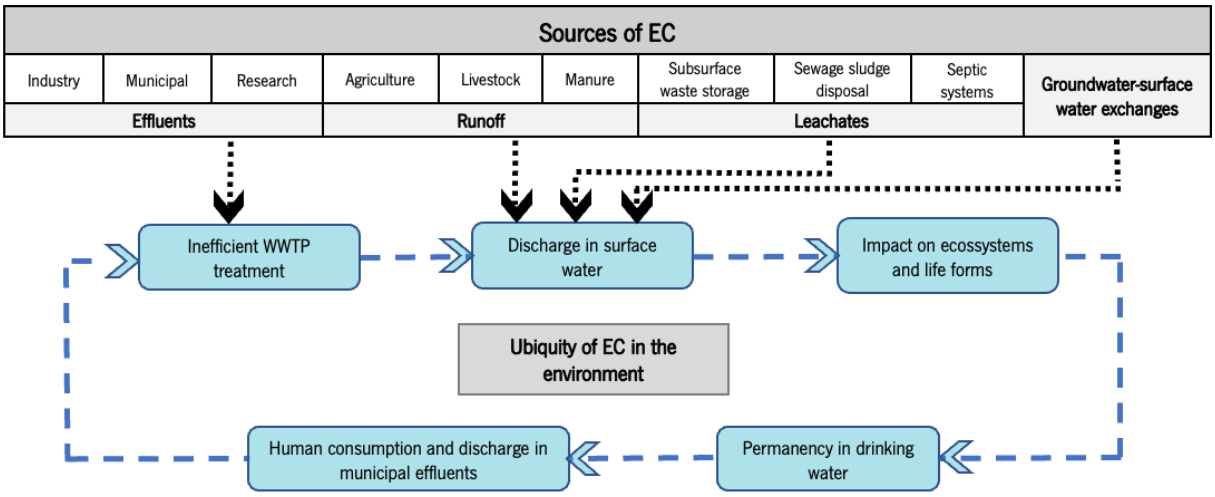


Figure 1. Ubiquity of EC (adapted from [14]).

Several molecules are listed nowadays as EC, including their metabolites and transformation products. They are pharmaceuticals and personal care products, "lifestyle" compounds, various proven or suspected endocrine disrupting compounds, metabolic regulators, contrast media, pesticides, veterinary products, engineered nanomaterials, industrial compounds/by-products as food additives, plasticisers, and preservatives, among others [3]. Characteristics such as polarity, water solubility, and persistence will determine the degradation of EC in sewerage systems, whether they become retained in the sludge or are released to surface water.

2.1.1 Pesticides

Different types of pesticides are applied worldwide every day to kill or control unwanted pests and their usage dates back hundreds of years, including herbicides, insecticides, fungicides, nematicides, rodenticides, molluscicides and plant growth regulators [15]. For a sustainable production of high-quantity agricultural food, the consumption of pesticides increased dramatically over the last decades. However, only a small part of the released agrochemicals reach the specific target while the rest moves into soils, reaching water resources [16]. Pesticides contamination of surface water and groundwater is a real environmental problem because of their toxicity, even at trace levels. In fact, according to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most dangerous and persistent organic chemicals are organochlorine pesticides [17]. Low concentrations of pesticide residues are frequently detected in natural water becoming a concern for the future production of potable water. The European Community of Drinking Water Directive referred that the level of pesticides in drinking water should not

be higher than 0.1 μL^{-1}. Besides the toxic effects, the long-term health risks of pesticides include different types of endocrine malfunction, interaction with estrogen and androgen receptors and thyroid function [18].

Herbicides are a type of pesticides specifically used to control undesirable or noxious plants growth (named weeds), in the crop production or where it is necessary to regulate plant growth. ATZ (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) (**Figure 2**) is an organonitrogen herbicide extensively used to control broadleaf and grass weeds [19].

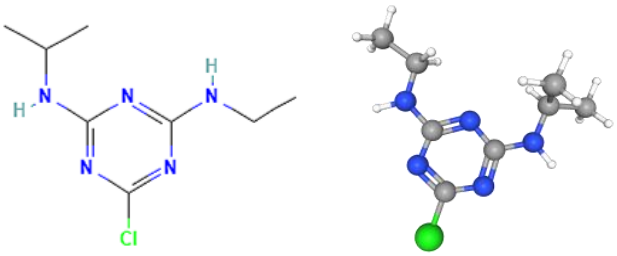


Figure 2. 2D and 3D structure of ATZ (from [20]).

This herbicide does not tend to bioaccumulate, but its permanency on environment (with a half-life from 35 to 125 days) and mobility on some soils, can led to surface and groundwater contamination [21]. In fact, it is commonly found at different water streams, because of its massive use in agriculture. It is registered in more than 70 countries, being in the top ten herbicides more used in USA and Europe [22]. As well as ATZ, deethylatrazine and deisopropylatrazine (degradation products) are recurrently detected in different environmental matrices as soils, sediments, groundwater and surface water [23]. The frequency with which it was detected at levels exceeding 0.1 µg/L in drinking water was one of the principal reasons for its ban in many EU countries, besides the potential threat to human health, because of its endocrine-disrupting nature and embryotoxic and embryolethal functions [24]. In Portugal, ATZ is also frequently detected in surface waters of agricultural areas [25] and drinking water [26].

Although there are different conventional processes that can be applied for the treatment of these chemicals, as chemical coagulation-sedimentation, photochemical degradation, adsorption on AC and oxidation [27], their high operation cost turns into expensive methods when applied at large scale.

2.1.2 Active pharmaceutical ingredients

Thousands of different active compounds present on pharmaceuticals are currently consumed globally every day to treat or prevent human and animal diseases. Over the last 20 years these molecules have been recurrently found in soil and water systems [28], becoming increasingly problematic on these environmental matrices. Pharmaceuticals are considered emerging micropollutants once their presence in environment is becoming ubiquitous and due to their toxic and recalcitrant characteristics [29]. They can enter into environment by a number of different routes, including human or animal excretions, wastewater of hospitals and pharmaceutical industries, agriculture run-off and landfill leachate [30,31]. Usually, household pharmaceuticals can reach sewer system after human excretion, via urine and feces, and washing-off by tap water via sinks or toilets [32]. Kümmerer [33] reported that from 10 % to 90 % of the administered dose of pharmaceuticals is excreted in their parent form, while the rest is excreted as metabolites and/or conjugated forms.

In aquatic environments, pharmaceuticals may be toxic to certain aquatic organisms and may cause adverse effects such as feminization of male fish [34], alteration of the reproductive function of male and female fishes [35], impairment of renal, gill, and liver in fish [36], and decrease in plankton diversity [5]. Even low levels of pharmaceuticals in aquatic environments may have toxic impacts on human health such as degenerative and inflammatory reactions in the liver of humans, for instance, when exposed to diclofenac [37]. The most frequently identified pharmaceuticals in surface water belong to non-steroidal anti-inflammatory drugs, beta-blockers group, drugs affecting nervous system, estrogens, lipid regulators and antibiotics [31,38]. Most of these commonly used drug classes are used in quantities similar to those of pesticides and other organic micropollutants [39].

A group of antidepressants that has been raising serious concern due to its wide use and mode of action, i.e., modulation of the levels of the neurotransmitter serotonin, are the selective serotonin reuptake inhibitors. It is the case of the antidepressant FLX (N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine) (**Figure 3**), that has been widely used to address a variety of mental health problems such as depression, panic, anxiety, or obsessive-compulsive symptoms.

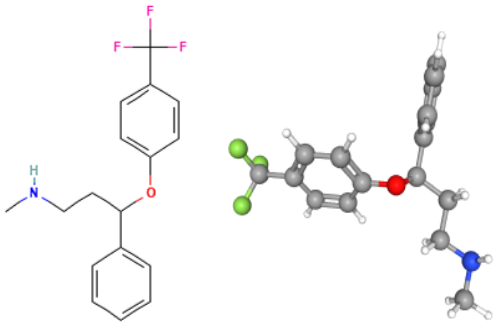


Figure 3. 2D and 3D structure of FLX (from [20]).

There was a 165 % increase in the prescribing of antidepressant drugs in England between 1998 and 2012 (an average of 7.2 % per year). Indeed, FLX is the fourth most prescribed antidepressant in England, and accounts for 11.3 % of all antidepressant drug use [40]. However, plenty of researchers have shown that such compounds performed both acute and chronic toxicity to aquatic ecosystems [41]. FLX is extensively metabolized to norfluoxetine and several other metabolites such as FLX glucuronide, norfluoxetine glucuronide, *para* - trifluoromethylphenol and hippuric acid. Due to (un)treated communal wastewater, FLX enter the aquatic environment, being detected in wastewater and receiving waters at levels ranging from ng L^{-1} to µg L^{-1} [36,42]. FLX and norfluoxetine remain biochemically active in the environment and can have marked effects on the morphology, physiology, and behavior of different species [43,44].

Antiepileptics and anticonvulsants are medicines that help to reduce the unnatural firings of nerves in the central nervous system and in the brain. Among this class of pharmaceuticals, CRB (5H-dibenzo[b,f]azepine-5-carboxamide) (**Figure 4**) is the most frequently detected pharmaceutical in various water bodies [45,46].

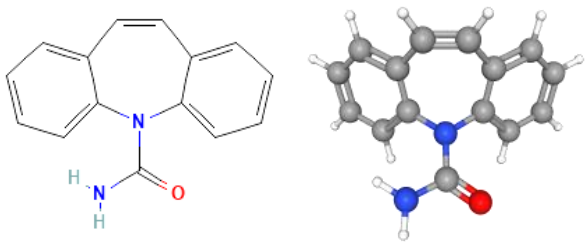


Figure 4. 2D and 3D structure of CRB (from [20]).

Clara *et al.* [47] classified CRB as a suitable marker for anthropogenic influences in the aquatic environment. Classified as an emerging recalcitrant organic pollutant, CRB is also found to be extremely persistent during chlorination procedures usually employed in water treatment systems [48,49]. CRB is hardly biodegraded or adsorbed onto solid surfaces which results in a relatively low removal performance, mostly below 10 %, through conventional WWTP processes [50–52]. Studies in North America and in Europe have revealed that CRB is one of the pharmaceuticals most commonly detected in river water, WWTP effluents, final sewage effluents, surface water, drinking water, and groundwater [45,53–55]. This scenario can be aggravated if metabolites are also present since besides of being able to persist in the environment for long periods of time, their effects and impact on living beings and on ecosystems are little known.

Several processes can be applied for the removal of active pharmaceutical compounds as biodegradation, adsorption on AC, flocculation, coagulation, photolysis and ozonation, although they are not regularly applied in WWTP [31]. On the other hand, processes as chlorination and chemical coagulation-sedimentation operated conventionally in WTP also do not eliminate efficiently pharmaceuticals [56].

2.2 TECHNOLOGIES FOR EMERGING CONTAMINANTS REMOVAL

The conventional treatments applied on WWTP and WTP are not sufficient to eliminate residual pharmaceuticals and herbicides. The presence of these micro pollutants in water bodies could remain in the course of drinking water, persisting in tap water. It is easy to understand that preventing water resources from pollution is wiser and friendlier than “repairing” contaminated aquifers. The impact of these pollutants, not only towards the different forms of life but also and especially towards public health has stimulated and even demanded the development and application of stricter environmental legislation, more efficient detection [57] and greener rehabilitation techniques namely those that may use innocuous microorganisms or their aggregates [58,59].

Different water treatment technologies are described in literature, however the design and an affordable operation can be still seen as a challenge. Abiotic techniques include physical-chemical processes as ion exchange, adsorption, precipitation, electrochemical and membrane technologies [58], oxidation with or without flame, air-striping, thermal degradation, condensation and incineration [60,61]. They are usually very expensive, from operational, maintenance and equipment perspectives, not eco-friendly, with pollutants concentration dependent efficiency and require a secondary treatment due to the

production of toxic sludge and toxic gases such as NO_x [62,63]. Some of them do not alter the chemical structure of the organic pollutants, but simply transfer them to a different phase [64]. On the other hand, biotic techniques cover processes like biodegradation, biosorption, bioremediation [65] and bioaccumulation [58] and have been used for several decades in standard sewage and water treatments, as well as in auxiliary reed bed and wetlands approaches [57,66]. They present important advantages over the abiotic ones, namely they can be carried out *in situ*, have an eco-friendly character and are cost-effective [65,67].

2.2.1 Adsorption process

Adsorption is considered a surface phenomenon defined by the physical adherence or chemical bonding of ions and molecules (adsorbate) onto a solid surface (adsorbent) or interface between two phases [68,69]. The equilibrium stage between sorbate and adsorbent is reached when the remaining concentration of adsorbate in solution is constant. The adsorption process can be physical or chemical. In physical adsorption (physisorption) the adsorbent attachment involves intermolecular interactions of attraction between the relatively weak species, which can be attributed to Van der Waals forces. Physisorption is a reversible process and presents a low value of enthalpy of adsorption. It occurs with possibility of multilayer formation of molecules in the adsorbed phase [70]. On chemical adsorption (chemisorption), the bounding between adsorbent and adsorbate implies a chemical interaction, by electron transfer, creating bonds more stronger than those observed in physisorption [71]. This chemical bond generates a monolayer on the surface of the adsorbent. **Figure 5** shows a representative scheme of the adsorption process.

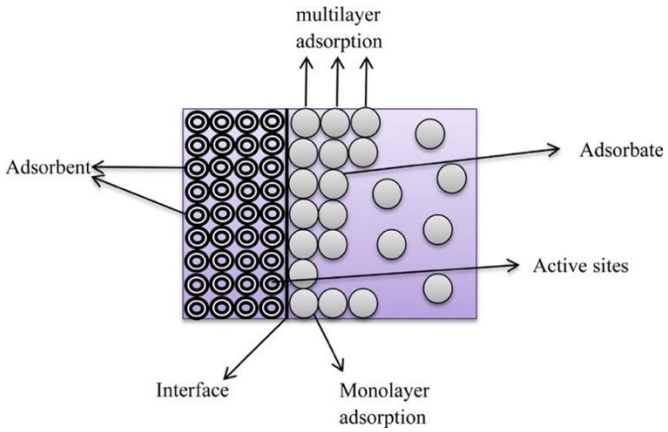


Figure 5. Schematic diagram of the adsorption process (from [72]).

Adsorption parameters can be assessed by batch and/or continuous mode assays, at lab scale. Adsorption kinetics and equilibria are characterized through batch experiments and mechanistic speculations on the interactions between adsorbate and adsorbent may be forwarded. On the other hand, treatments of high volumes of solutions, in short operating periods, can be performed in packed-bed lab size columns. This configuration allows the determination of important scale-up parameters as breakthrough and saturation times, the capacity and the maximum performance of the bed, mass transfer values and to understand the dynamic operation [71,73]. After the optimization step, the process can be simulated at pilot and/or at industrial scales.

This process is recognized for many researchers as a well-established, viable and cost-effective technology to treat industrial effluents, domestic wastewater, groundwater and drinking water [73], becoming appealing and promising to remove micro pollutants. The simple use, flexibility, low implementation costs and low-energy requisites make this technique very advantageous to remove emerging molecules [74].

On the other hand, biosorption is considered a subcategory of adsorption, where the sorbent is a biological matrix, denominated as biosorbent. The entails of dead or inactive biological material derived from biological sources allow the passive uptake of sorbates onto their structure [69,75], resulting in their concentration reduction in solution. The application of available biomaterials, with relevant selectivity and regenerative properties, allows the extension of waste materials life [76], promoting circular economy. In developing countries, the application of biosorption to remove pollutants is already seen as promising and attractive due to: i) vast quantities of biomass and waste bio-products for be used as biosorbents; ii) lack of advanced effluent treatment systems to remove organic micro contaminants and iii) relatively low cost when compared with others advanced methods often used in developed countries [76].

Great efforts have been made in this field in order to obtain increasingly effective biomaterials to apply in water and wastewater treatment [69]. However, despite the fact that this biotechnology has been highly studied, there is no substantial information about successful exploitation in an industrial context [69]. Research has shown that different sorbents are being applied for herbicides and pharmaceuticals removal from water and wastewater. As biosorbents sand, sediment and soil [77,78], natural clays [19,79], dairy manure [76], agricultural and industrial wastes as rice husk, pine bark, corn cob, wood charcoal, chitosan [80], AC derived from biomass and wastes [29,80,81], bottom ash [82] are among many other possibilities. As adsorbents rubber granules [82], commercial AC [30,58], nano - adsorbents, [74], synthetic clays [83], graphene and graphene oxide, carbon nanotubes [29], zeolites [74] may be used among other options.

The opposite process of sorption is desorption, which allows the recovery of sorbates and their possible reuse in new applications. So, when saturation of adsorbents is reached, materials can be regenerated applying desorption processes. This can be performed by different techniques, although after the process sorbents may improve or loss their sorption capacity. Thus, in the case of using extraction eluents they must be nontoxic, cause no damage to the sorbent, allow its reuse, and enable maximum recovery at the lowest possible eluent concentration and contact time. The possibility of regeneration and reuse of adsorbents is an important added value for the overall process [84].

One of the most pertinent questions pointed to adsorption and biosorption processes is about the ultimate safe disposal of the loaded sorbent, since occurs the transfer of the sorbate from the aqueous solution to a solid phase, as well as sorbate recovery occur. Although some laboratory studies have demonstrated the regeneration of sorbents using acids, alkalis and complexing agents, the viability of the whole process at industrial scale remains questionable [76]. Alternative uses for contaminated materials as phytoremediation disposal, soil amendment or construction applications can be taken into account because of the associated problems with incineration or disposal in engineered landfills [76].

Notwithstanding the relatively high publication of laboratory studies, large-scale commercial applications of the biosorption technology is still lacking globally. Besides the use of AC in water and wastewater treatment, the use of large-scale systems based on different materials of biological origin is extremely scarce [30,85], creating a serious gap between the fundamental knowledge and the laboratory-optimized techniques and systems, and the actual needs of either WWTP and industrial facilities.

2.2.1.1 Sorbents: from commercial to low-cost and waste materials

AC has undoubtedly been the most popular and widely used adsorbent in wastewater treatment throughout the world. It is produced by raw material dehydration and carbonization followed by activation [86]. This product is known for having a very porous structure with a large surface area. AC has been found to be a versatile adsorbent, which can remove diverse types of pollutants. Despite its abundant uses, its high cost, adsorption regeneration capacity, and disposal at end-of-life calls into question the prospect of its use for the treatment. For these reasons, adsorption on low-cost or wastes materials is considered an alternative method for wastewater treatment, because it is competitive and easy to operate [87].

The term “low-cost” adsorbents suggests affordability when these materials are easily and locally available in large quantities. Their usage depends upon many factors: the precursor should be freely

available, inexpensive, and nonhazardous in nature. Other necessary characteristics include high abrasion resistance, high thermal stability, and small average pore diameter, which results in higher exposed surface area and, hence, high surface capacity for adsorption [87,88]. The preparation of sorbents from waste materials has several advantages, mainly of economic and environmental nature. The use of wastes provide a positive impact on pollution abatement because their volume could be partly reduced, while allowing the development of adsorbents at a reasonable cost [89].

To improve the adsorption properties of low-cost or wastes, materials can be modified using various methods, which include chemical treatments as acid or alkali, etherification, esterification, and other modifications, as thermal [86].

2.2.1.2 System and modeling

Adsorption process can be performed in two main modes, at batch or continuous flow. Batch adsorption is frequently employed to carry out adsorptive processes in laboratory, industrial or pilot scale batch reactors. The system entails a suspension of an adsorbent in the presence of an adsorbate that is mixed during a time interval. Different process variables, such as agitation, temperature, adsorbate/solute concentration, adsorbent dose and particle size can be changed to enable the system to attain equilibrium. The batch system is useful to evaluate adsorbent efficiency, although comprehends some limitations, as the solid/liquid ratio and the hydrodynamic conditions.

The adsorption modelling is vital especially in water treatment processes. To ensure that sorbent is used with good efficiency, at the right rate and in the right conditions, mathematical models are employed. Batch flow adsorption models are used to deduce the adsorption capacity of an adsorbent, which is very important for the reliable prediction of adsorption parameters [90].

Adsorption kinetics represents the uptake of solutes within a contact time period at various experimental conditions, depending on the experimental (solute ion concentration, temperature, and pH) and material factors (sorbent and adsorbate type). On the other hand, equilibrium isotherm models are the fundamental modes of expressing the equilibrium limitation(s) of the system. Equilibrium isotherm equation accounts for the distribution pattern of the adsorbate onto an adsorbent. Various adsorption kinetic and equilibrium models have been used by researchers in the past to analyse the effectiveness of the process [91].

2.2.2 Biological remediation

Biological processes have been used for a long time due to the remarkable ability of microorganisms to detoxify organic and inorganic pollutants [69]. The use of naturally occurring or modified microorganisms and biological reactions to degrade toxic contaminants present in polluted environmental matrices is seen as a promising, friendly and viable technology [92,93]. This type of biotic approach has become in recent years a well acceptable technique within the research community since it is able to sustainably re-establish the initial conditions of different environmental matrices. Microbial remediation has many advantages such as the possibility to be applicable on a broad scale, the relatively simple procedure, low cost, and the absence of secondary contamination. Simultaneously, various factors, including ambient temperature, salinity, pH, nutrient content, toxic substances, and other factors, limit the efficiency of microorganisms. Therefore, using microorganisms with better performance and higher adaptation to the environment will have a more favorable result.

Biodegradation can be defined as the transformation of organic substances into smaller and simpler molecules by the action of enzymes naturally produced by living microbial consortia. Biodegradation processes can either be complete, with mineralization of the substrate into the final product CO_2 or it can be incomplete with the substrate transformation into intermediates/metabolites. Although many microorganisms are able to adapt to new environmental conditions, eventually degrading organic compounds of anthropogenic origin in the process [57,94], not all hazardous compounds are biodegradable, limiting the application of biodegradation.

Toxicological evaluation and biodegradation assays by bacteria can be performed in batch or continuous mode. The complete and/or partial degradation and mineralization of some compounds, such as ATZ [93,95] produce simple compounds as CO_2, water, nitrogen or other organic molecules. Biodegradation processes become thus one of the most effective option for removing these pollutants from the environment [93]. There are several reports on various microorganisms that have been identified for utilizing the target pollutant as a carbon, nitrogen or phosphorus source. ATZ-degrading bacterial strains can catabolize ATZ as the sole source of nitrogen and release the ring carbon atoms as CO_2. *Pseudomonas* species, *Arthrobacter sp.*, *Sinorhizobium sp.*, *Polaromonas sp.*, *Acidovorax sp.*, *Ralstonia eutrophus*, *Erwinia tracheiphila*, *Entrobacter agglomerans* and *Micrococcus varians* are some examples of reported bacteria able to degrade ATZ. However, *Arthrobacter sp.* is the mostly isolated bacteria in different soil samples contaminated with this pollutant [95].

2.2.3 Permeable reactive barriers/biobarriers

PRB emerged as most promising and widely accepted remediation of contaminated groundwater technology [96] and has been growing on its development and implementation from lab to full-scale since its first field application in 1991 [97]. PRB entails the emplacement of an engineered reactive material in the subsurface [92,98], filled perpendicular to groundwater flow direction [96], to degrade or immobilize contaminants *in situ* transported by the natural water flow [99]. Contaminants are removed and transformed into less toxic and harmful forms by different processes/mechanisms as degradation, biodegradation and reductive dechlorinating of mostly organic molecules, sorption of organic, inorganic molecules and heavy metals, reduction and/or precipitation of heavy metals, ion exchange, surface complexation [100], among others.

To select the best material to use as a barrier is important to take into account the type of pollutants to treat and the hydrogeological conditions of the local [92,101]. However, is necessary to previously evaluate the retention capacity of the material to be used towards the target hazardous molecule(s), how often the material should be replaced and the influence of external constituents. PRB optimization is usually performed in batch experiments and in continuous column assays, under the same remediation environments to reproduce groundwater flow conditions [101,102]. Because of pollution swapping, the use of a single material can turn this technology less efficient. To exceed this problem, multi-barriers can be applied, with two or more barriers composed with the same or a mixture of different geochemical and geophysical materials in a single structure [100,103]. Researchers have investigated their effectiveness reporting a good removal performance with different contaminants [104,105] as well as the increase of the barrier permeability [100] with combined materials.

To remove pollutants by adsorption in PRB, various materials can be used. The more common are granular iron (zero valent iron). Others, including granular activated carbon (GAC), zeolites, oxygen releasing compounds, peat, and sawdust are also extensively studied. Although they are efficient towards a large spectrum of contaminants, they present disadvantages as high costs and availability [101]. Replacing these materials for sustainable and cost-effective alternatives is seen as increasingly important nowadays. Wastes, by-products from industries or even modified products by chemical treatments are being used as promising sorbents to remove different contaminants [106]. In laboratory some attempts using clays [99], fly ash [106], soil, biochar, brick [103] to remove different contaminants in PRB have already been performed.

Biological activity in the permeable barrier may increase the effectiveness of the reactions that occur there [96], by direct transformation of organic pollutants or accumulation/precipitation of inorganic compounds by microorganisms. Barriers that use materials to stimulate contaminants abatement by anaerobic or aerobic microbial processes are denominated as PRBB [100]. The use of organic materials allows the growth of inoculated microorganisms improving contaminant degradation. Thus is crucial to work with the right microbial population or consortium, chosen according the pollutant degradative ability [96,100].

To create PRBB, microorganisms should be specifically placed on a porous reactive support (carrier) to form biofilm on its surface, creating a symbiotic relationship and increasing the performance of the rehabilitation process. Biofilms have proven to be efficient in the formation of biobarriers to control contamination plumes, due to the increase of microbial degradation rate when combined with the barrier material [96,107]. Biofilms allow the reduction of the permeability of the subsurface environment promoting the containment of such plumes [107]. PRBB also evidence increased lifetimes, whilst providing contaminant degradation [107]. Nonetheless, this synergy can affect microorganisms viability by the production of reactive oxygen species (ROS), creating oxidative stress, DNA deterioration and loss of permeability, leading to a decrease in degradation over time [96].

To accomplish degradation via aerobic it is necessary to have terminal electron acceptors (TEA) to receive the electrons from pollutants. Although O_2 is the preferred TEA used by microbes in metabolic processes, the aquatic environment where they are inserted is usually anaerobic, being necessary an external source to release oxygen. In PRBB, solid oxygen and nitrate-releasing compounds (as calcium peroxide, magnesium peroxide and hydrogen peroxide) are preferential over oxygen diffusers and compressed air due to lower cost [100].

Column studies and geochemical modeling make the prediction of barrier life time possible [100]. In fact, efforts have been made by researchers to understand these mechanisms, however for the long-term performance of PRBB with new reactive materials, more data is necessary. By the introduction of new and emerging molecules in the environment more studies to increase the effectiveness of the rehabilitation processes using PRBB must be performed. The use of adsorption/biosorption combined with biofilms needs to be seen as an environment friendly technology to be further explored. Different microorganisms and reactive media combinations, especially low-cost materials and/or reusable wastes, should be taken in consideration to establish better clean-up strategies.

2.2.3.1 Cell adsorption mechanisms and biofilm development

Biofilms are complex biostructures formed from the aggregation of multiple microbial cell populations and embedded in a hydrated extracellular matrix of polymers [108,109]. This complex matrix is developed with or even without a surface, but the presence of a solid support is beneficial for biofilm formation and acts as a strong base for microorganisms attachment [110,111]. Inside the biofilm, microorganisms grow protected from environmental stresses such as desiccation, reaction from the immune system, protozoa ingestion and the presence of biocides or toxic contaminants [112]. They adapt and survive better than free-floating (planktonic) microorganisms [113]. These matrices exhibit structural integrity, defence against sensitive microorganisms, intercell communication, formation and preservation of the microcolony and nutrient intake, essential for the biofilm system performance [114,115].

In wastewater treatment processes, the provided nutrients are used for the synthesis of new microorganisms and metabolic consumption, leading to contaminants degradation or bioaccumulation. Studies have shown that cell immobilization is an effective and promising technique for improving biological wastewater treatment. A recent publication has reported that immobilized cells can increase the efficiency of decontamination processes, especially when contaminants act as nutrients, by more than 60 % [116]. The most important properties of biofilms associated with wastewater treatments include stability, resistance to toxic compounds, efficient management of heavy loads and the possible coexistence of aerobic and anaerobic metabolic activity within the same process [117].

Different techniques for artificial cell immobilization on carriers have been applied to biomass. Adsorption is the most used cell immobilization technique applied in wastewater treatment due to its simplicity and reversibility [116]. In this conventional immobilization, a high concentration of biomass suspension is used to induce attachment on the surface by the action of extracellular polymeric substances (EPS). Between the surface of a water-insoluble carrier and the microorganism to be immobilized occurs physical binding through van der Waals interactions, ionic forces and hydrogen bonding [116,118]. This technique allows a simple, inexpensive, and effective operation, without chemical additives and allowing support reloading. On the other hand, due to the weak binding forces involved, the leaching rate of the adsorbed cells from the matrix is high [118]. To overcome this issue, suitable material carriers with strong adsorption affinity to cells should be selected. Properly adsorbed substrates not only protect cells from unfavourable media but also ensure their physiological activity [119].

Nonetheless, the immobilization via adsorption can be affected by different variables such as environmental factors and cell and carrier surface properties [116,120–122]. The bacterial cell surface hydrophilicity can be an important parameter for predicting the adherence of bacteria to a substratum according to its wettability. Hydrophobic bacteria have a higher ability to adhere to surfaces than hydrophilic ones [123]. Biofilm adhesion occurs readily on rough and hydrophobic surfaces and on those coated by surface conditioning films [124]. In aquatic environments, the microbial attachment rate may increase by increasing the velocity flow [125] and also be affected by water temperature [126] or nutrient concentrations [127,128]. **Table 1** illustrates the factors that influence microbial cell adhesion through the adsorption technique.

Table 1. Factors affecting microbial cell adhesion through adsorption immobilization (based on [116,120–122,129])

Environmental bulk liquid	**Carrier surface**	**Cell surface**
pH temperature nutrients availability hydrodynamic conditions	wettability roughness charge	hydrophobicity flagellation motility EPS

Biofilm formation is a complex and dynamic process involving several steps. Firstly, planktonic cells attach reversibly to a solid surface. This initial adhesion is mainly induced by the physicochemical properties of both cells and carrier surfaces and by the action of electrostatic and Brownian motion forces and acid-base interactions [117]. Regarding unicellular adhesion, attention should be paid to three adhesion factors: the physicochemical composition of the aqueous medium, the solid surface and the transitioning microbiota [130]. The adhesion step is crucial for biofilm performance since it assures the long-term stability of the system [131]. After adhesion, the cells start to multiply and form multilayer cell clusters. Gradually, tight, and irreversible connections between organisms and the substratum are established. This synchronization occurs through a mechanism of cell-to-cell communication called quorum sensing (QS). QS provides the ability to sense population density by measuring the accumulation of specific signalling molecules secreted by members of the community. This response is only activated in high population densities because of the appropriate accumulation of the signals in the extracellular

environment. When population density increases quorum signals reach sufficient levels to activate the maturation of the biofilm in a coordinate manner [112]. In the multiplication step, quorum signals are released to underlying EPS production [132]. This mixture is composed of polysaccharides, proteins and extracellular DNA. It is recognized to be important for biofilm cells intercommunication, 3D biofilm structure formation and multicellular living [132,133]. Irreversibly attached cells start the multiplication process forming small aggregates called microcolonies. Eventually, they develop into large cellular aggregates enclosed in an EPS matrix divided by water channels. The biofilm maturation is then initiated when the conditions support growth [132,134]. As the maturation process continues the sustainable biofilm growth can be inhibited by some factors, triggered by limited nutrient supply or reduced oxygen levels. The process of biofilm formation can naturally reverse by cell dispersion into the bulk liquid. The released biomass (individual cells or lumps of cells) can attach to new surfaces and colonize to form new microcolonies [130]. Biofilm detachment can be influenced by strong fluid dynamics, hydrodynamic shear forces, environmental conditions such as toxic chemical exposure and QS regulation [132,135]. The decoupling of aging biofilm is vital to the recovery of biofilm activity, once it allows to balance the attached biofilm quantity at steady-state conditions [114,130].

2.2.3.2 Biofilm carrier media

Immobilized bacterial biofilms for wastewater bioremediation are a cost-effective and attractive tool for the removal of various hazardous environmental pollutants. The crescent concern regarding environmental sustainability have encouraged researchers to look for ecological materials for bacterial adhesion, able to compete with the conventional used carriers.

Biofilm carriers have been reported to enable microbes colonization and to increase solid retention [136]. Traditionally, they have a high specific area and porosity, durability, and high mechanical strength. The selection of a carrier is a crucial step because it impacts the cost-effectiveness of the process, the durability of the biofilm and the treatment performance. Carriers could be prepared from a variety of materials. To date, various carriers have been presented for supporting microbial adhesion, from the most common used commercial carriers to more sustainable alternatives, such as natural materials and waste-based materials.

2.2.3.2.1 Commercial and synthetic supports

The traditionally used carriers are based on commercial and synthetic materials, namely AC [137–140], ceramics [141,142], glasses [110,143], polymers [144–146], carbon fibers [147–149] and organic materials such as biodegradable polymers [150–152]. However, only a few are commercially applied in full-scale systems. In general, the most used carriers for biofilm attachment are made of plastics due to their low density and high mechanical resistance [153]. Polyurethane sponge, polyethylene, polyvinyl alcohol, poly propylene and polyvinyl chloride are examples of the most common fabricated biofilm carriers. The flexible and porous polyurethane carrier is considered the most popular as it is an optimal microbial growth medium due to its large specific surface area, rough surface, high mechanical strength, and enhanced adhesion of microorganisms. In a recent report [154], conventional biofilm carriers were ranked based on their ability to reduce chemical oxygen demand (COD) as following: polyvinyl alcohol > polyurethane > polyethylene > polypropylene. Nacheva *et al.* [155] investigated seven different types of carriers (ceramic spheres, crushed tezontle, grains of high and low density polyethylene and of polypropylene, cubes of polyurethane and polyethylene tape) in continuous downflow reactors. The best phosphorus and nitrogen removal was achieved using polyurethane carriers, while polyethylene provided the best final COD. Tarjányi-Szikora *et al.* [156] investigated the development of biofilms on two different supports, Biolite™ and Perl™, for the treatment of municipal wastewater in terms of nutrient removal efficiency and dehydrogenase enzyme activity. The commercial Perl™ and Biolite™ carriers were manufactured from glass and ceramics, respectively. The authors reported that biofilms on Perl™ were very effective in removing organic matter, while those on Biolite™ had the best dehydrogenase enzyme activity. Moreover, the authors concluded that the artificial carriers were more promising than natural zeolites for wastewater remediation. Nguyen *et al.* [157] investigated different sizes of polyurethane sponges as biofilm carriers. The medium size sponges showed the best pollutant removal efficiency [90 % total phosphorus (TP), 95 % COD, 65 % total nitrogen (TN)] and biomass growth under aerobic conditions. In 2018, Chunyan *et al.* [158] modified polyethylene carriers to have positive charged surfaces to promote bacterial adherence and biofilm growth inside a moving bed biofilm reactor (MBBR). The process removed 392.6 mg/L of organic cyanide ions as initial concentration of for 24 hours.

Besides the ideal characteristics as structural strength, chemical stability [117], bio-affinity, resistance to biodegradation and slow aging [159], an ideal biofilm carrier should also be available at a low cost and have a low environmental impact [160]. However, the commercial and specifically designed supports are not cost-effective, present a non-eco-friendly character and their application becomes not

challenging [160,161]. From the perspective of circular bioeconomy (CBE) and green development, the utilization of low-cost materials and/or waste materials in the treatment of wastewater is the most thrust area for researchers and environmental scientists [162]. There are a variety of green and low-cost materials with different nature to be utilized in pristine or in modified structures for biofilm development.

2.2.3.2.2 Sustainable carriers

Researches are more oriented towards the utilization of cheap, reusable, low-impact and easily available materials that can be used to support biofilms [161]. They comprehended materials from natural origin [163–168] and wastes [160,167,169–171] as an attempt to substitute commercial and synthetic ones. They can be easily obtained and their use contributes towards the CBE [172].

Natural raw materials are economical, environmentally friendly, highly abundant, presenting high porosity and durability [162]. Wastes are generated worldwide and comprise a diverse variety of feedstocks such as food wastes (44 %), plastic wastes (12 %), glass wastes (5 %), metal wastes (4 %), wood wastes (2 %) and rubber and leather wastes (2 %) [173]. Wastes from the food industry, for example, are rich in organic matter and can provide great energy for the growth of anaerobic microorganisms. These wastes can provide plenty of carbon and nitrogen for biofilm nutrition, which may reduce the extra input for bacterial culture to give full play of its value [169].

Biofilm carriers based on inorganic materials exhibit excellent mechanical strength [142], have stable chemical properties and tend to have a large specific surface area. Furthermore, their rough surface and wide pore structure allow the protection of microorganisms from shock loads, providing an excellent nature for biofilm attachment. But they may also present some problems as slow biofilm formation, poor permeability, large flow resistance and easy clogging [174–178]. Clogging leads to backwashing, short circuit and excess head loss (biofilm detachment). On the other hand, organic based biofilm carriers are not toxic to cells and are efficient materials for bacteria adhesion due to their higher specific surface area [160]. They promote a good biofilm formation, especially the ones that can work as electron donors [179,180], but are often associated to low bio-affinity caused by their smooth surfaces [159]. After use, they can be easily handled (in soil remediation and/or for energy co-production [181]) and do not cause environmental pollution [159]. For example, cellulosic materials contain the glucose group, with the easily substituted hydroxyl groups, that provide weakly basic and acidic ion exchange conditions enhancing bacterial attachment. Cellulose has a porous structure and hence potentially high surface area, available for bacterial adherence [170,182–184]. Besides, it is cheap and stable at different

operating conditions such as temperature and pH [185]. Organic materials-based carriers are considered effective carbon sources for biological denitrification for application in wastewater treatment [150,170,186–192]. For example, Guan *et al.* [191] found that corn and rice straw can functioned both as high-quality carbon sources as well as biofilm carriers for a denitrification process in a drawer-type biological filter system. Also, Yang *et al.* [170] reported an enhancement in denitrification and TN removal of around 40 %, when using rice straw, luffa or corncob as carbon source biofilm carrier. This type of material can often exhibit low mechanical strength, poor mass transfer performance and rapid degradation by microorganisms. The reusability of this kind of carrier is more limited when compared to inorganic materials [159] and in a biological wastewater treatment application they can led to ammonia formation, dissolved organic carbon and to color problems [193].

The evaluation of carriers for biological wastewater remediation can be accomplished in batch experiments [160,164,194,195] with eventual optimization of operational conditions and systems modulation to their further upscale to pilot dimensions [163,167,196,197]. Biofilm reactors are increasingly employed for bioremediation, representing a proficient and appealing strategy for assessing operational parameters [161]. They provide a high active biomass concentration and an automatic and easily liquid-solid separation [198,199]. In fact, they present several advantages when compared to suspended systems, comprising flexibility and adaptability in operation, reduced hydraulic residence time, resilience to environmental changes, enhanced ability to degrade recalcitrant compounds and limited space requirements.

As an important part of the biofilm process design, the selection of carriers is crucial because they will directly influence the effectiveness of the wastewater treatment. This selection involves many aspects including the size of the equipment and the operation of the system. The properties of the carrier are strongly affected by the hydraulics of the reactor and the thickness of the biofilm. Prior to the selection of carrier materials, both physical parameters of the support (particles size, shape and density, hardness, specific surface area and resistance to physical erosion) and chemical properties of the media should be considered [200,201]. The cost of the material is obviously another aspect to have into consideration because it determines the economy of the system [202].

All over the years several reactor configurations have been developed and applied to biological wastewater treatment. The option between fixed bed biofilm reactors and moving bed biofilm reactors may be based on the condition of the supporting material. Configurations range from fixed bed systems, where biofilms are formed on static supports, and moving bed systems, that also comprise suspended and fluidized biofilms, maintained by air, water flow or mechanical stirring [203]. Reported literature using

sustainable carriers comprehended the following biofilm reactors: PBBR [163,167,209,183,188,196,204–208], biological trickling filter (BTF) [174,197,210–215], biological aerated filter (BAF) [216–220], sequencing batch biofilm reactor (SBBR) [221–225], fluidized bed reactor (FBR) [201,226–229], MBBR [230–232], membrane biofilm reactor (MBR) [170,233], up-flow anaerobic sludge blanket (UASB) [234,235], bio-contact oxidation reactor (BCOR) [168], biofilm airlift suspended reactor (BASR) [236,237], fixed bed sequence batch reactor (FBSBR) [238], biological contact biofilm reactor (BCBR) [239], integrated fixed-film activated sludge reactor (IFAS) [240,241] and some other configurations.

The following section deals with the exploitation of sustainable materials (without chemical or thermal treatments) used as bacterial or activated sludge consortia support for wastewater treatment, applied on batch experiments and laboratory or pilot scale biofilm reactors. Chemically and thermally modified materials were not included as sustainable carriers due to waste production and associated costs involved in both processes. For comprehensive reading, the discussion is divided by natural (made with raw materials) and waste-based carriers.

2.2.3.2.2.1 Natural resources

Table 2 summarizes selected studies that highlight the ability of raw materials to remove several pollutants and nutrients from different wastewater streams. Several studies have investigated natural inorganic materials as biofilm carriers in biological wastewater treatment processes such as kaolin [194,195], vermiculite [164,195,196,242], sepiolite [163,195], bentonite [195], zeolites [156,174,204,216,217,221,227], volcanic rocks [222,227–229,243–245], perlite [164,201,226,246], shungite [164], stone/pebbles [200,205,210–212] and sand [205,206].

Clays are naturally available materials with reasonable chemical and mechanical stability. They are ubiquitous phyllosilicate minerals found principally in geological and marine deposits [247]. They are widespread and abundant on earth and can be obtained at a low cost. When considering the economic and technological constraints, their use as biofilm carriers may be quite effective [248]. To date, it has been reported that clays and minerals-based biofilm carriers appear to be effective in the removal of heavy metals, nutrients, and organic pollutants. For example, Quintelas *et al.* [195] investigated four different clays with respect to their ability to function as *S. equisimilis* or *Arthrobacter viscosus* support to remove diethylketone (DEK). All the synergetic combinations between clays and bacteria were revealed to be efficient. In a recent study, Silva *et al.* [163] used sepiolite as *S. equisimilis* carrier in a pilot PBBR

to treat a complex industrial wastewater containing high concentrations of metals and spiked with DEK and methylethylketone (MEK). The addition of the biofilm improved the removal of heavy metals, besides preventing the leaching of some ions from the carrier structure, an extremely important advantage when compared to the system without biofilm.

Natural zeolites are negatively charged microporous aluminosilicate minerals, and are, in fact, some of the most common sedimentary rocks. They occur naturally in the environment and so they are readily available and inexpensive. Pores sizes can vary from 0.3 to 1 nm, resulting in porosities between 0.1 and 0.35 cm^3/g [249]. Their structure provides both high filtration and sorption properties, due to the high specific surface area and physicochemical properties [164]. Zhang *et al.* [174] used natural zeolite as activated sludge carrier in a BTF to treat a domestic wastewater. Results showed that the biofilm on zeolite grew slowly but its quantity was lower when compared to a synthetic sponge support, which may be due to the falling of biofilm under the souring effect in the reactor. Valdez *et al.* [204] compared the performance of a nitrifying consortium immobilized in a mixture of zeolites and in a synthetic carrier (polyurethane) in a lab-scale PBBR for trichloroethylene bioremediation. No significant differences between degradation percentages were obtained but the biomass concentration in each carrier was different in the end of the process. In zeolites the biofilm was formed primarily on the exterior surface of the minerals and in the synthetic carrier it was concentrated in the inside. In earlier studies, removal efficiencies of NH_4^+-N in wastewater were found to be higher than 90 % or even up using zeolite as biofilm carrier [216,221].

Volcanic rocks can be used as solid support media in wastewater treatment applications with excellent removal efficiencies, while maintaining their structural integrity [222,244]. For example, Shafai *et al.* [244] used black scoria, a volcanic rock, as microbial carrier in an ASFFR to treat a municipal wastewater. The reactor achieved a sludge volume index (SVI) of 46–74 ml/g for aerobic cycles and 18–50 ml/g for anaerobic/aerobic cycles. The average sludge production was 0.145 g TSS/g COD removed. Also, Koupaie *et al.* [222] operated an anaerobic/aerobic fixed bed SBBR with volcanic pumice stones in order to evaluate the biodegradation of azo dye Acid Red 18. The performance of the volcanic rock as biofilm carrier was compared to a plastic media made of polyethylene. In terms of COD and dye removal the reactor filled with the synthetic material achieved significantly higher values. The prevailing species of bacteria biofilm grown on the rock surface were rod shaped, while the biofilm on surfaces of the polyethylene exhibited a fluffier structure. In a study of Thaiyalnayaki *et al.* [201] perlite, a type of volcanic glass, was used as carrier material in an inverse anaerobic FBR to treat high strength organic wastewater. The support material proved to be a good and suitable carrier for high biomass retention and improvement

of the reactor performances in a short time of operation. In fact, perlite was found to be effective when compared to other tested materials due to its density and high biomass hold-up, with minimum particle washout. In a recent study, Safonov *et al.* [164] compared the sorption characteristics of perlite and shungite and evaluated the possibility of their use as biofilm support to be applied as a biofilm permeable barrier towards Cd (II) and Cr (VI) removal from a domestic waste landfill. Shungite consists of individual crystallites with a size of about 400–500 nm and present high porosity and specific surface area [164]. After biofilm formation, the removal efficiency of shungite was not affected by the biofilm, while in perlite it increased. Shungite can be used in permeable reactive barriers without changing its characteristics under the effects of biogenic processes.

The use of stone media, pebbles and sand seems to be a viable option for municipal wastewater treatment due to their economic advantage, availability and high potential for biofilm attachment [200]. Tilley *et al.* [250] recommended the uniformization of these support media, such that 95 % of the particles have a diameter between 7 and 10 cm. In an earlier study of Khan *et al.* [205] the efficiency of two PBBR was evaluated, with stone and sand media as activated sludge support for domestic sewage treatment. The removal of biochemical oxygen demand (BOD) and of COD was 89.67 % and 89.62 %, respectively, for the stone media reactor, while 97.12 % and 97.15 %, respectively, were attained for the treatment with sand support. In 2014, Naz *et al.* [212] concluded that attached-growth systems as the BTF with natural media can reach higher rates of nitrogen removal as compared to other attached-growth reactors using synthetic media. They concluded that the studied BTF system could be easily transferred to field scale for treating sewage for small communities in developing and underdeveloped countries. The removal of pharmaceutically active compounds from wastewater in onsite sewage facilities was evaluated by Dalahmeh *et al.* [206] in a PBBR, using sand as biofilm carrier. The immobilized activated sludge was efficient in the biodegradation of ranitidine (97-99 %), caffein (99 %) and COD (95 %).

Organic materials-based carriers are also widely used as microbial carriers in wastewater treatment. The most reported studies comprehended the use of luffa [160,167,170,188,190,213,223,230,251], followed by bamboo [168,218,236,252,253] and cactus fiber [167]. Luffa is a lightweight, highly porous and large surface area herbaceous plant composed mostly of α-cellulose (60 %) and hemicellulose (22 %) [230]. It has unique properties such as renewability, availability, low-cost, good hydrophilicity, biocompatibility, porous structure, non-toxicity to microorganisms and long-term sustainability to the natural environment [188]. These characteristics makes this material a suitable biofilm carrier for wastewater treatment. So far, the most common research interest for luffa sponges/fiber carriers is for the removal of nutrients [160,167,170,188,223,230],

pharmaceuticals [213], volatile organic compounds (VOC) [251] and water soluble polymers [190] present in wastewater. Dang *et al.* [230] compared the performance of pristine and modified luffa sponges as eco-friendly and renewable supports as carriers in a pilot scale MBBR similar reactor for the treatment of municipal wastewater. The tests using raw and modified luffa sponges achieved 79 % and 83 % of organic removal and 53 % and 71 % of TN removal, respectively. In another study, a lab scale BTF was operated with *Bacillus thuringiensis* B1 (2015b) immobilized in sponges of luffa to degrade Naproxen [254]. Luffa revealed to be a suitable material for the attachment of the biofilm due its high porosity. The bacterial biofilm was characterized by a large amount of extracellular matrix and even after 15 days of assay the sponges were almost completely covered by the bacteria. In a recent work of Zainab *et al.* [160] the density of bacterial cells attached to different natural carriers was evaluated and it followed the order of luffa sponge > coconut husk fiber > wood chips. A high microbial density in luffa was observed, mainly occupying the uneven ravine structure. This observation could be attributed to the presence of carboxyl, hydroxyl and amino groups in the surface of the organic support. These groups are responsible for providing a certain amount of nutrients that enhances bacterial growth and adsorption to the surface [255]. In another study, two different lignocellulosic biomass carriers were compared, cactus fiber and luffa, used as both biofilm carriers and substrates for the treatment of wine-based reconstituted wastewater in a anaerobic PBBR type reactor [167].

Bamboo fiber is a natural fiber material, with high specific surface area (around 5393 $m^2.m^{-3}$ [168]), good hydrophilicity and biocompatibility. When compared to the traditional synthetic fiber, bamboo is more appellative due to its low price, durability, sustainability and biodegradability character [168]. On the other hand, bamboo rings have low porosity and specific surface, consequently, require a larger reactor volume. However, their costs in underdeveloped countries are less than 5–10 % of the costs of the commercialized media [253]. This organic material type has been well investigated as biofilm carrier for the removal of nutrients from wastewater. Xiao *et al.* [168], for example, prepared bamboo fiber as raw material carrier and compared to a conventional combined carrier (polyester fiber) for the removal of organics, nitrogen and phosphor using a BCBR. The reactor filled with bamboo started up more rapidly than the one with polyester and the removal efficiencies for all nutrients were significantly higher using the bamboo carrier during start-up period. Moreover, the reactor packed with bamboo showed higher resistance to pollutants load impact. In a recent study of Nilawati *et al.* [252], the anaerobic treatment of acidic tofu processing wastewater was investigated using a fixed-bed reactor, as a PBBR, employing cut bamboo as carrier. The cut bamboo provided stable treatment under continuous operation, reveling to be an appropriate support for cell immobilization.

Table 2. Wastewater treatment by biofilm technology using natural carriers

Type of material	Biofilm carrier	Microorganism	Biofilm growth	Experimental setup	Target pollutants	Max removal (%)	Reference
Inorganic	**Kaolin**	*Escherichia coli*	n.r.	Erlenmeyer flasks/ SWW	Cd (II) / Cr (VI) / Fe (III) / Ni (II)	78.9 / 100 / 100 / 74.2	[194]
		Streptococcus equisimilis	n.r.	Erlenmeyer flasks/ SWW	DEK	98.6	[195]
		Arthrobacter viscosus	n.r.			98.3	
	Vermiculite	*Streptococcus equisimilis*	n.r.	Erlenmeyer flasks/ SWW	Ni (II) / Cd (II)	100 / 98.85	[242]
			Well-established biofilm at the end of operation	Pilot scale PBBR/ SWW	Al (III) / Cd (II) / Ni (II) / Mn (II)/ DEK	99.85 / 41.75 / 49.03 / 52.03 / 100	[196]
			n.r.	Erlenmeyer flasks/ SWW	DEK	98.6	[195]
		Arthrobacter viscosus	n.r.			98.3	
		Microbial anaerobic community	Stable biofilm after 30 days	Penicillin vials/ SWW	Cd (II) /Cr (VI)	84.0 / 14.5	[164]
	Sepiolite	*Streptococcus equisimilis*	Well-established biofilm at the end of operation	Pilot scale PBBR/ IWW with MEK and DEK addition	Total nickel/ Total chromium/ Total copper/ Total iron/ MEK/ DEK	30.6 / 83.3 / 84.8 / 76.1 / 86.0 / 96.0	[163]
			n.r.	Erlenmeyer flasks/ SWW	DEK	98.6	[195]
		Arthrobacter viscosus	n.r.			97.8	

Bentonite	*Streptococcus equisimilis*	n.r.	Erlenmeyer flasks/ SWW	DEK	98.6	[195]
	Arthrobacter viscosus	n.r.			98.2	
Natural zeolite	Activated sludge	-2 – 2.5 g TSS/ L -Covered with biofilm after 97 days	Laboratory scale reactor/ MWW	TOC / TC / TN / NH_4^+-N / TP / PO_4^{3-}-P	37.2 / 26.7 / 12.3 / 10.4 / 79.9 / 82.9	[156]
		Biofilm grew slowly compared to a sponge carrier	BTF/ DWW	COD/ NH_4^+-N/ NO_3^--N	69.5/ 9.2/ 17.8	[174]
		n.r.	Laboratory scale BAF/ MWW	NH_4^+-N	91.2	[216]
		n.r.	Laboratory scale BAF/ IWW	NH_4^+-N	40.2	[217]
	Anaerobic sludge from WWTP	The zeolite surface facilitated the adherence of negatively charged microbes	Laboratory scale anaerobic SBBR/ SWW	COD	>93	[221]
	Activated sludge from WWTP	0.120 g VSS/g COD	Laboratory scale denitrifying FBR/SWW	COD/ NO_3^--N	87 / 99	[227]
Zeolite mixture (chabazite /clinoptilolite)	Activated sludge from WWTP (nitrifying bacteria)	Carrier efficiently covered by bacteria	Laboratory scale PBBR/ SWW	Trichloroethylene	62.7	[204]

Volcanic rock	Granular sludge from WWTP	Spherical and rod-shaped bacteria as dominant species of biofilm	Laboratory scale anaerobic / aerobic fixed bed SBBR/ SWW	Azo dye Acid Red 18/ COD	96.6-96.9 / 92.9-94.6	[222]
	Activated sludge from WWTP (anoxic and aerobic process)	0.0082 – 0.0125 g VSS/ gcarrier	Laboratory scale liquid–solid circulating FBR/ SWW	COD/ PO_4-P / NH_4^+-N / TN	> 90 / 30 / 90 / 70	[228]
	Activated sludge from WWTP	0.0062 –0.0155 g VSS/ g carrier	Pilot scale liquid–solid circulating FBR/ SWW	COD / PO_4-P / TN	90 / 70 / 80	[229]
	Activated sludge from WWTP	0.19 g VSS/ g COD	Laboratory scale denitrifying FBR/SWW	COD / NO_3-N	77 / 94	[227]
	Activated sludge from MWW	-0.145 g TSS/ g COD -SVI: 46–74 ml/g for aerobic cycles and 18–50 ml/g for anaerobic/aerobic cycles	Laboratory scale up-flow submerged biofilm reactor/ MWW	COD / NO_3-N / Total kjeldahl nitrogen/ TN/ TP	89–93 / 57–99 / 66–96 / 50–80 / 17–37	[244]
	Activated sludge	Good biofilm formation	Biofilter reactor / MWW	Ammonia / H_2S	90 / 98	[245]
		Good biomass attachment	Aerobic submerged filter / MWW with sugar addiction	COD	80	[243]
Perlite	Inoculum from rumen by-product	-Uniform and thin biofilm -0.66 g AVS/ g carrier	Inverse anaerobic FBR / SWW	COD	55	[201]

	Anaerobic sludge	Thin biofilm of uniform thickness	Anaerobic FBR / SWW	COD / BOD / TOC	80–92 / 90–96 / 75–92	[226]
	Denitrifying biofilm	Biofilm thickness (< 1mm)	Aerobic biofilm reactor / SWW	BOD / NO_3-N / NH_4^+-N	98.67 / 100 / 95.29	[246]
	Microbial anaerobic community	Low strength of biofilm	Penicillin vials / SWW	Cd (II) / Cr (VI)	68.0 / 100	[164]
Shungite	Microbial anaerobic community	Biofilm poorly expressed			84.0 / 87.0	
Stone and pebbles	Activated sludge from WWTP	n.r.	Laboratory scale PBBR / MWW	COD / BOD / PO_4^{3-}-P / SO_4^{2-} / NO_2^- / NO_3^-	89.62 / 89.67 / 38.12 / 69.6 / 18.86 / 58.7	[205]
		n.r.	Pilot scale BTF / MWW	COD / BOD / PO_4^{3-}-P / SO_4^{2-} / TN	85.6 / 85.6 / 63.22 / 28.5 / 34.4	[210]
		n.r.	Laboratory scale BTF / MWW	COD / BOD	93.4 / 95.7	[211]
		n.r.	Pilot scale BTF / MWW	COD / BOD / NH_4^+-N	98.1 / 98.6 / 93.5	[212]
	Aerobic activated sludge from WWTP	Carrier showed a constant increase in biofilm weight up to the fifth and seventh week time under both conditions	Glass jars reactor / MWW	COD / BOD	41.0 / 47.7	[200]
	Anaerobic activated sludge from WWTP				27.2 / 14.2	

Organic	**Sand**	Activated sludge from WWTP	n.r.	Laboratory scale PBBR / MWW	COD / BOD / PO_4^{3-}-P / SO_4^{2-} / NO_2^- / NO_3^-	97.15 / 97.12 / 41.58 / 79.69 / 62.26 / 71.74	[205]
			n.r.	Laboratory scale PBBR / MWW	CRB / Metoprolol / Ranitidine / Caffein / COD/ TN	31-46 / 36-73 / 97-99 / 99 / 95 / 3	[206]
	Luffa (fiber/pices)	Activated sludge from WWTP	-Coverage biofilm value was more than 0.99 -Thicker biofilm formed	Aerobic biofilm reactor / SWW	Hydrolysed polyacrylamide / TN	54.3 / 60.1	[190]
			The microbial growth was twenty to forty times higher than control	Pilot scale MBBR / MWW	COD / TN	79 / 53	[230]
		Industrial sludge	Biofilm conserved its activity even after a non-feeding period of 3 months	Laboratory scale anaerobic PBBR / IWW with nutrients addiction	COD	87	[167]
		Activated sludge	Biofilm developed discontinuously on the carrier surface; the pores were rarely covered by the biofilm	Laboratory scale SBBR / SWW	COD / TN / NH_4^+-N	80 / 70 / 90	[223]
		Microbial anaerobic consortia	-High microbial density observed, mainly	Glass bottles / IWW	COD	88	[160]

		occupied the uneven ravine structure -Homogenous cellular morphology reveled				
	Microbial community	Total bacteria (2.36×10^9/mL), more than achieved by control	Laboratory scale MBR / SWW	TN	68.92	[170]
	Activated sludge from WWTP (anaerobic)	n.r.	Glass bottles / SWW	1,1,1-trichloroethane	88	[251]
	Bacillus thuringiensis B1(2015b)	After 15 days carrier was almost completely covered by the microflora	Laboratory scale BTF / SWW	Naproxen / COD	90 / 82.65	[213]
	Anoxic seed from WWTP	The fibrous structure and surface of fibrous threads were completely covered by bacteria	Laboratory scale PBBR / SWW	TN	98	[188]
Cactus fiber	Industrial sludge	Biofilm conserved its activity even after a non-feeding period of 3 months	Laboratory scale anaerobic PBBR / IWW with nutrients addiction	COD	82	[167]
Bamboo (fiber/rings)	Activated sludge from WWTP	n.r.	BCOR / SWW	COD / NH_4^+-N / TN / TP	74.93 / 74.2 / 40.2 / 28.1	[168]
		n.r.	BAF / SWW	COD / NO_3^--N	95.87 / 96.12	[218]

	n.r.	Laboratory scale BASR / MWW	TN / NH_4^+-N / NO_3^--N / NO_2^--N	66.58-75.23 / 50.96 / 66.43 / 75.6-85.6 / 85.30-87.79	[236]
Seed sludge	Biofilms were formed in the surface and inside the bamboo rings	Anaerobic fixed-bed reactor / SWW	TOC	95	[252]
Inoculum from a tank reactor (anaerobic)	n.r.	Pilot scale anaerobic fixed-bed reactor / IWW	COD	95	[253]

IWW industrial wastewater; MWW municipal wastewater; DWW domestic wastewater; SWW synthetic wastewater; TOC total organic carbon; TC total carbon; TSS total suspended solids; VSS volatile suspended solids; AVS attached volatile solids; biomass yield (mg (VSS/TSS)/mg COD); attached biomass (mg/L); average sludge production (g TSS/g COD); Average attached biomass concentration (g AVS/g carrier); n.r: not reported.

2.2.3.2.2.2 Waste-based materials

Table 3 summarizes selected studies that highlight the ability of waste materials as biofilm carriers to remove pollutants and nutrients from different wastewater. A huge variety of disposed wastes of different nature used for biofilm development are reported.

Agroforestry wastes, i.e., natural lignocellulosic materials can be used as sustainable alternatives to commercial carriers. Among these natural organic materials wood husk, pine bark, cotton sticks, coconut husk fiber, sugarcane bagasse, cypress cones, date palm fiber, wheat straw, among others have been reported as environmentally friendly and inexpensive carriers with potential application for microbial growth [160,167,183,184,197,215,234,256,257]. Zakaria *et al.* [183] reported the ability of an *Acinetobacter haemolyticus* biofilm supported on wood-husk to detoxify Cr (VI) to Cr (III), without the need to resupply fresh cells. The authors described wood-husk as a promising support material for biofilm development due to its abundance and reduced operating costs. Zainab *et al.* [160] investigated the potential of wood chips as biofilms carriers for anaerobic digestion of high load industrial wastewater. However, the authors concluded that the performance of the bioreactor containing wood chips was inferior to other bioreactors containing luffa sponge and coconut husk fiber in terms of methane concentration and organic matter removal efficiency. Other forest wastes such as cotton sticks were reported by Aslam *et al.* [215] as an alternative filter media to be used in a BTF wastewater treatment system. The use of these wastes as filter media allowed to reach 69-78 % of BOD removal and 65-80 % of COD removal. The authors concluded that cotton sticks appeared to be a degradation-resistant alternative filter media for the TF system and its use proved that municipal wastewater can be handled in an environmentally friendly and cost-effective manner.

Agricultural and food wastes have specific characteristics that make these materials interesting potential carriers for biofilm development, namely high porosity, high surface area and presence of specific chemical groups (amino, hydroxyl, carboxyl, thiol and phosphate groups) [258]. Since these functional groups can be involved in different mechanisms such as adsorption, ion exchange, complexation and microprecipitation, these wastes have been used as biosorbents of xenobiotic compounds as reported in several works [259,260]. The use of agro-industrial wastes as carrier materials for biofilm development have recently been reported in different studies [160,170,182–184]. Since these materials are mainly composed of cellulose, hemicellulose and lignin, they constitute a sustainable source of carbon besides being a suitable structure for the growth of microbial community. Some agricultural wastes such as rice husk, rice straw, corn straw and corncob have been reported as efficient external

carbon sources for enhancing bacterial denitrification [170,184,186,187,191,197,233,261]. Sun *et al.* [261] studied the use of corncobs as carbon source and biofilm carrier for the pre-treatment of coking wastewater. The authors concluded that alkali-pretreated corncobs performed a significative improvement on biodegradability and accomplished the simultaneous removal of nitrate and refractory organic pollutants from coking wastewater. Other work reported by Guan *et al.* [191] studied the feasibility of different agricultural organic wastes used as solid carbon sources and biofilm carriers on improving nitrogen removal from simulated wastewater. The authors concluded that the rice straw and the corn straw worked both as favorable solid carbon sources and biofilm carriers, achieving a COD removal rate ranging from 47.3 to 50.2 % and a TN removal rate between 21.8 and 24.8 % for wastewater with low C/N ratio. Recently, Hasanzadeh *et al.* [231] presented a study showing the feasibility of using walnut shell as an inexpensive and available carrier for the immobilization of halophilic microorganisms for the treatment of oilfield produced water. The authors reported that at higher total dissolved solids the presence of walnut shell enhanced the resistance of the system to shocks of salinity and organic loading rates in terms of effluent quality and sludge characteristics, due to combination of oil adsorption by the carrier and biofilm formation by microorganisms.

Apart from agro-food wastes, industrial and urban wastes such as waste tire rubber, cigarette filter rods, cotton cloth and textile wastes, disposed plastic bottles, coal cinder, disposed rubbers and some other materials have been introduced as novel biofilm carriers [171,207,211,214,220,224,225,232,235,237–241,262]. Waste tires seem to be a valuable option for wastewater treatment since they can be obtained easily at low costs and in large quantities. They are comparatively cheaper than any other usable packing material [263]. The utilization of these wastes can significantly reduce the accumulation of the entire raw materials [264]. In 2012, Han *et al.* [220] investigated the efficiency of a laboratory-scale up-flow BAF packed with tire chips for the treatment of 1,4-dioxane. The bioreactor was reported to have average removal efficiencies of 1,4-dioxane of 71.4 % in continuous mode, while the decrease on COD and BOD achieved was 58.2 % and 95.1 %, respectively. Naz *et al.* [262] evaluated the effectiveness and durability of tire derived rubber for biofilm development. It was reported a decrease of 73.3 % and 69.9 % of COD after 9 weeks of operation by aerobic and anaerobic biofilm, respectively. These authors reported that the rubber media showed durability under aerobic conditions and that this waste material can be employed as biofilter media in aerobic fixed biofilm reactors for wastewater treatment. More recently, in 2017, Sharafat *et al.* [171] reported the successful application of waste tires as a cost-effective support for biofilm development on static batch bioreactors that were run under anoxic conditions. Other study conducted by Derakhshan *et al.* [238] evaluated the

feasibility of using fixed bed biofilm carriers produced from existing waste tires for wastewater treatment. This study reported that the removal efficiency of soluble COD ranged between 76 and 98 % for the FBSBR. Other environmental concern is the generation of wastes during cigarette production. It is estimated that more than 1 % of the filter rods are wasted during cigarette manufacturing process. While the non-biodegradability of cigarette filter rods is an environmental concern, the characteristics and high surface area and entrapment ability for microbial cells, make cigarette filter wastes a potential media to be used in biofilm reactors as a carrier. In 2011, Sabzali *et al.* [241] conducted a research to evaluate the ability of cigarette filter wastes as sustainable carriers using an anaerobic MBBR. The results of this work revealed a COD average removal efficiency between 61.3 % and 64.5 % for AMBBR filled with cigarette filter rods, while the removal average of phosphorus was 67.7 %. In 2013, the same authors [240] studied the capability of cigarette filter rods as carriers in an IFAS process. The authors evaluated two similar laboratory-scale IFAS systems, comparing the performance of commercial carriers (Kaidnes-K3 plastic) and cigarette filter rods. The COD average removal efficiencies were 89.3 % and 93.9 % for Kaidnes-K3 and cigarette filters, respectively. Therefore, the authors demonstrated that the performance of the IFAS reactor containing cigarette filters was comparable to the reactor using Kaidnes-K3 plastic, which made the cigarette filters a good alternative to commercial carriers to be used in IFAS systems. More recently, Dorji *et al.* [235] demonstrated the ability of shredded waste plastic bottles to be used as a novel biofilter media in an on-site wastewater treatment system consisting of a combined UASB and an anaerobic biofilter in sequence. The pilot system produced a final treated effluent quality that met three out of four of the effluent discharge limits of Bhutan, being the cost of the pilot wastewater treatment system 7 % to 10 % lower than the conventional septic tanks.

Table 3. Wastewater treatment by biofilm technology using waste carriers

Type of material	Biofilm carrier	Microorganism	Biofilm growth	Experimental setup	Target pollutants	Max removal (%)	Reference
Forest wastes	**Wood husk / chips**	*Acinetobacter haemolyticus*	After 30 days of operation, biofilm-containing microorganisms had grown massively and filled the carrier substratum cavity	Laboratory scale PBBR / SWW	Cr (VI)	55.33	[183]
		Pseudomonas putida F1 (DMSZ 6899)	n.r.	Glass bottles / SWW	Toluene	78	[265]
		Bacillus cereus	n.r.			58	
		Microbial anaerobic consortia	Not very suitable for biofilm formation	Glass bottles / IWW	COD	57.35	[160]
	Pine bark	Activated sludge from WWTP	n.r.	Glass unit / SWW	Phenol / COD	100 / 88.5	[184]
	Cotton sticks	Activated sludge	n.r.	Pilot scale BTF / MWW	COD / BOD	65–80 / 69–78	[215]
	Date palm fibre	Activated sludge	n.r.	Pilot scale BTF / MWW	COD / BOD / TN / TP / SO_4^{2-}	79.6 / 78.7 / 22.7 / 32.1 / 24.3	[197]
	Cypress cones	Industrial sludge	Biofilm conserved its activity even after a non-	Laboratory scale anaerobic PBBR / IWW with nutrients addiction	COD	90	[167]

			feeding period of 3 months				
	Wheat straw	Industrial sludge	Biofilm conserved its activity even after a non-feeding period of 3 months	Laboratory scale anaerobic PBBR / IWW with nutrients addiction	COD	86	[167]
Agro-food wastes	**Coconut husk fiber**	Microbial anaerobic consortia	The density of anaerobes was not predominant	Glass bottles / IWW	COD	77.5	[160]
		Mixed Rhodopseudomonas sp. DD4, DQ41 and FO2	Biofilm was about 20 µmm thick	Erlenmeyer flasks / IWW	Anthracene / Naphthalene / Phenanthrene / Pyrene / Crude oil	95.3 / 94.2 / 91.4 / 96.2 92.6	[256]
		Anaerobic bacterial biomass	Biofilm shape: cylindrical Mean length: 70.35 mm Mean diameter: 0.275 mm	Laboratory scale anaerobic biofilm reactor / SWW	COD	80–90	[257]
	Agave fiber	Microbial community	n.r.	Laboratory scale biofilter reactor / MWW	COD / BOD	79.7 / 92	[182]
	Ridgegourd fiber	Anaerobic bacterial biomass	Biofilm shape: cylindrical Mean length: 48.8 mm Mean diameter: 0.233 mm	Laboratory scale anaerobic biofilm reactor / SWW	COD	80–90	[257]
	Sugarcane bagasse	*Candidatus Brocadia sinica*	n.r.	UASB / SWW	TN	85	[234]

Corncob	Microbial community	Total bacteria (2.09 × 10^9/mL), more than achieved by control	Laboratory scale MBR / SWW	TN	62.97	[170]
	Activated sludge	n.r.	Pilot scale BTF / MWW	COD / BOD / TN / TP / SO_4^{2-}	92.7 / 87.6 / 32 / 38.5 / 28.2	[197]
		After 100 days operation, the biofilm of the carrier was comparably less richer and thicker than the chemically modified carrier	Up-flow fixed-bed bioreactors / IWW	NO_3^--N	48	[261]
	Activated sludge from WWTP	n.r.	Glass unit / SWW	Phenol / COD	100 / 80.31	[184]
		n.r.	Up-flow laboratory reactors / SWW	NO_3^-	90	[186]
Corn straw	Activated sludge from WWTP (anaerobic)	n.r.	Drawer-type biological filter / SWW	COD / NO_3-N / TN / TP	51.4 / 22.3 /24.3 / 10	[191]
Sunflower stalk	Industrial sludge	Biofilm conserved its activity even after a non-feeding period of 3 months	Laboratory scale anaerobic PBBR / IWW with nutrients addiction	COD	94	[167]
Rice straw	Microbial community	Total bacteria (3.02 × 10^9/mL), more than achieved by control	Laboratory scale MBR / SWW	TN	82.97	[170]

		Activated sludge from WWTP (anaerobic)	n.r.	Drawer-type biological filter / SWW	COD / NO_3-N / TN / TP	38.7 / 20.1 / 22.1 / 10	[191]
	Rice husk	Activated sludge from WWTP	n.r.	Up-flow laboratory reactors / SWW	NO_3^-	95	[187]
		Activated sludge	Good microbial growth on the carrier surface, indicating favorable conditions for their growth and reproduction	Pilot scale MBR / MWW	COD / NO_3-N / TN	91.01 / 95.69 / 46.33	[233]
	Grape stalk	Industrial sludge	Biofilm conserved its activity even after a non-feeding period of 3 months	Laboratory scale anaerobic PBBR / IWW with nutrients addiction	COD	92	[167]
			n.r.	Anaerobic biofilm reactor / SWW	COD	92	[266]
	Walnut shell	Halophilic consortia	SVI: 38.8 ml/g	MBBR / SWW	COD / BOD / NH_3-N / TP	94.6 / 94.3 / 98.5 / 97.6	[231]
	Oyster shell	Activated sludge	n.r.	Laboratory scale BAF / MWW	COD / NH_4^+-N /TP	85.1 / 98.1 / 79.9–90.6	[219]
Industrial / urban wastes	**Cotton cloth**	Activated sludge	Thickness of matured biofilm (13th day of operation)	Laboratory scale BCBR / MWW	COD / NH_4^+-N / TP	93.4 / 82 / 56.8	[239]

		was 1.8 ± 0.2 mm and the relative density was 47.33 ± 1.2 mg/cm^3				
Geotextile	Activated sludge	The biofilm allowed the creation of stable zones with different O_2 concentration profiles, resulting in the growth and development of different types of microbial cultures	Laboratory scale reactor as BASR concept / SWW	COD / TN / TP	98.9 / 98.3 / 94.1	[237]
Polypropylene fibers	Activated sludge	SVI: 85 - 115 ml/g	Laboratory scale SBBR / MWW	COD / BOD / TN / TP	93 / 98 / 82 / 44	[224]
Cigarette filter rods	Activated sludge	n.r.	Laboratory scale anaerobic MBBR / SWW	COD / TP	61.3 / 67.7	[232]
	Activated sludge from WWTP	n.r.	Laboratory scale IFAS / SWW	COD / NO_3-N /TP	81.62–94.55 / 96.34–97.40 / 60.64	[241]
		Biofilm formation on the carrier was faster than on a commercial carrier	Laboratory scale IFAS / SWW	COD / NH_4-N / PO_4-P	93.9 / 79.2 / 80.4	[240]
Plastic bottles	Activated sludge (anaerobic)	n.r.	Pilot scale UASB / IWW	COD / BOD	29–72 / 60–70	[235]

Wire hose cover	Activated sludge from WWTP	A thin layer of biofilm and EPS was observed over the internal surface of the carrier. Biofilm coverage on the outer surfaces of the carriers was sparse due to abrasion losses (particle-to-particle collisions) and shear forces	SBBR / SWW	COD / NH_4^+-N / TN	86.5 / 98.4 / 71.4	[225]
Tire rubber/ships	Aerobic activated sludge	A higher biofilm development (0.51 g) under aerobic conditions was obtained than under anaerobic (0. 42 g) after 7 weeks	Glass jar type reactor / MWW	COD / BOD	73.3 / 72.9	[262]
	Anaerobic activated sludge			COD / BOD	69.9 / 69.5	
	Activated sludge	Maximum bacterial biomass: 4.73E + 10 CFU/ml cm^2	Laboratory scale batch reactor / MWW with Fe (III) addiction	COD / NO_3-N	37.9 / 17.7	[171]
	Activated sludge from WWTP	In the 69th day biofilm shows fibers and other peculiar structures with different morphologies	Laboratory scale up-flow BAF / IWW	1,4-dioxane / COD / BOD	71.4 / 85.2 / 95.1	[220]

			0.16–0.47 kg TSS/kg COD SVI: 83.78–143.61 ml/mg	Pilot scale FBSBR / SWW	COD	76–98	[238]
	Coal cinder	Activated sludge	n.r.	Laboratory scale BTF / MWW	COD / NH_4^+-N	91 / 78	[214]
	Rubber	Activated sludge from WWTP	n.r.	Laboratory scale BTF / MWW	COD / BOD	81.9 / 86.4	[211]
Mix of wastes	**Mix of scrap tire chips, sulfur pellets and oyster shell**	Activated sludge from WWTP	n.r.	PBBR / SWW	NO_3^--N	94	[207]
	Mix of sugarcane bagasse and coconut, aegle and groundnut shell	Denitrifying consortia	n.r.	PBBR / DWW	COD / BOD / NH_4^+-N / NO_3–N / NO_2^--N / PO_4^{3-}-P	100 / 100 / 79.40 / 84.66 / 59.45 / 90.13	[208]
	Peanut shell (with sand)	*Pseudomonas aeruginosa* PF2	Bacteria increased from approximately 8–11 log CFU/g peanut shell at the fifth day operation while biofilm formation also increased and reached steady state at the same time	Laboratory scale PBBR / SWW	Profenofos pesticide	90–96	[209]

IWW industrial wastewater; MWW municipal wastewater; DWW domestic wastewater; SWW synthetic wastewater; TOC total organic carbon; TC total carbon; TSS total suspended solids; VSS volatile suspended solids; AVS attached volatile solids; biomass yield (mg (VSS/TSS)/mg COD); attached biomass (mg/L); average sludge production (g TSS/g COD); Average attached biomass concentration (g AVS/g carrier); n.r: not reported

2.2.3.3 Environmental and economic impact assessment

The widespread use of polymeric and AC carriers becomes disadvantageous and not challenging when it comes to the criteria of economic (initial capital cost) and environmental (disposal and regeneration of carriers) aspects [160,161]. Besides, most of the manufactured carriers are derived from non-renewable oil resources hard to degrade once wasted, causing secondary pollution [168]. Industrial AC is usually produced by carbonization and activation of non-renewable precursors carbonaceous materials such as coal, wood, coconut shell, peat, and lignite, with production costs ranging from $ 1000 to $ 1500 t^{-1} [267]. Its use induces high environmental impacts such as ecotoxicity, acidification, ozone and fossil fuel depletion [268]. AC exhibits negative environmental impacts in material generation, delivery, storage and disposal that includes dewatering and landfill hauling [268]. Its production foments smog formation due to the emissions from electricity consumption during its generation. In the same way, supports based on plastics might present impediments on a widespread scale in some low-income countries due to high transportation or manufacturing costs [269]. Plastic pollution has become one of the most stressing environmental issues. Reducing plastic use is urgent because its production requires an enormous amount of energy and resources, including manufacturing, transporting and waste disposal. Natural sources like water, oil, natural gas and coal are required for its production. This causes carbon emissions and contributes to global warming. Reducing plastic waste will reduce the contamination of the environment [270].

The application of the traditional carriers in biological wastewater rehabilitation is not feasible or cost-effective in the context of our recent world situation. Wastewater treatments must be easy to perform with low operating and maintenance costs and low environmental impact. Obviously, cost-benefit analysis and life-cycle assessment should be performed to evaluate the feasibility of water and wastewater treatment systems [271]. On this basis, it is important to explore and find sustainable alternatives, with low cost, preferably locally available products or reusable materials that do not require additional energy input or huge modifications. They should be selected over an industrial product, to reduce the overconsumption of petrochemicals and the secondary pollution after disposal, establishing a sustainable and clean biofilm system.

Sustainable material sources with suitable properties and availability are attractive biofilm carriers [272]. Accessible materials from natural origin are available on a sustainable basis and may be obtained at low cost. They have a biodegradability and reduced toxicity character and require limited technology to

integrate them as carriers. Also, special attention should be paid to wastes and other residual by-products that are abundant in developing countries and in countries with significant industrial activities. This is the case of food waste, the most impactful waste in terms of quantity. The explosive population growth has generated up to 1.6 billion tons of food waste annually worldwide [273]. If not appropriately handled as it happens when disposed in open dumping, large quantities of greenhouse gases will be released into the atmosphere [171,274]. Another example is the disposal of wastes from the agricultural sector, allowing them to rot at the source or in stockpiles [275]. The lack of proper treatment of wastes severely affects the ecosystem [162]. For this reason, their incorporation in carriers for biological wastewater treatment is potentially environmentally sustainable as it will reduce their disposal and recover the materials for a new purpose [276]. Carbonized waste biomass can also integrate a potentially more competitive and friendlier alternative to AC because it costs less on a mass basis. Biochar can be easily produced, especially in agrarian economies with easy access to abundant biomass in the form of crop residues and organic wastes [277]. For example, biochar production from food wastes can not only significantly reduce the accumulation of raw materials but also show good prospect of application in environmental restoration such as soil remediation (as fertilizer), carbon fixation, energy co-production and wastewater treatment [169,181,268].

With the reuse of residues an increasing life cycle of materials is promoted, while energy requirements, resources depletion, emissions and waste disposal are decreased. A circular and sustainable economy is promoted with waste valorisation. This is in fact an actual prevailing priority in the European Union, that is to achieve a continuous decrease in the negative environmental effects of services and products throughout their life cycle [278]. To achieve an efficient biomass usage, European bioeconomy strategies increasingly consider the concept of a CBE. A bioeconomy can be defined as the *"production of renewable biological resources and the conversion of these resources and waste streams into value added products, such as food, feed, bio-based products and bioenergy"* [172]. With a CBE the dependence on using natural resources is reduced by converting biomass and organic wastes into diversified end-products and materials, providing competitive goods in an environmentally friendly way [279]. CBE is crucial and possesses vast potential towards a sustainable green world. Besides, its implementation contributes to food security, climate change mitigation, job creation, biodiversity preservation and, eventually, sustainability [280]. The sustainable use of natural origin and waste-based carriers can contribute towards the CBE (**Figure 6**).

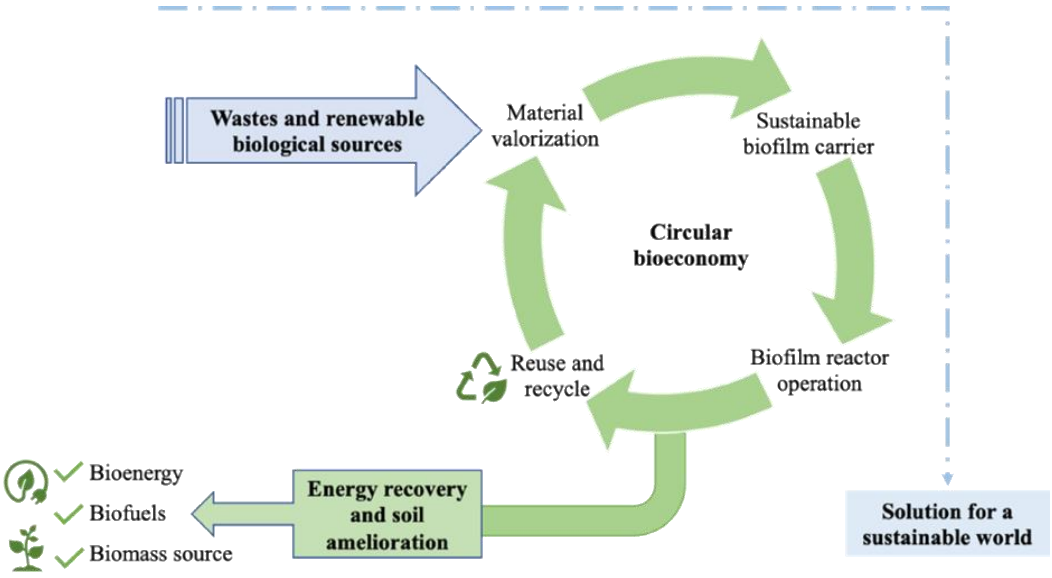


Figure 6. Schematic view of the contribution of sustainable carriers towards a circular bioeconomy.

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CHAPTER III

SORPTION POTENTIAL OF WASTE AND NATURAL ORGANIC MATERIALS FOR PESTICIDE REMOVAL

In the present chapter the rehabilitation of water contaminated with ATZ is evaluated by a permeable and sustainable barrier constructed with waste materials (sepiolite) and with biomaterials (cork and pine bark). ATZ adsorption is firstly assessed by kinetics and equilibrium assays and desorption is tested with different extraction eluents. Continuous fixed-bed experiments using those sorbents as PRB are performed to evaluate ATZ removal for up-scale applications. Recyclability of sorbents is evaluated by adsorption-desorption cycles. Analyses confirm no significant differences in material morphology and structure along the processes. This study demonstrates that recycling waste/biowaste is a sustainable option for wastewater treatment, with waste valorization and environmental protection.

This chapter is based on the following publication:

Lago, A.; Silva, B.; Tavares, T. Cleaner Approach for Atrazine Removal Using Recycling Biowaste/Waste in Permeable Barriers. *Recycling* **2021**, *6*, doi:10.3390/recycling6020041

3.1 INTRODUCTION

Recently, technologies that use adsorbents produced from biomass have attracted much attention of researchers since they are biodegradable, have a good mechanical and chemical resistance, being widely available in nature. The usage of biosorbents defines a low-cost technology and viable approach to remove herbicides from water due to their physical and chemical affinity [1]. Also, waste-based materials, whether organic or inorganic, are seen as promisors adsorbents for water and wastewater treatment due to their local availability, cost effectiveness, technical feasibility and applicability [2]. By recycling wastes, their life cycle is increased, and circular economy is promoted. Studies on the use of wastes (biosorbents or adsorbents) to remove pollutants are gaining more importance within the research community, as revealed by the growing number of published works [2–5].

Different waste-based materials can be used for herbicides removal such as sand, sediment and soil, clays, dairy manure, agricultural and industrial wastes, AC derived from biomasses and wastes, rubber granules, among others [6–10]. Clay minerals have been widely applied to adsorb different molecules due to their availability, large specific surface area, low-cost, chemical and mechanical stability [11–14]. Among clays, the relevant chemical properties of sepiolite, a naturally occurring hydrated magnesium silicate of sedimentary origin, have encouraged several authors to assess its efficiency on the adsorption of different organic and inorganic pollutants [15–17]. Its structure is responsible for its relevant specific surface area and high porosity, providing substantial surface sites for adsorption processes [18]. Sepiolite is widespread and abundant on earth and can be purchased at a low cost. It can also be classified as an inorganic waste from its exploitation deposits or from its processing and purification processes [19]. Bark from *Pinus pinaster* generated by the forestry industry is one of the most abundant agro-forestry lignocellulosic wastes in Europe. Due to its high production volume and biodegradability, new technologies for its reutilization are currently taking place. Pine bark has been proposed as biosorbent to remove different pollutants from contaminated water and wastewater due to its high potential sorption capacity by ion exchange and chelating processes [2,20]. Also cork, the outer bark of the oak tree (*Quercus suber*), has been valued as a natural adsorption substrate for the removal of a broad range of pollutants [2,21,22]. Cork has interesting properties as low density, buoyancy and important binding sites present on its structure [23]. Due to its unique characteristics, its availability in cork-producing areas or the low cost acquisition of used cork stoppers and cork powder rejected by industry, the use of cork as adsorbent has become an interesting alternative in the field of wastewater treatment [2]. Often spent tires are dumped in open or landfill sites, however, because of their abundance and low cost they could be

recycled and turned into a viable alternative adsorbent [24]. The structural characteristics of sorbents can be changed by physical and chemical treatments and their adsorption capacity improved. Pyrolysis processes allow the increase of the specific surface area of the waste rubber and the use of an activation agent favors the porosity of AC [25]. Activated chars produced from tire rubber wastes have been studied by the research community, to adsorb different compounds from wastewater [25,26].

Besides the low cost and the availability of waste materials, the economic feasibility of an adsorption process will also depend on the adsorption capacity of the material and recyclability over time. It is important to consider desorption of contaminants and regeneration capability in the selection of an adequate adsorbent. Regeneration over adsorbent replacement can significantly improve the lifetime of the PRB. The use of solvents to chemically regenerate adsorbents and recover adsorption capacity becomes feasible and attractive. The main advantages of this process are the possibility to extract/recover target substances and the low mass loss of the adsorbent [27]. There are several reported waste based materials with proven sorption capacity, however very few studies report their regeneration proneness [28]. Some of the reported materials are not easily regenerated, being their morphology and adsorption capacity affected after desorption processes [20]. The selection of an effective, but also eco-friendly and competitive desorption process that enables adsorbents reusability is a crucial step of the overall project.

Previous studies reported the adsorption characteristics of ATZ onto some reusable biomaterials/materials without pre-treatments in laboratory experiments [1,6,29,30], but the novelty of this study is the assessment of the capacity of sepiolite, cork and pine bark particles without previous resort to chemicals, to function as reactive constituents of PRB to remediate ATZ contaminated aquifers. It was possible to produce data from batch experiments, on adsorption behavior and capacities, rates and regeneration proneness for the tested materials, crucial to evaluate the performance of PRB columns to remediate ATZ contaminated plumes. Besides, the recovery effectiveness and recyclability of the selected biomaterials by an optimized desorption process was demonstrated. Adsorption of ATZ on activated waste rubber char was also performed in batch mode for comparison with other materials without pretreatment. The present research is in line with the growing concern and demand for information on herbicide removal with the use of sustainable waste-based biomaterials/materials with their recovery after usage and proven recycling ability, expected by environmental policies.

3.2 MATERIALS AND METHODS

3.2.1 Chemicals

ATZ (PESTANAL® analytical standard, 99.1 %) was purchased from Sigma-Aldrich. **Table 4** summarizes the major physicochemical properties of ATZ. To increase water solubility, ATZ was dissolved in acetonitrile (HPLC grade) and a stock solution of 1g/L was stored. Diluted solutions of ATZ were prepared from the stock solution using deionized water. For the determination of pH of zero point of charge (pH_{zpc}), 1.0 M H_2SO_4 (95 %, Fisher Chemical), 1.0 M NaOH (≥ 97 %, Fisher Chemical) and 0.01 M NaCl (99.5 %, Panreac) solutions were prepared. HPLC-grade acetonitrile (Fisher Chemical) and ultra-pure water, obtained from a Milli-Q Millipore system, were used to prepare the mobile phases for ATZ quantification by UHPLC-DAD. Acetate buffer was prepared by diluting sodium acetate (≥ 99 % Fisher Chemical) and acetic acid (≥ 99.7 %, Fisher Chemical) in water and potassium phosphate buffer by diluting di-potassium hydrogen orthophosphate (≥ 99 %, Fisher Chemical) and potassium di-hydrogen phosphate (99 %, Panreac) in water.

Table 4. Selected physicochemical properties of ATZ

Chemical name	**Molecular formula**	**Molecular weight (g/mol)**	**Water solubility (mg/L at 25°C)**(a)	**pKa**(b)	**Log K_{ow}**(b)	**Molar volume (cm^3/mol)**	**Width (Å)**(c)	**Depth (Å)**(c)	**Thickness (Å)**(c)
Atrazine	$C_8H_{14}ClN_5$	215.69	33	1.68	2.6-2.71	169.8	9.6	8.4	±3

(a) PubChem; (b) [32]; (c) [33]

3.2.2 Preparation of waste-based adsorbents/biosorbents

Natural sepiolite was supplied by Tolsa S.A. located in Madrid, Spain. Firstly, the clay mineral was sieved to obtain a particle size with equivalent diameter between 1 and 2 mm and then it was washed with deionized water to remove dust. It was placed in an oven at 60 °C during 24 h and stored in a desiccator for further use.

Cork stoppers were supplied by a cork producing industry, Corticeira Amorim, SGPS, S.A. located in Aveiro, Portugal. The stoppers so received were cut into small pieces and milled. The grains were sieved to a size with equivalent diameter between 1 and to 2 mm and then washed with deionized water to remove dirt and particulate materials. The cleaned cork was placed in an oven at 60 °C during 24 h and then stored in a desiccator.

Pine bark was collected from a pine forest located in Braga, Portugal. It was washed with deionized water to remove mud, other materials and impurities and then placed in an oven at 60 °C to be dried. After, it was ground into smaller particles, sieved in the range 1 mm to 2 mm and stored in a desiccator.

Activated rubber char material was prepared by the pyrolysis of waste rubber granules provided from a truck tires and rubber waste recycling company, Eco-recycling LTD, located in Novi Sad, Serbia. Pyrolysis and activation processes were performed at the Department of Materials, Vinča Institute of Nuclear Sciences, Belgrade, Serbia. The waste rubber granules were pyrolyzed into a quartz tube furnace under an N_2 flow at 800 °C for 4 h (heating rate was 5 °C/min). After this procedure the char was washed with 2 M HNO_3 to clean the sample surface from metals. Carbonized granules and the solution of 2 M HNO_3 were mixed on a magnetic stirrer for 2 h at room temperature, filtered, washed with distilled water and dried in an oven at 110 °C. After that, the material was mixed with KOH pellets (sample/ KOH = 1/4) and activated at 800 °C for 4 h under N_2 atmosphere. In order to remove the impurities, the resulting activated carbon-based powder was washed with 0.5 M HCl solution (mixed in a magnetic stirrer at 85 °C for 30 min). Lastly, the sample was washed with deionized water and dried in an oven at 110 °C.

3.2.3 Materials characterization

The pH_{zpc} of materials was determined as it helps to explain the electrostatic interactions between adsorbate and sorbent. A solution of 0.01 M NaCl was prepared, previously bubbled with nitrogen to avoid the dissolution of atmospheric CO_2 and to prevent the change of the solution pH. Solutions with pH values from 1 to 9 were prepared by adding 1.0 M H_2SO_4 or 1.0 M NaOH. For each pH value, 0.1 g of adsorbent was added to 25 mL of NaCl solution in 50 mL falcons. Flasks were sealed to avoid contact with air and were kept under moderate agitation in an incubator at 25 °C during 48 h. After, the adsorbent was filtered and the pH of the remaining solution was measured and plotted against initial pH. The pH_{zpc} is defined as the pH at which the curve crosses the line $pH_{initial} = pH_{final}$.

The surface morphology of materials (before ATZ adsorption and after adsorption-desorption cycles) was examined by scanning electron microscopy (SEM). Samples were evaluated by a Phenom

ProX SEM (Phenom-World BV, Netherlands). All data were processed using the ProSuite software. The observation of samples was performed with gold coating.

The presence of functional groups in the surfaces (in their natural form and after the first and the second desorption steps) was evaluated by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy using an ALPHA II- Bruker spectrometer (Ettlingen, Germany) with a diamond-composite cell. The measurements were recorded over the range from 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1} and 24 scans per sample.

3.2.4 Quantification of ATZ

ATZ quantification was performed by UHPLC-DAD, using a Shimadzu Nexera X2 and a Kinetex C18 column (Phenomenex) (1.7 µm x 100 Å x 2.1 mm) operating in isocratic mode. Eluents used were acetonitrile and ultrapure water (45:55 v/v %) at a flow rate of 0.2 mL/min. The autosampler and column temperature was kept at 25 °C and an injection volume of 5 µL was used. The chromatograms were registered at ATZ maximum adsorption wavelength at 225 nm. The linearity of the method was assured by setting a calibration curve over the concentrations range of 0.3–4 mg/L. Detection limit (0.1 mg/L) was determined as the minimum detectable concentration of the analyte in the test sample that can be reliably distinguished from zero. The quantification limit (0.3 mg/L) was set as the concentration above which the analytical method operates with precision. The average peak area of each standard was used for quantification. Data were processed by Lab Solutions software (version 5.71, Shimadzu Corporation).

3.2.5 Batch experiments

Kinetics and equilibria experiments were performed to evaluate the ATZ adsorption capacity of different adsorbents/biosorbents. A predetermined mass of each material was placed in amber Erlenmeyer flasks and a fixed volume of the ATZ stock solution with 2 mg/L was added. Desorption experiments were carried out to evaluate the desorption capacity of ATZ from sepiolite, cork and pine bark using different extraction solvents. To assure the reproducibility of the results, each assay was performed in triplicate and the average of the three assays was reported for each test. The experiments were conducted in the absence of light to avoid the photo degradation of the herbicide [34].

Experiments with activated rubber char were performed as a comparative term. Due to the high material production costs, reduced availability, and different particle size, only batch kinetics and equilibria tests were performed with this material.

3.2.5.1 Adsorption kinetics

Assays were performed to determine the equilibrium adsorption time and to evaluate the adsorption rate of ATZ onto the sorbents. Adsorbent doses of 1 g/L of activated rubber char, and 5 g/L for sepiolite, cork and pine bark were used for equilibria assessment. Each mass of adsorbent was placed in contact with 50 mL of 2 mg/L of ATZ solution in Erlenmeyer flasks and these were shacked in a temperature-controlled incubator at 140 rpm and 25 °C until equilibrium was reached (14 h for the activated rubber char, 56 h for sepiolite, 72 h for cork and 8 days for pine bark). For the kinetics, samples were taken at different time intervals from another set of erlenmeyers, filtered through 0.22 μm nylon syringe filters, being the filtrate analyzed for ATZ concentration by UHPLC-DAD. A blank test, with 2 mg/L of ATZ without biosorbent/adsorbent material, was performed in an Erlenmeyer flask and analyzed during the experiment. To determine the adsorption capacity, q_t (mg/g), the following Equation 1 was used:

$$q_t = \frac{(C_0 - C_t)\,V}{m} \quad (1)$$

where C_0 (mg/L) is the initial concentration of ATZ in solution, C_t (mg/L) is the remaining concentration of ATZ in solution at time t, V (L) is the initial volume of the solution and m (g) is the mass of the adsorbent material.

The experimental data were fitted by Lagergren pseudo-first order (PFO) [35] (Equation 2) and pseudo-second order (PSO) [36] (Equation 3) models so to describe ATZ adsorption kinetics. Origin Pro 8.0 software was used to perform the calculations.

$$q_t = q_e\,[1 + \exp\,(-k_1.t)] \quad (2)$$

$$q_t = \frac{k_2.q_e.t}{1 + k_2.q_e.t} \quad (3)$$

where q_t is the adsorption capacity at time t (mg/g), q_e is the adsorption capacity at equilibrium time (mg/g) and k_1 (min^{-1}) and k_2 (g/mg.min) are the adsorption rate constants of PFO and PSO, respectively.

3.2.5.2 Adsorption equilibrium

Once the equilibrium time for each sorbent was established, equilibrium experiments were performed to build up the adsorption isotherms and to determine the maximum uptake value for each material. Activated rubber char concentrations tested ranged between 0.05 and 0.75 g/L, while for sepiolite, cork and pine bark they ranged from 1 to 15 g/L. Different amounts of each adsorbent were placed in contact with 2 mg/L of ATZ solution in amber Erlenmeyer flasks. The flasks were kept under continuous stirring at 140 rpm and 25 °C, during the period of time needed to attain the equilibrium. Samples were then taken, filtered and analyzed by UHPLC-DAD to determine the remaining ATZ concentration. The adsorption capacity of sorbents at equilibrium q_e (mg/g) was determined using Equation 1, where C_i is substituted by C_e (mg/L), equilibrium concentration of ATZ in solution.

Freundlich [37] and Sips [38] isotherm models (Equation 4 and 5, respectively) were applied to equilibrium results to evaluate adsorption behavior:

$$q_e = K_F . C_e^{\frac{1}{n}} \quad (4)$$

K_F represents the relative adsorption capacity [(mg/g) (L/mg)$^{1/n}$] and n is a constant (dimensionless) related with the degree of non-linearity of the equation.

$$q_e = \frac{q_{max} . K_S . C_e^{\frac{1}{m_S}}}{1 + K_S . C_e^{\frac{1}{m_S}}} \quad (5)$$

K_S is the affinity constant related to the adsorption energy (L/g) and m_s is a parameter related with the heterogeneity of the system.

3.2.5.3 Desorption behavior

Batch assays were performed using 0.75 g of sorbents in 50 mL of 2 mg/L ATZ solution (solid/liquid ratio of 15 g/L) at 25 °C, during the time needed to reach equilibrium for each material (56 h for sepiolite, 72 h for cork and 8 days for pine bark). Then, samples were taken, filtered and analyzed by UHPLC-DAD to quantify the ATZ remaining in solution. The suspensions were filtered, the materials were recovered and dried at 30 °C. ATZ desorption assessment was then conducted by using the following extraction solvents: acetate buffer at pH 5, potassium phosphate buffer at pH 7, aqueous solutions with 5 %, 20 % and 40 % (except for sepiolite) of acetonitrile at 25 °C and deionized water at 25 °C, 40 °C and 60 °C. In detail, sorbents loaded with ATZ were mixed with 50 ml of each extraction solvent (using 15 g/L of sorbent concentration) under continuous agitation at 140 rpm for 24 h. Liquid samples were collected at regular intervals, filtered and the efficiency of ATZ desorption was evaluated through UHPLC-DAD analysis as described previously. The desorption percentage, D (%) was calculated by using the Equation 6:

$$D(\%) = \frac{C_{des}}{C_{ads}} \times 100 \quad (6)$$

In here C_{des} is the concentration of desorbed ATZ along the time and C_{ads} the initial concentration of adsorbed ATZ on adsorbent.

3.2.6 Column adsorption experiments

Column adsorption using a PRB at lab scale in continuous flow to treat 2 mg/L aqueous solution of ATZ allowed to determine operational parameters as breakthrough and exhaustion time, useful for process design and upscale. Regeneration and reusability assays and PRB simulation with sepiolite, cork and pine bark as supports were performed in column systems at lab scale. Consecutive adsorption of ATZ from 2 mg/L solution and desorption processes (using the extraction solvent with the best desorption capacity obtained for each material) were carried out to establish the adsorption capacity profile of each material after several adsorption-desorption cycles. To assure the reproducibility of the results, each assay was performed in duplicate and the average of both assays is reported for each test. The experiments were conducted in the absence of light to avoid the photo degradation of the herbicide.

3.2.6.1 Regeneration and reusability

The viability of adsorbents depends on their effective reuse, so the evaluation of adsorbent recyclability becomes necessary. The operation of a column with a fixed barrier for cyclic adsorption/desorption processes is an effective technique for the purpose as it makes the best use of the concentration difference driving force for adsorption and a more efficient utilization of the adsorbent capacity. The repetitive adsorption-desorption cycles were performed to determine the loss in the uptake capacity of the adsorbents. 20 g of each material were placed inside an acrylic column (height: 30 cm, internal ø: 4 cm) and a predetermined volume of 2 mg/L ATZ solution (using a solid/liquid ratio 15 g/L) was added. The pollutant influent was introduced into the column from the bottom to the top in closed-loop mode at a controlled flow rate of 1 mL/min for 96 h, 72 h and 83 h for sepiolite, cork and pine bark, respectively. When equilibria were reached, columns operation stopped and the excess of ATZ solution inside the columns was withdrawn. The regeneration of the adsorbent by desorption processes initiated in continuous mode during 48 h with the selected extraction solvents, aqueous solution with 20 % of acetonitrile for sepiolite and 40 % of acetonitrile solution for cork and pine bark. Materials were regenerated twice and reused other 2 times. During adsorption and desorption cycles, samples were collected periodically from the outflow of the column to determine ATZ concentration by UHPLC. To assure the reproducibility of the results, each assay was performed in duplicate and the average of two assays was reported for each test.

3.2.6.2 PRB simulation at lab scale

The use of PRB in a continuous flow column is a practical and efficient technique to remove pollutants from real wastewater. The ability of the waste-based sepiolite, cork and pine bark to function as effective PRB for the removal of ATZ at 2 mg/L was evaluated in open system experiments (continuous flow) at laboratory scale. The continuous column experiments were operated using three individual acrylic columns (height: 30 cm, internal ø: 4 cm), filled each one with 20 g of each material. ATZ solution was fluxed upwards through the columns using a peristaltic pump at 1 mL/min for 76 h without recirculation. Periodically, samples were collected in the outflow to determine ATZ concentration by UHPLC until exhaustion time was reached. The assays were conducted in duplicate and the results are an average of duplicates. Breakthrough curves were obtained by plotting the normalized concentration values (C_t/C_0) versus time.

Adsorption dynamics acquaintance and modelling are essential because they provide valuable information on some practical aspects such as sorbent capacity and prediction of the time necessary for the effective operation of a PRB column. Among the different mathematical models that can be used to describe the removal behavior of a certain pollutant, the modified dose-response [39] (Equation 7) and Yoon-Nelson [40] (Equation 8) models were selected to predict breakthrough curves and to determine the characteristic parameters of the column useful for process design, with nonlinear regression fitting. In this model α is the parameter that is associated to the shape of the breakthrough curve. When $\alpha \leq 1$, the model displays a parabola-like curve and for $\alpha > 1$ the curve assumes a sigmoidal shape [17].

$$\frac{C}{C_0} = 1 - \frac{1}{1+(\frac{C_0.Q.t}{q_0.\mathrm{m_B}})^{\alpha}} \quad (7)$$

Q is the flow rate (mL/min), q_0 is the adsorption capacity of the bed (mg/g), m_B is the mass of the adsorbent (g) and α is a dimensionless empirical constant.

$$\frac{C}{C_0} = \frac{1}{1+\exp\left(k_{YN}.\tau - k_{YN}.t\right)} \quad (8)$$

k_{YN} (h^{-1}) is the Yoon-Nelson rate coefficient, τ (h) is the time required to retain 50 % of the initial adsorbate and t is the operating time (min).

3.2.7 Statistical analysis

GraphPad Prism® software (version 8.0; GraphPad Software, Inc., San Diego, CA, USA) was used for statistical analyses. The level of significance was determined by two-ways ANOVA followed by Tukey's test for multiple comparisons. Significance was accepted at $p < 0.05$.

3.3 RESULTS AND DISCUSSION

3.3.1 Characterization of the adsorbents/biosorbents

The pH_{zpc} is of fundamental importance in surface science applied to environmental rehabilitation since it allows to determine the ability of a substrate to retain the molecules of interest. This parameter

is relevant in adsorption processes since it influences the solution pH and the electrostatic interactions between adsorbate and adsorbent. This parameter is described as the pH at which the net charge of the adsorbent surface is equal to zero. At a pH lower than pH_{zpc}, the surface of the adsorbent is positively charged, favoring anions species to be adsorbed. On the opposite, when the pH is higher than pH_{zpc}, the surface of the adsorbent is negatively charged and cations will be preferentially adsorbed. In **Figure 7** the results obtained for pH_{zpc} determination by the pH drift method are shown. The pH_{zpc} is identified as the point where the line $pH_{initial} = pH_{final}$ crosses the experimental pH curve. Sepiolite presents a pH_{zpc} value around 8, while for cork and pine bark the obtained pH_{zpc} values are 3 and 5, respectively.

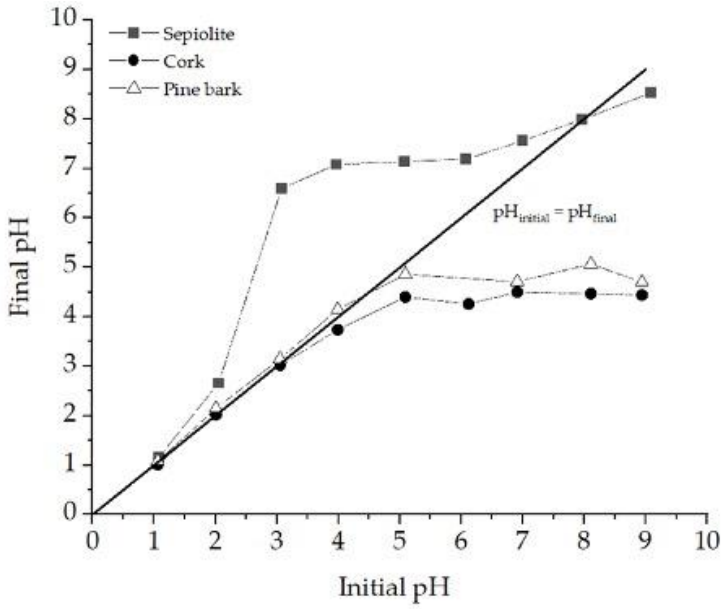


Figure 7. Determination of pH_{zpc} of sepiolite, cork and pine bark using the pH drift method.

ATZ is considered a very weak base with a pKa value of 1.68 and an isoelectric point of 9.23. Under the conditions of the study, the pH of ATZ solution at 2 mg/L has a value of 5.7. When ATZ molecules are in contact with the sorbent materials its pH changes because of the ionic interactions between adsorbate and adsorbent. **Figure 8** shows the charge distribution of ATZ at different pH. Increasing pH led to progressive deprotonation of the functional groups from the surface. ATZ molecules are in the neutral form in the range of pH between 6.5 and 11.5. ATZ is positively charged for pH values lower than 6.5 and negative charged at pH higher than 11.5.

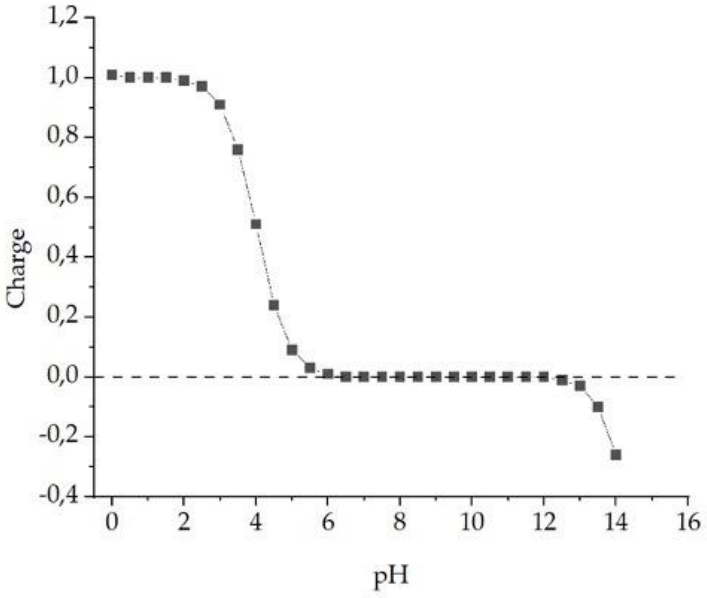


Figure 8. Charge distribution of ATZ as a function of pH (plot obtained using Marvin Sketch tool).

According to pH_{zpc} study, cork surface assumes a negative charge when in contact with ATZ solution, once the solution pH is higher than its pH_{zpc}. On the other hand, pine bark surface becomes more neutral, once solution pH is similar to its pH_{zpc} value. By ion-exchange mechanism, ATZ molecule is favorably adsorbed by cork. **Table 5** summarizes the charges distribution interactions of sorbents materials, when in contact with ATZ solution.

Table 5. Charges distribution of ATZ molecule and sorbents surface through solution pH

Waste material	pH variation	Atrazine molecule charge	Sorbents surface charge
Cork	5.7 - 5.39	+	-
Pine bark	5.7 - 4.62	+	Neutral/-
Sepiolite	5.7 - 9.2	Neutral/+	+

SEM has been used as a primary tool to characterize the surface morphology and the essential physical properties of the adsorbents, such as porosity and structure. Sepiolite, pine and cork (before adsorption experiments and after two regeneration cycles), as well as activated rubber char samples were analyzed by SEM (**Figure 9**). Raw sepiolite (**Figure 9A**) exhibits a micro-fibrous texture, with inter-fibers/bundles spaces. Its textural properties were reported in previous works by this research group:

surface area (S_{BET}) of 181.35 m^2/g, pore volume of 0.30 cm^3/g and average pore size of 65.8 Å [17]. **Figure 9C** shows a radial view of a ray of *Pinus pinaster* bark constituted of small pits. *Quercus suber* (**Figure 9E**) shows a porous surface formed from adjacent hollow polyhedral prismatic cells, similar to a honeycomb structure. Cork and pine bark present a very low specific surface area (< 4 m^2/g [17]). **Figure 9G** exhibits a particle of rubber char (dark area) activated with KOH (white area). This activated char has essentially mesoporous with high S_{BET} value and pore volume. For adsorbents with small micropores the adsorption mechanism is mainly chemical, once a stronger binding of the adsorbate to the adsorbent pore wall occurs. For larger molecules that cannot access to pores, this enhanced adsorption does not occur. As the pores increase in size, adsorption becomes continuously a physical (or multi-layer) process [33]. The morphology of adsorbent/biosorbent after sorbate second leaching was also observed in order to evaluate the effect of those processes on it. Sepiolite (**Figure 9B**) becomes more fibrous, with a rougher surface, meaning that the exposure to the extraction solvent (aqueous solution with 20 % of acetonitrile) may have worn out its surface, becoming more polished. For pine bark (**Figure 9D**) and cork waste (**Figure 9F**) differences in morphology were not observed.

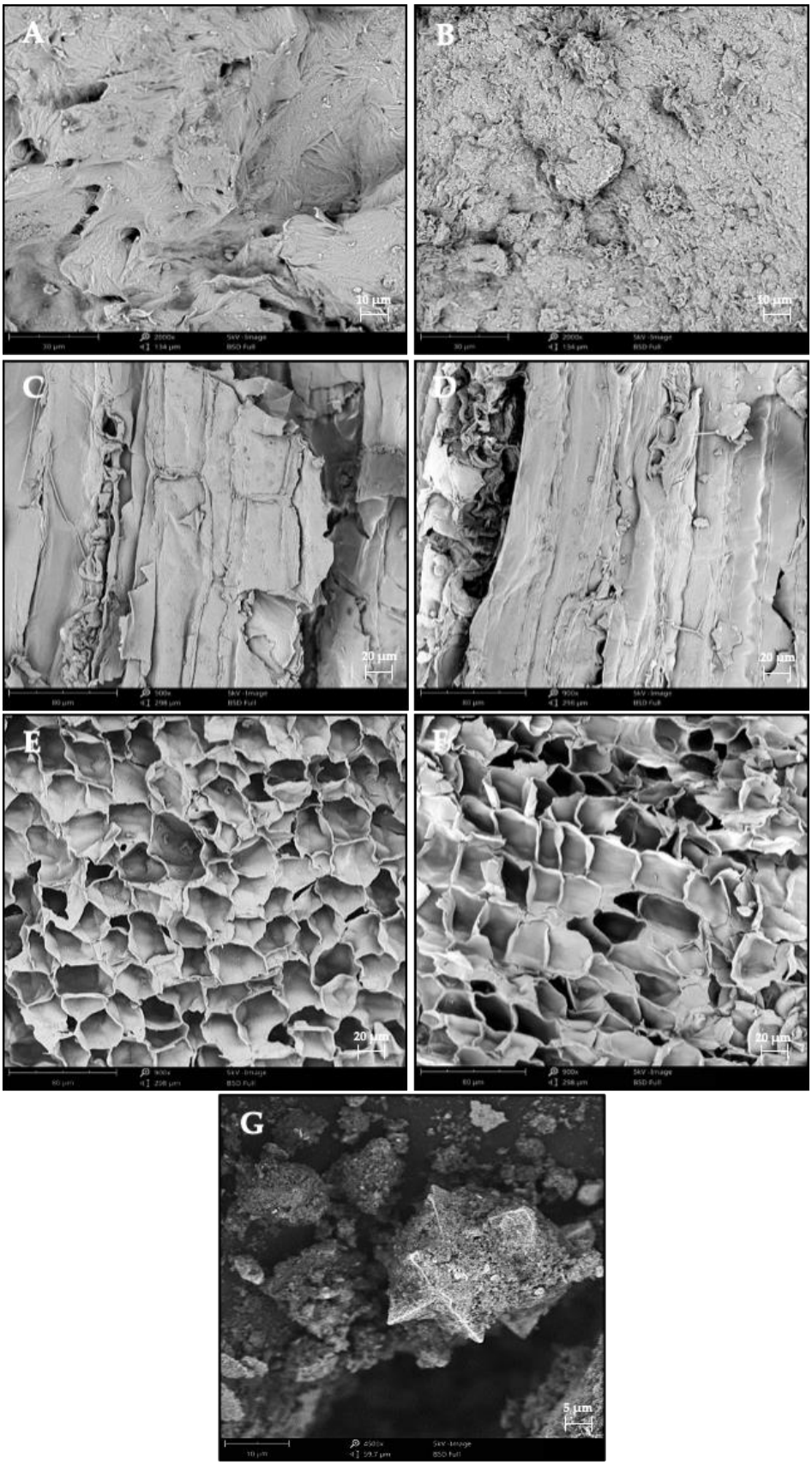


Figure 9. SEM micrographs at various magnifications of raw sepiolite (A), pine bark (C), cork (E), activated rubber char (G) and sepiolite (B), pine bark (D) and cork (F) after two regeneration cycles.

The ATR-FTIR spectra of sepiolite, cork and pine bark in their natural form, as well as after the first and second desorption steps, were recorded in the region of 400-4000 cm^{-1} and are shown in **Figure 10**, **Figure 11** and **Figure 12**, respectively. Analyses were performed to evaluate possible changes on surface functional groups after the regeneration process and therefore to clarify ATZ adsorption mechanism. In **Figure 10** the spectrum of raw sepiolite is shown and the characteristic stretching vibration of -OH groups attached to octahedral Mg^{2+} located in the interior blocks are evidenced, at 3690 cm^{-1}. At 3581 cm^{-1}, the Si–O band is verified. From 3000 to 4000 cm^{-1} the broad band is due to H–O–H vibrations. In addition, the band at 1658 cm^{-1} is related to the –OH bond and at 1197 cm^{-1}, as well as at 940 cm^{-1}, vibrations can be attributed to the Si–O combination. The 1024 cm^{-1} band corresponds to the Si–O–Si vibrations, the one at 450 cm^{-1} to the Si–O–Mg link and at 653 cm^{-1} there is the bending vibration of Mg-OH. Sepiolite impurities derived from dolomite are also detected at 1443 cm^{-1} [41,42]. Comparing the bands between the spectra of the raw sepiolite with the ones after exposure to the desorbing solvent, for both leaching steps, no substantial differences were detected (the 1443 cm^{-1} band from dolomite is the one that does not appear). So, it can be considered that the chemical structure of sepiolite is not affected after 2 regeneration cycles. Natural cork spectra (**Figure 11**) display several absorption peaks, indicating the complex nature of this biomaterial. The band at 3400 cm^{-1} represent –OH groups and the bands observed at 2923 cm^{-1} to 2853 cm^{-1} could be assigned to the C–H stretch. The peaks around 1760 cm^{-1} and 1638 cm^{-1} correspond to the C=O stretching. The absorption peak resulted from C–O bond is observed at 1036 cm^{-1} [22]. The principal constituents of Portuguese *Quercus suber L.* can be identified in this cork spectra as suberin, at 2923, 2853 and 1760 cm^{-1}, guaiacyl-type lignin at 1513, 858, and 816 cm^{-1}, polysaccharides at 1100 and 1036 cm^{-1} and extractives at 1603 and from 1457 to 1300 cm^{-1} [43]. Comparing the bands between the spectra of the cork in its natural form and after two desorption processes, the initial peaks are still observed, meaning that cork material after 2 regeneration cycles remains chemically unalterable, not affected by the desorption processes. As it can be seen in **Figure 12** raw *Pinus pinaster* bark spectra evidence at 3400 cm^{-1} the stretching vibration of the bond between oxygen and hydrogen. The bands at 2920 cm^{-1} and 1450 cm^{-1} can be assigned to the stretching of the C-H bond in the aromatic and aliphatic structures. The bands at 1610 cm^{-1} and 1510 cm^{-1} are attributed to the aromatic C-C skeletal vibrations and the band around 1733 cm^{-1} results from the stretching vibrations in C-O carbonyl structure. At 1160 cm^{-1} and 1210 cm^{-1} the stretching of C-O-C in the cellulose and hemicellulose is detected [44,45]. The phenolic –OH and aliphatic –CH stretch vibrations can be identified at 1364 cm^{-1}. Vibrations of –CO and aromatic –CH are detected at 1100 cm^{-1}. The 860, 820 and 773 cm^{-1} bands indicate the –CH bending vibrations from aromatic rings, related to phenolic

compounds [46]. In this spectrum the distinct signal at 1036 cm^{-1} is identified, produced by the aliphatic C–O stretching [44]. When comparing raw pine spectra with the ones obtained after two desorption steps, no substantial differences occur as it happens with the other materials.

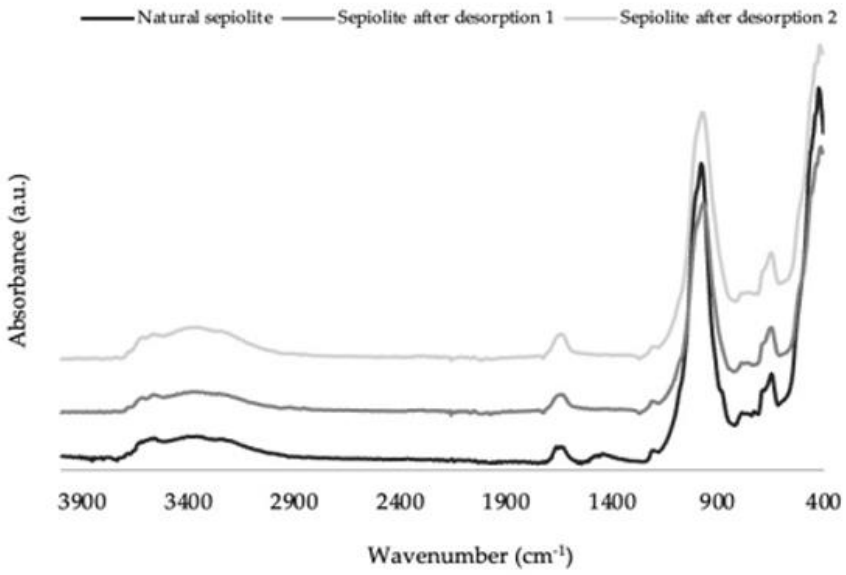


Figure 10. ATR-FTIR spectra of natural sepiolite and after 2 cycles of adsorption-desorption with a solution with 20 % of acetonitrile.

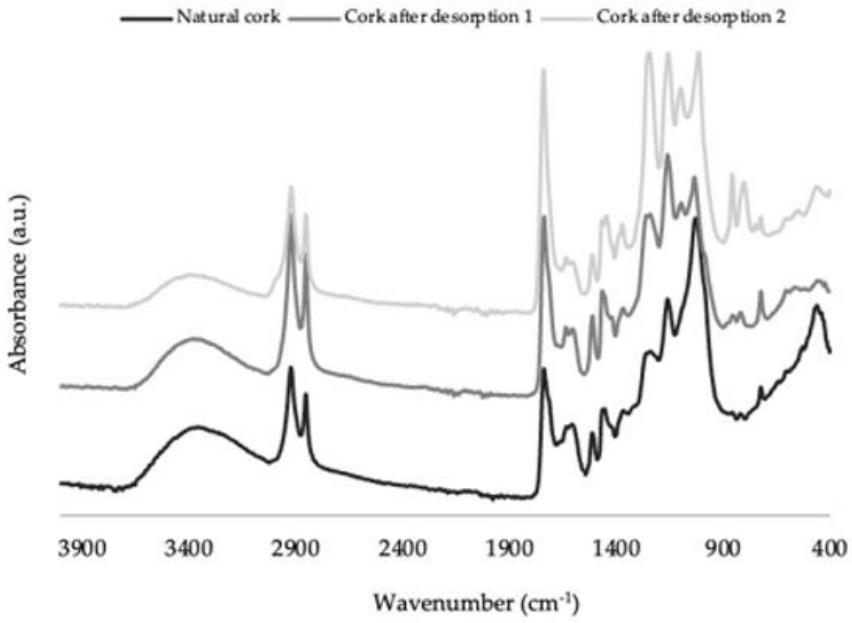


Figure 11. ATR-FTIR spectra of natural cork waste and after 2 cycles of adsorption-desorption with a solution with 40 % of acetonitrile.

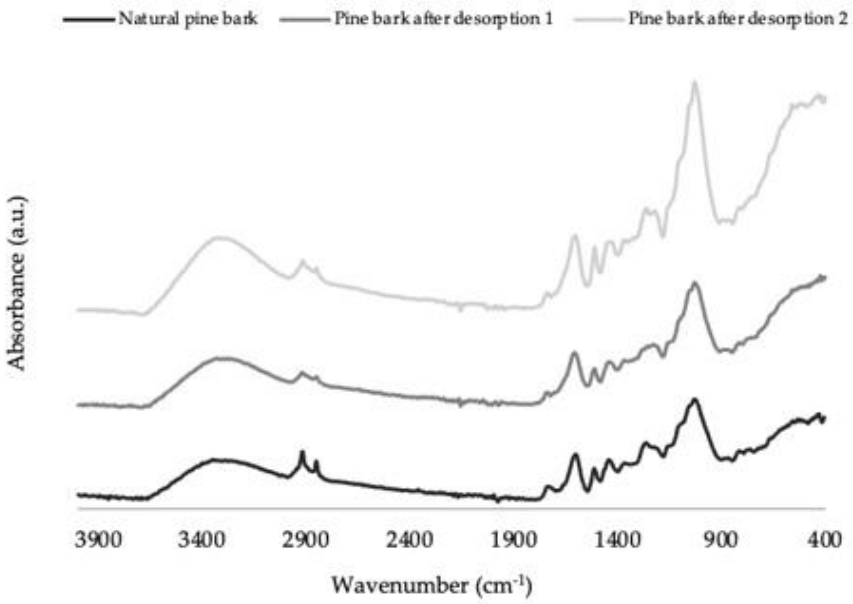


Figure 12. ATR-FTIR spectra of natural pine bark waste and after 2 cycles of adsorption-desorption with a solution with 40 % of acetonitrile.

3.3.2 Adsorption kinetics modelling

The assessment of adsorption kinetics can support the definition of the adsorption pathway and probable mechanism involved. **Figure 13** shows the experimental data and the fitting kinetics models for the adsorption of ATZ (at 2 mg/L) onto the waste biomaterials/materials. ATZ concentration measured on blank test remained unchanged during the experiment. Equilibrium is reached after 56 h for sepiolite, 72 h for cork and 8 days for pine bark, while for the powder activated rubber char this stage is attained after 14 h. The percentages of ATZ adsorbed at equilibrium time were approximately 22 %, 36 % and 50 % for sepiolite, cork and pine bark at 5 g/L, respectively, and 100 % for activated rubber char at 1 g/L. Results indicate that the adsorption of ATZ is initially fast (due to the availability of adsorption sites) and after a period of time the retention becomes slower until equilibrium is reached. The most common kinetics models used to explain the sorption processes were used to evaluate the kinetic parameters, as PFO and PSO. For both models the experimental q_e values are in agreement with the calculated ones. Although both models fit experimental data with high correlation coefficient values (R^2), the PSO model showed the best fit for the generality of the sorbents. Comparing the k_2 value (**Table 6**) for the studied materials, the adsorption rate of ATZ follows this sequence: sepiolite > cork > rubber char > pine bark. The waste-based materials without pretreatment, sepiolite, cork and pine bark have the capacity to retain effectively ATZ, in their natural form without resort to chemical pretreatments.

The development of materials with high carbon content as the rubber char, with mesoporosity, is important to achieve higher adsorption capacities, as well as faster adsorption kinetics. Besides that, the particle size of an adsorbent also plays a significant role in adsorption capacity and rate. This rate tends to increase when particle size decreases by increasing mesopore volume and S_{BET} [47] and reducing interior diffusional rate limitations. In this study, the rubber char particle has a substantial smaller size than the other materials and is, in fact, the one that achieves an equilibrium stage faster and a higher uptake value, suggesting that ATZ can be easily adsorbed on the outer surface of the material [48].

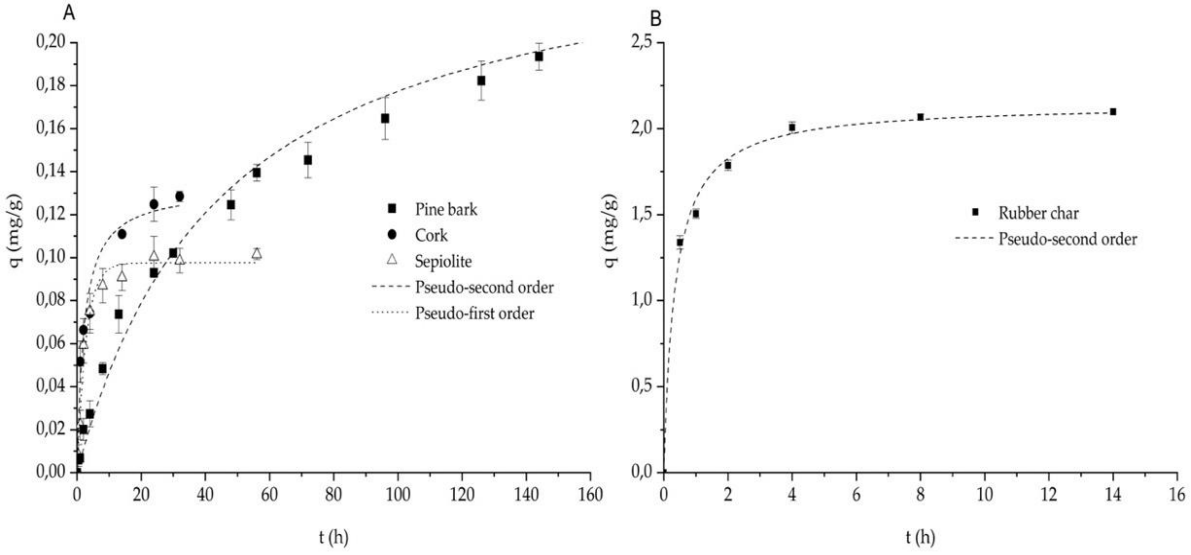


Figure 13. Adsorption of ATZ along time and fittings by the PFO and PSO kinetic models: (A) pine bark, cork and sepiolite; (B) activated rubber char. Experimental conditions: C_{ATZ}= 2 mg/L, biosorbent/adsorbent dose (A) 5 g/L, (B) 1 g/L.

Table 6. PFO and PSO model fittings for the adsorption of ATZ onto the different wastes

Waste	PFO			PSO		
	q_e	k_1	R^2	q_e	k_2	R^2
Cork	0.351 ± 0.074	0.120 ± 0.007	0.932	0.133 ± 0.006	3.514 ± 0.817	0.973
Pine bark	0.208 ±0.009	0.021 ±0.003	0.972	0.257 ± 0.011	0.086 ± 0.014	0.986
Sepiolite	0.098 ± 0.003	0.354 ± 0.043	0.977	0.108 ± 0.005	3.976 ± 0.884	0.970
Rubber char	2.000 ± 0.070	1.760 ± 0.285	0.968	2.143 ± 0.039	1.343 ± 0.167	0.994

q_e - adsorption capacity at equilibrium (mg/g); k_1 constant of the PFO model (min^{-1}); k_2- constant of the PSO model (g/(mg.min); R^2- coefficient of correlation.

3.3.3 Adsorption equilibrium modelling

Adsorption isotherms are useful to establish the adsorption capacity of sorbents and to describe their interaction with the adsorbate. In this study the relation between q_e and C_e was fitted by several different isotherms for water and wastewater treatment, being presented in **Table 7** just the best fitting ones for the selected adsorbents/biosorbents, as Freundlich and three-parameter Langmuir–Freundlich (Sips). The nonlinear regression was used in this analysis to avoid the errors associated to the commonly used linearized models. **Figure 14** shows the results obtained from the equilibrium adsorption experiments fitted with the isotherm models. According to **Table 7** the Freundlich model is the one that best fits the isothermal adsorption onto cork, sepiolite and activated rubber char, while the Sips model is the one that fits more adequately the isothermal adsorption onto pine bark.

Freundlich model is suitable for heterogeneous adsorbent surfaces and can be applied to non-ideal or multilayer adsorption by surfaces of varied affinities. n_F and K_F are Freundlich constants which denote the adsorption intensity and capacity, respectively. When n_F <1 the adsorption process is chemical, but if n_F > 1 adsorption becomes a favorable physical process [48]. According to **Table 7** sepiolite and rubber char present n_F > 1 suggesting a physical adsorption process, while cork (with n_F < 1) retains ATZ chemically. The highest K_F value was attained for rubber char, meaning its higher adsorption capacity. Sips model results from the combination of Langmuir and Freundlich and predicts a heterogeneous adsorption system, thereby avoiding the limitations associated with the increased adsorbate

concentration. When the concentration of the adsorbate is low, the model reduces to Freundlich isotherm, while for high concentrations, it predicts a monolayer adsorption process on a homogeneous surface, Langmuir model [49]. The m_s parameter *gives* information about the degree of surface non-homogeneity. Heterogeneous adsorbents present m_s values close to 0, while m_s values closer to 1 indicate a material with relatively homogeneous binding sites [50]. Pine bark presents a m_s value closer to 1, suggesting that its binding sites have the same adsorption affinity, homogeneously distributed over its surface. In terms of maximum adsorption capacities, q_{max}, activated rubber char is the one that presents the highest value, followed by cork, sepiolite and pine bark, sequentially. Rubber char is the adsorbent with the highest ATZ adsorption capacity since has enhanced physical properties as described in the previous section (higher surface area and micropore volume). It is reported that adsorbents/biosorbents without any kind of physical or chemical treatment normally present lower adsorption capacities when compared with the commercial/synthetic ones [2]. Among the tested biomaterials/materials without pretreatment, cork was the one that showed the best performance in terms of ATZ retention, 0.456 ± 0.034 mg/g. Adsorption of ATZ on cork has a strong concurrency with adsorption of water on this biomaterial. On cork, water is firstly adsorbed on hydrophilic sites constituted by hydroxyl and methoxyl groups and then water adsorption continues by clusters formation around the hydrophilic sites. Water adsorption isotherms in cork present the same profile as ATZ [51,52]. Statistical analysis shows no significant differences between biowastes/wastes performance. Comparing the adsorption capacity of biowastes/wastes (without pretreatment) with activated rubber char, a significant difference was found ($p < 0.0001$). The adsorption capacity of these wastes can be improved through surface modification treatments, which results in a higher affinity for the target pollutant. The methods used to modify the materials can include physical, chemical and biological methods.

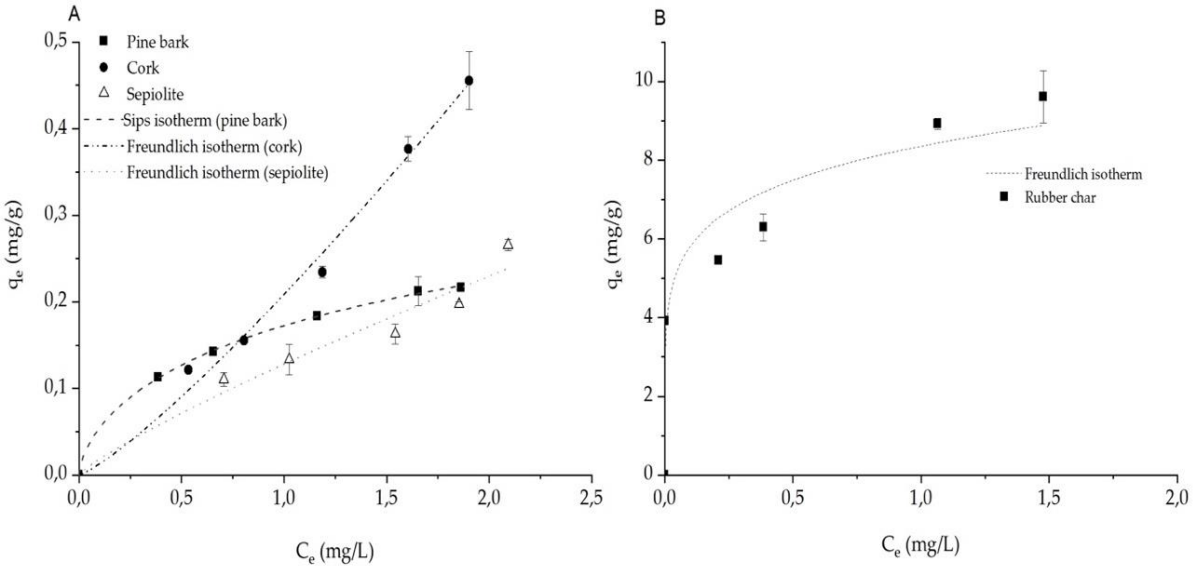


Figure 14. Adsorption of ATZ together with the fittings by the Freundlich and Sips models: (A) pine, cork and sepiolite; (B) activated rubber char. Experimental conditions: C_{ATZ}= 2 mg/L, biosorbent/adsorbent dose in the range (A) 1–15g/L and (B) 0.05–0.75g/L.

Table 7. Isotherm parameters obtained from the fitting to experimental results on the adsorption of ATZ onto the different wastes

Waste	**Sips**			
	q_{max}	K_S	R^2	m_S
Pine bark	0.477 ± 0.177	0.567 ± 0.338	0.999	0.64 ± 0.132
Waste	**Freundlich**			
	K_F	n_F	R^2	
Cork	0.209 ± 0.010	0.832 ± 0.062	0.999	
Sepiolite	0.128 ± 0.013	1.880 ± 0.246	0.946	
Rubber char	8.356 ± 0.548	6.344 ± 1.807	0.924	

3.3.4 Desorption evaluation

The selection of a suitable desorption eluent, with high affinity for the sorbate and able to remove it from the surface of the sorbent, without substantially changing its morphology and efficiency while competitive and environment friendly is seen as challenge in the field of biosorption [20].

Desorption of ATZ from sepiolite, cork and pine bark was evaluated, aiming their regeneration/recycling. After one adsorption step, each material loaded with ATZ was placed in contact with different leaching agents. The increase of ATZ concentration in the solution was measured over time during 24 h. **Figure 15** shows the ATZ desorption efficiency for waste biomaterials/materials using different eluents and different operation conditions (temperature and percentage of acetonitrile in aqueous solution). After 24 h of operation, around 100 % of ATZ was recovered from sepiolite using an aqueous solution with 20 % of acetonitrile, while for cork and pine bark, recoveries of 94 % and 98 %, respectively, were attained using an aqueous solution with 40 % of acetonitrile. These results evidence the reversible character of ATZ adsorption onto the materials. An increase of acetonitrile content was needed in the case of cork and pine bark to reach similar desorption values to those attained for sepiolite.

An organic solvent like acetonitrile increases ATZ solubility in water, justifying its selection for this desorption study. The use of methanol as the most adequate extraction solvent for ATZ desorption from synthetic polymers was demonstrated in a recent work, achieving 73.5 % as maximum ATZ desorption percentage [53]. Still in this study, it was concluded that higher efficiency values are reached for all materials when using a lower content of organic solvent, which minimizes the environmental impact of this process. Thus, ATZ recovery from these biomaterials/materials can be easily and successfully obtained through extraction with aqueous acetonitrile solutions, which is a rapidly and highly biodegradable solvent.

For sepiolite, a considerable higher desorption efficiency was reached using water at 60 °C, in comparison to the recoveries attained at 25 °C and 40 °C. These results confirm the reversible nature of the exothermic adsorption process. However, this tendency was not so evident for cork and pine bark as observed for sepiolite.

The desorption efficiency increased for all materials with the increase of the pH of the buffer from 5 to 7. With pH 5 buffer as acidic solvent, ATZ desorption reached 30 %, 20 % and 5 % of the original load from sepiolite, cork and pine bark, respectively. Statistical analysis of desorption data is expressed in **Figure 15**. In the case of sepiolite all the extraction solvents used for desorption differ from each other

($p < 0.05$). For pine bark and cork the 40 % of acetonitrile aqueous solution revealed to be the one with the highest difference compared to the other studied solvents ($p < 0.001$).

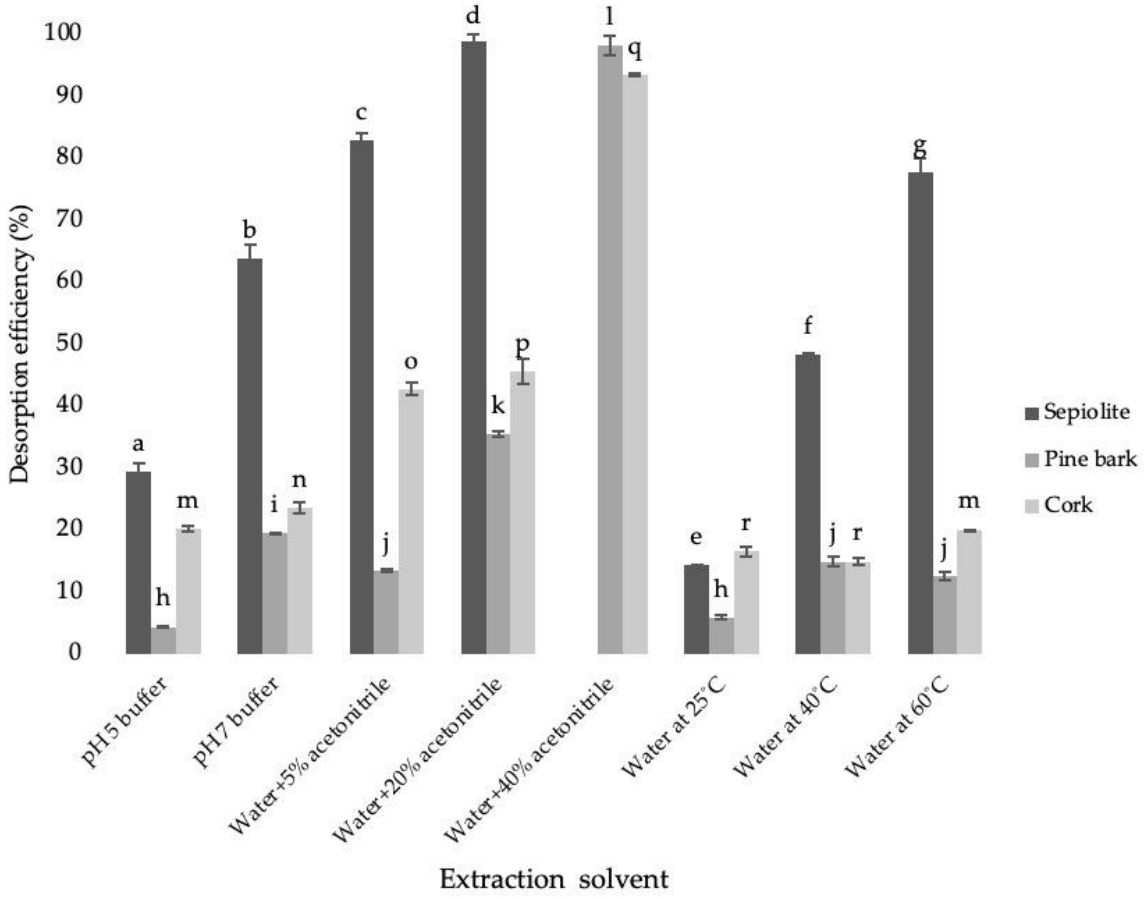


Figure 15. ATZ desorption efficiency for sepiolite, pine bark and cork, using different solvents and operation conditions, after 24 h of experiment. Different letters show significant differences ($p < 0.05$) between extraction solvents for the same waste biomaterial/material. Sepiolite (a-g), pine bark (h-j) and cork (m-r).

3.3.5 PRB breakthrough modelling

Assays with continuous flow in fixed-bed columns were carried out as a simulation of an up-scale PRB to evaluate the dynamic removal of ATZ. **Figure 16** shows the laboratory set-up using sepiolite, pine bark and cork as PRB. The overall performance of fixed-bed columns and some operational parameters such as breakthrough time, saturation time, adsorption capacity and breakthrough curve shape were evaluated. These parameters can be determined by plotting the experimental results in normalized concentration, C/C_0 over time, t. **Figure 17** shows the breakthrough curves obtained with

the column set-up experiments with a fixed mass of 20 g of cork, pine bark or sepiolite, when exposed to a continuous flow of a solution with 2 mg/L of ATZ.

Mathematical modelling allows the up-scale design and optimized operation, avoiding unnecessary investments and high operational costs by eventual underutilization or oversaturation of beds. Yoon-Nelson and modified dose-response model were applied to describe the fixed-bed column behavior and the fitting results are shown in **Table 8**. The modified dose-response was the model that best fitted experimental data ($R^2 > 0.96$ for all the materials), represented in **Figure 17**, suggesting its suitability to be used for the scale-up purpose. The bed adsorption capacity, q_0, is a critical indicator of its performance and may be calculated from the breakthrough curve. According to the modified dose-response model, the higher adsorption capacity was attained for sepiolite (23.3 ± 0.8 mg/g), followed by pine bark (14.8 ± 0.6 mg/g) and cork (13.0 ± 0.9 mg/g). Using the Yoon-Nelson model it was possible to predict the time required to retain 50 % of the initial ATZ concentration (τ). Sepiolite achieved the highest value of τ, followed by pine bark and cork, which is in agreement with the adsorption capacity performance predicted by the modified dose-response model. Sepiolite showed the best adsorption performance and, for being the densest material, a smaller bed length is occupied, which becomes an advantage in terms of experimental operation. Continuous treatments become more feasible than batch processes, allowing an intra-particle diffusion of a bigger amount of ATZ molecules into the pores of the materials. Although batch processes are often used in adsorption studies, the collected data from those experiments are not necessarily applicable to a continuous adsorptive system. Continuous adsorption studies with PRB columns, at lab and pilot scale, are still needed to give real information for a trustful scale up.



Figure 16. Fixed-bed columns using sepiolite, pine bark and cork as PRB.

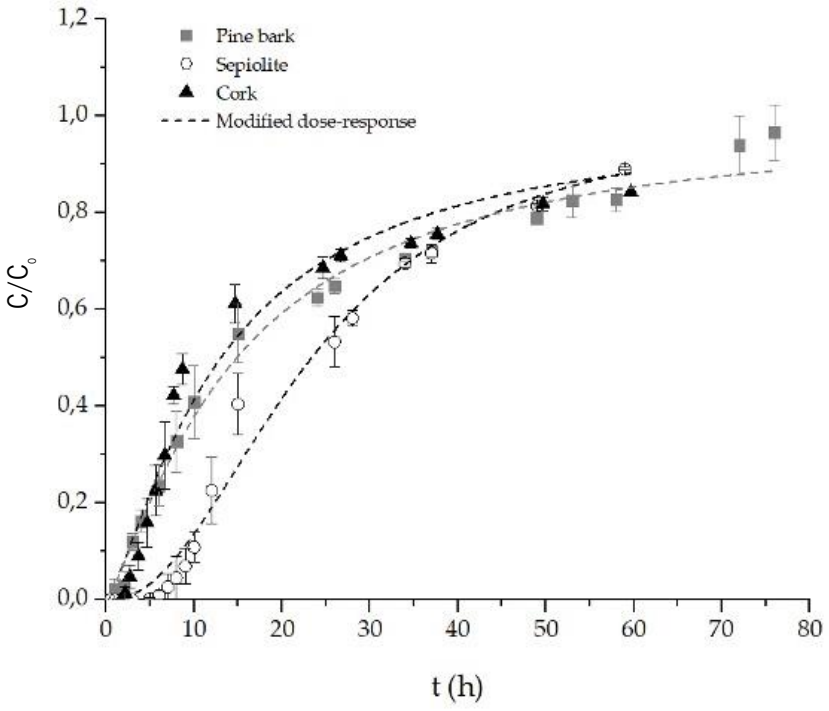


Figure 17. Breakthrough curves of pine bark, sepiolite and cork in fixed-bed columns (flow rate = 1 mL/min).

Table 8. Breakthrough parameters obtained from the fitting models for each biosorbent/adsorbent

	Yoon-Nelson			Modified dose-response		
	K_{YN} (h^{-1})	τ (h)	R^2	α	q_o (mg/g)	R^2
Pine bark	0.078 ± 0.011	21.6 ± 2.0	0.902	1.26 ± 0.06	14.8 ± 0.6	0.989
Cork	0.093 ± 0.018	18.2 ± 2.3	0.816	1.32 ± 0.11	13.0 ± 0.9	0.961
Sepiolite	0.118 ± 0.013	26.5 ± 1.3	0.940	2.17 ± 0.13	23.3 ± 0.8	0.984

3.3.6 ATZ retention and regeneration/recycling

After the selection of the best desorbing eluent, it is essential to evaluate the number of cycles of adsorption/regeneration that selected materials can cope, as they may reduce the overall operational costs. These processes can be performed either in batch or continuous procedures, however when the pollutant retention is conducted in PRB columns, it is better to perform the regeneration step in continuous mode [20]. When the PRB column becomes saturated, the adsorption process is stopped and switched to the desorption/regeneration step. In order to assess the recycling potential of sepiolite, pine bark and cork, successive cycles of adsorption and desorption were performed. Experiments were conducted in columns with the materials placed as PRB. Based on the results of section 3.3.4, aqueous solutions of 20 % of acetonitrile (for sepiolite) and of 40 % of acetonitrile (for pine bark and cork) were used for sorbents regeneration. The assays were initiated by 1 step of adsorption from 2 mg/L of ATZ solution in close-loop (until equilibrium was reached for each material) and then followed by one desorption step in continuous mode during 48 h. The equilibrium of the adsorption process using sepiolite as PRB was reached in 96 h, with pine in 82 h and with cork in 48 h. Three adsorption and two desorption steps were performed and the values of ATZ removal (%) and respective standard deviation (SD) for each step and each material are presented in **Table 9**. The percentage of ATZ removal after the adsorption processes is calculated based on the difference between the influent ATZ concentration and its outflow concentration, at equilibrium stage. Additionally, ATZ desorption is calculated based on the concentration of ATZ that was retained in the PRB column on each process and its concentration in the desorbing eluent at the end of 48 h of desorption. **Figure 18**, **Figure 20** and **Figure 22** show the output concentrations (C_{out}) profile of ATZ over time during adsorption using sepiolite, pine bark and cork in PRB, respectively. **Figure 19**, **Figure 21** and **Figure 23** exhibit the concentration profile of ATZ during desorption on the outflow of

the PRB of sepiolite, pine bark and cork, respectively. The results presented on **Table 9** show that desorption was performed effectively after the two cycles for sepiolite and pine bark, with performance percentages varying from 85 % to 96 %. Cork achieved 73 % and 55 % of desorption capacity after the first and after the second process, respectively. The results clearly show that the use of the selected desorption solvents, besides promoting an effective extraction of the retained pollutant, also promote, in a general way, an improvement of the adsorption capacity during the second adsorption step. After 2 regeneration cycles, removal percentages increased 30 % for sepiolite, 15 % for pine bark and remained practically unchanged for cork. This can be explained by the incomplete desorption of ATZ from cork after 48 h, remaining some adsorption sites unavailable for the second adsorption step. Sepiolite presented the best performance in terms of desorption efficiency, as well as good adsorption stability after regeneration, with the advantage of a smaller environmental impact of the selected elution solvent.

Previous studies demonstrated the performance of different solvents on the regeneration of adsorbent materials. Mashile *et al.* [54] demonstrated the efficient use of methanol (up to 95 %) to desorb methylparaben and propylparaben and to regenerate waste tire activated carbon-chitosan composite without affecting adsorption capacity. García *et al.* [55] selected pure acetonitrile as organic desorption agent to regenerate spent biochar, almost achieving an complete desorption of the pollutants, 96.8 % and 98.0 %, for methyl paraben and sulfamethoxazole, respectively. In the present study high desorption capacities are also reached but lower environmental impact solvents are used while maintaining the adsorption capacity of the materials. On this basis, it is possible to conclude that sepiolite, cork and pine bark can be regenerated and reused at least three times, without any loss in their adsorption efficiency, while acquiring an added value and favoring circular economy.

Table 9. ATZ removal for 3 sorbents after the adsorption and desorption cycles

Process	Sepiolite	Pine	Cork
	ATZ removal (%)	ATZ removal (%)	ATZ removal (%)
1st adsorption	51.8 ± 0.7	62.5 ± 1.1	55.7 ± 2.6
1st desorption	84.8 ± 2.9	95.5 ± 0.6	72.9 ± 1.0
2nd adsorption	82.6 ± 2.2	67.9 ± 1.3	56.4 ± 2.0
2nd desorption	96.1 ± 0.9	96.2 ± 0.3	55.3 ± 2.7
3rd adsorption	81.3 ± 1.2	77.8 ± 0.1	54.38 ± 0.3

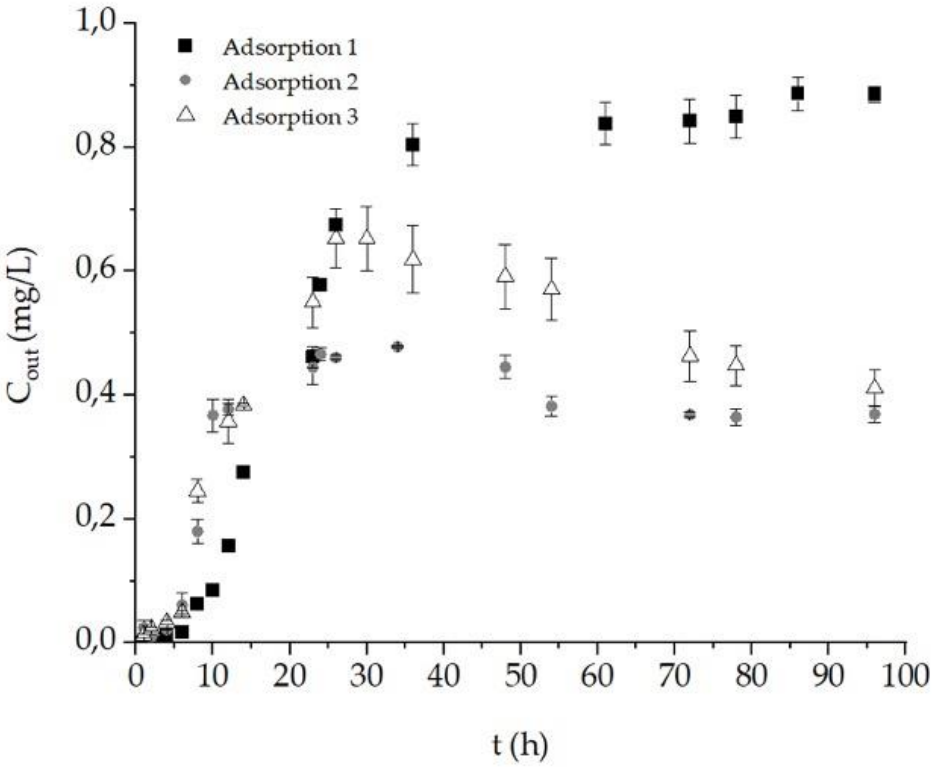


Figure 18. Concentration profiles of the outflow during 3 consecutive ATZ adsorption steps over time, using sepiolite as PRB.

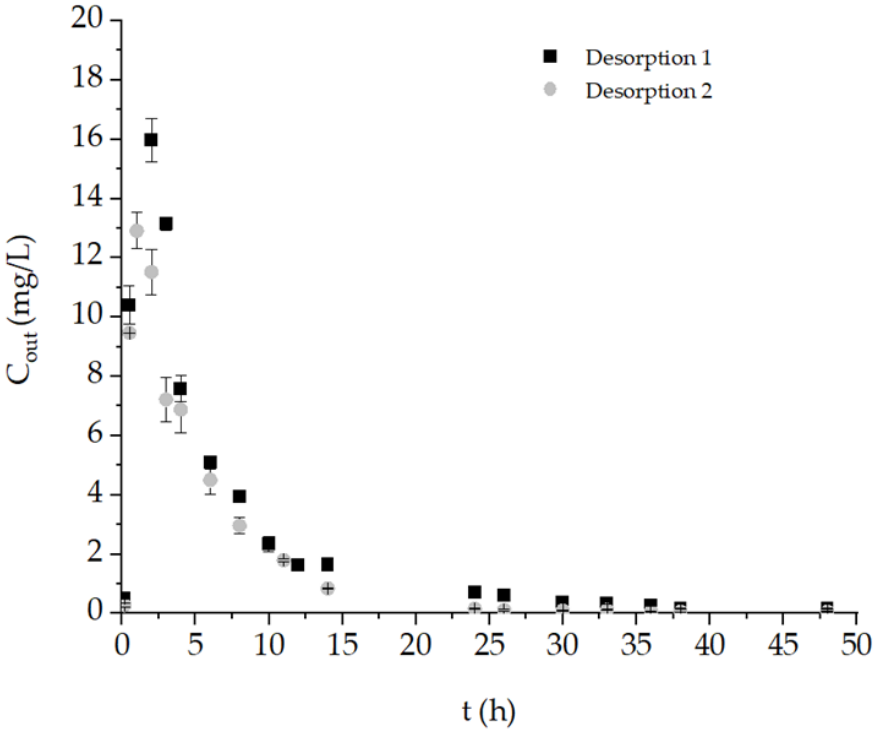


Figure 19. Concentration profiles of the outflow during 2 consecutive ATZ desorption steps over time, using sepiolite as PBR and 20 % of acetonitrile as extraction solvent.

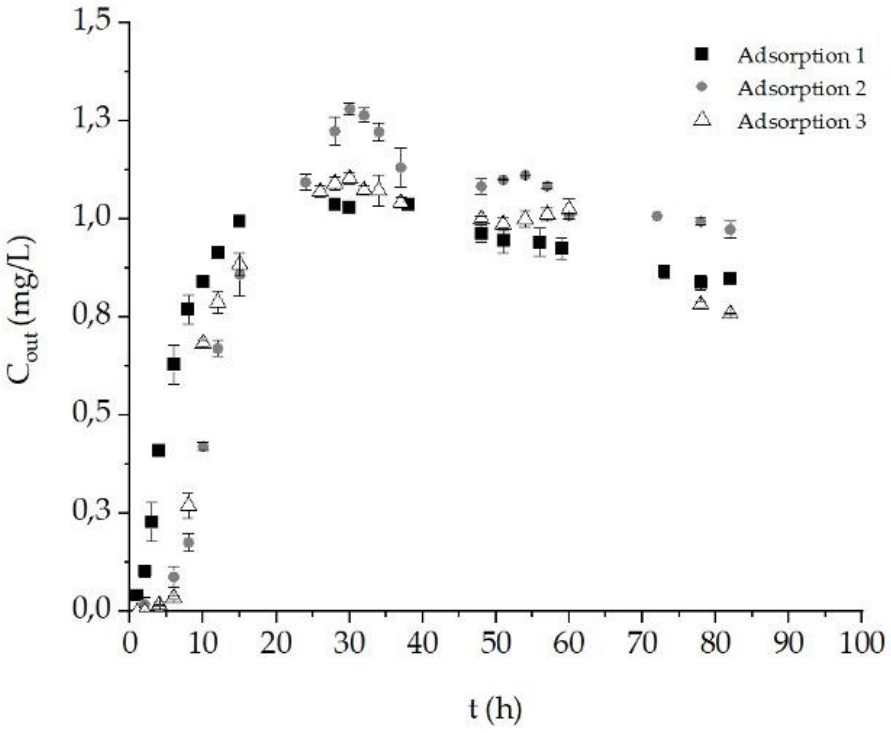


Figure 20. Concentration profiles of the outflow during 3 consecutive ATZ adsorption steps over time, using pine bark as PRB.

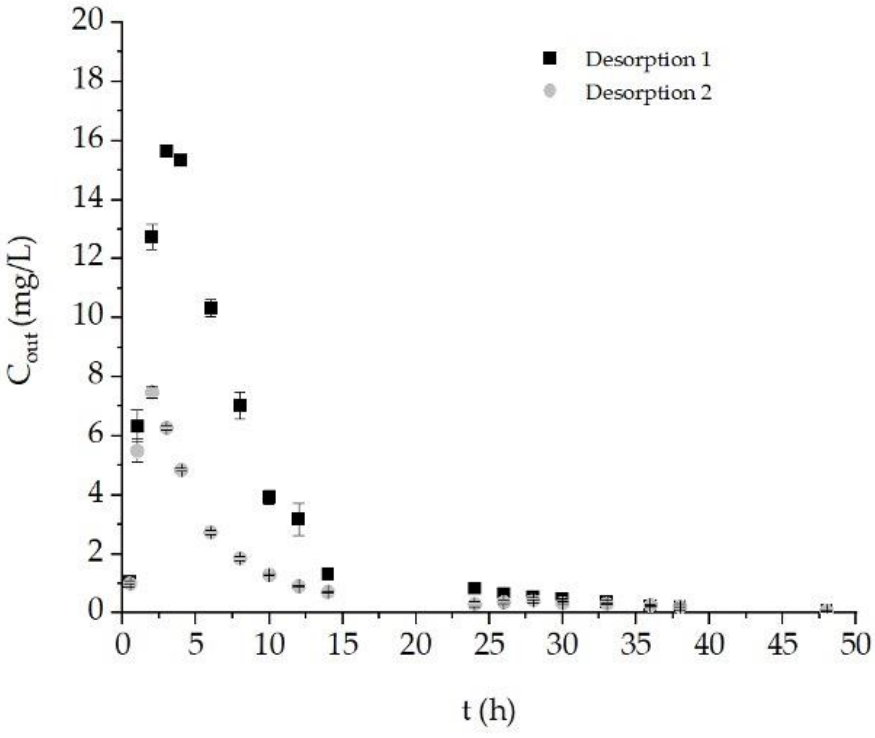


Figure 21. Concentration profiles of the outflow during 2 consecutive ATZ desorption steps over time, using pine bark as PBR and 40 % of acetonitrile as extraction solvent.

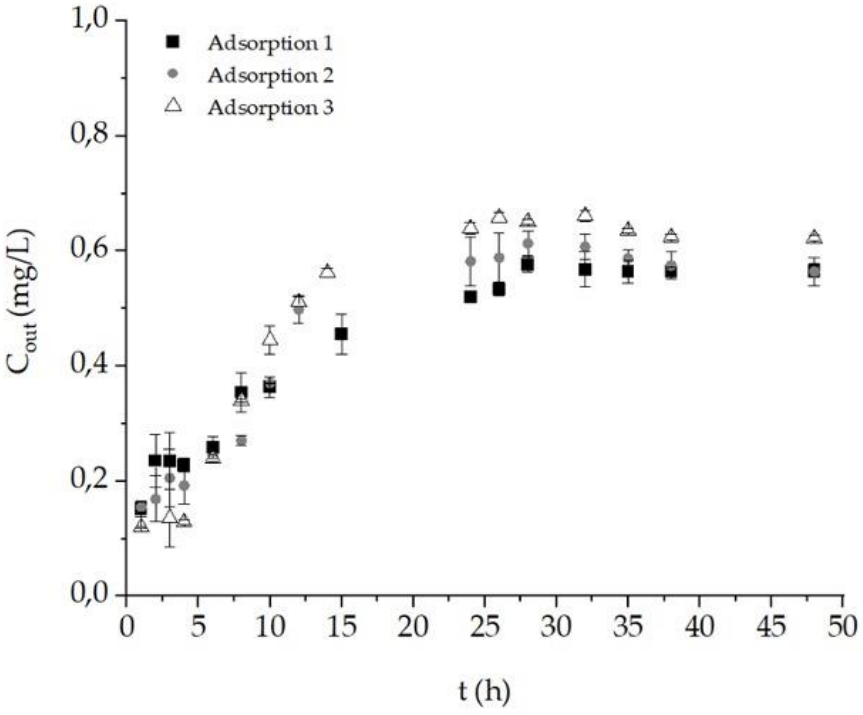


Figure 22. Concentration profiles of the outflow during 3 consecutive ATZ adsorption steps over time, using cork as PRB.

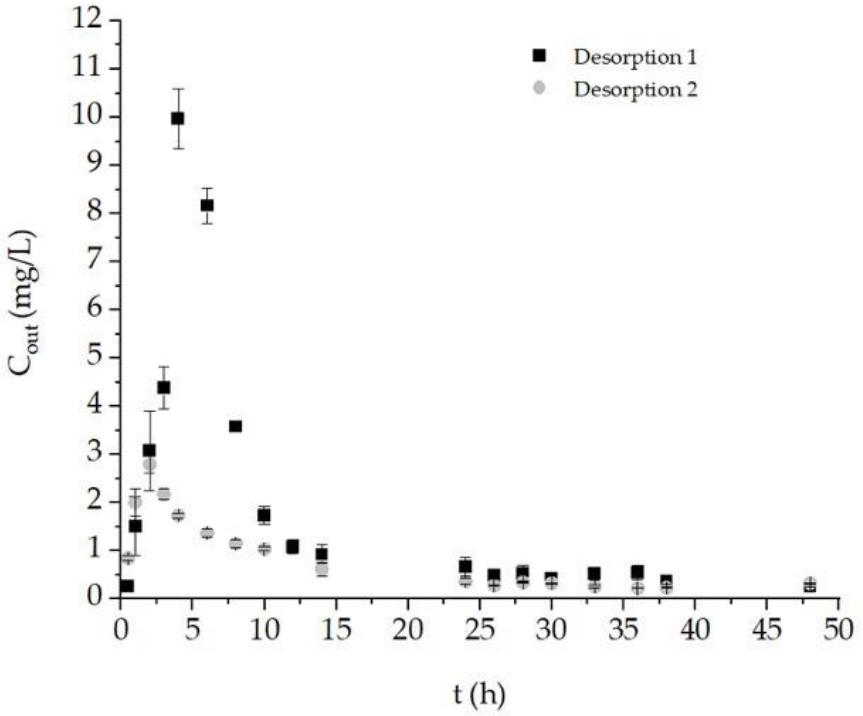


Figure 23. Concentration profiles of the outflow during 2 consecutive ATZ desorption steps over time, using cork as PBR and 40 % of acetonitrile as extraction solvent.

3.3.7 Sustainability assessment and lifecycle perspective

It is common knowledge that AC is the most adopted adsorbent in the field of wastewater treatment since it has the capability to retain efficiently a wide range of pollutants. However, its widespread use becomes often disadvantageous due to its high production cost (unbearable to underdeveloped countries), difficult regeneration and end-of-life disposal options [58]. Besides, the use of AC induces high environmental impacts as ecotoxicity, acidification, ozone depletion and fossil fuel depletion [57]. Biosorbents have been recognized as an environmentally sustainable solution for wastewater treatment. Besides their capacity to retain different pollutants and being available on a sustainable basis, their low-cost, biodegradability and reduced toxicity character are important advantages to consider. The use of waste-based biomaterials/materials contributes to an environmental sustainable growth, since it reduces wastes, as well as the recovery and reuse of materials [2]. The reuse of residues allows the increase of their life cycle and the decrease of energy requirements, resources and emissions [58]. Besides, it allows to reduce the waste disposal problem and the costs for producing new adsorbents for water treatment [20]. Through waste valorization a circular and sustainable economy is promoted, a prevailing priority in the EU for the continuous decrease of the negative environmental effects of services and products throughout their life cycle [59]. Bioeconomy is meant to reduce the dependency on natural resources by converting biomass and organic wastes into diversified end-products and materials and providing competitive goods in an environment friendly way [60]. This study is focused on the recycling of biological and natural resources wastes which is in line with the expected environmental policies. The sorbent materials, sepiolite, cork and pine bark were tested without any previous chemical treatment, with reduced waste generation (only the one produced by milling and washing steps) so they are good choices to be used as sorbents. Besides of being non-toxic materials, they are selective towards the target pollutant, are easy to operate in PRB due to their particle size and are regenerable and reusable. Exhausted sorbents can be also regenerated by successive adsorption/desorption cycles using acetonitrile aqueous solutions, which allows the further transference of ATZ into new solutions, for a possible reuse. An economic and environmental evaluation should be taken in consideration to select the material to perform an adsorption process.

3.4 CONCLUSIONS

This work evidences the adsorption potential of waste-based biomaterials/materials without resort to chemicals for the removal of ATZ from water.

Process kinetics were satisfactorily described by the PSO model for sepiolite, cork and pine bark. Freundlich model fits the adsorption isotherms for cork and sepiolite, while Sips model fits adsorption on pine bark more adequately. Kinetics and equilibrium of ATZ adsorption onto activated rubber char from waste rubber were also evaluated as a comparative term for the other materials without treatment.

ATZ desorption feasibility was evaluated in batch systems with different extraction solvents. In 24 h 100 % of ATZ was recovered from sepiolite using an aqueous solution of 20 % of acetonitrile, while from cork and pine bark the highest values of 98 % and 94 % were reached, respectively, using solutions of 40 % of acetonitrile. These desorbing agents were selected to regenerate sorbents in column recyclability assays.

Continuous adsorption of 2 mg/L of ATZ using sepiolite, cork and pine bark as PRB was performed in columns at lab scale. Breakthrough curves were best fitted by the modified dose-response model for all the tested materials ($R^2 > 0.96$), indicating its suitability for process design and upscale. The maximum adsorption capacity for sepiolite was 23.3 (± 0.8) mg/g, 14.8 (± 0.6) mg/g for pine bark and 13.0 (± 0.9) mg/g for cork and these values were determined by mathematical modelling. Sepiolite revealed to be the best material in terms of adsorption capacity, with the longest breakthrough time of 11 h, followed by 5 h for cork and 4 h for pine bark.

The recycling potential of sorbents in PRB was assessed by consecutive cycles of ATZ adsorption-desorption in column assays. After two regeneration cycles, the best performance in terms of ATZ recovery was measured for sepiolite with 81 %, followed by pine bark and cork with values of 78 % and 54 %, respectively. The desorption efficiency obtained in the second step of 48 h, reached 96 % for sepiolite and pine bark, and 55 % for cork. Results evidence that sepiolite is the best material in terms of ATZ adsorption capacity and stability after regeneration, with high potential for reuse and depending on a leaching agent with smaller environmental impact. The characteristics of the selected sorbents, before and after 2 cycles of sorption/regeneration, were evaluated by SEM and FTIR analyses and no substantial differences were found in structure or morphology.

Adsorption technology employing waste-based materials/biomaterials can be a sustainable option for wastewater treatment, once it promotes waste valorization, pollution mitigation and environmental

protection by reducing natural resources dependency. Through waste valorization, a circular and sustainable bioeconomy is promoted by the increased waste life cycle, a prevailing priority in the EU.

The application of sorbents able to compete with commercial ones is still seen as an emerging research challenge. The present study is in line with the growing concern on herbicide removal and uses reusable wastes with recovery capacity and recycling ability, expected by environmental policies.

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CHAPTER IV

BIOWASTE VALORIZATION ON EMERGING POLLUTANTS ABATEMENT IN AQUEOUS PHASE

The rehabilitation of water contaminated with FLX, CRB and ATZ (multi solute solution) by sustainable biosorbents is evaluated in this chapter. Raw, chemically and thermally modified biosorbents derived from pine bark are evaluated in terms of biosorption efficiency and economic feasibility. Pollutants extraction from raw pine is accomplished by testing different desorption eluents. The recyclability of pine is then evaluated by adsorption-desorption cycles at batch mode using the best extraction eluent. The effectiveness of pollutants biosorption by pine under dynamic flow conditions in an air-lift type loop reactor is also investigated. Biowaste valorisation for emerging pollutants biosorption can be considered a sustainable water treatment, by promoting biomaterials life cycle, circular bioeconomy and environmental protection.

This chapter is based on the following publications:

Lago, A.; Silva, B.; Tavares, T. Biowaste Valorization for Emerging Pollutant Abatement in Aqueous Phase. *Environ. Sci. Proc.* **2022**, *21*, 51.

Lago, A.; Silva, B.; Tavares, T. Biowaste valorization on emerging pollutants abatement in aqueous phase **(under submission)**

4.1 INTRODUCTION

Forest residues are a relatively abundant and important waste that is produced during harvesting and silvicultural activities [1]. Amongst them, pine bark has encouraged several authors to assess its efficiency as biosorbent due to its high potential sorption capacity by ion exchange and chelating processes [2–6]. However, this kind of materials often presents poor surface functionality (limited C–O, CO and OH surface groups), reduced porosity and surface area (usually lower than 150 $m^2 g^{-1}$) [7,8].

The surface functionality of materials can be tailored by chemical or thermal treatments to enhance their adsorptive capacities [1,9]. Hydrothermal carbonization (HTC) may be adopted to convert organic residues into porous carbonized materials with potential towards adsorption applications [10]. HTC is energetically and economically more favourable than pyrolysis, once it uses milder conditions, avoid gas emissions and the formation of tars [11].

Cost analysis is a requisite in decision-making over the large-scale use of adsorption processes. Besides adsorbents uptake capacity and recyclability, the economic feasibility analysis is crucial for the selection of adsorbents [4,12,13]. Regeneration of spent adsorbents is an appealing option to modern post-treatment practices as incineration, disposal in landfills or reactivation [14]. The use of low-impact solvents to chemically regenerate and recover adsorbents capacity is becoming feasible and attractive. The extraction and/or recovery of target molecules can be accomplished with a low mass loss of adsorbent [15,16].

The most popular type of flow reactors is a fixed bed column [2,5,17,18]. However, the risk of having regions with increased or reduced liquid flow and the potential risk of sorbent damage by gravitational pressure [19] are their major drawbacks. The air-lift reactor is a viable alternative to fixed bed reactors [20], once the adsorbent is forced to move by gas flow, leading to a more effective contact with the sorbate [21]. The adsorbent is efficiently used due to a homogeneous sorbate concentration within the whole reactor volume.

Most of the research conducted so far on pharmaceutical ingredients and pesticides concerned their adsorption using mono-component aqueous solutions. Therefore, for a more realistic evaluation of the adsorption capacity of pine bark, the present research investigates the adsorption process in multi solute aqueous solutions (FLX, CRB and ATZ in mixture as model compounds). The goal is to compare the adsorption capacity of raw, of chemically and thermally modified pine barks in batch experiments. The economic feasibility of the biosorbent usage is examined through a detailed cost analysis. The desorption capacity of the target molecules and reuse ability of raw pine bark were also assessed. The

operation of a pre-pilot air-lift reactor was also investigated as an effective adsorption rehabilitation system for up-scale use.

4.2 MATERIALS AND METHODS

4.2.1 Chemicals

FLX (Purity > 98 %), CRB (98 %) and ATZ (PESTANAL® analytical standard, 99.1 %) were purchased from Sigma-Aldrich. **Table 10** summarizes the major physicochemical properties of selected compounds. For the determination of pH_{zpc}, 1.0 M H_2SO_4 (95 %, Fisher Chemical), 1.0 M NaOH (≥ 97 %, Fisher Chemical) and 0.01 M NaCl (99.5 %, Panreac) solutions were prepared. HPLC-grade acetonitrile (Fisher Chemical), orthophosphoric acid (analytical reagent grade, Fisher Chemical) and ultra-pure water obtained from a Milli-Q Millipore system, were used to prepare the mobile phases for FLX, CRB and ATZ quantification by UHPLC-DAD. 2.5 M NaOH (98 %, Labkem), 1 M H_2O_2 (30 %, Fisher Chemical) and HNO_3 (69 %, Fisher Chemical) were prepared for chemical surface modification procedures. 1.0 M and 3.0 M NaCl, 50 % HPLC-grade methanol (Fisher Chemical), acetonitrile or ethanol (99.8 %, Fisher Chemical) aqueous solutions were prepared for desorption assays.

Table 10. Selected physicochemical properties of FLX, CRB and ATZ

Chemical name	**Molecular formula**	**Molecular weight (g/mol)**	**pK_a**	**Log K_{ow}**	**Water solubility (mg/L at 25°C)** [22]
Fluoxetine hydrochloride	$C_{17}H_{18}F_3NO.HCl$	345.79	9.8 [4]	1.22 [4]	14000
Carbamazepine	$C_{15}H_{12}N_2O$	236.269	13.9 [22]	2.45 [22]	18
Atrazine	C8H14ClN5	215.69	1.68 [23]	2.6-2.71 [23]	33

4.2.2 Preparation of waste biosorbents

Pine bark (*Pinus pinaster*) collected from a pine forest located in Braga (Portugal), was first ground into small particles, sieved (ranging from 1 mm to 2 mm) and washed with deionized water to remove impurities and powder particles. Afterwards, it was placed in an oven at 30°C for 24 h to dry and then stored in a desiccator.

Pine bark surface was chemically modified with alkali and acid modifying agents for further adsorption capacity evaluation. 2.5 M NaOH aqueous solution was used as reducing agent for alkali modification and 1 M HNO_3 and 1 M H_2O_2 aqueous solutions were used for acidification and oxidation, respectively. For both reduction and acidification treatments with HNO_3, 10 g of raw pine was weighed and transferred to 1 L beaker with 300 mL of the respective modifying agent solution. For the treatment with 1 M H_2O_2 a 1 % of solid ratio was used instead. Mixtures were stirred thoroughly for 24 h at 180 rpm and 25 °C. Then, modified materials were filtered and washed with distilled water until no color was observed on supernatant and a constant pH was reached. The solid was filtered and dried in an oven at 50 °C for 48 h. The material losses were determined by weight for each treatment.

Pine bark hydrochar was prepared in a 500 mL reactor loaded with 25 % of solid ratio and deionized water, in a final volume of 200 mL. The temperature of the reaction was set at 220 °C for 150 min. The experimental conditions were established based on reported literature concerning agricultural residues hydrochars for adsorption process purposes [10]. The reactor was cooled to room temperature, the solid was collected and washed with deionized water. Hydrochar was recovered by filtration and finally dried 12 h at 105 °C and the solid yield was determined by weight. **Figure 24** shows the steps involved in the preparation of pine bark hydrochar.



Figure 24. Steps involved during the production of pine bark hydrochar, A) reactor, B) reactor placed in the oven, C) hydrochar product after HTC and D) washing and filtration process.

4.2.3 Biosorbents characterization

The pH_{zpc} of selected biosorbents and surface chemistry by ATR-FTIR were evaluated following the procedures given in Chapter 3 (section 3.2.3).

4.2.4 Sorbates quantification by UHPLC-DAD

To increase total water solubility, FLX, CRB and ATZ were dissolved separately in acetonitrile (HPLC grade) and individual stock solutions of 1 g/L were stored. Diluted solutions of each chemical were prepared from the respective stock solution adding ultrapure water. The quantification of the components of the mixture was performed by UHPLC-DAD, using a Shimadzu Nexera X2 and a Kinetex C18 column (Phenomenex) (1.7 µm x 100 Å x 2.1 mm) operating in gradient mode. Eluents used were 0.1 % phosphoric acid in ultrapure water (eluent A), acetonitrile (eluent B) and ultrapure water (eluent C) at a flow rate of 0.5 mL/min. The autosampler was operated at 4 °C and the column temperature was kept at 50 °C, with an injection volume of 5 µL. The gradient elution started with 10 % of eluent B, increasing to 100 % B in 6.5 min, decreasing to 10 % B in 1.0 min and then, maintained the initial conditions during 1.5 min. The chromatograms were registered at a maximum adsorption wavelength of 227 nm. The linearity of the method was assured by setting a calibration curve over the concentration range of 0.3–10 mg/L. Detection limits (0.1 mg/L) were determined as the minimum detectable concentration of analytes in the test sample that can be reliably distinguished from zero. The quantification limits (0.3 mg/L) were set as the concentration above which the analytical method operates with precision. Two replicates of each standard were obtained, and the average of peak areas was used for quantification. Data were processed by Lab Solutions software (version 5.71, Shimadzu Corporation). **Figure 25** displays the obtained chromatogram of 5 mg/L of the multi solute solution of CRB (ret. time at 4.927 min), FLX (ret. time at 5.074 min) and ATZ (ret. time at 5.200 min) by UHPLC-DAD.

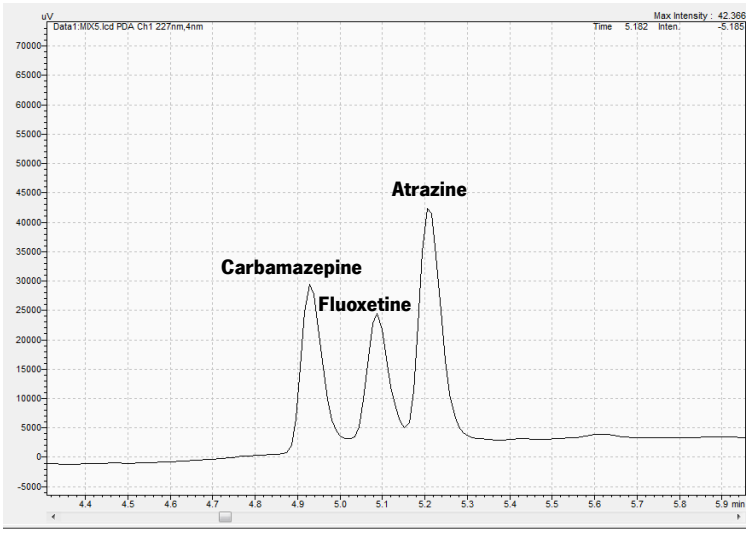


Figure 25. Chromatogram of 5 mg/L multi solute solution of CRB, FLX and ATZ by UHPLC-DAD.

4.2.5 Batch experiments

Kinetics experiments were performed to evaluate FLX, CRB and ATZ (in mixture) adsorption capacity by raw pine bark. A predetermined mass of the biosorbent was placed in amber Erlenmeyer flasks and a fixed volume of the target pollutants stock solutions with 5 mg/L each was added. The performances of the modified surfaces as well as of the hydrochar biosorbents were compared to raw pine bark at the equilibrium time previously established. Desorption experiments with extraction eluents were carried out to evaluate the desorption capacity of the target pollutants from raw pine bark (selected as biosorbent model due to sustainability and competitiveness). After the selection of the best performing extraction eluent (50 % acetonitrile solution), pine bark recyclability was evaluated by adsorption-desorption cycles. To assure the reproducibility of the results, assays were performed in triplicate and average values were reported. The experiments were conducted in the absence of light to avoid the photodegradation of compounds [24].

4.2.5.1 Adsorption kinetics

FLX, CRB and ATZ adsorption rates and respective equilibria time onto raw pine bark were established. Biosorbent doses of 10 g/L each were used in amber Erlenmeyer flasks with 50 mL of a mixture of FLX, CRB and ATZ at 5 mg/L each. The flasks were rotated in a temperature-controlled incubator at 140 rpm and 25 °C until equilibria were reached. For the kinetics, samples were taken at different time intervals from another set of erlenmeyers with the same operational conditions, filtered

through 0.22 μm nylon syringe filters, being the filtrate analysed for FLX, CRB and ATZ concentration by UHPLC-DAD. A blank test at the same operational conditions but without biosorbent material was also monitored during the experiment.

To determine the adsorption capacity of the sorbent, q_t (mg/g), the Equation 1 from Chapter 3 was used. C_0 (mg/L) is the initial concentration of the mixture FLX, CRB and ATZ in solution, C_t (mg/L) is the remaining concentration of FLX, CRB and ATZ in solution at time t; V(L) is the initial volume of the solution and m (g) is the mass of the biosorbent.

The experimental data were fitted by PFO [25] (Equation 2 from Chapter 3) and PSO [26] (Equation 3 from Chapter 3) models so to describe the adsorption kinetics. Origin Pro 8.0 software was used to perform the calculations.

4.2.5.2 Removal capacity of modified biosorbents

The surface functional groups of biosorbents can be significantly designed by chemical agents or thermal treatments. After modification procedures, the adsorption potentiality of those biosorbents was evaluated to compare them to the raw biomass. Experiments were conducted in the same experimental and operational conditions as in section 4.2.5.1. during the time needed to attain the equilibrium (previously established for each target molecule). At equilibrium time, samples were taken to determine the remaining FLX, CRB and ATZ concentration. The adsorption capacity of biosorbents was calculated using Equation 1 from Chapter 3, where C_t is substituted by C_e (mg/L), equilibrium concentration of FLX, CRB and ATZ in solution.

4.2.5.3 Desorption study

To investigate the possibility of repeated use of the raw pine biosorbent, desorption assays were performed. Batch assays were performed using 0.75 g of sorbent in 50 mL of 5 mg/L pollutants mixture solution (solid/liquid ratio of 15 g/L) at 25 °C, during the time needed to reach equilibrium for each pollutant (as previously established). The loaded biosorbent was then separated from solution by filtration, washed gently with water to remove any unadsorbed sorbates and dried at 30 °C. To assess the desorption of FLX, CRB and ATZ, the spent biosorbent was then mixed with different extraction eluents, as 1.0 M and 3.0 M NaCl solutions, aqueous solutions with 50 % acetonitrile, methanol or ethanol and deionized water at 25 °C. The solid/liquid ratio used was maintained (15 g/L) and flasks were agitated

for 48 h at constant temperature. At regular intervals samples were collected to identify the suitable time for desorption and analysed through UHPLC as described previously. The desorption percentage, D (%) was then calculated by using the Equation 6 from Chapter 3. C_{des} is the concentration of desorbed FLX, CRB and ATZ along time and C_{ads} is the initial concentration of adsorbed FLX, CRB and ATZ on pine bark.

4.2.5.4 Biosorbent regeneration and reuse

The viability of biosorbents depends on their effective reuse, so the evaluation of their recyclability becomes necessary. From the previous experiments, described in 4.2.5.3, the best extraction eluent was selected and used for the recyclability evaluation. Repetitive adsorption-desorption cycles were performed to determine the loss in the uptake capacity of pine bark. The first adsorption of FLX, CRB and ATZ was conducted as described in 4.2.5.1, but with a biosorbent dose of 15 g/L, until the sorption equilibria was reached. The concentration of the remaining pollutants was determined. Then, the spent biosorbent (15 g/L) was regenerated with 50 mL of 50 % acetonitrile solution for 24 h and reused in another adsorption step. The loading and regenerative cycles were repeated three times. On each cycle the concentration of the desorbed pollutants was evaluated.

4.2.6 Up-scale rehabilitation system

FLX, CRB and ATZ adsorption effectiveness, starting from a mixture with 5 mg/L of each sorbate, was assessed with raw pine under flow conditions in an air-lift type acrylic reactor. The reactor (total volume = 5 L) has a circular cross-section (Φ = 0.07 m) and is 1 m high. A 0.6 m long partition was mounted inside the reactor. The air supplied ensured the circulating motion of the contaminated solution and of the biosorbent around the partition. The scheme of the air-lift type reactor is established in **Figure 26**.

In prior assays, different air flows were tested (2, 5 and 7.5 L/min) using ATZ (at 2 mg/L) as a model molecule to select the best flow in terms of mixing and adsorption capacity. The assay was conducted at batch mode for 6 days, using 3 L as working volume, a biosorbent concentration of 15 g/L and the airflow was fixed at 2 L/min (optimized value). This flow rate enabled the biosorbent mixing in the entire volume available and prevented sedimentation at the bottom of the reactor. Periodically, samples were taken from the middle of the reactor height for UHPLC determination of each pollutant concentration.

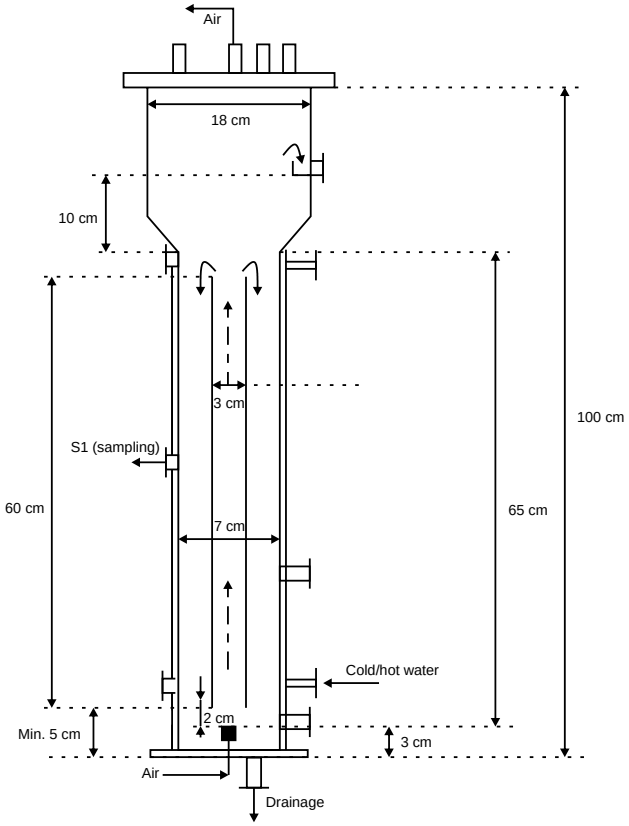


Figure 26. Air-lift type reactor design.

4.2.7 Statistical analysis

GraphPad Prism® software (version 8.0; GraphPad Software, Inc., San Diego, CA, USA) was used for statistical analyses. The level of significance was determined by two-ways ANOVA followed by post run test for multiple comparisons (Tukey's or Dunnett's test). Significance was accepted at $p < 0.05$.

4.3 RESULTS AND DISCUSSION

4.3.1 Characterization of the biosorbents

The solid yield is defined as the percentage of the original precursor weight that remains after treatment. The yield value for the alkali modified pine was 60 %, and for the biosorbents treated with HNO_3 and H_2O_2, values of 87 % and 68 % were obtained, respectively. The yield value of pine hydrochar was 70 %. Therefore, pine bark treated with NaOH suffered the highest weight loss (40 %) during the

procedure. The acid treatment led to the lowest effect in terms of weight loss. The solid yield was 95 % for raw pine after the washing step.

As explained in Chapter 3, when pH > pH_{zpc}, the adsorption of cation species is favored based on the negative charge of the adsorbent, while the adsorption of anionic species is favored on the positively charged adsorbent surface at pH < pH_{zpc}. The pH_{zpc} of natural pine bark (around 5) was determined and defined in the previous Chapter 3. The alkali modified pine presented a pH_{zpc} of 6.5 and values of 3 and 3.8 were obtained for the pine treatment with HNO_3 and the H_2O_2, respectively. The pH_{zpc} of the hydrochar was determined to be 2.8.

K_{ow} value is directly correlated to the hydrophobicity of a compound, thereby representing the tendency to be adsorbed by a solid or particulate phase rather than remaining in the aqueous phase [27]. According to K_{ow} of FLX, CRB and ATZ they are hydrophilic compounds, depicting a tendency to be adsorbed.

In comparison with the solution pH, the pK_a of the target molecules and the pH_{zpc} of the biosorbent are useful to explain the adsorption processes. FLX, CRB and ATZ speciation in solution depends on their pK_a values and the actual pH of the medium. **Figure 27** presents the charge distribution of the target pollutants in the pH range from 0 to 14. The plots were obtained using the software Marvin Sketch (version 18.11). Increasing pH led to progressive deprotonation of the functional groups of FLX and ATZ molecules. While ATZ is in its neutral form in the range of pH between 6.5 and 11.5, FLX is neutral above 12. Once pK_a of FLX is 9.8 and ATZ is 1.68, below that values both molecules take mainly the ionized state (positive charge), as can be seen in **Figure 27**, CRB is in its neutral form over the pH range from 0 to 14.

Surface charges of biosorbents change according to the pH of the solution. Under the conditions of the study, the pH of pollutants medium, at 5 mg/L each, has a value of 6.07. According to pH_{zpc} evaluation, raw, acid and oxidant modified pine bark surfaces assume a negative charge when in contact with the solution, once its pH is higher than their pH_{zpc}. On the other hand, the alkali-modified pine bark surface becomes positively charged, once the solution pH is lower than its pH_{zpc}. By ion-exchange mechanism, FLX adsorption is electrostatically favorable by raw, acid and oxidant modified biosorbents. Using the same biosorbents, the electrostatic attraction of ATZ is significantly weaker, because the positive charge of the molecule decreased with increasing pH (until the solution pH). Because CRB keeps neutral molecules within pH 0-14, very weak electrostatic reactions between adsorbate and biosorbents were expected [28]. **Table 11** summarizes the charges distribution of biosorbent materials, when in contact with the solution with FLX, CRB and ATZ.

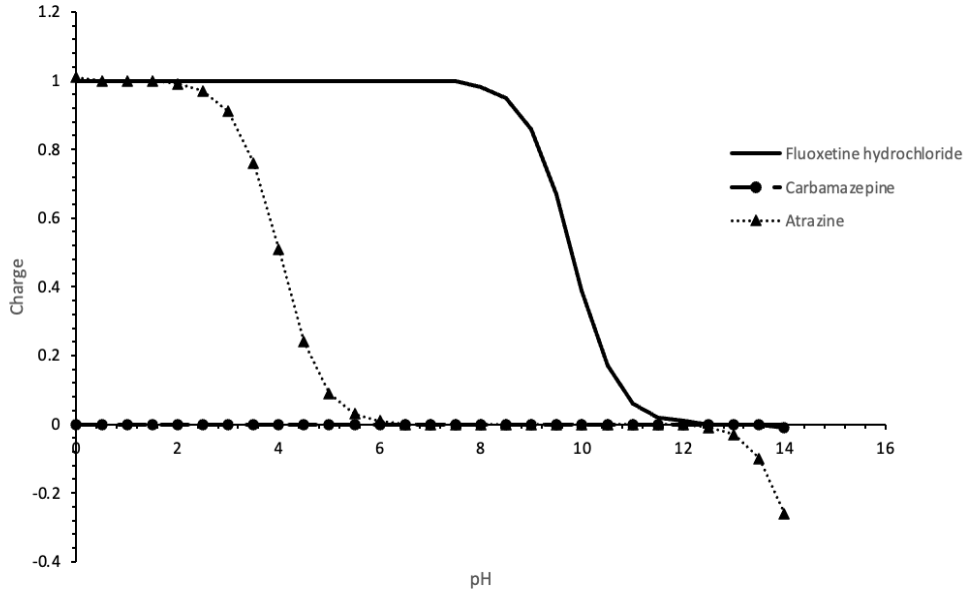


Figure 27. Charge distribution of pollutants as a function of pH (plot obtained using Marvin Sketch tool).

Table 11. Charges distribution of FLX, CRB and ATZ and of biosorbents surface as function of solution pH

Type of biosorbent	pH variation	FLX charge	CRB charge	ATZ charge	Biosorbents surface charge
Raw	6.07-4.64	+	neutral	neutral/+	-
Modified with NaOH	6.07-7.42	+	neutral	neutral	+
Modified with H_2O_2	6.07-4.13	+	neutral	neutral/+	-
Modified with HNO_3	6.07-3.42	+	neutral	neutral/+	-
Hydrochar	6.07-4.07	+	neutral	neutral/+	-

The ATR-FTIR spectra of the biosorbents were recorded in the region of 400-4000 cm^{-1} and are shown in **Figure 28**. FTIR measurements revealed the availability of functional groups on the cell walls of *Pinus pinaster* in comparison with the ones subjected to chemical and treatment processes. The FTIR spectra of the original samples is described in Chapter 3 (section 3.3.1). After the introduction of activating agents for surface modification, significant changes in the materials surface can occur in terms of band shift, bands disappearance and new bands formation [9]. All samples exhibit a vibration at 1600 cm^{-1} that can be assigned to an aromatic ring stretching mode, representative of the aromatic nature of the samples. The FTIR spectra of the sample oxidized with H_2O_2 did not show relevant differences in terms

of functional groups compared to the raw material. The raw pine presents bands at 2910 and 2844 cm^{-1} due to asymmetric and symmetric C–H stretching vibrations in aliphatic chain –CH, $–CH_2$, $–CH_3$. The oxidized pine with HNO_3 do not reveal the existence of these bands. In the alkali modified pine the carbonyl group (band at 1700 cm^{-1}) is not as evident as in the other samples. Pine hydrochar retained considerable oxygen-containing functional groups after hydrothermal treatment. Peaks between 3200-3600 cm^{-1} detected on raw pine bark correlated with O–H stretching of hydrogen-bonded hydroxyl groups were still evident in the hydrochar. The peak at approximately 2906 cm^{-1}, which correlates to –C–H stretching of the methylene groups, is also present [29]. Peaks at about 1100 and 1200 cm^{-1} appear in the hydrochar and not in the raw biomass. The peaks at 1100 and 1200 cm^{-1} correspond to C–O stretching and to O–H bending modes of alcoholic, phenolic and carboxylic groups [30]. The bands at 1719 cm^{-1} can be assigned to the C=O stretching vibration from ketones, aldehydes or carboxyl groups. However, this band is not so evident on hydrochar (the carboxylic acid groups are thus not so predominant in this biosorbent).

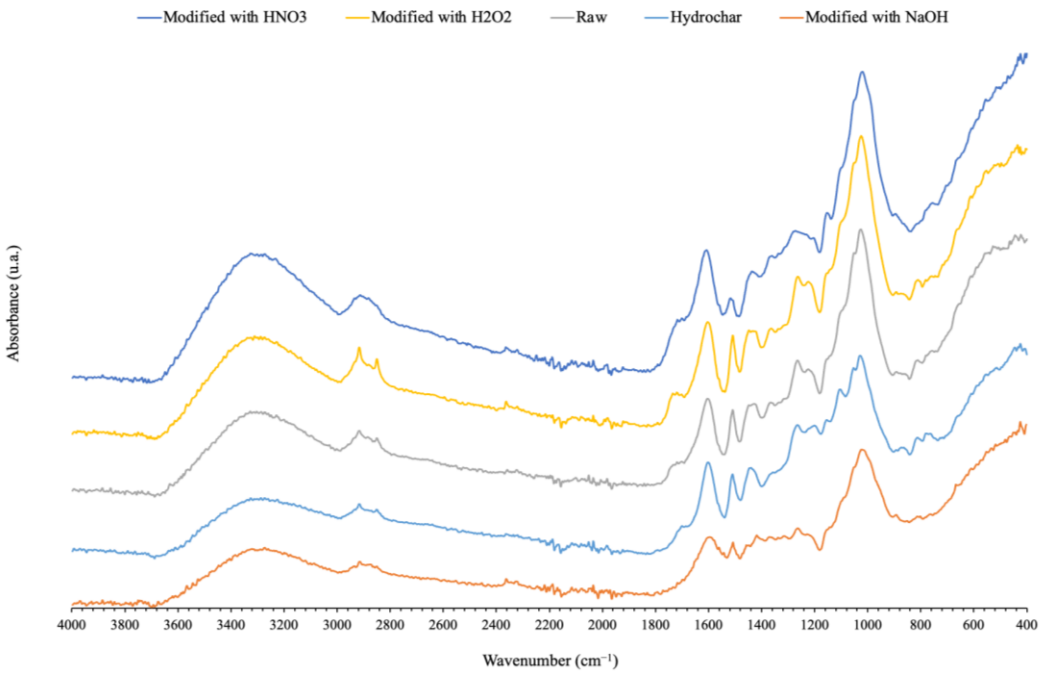


Figure 28. ATR-FTIR spectra of raw pine and modified biosorbents.

4.3.2 Kinetics adsorption modelling

Adsorption kinetics evaluation gives information about the rate at which the retention takes place and the minimum contact time required to reach the equilibrium [31]. Also, gives valuable information to

design and to model the process. The respective kinetics curves of FLX, CRB and ATZ in mixture (5 mg/L each) onto raw pine (10 g/L) are presented in **Figure 29**. The concentration of pollutants measured on blank test remained unchanged during the experiment. The adsorption of FLX was very fast in the first minutes of the assay, taking around 12 h to reach equilibrium. This behavior was not verified with CRB and ATZ, where equilibria were achieved in 192 h. At this point, the amounts of FLX, CRB and ATZ adsorbed per gram of biosorbent were 0.652, 0.144 and 0.522 mg/g, respectively.

The differences between the uptake values could be related to the distinct electrostatic interactions between the net surface charge of the biosorbent and the electric charge of the molecules (**Table 11**). The adsorption mechanism of FLX is based on attractive forces (strong electrostatic attraction) between FLX (positive charge) and pine bark (negative charge). This was demonstrated by the fast adsorption kinetics of the pollutant and the high affinity of the biosorbent. The same behavior was described by Silva *et al.* [4] who reported accelerated sorption kinetics of FLX (around 10 h to reach equilibrium) by pretreated forestry waste, as pine bark and cork. The adsorption kinetics of ATZ molecule is in agreement with results from section 3.3.2 from Chapter 3, where it was also verified an equilibrium time of 8 days by raw pine bark using a mono-component solution of 2 mg/L of ATZ. The preferentially neutral and positive ATZ species led to a lower electrostatic attraction between this molecule and the biosorbent. The neutral molecules of CRB led to very weak electrostatic reactions with the sorbent. This is consistent with the substantially lower adsorption ability of the biosorbent towards CRB.

Two kinetic models, namely PFO and PSO were selected to be fitted to the experimental data. The fitting parameters and the correlation coefficients obtained for each model are listed in **Table 12**. The high correlation coefficients showed that both models tailored well the experimental data ($R^2 > 0.97$). The experimental q_e values are in agreement with the calculated ones, but for ATZ the PFO presents a calculated q_e value closer to the experimental one (0.522 mg/g). It is not possible to conclude with certainty about the mechanisms involved in the adsorption of pollutants since these mechanisms are hardly assigned based on observed kinetics experiments or by fitting kinetics models [32,33]. Comparing the k_2 values, the adsorption rate of pollutants follows this sequence: FLX > CRB > ATZ.

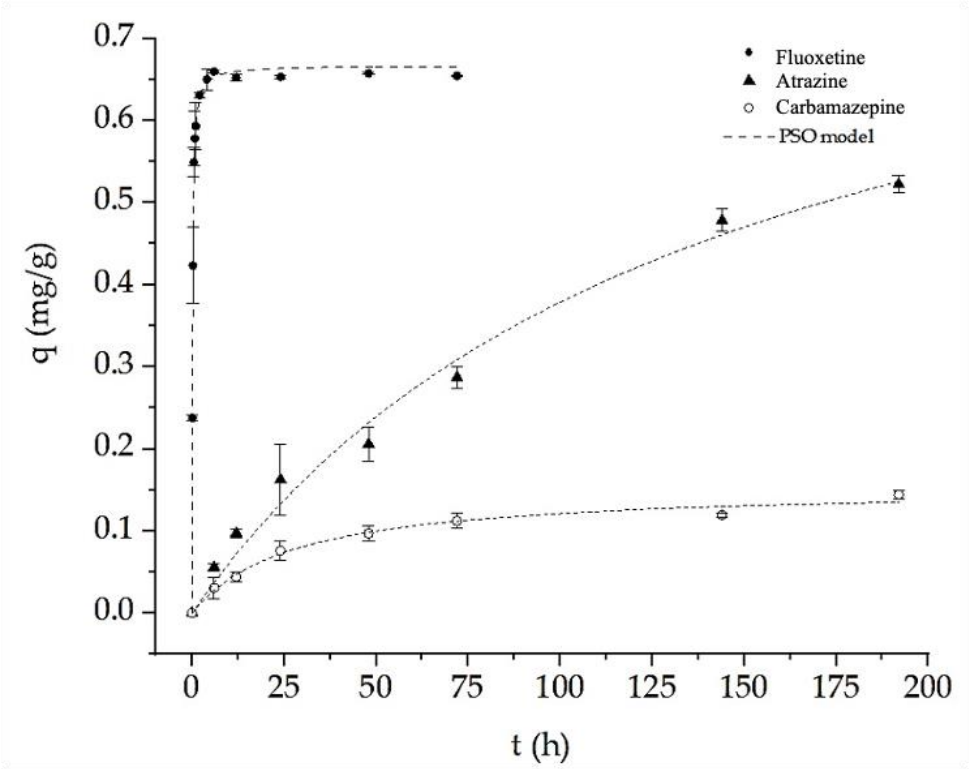


Figure 29. Adsorption of FLX, CRB and ATZ along time. Experimental conditions: C = 5 mg/L each, biosorbent dose 10 g/L.

Table 12. PFO and PSO model fittings for the adsorption of FLX, CRB and ATZ onto raw pine bark

Pollutant	PFO			PSO		
	q_e	k_1	R^2	q_e	k_2	R^2
Fluoxetine	0.640 ± 0.00838	4.32 ± 0.343	0.986	0.667 ± 0.00473	11.147 ± 0.640	0.996
Carbamazepine	0.130 ± 0.0060	0.0328 ± 0.0048	0.974	0.154 ± 0.00691	0.238 ± 0.0447	0.987
Atrazine	0.624 ± 0.0602	0.00952 ± 0.00178	0.983	0.909 ± 0.112	0.00786 ± 0.00276	0.985

q_e - adsorption capacity at equilibrium (mg/g); k_1 constant of the PFO model (min^{-1}); k_2- constant of the PSO model (g/(mg.min); R^2- coefficient of correlation.

4.3.3 Modified biosorbents performance

The success of an adsorbent/biosorbent depends on its surface chemistry. Therefore, modification processes can be performed through physical and chemical treatments to improve the quality of the material surface. In chemical processes, the modifying agents improve the surface functional groups of

a material, eventually converting it into a highly valuable product with high adsorptive capacities. The thermal processes enhance the physical characteristics such as density and solubility [9].

Figure 30 shows the maximum adsorption capacity of selected biosorbents. A simple comparison between adsorption capacities allows to infer about the changes introduced on surface accessibility and on affinity towards the sorbate. The performance sequence of the sorbents in terms of global adsorption capacity was as follows: HNO_3 (1.95 mg/g) treatment > hydrochar (1.91 mg/g) > raw = H_2O_2 (1.32 mg/g) treatment > NaOH (1.01 mg/g) treatment. The data in **Figure 30** clearly shows that the adsorbed amount of FLX was independent of the type of sorbent. FLX belongs to the family of cationic amphipathic drugs with a polar head and a hydrophobic body [34]. The electrostatic attraction between the positively charged FLX molecules and the negatively charged biosorbent surface (except the alkali modified one) promotes the adsorption of this analyte. On the alkali modified biosorbent the sorbate repulsion did not have a relevant impact on the retention mechanism. This less important electrostatic repulsion is due to a small difference between solution pH and the pH_{zpc} value, which produces a not so intense positive charge on the biosorbent surface [35].

The alkali treatments tend to improve the non-polar surface, hence, enhance the adsorption capacity of the material for non-polar substances. A sorbent after an alkali treatment assumes a positive charge on the surface promoting the adsorption of negatively charged species [9]. The modification of pine with NaOH decreased the capacity for CRB and ATZ adsorption, compared to the raw material and these results can be explained by the hydrophilicity of CRB and ATZ molecules and the very weak attraction forces assumed by CRB and ATZ neutral charges and the positive charge of the surface.

On the other hand, the acidification of sorbents surface with oxygen-containing functional groups enhances their acidic behaviour and hydrophilic nature by reducing the mineral content [36]. An acid treatment induces a negative charge on the surface, promoting the adsorption of positively charged analytes. The biosorbent treated with HNO_3 was more efficient than the one treated with H_2O_2 due to the lowest pH_{zpc} value that promoted stronger electrostatic attraction between the negative biosorbent surface and the sorbate molecules. An increment in adsorption capacity of 108.62 % for ATZ and 50.05 % for CRB was reported with the HNO_3 treated biosorbent.

The CRB molecules are mainly neutral along with pH. Thus, even though the surface of biosorbents change, their adsorption ability towards neutral molecules will remain low due to very weak electrostatic attraction between sorbate and biosorbent. Results indicated that pine hydrochar shows high adsorption capacity for all pollutants. The maximum increase was obtained for CRB (227.45 %), followed by ATZ and FLX in more 43.66 % and 4.8 %, respectively. This can be correlated with the abundant functional groups

on hydrochar surface, confirmed by the FTIR measurements. The HTC reaction occurs through water breakage of the carbon-oxygen and, to a lesser extent, of the carbon-carbon bonds between the organic structures present in the materials. As a result, various interactions with surface functional groups are promoted, leading to increased adsorption capacity [37]. In the work of Jian *et al.* [29] it was proved that even though rice husk hydrochar presents relatively low surface area, its adsorption capacity for methylene blue, iodine and copper ions is much higher than the one of pyrochar due to its ion-exchange and complexation ability.

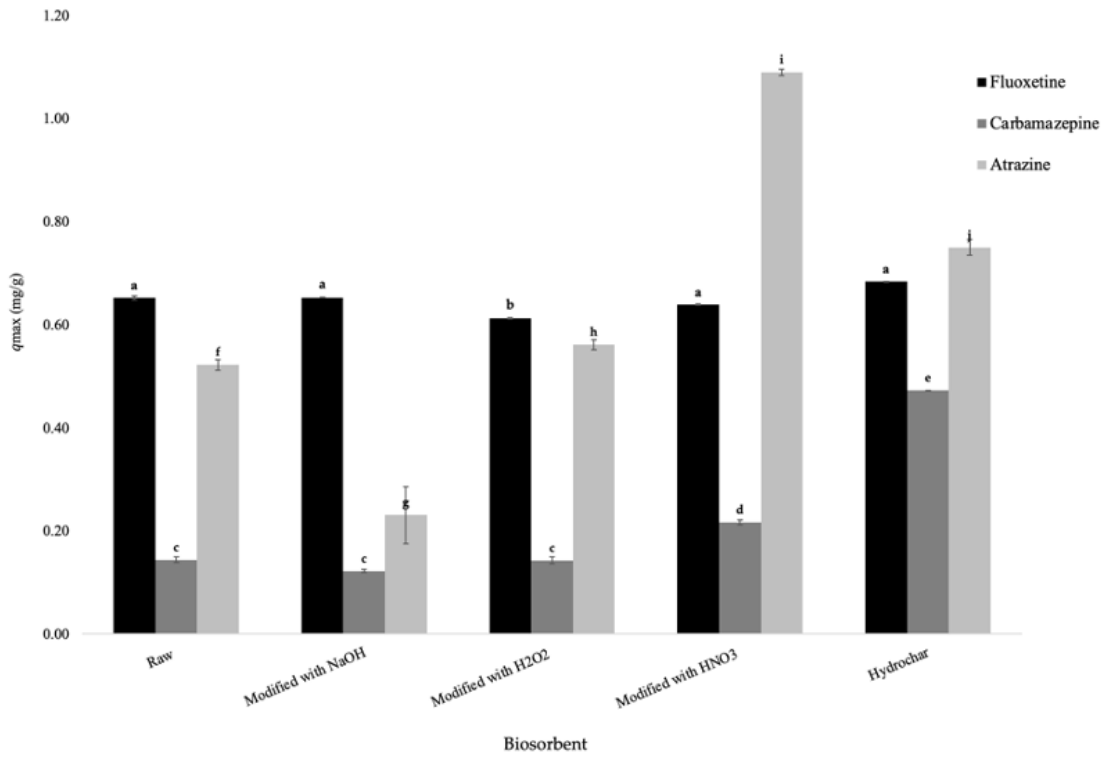


Figure 30. Maximum adsorption capacity of FLX, CRB and ATZ by different biosorbents. Experimental conditions: C = 5 mg/L each, biosorbent dose 10 g/L. Different letters show significant differences ($p < 0.05$) between biosorbent against raw pine performance for the same pollutant using the Dunnett's test for multiple comparison. FLX (a-b), CRB (c-e) and ATZ (f-j).

4.3.4 Cost analysis and feasibility

The process of removing pollutants from wastewater has a growing economic importance. The treatment of wastewater should be effective within environmental and financial standards, since one of the most critical parameters for the evaluation of an adsorbent and the corresponding adsorption process

is its cost [38]. The cost of sorbents is thus a core parameter for their industrial use, once the adsorption process is mainly dependent on that particular element [4]. The total cost of the process covers operational items including manufacturing, maintenance, feedstock, transportation, labour and distribution costs [39].

In the present work, an attempt is made to compare the use cost of raw pine biosorbent and the chemically and thermally modified biosorbents. **Figure 31** presents the different steps performed to produce 1 kg of each biosorbent under study (milling/sieving, washing, drying, chemical and thermal treatments and filtration procedures). Only the production costs of biosorbents were considered for the final treatment cost. The cost associated with the raw material was not considered once it is obtained for free. The solid yield of each biosorbent (in section 4.3.1.) was considered for the correct determination of the associated cost of each biosorbent. For each step, the input flows of energy, water and reagents were identified. The cost of each step, the total preparation cost for 1 kg of each biosorbent and the total cost of the removal of 1 mmol of pollutants mixture are given in **Table 13**. The cost of reagents and energy was defined considering average values practiced in the region of Portugal where preparations were carried out.

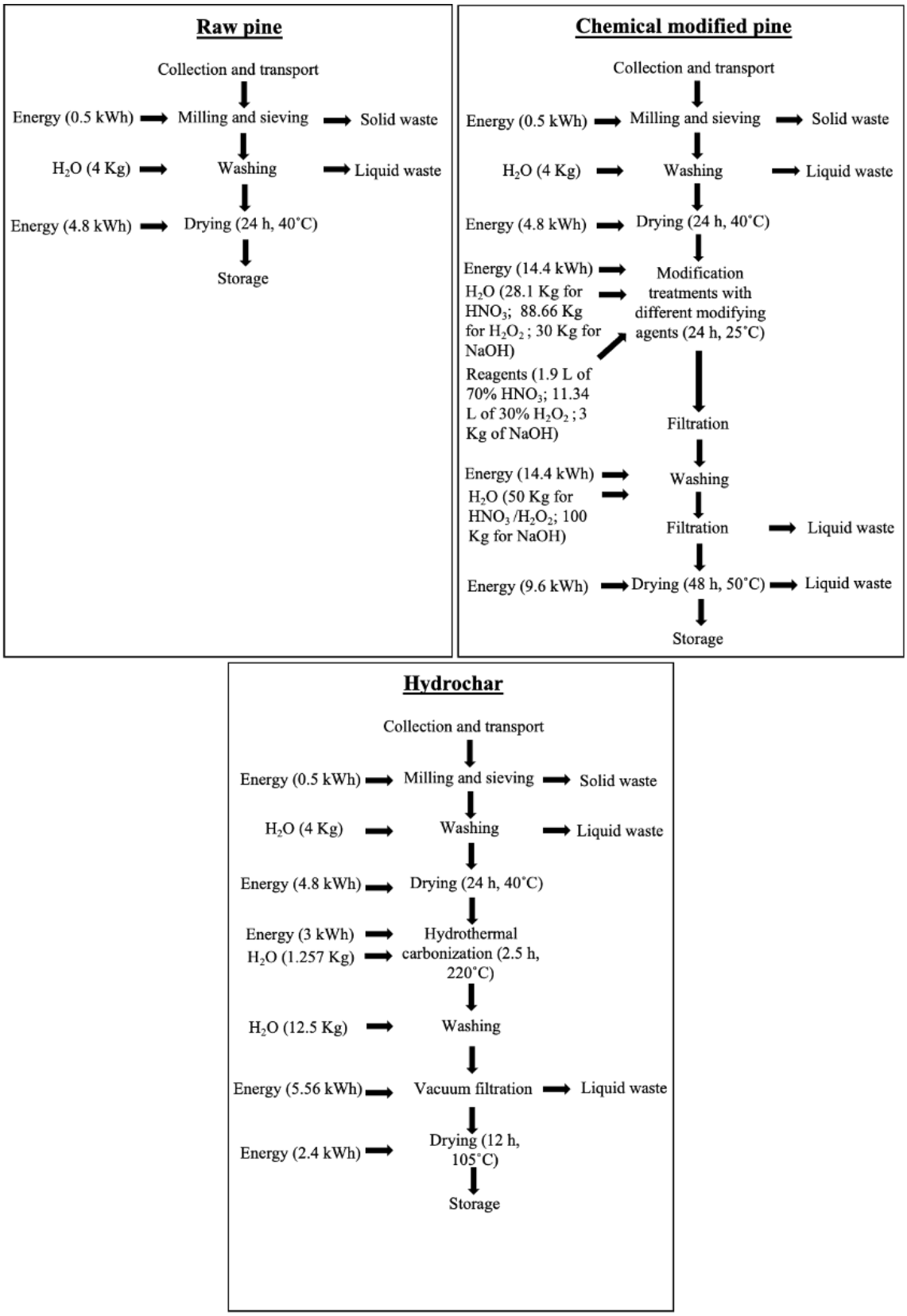


Figure 31. Flowchart for the preparation of 1 kg of raw pine and of modified biosorbents.

Table 13. Cost and uptake of selected biosorbents (1 kg basis)

Biosorbent	Pre-treatment cost (€/kg)						Weight loss (%)	Net cost (€/kg)	Total cost (€/kg)	$q_{máx}$ (mmol/g)	Treatment cost (€/mmol)
	Washing[a]	Surface treatment[b]	Shaking[c]	Drying[d]	Milling[e]	Filtration[f]					
Raw	0.003	-	-	0.77	0.08	-	5.0	0.85	0.90	$5.14x10^{-3}$	0.20
Modified with HNO_3	0.003	50.3	2.30	0.77	0.08	-	13	53.4	61.4	$8.03 x10^{-3}$	7.60
Modified with H_2O_2	0.038	102	2.30	2.30	0.08	-	12	107	123	$5.18 x10^{-3}$	23.7
Modified with NaOH	0.073	30	2.30	2.30	0.08	-	40	34.8	57.9	$3.69 x10^{-3}$	15.7
Hydrochar	0.012	-	-	1.63	0.08	0.89	30	2.6	8.7	$7.69 x10^{-3}$	1.1

[a] Considering a cost of 0.70 €/m^3 of deionized water

[b] Considered costs for washing pre-treatment steps: 10 €/kg for NaOH, 26.5 €/L for 1 M HNO_3 solution, 9 €/L for 1 M H_2O_2

[c] [d] It was considered the use of a 200 W oven in heating and drying operations up to 105 °C, and 1000 W for operations up to 250 °C and an energy cost of 0.16 €/kWh

[e] It was considered the use of a 3000 W laboratory mill and an energy cost of 0.16 €/kWh

[f] It was considered the use of a 418 W laboratory vacuum pump and an energy cost of 0.16 €/kWh

For comparison purposes, the cost of the removal of 1 mmol of pollutants mixture from water (€/mmol) was calculated dividing the total cost of the biosorbent by the total value of q_{max} (**Table 13**). The total cost of the biosorbent included the weight loss of each material during treatment. The treatment cost (€/mmol) of FLX, CRB and ATZ, starting with a 5 mg/L aqueous solution, as well as the total maximum adsorption capacity (mmol/g) for each biosorbent are listed in **Table 13**.

As it can be observed, chemically modified biosorbents present substantially higher costs when compared to the thermal treatments. The biosorbent with oxidizing modification with H_2O_2 is the most expensive procedure, 23.7 €/mmol, and hydrochar production is the less expensive, 1.1 €/mmol, amongst the performed modifications. The thermal treatment comprises a greener choice, once the chemical treatment involves the use of acid aqueous solutions, resulting in the production of wastewater, which should be avoided.

The raw material obviously presents the lowest cost per mmol of FLX, CRB and ATZ removed, 0.20 €. Besides its lower production price, the solid and liquid wastes associated to its preparation are substantially smaller than for other biosorbents, presenting a reduced environmental impact. For real applications, when costs and environmental impact are important to consider, this kind of analysis gives a clear idea of the most feasible and suitable sorbent material.

4.3.5 Desorption evaluation by solvent extraction

The regeneration process is essential to manage the waste of used sorbents, because when it is not possible, pollutants may end up disposed into the environment or requiring storage [38]. The desorption capacity justifies the usefulness of recycling a sorbent and influences the total cost of the treatment [12]. Chemical regeneration with extraction eluents is a potentially safe method to regenerate biosorbents [14]. As referred in Chapter 3, it is important that eluents do not cause damage or change the morphology of the sorbent, may be competitive, environmentally sound and that may enable a maximum recovery at the lowest possible eluent concentration and contact time. They should also avoid substantial mass loss or accumulation of micropollutants on the material over time [40].

In this work, the desorption efficiency of different eluents on retained FLX, CRB and ATZ was investigated, aiming biosorbent regeneration. As solvents polarity influences contaminant desorption, polar protic solvents (50 % methanol and 50 % ethanol solutions) and a dipolar aprotic solvent (50 % acetonitrile solution) were selected to create a highly solubilizing environment. Pure solvents were not considered due to their high toxicity character and environmental impact. The selected salt (for ion

exchange and/or competition evaluation) was sodium chloride since it is a simple and non-toxic substance, usually found in typical wastewater. Deionized water was considered to verify the risk of desorption due to equilibrium.

After one adsorption step, the loaded pine was placed in contact with the different leaching agents. The desorption profile of FLX, CRB and ATZ was evaluated over time for 48 h. **Figure 32** shows the maximum extraction recovery values of the target contaminants using different eluents. Around 100 %, 98 % and 30 % of FLX, CRB and ATZ were recovered, respectively, using an aqueous solution with 50 % of acetonitrile. Acetonitrile solution was selected as the best extraction solvent, followed by 50 % ethanol and 50 % methanol solutions. Thus, FLX and CRB extraction from pine can be easily and successfully obtained using aqueous acetonitrile solutions, which is a rapidly and highly biodegradable solvent. However, higher concentration of acetonitrile solutions is needed to enhance the solubility of ATZ in the extraction solvent. In previous works [41] the use of methanol for ATZ desorption from synthetic polymers achieved 73.5 % as maximum desorption. A combination of toluene and methanol proved to be the best extraction solvent mixture to recover CRB from spent activated carbonaceous adsorbents [14]. Methanol was used for the recovery of FLX from nanofibers [42], achieving 100 % of desorption.

The concentration of salt (1 M and 3 M) was also tested to verify its effect on desorption. High salt concentrations reduced desorption of all molecules tested. This might be due to the saturation of the solution in which FLX, CRB and ATZ cannot be dissolved. On the other hand, a low recovery was obtained using deionized water, which means that the strength of the surface bonds was sufficiently strong to prevent a new equilibrium.

Future scope includes the selection of other suitable regeneration techniques (thermal, solvent and ultrasound) with even lower chemical impact than the use of organic solvents for regeneration of spent pine for resource reclamation and cost reduction.

Statistical analysis of desorption data is presented in **Figure 32**. For FLX and CRB the performance of all the extraction eluents differs from each other ($p < 0.05$). For ATZ the 50 % of acetonitrile aqueous solution revealed to be the one with the highest recovery achieved compared to the other studied solvents ($p < 0.0001$).

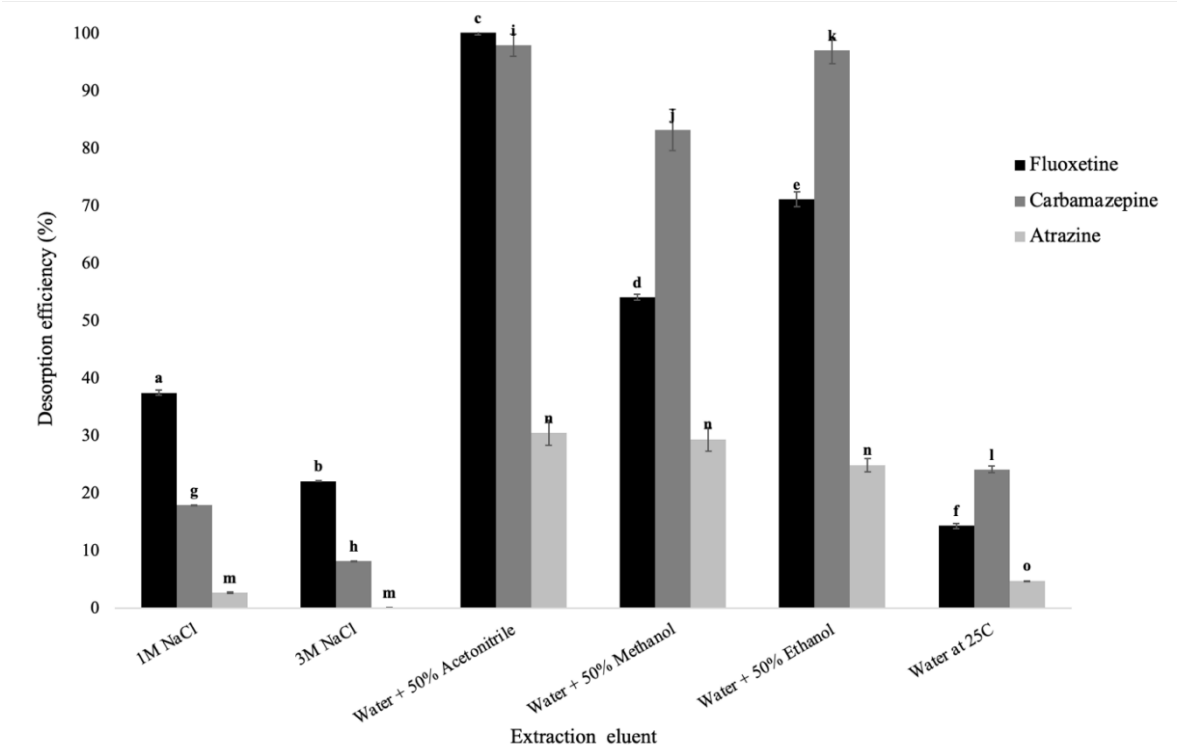


Figure 32. FLX, CRB and ATZ desorption from raw pine using different eluents over 24 h of experiment. Different letters show significant differences ($p < 0.05$) between extraction eluents for the same pollutant using the Tukey's test for multiple comparison. FLX (a-f), CRB (g-l) and ATZ (m-o).

4.3.6 Biosorbent recyclability

Besides the process cost and its potential environmental effects, the reuse cycle is also characterized by the level of sorbent reusability. With continuous cycles of adsorption and desorption, considerable financial and environmental benefits may be achieved [38].

The recycling efficiency of raw pine in the removal of FLX, CRB and ATZ was attested in three cycles. In this process, the biosorbent was dried and weighed before each adsorption or desorption tests to observe possible mass loss. At the end of three cycles, no significant mass losses were measured. **Figure 33** shows the achieved uptake of FLX, CRB and ATZ over the three consecutive adsorption steps, interleaved by desorption using 50 % of acetonitrile aqueous solution. It is interesting to observe that the adsorption capacity of all pollutants increased after the first desorption, around 32 % for CRB, 11 % for FLX and 8 % for ATZ. This can be due to a slight increase in porosity of pine due to the use of an organic solvent. The third adsorption cycle gave a similar efficiency trend for CRB, while for FLX and ATZ a decreased of around 30 % and 14 %, respectively, was observed. **Figure 34** represents FLX, CRB and ATZ desorption efficiency over the three desorption cycles. The third desorption cycle guaranteed an

extraction of a smaller amount of pollutants, around 50 % for FLX, 18 % for CRB and 52 % for ATZ. On this basis, it is possible to assert that pine can be regenerated and reused at least for two times, without any remarkable loss in its adsorption efficiency.

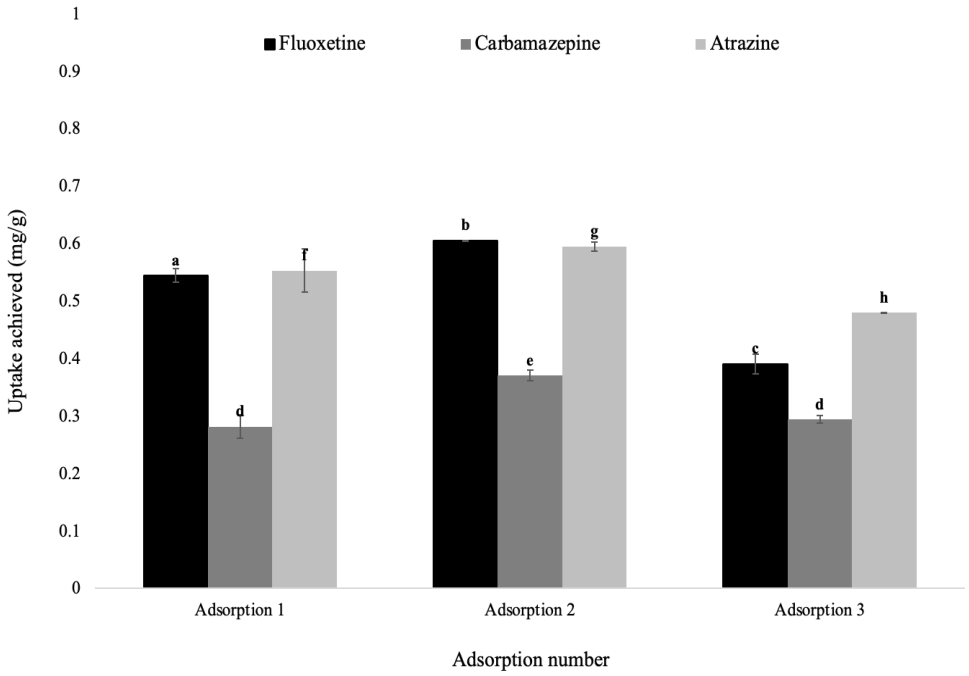


Figure 33. Maximum uptake achieved of FLX, CRB and ATZ over the three cycles of adsorption-desorption. Different letters show significant differences ($p < 0.05$) between the same pollutant over each adsorption process using the Tukey's test for multiple comparison. FLX (a-c), CRB (d-e) and ATZ (f-h).

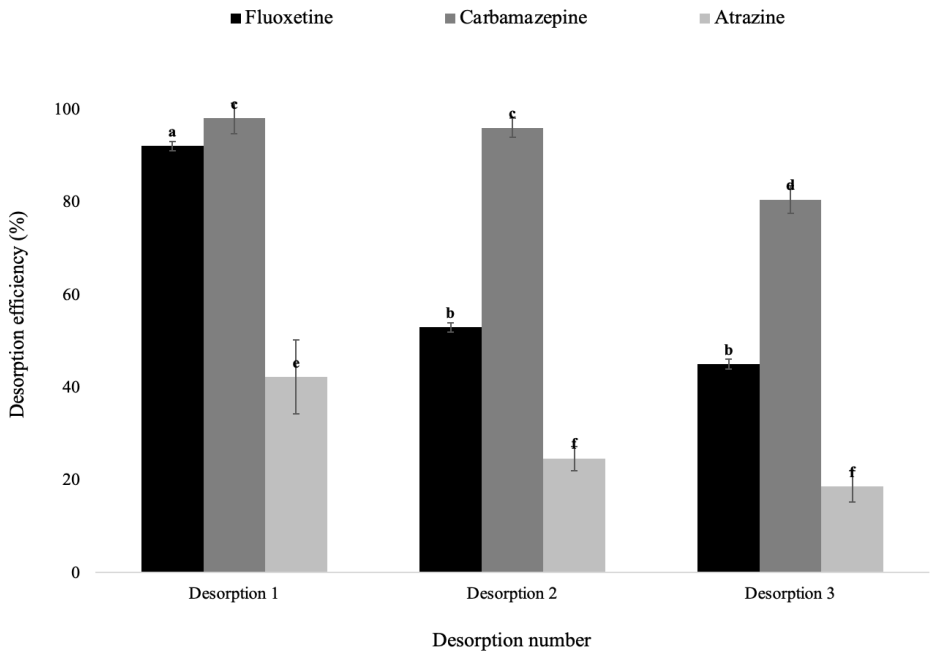


Figure 34. FLX, CRB and ATZ desorption over the three cycles of adsorption-desorption. Different letters show significant differences ($p < 0.05$) between the same pollutant over each desorption process using the Tukey's test for multiple comparison. FLX (a-b), CRB (c-d) and ATZ (e-f).

4.3.7 Air-lift reactor performance

To evaluate the behavior of the biosorbent obtained from *Pinus pinaster* biomass waste, in an up-scale adsorption system, a discontinuous experimental assay was performed using an air-lift reactor. In this experiment, the air-lift reactor was used as a batchwise homogenizer where pine bark is fluidized in the liquid medium with the gas input. The active contact between adsorbent and adsorbates is a prerequisite for high retention efficiency. The air-lift system has several advantages such as stable fluid motion due to separation of upward and downward flows, and high mass transfer efficiency of suspending particles due to fast fluid flow [43]. To the best of authors' knowledge, this is the first study in this kind of reactor using pine bark for the adsorption of FLX, CRB and ATZ.

The results obtained from the assay in the air-lift reactor are shown in **Figure 35**. The sorbent reached saturation in FLX after approximately 6 h, while it saturated in CRB in 124 h and in ATZ in 144 h. The adsorption capacity of FLX, CRB and ATZ in this system at equilibrium were 0.462, 0.275 and 0.447 mg/g, respectively. PFO and PSO models were selected to be fitted to the experimental data (**Table 14**). The assessment of the models tested revealed that the PSO equation provided the best

fitting to the experimental data for all investigated analytes considering the R^2 values (0.909–0.996). The calculated q_e values are also in agreement with the experimental ones. FLX has the highest adsorption rate, 70.05 min^{-1}, followed by CRB and ATZ, 0.876 and 0.197 min^{-1}, respectively. When comparing these k_2 with the ones obtained in the batch adsorption kinetics a significant increase was verified for all pollutants. Results support the effectiveness of FLX, CRB and ATZ adsorption under dynamic flow conditions. The air-lift reactor represents thus a viable up-scale rehabilitation design to treat water contaminated with pharmaceuticals and pesticides.

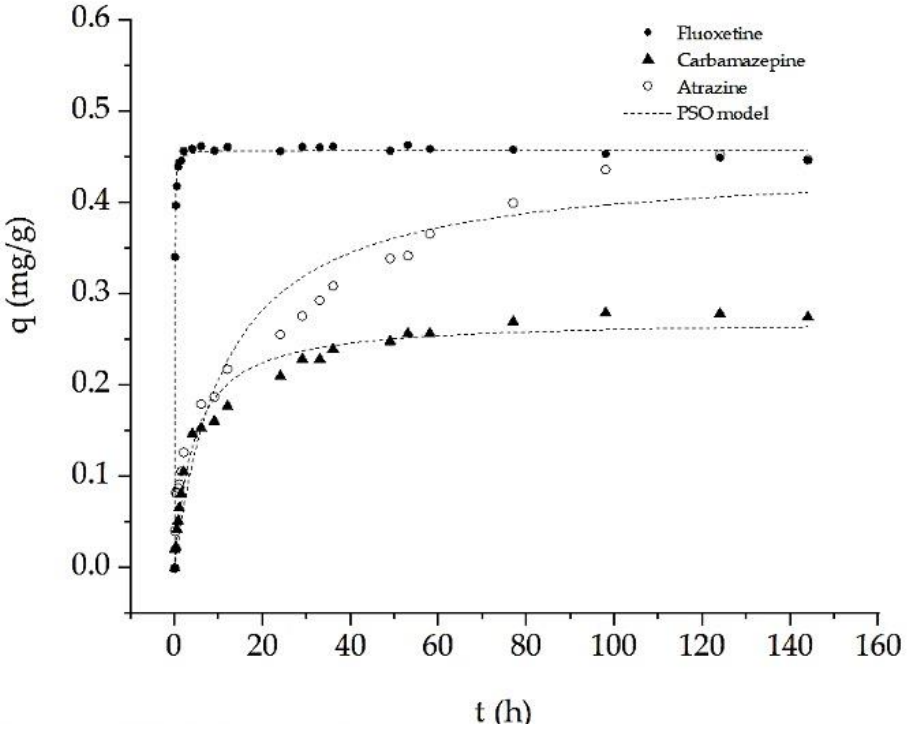


Figure 35. Adsorption of FLX, CRB and ATZ along time using an air-lift reactor. Experimental conditions: C= 5 mg/L of each pollutant, biosorbent dose 15 g/L, air flow 2 L/min.

Table 14. PFO and PSO model fittings to the adsorption of FLX, CRB and ATZ onto raw pine bark in an air-lift reactor system

Pollutant	PFO			PSO		
	q_e	k_1	R^2	q_e	k_2	R^2
Fluoxetine	0.451 ± 0.00321	15.8 ± 1.449	0.977	0.458 ± 0.00141	70.05 ± 4.319	0.996
Carbamazepine	0.249 ± 0.00736	0.174 ± 0.0246	0.938	0.272 ± 0.00572	0.876 ± 0.109	0.979
Atrazine	0.405 ± 0.0249	0.0514 ± 0.0109	0.859	0.444 ± 0.0273	0.197 ± 0.0609	0.909

q_e - adsorption capacity at equilibrium (mg/g); k_1 constant of the PFO model (min^{-1}); k_2- constant of the PSO model (g/(mg.min)); R^2- coefficient of correlation.

4.4 CONCLUSIONS

The present work displays pine bark as a promising biosorbent for the removal of active pharmaceutical ingredients and pesticides from aqueous solutions. The sequence of adsorption efficiency for targeted pollutants onto raw pine was as follows: FLX > ATZ > CRB, which correlated well with their pKa values and the electrostatic interactions with biosorbent. The kinetics of the adsorption process was well described by both PFO and PSO models. Rapid adsorption with quick attainment of equilibrium was verified for FLX (12 h). The same behavior was not verified for CRB and ATZ, for which equilibrium was achieved in 192 h.

Chemically and thermically modified pine sorbents were investigated to compare adsorption capacities, based on surface accessibility and affinity to adsorbates. Pine modified with HNO_3 revealed to be the best biosorbent in terms of overall pollutants removal (1.95 mg/g). The economic feasibility of raw and modified biosorbents was examined in the present study by a detailed cost analysis. The chemically modified biosorbents present substantially higher costs when compared to the ones thermally treated. The surface modification with H_2O_2 is the most expensive procedure, 23.7 €/mmol and hydrochar production the less expensive, 1.1 €/mmol, amongst the evaluated alternatives. The raw material presents the lower cost, 0.20 €/mmol and it is also the one that produces fewer solid and liquid wastes associated to its preparation, with a reduced environmental impact.

Pollutants desorption was evaluated with different extraction eluents, revealing an aqueous solution of 50 % of acetonitrile as the best desorbing agent. Pine bark recyclability potential was then assessed by consecutive cycles of adsorption- desorption in batch mode using the best desorbing agent for biosorbent regeneration. Results reveal that pine can be regenerated and reused as biosorbent at least twice, without any remarkable loss in its adsorption efficiency.

The adsorption of FLX, CRB and ATZ by the raw biomass was also evaluated under dynamic flow conditions in a pre-pilot air-lift type reactor. Results allowed to confirm the effectiveness and viability of the system to treat contaminated water for up-scale propose.

The study demonstrates that waste biosorbents may be successfully applied and be cost-effective for the removal of emerging pollutants from water. With the exploitation of forest wastes for adsorption applications, an environmental protection technology is promoted by pollution mitigation and waste valorization. It is expected that the information gathered in the present study contributes to the continuous search for sustainable and economical alternative biosorbents with possible recycling, able to compete with the commercially available sorbents for the removal of emerging pollutants.

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CHAPTER V

EVALUATION OF ATRAZINE ENTRAPMENT USING BACTERIAL BIOFILM SUPPORTED ONTO BIOWASTE IN LAB-SCALE PACKED BED BIOFILM REACTORS

The synergy between a bacterial biofilm supported by a sustainable material is studied, working both as a biobarrier for the treatment of water contaminated with ATZ. The rehabilitation system is evaluated in a PBBR using raw pine as biocarrier/biosorbent and *P. putida* or *S. equisimilis* biofilms. Bacterial adhesion onto biocarrier is firstly optimized to maximize the biofilm attachment and establishment. The ATZ toxicity towards *P. putida* and *S. equisimilis* and the relative growth rates are established. The bioremoval of ATZ by *S. equisimilis* per se is also assessed. Experiments using supported *P. putida* or *S. equisimilis* biofilms as biobarriers indicate an added beneficial effect towards the bioremoval of the selected herbicide. The process of elimination of the pollutant is attributed to the adsorption by the biofilm and by the carbon material, as well as to the degradation by bacteria.

This chapter is based on the following publication **(under submission)**:

Lago, A.; Silva, B.; Tavares, T. Sustainable permeable biobarriers for atrazine removal in packed bed biofilm reactors

5.1 INTRODUCTION

In parallel with the exploitation of sorption processes, the interest in biological procedures have increased due to their cost-effective and environment friendly character [1]. Previous studies have shown that the presence of bacteria in effluents favors the adsorption of organic compounds, through the adhesion of bacteria to the adsorbent surface. The establishment of a bacterial biofilm on a carbon sorbent carrier is reported to produce changes in the textural properties of the material and in its surface charge, as well as an increase of its hydrophobicity [2–6]. Besides, bacteria present a good potential for hazardous contaminants biodegradation and/or bioremoval, due to their ability to adjust to environmental changes [7]. With the conversion of biodegradable organic matter into biomass, CO_2 and residual products by microorganisms, the life of the carbon beds can be prolonged [8].

The performance of sorbents in the presence of a bacterial biofilm can be assessed in PBBR. The PBBR are filled with a support material followed by culture inoculation to form biofilm. Research with commercial carbon materials combined with bacteria to remove pollutants from solutions in PBBR has proven its efficiency and has numerous advantages [9]. However, the use of carbon sustainable materials as sorbents of emerging pollutants from aqueous solutions in the presence of bacteria may be limited [10–13].

With this background, the aim of this work is to investigate the behavior and efficacy of pine bark waste in the removal of ATZ from water, in the absence and in the presence of bacteria in lab-scale PBBR. Chemical and textural characteristics of the carbon sorbent carrier were considered in order to elucidate the process mechanism. In a first stage, batch experiments were performed to evaluate ATZ toxicity towards *P. putida* and *S. equisimilis*, when exposed to high concentrations of the herbicide and to establish the relative growth rates. The bioremoval of ATZ by *S. equisimilis* was then assessed with concentrated biomass aiming to explore its ability to significantly entrap the pollutant. In a second stage, a supported *P. putida* or *S. equisimilis* biofilms in lab-scale PBBR were tested as a bioremediation tool to increase the removal of ATZ, based on combined biosorption and biodegradation processes.

Results obtained contribute with novel data on: i) the adsorption mechanisms of ATZ on sustainable biomaterials; and ii) the effect of the presence of *P. putida* and *S. equisimilis* on the retention process. The present study is in line with contemporary bio-economy trends since it explores the use of biowaste materials and biological processes to rehabilitate effluents with an emerging pollutant.

5.2 MATERIALS AND METHODS

5.2.1 Bacterial strains, culture conditions and reagents

The bacterial strains *Streptococcus dysgalactiae subsp. equisimilis* (CECT 926) and *Pseudomonas putida* S12 were obtained from the Spanish Type Culture Collection — University of Valencia. Sterilized Brain Heart Infusion (BHI) and luria–bertani broth (LB) culture media were used for bacterial growth of *S. equisimilis* and *P. putida,* respectively. BHI and LB were selected as optimal growth media for each bacteria as reported in the literature [14,15].

Diluted aqueous ATZ solutions were prepared from a stock solution of 1 g/L of ATZ (PESTANAL® analytical standard, 99.1 %, Sigma-Aldrich, Missouri, EUA), prepared from powdered substance in acetonitrile (HPLC-grade, Fisher Chemical, Leicestershire, UK). This stock solution was stored at 3 °C before dilution to the working concentrations. Calibration curves in *S. equisimilis* bacterial culture media (for the evaluation of ATZ bioremoval by concentrated biomass) and in deionized water (for ATZ bioremoval evaluation in lab-scale reactors) were made. The mobile phases were prepared, HPLC-grade acetonitrile (Fisher Chemical) and ultra-pure water, obtained from a Milli-Q Millipore system (Merck, Darmstadt, Germany) for ATZ quantification by UHPLC-DAD, Shimadzu Corporation, Kyoto, Japan).

5.2.2 Preparation and characterization of biofilm carriers

Pine bark waste, obtained from a pine forest located in Braga, Portugal, was used in this study. It was previously ground and sieved in the range 1 mm to 2 mm. Then, it was washed with distilled water to remove impurities and dried at 60 °C. It was sterilized for 30 min at 121 °C before use as biofilm support.

To assess the bacterial adhesion to the pine material, its surface morphology was examined before and after the biofilm formation step, using a desktop SEM coupled with energy-dispersive X-ray spectroscopy (EDS) detector (Phenom-World BV, Netherlands)). All data were acquired using the ProSuite software integrated with Phenom Element Identification software, allowing the quantification of the elements present in the samples, expressed in either weight or atomic concentrations. Samples were added to aluminium pin stubs with electrically conductive carbon adhesive tape (PELCO Tabs™) on a Phenom Standard Sample Holder (SH) at 5 kV and a spot size of 3.3. Different points were analysed for

elemental composition. EDS analyses were conducted at 15 kV and spot size of 5.1. For SEM characterization, samples were observed with gold coating.

5.2.3 Quantification of ATZ

ATZ concentration was quantified by UHPLC-DAD, following the same procedure and conditions described in section 3.2.4 of Chapter 3. The linearity of the method was assured by setting two different calibration curves performed in bacterial media (BHI) and deionized water over the concentration ranges of 0.3–12 mg/L.

5.2.4 Measurement of bacterial cell growth

The growth of *S. equisimilis* and of *P. putida* was established by the intensity of optical density at 620 nm (OD_{620}) using a T60 UV-Visible Spectrophotometer (PG Instruments, Leicestershire, UK). The OD_{620} values were converted into biomass concentration (g/L) by using a bacterial calibration curve for each strain, obtained by the dry weight method [16]. A triplicate of 5 mL of bacterial suspension collected in the middle of the exponential growth phase was filtered through sterile 0.2 µm filter paper (Whatman, Maidstone, UK) and dried during 48 h at 105 °C. After cooling to room temperature, the dry weight of bacteria was calculated by the difference between the weights of blank filter papers (wetted with deionized water) and filter papers with bacteria cells. The relationship between OD_{620} and the biomasses of *S. equisimilis* (**Figure 36**) and *P. putida* (**Figure 37**) was established using the following equations 1 and 2, respectively:

$$S.equisimilis \text{ biomass (g/L)} = 0.2131 \times \mathrm{OD}_{620} - 0.00127 \ (\mathrm{R}^2 = 0.9995) \quad (1)$$

$$P.putida \text{ biomass (g/L)} = 0.431 \times \mathrm{OD}_{620} + 0.0046 \ (\mathrm{R}^2 = 0.9932) \quad (2)$$

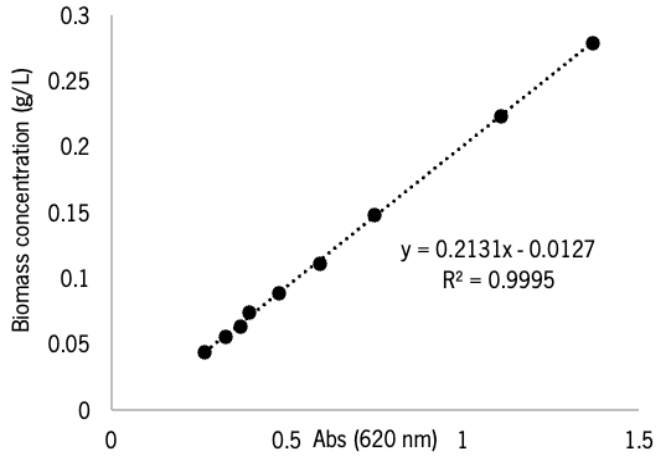


Figure 36. *S. equisimilis* calibration curve (OD_{620} versus biomass concentration (g/L)).

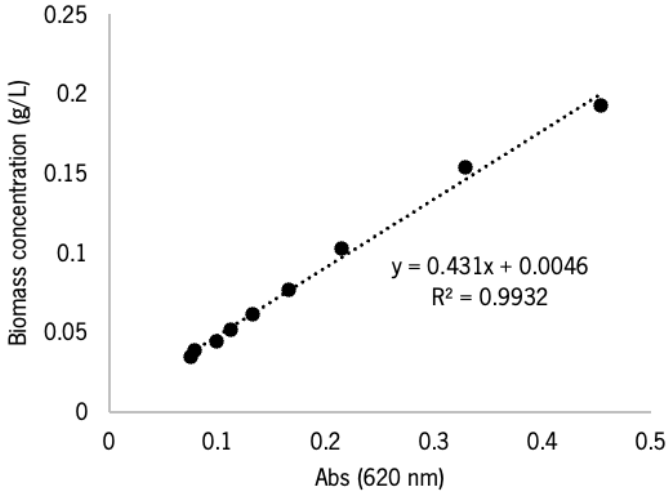


Figure 37. *P. putida* calibration curve (OD_{620} versus biomass concentration (g/L)).

5.2.5 Toxicity evaluation

To elucidate about the toxicity of ATZ towards *S. equisimilis* and *P. putida*, the bacterial growth (g/L) in the presence of the xenobiotic was monitored over time by OD_{620} measuring.

For bacterial pre-inoculum preparation, *S. equisimilis* and *P. putida* strains were transferred from Petri dishes cultures to 250 mL Erlenmeyer flasks containing BHI and LB broth media (100 mL), respectively. Bacterial suspensions were grown for 12 h at 30 °C and 140 rpm in a rotatory shaker. Erlenmeyer flasks containing BHI and LB broth media were inoculated with 6.6 % (*v/v*) of *S. equisimilis* and 13.3 % (*v/v*) of *P. putida* pre-inocula (0.1 g/L of biomass), for final volumes of 150 mL of the

suspensions. The target herbicide (from the stock solution in acetonitrile) was then spiked to suspensions to obtain initial concentrations of 10 mg/L, 25 mg/L, 50 mg/L, 80 mg/L and 100 mg/L.

Flasks were incubated at 30 °C under constant shaking during 48 h. Periodic samples were collected in sterile conditions and centrifuged at 13000 rpm (Eppendorf MiniSpin 9056, Merck) for 8 min, being the pellet resuspended in deionized water for OD_{620} quantification. Absorbance values were then converted into biomass concentration. Flasks inoculated with *S. equisimilis* and *P. putida* cultures and non-inoculated flasks were used as biotic and abiotic controls, respectively. The abiotic control set was used to investigate possible volatilization and hydrolysis of herbicide in the absence of bacterial cells [17]. Each assay was conducted in triplicate and the average and SD were reported for each test. The experiments were conducted in the absence of light to avoid possible photolysis of the herbicide [18] and the Erlenmeyer stoppers were covered with aluminum foil to avoid possible evaporation.

5.2.6 Bioremoval capacity of ATZ by *S. equisimilis*

5.2.6.1 Preparation of the concentrated biomass

S. equisimilis pre-inoculum was prepared following the same procedure mentioned in 5.2.5, with the difference of supplementing the media with 10 mg/L of ATZ during 12 h. Then, 6.6 % (*v/v*) of *S. equisimilis* was transferred to 150 mL of BHI media and kept growing for 24 h. The cells were harvested by centrifugation at 13000 rpm for 8 min and washed with a saline solution (0.9 %) to remove traces of ATZ from the pre-inoculum and then centrifuged again. The biomass pellet was then re-suspended in a pre-established volume of the culture medium in order to obtain the desired concentrated suspension to be used in the bioremoval assays.

5.2.6.2 Bacterial bioremoval assessment

Experiments with concentrated biomass were performed to determine the percentual bioremoval of ATZ by *S. equisimilis*. A working volume of 150 mL of sterilized BHI media was supplemented with 7.5 mg/L of ATZ. Flasks were inoculated with 4-5 g/L of biomass pellet and kept in a rotary shaker incubator during 15 days under the same incubation conditions mentioned in the previous section. Samples were withdrawn from the suspensions to measure ATZ decay (%). The abiotic control was also monitored during experiment time. Assays were conducted in triplicate and the average and SD were reported for each test.

At the end of the assay and after being exposed to ATZ, the viability of *S. equisimilis* was verified by sampling it in Petri plates with BHI medium. The presence of possible microbial contaminations was also scanned.

5.2.7 PBBR experiments: start-up and operation conditions

5.2.7.1 Optimization of microbial cell adhesion onto biocarrier

The immobilization of bacteria via adsorption can be disturbed by different variables, such as environmental factors and cell and carrier surface properties [19–22]. *S. equisimilis* was used as a bacteria model for this optimization process. Also, pine bark carrier surface properties were kept unchanged. The mass of the biocarrier (53 g) in the column was fixed to ½ of the column height (30 cm).

To enhance bacteria adhesion onto the biocarrier, different operational conditions were tested. In aquatic environments, the microbial attachment depends on the velocity flow [23], on water temperature [24] and on nutrient concentrations [25,26]. In this study, pH and temperature were settled at the optimum bacteria growth values. Parameters as initial biomass concentration, inoculum growth time and hydrodynamic conditions, as contact time and recirculation flow of bacterial suspension into the PBBR were tested. The recirculation of 1 L of diluted BHI media at 5 mL/min, added to the bacterial suspension on the PBBR was performed during 24 h in each tested condition. **Table 15** describes the conditions tested for each assay. The bacterial flow rates established in this optimization procedure (10 and 15 mL/min) were selected since high values allow the formation of a compact biofilm, more resistant to the erosion stress promoted by hydrodynamic forces.

The best operational parameters that allow the strongest biofilm attachment were selected for the sequential bioremoval assays with *S. equisimilis* and with *P. putida* in the PBBR. Samples of the different biocarriers were collected at the end of the operation and visualized by SEM, for a qualitative analysis.

Table 15. Operational conditions tested for S. equisimilis growth and biofilm establishment on the support, in a PBBR

Operational variables	Condition 1	Condition 2	Condition 3	Condition 4
1°) Initial biomass concentration	1 g/L	2 g/L	2 g/L	2 g/L
2°) Inoculum growth time	12 h	24 h	36 h	24 h
3°) Contact time of the bacterial suspension with the biocarrier	60 h	48 h	48 h	72 h
4°) Recirculation flow of bacterial suspension on the PBBR	10 mL/min	15 mL/min	15 mL/min	15 mL/min

5.2.7.2 Bioremoval in descontinuous flow at lab-scale reactors

After the biofilm optimization process, ATZ bioremoval assays with pine bark as fixed bed sorbent supporting *P. putida* or *S. equisimilis* biofilm in PBBR were carried out. Control assays were performed just with the support. The experimental setup consisted of six acrylic PBBR columns (height: 30 cm, internal ø: 4 cm), two columns filled with 53 g of pine and the other four with the same amount of pine covered with supported biofilm, either *S. equisimilis* or *P. putida*. The operational condition 4, as previously described in 5.2.7.1. was selected for the biofilm development. An inoculated culture medium (with 2 g/L of initial bacterial concentration) was fed from a container maintained in a water bath, kept in shaking at 37 °C for *S. equisimilis* and at 30 °C for *P. putida*, and passed through each column in closed-loop for 72 h at 15 mL/min using a peristaltic pump. After this time 1 L of diluted BHI and LB media for *S. equisimilis* and *P. putida*, respectively were added to the culture suspension and let for more 24 h in closed-loop, after which the media were completely washed out. **Figure 38** shows the laboratory set-up of the PBBR columns.

ATZ solutions of selected concentration were then loaded upwards (C_0 = 5 mg/L) through the six columns at 1 mL/min. The assay was conducted in discontinuous mode for 15 days. The ratio of solid support and pollutant solution was fixed at 15 g/L. The pollutant concentration in the outflow was periodically assessed by UHPLC-DAD. The experiments were conducted in duplicate and the results are an average of duplicates. At the end, the column reactors were washed out and samples of biocarrier covered with *P. putida* or *S. equisimilis* biofilm were seeded in Petri plates with BHI or LB media,

respectively, in order to evaluate the viability of the bacteria after being exposed to ATZ and to scan for the presence of possible microbial contaminations.



Figure 38. Laboratory set-up of the PBBR columns using pine as support, during biofilm formation.

5.2.8 Statistical analysis

Statistical analysis of the experimental data was carried out using GraphPad Prism® software (version 8.0; GraphPad Software, Inc., San Diego, CA, USA). One-way ANOVA through the Tukey's test multiple comparison was used to compare tests between the control and parameters. The variations were considered statistically significant at a confidence interval of $p < 0.05$.

5.3 RESULTS

5.3.1 Characterization of the biological samples

Figure 39A and **Figure 39B** show the micrographs obtained by SEM of raw pine. Pine samples present a fiber structure, constituted of small pits with different shapes and sizes, and irregular surfaces.

The existence of several waxy, suberized layers may contribute to the hydrophobic character of pine. Also, some diethyl ether and methanol extractables interfere with water penetration [27].

The viability of an adequate biofilm to be formed on subtracts depends mainly on the chemical nature of the surfaces, as well as on their extension since bacteria cannot reach the narrower porosity but just the larges pores [28]. It is also referred that biofilm adhesion occurs readily on rough and hydrophobic surfaces [29]. Pine have thus an appropriate external surface morphology to accommodate bacteria.

The first step for the formation of a biofilm on a carrier is the fixation of the bacteria on the surface of the support. For that, different operational conditions for *S. equisimilis* biofilm adhesion and development were tested in this study. SEM images (**Figure 39C**, **Figure 39D**, **Figure 39E** and **Figure 39F**) show that the biofilm is present on the external surface and on the wider porosity of the material. The sample from the operational condition 4 (**Figure 39F**) has the biggest amount of *S. equisimilis*, retained from the initial suspension after 72 h of contact. As can be observed the bacterial adhesion and development in the support structure is more uniform, with a well-developed and established biofilm over the pine surface. The textural properties of the materials seem to be modified by the obstruction of the porosity by the bacteria. **Figure 39F** also shows that large cellular aggregates of *S. equisimilis* are enclosed in an EPS matrix [30,31]. This mixture is composed of polysaccharides, proteins and extracellular DNA. These matrices are important for biofilm cells intercommunication, 3D biofilm structure formation and multicellular living [30,32]. The initial concentration of biomass was duplicated from condition 1 (**Figure 39C**) to condition 2 (**Figure 39D**). In fact, a suspension with high concentration of biomass is used to induce the attachment on the surface by EPS. On condition 3 (**Figure 39E**) the irreversibly attached bacteria cells are going through the multiplication process, forming small aggregates called microcolonies. During this stage, quorum signals are released to underlying EPS production [30].

After selected condition 4 as the most adequate to fixe *S. equisimilis,* the adhesion of *P. putida* at the same conditions was also evaluated (**Figure 39G**). According to the micrographs *P. putida* has colonized onto pine and an established biofilm was also created.

Figure 39H and **Figure 39I** show the *S. equisimilis* and the *P. putida* biofilm, respectively, after the ATZ bioremoval. SEM analyses revealed still the presence of a well-established biofilm at the end of the lab-scale experiment for both bacteria, indicating that the total amount of ATZ retained was not enough to deteriorate bacteria, as predicted by the toxicity tests. The resilience of *S. equisimilis* to grow in very hazardous and toxic conditions was also observed by Costa and Tavares [33] and Silva *et al.* [1], when

evaluating its bioremoval capacity towards multi-component solutions containing inorganic and organic solutes.

The pine cellular wall keeps its original shape during these assays, although it could be selectively degraded by the bacteria decomposition. Therefore, physical, chemical and morphological alterations in the lignocellulosic material can occur, making the cellular wall components accessible to aqueous solutions and providing a broad surface area for the biosorption process [34].

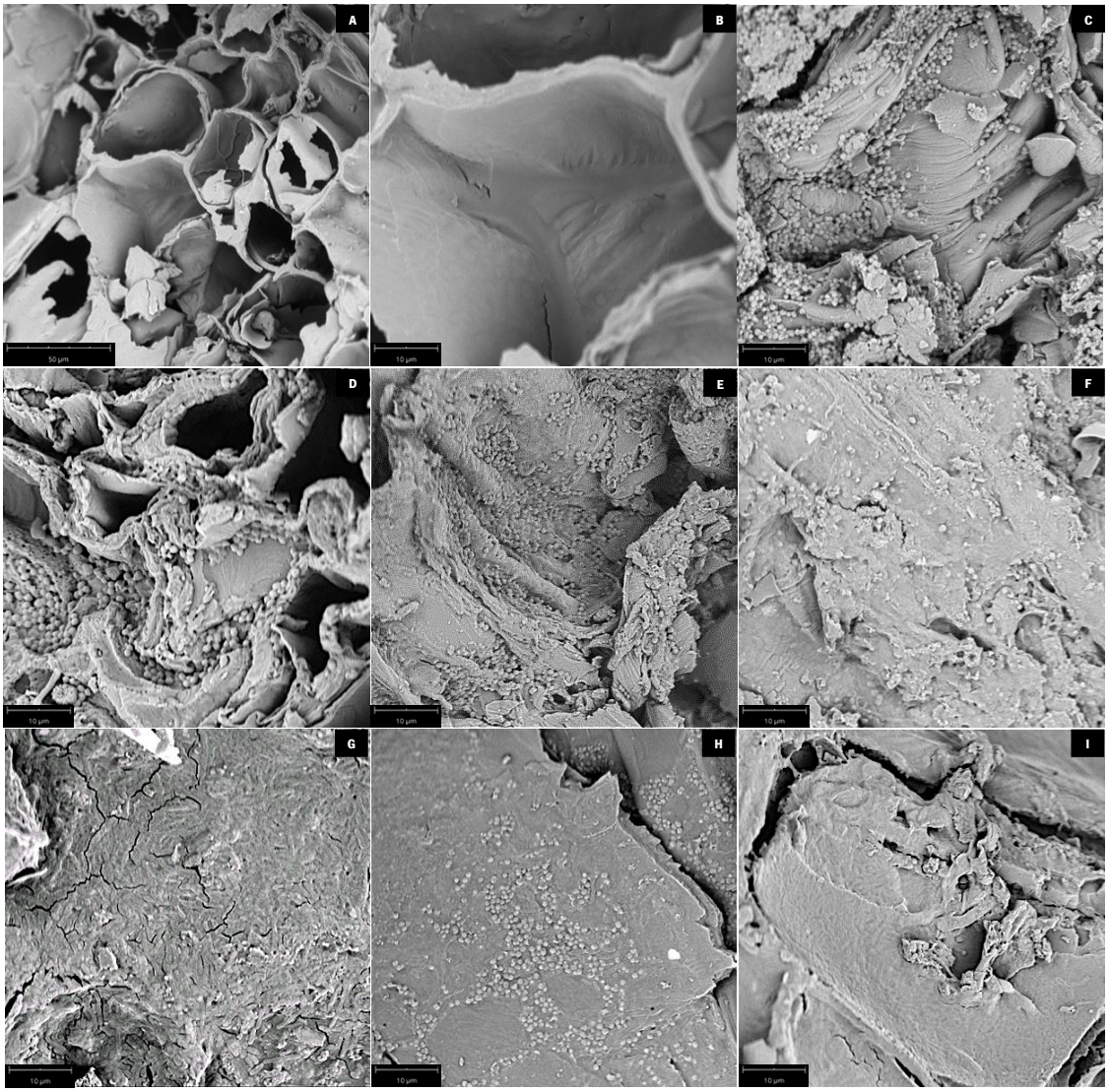


Figure 39. SEM micrographs of raw pine at 1500x (A) and 4500x (B) of magnification; pine covered with *S. equisimilis* at condition 1 (C), condition 2 (D), condition 3 (E) and condition 4 (F); pine covered with *P.putida* at condition 4 (G); *S. equisimilis* (H) and *P. putida* (I) biofilm on pine after ATZ bioremoval studies on lab-scale reactors, at 4500x of magnification.

The semi-quantitative elemental analysis (wt %) of a pine sample is shown in **Table 16**. The carbon and oxygen relative contents are due to the nature of the biowaste, since being a natural lignocellulosic biomass it has cellulose, hemicellulose and lignin units in its constitution.

Table 16. Weighted average elemental composition (wt %) of pine biocarrier

Element	wt %
C	56.84
O	42.38
Ca	0.40
K	0.09
S	0.06
Mg	0.06
Na	0.06
Al	0.04
P	0.03
Si	0.03
Cl	0.01

5.3.2 Effects of ATZ on bacterial growth

Bacterial toxicity measurements based on growth are widely used for providing information regarding the harmfulness of a compound according to the growth inhibition of the bacteria [35]. To elucidate on the toxicity and effect of ATZ (in the presence of acetonitrile) towards *S. equisimilis* and *P. putida,* the bacterial growth rates were assessed in the presence of a range of high concentrations of ATZ (from 5 mg/L to 100 mg/L). The experiment allowed to evaluate the effect of the pollutant on the bacteria cell growth and to assess the eventual growth stimulation or inhibition profile. Assays for tolerance evaluation are usually the first step to access the microbial biodegradation capacity, once microorganisms need to overcome the physiological stress caused by the xenobiotic [14,36]. *P. putida* is reported as a degrading-ATZ bacterium [37] and was used as a comparison organism.

S. equisimilis and *P. putida* growths in the presence of different amounts of ATZ were assessed by measuring biomass concentration (g/L) over 48 h and are represented in **Figure 40** and **Figure 41**, respectively. Both bacteria showed variations in resistance towards the xenobiotic. **Figure 42** shows the

relative growth rate (based on each control) achieved for both bacteria and for the tested toxicant amounts. Bacteria frequently endure stress conditions caused by herbicides application, but they developed the ability to sense the stress signal through a process in which many enzymes are involved [38]. The application of 5 mg/L of the herbicide induced a stimulation on microbial biomass on both species, 3.37 % in *S. equisimilis* and 28 % in *P. putida*. Still, the growth rate was increased in 47 % for *S. equisimilis* and in 74 % for *P. putida*. At 10 mg/L the growth rate was similar to control for *S. equisimilis*, however for *P. putida* was improved in 14 %. For concentrations of 25 mg/L the growth was inhibited in 16 % for *S. equisimilis* and in 39 % for *P. putida.* This bacterium is significantly disturbed by ATZ doses of 50 mg/L ($p \leq 0.001$) and superior ($p \leq 0.0001$). The growth of *S. equisimilis* was significantly affected at 80 mg/L ($p \leq 0.01$) and 100 mg/L ($p \leq 0.001$). At 48 h of assay, 95 % and 96 % (**Figure 40** and **Figure 41**) of growth inhibition at 100 mg/L is achieved for *S. equisimilis* and for *P. putida,* respectively. For concentrations equal and higher than 25 mg/L, the growth performance of *P. putida* is considerably minor, although it revealed a better tolerance with 5 and 10 mg/L of the herbicide. Under these experimental conditions *S. equisimilis* tolerates higher amounts of ATZ than *P. putida*.

It is true that not all species have equal sensitivity to a toxicant. The same compound can produce a distinct response in Gram-positive and Gram-negative bacteria, as the antioxidant enzyme levels differ greatly between both. Zhang *et al.* [38] reported Gram-negative bacterium *E. coli* K12 to have higher sensitivity to ATZ stress in comparison to Gram-positive bacterium *B. subtilis* B19, becoming thus the Gram-positive a more suitable organism for studies concerning the effect of ATZ tolerance. The relative less sensitivity of *S. equisimilis* to ATZ can be explained by its Gram-positive character over the Gram-negative *P. putida.* ATZ exhibits genotoxicity by causing single- and double-strand breaks in DNA through the formation of ROS [39]. The ROS generated by ATZ and its metabolites can damage bacterial cells and decrease bacterial growth. Polyunsaturated fatty acids are attacked directly by ROS, resulting in lipid peroxidation [40] and causing decreased membrane fluidity, specific ion permeability and activity of membrane receptors and enzymes [41]. Besides, oxidative stress might cause severe damage to DNA by specific disruption of carbohydrate components and nitrogen bases, resulting in breakage of the DNA backbone, formation of adducted nucleotides, genetic alterations, molecular cross-linking, chromosomal abnormalities and blockage of DNA replication [42].

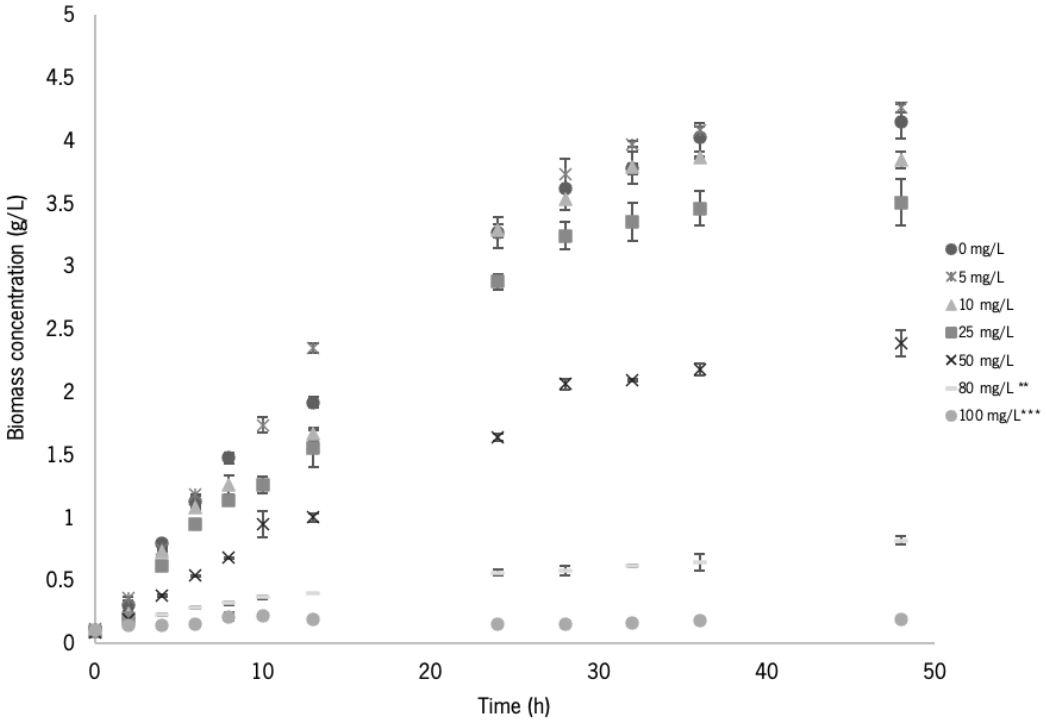


Figure 40. Growth kinetics of *S. equisimilis* presented in biomass concentration (g/L) at different ATZ concentrations (5 - 100 mg/L). Error bars represent the SD of the mean (n = 3); Asterisks represent statistical significance compared to control, ** — $p \leq 0.01$, *** — $p \leq 0.001$.

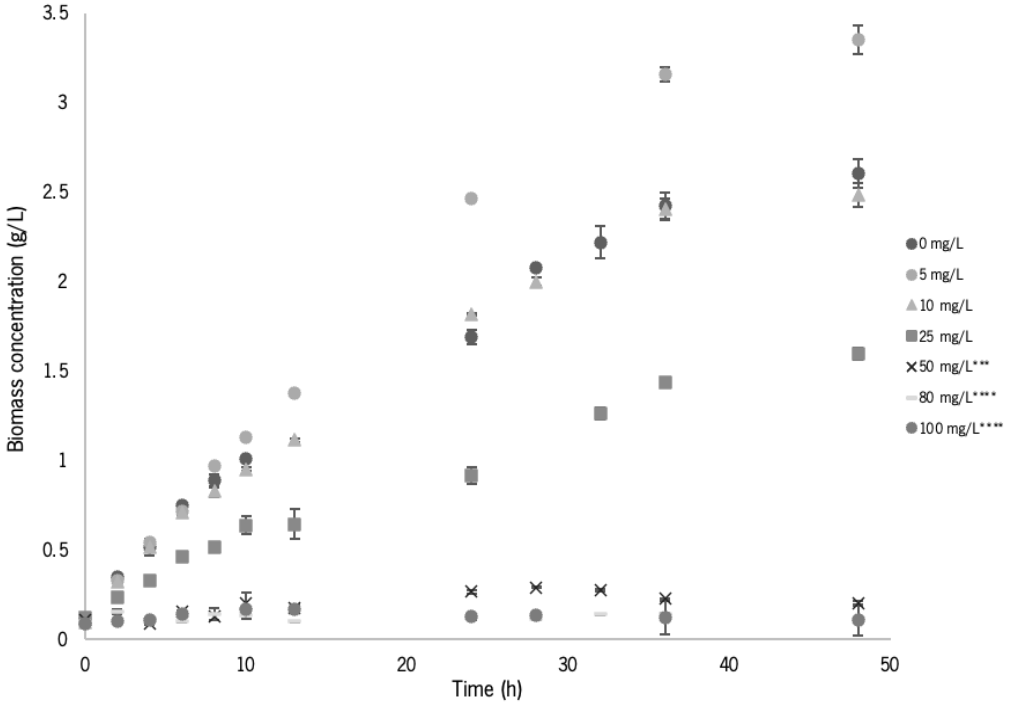


Figure 41. Growth kinetics of *P. putida* presented in biomass concentration (g/L) at different ATZ concentrations (5 - 100 mg/L). Error bars represent the SD of the mean (n = 3); Asterisks represent statistical significance compared to control, *** — $p \leq 0.001$, **** — $p \leq 0.0001$.

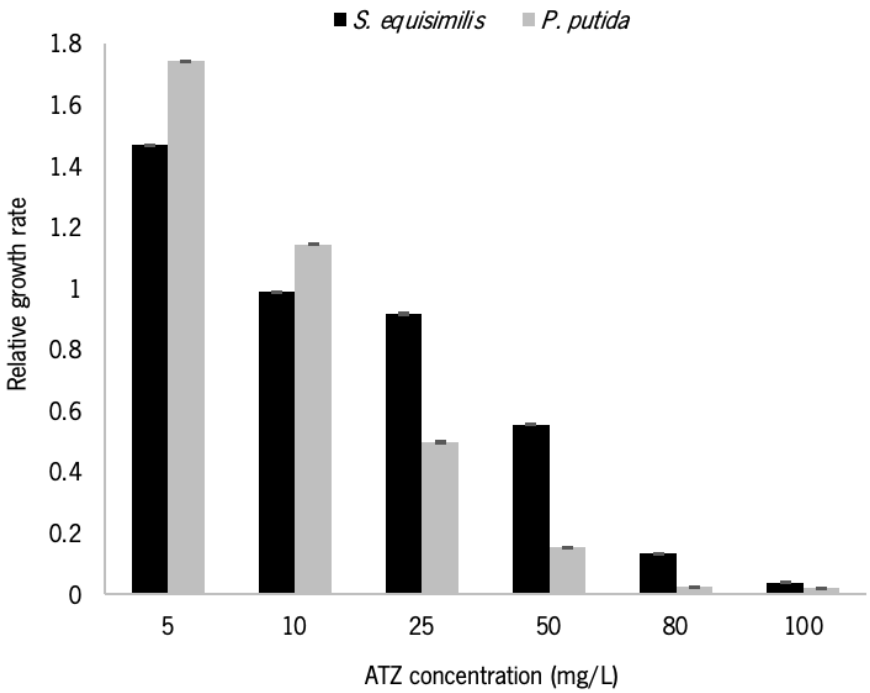


Figure 42. Relative growth rate of *S. equisimilis* and *P. putida* at different concentrations of ATZ. Error bars represent the SD of the mean (n = 3).

5.3.3 ATZ bioremoval potencial by concentrated *S. equisimilis* biomass

The present study evaluates the bioremoval capacity of *S. equisimilis* biomass when exposed to 7.5 mg/L of ATZ. The mechanisms involved in the removal of pesticides by microorganisms include biodegradation, biosorption, volatilization, hydrolysis and photolysis. ATZ photolysis is not expected to occur as although the molecule degrades under the influence of sunlight to a range of dechlorinated and dealkylated products, the reaction is very slow with an estimated half-life in water of about a year. ATZ may be transformed to hydroxyatrazine by hydrolysis, but the reaction is also very slow in sterile water. This transformation is more rapid in soils, where hydrolysis is believed to be catalyzed by acidic groups present within the soil organic matter to which ATZ adsorbs. The loss through volatilization is also expected to be negligible, due to its low Henry's Law constant that can be calculated to be 2.63 x 10^{-9} atm-cu m/mole, which indicates ATZ volatilization from water will not be considerable important [43]. In fact, some studies [44,45] defended that this herbicide is not removed via volatilization and hydrolysis. To corroborate this thesis, no removal of ATZ was observed in the control (data not shown) during the experimental period, suggesting that the main mechanisms involved in the removal of ATZ are biodegradation and biosorption.

Biodegradation of ATZ by microorganisms is a multistep process leading to the formation of hydroxy, dechlorinated or dealkylated by-products like hydroxyatrazine, deethyl, or deisopropyl ATZ, which can be further transformed into ammeline, ammelide or cyanuric acid and completely mineralized. Microbes have been used to remove ATZ and 14 strains of bacteria that biodegrade ATZ were identified so far [46]. On the other hand, biosorption is the process of adherence of contaminants to the cell wall of biological agents and it does not involve oxidation via aerobic or anaerobic metabolism. Both living and dead cells can serve as biosorbents [47].

The results in **Figure 43** show that the bioremoval of ATZ (7.5 mg/L) by *S. equisimilis* biomass can reach circa 83.5 % after 15 days of assay. ATZ could be potentially degraded by the biomass and biosorbed to the solids. Indeed, both mechanisms are expected to be responsible for the ATZ bioremoval by *S. equisimilis* biomass. In the work of Jan *et al.* [48] ATZ degrading microorganisms were isolated from soil and identified and in fact, among the bacterial isolates the highest degradation potential (62.77 %) was shown by *Streptococcus* specie. The biodegradation ability of concentrated *S. equisimilis* for the treatment of aqueous solutions containing organic solvents was also accessed by other authors [1,14], demonstrating its biodegradation capacity for other kind of pollutants.

S. equisimilis is reported as a facultative anaerobe [49] so, even experiments were conducted in Erlenmeyers with stoppers and covered with aluminum foil bacteria was able to growth and maintained viable at the end of the assay. **Figure 44** shows the viability of *S. equisimilis* without and after being exposed to ATZ during 15 days of assay. No microbial contaminations of the bacterial samples were detected.

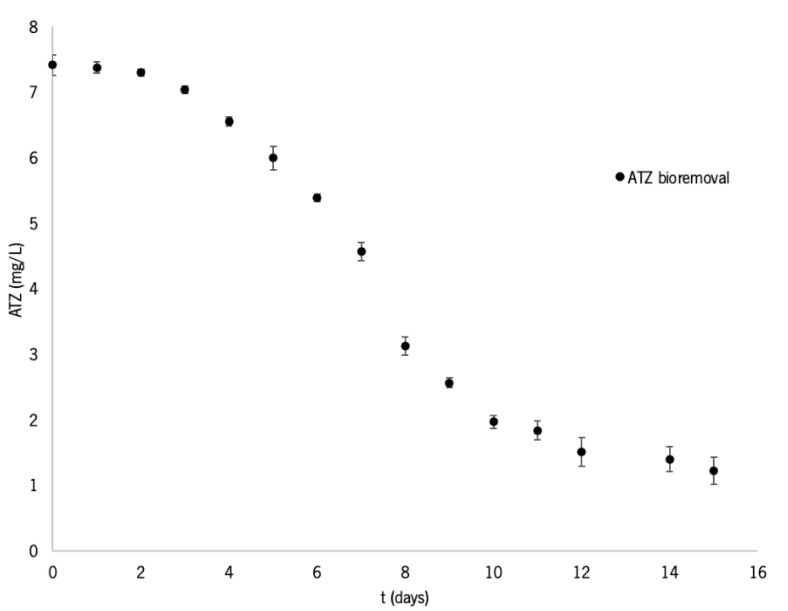


Figure 43. Bioremoval of ATZ (7.5 mg/L) by concentrated *S. equisimilis* biomass (5 g/L) for 15 days. Error bars represent the SD of the mean (n = 3).

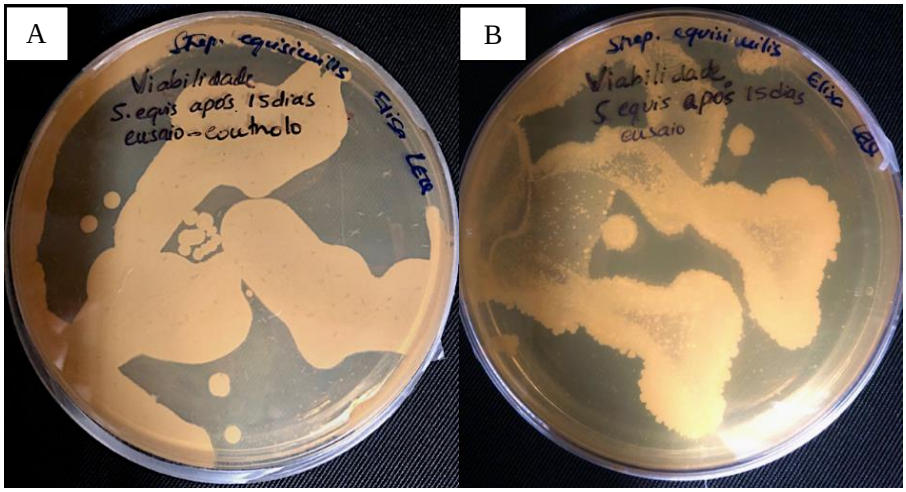


Figure 44. Viability of *S. equisimilis* after 15 days of assay, A) control without ATZ and B) exposed to ATZ.

5.3.4 PBBR performance evaluation

Fixed biofilm systems have been used widely in the treatment of wastewater, particularly in the attainment of secondary effluent standards. In fact, bacterial cell attachment to an adsorbent facilitates the removal of chemicals [2]. The reactors using biofilms are an advantage compared to the conventional treatment since contaminants in highly concentrated solutions are treated over long periods. In the PBBR, the necessary oxygen is supplied to the biofilm through the water. The support is fixed within the reactor and the biofilm is allowed to grow on it. There exists a strong interface between the liquid and the biofilm [50]. The contaminants present in the water are removed as the biofilm entrap them.

The bioremoval curves (C/C_o versus t) obtained with C_0 = 5 mg/L were measured with a bed height of 30 cm. The assays were performed on discontinuous mode as a starting evaluation of the system. Experiments using pine (**Figure 45**) and pine supporting *S. equisimilis* or *P. putida* biofilms (**Figure 46**) were performed until equilibria were reached. Results indicate that the best performing system was the one with supported *S. equisimilis* biofilm, reaching 90.42 % of ATZ removal, followed by the sorbent with supported *P. putida,* with 79.71 % removal and lastly just the pine, achieving 58.31 % removal. Equilibrium time for sorption on pine was attained in 96 h and using bacterial biofilms, it was reached in 264 h for *S. equisimilis* and 288 h for *P. putida.*

The removal rate of ATZ with pine sorbent is faster at the beginning of the process and it slows down as it approaches equilibrium, meaning that the adsorption is carried out early and quickly on the accessible external surface and then slower in the internal porous structure, less accessible. On the bioremoval studies with the bacterial biofilms supported on pine, significant changes in the rate of ATZ removal are observed. In both cases, the bioremoval can be described in three stages. Firstly, the process of biosorption occurs onto the pores that remained available after the biofilm formation. Then, once these sites became saturated, a second stage occurs with an exponential behavior attributed to the entrapment of the herbicide into the biofilm, in such a way that the bacteria begin to swell to the point where they become saturated. Lastly, the degradation of the compound by bacteria occurs to keep its continuous growing. These experiments revealed that *S. equisimilis* and *P. putida* are able to efficiently grow as well as to retain and degrade the herbicide, as it may be observed by their metabolic viability at the end of 15 days of assay (**Figure 47**).

Thus, is suggested that the process of elimination of the pollutant can be attributed to a sorption process onto the surface of the supporting material, onto the biofilm and to the biodegradation by bacteria. In general, separation of the biodegradation step and the biosorption step is not feasible [17].

Actually, the coupling of biosorption/biodegradation favors the bioremoval process significantly. These results suggest that the bacterial system could be used to treat relevant concentrations of ATZ, although equilibrium is reached slower than just with pine which may become a disadvantage. The difference on the removal percentage of ATZ indicates that the presence of a bacterial biofilm enhances the rehabilitation process, representing a beneficial effect towards the retention of the selected herbicide.

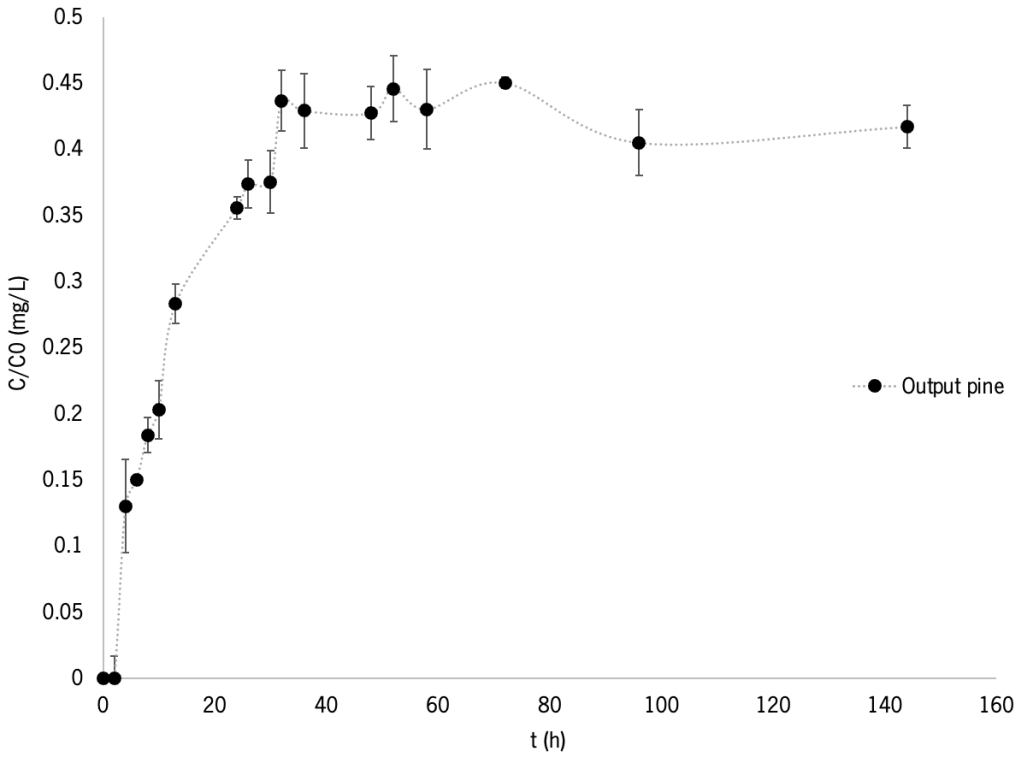


Figure 45. Bioremoval of ATZ using pine as sorbent in a discontinuous flow packed bed reactor.

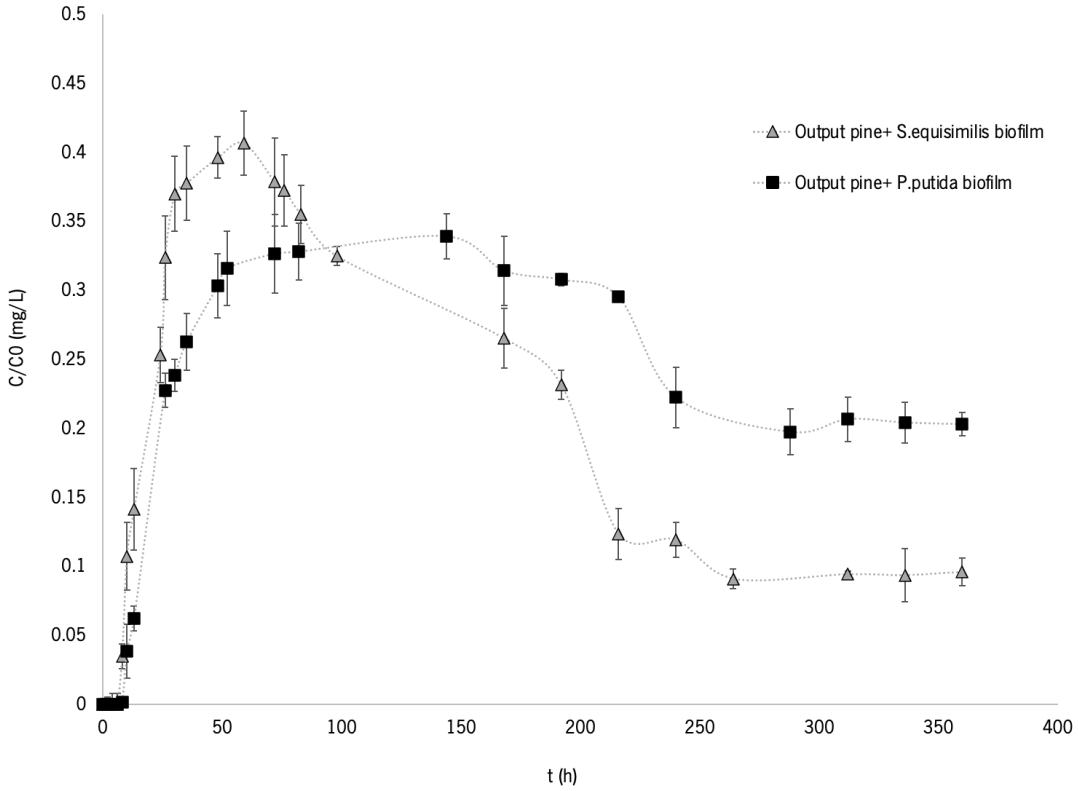


Figure 46. Bioremoval of ATZ using *S. equisimilis* and *P. putida* biofilms supported on pine in a discontinuous flow PBBR.

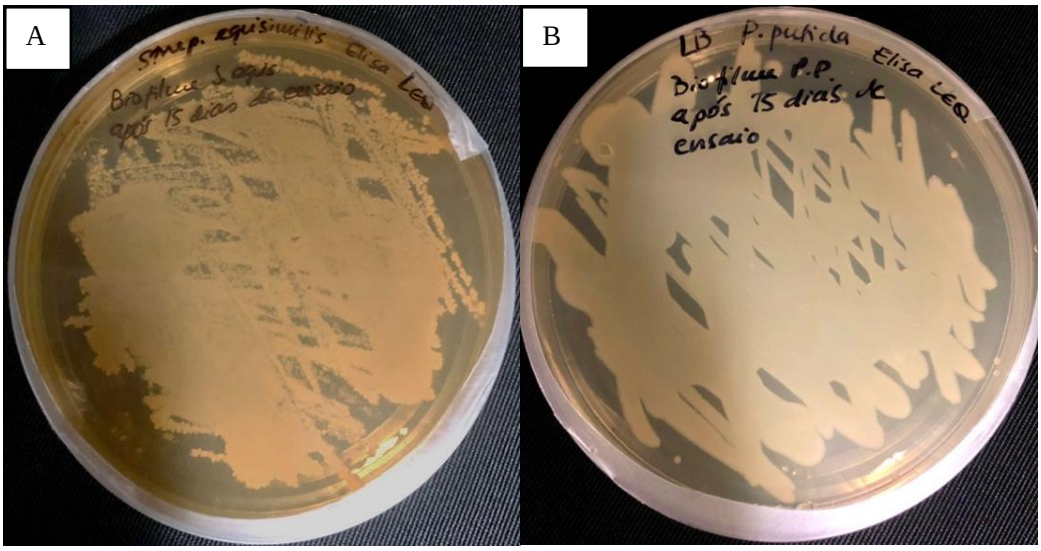


Figure 47. Viability of A) *S. equisimilis* and B) *P. putida* at the end of the assay.

5.4 CONCLUSIONS

This study explored the performance of a sustainable remediation material, a bacterial biofilm supported on pine on a PBBR operation to remove ATZ from water. Batch experiments were performed to evaluate the toxicity of growing concentrations of ATZ towards *P. putida* and *S. equisimilis* and to establish the relative growth rates. The bioremoval of ATZ by *S. equisimilis* biomass per se was assessed aiming to explore its ability to retain the pollutant. Then, *P. putida* and *S. equisimilis* biofilms supported on pine were evaluated as biosorbents to enhance the bioremoval of ATZ (based on combined biosorption and biodegradation processes). The present study is in line with contemporary bio-economy trends since it explores the use of biowaste and biological processes to rehabilitate effluents with EC.

Sorption and degradation of ATZ by *S. equisimilis* biomass were found to be the dominant elimination routes while volatilization, hydrolysis and photolysis may be considered irrelevant. The column experiments conducted in discontinuous flow at lab-scale showed to be efficient for the treatment of high concentrations of ATZ. The presence of any of the biofilms improved the ATZ removal performance, which stands as an important benefit. Is thus possible to use biological wastes that have specific textural properties as sustainable supports for *P. putida* and *S. equisimilis* biofilms. The results obtained show the potential use of this system for water or wastewater treatment processes.

These preliminary results indicate the need to test other sustainable biomaterials, pollutants and bacterial species to understand the mechanistic interactions between the different classes of pollutants and microbial species, aiming the definition, optimization and the upscale of such technology to be applied in real context scenarios.

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CHAPTER VI

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In this final chapter, a general overview of the work performed during the preparation of the thesis and the most relevant conclusions are presented. Some future perspectives on the field of sorption and bacterial biofilms for EC removal are also proposed.

6.1 CONCLUDING REMARKS

Water pollution caused by the presence of EC has become one of the most urgent environmental issues. Pesticides and pharmaceuticals occurrence has been recurrently reported due to their increasing use and inefficient elimination by WWTP. In the last years alternative technologies to conventional treatments have been considered. Among the technologies available, adsorption and biosorption/biodegradation by bacteria appear to be advantageous to reduce EC from water matrices.

In this research work, a sustainable approach based on adsorption/biosorption processes by waste materials and biosorption/biodegradation by bacterial biofilms, were found to reduce the presence of EC in water. Sorption processes were conducted using low-cost materials and wastes as alternative adsorbents/biosorbents to AC. Their adsorption capacity, regeneration and recyclability in batch and/or column reactors were evaluated in the abatement of one herbicide, ATZ and two active pharmaceutical formulations, FLX and CRB. This study presents an important step forward since it turns the treatment process greener, affordable and cost-effective. Besides with waste valorisation, the dependency on natural resources is reduced and circular bioeconomy is promoted. Moreover, the reduction ability of two tolerant bacteria supported in a biowaste, acting as biofilm carrier and biosorbent in a PBBR, allowed the removal of ATZ in a more efficient approach.

The use of low cost and available waste sorbents as naturally occurring clays, forest waste or inorganic residues has gained particular interest in the field of adsorption/biosorption of different kind of pollutants. This can be attributed to the disadvantages of using AC with a high cost of production and with a non-renewable source origin. Besides, according to the UN sustainable development goals for the 2030 Agenda competitive, renewable and sustainable alternative materials should be considered and evaluated for this purpose. The synergistic effect between sorption and biodegradation has been studied in recent years. In the present work, the presence of a bacterial biofilm allowed an improvement of the bioremoval of ATZ, an important benefit in comparison to the system without biofilm. The results obtained show the potential use of this system for water or wastewater treatment processes.

The studies developed during this thesis present an eco-friendly alternative rehabilitation process that allows the decontamination of pesticides and pharmaceuticals present in water. More detailed conclusions that were reached during this research regarding the adsorption/biosorption of EC and the synergic combination of sorption and bacterial biofilms, will be further summarized.

6.1.1 Sorption potential of naturally occurring and waste-based materials for pesticide and pharmaceuticals entrapment

The exploitation of using wastes and natural raw materials as sorbents/biosorbents for the removal of pesticides and pharmaceuticals was assessed in Chapters 3 and 4. In Chapter 3, the main goal was the rehabilitation of water contaminated with ATZ, entrapping it in a permeable and sustainable barrier constructed with waste materials (sepiolite) and with biomaterials (cork and pine bark). In Chapter 4, the focus was on the abatement of active pharmaceutical molecules (FLX and CRB) and pesticide formulations (ATZ) in solution using raw pine and chemically and thermically modified pine. In both approaches it was demonstrated that it is possible to increase the life cycle of materials and use them as sustainable remediation process supports.

Firstly, a screening was performed to assess the capacity of different low-cost naturally occurring and waste-based materials to remove ATZ. After the initial evaluation, sepiolite, cork pine bark and activated rubber char were selected for further research. In Chapter 3, ATZ adsorption by those target materials was assessed by kinetics and equilibrium assays. Activated rubber char was not further considered due to the high production costs, reduced availability and variable particle size. Then, ATZ desorption from the other three materials was tested with different extraction solvents. Adsorbed ATZ was recovered 100 % from sepiolite using a 20 % acetonitrile solution, while 40 % acetonitrile was needed to leach it from cork (98 %) and pine (94 %). After, PRB experiments with those sorbents were performed to evaluate ATZ removal for up-scale applications. The modified dose-response model properly described the breakthrough data. The highest adsorption capacity was achieved by sepiolite (23.3 (± 0.8) mg/g), followed by pine bark (14.8 (± 0.6) mg/g) and cork (13.0 (± 0.9) mg/g). Still in this Chapter 3, the recyclability of sorbents by adsorption-desorption cycles was evaluated. After two regenerations, sepiolite achieved 81 % of ATZ removal, followed by pine with 78 % and by cork with 54 %. Sepiolite had the best performance in terms of adsorption capacity/stability. SEM and FTIR analyses confirmed no significant differences in the material morphology and structure along the assays. The study demonstrated that recycling waste/biowaste is a sustainable option for wastewater treatment, with waste valorisation and environmental protection.

Adsorption kinetics and equilibria measurements to characterize the adsorption from a multi solute solution of FLX, CRB and ATZ (5 mg/L each) by raw pine bark (10 g/L) were described in Chapter 4. The maximum amount of adsorbed pollutants by mass of biosorbent followed the order: FLX (0.652 mg/g) > ATZ (0.522 mg/g) > CRB (0.522 mg/g), explained by the electrostatic attraction forces between the

biosorbent surface and the pollutants. Chemically and thermically modified pine sorbents were also investigated to validate the changes introduced on surface accessibility and affinity with such treatments. Pine modified with HNO_3 revealed to be the best biosorbent in terms of overall sorbate entrapment capacity (1.95 mg/g). The economic feasibility of raw and modified biosorbents was examined by a detailed cost analysis. The chemically modified biosorbents present substantially higher production costs when compared to the ones thermally treated. The surface modification with H_2O_2 is the most expensive procedure, 23.7 €/mmol and hydrochar production the less expensive, 1.1 €/mmol, amongst the evaluated alternatives. The raw material presents the lower cost, 0.20 €/mmol and it is also the one that produces fewer solid and liquid wastes associated to its preparation, with a reduced environmental impact. As performed in Chapter 3, desorption assays were also conducted with different extraction eluents. It was found that FLX, CRB and ATZ can be recovered in 100 %, 98 % and 30 % from raw pine, respectively, using 50 % acetonitrile solution. The recyclability of the natural biosorbent was then evaluated by adsorption-desorption cycles in batch mode using 50 % acetonitrile solution as the best desorbing agent. Pine can be regenerated and reused as biosorbent at least for two times, without losing its adsorption efficiency. Lastly, the adsorption of FLX, CRB and ATZ by the raw biomass was investigated under dynamic flow conditions in an air-lift type reactor. Results confirmed the effectiveness of the rehabilitation system to treat contaminated water, for up-scale purpose.

Data presented in Chapters 3 and 4 demonstrate that by using waste-based biomaterials/materials an environmentally sustainable growth can be fostered, since the approach reduces wastes and promotes the reuse of materials. Besides, their reuse increases their life cycle span and decreases the energy requirements, resources and emissions associated with the production of synthetic sorbents. The consideration of this kind of materials for emerging pollutants entrapment can be seen as a sustainable water rehabilitation pathway, by promoting biomaterials life cycle, circular bioeconomy and environmental protection.

6.1.2 Design and tailoring of biobarriers made of bacterial biofilms supported onto biowaste for pesticide treatment

After the evaluation of the sorption capacity of pine bark in Chapters 3 and 4, its synergetic interaction with bacterial biofilms was evaluated in Chapter 5, working together as biobarriers for the treatment of water contaminated with ATZ. The tested rehabilitation systems were carried out in PBBR, as most the promising and widely accepted remediation design for the treatment of contaminated water.

Biobarriers were constructed with raw pine supporting *P. putida* or *S. equisimilis* biofilms. Before the bioremoval assays in the PBBR, the maximization of the biofilm attachment and establishment was performed. In this way, the bacterial adhesion onto the biocarrier was improved using selected operational conditions. The sequential experiments were then performed at the best operational conditions that allow the strongest biofilm attachment. The ATZ toxicity towards *P. putida* and *S. equisimilis* and the relative growth rates were also established. Under the tested experimental conditions *S. equisimilis* tolerated higher amounts of ATZ than *P. putida*. This fact can be explained by *S. equisimilis* Gram-positive character over the Gram-negative *P. putida* as the antioxidant enzyme levels differ greatly between both. The bioremoval of ATZ by *S. equisimilis* per se was also assessed. Results revealed that ATZ could be potentially anaerobically degraded by the biomass and biosorbed by the solids, with overlapping of the biodegradation and the biosorption mechanisms. Lastly, experiments conducted using supported *P. putida* or *S. equisimilis* biofilms as biobarriers indicated an added beneficial effect towards the bioremoval of the selected herbicide. Results indicate that the best performing system is the one with supported *S. equisimilis* biofilm, reaching 90.42 % of ATZ removal, followed by the supported *P. putida,* with 79.71 % removal and lastly just the pine (control), achieving 58.31 % removal. The process of elimination of the pollutant using the bacterial biofilms supported on pine was attributed to adsorption by the biofilm and by the support, as well as to the biodegradation by bacteria. The coupling of biosorption/biodegradation favors the bioremoval process significantly. These results suggest that the bacterial system could be used to treat relevant concentrations of ATZ, although equilibrium is reached latter than just with pine which may become a disadvantage. The difference on the removal percentage of ATZ indicates that the presence of a bacterial biofilm enhances the rehabilitation process, representing a beneficial effect towards the retention of the selected herbicide.

6.2 FUTURE PERSPECTIVES

With a theme as broad and varied as the proposed in this thesis, numerous new approaches can be explored. Several interesting directions of research regarding the elimination of EC using sustainable treatment approaches combining sorption and biological procedures should be therefore continued.

The present work explores the treatment of aqueous matrices with ATZ, FLX and CRB, but for future, other several hazardous, persistent and of concern molecules can be contemplated due to the continuous and growing usage of a larger range of compounds by society. In fact, the problematic of

water crisis should push research community to find more efficient and low impact treatment alternatives considering different kinds of pollutants.

Also, the exploitation of different waste and low-cost materials as potential sorbents could be addressed with their eventual combination to enhance sorption process. Other different sorbents surface treatments can be explored to possibly enhance the overall treatment process. Moreover, using different extraction eluents and approaches may produce other results, even more sustainable or with better results concerning the recyclability and regeneration of materials.

The use of sorbent materials combined with biofilms needs to be seen as an environment friendly technology to be further explored. It would be interesting to design different sustainable biobarriers using other microorganisms, eventually genetically modified ones prepared for sequestration purposes, with alternative media combinations to establish better clean-up strategies.

Theorizing on pollutants degradation pathways should also be considered for future research, as well as the usage of contaminated aqueous matrices from industrial, agricultural and urban wastes in order to define and to establish the best strategies for up-scale applications.