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Original Research

## Enhanced removal of chiral emerging contaminants by an electroactive biofilter



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### ARTICLE INFO

#### Article history:

Received 12 February 2024

Received in revised form

14 October 2024

Accepted 20 October 2024

#### Keywords:

Chirality

Emerging contaminants

Microbial electrochemical technologies

Ecotoxicity

Capillary electrophoresis

### ABSTRACT

50% of pharmaceuticals and 25% of herbicides used worldwide are chiral. Each enantiomer has a unique toxicity and biodegradation profile, affecting differently to organisms. Chirality plays a key role in the behavior of these emerging contaminants (ECs) in terms of their pharmacological or herbicidal activity, but this peculiarity is often overlooked in environmental research. The complexity of chiral ECs is underestimated, as the varying sensitivity of biological systems to enantiomers is rarely considered. Biofilters can promote the activity of specific microbial communities, facilitating the degradation of ECs, due to the greater interaction between water and microorganisms and their compact design. Here, we show that an electroactive biofilter can alter the chirality of drugs and herbicides in wastewater treatment, impacting their removal and toxicity. The electrochemical biofilter (BioeF) removed 80% of pharmaceuticals and 50–75% of herbicides, outperforming the conventional filter (ConF). BioeF also showed greater chiral alterations and lower ecotoxicity. This work provides the first evidence of a relationship between changes in contaminant chirality and detoxification capacity, enhanced by electroactive systems. The increased microbial activity observed in the BioeF suggests that bio-electrochemical systems offer a valuable advance for ECs removal and ecotoxicity reduction, addressing the environmental challenge posed by ECs.

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## 1. Introduction

Modern lifestyles demand a wide variety of human-made chemical compounds, which are tailored to our needs in the form of materials, pharmaceuticals, personal care products, agrochemicals, and other commodities. These compounds are transported into waterways through water usage and natural runoff [1], where they find their way into ecosystems and organisms that are not accustomed to them, becoming xenobiotics. Their presence has caused emerging environmental concerns. Thus, they have been collectively designated emerging contaminants (EC), as they often

undergo limited biodegradation, and their combined interactions with organisms are often unpredictable a priori [2]. In particular, pharmaceuticals and biocides are designed to have biological effects, and the resulting disruptions of control mechanisms (hormone disruptions) are known to be effective at extremely low concentrations [3].

To avoid the environmental problems derived from these chemicals passing into wastewater, their release has been increasingly regulated, and adequate treatments have been declared mandatory for most discharge operations. Unfortunately, current sewage treatment plants are not optimized to remove xenobiotics [4,5], as pharmaceuticals exhibit removal rates ranging from 20% to 80% [6]. Wastewater is commonly cleaned up using biological treatments. However, the limited availability of suitable electron acceptors for these microorganisms' respiration limits biodegradation rates, so oxygen is usually artificially supplied. The

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discovery of bacteria that can use electroconductive materials as electron acceptors [7] has given rise to the field of microbial electrochemistry and several related applications, which are called microbial electrochemical technologies (METs).

Energy can be harvested from such electrochemical processes at the lab scale [8,9]. However, no system with full-scale capabilities for converting wastewater into electrical energy production has yet emerged. In contrast, several strategies designed to stimulate metabolic oxidation in bacteria using bioelectrochemistry have allowed the birth of a new field—the so-called electro-bioremediation [10]. The interaction between the bacteria and electrodes overcomes the conventional limitations due to electron acceptors' availability and pollutant biodegradation enhancement [11]. Among the various electrobioremediation strategies explored so far, the microbial electrochemical snorkel (MES) has been reported to be especially successful [10,12,13]. In an MES, the presence of a single piece of electroconductive material promotes closed-circuit conditions, and electrons flow from anodic zones to more oxidative environments (cathodic zones). Applying this configuration to wastewater treatment has yielded the so-called METland [11] electroconductive biofilter, which eventually achieved full-scale implementation to treat urban and industrial wastewater [14]. A flooded METland filter provides an unlimited electron acceptor for electroactive biofilms. Electrons are consumed in the upper layers, where atmospheric oxygen is more abundant. The efficacy of this electron flow process was demonstrated by researchers who measured the electric potential profiles at both lab scale [15–17] and full scale [18]. This microbial redox strategy promotes the interaction between electroactive bacteria through conductive-mediated interspecies electron transfer (CIET) [19]. CIET allows cells to exchange electrons directly through their metabolism, triggering microorganisms to expand their substrate profiles [20], regardless of their preference for more accessible nutrient sources [21]. Among these strategies are enhanced methanogenesis in anaerobic reactors [22,23] and complete mineralization of pollutants as herbicides [24,25] or antibiotics [26]. The expansion of substrate profiles facilitates new scenario to biologically degrading recalcitrant pollutants, such as ECs, through microbial electrochemical actors.

The efficiency of removing antibiotics and other pharmaceuticals by METs has been reviewed favorably compared to that achieved by other technologies, with the values reached recalling those of enhanced membrane bioreactors [27,28]. In addition, horizontal flow electroactive biofilters have been proposed to more efficiently remove and detoxify a mixture of pharmaceuticals from wastewater [18,29]. Electroactive bacteria developed in METland-like biofilters have frequently been demonstrated to biodegrade various pollutants typically classified as recalcitrant.

This work hypothesizes that a complex microbial community capable of sharing electrons on demand enables the breakdown of functional groups or bond cleavages through either oxidation or reduction mechanisms as required. In contrast, conventional biofilms developed on inert substrates, such as the gravel used in conventional biofilters, exhibit a limited ability to transfer electrons beyond the immediate vicinity of cells. This can restrict the electron availability of some bacteria, as was demonstrated by Rotaru et al. [19].

Further compounding the xenobiotic problem, biological environments have a chiral bias, which derives from the chirality of the basic molecules of life, such as amino acids and carbohydrates. Accordingly, most biological processes are sensitive to and selective for this property, and they are usually tailored to interact with one particular stereoisomer of chiral molecules. As stereoisomers differ in terms of their pharmacokinetic and pharmacodynamic properties, such as metabolites, bioavailability, affinity for plasma

proteins, and individual rates of uptake in organisms [30], the pharmaceuticals industry leverages this feature, and more than 60% of its products are commercialized as purified chiral compounds [31].

Similarly, many agrochemicals are chiral, with about 30% containing one or more chiral centers. Commonly, only one enantiomer has the desired biocide activity, so some are sold as pure active stereoisomers [32]. For economic reasons, many others are still sold as racemates, wherein both enantiomers appear in a stereometric ratio of 1:1. This applies to the family of phenoxy acid herbicides classified as phenoxyalkanoic acids. They are extensively used in agriculture, parks, and green areas, leading to their frequent presence in wastewater treatment plants (WWTPs) [21]. While removing herbicides in WWTPs has rarely been studied, a recent review by Muszyński, Brodowska, and Paszko [33] offers a good perspective on the general environmental behaviors of phenoxy acid herbicides. Their medium-to-low hydrophobicity keeps them in the aqueous solution rather than deposited in sludges or other solids, facilitating their dispersion into river systems and groundwaters, whereas they remain highly mobile in sediments [34].

Unfortunately, the lack of the desired properties in a stereoisomer does not equate to a lack of environmental effects. Each stereoisomer has its own toxicity and environmental degradation rate, which are heavily influenced by the nature of the matrix and the correlated microbial community [35]. Moreover, chiral compounds have been observed to change between enantiomers, particularly in biological systems, in a process called chiral inversion—which may result in the emergence of one particular enantiomer in the environment, even when it is not commercialized [30]. The use of capillary electrophoresis (CE) to quantify the enantiomeric concentrations of contaminants [36] is key because it offers great chiral discrimination potential, marked by high efficiency, enantioselectivity, short analysis times, and small volumes of reagents and samples.

To our knowledge, no previous reports have evaluated the chirality of pollutants subjected to MET-based wastewater treatment. Thus, this manuscript had two main objectives: (i) to study the enantiomeric profiles of contaminants treated with both a flooded electroactive biofilter and a conventional gravel biofilter and (ii) to analyze the changes in chirality together with the ecotoxicity data of the treated wastewater to determine relationships between removal and detoxification capabilities. Previous experiments have demonstrated that bioelectrochemical systems are more efficient than conventional systems in removing organic micropollutants. However, these studies did not consider chiral contaminants. Thus, the current research focused on this previously unexplored factor in bioelectrochemical systems: the treatment of chiral contaminants. The water used in our experiments was designed to simulate industrial wastewater regarding its organic and toxic load, as it contained high concentrations of pharmaceuticals and phenoxyacids.

## 2. Materials and methods

### 2.1. Chemical reagents

We purchased 85% orthophosphoric acid, sodium hydroxide (NaOH), sulfated  $\beta$ -CD (S- $\beta$ -CD), and heptakis(2,3,6-tri-O-methyl)- $\beta$ -CD (TM- $\beta$ -CD) from Sigma-Aldrich (St. Louis, MO, USA). Yeast extract, anhydrous sodium acetate (NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>), and (2-hydroxypropyl)- $\beta$ -CD (HP- $\beta$ -CD) were acquired from Fluka (Buchs, Switzerland). Potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>), iron citrate (FeC<sub>6</sub>H<sub>6</sub>O<sub>7</sub>), 37% hydrochloric acid (HCl), and methanol (MeOH) were obtained from Scharlau Chemie (Barcelona, Spain). Anhydrous D-fructose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>), sodium bicarbonate (NaHCO<sub>3</sub>),

ammonium chloride (NH<sub>4</sub>Cl), magnesium chloride (MgCl<sub>2</sub>•6H<sub>2</sub>O), calcium chloride (CaCl<sub>2</sub>•2H<sub>2</sub>O), and zinc sulfate (ZnSO<sub>4</sub>•7H<sub>2</sub>O) were acquired from Panreac (Castellar del Vallès, Barcelona, Spain). Milli-Q-quality (Millipore, Bedford, MA, USA) water was used.

Standard compounds with high purity (>99%) were purchased, including (R,S)-terbutaline (TER), (R,S)-propranolol (PRP), (R,S)-verapamil (VER), 97% (R,S)-2-(2,4,5-trichlorophenoxy)propanoic acid (fenoprop) (FEN), (R,S)-2-(4-chloro-2-methylphenoxy)propanoic acid (mecoprop) (MEC), (R,S)-2-(2,4-dichlorophenoxy)propanoic acid (dichlorprop), 2-(4-chlorophenoxy)propanoic acid (4-CPPA), and 2-(3-chlorophenoxy)propanoic acid (3-CPPA) from Sigma-Aldrich (St. Louis, MO, USA); 2-phenoxypropanoic acid (2-PP) from Chem Service (West Chester, PA); (R,S)-metoprolol tartrate (MTP) from Astra (Hässleholm, Sweden); (R,S)-betaxolol (BET) from Sanofi (Paris, France); and (R,S)-duloxetine (DUL) HCl from IS Chemical Technology (Shanghai, China).

## 2.2. Influent composition

The synthetic wastewater (SW) used in this study was the same as employed in our previous work [29]. All chiral xenobiotic pollutants (pharmaceuticals and herbicides) were individually dissolved in methanol at 4 g L<sup>-1</sup> and stocked at 4 °C. Toxic wastewater (TW)—0.25 L for each assay—was prepared daily by adding the required volumes of xenobiotic stock solutions to reach a final concentration four times greater than the enantiomer quantitation limit. The pharmaceutical enantiomer concentrations in the TW ranged from 4 to 13 mg L<sup>-1</sup>, while the herbicide enantiomer concentrations were between 6 and 20 mg L<sup>-1</sup>. The detailed composition of the wastewater used in this work can be found in Table 1.

## 2.3. Biofilter construction, operation, and sampling

Four vertical upflow biofilters were constructed in bottom-sealed polypropylene cylinders (height × diameter = 220 × 80 mm) (Fig. 1). Inlet flows were directly fed into a fiberglass gauze bundle to facilitate distribution before reaching the electroconductive bed. A polypropylene sheet was placed inside the bed at a 15 cm height and perforated by a silicone tube. An outlet port was drilled 100 mm from the inlet to act as an outlet for the silicone tube. Both silicone tube ends—for influent and effluent—were held above the water level by wire loops circling the bottle. The biofilters were covered with aluminum foil to avoid light interference but allow gas exchange at the top.

Two biofilters were designated as controls (ConFs) since they were made of siliceous gravel (750 mL, 1.2 kg, diameter: 5–12 mm, specific surface area: 3.807 m<sup>2</sup> g<sup>-1</sup>), reaching 170 mm of bed height. The other two systems were electroactive biofilters (BioeFs) made of electroconductive bed coke (750 mL, 0.54 kg, diameter: 3.5–10 mm, specific surface area: 4.554 m<sup>2</sup> g<sup>-1</sup>) that reached 16.5 cm above the entrance. The polypropylene sheet of each biofilter was adjusted to achieve the same hydraulic retention time (HRT), one day, on all systems. The biofilters were operated in an acclimatized laboratory with temperatures kept close to 22 °C.

All biofilters were inoculated with 50 mL of a culture of *Geobacter sulfurreducens* strain DL1 (optical density 0.6) pregrown in a freshwater medium [37]. To increase the diversity of the microbial communities, 100 mL of real urban wastewater was added to the inoculum. The biofilters were fed 100 mL of SW and incubated for two weeks to allow microbial colonization of the beds (marking Phase I of the experiment).

Subsequently, the systems were operated across different phases in a semicontinuous regime at a constant HRT of one day (in each sequential batch, the whole volume of 250 mL was substituted daily in each reactor). The difference between Phases II, III, and IV

was that the SW was not fed toxicants in Phases II and IV, while the TW contained mixtures of drugs or herbicides in Phase III.

Samples of all TW influents were obtained from the mixture prepared each day just before the feeding operation. The effluent samples from the bioreactors were taken from the homogenized volume recovered at each outlet. All samples were filtrated by nylon membranes with a pore size of 0.45 μm and then conserved at -20 °C in polypropylene Falcon tubes for analysis.

## 2.4. Enantiomeric analysis via capillary electrophoresis with ultraviolet detection

Capillary electrophoresis (CE) measurements were performed using an Agilent 7100 CE system (Agilent Technologies, Waldbronn, Germany) equipped with HP3D CE ChemStation software by employing an uncoated fused silica capillary with a 50-μm internal diameter, a 375-μm outside diameter, a total length of 58.5 cm, and an effective length of 50 cm (Polymicro Technologies, Phoenix, AZ, USA). The enantiomers in the influent and effluent of the biofilters were separated and determined using the chiral method described by Valimaña-Traverso et al. [38,39] for pharmaceuticals and herbicides, respectively. In these procedures, detection was carried out at the following wavelengths: 194 nm (MTP, BET, 4-CPPA, and 2-PPA), 200 nm (TER, VER, MEC, DIC, and 3-CPPA), 210 nm (FEN), 215 nm (PRP), and 220 nm.

## 2.5. Calculation of chirality parameters

A simple way to describe a mixture containing two enantiomers is with the enantiomeric ratio (ER). This can be calculated using equation (1), which indicates how they cause plane-polarizing light to deviate:

$$ER = \frac{A_+}{A_-} \quad (1)$$

where A<sub>+</sub> and A<sub>-</sub> are the concentrations of each enantiomer.

Although this formula is simple and often used for comparisons to a racemate (ER = 1), it has several drawbacks for practical calculations, as its possible values range from 0 to infinity. To solve this problem, the enantiomeric fraction (EF) is preferred as a parameter for describing the extent of deviation from racemates in chiral compound mixtures [40]. Equation (2) defines EF, A<sub>1</sub>, and A<sub>2</sub>, which correspond to the concentrations of the first and second enantiomers to elute after the mixture is separated by a chiral column:

$$EF = \frac{A_1}{A_1 + A_2} \quad (2)$$

As EF ranges from 0 to 1, with 0.5 corresponding to a racemate and equal deviations above and below corresponding to equivalent changes in proportion, this parameter is much more suitable for mathematical processing. Since, in this experiment, the initial mixture was racemic for all compounds studied, deviations from the initial value of 0.5 in the effluent indicate enantioselective processes. The change in EF (ΔEF) was interpreted as the difference between the EF values at the inlet and the corresponding outlet given an HRT of one day.

## 2.6. Xenobiotic removal

The removal capability of the systems for each compound was calculated based on the concentrations of both of its enantiomers, which were obtained according to the methods described in Section 2.4. The complex nature of similar systems, which can lead to

**Table 1**  
Composition of the SW and concentrations of xenobiotics added to TW.

Compounds for synthetic wastewater	Concentration (mg L <sup>-1</sup> )	Supplier
Yeast extract	56.0	Fluka (Buchs, Switzerland)
Anhydrous D-fructose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	172.0	Panreac (Castellar del Vallès, Barcelona, Spain)
Anhydrous sodium acetate (NaC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> )	460.0	Fluka (Buchs, Switzerland)
Potassium phosphate (KH <sub>2</sub> PO <sub>4</sub> )	44.0	Scharlau Chemie (Barcelona, Spain)
Sodium bicarbonate (NaHCO <sub>3</sub> )	310.0	Panreac (Castellar del Vallès, Barcelona, Spain)
Ammonium chloride (NH <sub>4</sub> Cl)	104.0	Panreac (Castellar del Vallès, Barcelona, Spain)
Iron citrate (FeC <sub>6</sub> H <sub>6</sub> O <sub>7</sub> )	36.9	Scharlau Chemie (Barcelona, Spain)
Magnesium chloride (MgCl <sub>2</sub> •6H <sub>2</sub> O)	50.0	Panreac (Castellar del Vallès, Barcelona, Spain)
Calcium chloride (CaCl <sub>2</sub> •2H <sub>2</sub> O)	74.0	Panreac (Castellar del Vallès, Barcelona, Spain)
Zinc sulfate (ZnSO <sub>4</sub> •7H <sub>2</sub> O)	0.4	Panreac (Castellar del Vallès, Barcelona, Spain)
Phenoxyacids added for toxic wastewater	Concentration (mg L <sup>-1</sup> )	Supplier
2-PPA (C <sub>9</sub> H <sub>10</sub> O <sub>3</sub> )	19.2	Chem servisse (West Chester, PA)
3-CPPA (C <sub>9</sub> H <sub>9</sub> ClO <sub>3</sub> )	11.2	Sigma-Aldrich (St. Louis, MO, USA)
4-CPPA (C <sub>9</sub> H <sub>9</sub> ClO <sub>3</sub> )	16.0	Sigma-Aldrich (St. Louis, MO, USA)
Dichlorprop (C <sub>9</sub> H <sub>8</sub> Cl <sub>2</sub> O <sub>3</sub> )	13.2	Sigma-Aldrich (St. Louis, MO, USA)
Mecoprop (C <sub>10</sub> H <sub>11</sub> ClO <sub>3</sub> )	10.4	Sigma-Aldrich (St. Louis, MO, USA)
Fenoprop (C <sub>18</sub> H <sub>14</sub> Cl <sub>6</sub> O <sub>6</sub> )	6.8	Sigma-Aldrich (St. Louis, MO, USA)
Pharmaceuticals added for toxic wastewater	Concentration (mg L <sup>-1</sup> )	Supplier
Duloxetine (C <sub>18</sub> H <sub>19</sub> NOS)	4.0	IS Chemical Technology (Shanghai, China)
Terbutaline (C <sub>12</sub> H <sub>19</sub> NO <sub>3</sub> )	80	Sigma-Aldrich (St. Louis, MO, USA)
Propranolol (C <sub>16</sub> H <sub>21</sub> NO <sub>2</sub> )	3.2	Sigma-Aldrich (St. Louis, MO, USA)
Verapamil (C <sub>27</sub> H <sub>38</sub> N <sub>2</sub> O <sub>4</sub> )	7.2	Sigma-Aldrich (St. Louis, MO, USA)
Metoprolol (C <sub>15</sub> H <sub>25</sub> NO <sub>3</sub> )	9.2	Astra (Hässleholm, Sweden)
Betaxolol (C <sub>18</sub> H <sub>29</sub> NO <sub>3</sub> )	10.0	Sanofi (Paris, France)

an undetermined distribution of xenobiotics between the different physical phases of the systems, has already been established [41]. Therefore, the removal of xenobiotics was evaluated by applying a mass balance in the liquid phase. For this purpose, we calculated the total mass of each xenobiotic feed into each system during the entire length of the experiment, along with the total mass recovered in the effluent for the same period. Similarly, the EF changes for each compound were determined based on the total mass of each enantiomer in the influent and effluent of the four reactors during the operation.

### 2.7. Ecotoxicity evaluation

Ecotoxicity assays were performed using the green alga *Raphidocelis subcapitata* and the microinvertebrate *Daphnia magna* as bioindicators. The algae ecotoxicity test was carried out according

to the modified Organization for Economic Cooperation and Development (OECD) Test Guideline 201 [42] as in previous works [43]. The mobility of *D. magna* was inhibited following OECD Test Guideline 202 [44]. In both tests, each sample and control was replicated four times.

## 3. Results and discussion

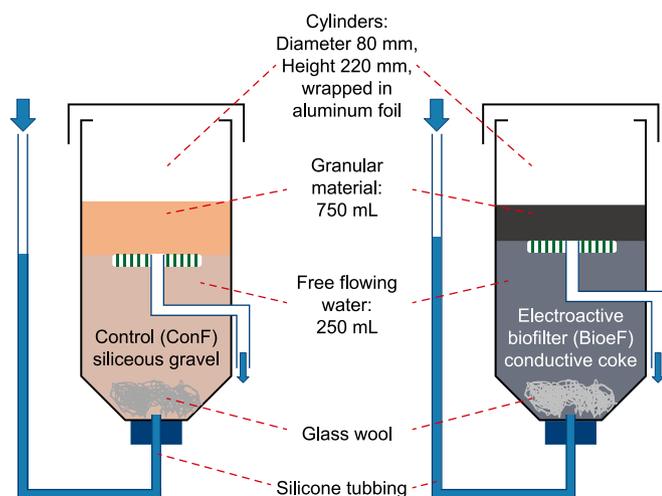
### 3.1. Analysis of pollutants

Some examples of the resulting electropherograms are presented in [Supplementary Material Fig. S1](#) for the pharmaceutical experiment and [Supplementary Material Fig. S2](#) for the herbicides. In both figures, line A corresponds to the influent wastewater containing the contaminants, line B represents the effluent of the conventional biofilter, and line C corresponds to the effluent of the bioelectroactive biofilter. Good resolution of the peaks can be observed in both figures, validating the choice of technique. Notable differences can also be observed in the size of the peaks, with those in the influent (line A) appearing visibly larger in both figures. Meanwhile, the peaks in the BioEF effluent (line C) are always the smallest, decisively confirming that the electroactive biofilter achieves the most effective removal of xenobiotics.

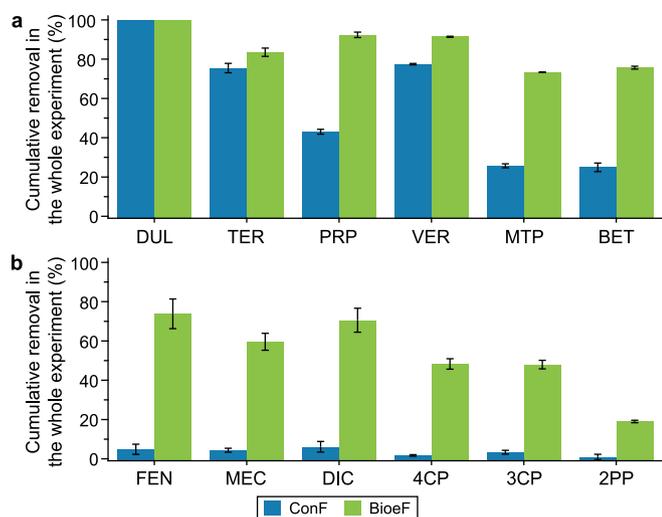
### 3.2. Removal of pharmaceuticals

[Fig. 2](#) displays the cumulative removal of each contaminant in both biofilters. [Fig. 2a](#) presents the removal of pharmaceuticals, while [Fig. 2b](#) presents the removal of herbicides. The results reveal that the BioEF outperformed the conventional biofilter (the ConF, made of inert materials) in terms of pollutant removal. Indeed, an average of 80% of the pharmaceutical pollutants tested were efficiently removed by electroactive microbial communities, compared with 50% by the ConF ([Fig. 2a](#)).

Special attention should be paid to β-blockers, such as PRP, which is usually considered a recalcitrant compound in WWTPs, so that more aggressive treatments are required for its degradation



**Fig. 1.** Schematics of the upflow biofilters.



**Fig. 2.** Cumulative mean removal of all contaminants throughout the experiment for pharmaceuticals (a) and phenoxyacids (b). Error bars indicate divergence in overall removal between both enantiomers.

[45–47]. In our study, however, 90% of the PRP was removed by the BioEF, while just 40% of the racemic drug was eliminated by the ConF. Turning to MTP, the second  $\beta$ -blocker studied, the literature has reported limited degradation in WWTPs through conventional and extensive treatments [48], particularly under the conditions studied here. The BioEF was 2.5-fold more efficient than the ConF in removing MTP, achieving an 80% removal rate at a one-day HRT under anaerobic conditions from an initial concentration of  $9.2 \text{ mg L}^{-1}$ . These results outperform those previously reported by Rubirola et al. [49] for MTP, where a membrane bioreactor under aerobic conditions removed 60% from a concentration of  $1 \text{ mg L}^{-1}$  at a similar HRT.

BET has been considered more biodegradable than other  $\beta$ -blockers in WWTPs, with removal rates between 45% and 67% [50]. These figures are higher than the results obtained with the ConF (25%). When we used the bioelectrochemical process, the removal was enhanced 1.5-fold above this reported value and three-fold compared to the ConF.

However, the antidepressant DUL was almost completely removed by both biofilters used in this work. Although this is logical, given the low concentrations detected in rivers and lakes [51,52], a study by Fick et al. [53] reported an average removal efficiency of around 29% for DUL in Swedish conventional WWTPs, suggesting that the complete removal is not attributable either to conventional treatment processes or to adsorption into sludge. According to Osawa et al. [54], this degradation process seems more likely attributable to ultraviolet (UV) exposure in waterways. The high removal obtained by both biofilters suggests that they are a valid option for DUL treatment, perhaps due to the differences in the biological activity in the biofilters compared to the usual activated sludge treatments in conventional WWTPs. However, further research is necessary for a proper comparison.

The ConF system achieved an 80% removal rate with the bronchodilator TER, similar to the values reported in extensive systems, such as conventional wetlands [55]. In turn, the BioEF achieved a rate 10% higher, closer to the 90% removal rate associated in the literature with advanced oxidation processes such as ozonation and UV treatment [56].

The antihypertensive VER followed the same trend, exhibiting a 77% removal yield in the ConF vs. a 91% rate in the BioEF (12% higher). Kovalova et al. [57] showed that VER is more readily

degraded by physicochemical processes. These authors found a removal yield greater than 88% using activated carbon adsorption, 40% using UV, and over 76% via ozone, while a membrane bioreactor achieved 80% removal. This points to the BioEF as the most effective among the reported techniques for removing this compound, as no extra energy cost is required.

### 3.3. Removal of herbicides

Regarding the removal of herbicides (Fig. 2b), the results from the ConF follow those described in the literature [34,58,59], with 1–6% reductions through the system for all compounds, as opposed to barely any removal under conventional conditions. On the contrary, the BioEF achieved much higher removal rates, at 50–75% for all compounds except 2 PP (19%).

Among the sparse studies available, Escolà Casas et al. [58] required ten days under anaerobic batch conditions to reach a similar removal rate of 50% for MEC, starting from 100 ppb. Better results were reported by Feld et al. [60], who removed up to 30% of the initial concentrations of 0.5–1.4 ppb of MEC, DIC, and 4 PP using a percolating sand filter. Meanwhile, the BioEF achieved 60% removal of an initial 10.4 ppm over a one-day HRT. Previous works have treated MEC and DIC in similar concentrations (10 and 40 ppm) with activated sludge but required seven days to achieve complete removal under aerobic conditions, with no degradation reported under anaerobic conditions [59].

Escolà Casas et al. [58] showed that MEC is barely affected by conventional WWTPs, while bioremediation (biofilms in soil) achieves mineralization in 30–50% under aerobic conditions. They emphasized that the structure of the microbial community is a key factor in the degradation of these herbicides, and different initial conditions can lead to very different removals.

Based on the literature, the behavior of the BioEF differs from that of other systems under anaerobic conditions, illustrating how the presence of electroconductive material acting as an electron acceptor overcomes the respiratory limitations usually seen in those systems. The removal rate of herbicides reported in our experiment is closer to those under aerobic conditions, sometimes even outperforming them. This is not unexpected, as similar results have been seen in previous works with METs [24–26]. Moreover, CIET has been shown to promote enhanced degradation capabilities [20,21], thus facilitating the formation of bacterial communities capable of degrading herbicides.

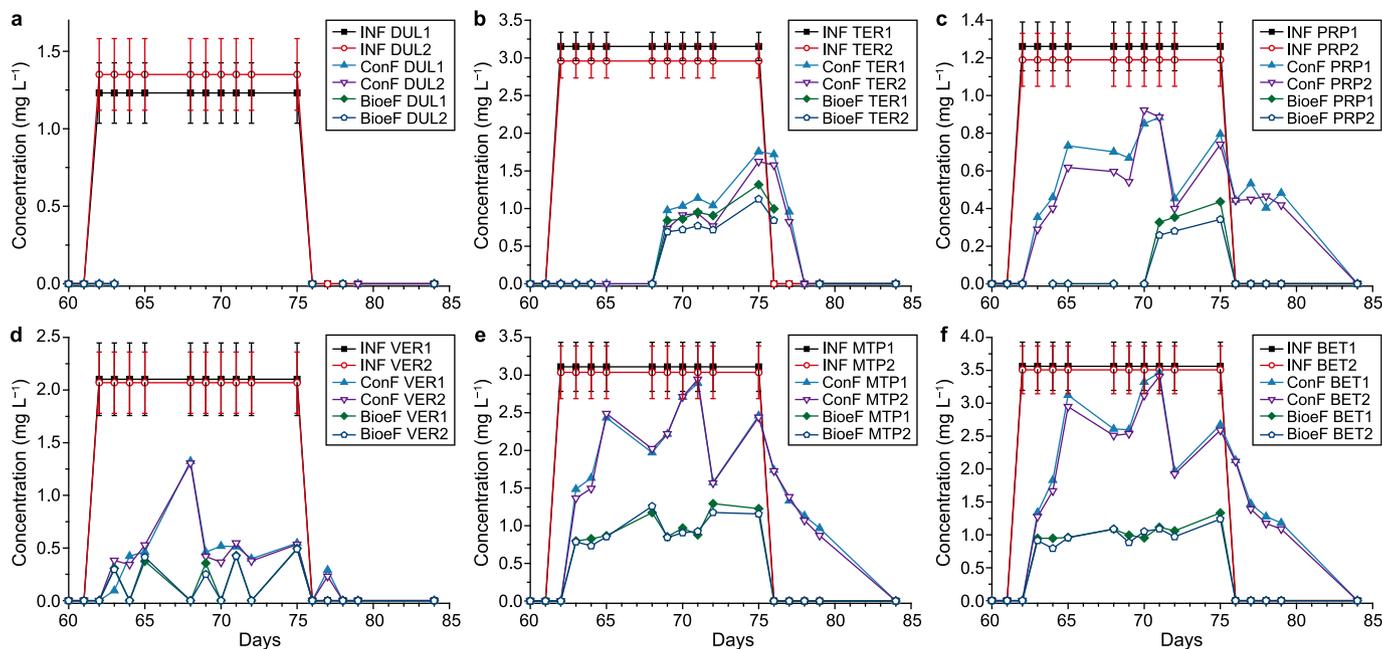
### 3.4. Fate of enantiomers in biofilters

The concentrations of stereoisomers throughout the experiment were obtained using CE to study the degradation patterns of enantiomers in both biofilter configurations (ConF and BioEF). The results were analyzed based on each bioreactor pollutant's concentration profiles of enantiomeric forms while considering the chirality parameters calculated for each compound (Figs. 3 and 4).

#### 3.4.1. Concentration profiles of enantiomers in biofilters

**3.4.1.1. Concentration profiles of pharmaceuticals.** Fig. 3 shows the enantiomeric concentration of each pharmaceutical studied in the biofilters' influent and effluent. The results point to three different behavioral patterns for drugs in vertical biofilters, with no appreciable differences observed between the fates of the enantiomers of each compound.

The first pattern—observed for DUL (Fig. 3a), with its complete disappearance from the effluent for both enantiomers—indicates total removal by both biofilter configurations throughout the experiment. Fick et al. showed that DUL is not removed by adsorption into WWTPs, which suggests that biodegradation is the



**Fig. 3.** Enantiomeric concentrations for pharmaceuticals during the experiment for duloxetine (DUL, **a**), terbutaline (TER, **b**), Propranolol (PRP, **c**), verapamil (VER, **d**), metoprolol (MET, **e**), and betaxolol (BET, **f**). Error bars correspond to confidence intervals of 95% around the median values, considering five samples.

main mechanism of DUL removal in biofilters.

In the second pattern (Fig. 3b), TER was not detected in the effluent during the first week of TW feeding. In the second week, the effluent showed slightly increasing concentrations of enantiomers, regardless of the biofilter construction. This pattern suggests that adsorption may be the main mechanism operative in biofilters until the sorbent materials are saturated. At longer operational times, the differences in concentrations of the influent and effluent can only be attributed to biodegradation, which appears to be enhanced in the BioeF. No appreciable differences were noted between the fates of the TER enantiomers.

Finally, Fig. 3c shows the third pattern for PRP, where the enantiomers have similar profiles. A significant fraction of PRP enantiomers crossed the ConF from the start and remained approximately constant during the TW feeding phase. With the return to SW feeding in Phase 4, the retained fraction of enantiomers was eluted. In contrast, the BioeF pattern differed, resembling TER's behavior. In Phase 4, no PRP enantiomers were detected in the BioeF effluent. This pattern suggests lower biodegradation in the ConF, as indicated by the release of the retained fraction of pollutants when the TW became SW in the last phase of the experiment.

VER, MTP, and BET exhibited the same enantiomeric pattern in both stereoisomers (Fig. 3d–f). The BioeF curves exhibited lower concentrations for both enantiomers and no elution in the last phase during SW feeding. In contrast, the ConF effluent behaved as described above for PRP, with significant elution of the stereoisomers in the final phase.

Previous works [46] have indicated that the biological degradation of pharmaceuticals, particularly beta-blockers such as MTP and PRP, is enantioselective in a biotic reactor. However, comparing the daily enantiomer concentration changes from the influent to the effluent revealed no appreciable differences for any pharmaceuticals between the systems. Thus, more thorough research is required.

### 3.4.1.2. Concentration profiles of herbicides. Turning to the

herbicides, Fig. 4 displays the enantiomeric concentration of each herbicide evaluated in this work for both biofilters (i.e., the conventional and electroactive systems). The curves in Fig. 4a–f follow a similar pattern comparable to the third pattern observed for VER, MTP, and BET, suggesting similar behavior.

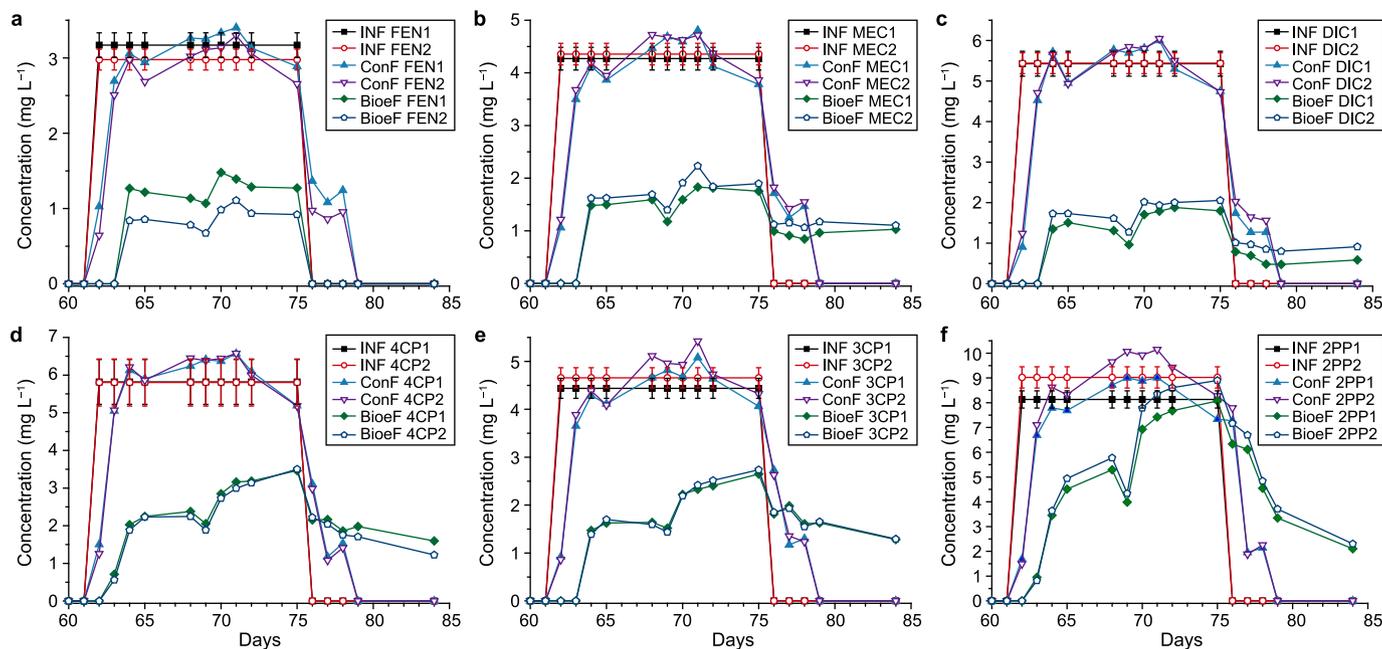
All enantiomers were lightly retained in the ConF with the herbicides, so their effluent concentrations soon reached their influent levels during Phase 3 of the biofilter operation. The transition to Phase 4 caused a brief elution of the enantiomers. In contrast, for the BioeF, the behavior of each herbicide enantiomer was similar, but much lower concentrations of enantiomers were measured in the effluent. The BioeF procedure in Phase 4 indicated elution for all enantiomers apart from those of FEN. This pattern highlights the effects of the absorption capabilities of the substrate, as, despite the phenoxyacids' low adherence to WWTP sludge [34] or soil [33], their residence time within the system was increased.

In the ConF, the profiles exhibited no biodegradation and little adsorption for all herbicides, consistent with previous results indicating their stability under anaerobic soil conditions [33]. Contrarily, the results for the BioeF indicate that both processes took place inside the biofilter. Unfortunately, in the literature, no comparable work was found regarding the concentrations of enantiomers during the operation of a biofilter, with their behavior in activated sludge serving as the closest analogs [61].

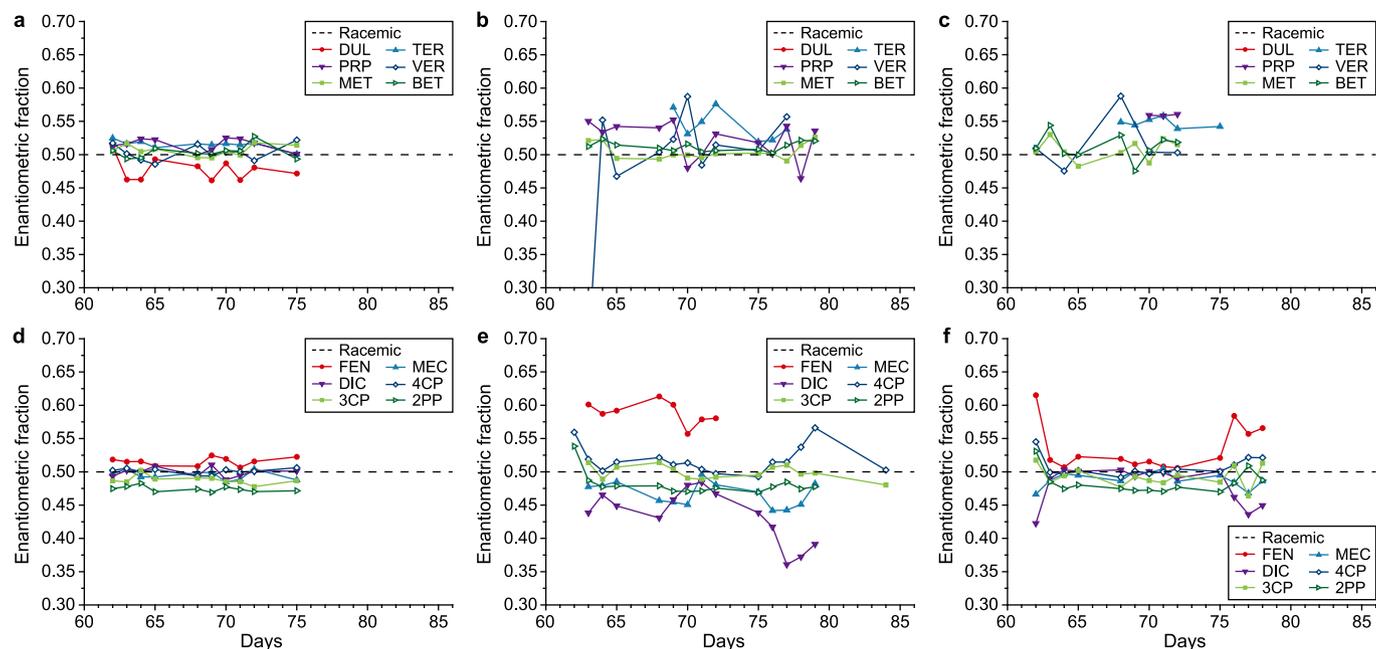
### 3.4.2. Changes in enantiomeric parameters

**3.4.2.1. Daily profiles of enantiomeric fractions of pharmaceutical compounds.** We present the daily evolution of the enantiomeric fractions during the experiment (Fig. 5), calculated according to equation (2).

Regarding the daily EF values of the compounds (Fig. 5), it is important to point out that the influent TW was prepared from racemate stock solutions of xenobiotics, but the experimental calculations of EF values deviated slightly, up to 5%, for all drugs and herbicides. The EF values calculated in this work are within the range expected (under 0.6–0.7) given the environmental setting of water and soil ecosystems [62].



**Fig. 4.** Enantiomeric concentrations for phenoxy acids during the experiment for Fenprop (FEN, a), Mecoprop (MEC, b), Dichlorprop (DIC, c), 4-CPA (d), 3-CPA (e), and 2-PPA (f). Error bars correspond to confidence intervals of 95% around the median values, considering five samples.



**Fig. 5.** Enantiomeric fractions in pharmaceuticals influent (a), pharmaceuticals effluent from conventional filter (b), pharmaceuticals effluent from bioelectrochemical filter (c), phenoxyacids influent (d), phenoxyacids effluent from conventional filter (e), and phenoxyacids effluent from bioelectrochemical filter (f).

For all pharmaceuticals, the EF values in the effluents of the ConF and the BioeF deviated further from the racemic mixture than the values in the TW, however slightly, indicating preferential elimination of one of the two chiral species of the pharmaceutical. Higher deviations from the racemate were observed for TER and PRP in the effluents of the BioeF, although only in the case of PRP was there a significant change in EF in the electroactive biofilter relative to the ConF.

As far as we know, this is the first time EF values have been

reported for TER in wastewater. The EF values obtained in this work for PRP align with those observed by Evans, Bagnall, and Kasprzyk-Hordern [46], who reported EF values for PRP of 0.41 in the effluent of activated sludge systems at full scale. These authors demonstrated that preferential stereoselective degradation yields a higher removal of the R(−) enantiomer of PRP. The differences in EF values between the works are probably due to the radically different nature of the wastewater treatments and are likely linked to biological transformation, as the EF of PRP has been noted as remarkably

stable under physicochemical treatments but can be strongly modified under biological treatment in WWTPs [63].

3.4.2.2. Daily profiles of enantiomeric fractions of herbicides. We present the daily evolution of the enantiomeric fractions during the experiment as calculated according to equation (2) (Fig. 5d–f).

As with the pharmaceuticals, the influent distribution was close to the theoretical racemic mixture, with all EF values hovering around 0.5. For herbicides, however, the detected changes in the enantiomeric fraction indicate a more clearly differentiated elimination of the enantiomers of these contaminants. In this context, our results show that the electroactive biofilter causes changes in enantiomeric fractions of between 4.17 and 5.94, greater than the changes observed in the gravel biofilter for five of the six herbicides studied. These results indicate that the BioEF reactor’s capacity to alter the chirality of herbicides is greater than the capacity of the conventional ConF system—noticeably so for FEN, DIC, and MEC. These changes in the EF are a clear indicator of enantioselective mechanisms inside the BioEF that are either absent or greatly diminished in the control system.

Table 2 presents the median EFs of the pharmaceuticals and herbicides measured throughout the experiment and their changes related to the influent. Here, the ΔEF value is the difference at the system outlet from the TW at the inlet. Negative values indicate an increase in the second enantiomer eluted from the chemical instead of the first.

Comparing the ΔEF values calculated for the ConF and BioEF, we see opposite trends for MTP and VER, which favor the removal of the first enantiomer eluted in the BioEF instead of the second, as is the case in the ConF. Changes in the EF of MTP have been previously observed in WWTPs and shown to be highly dependent on the WWTP, likely due to the specific microbial communities present [64]. Nevertheless, the EF change remained consistently in favor of the same enantiomer, which suggests that the differences in the microbial communities in the ConF and BioEF are more significant than those between the different WWTPs studied.

The last line of Table 2 presents the ratio of ΔEF in BioEF to ΔEF in ConF, thus comparing the influence of both systems in terms of EF. The ratio indicates little difference between the systems (under 6%) for most pharmaceuticals. Again, the main increases were seen in TER (13%) and PRP, which exhibited a six-fold change. These values suggest that the enantioselective processes present in a vertical biofilter are enhanced by MET for TER and PRP, resulting in an important difference for PRP. For this pharmaceutical, the changes in EF obtained in the bioelectrochemical system were almost six-fold higher than those yielded by the conventional biofilter.

Regarding the parameters obtained for the herbicides (Table 2), the EF values in the effluent of the ConF presented minor deviations from the initial racemate. Meanwhile, the effluent of the BioEF showed a marked deviation in EF from the TW values, particularly for FEN and DIC. In addition, the ΔEF followed the same trend independently of the nature of the biofilter. FEN, 4CP, 3CP, and 2 PP

increased the proportion of the first eluted enantiomer, while MEC and DIC showed the opposite tendency. Looking at the ratio of the ΔEF for the BioEF to that of the ConF (last line in Table 2), we can see that its values were consistently in the 4–6.5 range. The only exception is 2 PP, which exhibited barely half the ΔEF of ConF.

Zipper, Fleischmann, and Kohler [61] reported preferential removal in an aerobic batch for FEN, 3CP, and 2 PP, with a bias in favor of the (R) enantiomer in FEN and 2 PP, while in 3CP, (S) exhibited faster removal. Meanwhile, the removal of 4CP maintained the racemic mixture. Deviating from their results, the 4CP in our experiment showed a clear divergence from the racemic mixture in the effluent. The authors also mentioned the sequential elimination of 2 PP enantiomers, reporting a complete degradation of one before the degradation of the second started. This was not observed in the current experiment, as it would have resulted in progressive divergence of the enantiomer concentrations, given the sequential batch feeding, as well as a drastic drop in the concentration of the degraded form during Phase 4, which are not present in Fig. 6f.

When Müller and Kohler [59] reviewed data on herbicides’ enantioselective biodegradation, they found that (S)-MEC and (S)-DIC were preferentially removed under aerobic conditions, albeit slowly, while no degradation was observed under anaerobic conditions. More recently, Escolà Casas et al. [58] also reported enantioselective removal of MEC under aerobic batch conditions, which increased when methanol was used as a cosubstrate. Their systems preferred (S)-MEC removal with a very slow degradation time, but the authors did not report EF values from this process. Despite the anaerobic conditions in the BioEF, its effluent presented similar shifts in enantiomer concentrations, underlining that electroactive systems are not limited by the lack of electron acceptors and that their bacterial communities are comparable to those of aerobic systems.

Muszyński, Brodowska, and Paszko [33] reviewed phenoxy acid transformations in aquatic environments, including those of MEC and DIC. Their enantioselective degradation results were reported only under aerobic conditions and without a clear trend favoring one enantiomer over the other. Comparing this outcome to our results and previous work, microbial communities cannot be universally assumed to show one enantioselective preference.

The analysis of EF in this experiment revealed a clear enhancement of the enantioselective processes in the bioelectrochemical filter compared to the conventional biofilter. Specifically, all phenoxy acids and the pharmaceutical PRP were more strongly affected.

3.5. Changes in ecotoxicity during treatment

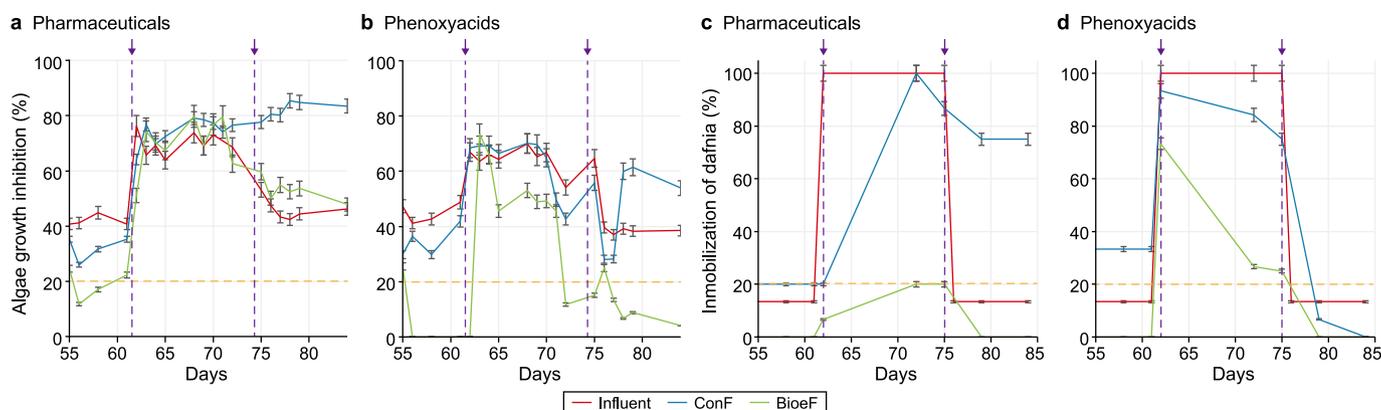
Fig. 6 presents the results of the ecotoxicity assays for the influent and effluent of all systems. The data shown in this figure correspond to Phases II, III, and IV of the continuous feeding of the biofilters.

Table 2

Median Enantiomeric Fraction of contaminants measured during the whole experiment and changes compared to the influent.

Parameters	Sample	DUL	TER	PRP	VER	MTP	BET	FEN	MEC	DIC	4CP	3CP	2 PP
EF	Influent TW	0.477	0.515	0.515	0.503	0.506	0.504	0.516	0.495	0.499	0.501	0.488	0.474
	Effluent of ConF	-	0.544	0.522	0.490	0.505	0.512	0.528	0.491	0.486	0.504	0.491	0.480
	Effluent of BioEF	-	0.548	0.559	0.517	0.508	0.512	0.589	0.467	0.435	0.515	0.499	0.476
ΔEF	Effluent of ConF	-	0.0288	0.0075	-0.0135	-0.0015	0.0084	0.0123	-0.0043	-0.0137	0.0029	0.0027	0.0054
	Effluent of BioEF	-	0.0325	0.0443	0.0143	0.0016	0.0082	0.0732	-0.0282	-0.0646	0.0141	0.0111	0.0022
ΔEF BioEF		-	1.13	5.91	-1.05	-1.06	0.98	5.94	6.51	4.70	4.81	4.17	0.41
ΔEF Control													

Note: ΔEF is the difference at the system outlet from the SW at the inlet. Negative values indicate an increase in the 2nd eluted enantiomer of the chemical instead of the 1st.



**Fig. 6.** Ecotoxicity values measured during the operation of biofilters for pharmaceuticals using algae (a), for herbicides using algae (b), for pharmaceuticals using microinvertebrates (c), and for herbicides using microinvertebrates (d). Arrows and the purple dotted line mark the beginning and the end of toxic feeding. The yellow dotted lines mark the toxic (above) and non-toxic levels (below).

### 3.5.1. Ecotoxicity of algae

The red lines in Fig. 6a and b represent the ecotoxicity values of the influent for *R. subcapitata*. Mid-level inhibition (about 45%) can be observed even for our baseline SW, and the addition of xenobiotics in TW raised this figure to 60–70%. With the return to SW, the toxicity diminished to about 50%, returning to its initial level. Turning to Fig. 6a, the effluent of both systems (represented by the blue and green lines, respectively) responded to the changes in influent composition. In Phase II (no pharmaceuticals), the toxicity values obtained corresponded to a detoxification yield from the inlet of 15% for the ConF and 20% for the BioeF. During Phase III (in which pharmaceuticals were continuously fed into the biofilters), the effluent of the ConF (the blue line) showed no detoxification and, as the experiment continued, an increase in toxicity compared to the influent. This tendency was maintained during Phase IV, despite the return to the initial influent composition. This does not match the enantiomer concentrations shown in Fig. 3, where, despite the reduced pharmaceutical concentrations measured, we observed persistent toxicity in the effluent of the ConF. These data suggest the toxicity came from the mixture of parent compounds and metabolites generated inside the system.

The effluent of the BioeF (represented by the green line in Fig. 6a) behaved similarly, with virtually no change in toxicity values in Phase III compared to the influent. Moving on to Phase IV, the effluent of the BioeF closely followed the toxicity values from the influent instead of maintaining the values from the previous phase, as the ConF did. Given the concentrations present and the toxicities described in Ref. [63], only PRP seems to exhibit a noteworthy match with the algal toxicity, at least in the ConF effluent. It is noteworthy that PRP was also the pharmaceutical with the highest change in EF, as seen in Table 2. Unfortunately, in this case, the results suggest that the high toxicity in the algae populations came from the metabolites generated in both systems. BioeF managed at least to retain and remove both pharmaceuticals and their metabolites in sufficient quantities to maintain toxicity levels similar to those of the influent. Meanwhile, the ConF compounded the toxicity of the original eluted drugs with that of their metabolites, resulting in a negative detoxification of this influent, probably with a progressive buildup of metabolites inside the biofilter that resulted in the increased effluent in Phase IV. In this light, no clear connection between toxicity and any particular enantiomer can be established, as the metabolites' contribution in the algae toxicity appears to have shadowed that of the analyzed pharmaceuticals.

The results obtained for the herbicides are shown in Fig. 6b. In

Phase II, the toxicity of the effluent for algae behaved similarly to that of the pharmaceuticals, although the BioeF effluent reached nontoxic levels. Once the influent shifted to TW (Phase III), both effluents reached 70% inhibition of algae growth. Considering the detected concentrations of herbicides in the effluent of the ConF (Fig. 4), this is not unexpected, as the herbicides seem to have been unaffected by their treatment via the ConF. For the BioeF effluent, we see lower levels of toxicity than for the ConF—about 20% less toxic until day 70. After this point, the toxicity values of the BioeF effluent drop consistently, eventually dipping under 20%, which is the toxicity limit for environmental samples. Surprisingly, this also corresponds to an increase in 3CP, 4CP, and 2 PP concentrations, suggesting they do not play a significant role in algae toxicity.

With the return to SW (Phase IV), the toxicity of the BioeF effluent maintained nontoxic levels, while the ConF effluent showed an increase of up to 15% from the corresponding influent. Although the concentration curves in Fig. 4 indicate generally lower values for the BioeF effluent than that of the ConF, their profiles do not match those seen for the ecotoxicity of algae. The likeliest explanation is that the ecotoxicity represents the combined effect of the parent herbicides and their metabolites in the effluent, with the metabolites making a greater contribution.

Although no direct correlation between specific enantiomers and algae toxicity can be discerned, the remarkably different changes in EF between the ConF and the BioeF are paralleled in their detoxification of the herbicide mixture. An EF change five-fold higher than that of the control corresponds to a four-fold detoxification for algae. This correlation is likely due to both EF and detoxification originating from microbial metabolism and its enhancement, thus increasing both.

### 3.5.2. Ecotoxicity for microcrustacean

Ecotoxicity evaluations via *D. magna* assays indicated a much stronger sensitivity of this organism to mixtures of pharmaceuticals and herbicides. The influent's toxicity changed from a barely toxic level for SW (lower than 20% inhibition) to complete inhibition (100% immobilization of microcrustaceans at 24 h) for TW (Fig. 6c and d).

The effluents of the pharmaceuticals (Fig. 6c) for the ConF began at a nontoxic level in Phase II, increased during Phase III until the toxicity was almost equivalent to that of the influent, and remained high in Phase IV. This is the same toxicological profile observed for the algae (Fig. 6a), which can similarly be attributed to the mixture of parent pharmaceuticals and derived compounds. Meanwhile, the toxicity of the BioeF effluent always remained below 20% inhibition

in all phases, thus correlating more closely with the concentrations of pharmaceuticals observed in Fig. 3. Though they were probably not the only source, it should be noted that  $\beta$ -blockers (particularly PRP) and their mixtures have been reported as highly toxic to *D. magna*, and their metabolites often exhibit higher toxicity than the parent compounds [63].

Curiously, this does not seem to have been the case for BioeF, as the suspected metabolites did not significantly affect the *Dafnia* in the effluent; the toxicity presented a closer correlation to the concentrations detected in the effluent, particularly those of PRP and TER. As the chiral forms of both increased in lockstep, we cannot attribute this to any specific enantiomer. Given the divergence in toxicity levels for the ConF in Phase IV, it appears that the metabolites generated by MET differ significantly in toxicity to *D. magna* from those generated in the ConF. Whether this is a matter of the concentration or of the exact compounds generated is a question for future work. Nevertheless, the detoxification achieved with the BioeF was four times greater than that obtained with the ConF.

In the herbicide experiment, the toxic response of the ConF effluent in the *D. magna* assay closely followed that of the influent. This confirms the data on general removal (Fig. 2) and enantiomer concentrations (Fig. 4), which indicate little to no degradation of herbicides in the ConF. The toxicity profile when using crustacea for the BioeF effluent is comparable to that achieved using algae, with the toxicity decreasing as the experiment progressed, leading to similar conclusions. The toxicity values for the BioeF effluent were below those of the ConF in all phases, achieving detoxification rates between two and five times higher. This also aligns with the higher EF changes in the BioeF, reported in Table 2, which was likely also due to the enhanced metabolism activity promoted by MET [17].

Studies of ecotoxicity in bioelectrochemical systems are rare, and no published articles using comparable conditions were found by the authors. Previous work by our group [29], which used a horizontal biofilter feed under a continuous regime with a different combination of EC (with concentrations closer to those found in urban wastewater), reported complete detoxification of *Daphnia* from an initial influent with 40% toxicity. The toxicity of algae in the same system was reduced from 90% growth inhibition to an average of 30%. Another work [65] used a polarized fluidized bed system and achieved an average toxicity reduction of 50–70% for *Vibrio fischeri* corresponding to the electric potential applied. Although it has limits, the evidence thus supports the value of bioelectrochemical systems for toxicity removal applications.

#### 4. Conclusions

This work evaluates for the first time the chirality of pollutants in complex mixtures of xenobiotics treated with vertical electroactive biofilters. We compared the enantiomeric profiles of pharmaceuticals and herbicides subjected to MET-based wastewater treatment with those subjected to conventional treatment.

Regarding removing pharmaceuticals, we obtained greater differences in the system's performance for all the  $\beta$ -blockers, which appear highly biodegradable under bioelectrochemical processes but poorly removable using conventional biological filtration processes. Interestingly, PRP, considered the most ecotoxic of the  $\beta$ -blockers [63], was also the one with the strongest contrast in EF between the ConF and BioeF, suggesting that PRP is also the pharmaceutical most acutely affected by MET. The ecotoxicity assays reinforced this conclusion: considering the inhibition values of *D. magna*, the detoxification achieved with the BioeF was four times greater than that obtained with the ConF, indicating a clear advantage of the BioeF over the ConF.

The rest of the pharmaceuticals, whose structures differ from

those of the  $\beta$ -blockers, achieved improved removal in the BioeF compared to the control system, and in all cases, the removal rates were in the upper tier or above compared to the literature. In some cases, such as TER and VER, these changes corresponded to advanced oxidation processes, suggesting a higher removal efficiency, at least from an energy perspective. These mechanisms may be inherent to the bacterial communities of biofilters and merely enhanced by the increased metabolic rate provided by MET, as no significant deviations in  $\Delta$ EF were observed between the BioeF and the ConF for these ECs.

These results characterize electroactive biofilters as an effective treatment for pharmaceutical mitigation.

Their effect on phenoxy acid herbicides was stronger, with a notable increase in the removal and detoxification of the effluent. The bacterial activity on the herbicides was apparent in the transformations of enantiomers in both systems. The marked changes in EF point to strong enantioselective processes occurring in the BioeF for phenoxy acid herbicides. Given the low removal rates observed, it is likely that the changes in EF correspond to transformations from one enantiomer into the other, although this is far from the only possibility. Nonetheless, the ecotoxicity reduction shows that the microbial activity in the electroactive biofilter strongly dampened the toxic effect of the phenoxy acid herbicides, facilitating faster adaptation than conventional systems and reducing toxicity in the effluent.

The observed changes in chirality in the pharmaceutical and herbicide mixtures correlate with the biodegradation capacities of the studied systems, which were higher in the bioelectrochemical reactors. In this context, the changes in enantioselectivity of the micropollutants obtained in the biofilters with their capacity to reduce water toxicity seem to have followed the same trend, which was more evident in the case of herbicides.

Our work presents the first evidence of a relationship between changes in contaminant chirality and detoxification capacity. It should be noted that the contaminants studied in this work formed a multicomponent mixture in which, toxicologically, the mixtures consisted of each of the two enantiomers of the studied pharmaceutical or herbicide. Additionally, the complexity of the mixtures increased in the biological reactor due to the appearance of unknown metabolites, which also contributed to the measured toxic response. It is very difficult to identify a clear pattern when working with complex mixtures and, therefore, accurately assess the degree of interaction between chirality changes and observed detoxification capacity. In this context, further studies are needed to analyze chiral micropollutants individually and to measure the toxic response of each enantiomeric species to minimize the interference of other chemical species that appear during the reaction.

In a nutshell, the increased microbial activity provided by MET results in an overall improvement of the treatment capabilities of biofilters, both in EC removal and in the reduction of its ecological effects, implementing MET systems a promising technological advance for addressing the growing problem of EC presence in the environment.

#### CRedit authorship contribution statement

**Álvaro Pun:** Writing - Review & Editing, Writing - Original Draft, Methodology, Investigation, Data Curation. **Jesús Valimaña-Traverso:** Writing - Original Draft, Methodology, Investigation, Data Curation. **María Ángeles García:** Writing - Review & Editing, Supervision, Resources, Project Administration, Methodology, Investigation, Funding Acquisition, Formal Analysis. **María Luisa Marina:** Writing - Review & Editing, Validation, Supervision, Project Administration, Investigation, Funding Acquisition, Formal Analysis. **Abraham Esteve-Núñez:** Writing - Review & Editing,

Supervision, Resources, Project Administration, Funding Acquisition, Formal Analysis, Conceptualization. **Karina Boltes:** Writing - Review & Editing, Writing - Original Draft, Supervision, Software, Project Administration, Methodology, Investigation, Funding Acquisition, Formal Analysis, Data Curation, Conceptualization.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgments**

This work was supported by the Spanish Ministry of Science, Innovation and Universities research project mobiMET. Ref: CPP2021-008936. M.A.G. and M.L.M. thank the Ministry of Science and Innovation (research project PID2022-140512NB-I00) and the University of Alcalá (research project PIUAH23/CC-041).

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ese.2024.100500>.

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