Detoxification of wastewater containing pharmaceuticals using horizontal flow bioelectrochemical filter

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**Abstract**

Removal of a mixture of 13 pharmaceutical compounds was investigated in this work in a horizontal subsurface flow electroconductive filter. Biofilter was constructed using electroconductive carbon with capacity for allowing the electron flow between microbial communities at different redox environments; under a configuration called snorkel. Stable operation was observed during 200 days, with medium values for COD and N-NH4 removal of 92±3% and 70±10%, respectively under different organic loading rates. Most of the pharmaceuticals tested were removed above 90% (seven compounds) at HRT of 1 day while 65% were removed at HRT as low as 0.5 days.

These results were corroborated by analysing the detoxification efficiency of the biofilter using microcrustaceans and a green alga as bioreporters. Results revealed that effluents were detoxified in 80% for HRT of 0.5 days. Electroconductive biofilter was proved as a promising technology for the elimination of emerging pollutant from wastewater with minimum operational requirements.

**Keywords:** microbial electrochemical technology; pharmaceuticals; mixture toxicity; biofilter.

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**1. Introduction**

A growing environmental issue that modern society has to face is the expansion of daily use of synthetic substances, these constitute a new type of emerging contaminants (EC). Of these, pharmaceuticals are an important and diverse group in surface waters due to their increasing release near population centers and their proven toxicological effects on different organisms even at trace levels (Mezzelani et al., 2018; Noguera-Oviedo et al., 2016). Conventional sewage treatment plants do not completely remove many of these compounds, so their continuous release makes them persistent in the environment. It is therefore essential to either adapt current processes or implement new ones to eliminate these ECs.

All biological treatments of wastewater require a suitable electron acceptor as a sink for the electrons generated by the microbial oxidation of organic matter; very commonly, this is artificially supplied oxygen. In contrast to this classical approach, in early 2000s a new discipline of microbial electrochemistry emerged using electrically conductive materials as an electron acceptor for electrons from bacterial metabolism (Ramírez-Vargas et al., 2018). This process was then codified into the field of microbial electrochemical technologies (METs). Although electrical current can be harvested through METs (Borjas et al., 2015), it is even more remarkable that using electroconductive material, bacteria are not limited by the availability of electron acceptors. This allows microbial oxidative rates to be enhanced (Aguirre et al., 2016). On top of that, the presence of conductive material promotes a novel syntrophic metabolism called Direct Interspecies Electron Transfer (DIET). This microbial redox strategy allows cells to directly exchange electrons from differing metabolisms, allowing microorganisms to utilize an expanded profile of substrates (Chen et al., 2014). The immediate consequence of this metabolic expansion may be a methanogenesis using an “electron charged” bacteria as sole electron donor for the methanogens, and also the complete mineralization of recalcitrant pollutants (Dominguez et al., 2018; Rodrigo Quejigo et al., 2016 and 2019). Such an expansion of substrate profile opens the possibility to biologically degrade recalcitrant pollutants like ECs through electroactive microbes.

Removal efficiency of antibiotics and other pharmaceuticals by METs have showed promise as compared to other technologies (Cecconet et al. 2017, W. Yan et al., 2019). Until now, previous work mainly studied the treatment of single compounds using conventional one or two chamber Microbial Fuel Cells (MFCs) reactors or Microbial Electrolysis Cells (MECs). The systems all have similar designs including graphite electrodes in the form of bars, plates, and/or felts. Antibiotics, specifically sulfamethoxazole, was reported as degradable in both bioelectrochemical systems (Wang et al., 2016; Miran et al., 2018), reaching removal efficiencies from 85% to more than 99%.

Conversely, some studies on the treatment of mixed organic micropollutants, including pharmaceuticals, using bioelectrochemical reactors presented controversial results. Werner et al., 2015 did not find any advantages in the removal of a mixture of ten pollutants (including five pharmaceutical compounds) as compared to conventional wastewater treatment. In contrast, Chang et al., 2014 found removal higher than 90% for a mixture of 3 pharmaceuticals in a MFC coupled with an aerobic reactor. In the same vein, Zhou et al., 2018 reported a complete elimination of a mixture containing four antibiotics.

Studies can be also found in the literature on the use of granular active carbon as the electrodes for a bioelectrochemical reactor to remove antibiotics. Zhang etal., 2016; Song et al., 2017 and Yang et al., 2018 reported good removal of organic matter, sulfamethoxazole, tetracycline or sulfadiazine using 3D biofilm electrode reactors operated under potentiostatic control. Here, the fixed bed was formed by four layers of different materials (active carbon fiber, titanium mesh, granular-activated carbon, and gravel). In the same way, coupling of constructed wetland (CW) and a MFC was also reported by Zhang et al., 2017 and 2018, and Li et al., 2018 for the efficient elimination of the same antibiotics. In these works, the bioelectrochemical system was embedded into a fixed bed and also operated in line with one or two external MFCs and MECs, illustrating the possibility of multiple operational schemes and reactor configurations. Moreover, an attractive combination of a membrane bioreactor (MBR) and a MFC was used, using an expanded cathode of granular activated carbon to synthetize H2O2 which increased the removal of tetracycline in water solutions (Li et al., 2017).

All these previous reports show the great potential for ECs degradation by bioelectrochemical processes, but so far all these strategies have been tested at the lab scale, with reactor configurations that are difficult to scale-up. In order to maximize the impact of this new strategy it makes sense to test configurations with the ability to be scaled-up. In that sense, the only MET-based system already applied at full scale is the METlands ([www.imetland.eu](http://www.imetland.eu)). These systems integrate a microbial electrochemical biofilter with a constructed wetlands (CW) (Ramírez-Vargas et al., 2018).

In standard CW or biofilters, the wastewater is treated as it flows through a porous medium that acts as support for bacterial communities. The fixed bed is either gravel or synthetic materials (plastic or ceramics) specifically designed for improve flow through the bed and offer a high active surface for biofilm attachment. METlands substitute these inert materials with conductive ones, turning the bed into an active part of the degradation process through its link to electroactive microorganisms. This combination of technologies provides a great enhancement to COD, BOD, and nitrogen removal in comparison with standard CW made of inert gravel (Aguirre-Sierra et al., 2016). Full-scale applications (2-20 m3/day) of such METlands demonstrated the feasibility of the technology for treating urban wastewater. Conceptually, METland systems operate similarly to a microbial electrochemical snorkel composed of a single conductive material that allows electron flow between areas rich in either electron donors or electron acceptors (anoxic and oxic zones). The metabolism of the electroactive biofilm can be correlated to the electron flow in the electroconductive bed, or to the ionic current in the water matrix, both resulting in a high COD removal activity (Ramírez-Vargas et al., 2019).

The filter bed used in this work, similarly to the METland, was made of electroconductive material. Unlike to all previously published work, we used a carbon waste, namely graphitized coke. This material, which is generated by high temperature treatment of residues from steel foundries, has been extensively tested in a previous work. Prado et al., 2019**,** reported an electrical conductivity at 0.38 S cm-1, a highly irregular surface, with an abrupt morphology, large crater holes, and pores between 10 and 100 µm.

Biofilters are low energy systems, which make them an ideal technology for small population communities**.** According to the 2019 report of the European Environmental Agency (https://www.eea.europa.eu), in central Europe almost all wastewater is collected and treated but in northern regions 15% is directly released into waterways. This percentage reaches almost 25% in southern regions, and worsens for the southeast with 57% of wastewater discharged without secondary treatment. This is mainly associated with small and rural populations (<2000 eq-hab) where conventional water treatment plants are not considered cost effective. In turn there is an increased release of ECs into nature from these rural communities. A potential solution is different configurations of treatment systems/plants. CWs are widely used worldwide, especially for secondary and tertiary wastewater treatment. Their performance and effectiveness for the removal of pharmaceuticals is persuasive. In general, there is a wide variation in pharmaceutical removal efficiencies among CW due to the diversity of their configurations and operation (Zhang et al., 2014, Gorito et al., 2017).

No previous studies were found on EC removal using horizontal subsurface flow bioelectrochemical CW. In this work, we investigated for first time the capability of a lab scale microbial electrochemical system based on a horizontal subsurface flow METland for removing a mixture of 13 pharmaceutical compounds at trace levels. The mixture contained four analgesics/anti-inflammatories, an antibiotic, a beta-blocker, a stimulant, an anti-depressant, three metabolites from analgesic drugs, and two metabolites from stimulants. Wastewater containing pharmaceuticals presents an environmental danger due to the toxicity of the parent compounds and their derived metabolites, which if not eliminated during treatment are continuously discharged into our ecosystems. In order to evaluate the environmental risk of the wastewater treated in the horizontal subsurface flow MET-based biofilter, we also analyzed the efficacy of the detoxification of the filter using two of the most common toxicological indicators of aquatic ecosystems: green algae and microcrustaceans. Additionally, the effect of the biological degradation versus physicochemical sorption was also studied by measuring the removal capability of inert carbon materials for the same pharmaceutical mixture.

**2. Materials and methods**

***2.1. Chemicals***

The following pharmaceuticals were all purchased from Sigma Aldrich, with a 99.9% purity: Ampyrone (4-AA, CAS 83-07-8), N-acetyl-4-aminoantipyrine (4-AAA,CAS 83-15-8), Aminophenazone (4-DAA, CAS 58-15-1), N-formyl-4-aminoantipyrine (4-FAA, CAS 1672-58-8), Acetaminophen (PAR, CAS 103-90-2), Atenolol (ATE, CAS 29122-68-7), Caffeine (CAF, CAS 58-08-2); Carbamazepine (CBZ, CAS 298-46-4), Cotinine (COT, CAS 486-56-6), Ketoprofen (KET, CAS 22161-81-5), Naproxen (NPR, CAS 22204-53-1), Paraxanthine (PXA, CAS 611-59-6). and Sulfamethoxazole (SMX, CAS 723-46-6). The rest of the chemicals were also analytical grade and acquired from Sigma-Aldrich. Ultrapure water was generated from a Direct-Q™ 5 Ultrapure Water Systems from Millipore (Bedford, MA, USA) with a specific resistance of 18.2 MΩ cm.

***2.2. Systems construction and operation.***

A horizontal subsurface flow biofilter was constructed inside a polypropylene box (LWH: 315 × 215 × 155 mm), with a perforated inlet at the middle top and an outlet at the middle bottom on the smaller sides. The electroconductive biofilter was built with graphitized coke provided by METfilter SL. resulting in a bed with a depth of 7 cm, and a volume 5.29 L weighting 3.41 kg.

The hydraulic volume of the system was 2 L with the height of the water table at 6 cm. A T-pipe placed at the outlet was open to the atmosphere to maintain the water table at the correct height. All sides of the box were covered with opaque plastic to avoid light exposure. The top of the box was loosely covered with aluminum foil, allowing gas exchanges but limiting light exposure of the top layer of the biofilter. The operation of the system took place in an acclimatized laboratory with temperatures kept close to 22 ºC.

For phase I, the biofilter was initially kept under batch conditions for three weeks, inoculated with 200 mL of *G. sulfureducens* culture added to 1.5 L of Fresh Water medium (Lovley and Phillips, 1988). An inoculum of 100 mL of a real urban wastewater from Carrión de los Céspedes (Seville, Spain) was added two weeks into phase 1. Afterwards, a pump was used to continuously feed the biofilter at a hydraulic retention time (HRT) of 1 day. This HRT was maintained for 2 weeks for biomass activation and biofilm formation. The system was fed with autoclaved synthetic wastewater (SW), which was maintained at 4C and sparged with nitrogen gas.

In phase II, the biofilter was operated for a total of 11 weeks. The system was tested under HRT of 2, 1, and 0.5 days, each condition maintained for a minimum of 6 times the current HRT. Steady state was determined by the stable removal of total organic carbon (TOC). Phase III totaled 10 weeks under the same hydraulic conditions as phase II. The reactors were fed the same SW spiked with a cocktail of ECs. The exact time for the third step of operation was: 4 weeks at HRT 2 days, 3 weeks at HRT 1 day, 3 weeks at HRT 0.5 days. Finally, in the phase IV, the feeding was returned to SW without ECs. In phase IV, over 6 weeks, the HRT was gradually increased from 0.5 to 2 days to assess changes in the system as a result of the exposure to the ECs in phase III.

***2.3. Influent composition.***

The SW (Table 1) was prepared based on Osachoff et al. 2014. The 13 ECs and their concentrations were chosen based on a sampling campaign of real wastewater in a rural area of Seville, Spain. Contaminants stocks were prepared with deionized water:methanol (2000:1) at 4ºC and used to produce the desired concentration in the SW.

***2.4. Analytical methods.***

The sampling of the effluent took place daily at the outlet, while the influent was collected at the end of the inlet pipe. Samples were filtered with a 0.45 µm nylon membranes prior to storage at -20 ºC. TOC was determined daily in the fraction of non-purgeable organic carbon (NPOC) with dilution rate of 1:5 in a TOC-VCSH Shimadzu analyzer. TOC was used as the main indicator for treatment efficiency and steady state achievement. Nitrogen species were monitored by ion chromatography using a Metrohm 930 Compact Ion Chromatograph Flex. Pharmaceutical concentrations were determined with samples concentrated by SPE with OASIS HLB extraction cartridges and analyzed with HPLC-QTOF according to Martínez-Hernández et al., 2017.

***2.5. Toxicity.***

Ecotoxicity assays were performed using the green alga *Raphidocelis subcapitata,* and the microcrustacean *Daphnia magna* as bioindicators. The green algae inhibition growth test was done according to a modified OEDC TG 201 open system (OECD, 2006) as in previous work (González-Pleiter et al., 2013). Each sample tested had four replicates. Tests were performed with a randomized placement in every run including blanks and controls without pollutants.

The mobility of *D. magna* assay followed strictly OEDC Guideline 202. Each sample and control had four replicates. Incubation was under continuous illumination at 20ºC and youths’ mobility was visually assessed at 24 h. Both the effluent and adsorption controls were evaluated for toxicity.

All nutritive solutions and organisms for ecotoxicity test were purchased from MicroBio Test Inc. (Belgium).

***2.6. Adsorption assay.***

The ability of the electroconductive material to retain the pharmaceuticals used was assessed under sterile conditions by contact with 2, 5, and 10 g of coke with 500 mL of TW in closed glass bottles. Three replicates of each condition were kept at 25 ºC during 24 h in an orbital shaker. Initial and final concentrations of contaminants were determined as described in sections 2.4.

**3. Experimental results and discussion**

***3.1. Removal of organic matter and nitrogen.***

The horizontal subsurface flow METland was operated over 200 days under continuous flow with differing HRTs, as stated in section 2.2. Once steady state was reached for each defined condition, the concentration of total organic carbon (TOC) and nitrogen species were evaluated.

Fig. 1 shows the concentrations of TOC and nitrogen species at the influent and the effluent of the biofilter, while operated at different HRTs in the presence of the ECs. No significant differences in bioreactor performance was observed for the elimination of organic matter at HRTs between 2 to 0.5 days during the feeding of SW with pharmaceuticals in phase III (Fig. 1A). TOC removal appears to be independent of the presence of pharmaceuticals. In fact, TOC removal obtained in the phase II (before addition of EC) and also for the phase IV (after the EC was removed from the feeding) varies from 93.7 to 92.7% and 93.5 to 92.5%, respectively. Considering the 200 days of biofilter operation, the efficiency of organic matter removal was 92±3% under organic loading rates (OLR) between 3 and 14 TOC g m-2 day-1.

Similar removal efficiencies were obtained by Aguirre-Sierra et al., 2016 treating real urban wastewater. In this previous work with METlands, an organic matter removal efficiency of 91% COD at HRTs ranging from 4 to 0.5 days was reported. Ramírez-Vargas et al., 2019 further confirmed the performance of METlands with a removal efficiency of 90% for COD at 10-fold higher loading rates than those commonly used for CW.

Concentrations of nitrogen species in effluents through the study (Fig. 1B) showed N-NH4 removal at 70±10% under all conditions. Part of the N-NH4 was oxidized to N-NO3, in spite of the anaerobic conditions. Both oxidization and removal suffered by decreasing HRT. These values are in accordance with the values reported by Aguirre-Sierra et al., 2016 and Ramírez-Vargas et al., 2019.

Both organic matter and nitrogen removal reached in the electroconductive biofilters are higher than those typically described in the literature for conventional CW. Extended reviews of Valipour and Ahn, 2016 and Jahangir et al. 2016, reported a wider range of variation, with 60-80% removal of organic matter and 20-99% removal of nitrogen species when considering similar systems.

Regarding the removal of organic matter in MET based processes, Xu et al., 2016, Mansoorian et al., 2016, and Abassi et al., 2016 showed that the efficiency of COD removal varied from 75 to 95% at the lab scale. This was independent of reactor configuration and flow regimen for both real and synthetic wastewater. Comparison was difficult due to the different reactor configurations, HRTs, and the nature and concentration of the organic matter. Even so all previous published data confirms the advantage of bioelectrochemical treatment over conventional biological processes to remove COD.

In contrast to the extensive data published on organic matter treatment by MET-based processes, studies in which antibiotic elimination was evaluated did not report COD removal associated with the biological processes. Only the work of Zhang et al., 2016 and Li et al., 2017 showed COD removal in the presence of antibiotics at 98.9% and 90%, respectively. In addition, N-NH4+ elimination was 80% for Membrane Bioreactor-MFC coupled system treating sulfamethoxazole and tetracycline (Li et al., 2017).

***3.2. Removal of pharmaceuticals.***

*3.2.1. Abiotic sorption on materials*

The sorption capability of coke to remove the evaluated pharmaceuticals at 24 h of contact was studied. Fig. 2 shows changes in concentration for all compounds in contact with the inert material. The removal of pharmaceuticals by sorption on the material was between 13.1% to 18.5% per g of coke for all compounds, except for CBZ which reached 29.7%. The lowest removal efficiency, for SMX, is unsurprising in light of previous studies were SMX has been described as outcompeted at sorption sites by most comparable chemicals (Reguyal and Sarmah, 2018).

Moreover, the sorption profiles showed favorable adsorption for all drugs and metabolites on coke. In batch assays of this study the maximum sorption capability of material is not reached due to the low level of concentration of pharmaceuticals, but the results obtained in this work indicate the trend on the materials used.

*3.2.2. Operation of biological systems*

Concentrations of pharmaceuticals at the inlet and the outlet of the reactor, obtained for the higher organic loading conditions (0.5 day of HRT), are show in Fig. 3. Here, 5 of the EC were removed above 99% (SMX, PXA, CBZ, CAF and 4-AA) and another 2 above 95% (ATE, NPR). Several other EC were also removed at high percentages (85% for KET, 79% for 4-AAA) or medium high at 65-70% (PAR, 4-DAA, COT). Only one compound (4-FAA) presented a low removal efficiency at 34%.

SMX and CBZ are described as hard to degrade in purely biological systems (Zhang et al. 2014) but recent studies point to membrane and biolelectrochemical systems as effective in their removal at 100% and 80% respectively (Ceconet et al. 2017; Zhang et al. 2017). This study obtained similar results albeit lower removal efficiency for CBZ. While these systems had similar removal efficiencies, the systems described in previous works had high-energy demands for pumping or polarization. The MET biofilter offers better energy usage for pharmaceuticals treated due the lack of these high energy demands.

Traditional CW have been described as being able to remove ATE and NPR in similar quantities as this work (Zhang et al. 2014; Ceconet et al. 2017). Meanwhile KET is generally considered a problematic compound in conventional wastewater treatments, with a varying overall removal about 30-100% dependent on the particular system employed (Zhang et al. 2014; Martínez-Hernández et al. 2018). Nevertheless, the MET-based biofilter removed the 85% of this drug even at the lower residence time used.

Both PAR and COT are considered easy to degrade, with high removal efficiencies in conventional biological water treatments. 100% removal of PAR can be reached as was reported by Luo et al. 2014. The main human metabolite of nicotine, COT, is readily degradable by several specific bacterial strains (Gurusamy and Natarajan, 2013; Qiu et al. 2018). The relatively low elimination in our system is unexpected, and may suggest the lack of the proper bacteria for the degradation.

Primary and secondary metabolites related by degradative pathways should receive a special consideration for removal purposes; not only does the system gets an influx with the influent, but are also generated inside the biofilter by the degradation of their precursor. PXA is one of the first possible steps for CAF degradation (Ibrahim et al. 2014). When compared to the low removal of CAF and PXA seen in the adsorption assay, the high removal of both compounds denotes a highly active metabolic route for degradation, in accordance with the easy biodegradability of CAF (Zhang et al. 2014).

The compounds 4-DAA, 4-AA, 4-FAA, and 4-AAA are all closely related to the pharmaceutical Dipyrone, and are all described as part of its degradation pathway (Pieper et al. 2010), except for 4-DAA which differs by an extra methyl group from the first compound in the pathway, 4-MAA. 4-AA is the next step of degradation with both 4-FAA and 4-AAA as the final forms described in the pathway.

The literature mentions 4-FAA as the last step in the degradation pathway for human metabolism of Dipyrone, and is also considered as a persistent compound in waterways. In a conventional biofilter Pieper et al. 2010 proved that the described pathway occurs rapidly (12 h), with almost no 4-AA found and an increase (28%) of 4-FAA at the outlet, without appearance of 4-AAA. With a similar lack of 4-AA, the results obtained in the horizontal flow bioelectrochemical filter support this proposition. The 4-FAA concentration of the system does not increase but 34% is removed despite the added production by the degradations of precursors. For 4-AAA, Gyenge-Szabó et al. 2014 described higher removal efficiencies (80-95%) in aerated biofilters than the bioelectrochemical filter along with comparable results for 4-FAA (40%) and worse removal for 4-AA although highly variable (6-75%). This last value suggests a slower metabolic rates than our system, and thus a slower generation of 4-AAA and 4-FAA in their aerated biofilter.

Regarding to the removal profiles of EC related to the changes in the HRT of biofilter, it was observed that most of the EC were eliminated with almost constant efficiency, unaffected by HRT. Only 3 EC presented a relevant enhancement in removal with an increase of HRT: 4-DAA (20%), 4-FAA (38%) and PAR (26%) when comparing an HRT of 0.5 days to 2 days. Additionally, KET removal was also increased to 14% under these conditions.

An interesting response was obtained for PXA, which, contrary to the previous cases, showed complete removal at the lower HRT assayed, probably explained by some adaptive response of biomass to this compound.

According to the analytical measurements obtained for EC, the MET-based biofilter showed better removed pharmaceuticals, at higher efficiency than conventional systems. Comparison to others MET-based systems reported in literature was only possible for the antibiotic SMX; the most studied drug in bioelectrochemical process until now. In our system we obtained an almost complete removal of SMX, a similar result to those reported by Zhang et al., 2016, Song et al., 2017, and Yang et al., 2018. Unlike these studies, who relied on polarized electrodes, our system lacks any potentiostatic control, letting the conductive material reach its natural potential, and thus forgoing both complexity and the associated energy cost.

***3.3. Detoxification***

The toxicity of the feed synthetic wastewater and the effluent from the bioelectrochemical filter was determined for first time in this work. Two species typical as environmental ecotoxicity indicators were used: *R. subcapitata* and *D. magna.*

*3.3.1. Detoxification from abiotic sorption on materials*

Both organisms were negatively affected by the TW. The microcrustacean *D. magna* proved to be less sensitive than green algae. *R. subcapitata* growth was completely inhibited (100 % inhibition) by the initial TW, but in equilibrium with coke the inhibition decreased to 87%. These results showed a small removal of toxicity by the abiotic sorption on coke, and are in accordance to the low removal of EC reported in section 3.2.1.

*3.3.1. Operation of biological systems*

Fig. 4A shows the toxicity of the influent and effluent of the biofilter as measured by the percentage of immobilized daphnisneonates after 24 h. Here, it was observed that the system decreased toxicity for *D. magna* in all phases of the experiment. Toxicity values decreased from the initial 17.5% to 0% in phase 2 (background toxicity without EC) and also in phase 4, after EC were removed from feeding. Results confirm the almost complete detoxification of wastewater filtered by the biolectrochemical system.

Moreover, the toxicity of influent at phase 3 (with EC) is 2.4-fold higher the background toxicity for the invertebrate, so *D. magna* can be considered sensitive to the mixture of drugs used in this work.

Toxicity of the effluent did not change significantly with changes in HRT in phase 3. In fact, the ecotoxicity measured by *D. magna* inthe effluent was only 0-2.5%, values considered as non-toxic. According to this ecotoxicity profile, the MET-based biofilter remove more than the 95% of the associate toxicity with the pharmaceuticals, even at the lower HRT of 0.5 days. Biofilter maintained their high capability for detoxification even after a long exposure to the mixture of pharmaceuticals, as can be observed by toxicity values measured in phase 4.

The result obtained for the algae assays (Fig. 4B), showed that the base wastewater SW was already slightly toxic with a 35% inhibition of the growth rate of the algae. The addition of the pharmaceuticals increased the influent toxicity 2.8-fold over the background toxicity, so the green algae was more sensitive to the synthetic wastewater and EC than the daphnids.

Contrary to the assays using microcrustaceans, here changes in the toxicity of the effluent as response to the lowering of HRT were observed.

The effluent remains above the 20% threshold of non-toxic values for HRT of 1 and 2 days, showing an increment of 14% in toxicity due to the higher loading rate of the contaminants. Nevertheless, better toxicity removal at the lower HRT of 0.5 days was obtained, probably explained by an adaptive response of biomass to the toxic mixture used.

Only one study was found reporting bacterial biotoxicity reduction from SMX after treatment in a MFC (Wang et al. 2016). Wang et al. showed significant reduction of antibacterial activity by SMX towards two bacterial species, *Shewanella oneidensis* MR-1 and *Escherichia coli* DH5.

This persistent reduction in toxicity compared to the value obtained in the abiotic assay reinforces our idea of a strong degradation effect by the bacterial community in neutralizing the contaminants.

The toxicity of mixed chemicals is a hot spot in environmental chemistry. A lot of work was published evaluating the environmental risk of mixtures of pollutants on different ecosystems (González-Pleiter, et al., 2013; Melvin et al., 2014; Backhaus T., 2016), and also the detoxification capability of treatments (Wen et al., 2018; Bansala et al., 2019). But until now, no data has been reported for detoxification of a mixture of pharmaceuticals with bioelectrochemical reactors. The biofilter evaluated in this work reached detoxification efficiency higher than 80% for both biosensors used at the shorter HRT of 0.5 days, lowering the toxicity of the effluent at non-toxic level.

4**. Conclusions**

The horizontal flow bioelectrochemical filter is effective for the elimination of EC from wastewater, reaching almost complete removal of most of drugs, without sacrificing the removal yield of organic matter or nitrogen species. System effluent results non-toxic without polarization or complex reactor configurations. Given the minimal contribution of material sorption, in comparison to the overall removal, the biofilter mainly degraded pharmaceuticals through biological processes. Although deeper evaluation of these systems is required, this technology shows great promise as a low energy, high efficiently, and fully scalable method for addressing the growing EC problems in our waterways and ecosystems.

E-supplementary data of this work can be found in online version of the paper

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**Figure captions:**

Fig. 1. Concentrations of organic matter (A) and nitrogen species (B) at the influent and effluent of the systems, related to HRT in phase III. The error bars indicate 95 % confidence intervals.

Fig. 2. Adsorption assay on biofilters materials: Initial concentrations of pharmaceuticals and equilibrium concentration after 24 h of contact. The error bars indicate 95 % confidence intervals.

Fig. 3. Concentration of pharmaceuticals at the influent and effluent of the systems at HRT 0.5 days. The error bars indicate 95 % confidence intervals.

Fig. 4. Toxicity values to influent and effluent of the system. *D. magna* after 24h exposure (A) and *R. subcapitata* after 3 days exposure (B). The error bars indicate 95 % confidence intervals.