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Treatment of a simulated wastewater amended with a chiral pharmaceuticals mixture by an aerobic granular sludge sequencing batch reactor

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ABSTRACT

An aerobic granular sludge-sequencing batch reactor (AGS-SBR) was fed for 28-days with a simulated wastewater containing a mixture of chiral pharmaceuticals (CPs) (alprenolol, bisoprolol, metoprolol, propranolol, venlafaxine, salbutamol, fluoxetine and norfluoxetine), each at 1.3 μg L^{-1}. AGS-SBR exhibited the highest removal efficiency for norfluoxetine, with preferential removal of the (*R*)-enantiomer indicating that biological-mediated processes occurred. For all other CPs, removal was non-enantioselective, occurring through biosorption onto AGS. A gradual decline of CPs removal was observed, probably related to the decrease of AGS adsorption capacity. Moreover, chemical oxygen demand (COD) content in the bulk liquid after anaerobic feeding increased, and P-release dropped, probably because the polyphosphate-accumulating organism's activity was affected. Nitrification was also affected as indicated by the ammonium effluent concentration increase. Moreover, CPs exposure promoted AGS disintegration, with decreasing granule size. After stopping CPs feeding, the AGS started to recover its compact structure, and the system returned its normal performance concerning COD- and P-removal. N-removal seemed to be a more sensitive process, as while the ammonium levels were fully restored at the end of operation, nitrite reduction was only partially restored. Results provide useful information on AGS performance during the treatment of wastewater containing pharmaceuticals, a frequent scenario in WWTP.

1. Introduction

In the last decade, environmental contamination from pharmaceuticals has received great attention. Pharmaceuticals are generally highly recalcitrant compounds, and even at residual concentrations they may adversely affect non-target organisms (Kasprzyk-Hordern et al., 2010; Petrie et al., 2015). Most of the top-selling pharmaceuticals marketed are chiral pharmaceuticals (CPs), which are dispensed as racemic mixtures or in their enantiomerically pure form (Ribeiro et al., 2014a). Although enantiomers have similar thermodynamic properties, their biological effects can be

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different due to enantioselective interactions with biological systems (De Andrés et al., 2009; Sun et al., 2014). Unlike abiotic processes, CPs removal in wastewater treatment plants (WWTPs) can be enantioselective and racemization can also be biologically promoted (Pérez and Barceló, 2008). WWTPs are not designed to completely remove these contaminants, being unable to avoid their discharge into receiving waters. The occurrence of CPs in the environment is an issue of great concern due to the potential toxicological effects in aquatic life, and the enantioselective behavior of the enantiomers in biological processes (Kasprzyk-Hordern, 2010; Ribeiro et al., 2012). In the latest revision of the Water Framework Directive (European Community, 2000; Directive, 2000/60/EC), the European Union has already included the anti-inflammatory drug diclofenac in the watch list of substances to be monitored (Council, 2013; Directive, 2013/39/EU, 2013), pending a possible subsequent definition of Environmental Quality Standards. It is expected that in the future there will be an inclusion of parametric values of such micro-pollutants in water bodies and/or in WWTP discharges, providing a strong drive for the development of highly efficient biotreatment systems able to remove pharmaceutical compounds from effluents (Kümmerer, 2009).

Aerobic granular sludge-sequencing batch reactors (AGS-SBRs) are promising biotechnological systems for wastewater treatment, successfully applied in the treatment of domestic and industrial wastewaters (Gao et al., 2011). The excellent settling ability, the dense and strong microbial structure, the ability to withstand high organic loading rates, and the high tolerance to toxicity are unique features of AGS that contribute to the great potential of this technology (Adav et al., 2008). Currently, Portugal, Netherlands, South Africa and China have full scale AGS-SBRs in operation and it is expected that the use of this technology will increase rapidly worldwide (Giesen et al., 2013; Li et al., 2014). Removal of pharmaceuticals from wastewater has been mostly evaluated using other biological-based treatments, such as conventional activated sludge (Osachoff et al., 2014) and membrane bioreactors (Cheng et al., 2015), using non-biological treatments such as advanced oxidation processes (Monteagudo et al., 2014), or using integrated approaches that combine physicochemical and biological processes (Yahiat et al., 2011). Only a few studies have evaluated the potential of AGS technology to treat wastewater containing such micro-pollutants (Kong et al., 2015; Moreira et al., 2015; Shi et al., 2013). Therefore, further understanding of the impact of frequently detected micro-pollutants, e.g. CPs, on the treatment process is needed to provide guidance for a stable operation. CPs enantiomers are usually treated as a unique molecular entity. The present study aimed at evaluating the removal of CPs at an enantiomeric level, and to assess the effect of the CPs mixture on the performance of the main biological processes taking place in an AGS bioreactor. The impact of the CPs mixture on the AGS structure was also evaluated, contributing to the growing knowledge on the effect of micro-pollutants on process stability. To our knowledge, this paper reports for the first time the fate of a mixture of CPs enantiomers in AGS-SBRs.

2. Materials and methods

2.1. Chemicals

Fluoxetine hydrochloride (FLX), (*S*)-fluoxetine hydrochloride ((*S*)-FLX), norfluoxetine (NFLX), alprenolol hydrochloride (ALP), (*S*)-alprenolol L-tartrate hydrate ((*S*)-ALP), metoprolol tartrate (MET), propranolol hydrochloride (PHO), (*S*)-propranolol ((*S*)-PHO), salbutamol hemisulfate (SBT), (*R*)-salbutamol hydrochloride ((*R*)-SBT) and bisoprolol hemifumarate (BSP) were purchased from Sigma-Aldrich (Steinhein, Germany). Venlafaxine hydrochloride (VNF), (*S*)-venlafaxine ((*S*)-VNF), (*S*)-metoprolol ((*S*)-MET) and (*S*)-norfluoxetine ((*S*)-NFLX) were purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany). All reference standards were of >98% purity. Stock solutions with a concentration of 1300 μg mL^{-1} for the enantiomeric mixtures and of 650 μg mL^{-1} for the individual enantiomers were prepared in ethanol and stored at −20 °C.

2.2. AGS-SBR set-up

The experiment was carried out using a lab-scale AGS-SBR with a working volume of 2.5 L, a total height of 110 cm and an internal diameter of 6.5 cm. The AGS-SBR was operated for four successive treatments of 6 h-cycles per day. Each cycle consisted of four consecutive phases: filling (60 min) during which 0.95 L of a simulated wastewater was pumped into the bioreactor; reaction (292 min) providing aeration through the reactor bottom at 4 dm^3 min^{-1} (superficial air velocity of 84.8 m h^{-1}); settling (3 min) and effluent drawing (5 min). The reactor was operated at a volumetric exchange ratio of 40% and a corresponding hydraulic retention time (HRT) of 9.7 h. The Sludge Retention Time (SRT) was not controlled over the operational period. Dissolved oxygen and pH were measured online. The pH was maintained at 7.0 ± 0.8 by dosing with NaOH or HCl. The bioreactor was operated at room temperature (25 °C), and protected from light to prevent photolytic degradation of pharmaceuticals. Activated sludge collected from the aerobic tank of the municipal WWTP of Parada (Maia, Portugal) was used to inoculate the bioreactor. The AGS had been previously exposed to fluoroquinolones and fluoxetine (Amorim et al., 2014; Moreira et al., 2015), but before the present experiments, a steady state operation concerning chemical oxygen demand (COD) and nutrient removal was achieved.

2.3. Wastewater composition and operational conditions

The bioreactor operation was split into three phases. Firstly, during phase I (from day-0 to day-28), the bioreactor was fed with a simulated wastewater. Then in phase II (from day-29 to day-57), a mixture of CPs was added to the wastewater. Next, during phase III (from day-58 to day-86), the CPs mixture was removed from the influent feeding wastewater. The simulated wastewater consisted of two concentrated media as described by de Kreuk et al. (2005). From each media, a volume of 89 mL was dosed per cycle together with 772 mL of water. In summary, apart from micro-nutrients, the average influent concentrations of COD, ammonia and phosphate were 330, 46 and 18 mg L^{-1}, respectively. During phase II, the synthetic wastewater was amended with a mixture of CPs in order to reach a concentration in the inlet flow of 1.3 μg L^{-1} of each CP. Eight CPs, belonging to three different therapeutic classes, namely ALP, BSP, MET, PHO, VNF, SBT, FLX and its metabolite NFLX, were chosen as a model for this study. These are among most prescribed drugs, and those usually found in monitoring WWTP studies, and were used at concentrations similar to those found in wastewaters influents (Oliveira et al., 2015; Santos et al., 2013).

2.4. Analytical methods

Bioreactor influent and effluent samples were regularly withdrawn and filtered through nylon membrane syringe filters (0.45 μm pore-size) to remove biomass. Phosphate, ammonia, nitrate and nitrite concentrations in the filtrate were determined with photometric test kits (Spectroquant®, Merck Millipore), according to the manufacturer's instructions.

For the enantiomeric CPs quantification, sample preparation and HPLC-MS/MS analysis were performed as described elsewhere