Aerobic granular sludge sequencing batch reactor performance under fluorinated pharmaceuticals shock loadings

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Abstract

The widespread usage of pharmaceuticals is of increasing concern. Aerobic granular sludge sequencing batch reactors (AGS-SBR) constitute a promising technology for the treatment of wastewaters, however how the removal of carbon and nutrients can be affected by such micropollutants is largely unknown.

This study evaluates the impact of different fluorinated drugs (ofloxacin, norfloxacin, ciprofloxacin and fluoxetine) on the performance of an AGS-SBR. During 468 days, a sequence of intermittent, alternating and/or continuous shock loads of pharmaceuticals were apllied to an AGS-SBR and the effects on the main biological processes were evaluated. Here we report on the effect of fluoroquinolones on reactor performnce. The organic removal, measured by COD, was not markedly affected by pharmaceuticals shock loads. Ammonium and nitrite were practically not detected in the bioreactor effluent indicating that the presence of the pharmaceuticals did not inhibit nitrification, whereas accumulation of nitrate in the effluent was observed, indicating that denitrification was affected. Phosphate removal was affected to some extent. There was no evidence of biodegradation whereas adsorption of the target pharmaceuticals to the AGS was observed, which were gradually released into the medium after withdrawal from the inlet stream.

Keywords: Aerobic granular sludge; Sequential batch reactor; fluoroquinolones; fluoxetine

Introduction

The effects of fluorine on biological properties of molecules have had a marked impact on pharmacology. Fluorine is often considered the favourite substituent to modulate unfavourable pharmacokinetic drug properties. It can improve metabolic stability, bioavailability and interactions with the biological target (Wang et al., 2014). As a result, the number of fluorinated drugs is continually increasing. It is estimated that up to 20% of pharmaceuticals prescribed or administered in the clinic contain a fluorine atom and 30% of the leading 30 blockbuster drugs by sales contain fluorine (O'Hagan, 2010). However, the presence of the fluorine atom on the molecules also increases the resistance to biotic and abiotic degradation and, consequently, they accumulate in the environment (Iwai et al., 2009).

Pharmaceuticals have received increasing attention as emerging organic pollutants due to their frequent occurrence in the environment and potential adverse effects (Fent et al., 2006). Pharmaceuticals may not be completely metabolized in the human body and can enter municipal sewage systems as the parent drug and as their "biologically active" metabolites. Some of these compounds cannot be easily removed at wastewater treatment plants (WWTPs). Even low concentrations of these substances may lead to unwanted effects in aquatic systems, since they are designed to have a biological effect at low concentrations. They can affect non-target organisms with harmful effects (Daughton and Ternes, 1999). These compounds have been found to persist in the environment and exhibit bioaccumulative and endocrine disruptive activities (Caliman and Gavrilescu, 2009). Therefore, understanding their removal and impact on WWTPs is a topic of major concern.

The biological processes occurring in WWTPs - C, N and P removal - can be affected by these pollutants. Aerobic granular sludge sequencing batch reactors (AGS-SBR) constitute a promising technology for the treatment of effluents containing micropollutants (Adav et al., 2008). Aerobic granular sludge has several advantages over activated sludge, such as excellent settling properties, high biomass retention, and ability to deal with high organic loading rates and to perform simultaneously diverse biological processes, such as Chemical Oxygen Demand (COD), N and P removal (Adav et al., 2008, de Kreuk et al., 2005).

Fluoroquinolones (FQs) are broad-spectrum antibiotics, being ciprofloxacin (CPF), ofloxacin (OFL) and norfloxacin (NOR) amongst the most worldwide prescribed antibiotics (Sukul and Spiteller, 2007). Fluoxetine (FLX) is mainly indicated for treatment of depression and is also one of the most dispensed drugs in the world (Hiemke and Härtter, 2000). There is a clear evidence of environmental contamination with these drugs (Zorita et al., 2009). The effect of the target fluorinated pharmaceuticals on the main biological processes occurring in the AGS-SBR was evaluated in this study.

Material and Methods

Chemicals

OFL, NOR, CPF and FLX (>98% in purity) were purchased from Sigma–Aldrich (Germany). All other chemicals used in this study were analytical grade (Sigma-Aldrich, Germany; Merck, Germany; VWR International, France).

SBR set up and operation

The experiment was performed in a SBR with a working volume of 2.5 L, an internal diameter of 6.5 cm and a total height of 110 cm, at a volumetric exchange ratio of 40%. Activated sludge from a municipal WWTP (2 L) was used as inoculum for the start-up. The system was operated in cycles using an automatic timer (Siemens Logo! 230RC) to start and stop pumps for influent, aeration and effluent withdrawal. Aeration was performed at the bottom of the reactor (4 L min^{-1}; superficial air velocity of 84.8 m h^{-1}). Dissolved oxygen (DO) was measured online as percentage of the oxygen saturation concentration. The oxygen saturation level was monitored, but not controlled. The pH was maintained at 7.0 ± 0.8 by dosing 1 M NaOH or 1 M HCl.

AGS-SBR operation schedule

Pharmaceuticals shock loadings were applied under different operation conditions as described in Table 1.

Table 1 – AGS-SBR operation conditions

Phase	Days	[Inlet carbon source] (µM)		Cycle time (h)	Operation
		Acetate	FQs/FLX		
1	0-96	5900	0	3	
2	97-111	5900	32	3	CPF single cycle shock applied on days 97, 98, 104 and 105
3	112-131	5900	32	3	NOR single cycle shock applied on days112, 113, 125 and 126
4	132-145	5900	32	3	OFL single cycle shock applied on days 132, 133, 139 and 140
5	146-207	5900	0	3	
6	208-228	5900	9	8	OFL continuous shock applied from day 208 to 219
7	229-249	5900	9	8	NOR continuous shock applied from day 229 to 240
8	250-270	5900	9	8	CPF continuous shock applied from day 250 to 261
9	271-291	5900	9[a]	8	Mix FQs continuous shock applied from day 271 to 282
10	292-340	5900	0	8	
11	341-468	5900	0-4	6-12	FLX shock loads

[a] 3 µM of each tested FQ

The reactor was continuously operated in successive cycles of 3, 6, 8 or 12h. Each cycle consisted of 60 min influent feeding (which was introduced in the bottom of the reactor, no aeration), 112, 292, 452 or 612 min aeration (in the 3, 6, 8 and 12 h cycle, respectively), 3 min settling and 5 min effluent withdrawal. The bioreactor was operated at room temperature around 25 °C and was protected from light in order to prevent photolytic

degradation of pharmaceuticals and algal growth. Synthetic wastewater was used to feed the bioreactor as described by de Kreuk et al., 2005. During pharmaceuticals shock loads, the inlet was supplemented with the respective drug in order to reach the desirable concentration in the inlet flow (Table 1).

FQ/FLX biodegradation assays

Degradation of FQ/FLX by *L. portucalensis* strain F11 (Carvalho et al., 2005) was investigated in the presence of acetate as additional carbon source. Experiments were run in 1 L flasks filled with 0.2 L of mineral salts medium (MM) (Carvalho et al., 2005) supplemented with acetate (5.9 mM) and with different FQ/FLX concentrations. Experiments were conducted aerobically at 25 °C and 150 rpm.

Analytical methods

Samples from the influent and effluent streams of bioreactor were filtered on syringe nylon membrane filters (0.45 µm pore-size). The analysis of the pharmaceuticals was performed by high-performance liquid chromatography with fluorescence detection (HPLC-FD) (Maia et al., 2014 and Ribeiro et al., 2014). Previously described sequential injection methodologies were used for determination of nitrite, and nitrate (Mesquita et al., 2009), ammonium (Segundo et al., 2011) and phosphate (Mesquita et al., 2012). COD was determined according to Standard Methods for the examination of Water and Wastewater (APHA, 1998).

Results and Discussion

COD removal

AGS-SBR was operated for treating a synthetic wastewater containing fluorinated pharmaceuticals. The COD concentration observed in the effluent of the SBR reactor was low (0 - 60 mg/L) (Figure 1a). Almost all COD was consumed during the anaerobic feeding period. The removal of COD was not markedly affected by the feeding with the tested pharmaceuticals. The effluent COD concentration was always below the emission limit value of 125 mg O_2 L^{-1} (Council Directive 91/271/EEC).

Nitrogen and Phosphate removal

In each phase, ammonia was consumed during the aerobic phase and only residuals levels of ammonium and nitrite were found at the effluent of SBR (maximum 0.03 and 0.01 mM for NH_4^+ and NO_2^-, respectively) indicating that neither ammonia oxidizing bacteria (AOB) nor nitrite oxidizing bacteria (NOB) were inhibited by the presence of the target pharmaceuticals (Figure 1c and 1d). Nitrate was found to be the major product of the nitrification process appearing in the effluent, indicating a non-complete denitrification

(Figure 1d). The levels of phosphate in the bulk liquid after the anaerobic feeding were higher than expected taking in account the dilution factor, indicating that PAO released phosphate with concomitant uptake of COD in the anaerobic stage. However, a decline of both phosphate release (anaerobic feeding) and phosphate uptake (aerobic phase) was observed which could be due to an inhibitory effect on PAOs exerted by pharmaceuticals exposure (Figure 1b). The phosphate released into the bulk liquid by PAO during the anaerobic feeding period was not completely removed and the levels of phosphate in the bioreactor effluent increased during the period of feeding with FQ.

FQs removal

No evidence of biodegradation of the target pharmaceuticals was observed in the bioreactor. The results obtained are indicative of adsorption to the AGS. FQs have been shown to adsorb to activated sludge (Li and Zhang, 2010) and soil (Conkle et al., 2010). Adsorption to AGS can be advantageous for treatment of wastewaters on SBR, because of reducing the shock, via initial biosorption onto the biomass granules. It has been described that AGS play promising role in adsorption of toxic chemicals due to high surface area, porosity and good settling capability (Adav et al., 2008). The extracellular polymeric substances (EPS) in AGS have hydrophobic properties that are beneficial for organic pollutant adsorption (Sheng et al., 2010). Adsorption of pollutants to the AGS has also been reported by other authors, including pharmaceuticals such as tetracycline (Shi et al., 2011). AGS revealed the capacity to remove the tested pharmaceuticals through adsorption and a mechanism of adsorption and desorption occurred during reactor operation.

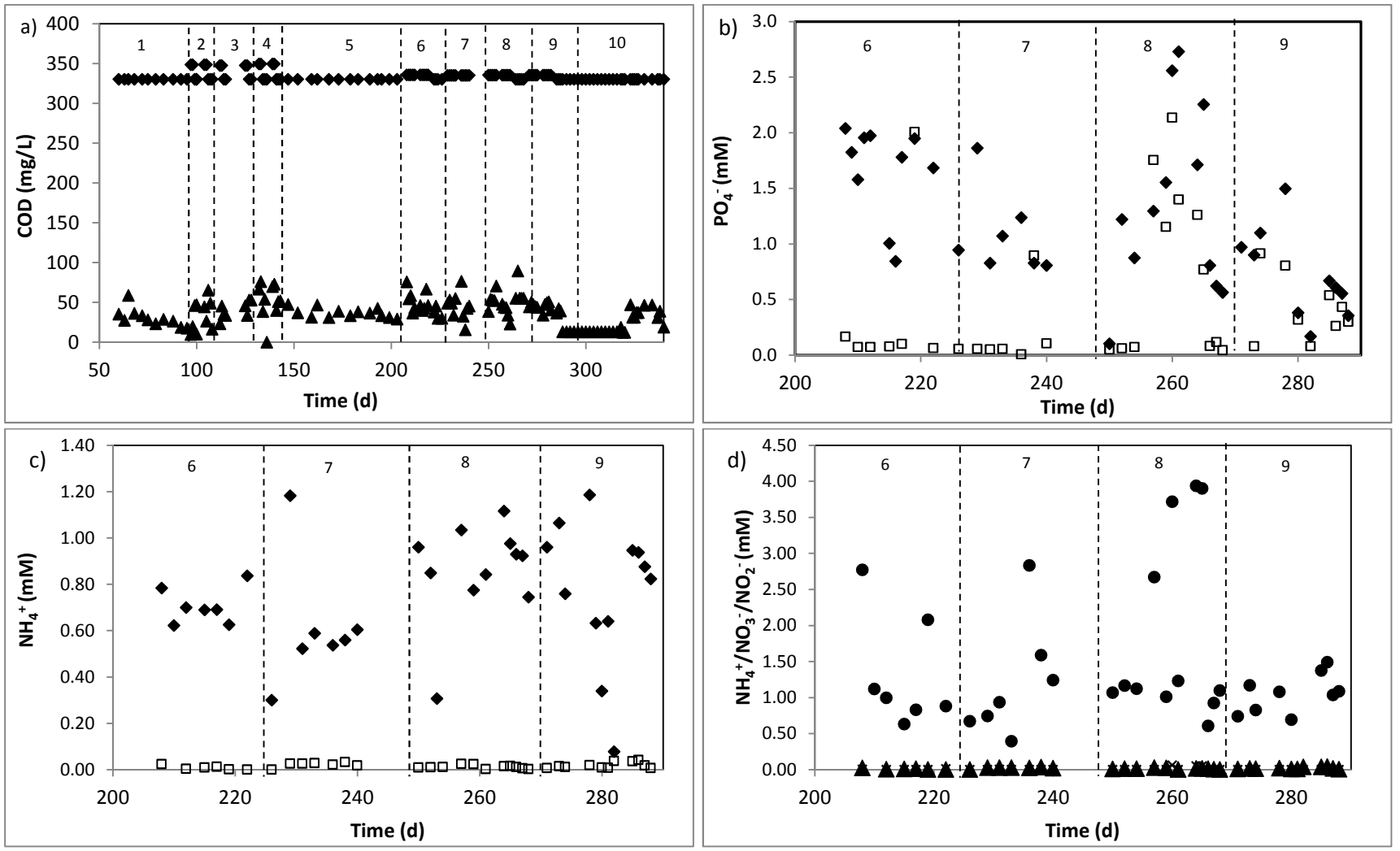

Figure 1. (a) COD, (b) PO_4^{3-} and (c) NH_4^+ concentration profiles in SBR along its operation. Concentrations in inlet (♦) and effluent (□) are shown. (d) N profile. Effluent concentration of NH_4^+ (▲), NO_3^- (●) and NO_2^- (x) are shown. Numbers are indicative of the different phases listed in Table 1.

Biodegradation of FQs and FLX by Labrys portucalensis F11

In parallel studies, the ability of strain F11 to biodegrade these pharmaceuticals was investigated. Strain F11 demonstrated to be able to degrade FQs, namely OFL, NOR and CPF (when supplied individually or as a mixture) and FLX, in the presence of an easy degradable carbon source. The absence of pharmaceuticals degradation in the bioreactor can be indicative of absence of indigenous FQs/FLX degrading organisms in the bioreactor biomass. However, the ability of strain F11 to grow using the readily available carbon source while maintain its ability to degrade these pharmaceuticals reinforce the potential of this strain in bioaugmentation processes. As the indigenous microbial communities in biotreatment processes may not be able to remove such contaminants, bioaugmentation strategies such as inoculation of the degrading strain as a suspension or immobilized on carrier material, or using a plasmid donor strain carrying the degradative genes, could be assessed to improve removal of the micropollutants.

There is a need to increase the knowledge about the fate and effect of pharmaceuticals during wastewater treatment for implementation of better removal techniques. AGS is a promising technology for the treatment of wastewater. In this study we have shown that FQs shock loads, at concentrations within the higher ranges reported for effluents, temporarily affected the reactor performance concerning nutrient removal. However, the system revealed to be robust and was able to resume performance when lower levels were present, accompanied by the recovery of the granules. In spite of the observed lower performance in nutrient removal, the emission limit values were not exceeded. AGS-SBR showed to be a robust system to deal with pharmaceuticals shock loads, since it was able to recover from the initial decrease on its performance and the levels of nutrients in the effluent were always below the emission limit values (Council Directive 91/271/EEC).

Conclusions

In this study the effects of fluorinated pharmaceuticals shock loadings on AGS-SBR performance was assessed. The main conclusions are summarized as follows:

- The organic removal, measured by COD, was not markedly affected by pharmaceuticals shock loads;
- The initial shock with pharmaceuticals resulted in a decline of removal efficiency of phosphate but this was recovered in the subsequent phases;
- Granular sludge AOB and NOB activities were not affected by the presence of FQs;
- The structure of the AGS seemed to protect the microbial population from toxicity, thus preserving most of the biological processes occurring within the granules;
- Adsorption/desorption of the compounds to the AGS occurred.

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