Journal of Hazardous Materials 287 (2015) 93–101

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Removal of fluoxetine and its effects in the performance of an aerobic granular sludge sequential batch reactor

Irina S. Moreira[a], Catarina L. Amorim[a], Ana R. Ribeiro[a,b,c], Raquel B.R. Mesquita[a,d], António O.S.S. Rangel[a], Mark C.M. van Loosdrecht[e], Maria E. Tiritan[b,c], Paula M.L. Castro[a,*]

[a] CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal
[b] Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[c] CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Rua Central de Gandra 1317, 4585-116 Gandra PRD, Portugal
[d] Laboratory of Hydrobiology, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Institute of Marine Research (CIIMAR), Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal
[e] Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628BC Delft, The Netherlands

HIGHLIGHTS

- Enantioselective removal of fluoxetine by aerobic granular sludge was evaluated.
- Sorption of fluoxetine to aerobic granules occurred.
- Bacterial community gradually changed during operation of sequential batch reactor.
- Main biological processes occurring within the granules were preserved.
- Overall performance of the reactor was recovered after initial fluoxetine shock loads.

ARTICLE INFO

Article history:
Received 7 October 2014
Received in revised form 5 January 2015
Accepted 6 January 2015
Available online 8 January 2015

Keywords:
Fluoxetine
Sequential batch reactor (SBR)
Aerobic granular sludge (AGS)
Removal efficiency
Microbial community

ABSTRACT

Fluoxetine (FLX) is a chiral fluorinated pharmaceutical mainly indicated for treatment of depression and is one of the most distributed drugs. There is a clear evidence of environmental contamination with this drug. Aerobic granular sludge sequencing batch reactors constitute a promising technology for wastewater treatment; however the removal of carbon and nutrients can be affected by micropollutants. In this study, the fate and effect of FLX on reactor performance and on microbial population were investigated. FLX adsorption/desorption to the aerobic granules was observed. FLX shock loads ($\leq$4 μM) did not show a significant effect on the COD removal. Ammonium removal efficiency decreased in the beginning of first shock load, but after 20 days, ammonia oxidizing bacteria became adapted. The nitrite concentration in the effluent was practically null indicating that nitrite oxidizing bacteria was not inhibited, whereas, nitrate was accumulated in the effluent, indicating that denitrification was affected. Phosphate removal was affected at the beginning showing a gradual adaptation, and the effluent concentration was <0.04 mM after 70 days. A shift in microbial community occurred probably due to FLX exposure, which induced adaptation/restructuration of the microbial population. This contributed to the robustness of the reactor, which was able to adapt to the FLX load.

1. Introduction

Technological advances in medicine and the increase in population longevity and their demands resulted in a drastic increase in pharmaceuticals consumption [1]. Most pharmaceuticals are not completely metabolized by human body, resulting in the excretion of some unchanged forms of these compounds and of their metabolites, which subsequently enter the ecosystems. The worldwide annual per capita consumption of drugs is 15 g with developed countries contributing three to ten times higher that value (50–150 g) [2]. Hence, it can be expected that the raw sewage from developed countries contains high amount of pharmaceutical compounds. Effluents from wastewater treatment plants (WWTPs) are considered relevant sources of pharmaceuticals to the aquatic

* Corresponding author. Tel.: +351 22 558 0000; fax: +351 22 509 0351.
E-mail address: plcastro@porto.ucp.pt (P.M.L. Castro).

http://dx.doi.org/10.1016/j.jhazmat.2015.01.020
0304-3894/

environment [3]. Annual rates of discharge of pharmaceuticals from municipal WWTPs into receiving waters may reach kilogram levels [4] which can contribute to water pollution due to their potential ecotoxicological impacts on aquatic organisms. Furthermore, human exposure to pharmaceuticals is also harmful and can occur via various routes. They can return to humans via drinking water [5]. Other exposure pathways to humans include food chain and wastewater reuse for household purposes [1,5]. The pollution of the environment by these emerging pollutants is a current topic of great concern and WWTPs have a limited capability to remove these micropollutants.

Fluoxetine hydrochloride (FLX) (*N*-methyl-(3-phenyl-3-(4-trifluoromethyl-phenoxy)-propyl)-amine) ($C_{17}H_{18}F_3NO$; molecular mass: 309.33 g mol^{-1}) is a selective serotonin reuptake inhibitor, one of the most frequently prescribed drugs to treat depression, eating disorders, and obsessive–compulsive disorder [6]. FLX is a chiral compound and the commercially available drug is marketed as a racemic mixture. FLX and its principal metabolite norfluoxetine (NFLX) are detected in surface waters due to their incomplete metabolization after therapeutic use and due to incomplete elimination in WWTPs [7]. Concentrations of FLX in tertiary wastewater effluents are in the range of 30–82 ng L^{-1} [8]. Brooks et al. [9] detected FLX and NFLX in tissues of three different fish species living in an effluent dominated stream. The occurrence of FLX in surface waters and in aquatic organisms has raised concern as ecotoxicological effects to aquatic organisms have been reported [10].

Enantioselectivity in fate and effects of chiral contaminants is being increasingly recognized, however this has largely been ignored for pharmaceutical contaminants. Enantiomers exhibit the same thermodynamic properties, but the interactions with chiral environments are usually different [11]. It is important to notice that even chiral pharmaceuticals that are distributed as racemates are frequently not found in the environment in the 1:1 ratio [11]. It is of utmost importance to understand the environmental fate and effects of each enantiomer of widely used racemic pharmaceuticals, such as FLX, during wastewater treatment.

Aerobic granular sludge offers an interesting alternative for conventional activated sludge systems due to its excellent physical characteristics as a biofilm composed of microbial self-immobilized cells [12]. In comparison to activated sludge, AGS has a denser and stronger microbial aggregate structure, a higher biomass concentration, a better settling capacity and the ability to withstand shock loads [13]. The stratification of conversion processes and redox zones within the granules provide aerobic and anaerobic/anoxic layers, which allow the simultaneous removal of carbon, nitrogen, and phosphorous [14]. Moreover, the systems based on aerobic granular biomass are known to perform better in front of inhibitory or toxic compounds compared to activated sludge system, because granule architecture causes diffusion gradients protecting sensitive bacteria [15]. The development of aerobic granules has been extensively reported using sequencing batch reactor (SBR) systems, using an anaerobic feeding period, followed by an aeration period in which simultaneous nitrification/denitrification takes place. In the anaerobic stage, most or all chemical oxygen demand (COD) is taken up by microorganisms, such as polyphosphate accumulating organisms (PAOs) and glycogen-accumulating organisms (GAOs), which store COD as intracellular polymers [16]. In the aerated stage, nitrification occurs at the oxygen-containing outer part of the granules while PAOs and GAOs oxidize their storage polymers in order to grow. This is done with oxygen in the outer part of the granules and with nitrate or nitrite in the inner part of the granules [14]. Operational flexibility of the SBR (ability to decrease settling time, initial reactor volume, etc.) played a key role to promote formation and maintenance of a compact granular biomass [17]. The system has been used to treat different municipal wastewater [18] and wastewater containing specific pollutants [19–21].

Table 1
Operating conditions tested in the SBR.

Phase	Length of operation (days)	Days of operation	Cycle time (h)	Inlet acetate concentration (mM)	Inlet FLX concentration (μM)
I	0–33	34	3	5.9	0
II	34–54	21	12	5.9	4
III	55–62	8	8	5.9	3
IV[a]	63–97	35	6	5.9	3
V	98–105	8	6	5.9	3
VI	106–124	19	6	5.9	0
VII	125–138	14	6	5.9	3
VIII	139–148	10	6	5.9	0

[a] Organic shock loadings with FLX applied 2 days/week.

The main objective of the current study was to investigate if aerobic sludge granular technology is able to handle shock loads of FLX and to assess the effect of the micropollutant on aerobic granular SBR performance. The performance of the AGS was evaluated in terms of FLX, carbon, phosphate, and nitrogen removal; the dynamic of the bacterial population of the granules was assessed. The behavior of *R* and *S* enantiomers of FLX on the aerobic granular system was also investigated.

2. Materials and methods

2.1. Chemicals and materials

Ethanol (HPLC grade) was purchased from Fisher Scientific UK Limited (Leicestershire, UK). Ammonium acetate and acetic acid 100% Chromanorm (HPLC grade) were purchased from Merck (Darmstadt, Germany) and VWR International (Fontenay-sous-Bois, France), respectively. Ultrapure water was supplied by a Milli-Q water system. HPLC grade solvents were filtered with 0.45 μm glass microfiber filters (Whatman™). Fluoxetine hydrochloride (FLX), (*S*)-(+)-fluoxetine hydrochloride ((*S*)-FLX), and (*R*)-(−)-fluoxetine hydrochloride ((*R*)-FLX) were purchased from Sigma–Aldrich (Steinhein, Germany). All reference standards were of >98% purity.

SBR influent media [14] were prepared with analytical-grade chemicals (Sigma–Aldrich Chemie, Steinheim, Germany; Merck, Darmstadt, Germany).

2.2. SBR operation

A 2.5 L SBR with 110 cm height and an internal diameter of 6.5 cm was previously established with activated sludge from a municipal WWTP, as described by Amorim et al. [22]. After the experiments reported in the previous study, the aerobic granules were let to regenerate for ca. 3 months under standard operating conditions described in this paper. The performance of SBR to degrade FLX was afterwards assessed. The operating conditions tested in the SBR are described in Table 1. The system was operated in cycles using an automatic timer (Siemens Logo! 230RC) to start and stop pumps for influent, aeration (4 L min^{-1}; superficial air velocity of 84.8 m h^{-1}) and effluent withdrawal. Dissolved oxygen (DO) and pH were measured online. DO was measured as the percentage of the oxygen saturation concentration. The oxygen saturation level was monitored, but not controlled, during the cycle. The pH was maintained at 7.0 ± 0.8 by dosing 1 M NaOH or 1 M HCl.

The reactor was operated in successive cycles of 3 h (during phase I), consisting of 60 min influent feeding (introduced in the bottom of the reactor), 112 min aeration, 3 min settling, and 5 min effluent withdrawal. During phases II, the aeration period was increased to 652 min in the 12 h cycle and then decreased to 412 min in the 8 h cycle (phase III) and, afterwards, to 292 min in the