ENANTIOMERIC FRACTION EVALUATION OF PHARMACEUTICALS IN AN AEROBIC GRANULAR SLUDGE SEQUENCING BATCH REACTOR

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INTRODUCTION

Pharmaceutical compounds are emerging environmental contaminants that have received growing attention over the last two decades. Regarding chiral pharmaceuticals (CPs) the pharmacokinetic, pharmacodynamics, toxicological and ecotoxicological properties can diverge between enantiomers. Therefore, the evaluation of the enantiomeric fraction (EF) of CPs during the removal processes occurring in the environment, is crucial for assessing the fate of these contaminants. Aerobic granular sludge (AGS) technology is one of the promising biotechnologies in wastewater treatment. However, due to its technological novelty, up to now there is a lack of information regarding the fate of pharmaceuticals on such technology. In this study, an AGS bioreactor was operated in order to assess its removal performance when treating a synthetic wastewater, containing a racemic mixture of several pharmaceuticals from three different therapeutic classes (betablockers, antidepressants and a beta$_2$-adrenergic agonist), under continuous feeding. The EF was access by LC-MS/MS with an Astec Chirobiotic™ V chiral stationary phase under reversed elution mode [1].

EXPERIMENTAL APPROACH

Table 1. Chiral compounds (CPs) supplied in the synthetic wastewater

Therapeutical classe	CPs
Antidepressants	Fluoxetine (FLX)
	Norfluoxetine (NFLX)
	Venlafaxine (VNF)
Betablockers	Alprenolol (ALP)
	Bisoprolol (BSP)
	Metoprolol (MET)
	Propranolol (PHO)
Beta$_2$-adrenergic agonist	Salbutamol (SBT)

Each CP was supplied as a racemic mixture of 1.3 µg/L of each enantiomer

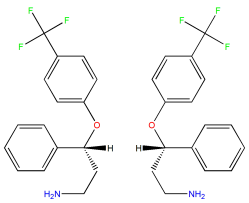

Figure 1. Enantiomers of norfluoxetine.

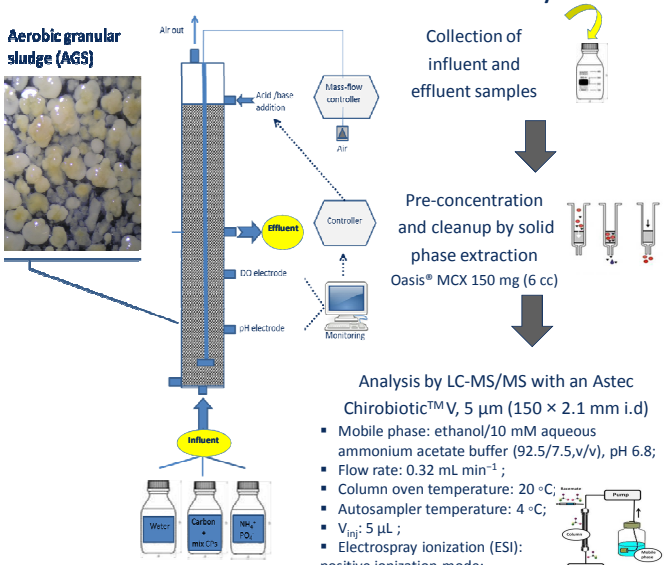

Figure 2. Schematic representation of the AGS bioreactor and its biomass.

Organic shock loading with a racemic mixture of CPs applied in continuous mode during 28 days

Collection of influent and effluent samples

Pre-concentration and cleanup by solid phase extraction

Oasis® MCX 150 mg (6 cc)

Analysis by LC-MS/MS with an Astec Chirobiotic™V, 5 µm (150 × 2.1 mm i.d)

- Mobile phase: ethanol/10 mM aqueous ammonium acetate buffer (92.5/7.5,v/v), pH 6.8;
- Flow rate: 0.32 mL min^{-1} ;
- Column oven temperature: 20 °C;
- Autosampler temperature: 4 °C;
- V_{inj}: 5 µL ;
- Electrospray ionization (ESI): positive ionization mode;
- Selected Reaction Monitoring.

RESULTS AND DISCUSSION

Table 2. Summary of AGS bioreactor performance for CPs removal

	CP mass balance (ng)		Removal
	CP $_{fed}$	CP $_{effluent}$	efficiency (%)
S-FLX	222022	34334	85
R-FLX	235130	31994	86
S-NFLX	33311	1411	96
R-NFLX	32378	713	98
S-VNF	44367	23310	47
R-VNF	42303	21779	49
R-SBT	48370	19075	61
S-SBT	43842	20333	54
S-ALP	43237	21434	50
R-ALP	42366	21905	48
BSP E1	46363	24760	47
BSP E2	47383	25320	47
S-MET	43107	23515	45
R-MET	39452	20796	47
S-PHO	41463	20165	51
R-PHO	39008	18855	52

Results from 84 analysed cycles during 28 days of feeding

- High removal efficiencies (>96%) for both (*R*)- and (*S*)-enantiomers were achieved for NFLX;
- For FLX, removal efficiency was also high (ca. 85% of each enantiomer being removed from the total influent load);
- All other CPs were reduced by about 47–61%.

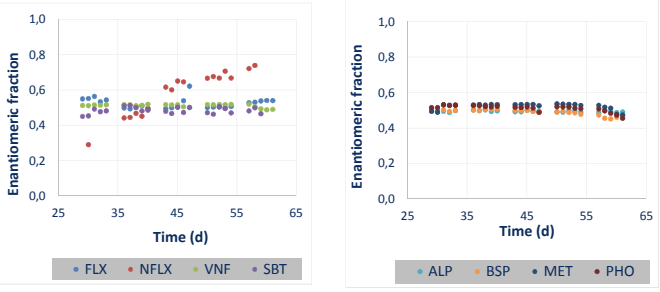

Figure 2. Changes in the EF during the CPs continuous feeding.

- NFLX at effluent exhibited EF values ranging from 0.44 to 0.74, indicating enantioselectivity in the processes, with preferential removal of the (*R*)-enantiomer;
- Non-enantioselective removal was observed for all other CPs.

EF evaluation of CPs could be an effective mean of gaining insights into the overall treatment performance.

REFERENCES

[1] Ribeiro AR, Santos LHMLM, Maia AS, Delerue-Matos C, Castro PML, Tiritan ME (2014b) Enantiomeric fraction evaluation of pharmaceuticals in environmental matrices by liquid chromatography-tandem mass spectrometry. J Chromatogr A 1363: 226–235

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