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The role of microbiological community on organic micropollutants biotransformation in anoxic conditions

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Abstract: Information about the biotransformation of organic micropollutants (OMPs) in biological systems is currently scarce, particularly in anoxic environments. A lab-scale reactor was set up to elucidate which is the biological mechanism driving OMPs biotransformation in heterotrophic denitrifying conditions. The influence of microbial composition on the OMPs removal was analysed. Increasing levels of nitrate loading rates were applied during the study in order to analyse cometabolism. In terms of OMPs, high removal efficiency was achieved for compounds such FLX and SMX, whereas moderate removal was achieved for some antibiotics (ERY, ROX, TMP), the anti-depressant drug CTL or the natural hormones. Other OMPs, like DCF or CBZ were recalcitrant. Removal due to cometabolism was detected for BPA, ERY, ROX and CTL.

Keywords: Anoxic process; cometabolism; organic micropollutants; microbial composition; heterotrophic denitrification

1. Introduction

The presence of organic micropollutants (OMPs) in wastewater have been well documented in different countries and continents around the world (Tran et al., 2018). Despite most of wastewater treatment plants (WWTPs) are not specifically designed for the removal of these pollutants, some of them can be (partially or completely) biotransfomed in the secondary treatment (Carballa et al., 2004).

The biological mechanisms for explaining the OMPs biotransformation in WWTPs remain unclear. Currently, there are three possible hypothesis to explain these processes: metabolism (microorganisms are able to degrade OMPs and obtain carbon and energy for their development), bacteria resistance (microorganisms are able to change the molecular structure of OMPs to reduce their biological effect) and cometabolism (while consuming growth substrates, microorganisms are able to biotransform OMPs due to the non-specific action of the enzymes released) (Rios Miguel et al., 2020). As the OMPs are commonly present in WWTPs at trace levels (µg/L or ng/L), the most probable removal mechanism is cometabolism.

One of the most important parameters driving organic micropollutants biotransformation in wastewater treatment plants is the redox condition (Alvarino et al., 2018). Aerobic and anaerobic conditions have been studied more than anoxic systems, like denitrifying heterotrophic reactors. These differences in the behaviour of OMPs has been evidenced for ibuprofen, which can be greatly removed in aerobic systems (> 80%) whereas it is almost recalcitrant (< 20%) in anoxic reactors (Suarez et al., 2010). However, the underlying reason for this different behaviour is still unknown.

Microbial composition has been suggested as one possible key factor determining OMP behaviour (Johnson et al., 2015). Operational conditions (SRT, nutrient load, redox condition, etc.) define this microbial community composed by a group of microorganisms (core community) which is the responsible of performing the metabolic activities in the biological reactor, as well as of the biotransformation of some of micropollutants (Wolff et al., 2018).

The aim of this research is to evaluate the relationship between the OMPs biotransformation and the microbial composition in a heterotrophic denitrifying system operated at different levels of metabolic activity.

2. Material and methods

2.1. Reactor conditions and operational strategy

A lab-scale reactor (5 L reactor + 2 L sedimentation tank) was set up to perform the study. It was inoculated with 1.5 g VSS/L of activated sludge taken from a WWTP close to Santiago de Compostela. The reactor was operated at room temperature, which varied between 18 and 24ºC during the whole operation.

The biological reactor was fed with a synthetic mixture composed by two solutions of chemicals simulating the composition of a medium loaded domestic wastewater. The first one provided nutrients and cofactors for the bacterial community growth and maintenance (macronutrients) and was fed at a flow rate of 4.5 L/d (Table 1). A mixture of sodium acetate and acetic acid was added to the system maintaining a C/N ratio around

5-6, with a pH adjusted to compensate the alkalinisation due to denitrification.

Table 1. Composition of macronutrients in the synthetic feed. Adapted from Suarez et al. (2010).

Compound	Concentration (g/L)
CH_3COONa	0.4-1[1]
CH_3COOH	10-33 mL[1]
$NaNO_3$	0.17-2.7[1]
KH_2PO_4	0.02
Na_2HPO_4	0.04
Trace solution[2]	2 mL

[1] The concentration of these compounds varied depending on the period of the experiment

[2] Trace solution composed of: $FeCl_2$, $ZnSO_4$, $CoCl_2$, $MnCl_2$, $CuSO_4$, KI and H_3BO_3

The second feed contained a mixture of different types of micropollutants dissolved in water, providing a continuous spike of these compounds to the media (Table 2). Such feed was added after a period of acclimation to the denitrifying conditions with a flow rate of 0.5 L/d. Thus, the HRT of the biological reactor was set at 1 d.

Table 2. List of OMP spiked to the reactor at concentrations of 100 and 10 ppb.

Compounds (100 µg/L)		Compounds (10 µg/L)
Erythromycin (ERY)	Ibuprofen (IBP)	Estrone (E1)
Sulfamethoxazole (SMX)	Carbamazepine (CBZ)	Estradiol (E2)
Fluoxetine (FLX)	Diazepam (DZP)	Ethinylestradiol (EE2)
Roxithromycin (ROX)	Triclosan (TCS)	
Diclofenac (DCF)	Naproxen (NPX)	
Bisphenol-A (BPA)	Trimethoprim (TMP)	

In order to evaluate the potential cometabolic effect, the reactor was operated at different levels of nitrogen load, being nitrate the limiting substrate. Five different periods (P) of metabolic activity were evaluated with a nitrate loading rate of: 25 (P1), 75 (P2), 200 (P3 and P4) and 400 (P5) mg $N\text{-}NO_3$ L^{-1} d^{-1}. In each period, OMPs sampling campaign was performed both in liquid and solid phase.

2.2. Analytical methods

Conventional parameters, like COD, nitrogen species or biomass concentration, were regularly followed. OMPs analysis were performed in a LC-MS-MS after a preconcentration step utilizing a solid phase extraction protocol in 3 mL Oasis Cartridges (Alvarino et al., 2015). DNA was extracted from biological samples taken in each period to analyse the bacterial community composition by 16S rRNA gene amplicon sequencing analysis.

3. Results and discussion

3.1. Reactor performance

The biological reactor operated under stable conditions after the application of the increasing nitrogen loading rates, being able to efficiently remove the nitrogen during the whole operational period (Figure 1). After a period of acclimation to anoxic conditions (30 days approximately), nitrate removal efficiency was above 99% throughout the entire operation, while COD removal efficiency fluctuated between 75 and 90%. No nitrite was detected in the effluent. VSS concentration varied between 1.4 and 2.5 g/L during the reactor operation.

3.2. Micropollutants removal

In terms of OMPs removal efficiency, FLX, TCS and SMX were almost completely removed (>80%); ERY, ROX, CTL, BPA, E1 and E2 were moderately removed (between 40 and 80%) and the rest of them (IBP, NPX, EE2, CBZ, DZP, DCF) were recalcitrant (<20%). Results correspond to the average removal considering the five periods analyzed (Figure 2).

In general, results are in accordance with other studies in similar denitrifying conditions (Suarez et al., 2010), except for ERY, ROX and CTL, whose behaviour was previously classified as recalcitrant, instead of being moderately biotransformed. However, Torresi et al., (2017) using methanol and ethanol as carbon source for performing the denitrification, reported that ERY was moderately and CTL highly removed. Such differences between studies can be explained by the specific microbial composition determined by the operational conditions (SRT, HRT, carbon source, etc.) applied to the systems.

The main mechanism for removing OMPs in this system was biodegradation. Volatilization was negligible, since the selected compounds are not volatile and the reactor is not aerated (like in conventional activated sludge treatments). However, sorption had a significant impact in the removal of some compounds like FLX or TCS (results indicated a contribution of up to 10%). These compounds were previously reported as lipophilic OMPs in anoxic environments (Pomiès et al., 2015).

In general, the OMPs removal efficiencies achieved in this heterotrophic denitrification reactor was lower compared to aerobic systems (Kennes-Veiga et al., 2021, Suarez et al., 2010). This behaviour was previously reported by Alvarino et al. (2018) and explained based on the assumption that the nitrate has a lower oxidation potential than oxygen.

3.3. Cometabolic effect

A clear evidence of cometabolic biodegradation in biological processes is the correlation of the pollutant removal rate with the growth substrate metabolic activity of the bacterial culture (Kennes-Veiga et al., 2021). However, in this study, results do not show a clear tendence between the OMPs and the nitrate removal rate (selected as the limiting growth substrate). Despite this, a difference has been observed between the second (75 mg $N\text{-}NO_3$ L^{-1} d^{-1}) and the last period (400 mg $N\text{-}NO_3$ L^{-1} d^{-1}) in terms of pollutants removal rate (Table 3).

Table 3. Comparison of the micropollutant specific removal rate between the second and the last period of operation

Compound	Micropollutant specific removal rate (µg $gVSS^{-1}$ d^{-1})			
Period	**ERY**	**CTL**	**ROX**	**BPA**
2	3.32	5.42	1.91	0.43
5	4.44	5.91	6.12	3.70

Additionally, sludge samples for the analysis of the microbial composition by 16S rRNA gene massive sequencing have been taken during the different operational periods. Data corresponding to microbial ecology are currently being processed, so in few weeks we expect to be able to complete the analysis that determined the influence of the microbial composition and OMPs removal.

4. Conclusions

A denitrifying heterotrophic reactor was able to achieve a stable operation and to denitrify more than 99% of the nitrate fed during the experiment. The removal of several OMPs was studied in this reactor through different levels of denitrifying activity. Results indicated that TCS, FLX and SMX were almost completely removed (>80%) and ERY, ROX, CTL, BPA, E1 and E2 were moderately removed (40-80%). A cometabolic removal was detected for some compounds like ERY, ROX, CTL and BPA between the second and the last period.

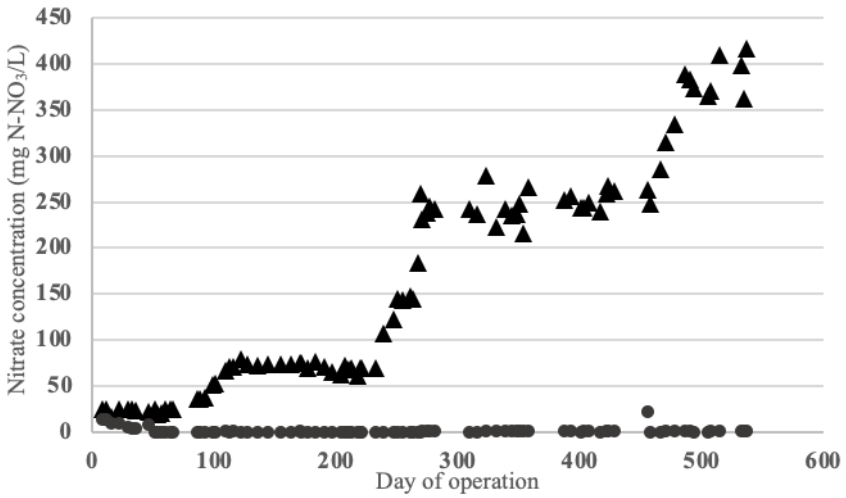

Figure 1. Nitrate concentration in the ▲ feed and the ● effluent during the operation.

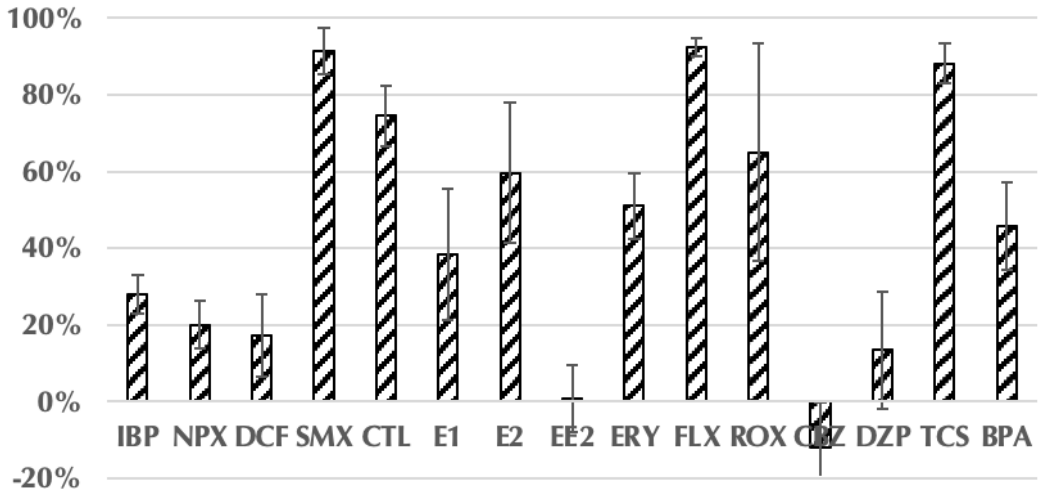

Figure 2. Average removal of the selected OMPs during the whole operation.

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