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Ciprofloxacin and sulfamethoxazole biotransformation products in anaerobic packed bed biofilm reactor applied to the sanitary sewage treatment

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Abstract. The presence of antibiotics in the environment has received a lot of attention in recent years due to their ability to promote and spread antimicrobial resistance. In this work, the biodegradation of the antibiotics ciprofloxacin (CIP) and sulfamethoxazole (SMX), as well as the concurrently generation of their biotransformation products (BTPs) were assessed in an anaerobic packed bed biofilm reactor (APBBR) treating sanitary sewage. Initially, anaerobic batch assays at high concentrations of the antibiotics (5 mg L^{-1}) were performed to identify the metabolites generated during the CIP and SMX biodegradation in methanogenic condition. From the mass transitions (m/z) of the identified BTPs, they were analyzed along the spatial profile of the APBBR fed with sewage (300 ngCIP L^{-1} and 400 ngSMX L^{-1} approximately). Three BTPs were identified: one related to the biodegradation of CIP (m/z 316) and two related to the biodegradation of SMX (m/z 256 and m/z 270). The molecular structures of the BTPs revealed that the biotransformation occurs mainly in the isoxazole ring of SMX and there was a dehydroxylation in the CIP molecule. Nonetheless, the sulfonamide and fluoroquinolone molecular structures remained intact in the anaerobic effluent stream, thus presenting residual antimicrobial activity in the environment.

Keywords: Anaerobic fixed bed biofilm reactor; Antibiotics; Emerging micropollutants; Fluoroquinolone; Sulfonamide.

1. Introduction

Among the emerging micropollutants, antimicrobial compounds cause great concern related to the development of resistance genes in bacteria. Furthermore, during sanitary sewage treatment, antibiotics are generally partially degraded, generating metabolites that may be more toxic than their parent compounds (Majewsky et al. 2014). Therefore, the identification of these biotransformation products (BTPs) plays an important role in the environmental and public health context. Achermann et al. (2018) emphasize the importance of focusing on the study of antibiotics' BTPs, since they may still have antimicrobial residual activity in the environment, with few changes in the original molecule.

The antibiotics ciprofloxacin (CIP – fluoroquinolone class) and sulfamethoxazole (SMX – sulfonamide class) are widely used in human and veterinary medicine, and are frequently detected in sanitary sewage. Studies on the biotransformation products of antibiotics usually apply initial concentrations of the analyte in the order of mg L^{-1}, not reflecting the levels found in the environmental matrices. Research on the metabolic pathways of anaerobic biotransformation of antibiotics is incipient (Alvarino et al. 2016) and there are few studies considering the typically concentrations found of these pharmaceuticals in anaerobic systems.

The application of fixed bed biofilm reactors as alternative for the anaerobic wastewater treatment presents some advantages such as its ability to maintain high solids retention time; low content of suspended solids effluent; and the biofilm formation can increase the resistance to toxic compounds (Carneiro et al. 2020). In this context, the main objective of this study is to identify the CIP and SMX BTPs formed along the spatial profile in an anaerobic fixed bed biofilm reactor, in order to elucidate their possible metabolic pathways during the anaerobic digestion of sanitary sewage.

2. Material and methods

The treatment system consisted of a bench-scale anaerobic packed bed biofilm reactor (APBBR) of 2.7 L – Figure 1. The fixed bed was filled with cylindrical matrices of polyurethane foam with a diameter of 0.8 cm and height of 1.2 cm, resulting in bed porosity of 59%. The seed biomass was a granular methanogenic sludge

from a full-scale UASB bioreactor (Tietê, São Paulo, Brazil). The reactor feeding was initially done with lab-made sewage (composition detailed in Carneiro et al. (2019)) and then with sanitary sewage. The physical-chemical characteristics of each bioreactor feeding streams are detailed in Table 1. The temperature was kept in mesophilic range (30 ºC) and the hydraulic retention time was set in 12 h. The spatial profile of CIP and SMX decay along the reactor was assessed and the kinetic model with best fit of the data was the first order model with residual concentration.

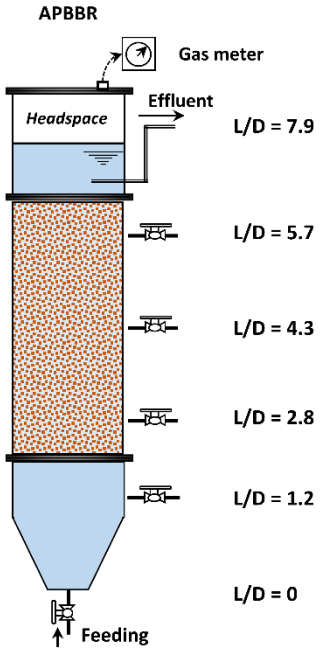

Figure 1. Schematic representation of the up-flow anaerobic packed bed biofilm reactor (APBBR) according to Carneiro et al. (2020). The sampling points correspond to the different length/diameter (L/D) ratios.

Table 1. Physical-chemical characteristics (average ± standard deviation) of each bioreactor feeding stream according to Carneiro et al. (2019).

Parameter	Lab-made sewage	Sanitary Sewage
COD* (mg L^{-1})	270 ± 25	393 ± 108
CIP (ng L^{-1})	333 ± 117	220 ± 99
SMX (ng L^{-1})	382 ± 60	428 ± 137
pH	7,47 ± 0,17	7,50 ± 0,26

* Chemical Oxygen Demand

To evaluate the BTPs, batch assays were initially carried out with 5 mg L^{-1} to identify the metabolites of the CIP and SMX anaerobic biodegradation. The batch assays were performed under the following conditions: 72h of incubation, 125 rpm, 30°C and 1.0 gVSS g^{-1} foam. From the mass transitions of the identified metabolites, they were analyzed along the spatial profile of the APBBR. For each reactor sampling point (Figure 1, length/diameter ratio ranging from 0 to 7.9), a sample volume of 400 mL was collected, which was filtered at 0.45 µm and then passed through the solid phase extraction (SPE) procedure followed by liquid chromatography-mass spectrometry (LC-MS/MS) - ABSciex QTRAP 5500. The positive ESI mode was used for all of the analytes.

3. RESULTS AND DISCUSSION

The APBBR presented quite a high performance in organic matter and antibiotics removal, as shown in Table 2.

Table 2. Results of APBBR performance (average ± standard deviation) regarding the COD, CIP and SMX removal according to Carneiro et al. (2019).

Parameter	Lab-made sewage	Sanitary Sewage
COD Removal (%)	90 ± 4	80 ± 7
CIP Removal (%)	88 ± 8	67 ± 19
SMX Removal (%)	91 ± 4	78 ± 9

Three BTPs were identified in the batch assays - one related to the CIP biodegradation (BTP1 - m/z 316.2154) and two related to the SMX biodegradation (BTP2 - m/z 256.0747 and BTP3 - m/z 270.094). Based on the mass spectra (MS/MS) obtained for each identified BTP, the molecular structures for these compounds have been proposed, as presented in Figure 2.

Regarding CIP, the BTP1 (m/z 316) has not been previously identified in the literature. It presented a cleavage in the carboxylic group, while the structures of the piperazine ring, cyclopropyl group and the C–F bond remained intact (Figure 2a). Pan et al. (2018) identified some BTPs from CIP biodegradation by a pure culture of *Thermus* sp. isolated from a pharmaceutical sludge and previously adapted to the fluoroquinolone. The authors found changes in the piperazine ring (BTPs of m/z 190, 223, 263, 306, 334, 348 and 360), cleavages in the cyclopropyl group (BTPs of m/z 190 and 223), and the C–F bond (BTP of m/z 190). Jia et al. (2018) also found cleavage in the piperazine ring (BTPs of m/z 306, 263) in anaerobic sulfate-reducing condition. In addition, they observed hydroxylation of the molecule (BTP of m/z 348), replacement of the fluorine atom by the hydroxyl group (BTP of m/z 330), and a cleavage of the carboxylic group and its replacement by –OH (BTP of m/z 304). In anoxic condition Liu et al. (2013) observed no cleavages in the molecule, only changes in the piperazine ring with the addition of the –NO bond (BTP of m/z 361) or three oxygen atoms (BTP of m/z 374).

The two BTPs (BTP2 and BTP3) from SMX biodegradation showed changes in the isoxazole ring and no changes were observed in the aniline ring, as highlighted in Figure 2b and 2c. Jia et al. (2017) also identified the BTP m/z 256 in anaerobic sulfidogenic condition and still found a BTP derived from the total cleavage of the isoxazole ring - BTP m/z 173. Furthermore, the authors observed the methylation and hydroxylation of the isoxazole ring. Other research has shown changes in the aniline ring, e.g. the loss of the benzene ring amino group in aerobic and anaerobic

conditions (Poirier-Larabie et al. 2016) and the replacement of the amino group by the hydroxyl group (Müller et al. 2013). The BTP m/z 270 found in this study was not identified in previous studies in the literature.

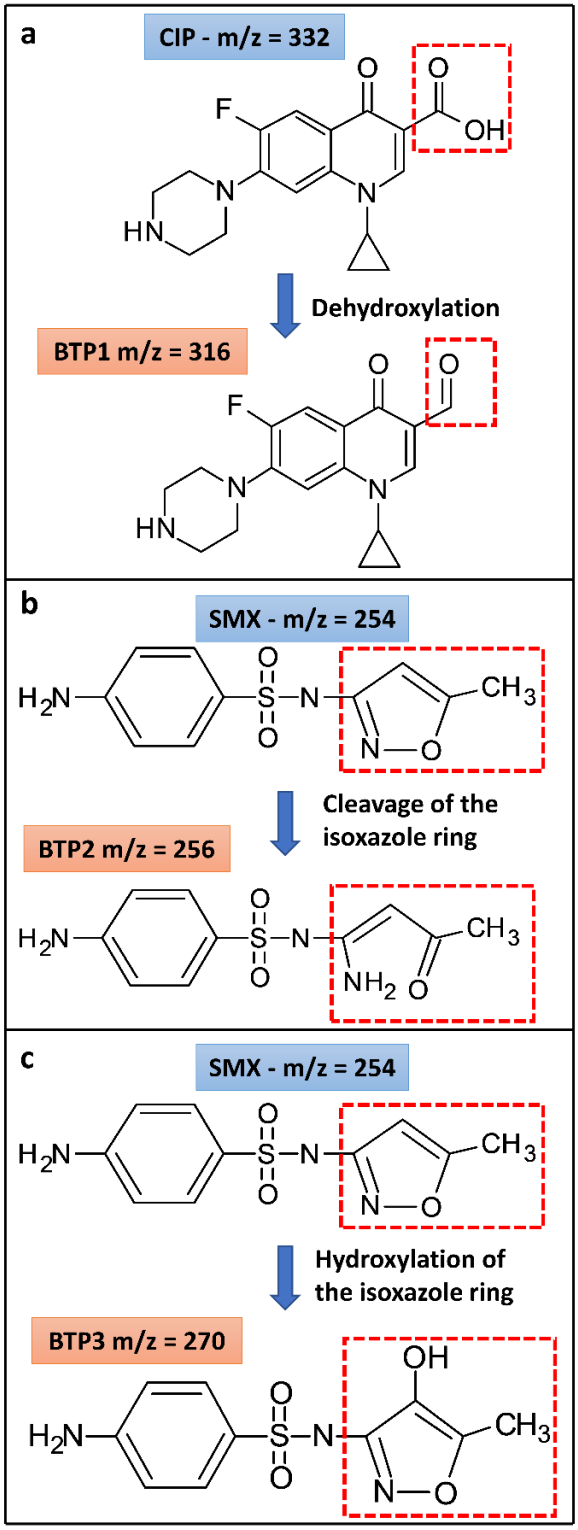

Figure 2. Diagrams of the possible biotransformation pathways of CIP (a) and SMX (b and c) during the anaerobic digestion in the APBBR.

Figure 3 shows the CIP and SMX decay profile together with the relative areas of the BTPs chromatograms detected along the APBBR spatial profile, represented by the L/D (length/diameter) ratio. It was found that the generation of BTPs occurred concurrently with the antibiotics' biodegradation. In addition, there was no significant biodegradation of the metabolite generated along the APBBR profile. The results found show that the differences in the substrate matrix (lab-made and sanitary sewage) can influence the biochemical reactions involved in the generation of BTPs.

4. CONCLUSIONS

The anaerobic packed bed biofilm reactor (APBBR) was able to remove the antibiotics CIP and SMX from sewage, presenting high removal efficiencies (81 ± 16% for CIP and 85 ± 10% for SMX). The analysis strategy for the identification of the BTPs of CIP and SMX during the anaerobic digestion was feasible to detect three metabolites (one BTP from CIP and 2 BTPs from SMX biodegradation). The molecular structures of the BTPs and their possible metabolic pathways along the bioreactor removal spatial profiles showed that the sulfonamide and fluoroquinolone molecules remained intact in the anaerobic effluent stream. Thus, the antibiotics might still be bioactive in the environment. Further studies are needed to predict the toxicological impacts of these metabolites generated on the aquatic biota and human health.

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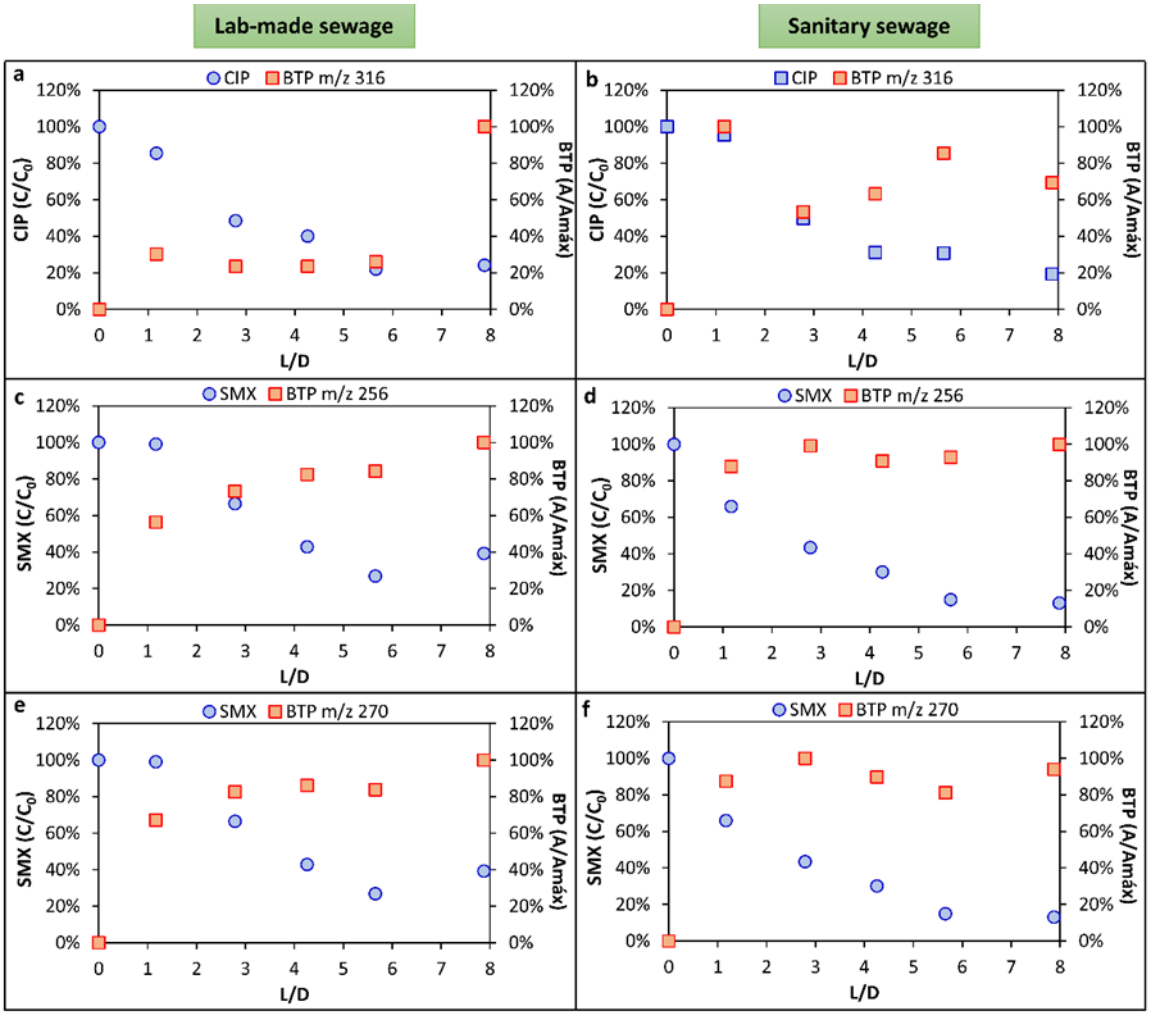

Figure 3. CIP and SMX BTPs identified along the APBBR spatial profile operated with lab-made and sanitary sewage – m/z 316 (BTP1 - a and b), m/z 256 (BTP2 c and d) and m/z 270 (BTP3 e and f).

References

Achermann, S., Bianco, V., Mansfeldt, C. B., Vogler, B., Kolvenbach, B. A., Corvini, P. F. X., & Fenner, K. (2018). Biotransformation of Sulfonamide Antibiotics in Activated Sludge: The Formation of Pterin-Conjugates Leads to Sustained Risk. *Environmental Science and Technology*, *52*(11), 6265–6274.

Alvarino, T., Nastold, P., Suarez, S., Omil, F., Corvini, P. F. X., & Bouju, H. (2016). Role of biotransformation, sorption and mineralization of 14C-labelled sulfamethoxazole under different redox conditions. *Science of the Total Environment*, *542*, 706–715.

Carneiro, R. B., Mukaeda, C. M., Sabatini, C. A., Santos-Neto, A. J., & Zaiat, M. (2020). Influence of organic loading rate on ciprofloxacin and sulfamethoxazole biodegradation in anaerobic fixed bed biofilm reactors. *Journal of Environmental Management*, *273*.

Carneiro, R. B., Sabatini, C. A., Santos-Neto, Á. J., & Zaiat, M. (2019). Feasibility of anaerobic packed and structured-bed reactors for sulfamethoxazole and ciprofloxacin removal from domestic sewage. *Science of The Total Environment*, *678*, 419–429.

Jia, Y., Khanal, S. K., Shu, H., Zhang, H., Chen, G. H., & Lu, H. (2018). Ciprofloxacin degradation in anaerobic sulfate-reducing bacteria (SRB) sludge system: Mechanism and pathways. *Water Research*, *136*, 64–74.

Jia, Y., Khanal, S. K., Zhang, H., Chen, G. H., & Lu, H. (2017). Sulfamethoxazole degradation in anaerobic sulfate-reducing bacteria sludge system. *Water Research*, *119*, 12–20.

Liu, Z., Sun, P., Pavlostathis, S. G., Zhou, X., & Zhang, Y. (2013). Inhibitory effects and biotransformation potential of ciprofloxacin under anoxic/anaerobic conditions. *Bioresource Technology*, *150*, 28–35.

Majewsky, M., Wagner, D., Delay, M., Bräse, S., Yargeau, V., & Horn, H. (2014). Antibacterial activity of sulfamethoxazole transformation products (TPs): General relevance for sulfonamide TPs modified at the para position. *Chemical Research in Toxicology*, *27*(10), 1821–1828.

Müller, E., Schüssler, W., Horn, H., & Lemmer, H. (2013). Aerobic biodegradation of the sulfonamide antibiotic sulfamethoxazole by activated sludge applied as co-substrate and sole carbon and nitrogen source. *Chemosphere*, *92*(8), 969–978.

Pan, L. jia, Li, J., Li, C. xing, Tang, X. da, Yu, G. wei, & Wang, Y. (2018). Study of ciprofloxacin biodegradation by a Thermus sp. isolated from pharmaceutical sludge. *Journal of Hazardous Materials*, *343*, 59–67.

Poirier-Larabie, S., Segura, P. A., & Gagnon, C. (2016). Degradation of the pharmaceuticals diclofenac and sulfamethoxazole and their transformation products under controlled environmental conditions. *Science of the Total Environment*, *557–558*, 257–267.