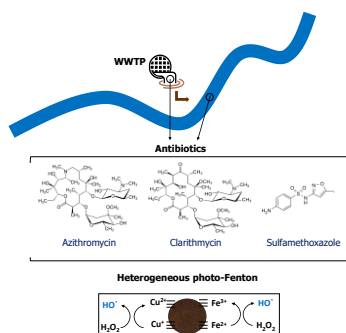


# Simultaneous degradation of azithromycin, clarithromycin, and sulfamethoxazole in WWTP effluent applying heterogeneous photo-Fenton process

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This study investigated the degradation of the macrolide antibiotics azithromycin (AZT) and clarithromycin (CLA) by the heterogeneous photo-Fenton process using copper oxide and copper modified iron oxide as catalysts. The macrolides degradation was compared to another antibiotic belonging to the sulfonamide class, sulfamethoxazole (SMX). Experiments involving hydroxyl radical inhibitors and WWTP effluents demonstrated the significance of matrix and  $\cdot\text{OH}$  radicals in macrolide degradation, while the contribution of other radicals to SMX degradation was observed. A comparison of SMX degradation with that of AZT and CLA in water and WWTP effluents highlighted the importance of the electronic transfer mechanism in macrolide degradation.

## Introduction

Macrolide antibiotics are the second most used class of antibiotics in Brazil [1]. Only a limited number of studies have reported the presence of azithromycin (AZT) and clarithromycin (CLA) in Brazilian surface waters, indicating macrolide concentration ranges between 35.9 and 158 ng L<sup>-1</sup> for AZT and 39.2 to 199 ng L<sup>-1</sup> for CLA [2]. However, these studies were conducted before the onset of the coronavirus pandemic, during which AZT was globally prescribed for COVID-19 treatment, increasing the prescription, which likely contributed to a rise in AZT occurrence in water, as observed in a Italian study [3].

In the present study, a catalyst based on iron and copper oxide was employed to investigate the degradability of macrolide antibiotics and sulfamethoxazole (SMX) to heterogeneous photo-Fenton process both in water and WWTP effluents.

## Material and Methods

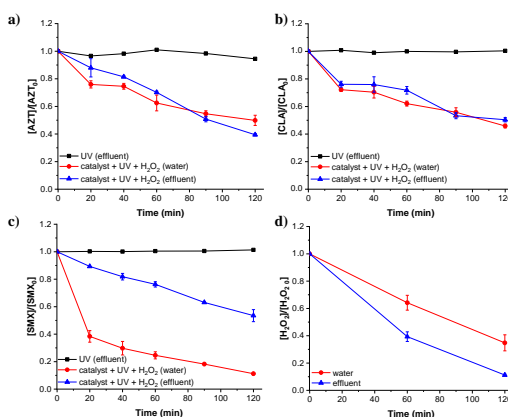
AZT and CLA stock solutions (500 mg L<sup>-1</sup>) were prepared using a 1:1 mixture of acetonitrile and ultrapure water. The SMX 100 mg L<sup>-1</sup> stock solution was prepared in ultrapure water. The WWTP effluent was collected in May 2023 in Araraquara, Brazil (21° 49' 28.3"S 48° 15' 07.5"W) using glass bottles and stored at 4 °C for maximum one week. The catalyst was synthesized using iron and copper in theoretical 1:1 iron-to-copper proportion through the coprecipitation method [4].

Degradation experiments were conducted in a lab-made glass photoreactor (9.3 cm diameter with a maximum capacity of 450 mL) using 300 mL of AZT, CLA, and SMX solution at a concentration of 6.6 μmol L<sup>-1</sup> each, under magnetic stirring. The pH was adjusted to predetermined values after the addition of the catalyst and H<sub>2</sub>O<sub>2</sub> to initiate the process. Aliquots were withdrawn at predetermined intervals

for analysis and were then filtered using a nylon 0.45 μm syringe filter. The photoreactor was equipped with two 15 W UV blacklight tubes (maximum emission at 365 nm), positioned 4.5 cm above the antibiotic solution.

## Results and Discussion

Simultaneous degradation of AZT, CLA, and SMX was conducted in a WWTP effluent to evaluate the effect of this complex matrix in a possible application of the heterogeneous photo-Fenton treatment. The pH was adjusted to 8, as was the degradation through photolysis in the effluent to investigate the influence of other photosensitizers and UV irradiation (Fig. 1).



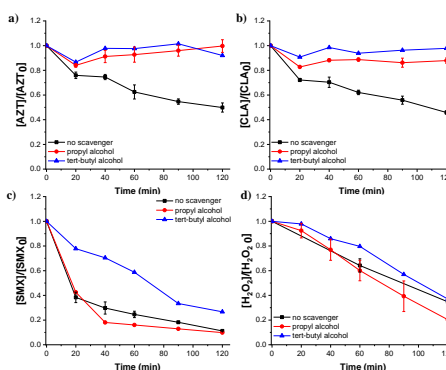
**Fig. 1.** Degradation of **a)** AZT; **b)** CLA and **c)** SMX by photolysis in effluent and by the heterogeneous photo-Fenton process in purified water and WWTP effluent; **d)** H<sub>2</sub>O<sub>2</sub> consumption. Initial conditions: CLA = AZT = SMX = 6.6 μmol L<sup>-1</sup>; [CAT] = 0.125 g L<sup>-1</sup>; [H<sub>2</sub>O<sub>2</sub>] = 15 mmol L<sup>-1</sup>; pH = 8.

No direct or indirect photolysis of the three antibiotics was observed in the effluent in the period of 120 min, indicating that no sufficient generation of radicals from photolysis of nitrate or other species present in the effluent occurs to promote the antibiotics degradation. The matrix did not affect the macrolides degradation, achieving almost the same degradation after 120 min in water and WWTP effluent. In contrast, the effluent significantly impaired SMX degradation, which decreased 42% after 120 min. The results when fitted to a pseudo or second order kinetic models demonstrate that the effluent did not influence AZT or CLA degradation, in opposite to SMX. As a result, it can be observed that SMX degradation in water was 11.4 times faster than in the effluent, indicating a significant influence of the matrix on SMX degradation.

SMX degradation depends on the electrophilic addition of the hydroxyl radical generated in the Fenton reaction to the aromatic ring, a process hindered in the presence of scavengers in the effluent. Furthermore, the high concentration of organics in the effluent contributes to the polarization of the electronic density of the medium, potentially reducing the degradation rate of pharmaceuticals reliant on electronic density for reaction with hydroxyl radicals. Consequently, SMX degradation is more susceptible than macrolide due to its rapid reaction with  $\cdot\text{OH}$  owing to its high nucleophilicity and the favorable acceptance of electrons by the hydroxyl radical [5].

The same effect is not observed for macrolides, as their primary degradation mechanism involves electron transfer [6]. In this scenario, pharmaceutical degradation kinetics are not fundamentally dependent on hydroxyl radical attack on a high electron density region of the molecule.  $\text{H}_2\text{O}_2$  consumption was 89% in the effluent and 66% in water. This outcome was expected due to the presence of a high concentration of organic matter in the effluent, which consumes generated radicals. Experiments done in the presence of hydroxyl radical scavengers (t-butanol and propyl alcohol)

demonstrated a dependence of macrolide degradation on the presence of hydroxyl radicals in the medium (Fig. 2), which are the main radicals involved in the electron transfer mechanism. However, the contribution of other radicals in the medium for SMX degradation is suggested. SMX degradation was inhibited only when tert-butanol was employed. This result suggests that in the presence of scavenger that does not react with the catalyst surface-bonded hydroxyl radical generated (propyl alcohol,  $k = 2.7 \times 10^9$ ), the reaction between the radical and SMX occurs swiftly and is not inhibited. Conversely, when a scavenger that reacts with the surface-bonded hydroxyl radical and with the free  $\cdot\text{OH}$  generated in the media (tert-butanol,  $k = 5.2 \times 10^9$ ) is used, a reduction in the degradation rate is observed during the application of the process [7]. The contribution of other radicals to SMX degradation is expected due to the different mechanisms involved in the photo-Fenton process.



**Fig. 2.** Effect of different hydroxyl radical scavengers on the degradation of **a) AZT;** **b) CLA** and **c) SMX;** **d)  $\text{H}_2\text{O}_2$  consumption.** [CLA] = [AZT] = [SMX] = 6.6 mmol L<sup>-1</sup>; [CAT] = 0.125 g L<sup>-1</sup>; [ $\text{H}_2\text{O}_2$ ] = 15 mmol L<sup>-1</sup>; [ACN] = 1% (v/v); pH = 8; [scav.] = 100 mmol L<sup>-1</sup>.

## Conclusions

The study of the degradation mechanisms and rate of AZT and CLA through heterogeneous Fenton process, revealed that primarily electron transfer with the contribution of hydroxyl radicals to the degradation occurs. Macrolides degradation were not influenced by the matrix, in opposite to SMX. These achievements combined with scavengers experiments and macrolides transformation products contributes to our understanding of the degradation mechanisms of macrolides to heterogeneous photo-Fenton process.

## Acknowledgments

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