Continuous-flow carbamazepine removal from contaminated wastewater by hybrid enzymatic-photo-oxidative treatment

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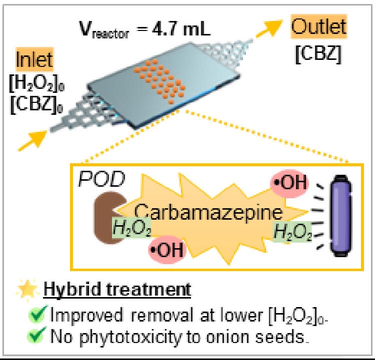
N. Klanovicz[1,2], *E.R. Bordin[3], L.C. Buba[3], A.F. Camargo[2], B. Ramos[1,4], M.M. Cestari[3], H. Treichel[2], A.C.S.C. Teixeira[1]. (1) Research Group in Advanced Oxidation Processes, Escola Politécnica, University of São Paulo, São Paulo, Brazil, nataliaklanovicz@usp.br. (2) Laboratory of Microbiology and Bioprocesses, Federal University of Fronteira Sul, Erechim, Brazil. (3) Department of Genetics, Federal University of Paraná, Curitiba, Brazil. (4) Department of Chemical Engineering, Centro Universitário FEI, São Bernardo do Campo, Brazil.*



This study presents, for the first time, carbamazepine (CBZ) removal by a hybrid continuous-flow treatment. The coupling of homemade peroxidase enzyme, immobilized onto Fe_3O_4 nanoparticles, and UVC/H_2O_2 in a single reactor was first studied with pure water. The hybrid reactions achieved 100% CBZ removal with a 10:1 H_2O_2:CBZ molar ratio, contrasting with the UVC/H_2O_2 system which requires molar ratios of over 40:1 for similar removal. The hybrid treatment efficiency results from the intensification of photo-oxidative reactions facilitated by peroxidase. An increase in the H_2O_2 dose was required for a real wastewater matrix, resulting in a maximum removal of 68% (hybrid treatment). Phytotoxicity in *Allium cepa* test system demonstrated the environmental compliance of the proposed hybrid continuous-flow treatment for carbamazepine degradation.

Introduction

Carbamazepine (CBZ) is the antiepileptic drug most frequently detected in water samples in its original form (active principle), reaching concentrations of mg L^{-1} in pharmaceutical industry wastewater [1]. Existing treatment technologies generally exhibit low efficiency in removing this compound [2].

The adoption of advanced and innovative degradation processes, such as hybrid treatment systems that integrate chemical and enzymatic reactions, is imperative for CBZ removal. The enzyme peroxidase (POD) is a promising candidate for use with UVC/H_2O_2, since both degradation mechanisms require hydrogen peroxide to generate free radicals [3].

In this context, this study aimed to study the hybrid continuous-flow peroxidase-photo-oxidative system for the removal of carbamazepine from pure water and spiked effluent. The phytotoxicity of the proposed treatment was also assessed using onion seeds.

Material and Methods

The continuous-flow device used in this study is an 3D-printed flat-plate reactor with geometry detailed in the Graphical Illustration. The reactor was operated with a precision syringe pump at a fixed space time of 10 min. Samples were periodically collected at the reactor outlet until a steady-state regime was reached. CBZ concentrations were monitored using an Ultra-fast liquid chromatography system. A reverse-phase C18 column was used, with a mobile phase of acetonitrile and acidified water (50:50 v v^{-1}, 1.0 mL min^{-1}, 40 °C).

The peroxidase enzyme used in this study was produced by submerged fermentation, yielding approximately 53 U mL^{-1} POD activity. The enzyme was used in its free form and immobilized onto magnetic Fe_3O_4 nanoparticles (MNPs). After immobilization, the POD_{imob} activity was 7 U mg^{-1}, and the obtained MNPs have a surface area of 189 m^2 g^{-1} and pore volume of 0.3 cm^3 g^{-1} [4].

Experiments for CBZ degradation were carried out at room temperature (~25 ºC) in a closed reaction cabinet equipped with 4 OSRAM Puritec lamps (λ_{max} = 254 nm) emitting a fixed irradiance of 4.4 W m^{-2} nm^{-1}. Firstly, the CBZ degradation study was performed with pure water ($[CBZ]_0$ = 5 mg L^{-1}) and separated POD_{imob}/H_2O_2, MNPs/UVC/H_2O_2 (photo-Fenton), and UVC/H_2O_2 reactions as controls.

The immobilized POD and MNPs material were distributed within the reaction volume (2 mg mL^{-1}) adhered to a magnet. An additional pump with a mixer was used for the free POD. The coupling of enzymatic and photo-oxidative reactions in a single reactor was studied at varying $[H_2O_2]_0$:$[CBZ]_0$ molar ratios for pure water and real effluent matrix. The $[H_2O_2]_0$ was varied based on the stoichiometric amount needed to complete the mineralization (40 mol H_2O_2 to 1 mol of CBZ).

The CBZ-spiked effluent at 5 mg L^{-1} was prepared using a real wastewater matrix collected from a municipal treatment plant of Campinas, Brazil. The detailed effluent characterization is presented in our previous study [4]. After adjusting the $[H_2O_2]_0$ for maximum CBZ removal, phytotoxicity in *Allium cepa* test system was performed according to Souza *et al.* method [5]. All the experiments were carried out in triplicate, and the data underwent analysis of variance (p-value < 0.05).

Results and Discussion

The ratio between the final and initial CBZ concentration in Fig. 1(a) shows that CBZ removal is

H_2O_2 dose-dependent. For the UVC/H_2O_2 system, the stoichiometric amount reached 96.7±0.6% removal. Excess H_2O_2 doses (100:1 and 200:1) were also tested, and the removal reached 100% for both doses. When the H_2O_2 is in deficit, the chemical process was inefficient, removing 74.1±1.1% for 10:1 molar ratio.

For the hybrid treatment, which combined chemical and peroxidase oxidation, the removal reached 100% for 10:1 molar ratio, and the treatment performance also decayed when lower H_2O_2 doses were used (see Fig. 1(a)). For phenomena comprehension, this same molar ratio was tested for photo-Fenton system (59.4±2.6%), enzymatic system with POD_{imob} (7.4±1.2%), and hybrid treatment with free POD (57.7±1.1%). A batch control with CBZ and MNPs showed that no adsorption in the pores occurred, eliminating the physical removal mechanism.

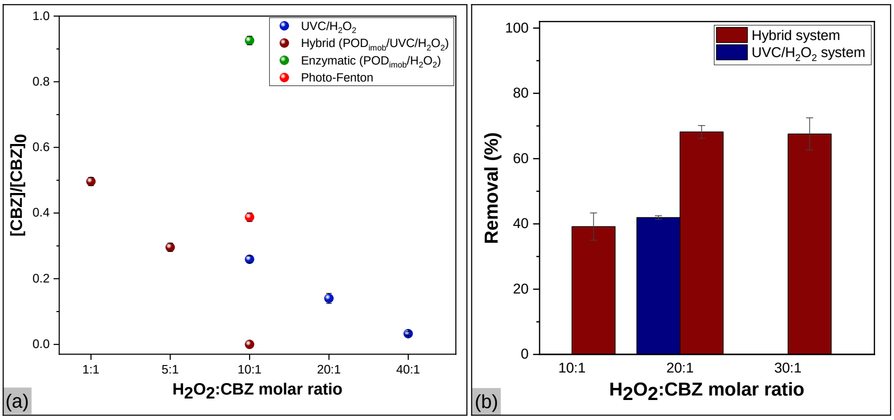


Figure 1. Results of continuous-flow experiments for (a) pure water and (b) wastewater spiked with CBZ.

These results reinforce that the degradation of CBZ by the proposed hybrid treatment results from the action of photo-Fenton reactions intensified by peroxidase presence. This enzyme is an oxidoreductase that produces free radicals responsible for the biotransformation of CBZ in subsequent reactions [3].

The lower H_2O_2 dose in the hybrid treatment compared to the UVC/H_2O_2 system is a significant highlight. The enzyme biocatalytic mechanism can explain this behavior, as peroxidases can be inactivated by excess hydrogen peroxide [3].

The hybrid POD_{imob}/UVC/H_2O_2 and UVC/H_2O_2 systems were also tested with CBZ-spiked effluent, as shown in Fig. 1(b). The maximum removal for the real water matrix was statistically equal when using 20:1 and 30:1 molar ratios for the hybrid treatment (~68%).

As for pure water, the hybrid reactions were more efficient at lower initial H_2O_2 concentrations for the real matrix. The removal efficiency decreased considerably for the real effluent compared to pure water and reached a maximum at low H_2O_2 doses.

The assays with *Allium cepa* seeds showed that both POD_{imob}/UVC/H_2O_2 and UVC/H_2O_2 systems did not result in phytotoxicity effects when analyzing germination and root growth processes.

Conclusions

The hybrid POD/UVC/H_2O_2 and UVC/H_2O_2 reactions are H_2O_2 dose-dependent. The hybrid system performed better at lower doses, while the chemical treatment required excess H_2O_2. The CBZ removal decreased considerably for the real effluent compared to pure water, reaching a maximum of 68% at 20:1 H_2O_2:CBZ molar ratio (hybrid treatment). The phytotoxicity assays with onion seeds proved the environmental compliance of the proposed continuous-flow treatment system.

Acknowledgments

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