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ENZYMATIC CATALYSIS WITH WASTEWATER TREATMENT PURPOSE: FROM BATCH TO HYBRID CONTINUOUS FLOW SYSTEM

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1. INTRODUCTION

Several batch treatment arrangements successfully fulfill their role in degrading or mineralizing contaminants currently reported in industrial and urban wastewaters. However, most of these effluents are continuously generated, and the current batch-mode approaches fail to meet the demands of wastewater treatment plants (KANAKARAJU; GLASS; OELGEMÖLLER, 2018).

While biological and chemical treatments alone can significantly reduce the imminent danger of micropollutants by different mechanisms, they can be potentialized by biological-chemical hybridization, considered a feasible strategy to improve contaminants removal performance and suitability (SAIDULU *et al.*, 2021). In this sense, the present work reports, for the first time, the behavior of the peroxidase-assisted oxidation of 2-methoxyphenol (guaiacol), selected as a model substrate, in a 3D-printed flat-plate reactor operating in continuous flow. We evaluated the system hybridization by combining UVC/H_2O_2 and enzymatic biocatalysis, aiming for its application in wastewater treatment.

2. MATERIALS AND METHODS

A crude enzymatic extract containing peroxidase (POD) was produced by submerged fermentation using *Trichoderma koningiopsis* MK860714 (REICHERT JÚNIOR *et al.*, 2019) and microalgal biomass as culture medium, both from noncommercial sources. Fermentation was carried out for 72 h on an orbital shaker at 120 rpm and 28 °C, with a medium composed of 10 g of fresh *Chlorella* biomass (89% humidity) and 90 mL of distilled water. After fermentation, the content was filtered, the liquid permeate was centrifuged, and the supernatant corresponded to the crude peroxidase extract.

A known reaction medium was used to study the transition from batch to continuous flow system, composed of the substrate guaiacol (93 mmol L^{-1}) and the cosubstrate H_2O_2 (75 mmol L^{-1}). The unit of POD specific activity was defined as the enzyme amount capable of causing a 0.001 increase in the absorbance unit (at 470 nm) per minute per milligram of the total protein. The following variables for continuous flow regime were investigated by a Plackett-Burman (PB) design: 2-methoxyphenol:H_2O_2 molar ratio (1-10), UVC dose (0-1860 mJ cm^{-2}, buffer pH (4.2-8.2), and reaction temperature (20-50 °C). The PB was conducted in the absence and presence of POD. The experimental arrangement is represented in Figure 1. The evaluated responses were relative absorbance (quotient between the outlet and inlet absorbance of the reaction medium, at 470 nm,

without enzymatic extract) and relative POD activity (quotient between the outlet and inlet enzymatic activity).

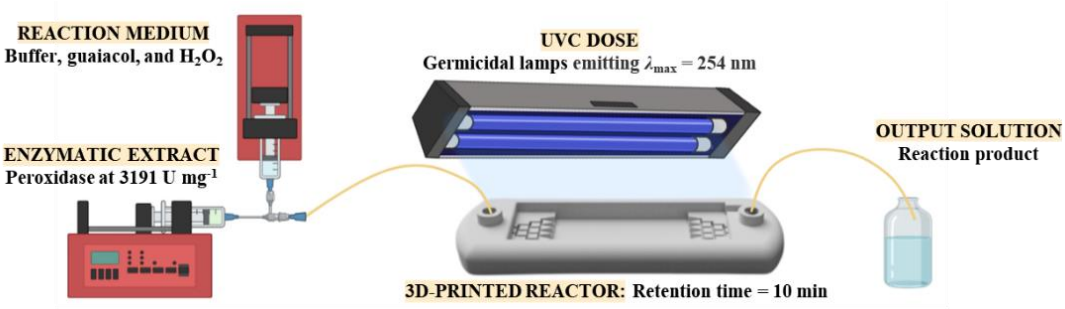

Figure 1. *Experimental setup for the hybrid continuous flow system experiments.*

3. RESULTS, DISCUSSION, AND CONCLUSIONS

Considering the interaction of UV radiation, H_2O_2, and 2-methoxyphenol, we initially quantified the reaction medium's relative absorbance under the PB design conditions without POD. At a p-value <0.05, only the UVC dose positively affected the relative absorbance, increasing up to 252%. The absorbance did not change in assays without UVC. The UV/H_2O_2 process was able to oxidize 2-methoxyphenol, possibly generating dihydroxylated rings (e.g., catechol, methoxyhydroquinone, methoxycatechol, and their quinonic forms) (SAMET; WALI; ABDELHÉDI, 2011).

Subsequently, the PB design was carried out with peroxidase, and only the enzymatic activity was quantified. The blank was the output oxidized reaction medium without enzymatic extract. Compared to the inlet activity (measured by the standard batch procedure), the POD activity decreased in all assays (39-60%); however, at a p-value <0.05, none of the variables showed a significant effect on the enzymatic response.

The current work indicated that system hybridization by combining UVC/H_2O_2/POD is efficient in oxidizing 2-methoxyphenol. Despite exposure to high UVC doses, the POD biocatalytic activity was partially maintained in a system with competition to assimilate H_2O_2 and 2-methoxyphenol. Therefore, the proposed hybrid continuous flow system has potential application in the degradation of contaminants, which will be investigated and optimized in further stages of this research.

4. REFERENCES

KANAKARAJU, D., GLASS, B.D., OELGEMÖLLER, M. 2018. Advanced oxidation process-mediated removal of pharmaceuticals from water: A review. J. Environ. Manage., 219:189–207. DOI: 10.1016/j.jenvman.2018.04.103

REICHERT JÚNIOR, F.W., SCARIOT, M.A., FORTE, C.T., *et al.* 2019. New perspectives for weeds control using autochthonous fungi with selective bioherbicide potential. Heliyon, 5(5):e01676. DOI: 10.1016/j.heliyon.2019.e01676

SAIDULU, D., GUPTA, B., GUPTA, A.K., *et al.* 2021. A review on occurrences, eco-toxic effects, and remediation of emerging contaminants from wastewater: Special emphasis on biological treatment based hybrid systems. J. Environ. Chem. Eng., 9(4):105282. DOI: 10.1016/j.jece.2021.105282

SAMET, Y., WALI, I., ABDELHÉDI, R. 2011. Kinetic degradation of the pollutant guaiacol by dark Fenton and solar photo-Fenton processes. Environ. Sci. Pollut. Res., 18:1497. DOI: 10.1007/s11356-011-0514-4