ABSTRACT

Antibiotics and synthetic dyes cause adverse effects on aquatic life, and contaminate drinking water resources. Therefore, to decrease the release of these pollutants into the environment, it is necessary to develop more efficient treatments for this issue. The objective of this research was to establish a methodology for the remediation of water contaminated with antibiotics and another for the remediation of water contaminated with synthetic dyes, based mainly on the native fungus *Leptosphaerulina* sp. (CECT 20913).

In the first phase of the research, the biotransformation of nine antibiotics: cephalosporins (cephalexin (CPX), cephradine (CED) and cephadroxyl (CPD)), fluoroquinolones (ciprofloxacin (CIP), norfloxacin (NOR) and levofloxacin (LEV)), Isoxazolyl-Penicillins (oxacillin (OXA), cloxacillin (CLX) and dicloxacillin (DCX)), and five synthetic dyes: azoic (Reactive Black 5 (RB5) and Novacron Red FN-2BL (NR)), phthalocyanine (Turquoise Blue (TB)), indigoid (Indigo Carmine (IC)), and triphenylmethane (Crystal violet (CV)), by *Leptosphaerulina* sp. (CECT 20913), and its ligninolytic enzymes (laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP), and versatile peroxidase (VP) was evaluated.

Leptosphaerulina sp. (CECT 20913) considerably eliminated the majority of antibiotics and dyes evaluated. The antibiotics and dye removal rate differences were associated with the different chemical structures. Antibiotics and dye biodegradation was associated to Lac and VP action. Assays with enzymatic extracts (crude and pre-purified) from *Leptosphaerulina* sp. (CECT 20913), commercial enzymes, and enzymatic inhibitors, confirmed the significant role of the enzymes in the transformation of pollutants. In addition, *Leptosphaerulina* sp. (CECT 20913)

eliminated antibiotics and dyes from simulated complex matrices (hospital wastewater and wastewater treatment plant).

In the second phase of this work, fungal co–culture was evaluated as an eliciting strategy for the production of ligninolytic enzymes by *Leptosphaerulina* sp. and for improving the degradation of antibiotics and dyes. Thus, *Trichorderma viride* and *Aspergillus terreus* were selected from other fungi of biotechnology interest (*A. niger*, *A. fumigatus*, *Fusarium* sp., and *Penicillium chrysogenum*) as the most compatible fungi based on its enzymatic and decolourising activity in solid media, respectively. Then, a response surface methodology (RSM) was applied to evaluate the effect of fungal co–culture, in liquid medium, to remove the pollutants. It was found that the co–culture significantly enhanced the enzymatic production (Lac 8–times, VP 36–times, and MnP 88–times during RB5 bio-treatment, and MnP ~1.3–times when the fungi were used to treat OXA) and pollutants removal (92% RB5 (1.2–times) and ~100% OXA) compared to *Leptosphaerulina* sp. monoculture.

The third phase included the evaluation of chemical oxidation processes as a coupled method for the removal of recalcitrant dyes that were not completely eliminated by *Leptophaerulina* sp. (CECT 20913). In this instance, RB5 and CV were chosen due to they were partiality and poorly removed, respectively. The decolourisation of aqueous solutions with RB5 was performed by TiO_2-photocatalysis, using a Box–Behnken experimental statistical design, to evaluate the effect of the combination of TiO_2 concentration, pH, and RB5 concentration on dye decolourisation. The highest decolourisation percentage of RB5 (99.51%) was obtained at 10 h of exposure to UV light, using 0.5 g L^{-1} of TiO_2, 50 mg L^{-1} of the dye, and a pH of 3.

In it turn, TiO_2-photocatalysis, sonochemistry and electrochemistry were combined with *Leptosphaerulina* sp. (CECT 20913) to eliminate the CV dye with successful results. After the process, the fungus produced ligninolytic enzymes in the final solutions from photocatalysis, sonochemistry and electrochemistry treatment. The electrochemical process was the most suitable pre-treatment for CV removal as it increased the enzymatic production in a short time (1.33 h), decreased the total organic carbon and low electrical energy consumption compared with the photocatalysis and sonochemistry treatments.

All results evidenced the *Leptosphaerulina* sp. (CECT 20913) potential for removing antibiotics and dyes of different chemical nature. *Leptosphaerulina* sp. (CECT 20913) and its ligninolytic enzymes is the most suitable methodology to remove antibiotics. Whereas, the use of fungal co–culture (*Leptosphaerulina* sp. (CECT 20913) with *T. viride* and *A. terreus*) is the appropriate and novel environmentally–friendly method to enhance *Leptosphaerulina* sp. (CECT 20913) enzymes production and dye removal. Finally, the synergism between chemical and biological process to remove non-biodegradable dyes (CV) from waters was also demonstrated. CV best removal method was the combination of electrochemical process and the *Leptosphaerulina* sp. (CECT 20913) fungus.

As outcomes from this research work, two international publications in the Q1 international journal, Science Of The Total Environment ("Elimination of Isoxazolyl-Penicillins antibiotics in waters by the ligninolytic native Colombian strain *Leptosphaerulina* sp. considerations on biodegradation process and antimicrobial activity removal and "Enhancement of ligninolytic enzymes production and decolourising activity in *Leptosphaerulina* sp. by co–cultivation with *Trichoderma viride* and *Aspergillus terreus"*) and one in the Q4 national journal, Revista Colombiana de Química ("Decolorization of Reactive Black 5 Dye by Heterogeneous

Photocatalysis with TiO_2/UV") were done. In addition, the results were presented in one national (I Congreso Colombiano de Procesos Avanzados de Oxidación, PAOx) and two international scientific events (IX Congreso Latinoamericano de Micología and XIII Congreso Argentino de Microbiología General).

Keywords: white-rot fungi, ligninolytic enzymes, antibiotics removal, dyes elimination, fungal co–culture, chemical oxidation processes.