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Bio-electro oxidation of indigo carmine by using microporous activated carbon fiber felt as anode and bioreactor support

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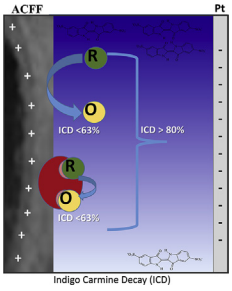
HIGHLIGHTS

- The combination of biochemical and electrochemical oxidation processes is proposed.
- An activated carbon fiber felt is used with dual function of anode and bioreactor.
- The efficiency of isolated and combined technologies is compared.

GRAPHICAL ABSTRACT



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ABSTRACT

The bioremediation and electro-oxidation (EO) processes are included among the most promising cleaning and decontamination mechanisms of water. The efficiency of bioremediation is dictated by the biological actuator for a specific substrate, its suitable immobilization and all involved biochemical concepts. The EO performance is defined by the anode efficiency to perform the complete mineralization of target compounds and is highlighted by the low or null use of reagent. Recently, the combination of both technologies has been proposed. Thus, the development of high efficient, low cost and eco-friendly anodes for sustainable EO, as well as, supporting devices for immobilization of biological systems applied in bioremediation is an open field of research. Therefore, the aim of this work was to promote the bio-electrochemical remediation of indigo carmine dye (widely common in textile industry), using new anode based on a microporous activated carbon fiber felt (ACFF) and ACFF with immobilized Laccase (Lcc) from *Pycnoporus sanguineus*. The results were discolorations of 62.7% with ACFF anode and 83.60% with ACFF-MANAE-Lcc anode, both for 60 min in tap water. This remediation rates show that this new anode has low cost and efficiency in the degradation of indigo dye and can be applied for other organic pollutant.

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1. Introduction

The humankind explosion allied to the tech evolution and social regression is translated by a progressive impact on the environment. As an example, since the ancient times the dyes has been used more and more on the clothes coloring. Therefore, many effluent treatment technologies have been proposed, such as, biological treatment using microorganisms or their enzymes, for the bioremediation of pollutants. Laccases (Lcc) are polyphenol oxidases that catalyze the electron transfer between substrate and the molecular oxygen, with the reduction of water (Spina et al., 2015). These enzymes are more active against on phenolic groups with low redox potential, but the use of mediators allows the indirect transfer of electrons to others compounds, promoting an overall pollutant degradation (Zeng et al., 2017). Thus, Lcc presents a diversity of environmental applications, such as dyes bioremediation (Pan et al., 2014), drugs (Lloret et al., 2013; Becker et al., 2016), endocrine disruptors (Spina et al., 2015), pesticides and herbicides (Zeng et al., 2017).

The immobilization of enzymes on ionic surface are processes with significant advantages, such as, the minimum modification of enzyme conformation that guarantee the preservation of functions and simple technology requirements. On the other hand, the main disadvantage is the loss of immobilized enzymes due to the presence of salts or others charged compounds by lixiviation, in the target solution (Zucca et al., 2016).

The remediation of pollutants have been also achieved by the employment of advanced oxidation processes (AOPs), due to ease operation, environmental compatibility, safety, versatility and high energy efficiency, they are a promising solution for this specific case (Sirés and Brillas, 2012; El-Ghenymy et al., 2015).

The electro-oxidation (EO) is a type of electrochemical remediation and it is the most known technology among the AOPs. It presents high capability of transform and/or mineralize a wide range of organic pollutants. EO is characterized by the generation of hydroxyl groups, a powerful oxidizing agent, from the electrolysis of water on the surface of the anode (El-Ghenymy et al., 2015; Ganiyu et al., 2016; Massa et al., 2017; Benito et al., 2017). The anodes are composed of diverse conductive materials, the best of them is polycrystalline boron-doped diamond electrode (BDD), however its high cost avoids their use in large scale. Metallic anodes modified with other metallic oxides are a good alternative, although they present short useful life due to the chemical leaching of the metals, for example, with lead and copper (Thiam et al., 2014; Ganiyu et al., 2016). Hence, the development of efficient and cheap electrodes is necessary to minimize the environmental contamination.

Carbon materials are widely used in electrochemistry as electrodes (Sirés and Brillas, 2012; Garcia et al., 2015; Ye et al., 2015).

In addition, carbon materials have high adsorptive capacity, as already reported in the literature: fixed beds of granular activated carbon, developed from fertilizer waste material (Gupta et al., 1998), alumina-coated carbon nanotubes (Gupta et al., 2011), multi-walled carbon nanotubes and titanium dioxide (Saleh and Gupta, 2012a), column with carbon nanotubes/magnesium oxide composite (Saleh and Gupta, 2012b).

In order to ensure the environmental conservation, it is mandatory to, covering a wide range of contaminants. Thus, the combination of different technologies may be a powerful strategy. In this context, the use of bio-electrochemical remediation has been rising. The microporous activated carbon fibers felt (ACFF) possess high surface area, great adsorptiveness and good electric conductivity, being useful in bioreactors as immobilization platform and as anodes for electro-oxidation (EO) (Huang et al., 2015; Marcuzzo et al., 2016). The immobilization of Lcc at electrochemical transducers is very usual in biosensor development (Garcia et al., 2015). Thus, owing to the characteristics of ACFF, the possibility to attain simultaneous electrochemical and biochemical oxidation is real and innovative. Moreover, the carbon felt can be easily produced from recyclable textile fiber. Hence, the aim of this work is the immobilization of Lcc at anionic surface of ACFF focusing the development of less specific and synergic cleaning processes. As first, an attempt, the indigo carmine dye was herein used as a target compound of bio-electrochemical remediation.

2. Materials and methods

2.1. Reagents

Malt extract and $CuSO_4 \cdot 5H_2O$ were from HiMedia and Cromoline, respectively. 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonate) acid (ABTS), syringaldazine, sodium periodate and 2,5-xylidine were from Sigma Aldrich Chemical Co. Indigo carmine ($C_{16}H_8N_2Na_2O_8S_2$ FD&C blue nº 2 indigotine) was from Sensient Colors. Ethylenediamine and sodium bicarbonate were from Vetec quimica fina Ltda. Sodium phosphate and acetate buffers were from Synth produces para laboratório Ltda. MS and HPLC grade solvents were purchased from J.T. Baker.

2.2. Biological remediation

2.2.1. Microorganism and Lcc production

The *Pycnoporus sanguineus* CCT-4518 was obtained at André Tosello Foundation, Campinas, São Paulo, Brazil. The microorganism was kept in potato dextrose agar plates (PDA) at 4 °C.

The production of the crude extract containing Lcc was performed by submerged bioprocess. The *P. sanguineus* mycelium was fractioned into 5 disks (7 mm diameter), then inoculated in 250 mL Erlenmeyer flasks with 50 mL of liquid culture medium composed of 12.8 g L^{-1} of Malt extract, 0.005 g L^{-1} of $CuSO_4.5H_2O$ and 50 mg L^{-1} of 2.5-xylidine. The erlenmeyers were maintained in orbital shaker at 150 rpm in the dark at 28 °C for 72 h (Valeriano et al., 2009). After this period, the mycelium was separated from the supernatant by filtration on paper filter and used as crude extract, source of Lcc.

The Lcc activity was determined by the oxidation of ABTS substrate (ε_{420nm} = 36000 mM^{-1} cm^{-1}). The reaction contained 50 μL of crude extract, 850 μL of 50 mM sodium acetate buffer (pH 4.0) and 100 μL of 1 mM ABTS. An enzyme activity unit (U) was defined as the amount of enzyme capable of oxidizing 1 μM of ABTS under standard assay conditions. The results were expressed as U mL^{-1}.

2.2.2. Discoloration assays with Lcc crude extract

The indigo carmine is a synthetic dye, selected for the discoloration assay with *P. sanguineus* crude extract. The dye stock solution (1% w/v in distilled water) was kept at 4 °C in the absence of light. To evaluate the optimal experimental conditions of discoloration, we tested several conditions: salts – 100 mM sodium acetate buffer pH 5.0 (control), 100 mM sodium phosphate buffer - PBS pH 7.0, 100 mM Na_2SO_4 solution pH 7.0 ± 0.5, and 10 mM NaCl solution, pH 7.0 ± 0.5; mediators - seryngaldazine and ABTS at different concentrations, 0, 0.1, 0.5 and 1 μM – and fixed conditions of 0.001% indigo carmine dye and crude laccase extract with final activity of 280 U mL^{-1}. The assays were performed at a final volume of 1 mL for at least 24 h in the absence of light without agitation at 30 °C. All experiments were carried out in triplicate, with due controls.