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BIOTREATMENT OF FRUIT PROCESSING WASTEWATER OF HIGH FUNGICIDE CONTENT IN A LABORATORY IMMOBILIZED CELL BIOREACTOR

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Abstract
The fruit processing industry is one of the most important agro-industrial sectors worldwide. To prevent damage of fruits by fungal infections during storage, various post-harvest fungicides are used in the fruit packing plants. Among the most common postharvest fungicides applied on fruits like apples, pears and citrus are imazalil and fludioxonil. However, the high concentration of these fungicides in the wastewater produced by their application practice and their recalcitrant nature make improper the use of conventional activated sludge systems for the treatment of these agro-industrial effluents. In this work, a laboratory immobilized cell bioreactor was set-up and operated to remove postharvest fungicides from a wastewater derived from the fruit packing industry. A range of physicochemical traits, such as pH, EC, COD, TKN and NH_4-N, were determined during biotreatment. The concentrations of the post-harvest fungicides were also determined by HPLC in the influent and the effluent of the biosystem. Analysis of the experimental data showed that the immobilized cell bioreactor could effectively remove fungicide content, determining removal efficiencies higher than 75%. It is concluded that the bioreactor configuration employed was capable of removing the high postharvest fungicide content of such wastewaters.

Keywords: fruit packing industry; postharvest fungicides; fungicide degradation; immobilized biomass

1 INTRODUCTION

The modern style of life has increased the demand of various crop fruits throughout the year, independently of their harvest period. In addition, the continued rise in global population has also increased the demand of fresh fruits. Thus, the fruit processing industry, a dynamic agro-industrial sector worldwide, has adopted several approaches for the postharvest management of crops. However, the main constraint of the fruit packing industry to prolong the lifetime of the fruits is the fungal infections, which deteriorates crop quality during postharvest storage, resulting in serious economic harms (Sepulveda *et al.*, 2015). In the last decades, various systemic and non-systemic postharvest fungicides have been developed by the agro-pharmaceutical industry to prevent fungal diseases and minimize economic losses during postharvest storage of the fruits (Yoshioka *et al.*, 2010).

Nowadays, imazalil and fludioxonil are among the most common postharvest fungicides applied to fruits. Fludioxonil is characterized by low water solubility (1.8 mg/L) and high hydrolytic and thermal stability (EFSA, 2007). On the other hand, imazalil is less thermally stable than fludioxonil, although it is more soluble in water (US EPA, 2005). Imazalil is a systemic imidazole fungicide, which is applied to fruits, such as citrus, apple and pears, to face post-harvest fungal infections induced by fungi like *Penicilium digitatum* and *Alternaria alternate* (Altieri *et al.*, 2016). Moreover, fludioxonil, a non-systemic postharvest phenylpyrrole fungicide, can be applied to citrus, pears, apples, strawberries, kiwis and mangos, preventing fruit decay from various fungal taxa, like *Botrytis*, *Penicillium* and *Alternaria* (Diskin *et al.*, 2019; Schirra *et al.*, 2005). Fludioxonil is often applied for the pre-harvest treatment of seeds and crops.

Concern has arisen for their toxicity against certain organisms. Velki *et al.* (2019) classified a mixture of difenoconazole and fludioxonil as supertoxic to the earthworm *Eisenia andrei.* Sub-lethal effects of imazalil on *E. andrei* have also been reported. Lethality of a mixture 2.5% fludioxonil and 1% metalaxyl-M applied to the amphibian *Rhinella arenarum* was found to be dose- and exposure time-dependent (Svartz *et al.*, 2018). Moreover, fludioxonil has been reported to cause malformations in *Xenopus tropicalis* embryos (Li *et al.*, 2016). In addition, Jin *et al.* (2016) stated the potential of imazalil to induce toxicity at early developmental stages and abnormalities in locomotor activity of zebrafish (Jin *et al.*, 2016).

Heterogeneous advanced oxidation processes can be applied for the treatment of imazalil-rich wastewaters produced by the fruit process industry (Santiago *et al.*, 2014). Fenton and photo-Fenton processes have been reported to be capable of mineralizing 50 mg/L imazalil, although high Fe(II) concentrations are required (Santiago *et al.*, 2018). Application of UV/TiO_2, UV/$K_2S_2O_8$ and UV/TiO_2/$K_2S_2O_8$ resulted in the mineralization of 25 mg/L imazalil in a period of 25, 4 and 2 h, respectively (Hazime *et al.*, 2012). Absorption of 100 mg/L imazalil on activated carbon led to high removal efficiency (Martín-González *et al.*, 2014). In addition, physicochemical methods have been performed for the treatment of fludioxonil-rich wastewaters. Photocatalytic oxidation using ZnO or TiO_2 resulted in complete depuration of a mixture of cyprodinil and fludioxonil (Fenoll *et al.*, 2011).

Moreover, biomixture approaches have been recently applied for the removal of fungicides. In particular, a mixture of fungicides, including fludioxonil, was treated in an organic biomixture containing pruning residues and wheat straw, achieving 50% fludioxonil reduction within a period of 70 days (Coppola *et al.*, 2011). A decrease by approximately 80% in fludioxonil concentration was also recorded during the treatment of this postharvest fungicide in a biomixture, consisting of 50% spent mushroom substrate, 25% wheat straw and 25% soil (Papazlatani *et al.*, 2019). Thus, biological methods are restricted regarding depuration of postharvest fungicides. In this work, an immobilized cell bioreactor was set up and operated to treat a fungicide-based wastewater consisting of both imazalil and fludioxonil.

2 MATERIALS AND METHODS

A 200 mL horizontal immobilized cell bioreactor was used to treat a wastewater consisting of a mixture of imazalil and fluodioxonil, 200 mg/L each fungicide. The bioreactor consisted of three compartments: in the first, the influent was introduced in the bioreactor, the second contained the plastic rings (25 mm x 12 mm, 500 m^2/m^3) for the immobilization of the biomass and in third, a recirculation stream was returned into the 1^{st} compartment, whereas part of the treated effluent was discarded. The hydraulic retention time was 10 days. The bioengineering system was operated for a period of three months under the continuous mode.

Physicochemical parameters were determined according to "Standard Methods for the Examination of Water and Wastewater" (Clesceri *et al.*, 1998). In particular, pH and electrical conductivity (EC) in both influent and effluent were determined by using a METROHM 632 and a CRISON pH and conductivity meter, respectively. The concentration of dissolved oxygen in the wastewater of the immobilized cell bioreactor was determined by using a WTW Oxi 320 meter, which was equipped with a CellOx 325 electrode.

To estimate chemical oxygen demand (COD), 2.5 mL of the influent or the effluent as appropriate were mixed into a durable glass vial with 2.5 mL of 0.1 N $K_2Cr_2O_7$ and 5 mL concentrated sulfuric acid/mercury sulfate solution to create a strong acidic environment The samples prepared were digested at 148 °C for a period of 2 h. A blank sample was also included for each series of samples, where wastewater was replaced with respective volume of deionized water. COD determination was performed by titrating samples with 0.02 N ammonium ferrous sulfate in the presence of 25 mM ferroin indicator.

To determine ammonium nitrogen (NH_4^+-N) concentration, 50 mL wastewater, 25 mL borate solution (12.5 mM $Na_2B_4O_7.10H_2O$/8.8 mM NaOH) and 25 mL 6 N $NaOH$ were added into a volumetric tube. The mixture was subjected to distillation and the ammonia vapor was entrapped in 50 mL 20 g/L boric acid solution. The distillate was titrated with 0.01 N H_2SO_4 in the presence of methyl red-methylene blue indicator until the development of purple-blue color. All measurements were performed against blank containing 50 mL deionized water instead of wastewater.

Total Kjeldahl Nitrogen (TKN) concentration was determined in Kjeldahl tubes filled with 50 mL wastewater (or water in the case of blank) and 25 mL digestion reagent (134 g K_2SO_4, 11.4 g $CuSO_4$ and 134 mL concentrated H_2SO_4 in 1 L dH_2O). After heat digestion for 4 h, 25 mL deionized water and 25 mL $Na_2S_2O_3$ alkaline solution (25 g $Na_2S_2O_3.5H_2O$ and 500 g NaOH in 1 L H_2O) were added in each tube and the ammonia content after distillation was fixed in 50 mL 20 g/L boric acid solution. Nitrogen content was measured after titration with 0.01 N H_2SO_4 in the presence of methyl red-blue indicator, as described above.

The determination of postharvest fungicides was performed against standards in HPLC-PDA apparatus (ECOM, Czech Republic) by using a C18 250 x 4.6 mm column (Fortis, UK), where acetonitrile/H_2O was used as the mobile phase.

3 ANALYSIS, RESULTS AND DISCUSSION

Determination of postharvest fungicides in the immobilized cell bioreactor showed a significant reduction in the concentration of both fludioxonil and imazalil (Figure 1). In particular, fludioxonil and imazalil concentrations were decreased by 96.6 ± 0.8% and 84.1 ± 2.6%, respectively. This indicates that the concentration of these compounds can be decreased in the immobilized cell bioreactor despite their recalcitrant nature. In addition, the concentrations of postharvest fungicides examined were among the highest examined in biological systems (Coppola *et al.*, 2011). Until now, a limited number of studies have been focused on the biodegradation of fungicide wastewater (Papazlatani *et al.*, 2019; Wu *et al.*, 2018).

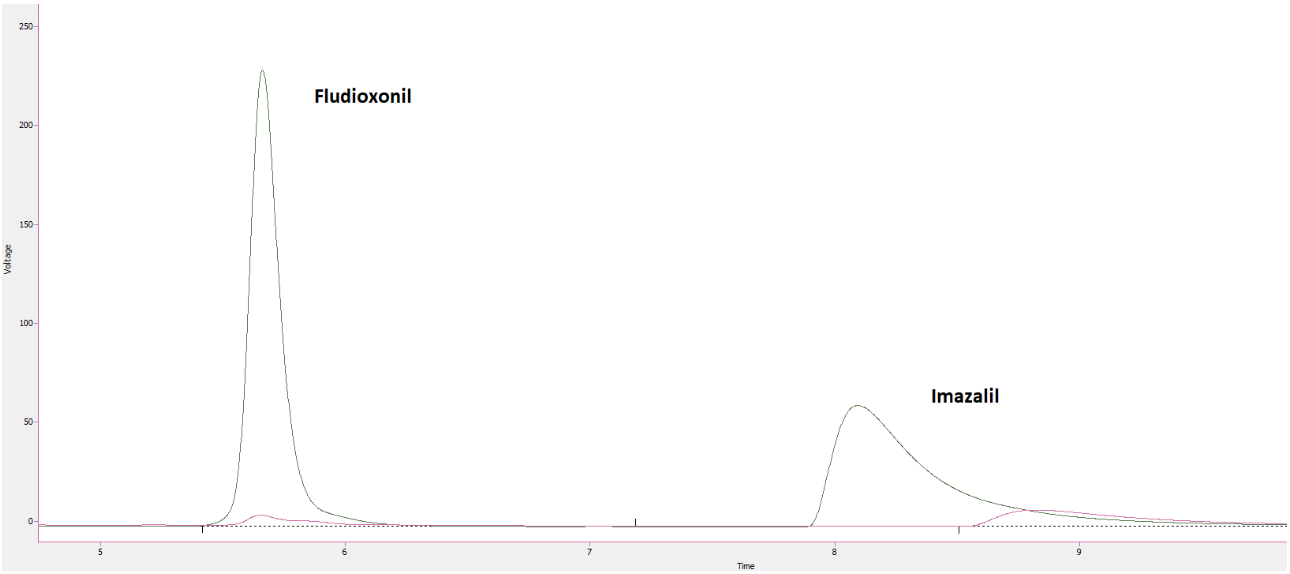

Figure 1: Reduction of fungicides mixture in the immobilized cell bioreactor.

Total COD reduced from 462 ± 51 to 108 ± 25 mg/L, resulting in COD removal of 74.9 ± 3.5%. The influent and effluent pH values were near pH 7, i.e. 6.9 ± 0.1 and 7.0 ± 0.1. Influent electrical conductivity (EC) was stable and equal to 3.1 mS/cm, while the electrical conductivity (EC) in the effluent increased up to 4.4 mS/cm. TKN, NH_4^+-N and NO_3^--N in the effluent were estimated to be 9.9, 5.9 and 0.7 mg/L during the first month of biotreatment, whereas no NO_2^--N was detected.

4 CONCLUSIONS

Fungicides removal efficiencies were high and equal to 96.6 ± 0.8 and 84.1 ± 2.6% for fludioxonil and imazalil, respectively. In addition, high COD removal efficiencies were recorded. It appears that the immobilized cell bioreactor was capable of effectively remove the fungicide mixture of high concentration.

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