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Introduction of advanced oxidative pre-treatment in the Biobed reactor system in the final disposal process of pesticide effluents

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Brazil has become the world's largest consumer of pesticides since 2008, which can lead to serious environmental, health and social risks. Practices to mitigate the impact caused by pesticide effluents in rural areas have increased in importance, given the impossibility of carrying out conventional treatment of these residues in industrial plants. Among these practices, the use of fixed-bed bioreactors, known as Biobed, stands out in reducing point contamination. It is known that this model of reactor faces difficulties in the biodegradation of some pesticides, including the fungicide tebuconazole, which can negatively interfere with its efficiency. This problem needs to be addressed in order for the biological system to meet the required environmental safety parameters. And one of these alternatives is the use of the UV/H_2O_2 Advanced Oxidation Process as a pre-treatment, so the pesticide effluent can reach lower concentrations before being disposed in a Biobed. The aim of this work was to evaluate the influence of the pre-treatment in relation to the Biobed. Pre-treated and untreated effluents were applied in the bioreactor in the form of columns, being evaluated at 1, 84 and 168 days after contamination, through gas chromatography that determined the concentration of the pesticide residues. The results showed that the pre-treatment fulfilled its role of reducing effluent pesticide concentration (68.1 %), without inhibiting the biological action of the Biobed reactor still serving to increase the efficiency of the tebuconazole degradation, in relation to the contaminated reactors without pre-treatment. These results allow the recommendation of Biobed systems usage with oxidative pre-treatment UV/H_2O_2.

1. Introduction

Pesticides are worldwide used on large scale for pest control in agricultural production, and Brazil is the world's largest consumer since 2008 (Carneiro et al., 2015). According to Gebler et al. (2015) these substances are considered as the main environmental contaminants generated in rural areas. Throughout its handling, when the effluent generation (accidental spills, equipment leftovers and its washing water) occur in places without adequate environmental management, it becomes a source of point contamination (Castillo et al., 2008), capable of affecting the soil, groundwater and surface waters (Tortella et al., 2012).
Reactors based on accelerated bioremediation processes, called Biobed, have been showing high efficacy for pesticides degradation, and used as a measure to mitigate point contamination. This system consists of a mixture of straw, peat and soil, and aims to efficiently reproduce the sorption and biodegradation activities that occur in the environment (Fogg et al., 2003). Some microbiological communities act on the pesticides biodegradation, and among them, lignolytic fungis have an important role (Diez, 2010). Despite this, some studies have demonstrated the difficulty of this reactor to establish an effective biodegradation for some types of fungicides, due to its inhibitory action to this microbiota, being pronounced for some fungicides of the triazole family, among them tebuconazole (TB) (Castillo-González et al., 2017; Murillo-Zamora et al., 2017). Triazoles are highly persistent in soil, with a half-life normally over 100 days (Lewis et al., 2016), and TB ((RS) -1-p-chlorophenyl-4,4-dimethyl- 3- (1H-1,2,4-triazole-1-ylmethyl) pentan-3-ol), despite having been commercialized for some decades (Storck et al., 2016), it still has gaps regarding its behavior in the

environment, their transformation reactions and the ecotoxicological impacts of their by-products (EFSA, 2014).
In this context, the inclusion of a pre-treatment to the bioreactor can be a feasible alternative to the triazole fungicide degradation problem, in order to increase the removal efficiency due the combination of treatments (Rodriguez-Narvaez et al., 2017). And given the difficulties of carrying out conventional treatments in pesticide effluents (Gogoi et al., 2018; Rodriguez-Narvaez et al., 2017), the Advanced Oxidation Process (AOP) by UV/H_2O_2 presents itself as a promising alternative. This technology can transform recalcitrant molecules into shorter and simpler organic compounds, being available to biodegradation processes (Boczkaj; Fernandes, 2017). Its mechanism of action is based on UV radiation as an activating agent and hydrogen peroxide (H_2O_2) as the oxidizer, resulting in photolysis of this substance and formation of hydroxyl radicals (•OH). These radicals are powerful oxidizing agents, highly reactive and practically non-selective, capable of acting on different organic molecules, including pesticides (Boczkaj; Fernandes, 2017; Rodriguez-Narvaez et al., 2017). UV/H_2O_2 has been addressed in the recent literature on the degradation of different active ingredients (a.i.), including tebuconazole (Celeiro et al., 2017; Chen et al., 2018; Djelal et al., 2016; Parker et al., 2017; Semitsoglou-Tsiapou et al ., 2016; Utzig et al., 2019).
This research aimed to study the Advanced Oxidative Process - UV/H_2O_2 as a pre-treatment of the Biobed reactor, in order to test whether the inclusion of the pre-treatment can help in the degradation of the tebuconazole fungicide in the Biobed reactors. Thus, some reactors were contaminated with raw effluent and others with pre-oxidized effluent, observing the degradation behavior of these systems over time.

2. Materials and Methods

The photochemical experiments, as well as the relevant chemical analyzes, were carried out at the Water Reuse Laboratory (LaRA). The bioremediation reactors were accommodated at the Educational Sewage Treatment Plant (CETESAN). Both facilities are located in Florianópolis, State of Santa Catarina, Brazil, and belong to the Department of Sanitary and Environmental Engineering at the Federal University of Santa Catarina (UFSC). This work was carried out in partnership with Embrapa Uva e Vinho, located in the municipality of Vacaria, State of Rio Grande do Sul, Brazil. The chromatographic readings of the analyzed pesticides were performed by Embrapa Uva e Vinho, in partnership with the Center for Research and Analysis of Residues and Contaminants (CEPARC) at the Federal University of Santa Maria (UFSM), state of Rio Grande do Sul, Brazil.

2.1 Chemicals and reagents

The commercial emulsifiable concentrate of tebuconazole, 20 % w/v (active ingredient), was provided by UPL (Ituverava, Brasil). Hydrogen peroxide (H_2O_2) 35 % w/v was provided by Dinâmica (Indaiatuba, Brasil). Chromatographic solutions are listed in Dias et al. (2017).

2.2 Effluent

The pesticide effluent generated in the rural area has a lower concentration than the spray solution applied in the plantations due to the dilution caused by the addition of the washing water of the equipment and floors of the area where these substances are handled, although the contaminant load is extremely more concentrated by area. However, for the worst environmental scenario to be tested, the TB concentration adopted for the treatment applied in this study was its recommended dose for application in apple plantations (100 $mg.L^{-1}$). Tap water was used to prepare the syrup to replace distilled / deionized water, in order to get closer to the reality of rural producers.

2.3 Photochemical pre-treatment with UV/H_2O_2

The photochemical degradation occurred in a 0.7 L laboratory reactor, with a double glass wall for the passage of water (temperature controlled at 22 ° C) and side openings for addition of reagent and sample collection. The inner tube, made of quartz, is responsible for coupling the high pressure mercury vapor lamp (125 W / OSRAM) to the reactor. The TB was diluted in water and then transferred to the reactor, then H_2O_2 (1,000 $mg.L^{-1}$) was added, the lamp was turned on together with the timer (oxidation time 40 min.) The system remained in constant agitation.

2.4 Biological treatment with Biobed reactor

The Biobed reactors were built on a pilot scale, composed of PVC tubes in the form of columns (height 1 m and diameter 100 mm), with the lower openings closed (watertight system). This conformation was based on Fogg et al. (2004). The tubes were filled with a biomix of wheat straw (pieces <1 cm), peat and soil in a 2:1:1 ratio (Tortella et al., 2012). The soil was collected at Embrapa Uva e Vinho Experimental Station, located in

the south of Brazil, in areas of apple cultivation, using layers of soil up to 10 cm deep with microbiota adapted to the tested pesticide (Sniegowski et al., 2011), and a grass cover over the biomix (Castillo et al., 2008; Gebler et al., 2015). The reactors were installed outdoors, under a plastic greenhouse covering structure (Figure 1), in order to avoid possible flooding of the systems in the event of intense rains (Gebler et al., 2015). The humidity control was carried out 3 times a week, with irrigation (tap water) whenever the reactors presented a dry aspect, with varying amounts according to the climatic conditions.

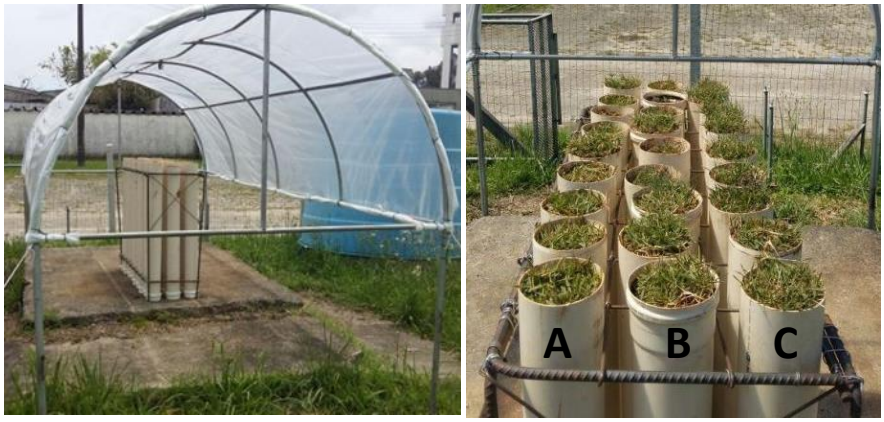

Figure 1: Test condition of columnar field reactors with grass cover detail, being row "A" reactors without contamination, row "B" reactors with contamination of pre-treated effluent and row "C" reactors with contamination of effluent without pre-treatment. Photo: The authors

2.5 Experimental set-up

After the maturation period (9 months) all reactors in rows "B" and "C" received effluents, while row "A" received only water. The reactors received a single dose of 7.85 mL of effluent (raw or pre-treated) or water (white reactor), simulating an accidental spillage of syrup with a proportion of 1 L in 1 m^2. And after application, three collection times were evaluated: 1, 84 and 168 days, with the complete dismantling of the reactor and the sending of all the solid substrate for chemical analysis (Figure 2).

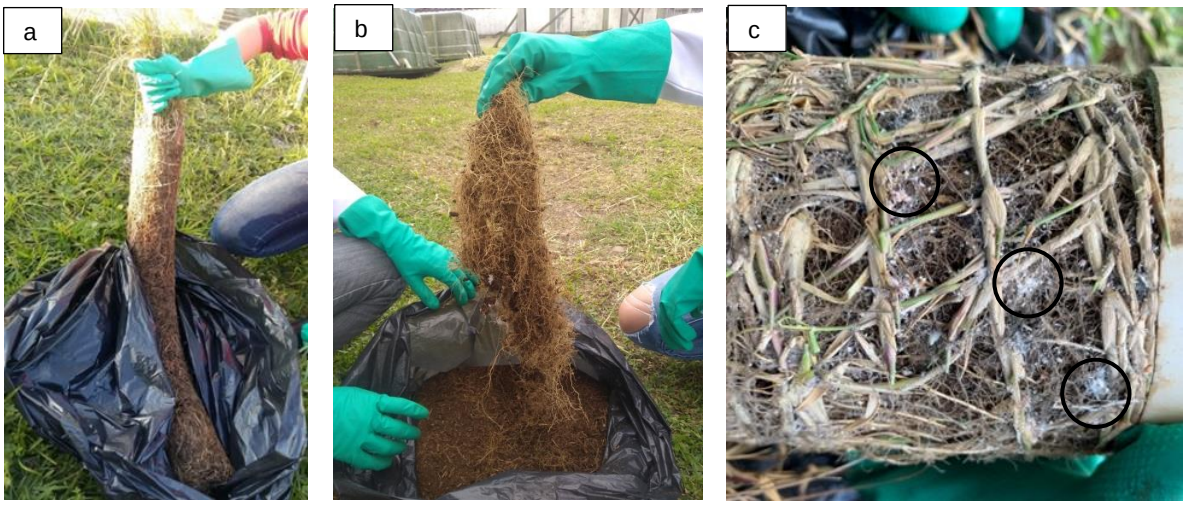

Figure 2: a) Reactor being disassembled during sample collection periods (1, 84 and 168 days) with details of the intense root formation; b) Amount of biomix recovered and sent to the laboratory; c) Detail of the colonization of lignolytic fungi (white rot fungi).

2.6 Analytical methods

Tebuconazole was determined as described in Dias et al. (2017) by Ultra Performance Liquid Chromatography (Acquity UPLC-MS/MS, Waters, EUA), with a flow rate of 0.45 $mL.min^{-1}$, at 60 ºC, with an injection volume of 2 µL, eluent A - water/ammonium formate 3 $g.L^{-1}$ and eluent B - methanol; coupled to Mass

Spectrometry (triple quadrupole Xevo TQS, Waters, EUA), with atmospheric pressure ionization by ESI mode positive, gas temperature 400 °C, gas flow 0.15 mL.min^{-1}, capillary voltage 3.0 kV. The solid matrix samples underwent a substrate extraction procedure developed by Vareli (2018). The detection limit (LOD) of the method for liquid matrix samples is 0.4 mg.L^{-1} and the quantification limit (LOQ) is 2 mg.L^{-1}. And for solid matrix samples, the LOD is 10 µg.kg^{-1} and the LOQ is 20 µg.kg^{-1}.

3. Results and Discussions

3.1 Photochemical pre-treatment with UV/H_2O_2

The pre-treatment achieved a significant removal of TB from the solution, reaching 68.1 % efficiency (Table 1). This is confirmed by Celeiro et al. (2017), who obtained 100 % removal for TB (10 µg.L^{-1}) with the same treatment process, in an effluent composed of 8 more fungicides, and also by Moreira et al. (2012), applying UV/H_2O_2 and solar radiation in biological effluent containing 19 pesticides, including TB (4.64 mg.L^{-1}), obtaining a reduction from 23 % to 0.5 % of carbonaceous contribution of the pesticides in question (in terms of dissolved organic carbon). These studies confirm how the UV/H_2O_2 method is effective for the degradation of pesticides, including in effluents with more than one active ingredient.

Table 1: Chromatographic readings of tebuconazole fungicide concentration (mg.L^{-1}).

Effluent	Untreated	Pre-treated	Removal (%)
TB	149.0 ± 0.4	47.5 ± 0.8	68.1

3.2 Biological treatment with Biobed reactor

In order to test whether the pre-treatment would interfere with the operation of Biobed reactors for the TB pesticide degradation, some systems were fed with raw effluent and others with pre-oxidized effluent. The results of the white reactors (Table 2) are below the detection limit of the reading method, so the biomix used had no previous TB contamination.

Table 2: Remaining concentration (µg.kg^{-1}) of the tebuconazole (TB) fungicide in Biobeds, for the opening times of 1, 84 and 168 days after application, and respective percentages (%) in reactors under white condition, with and without pre-treatment. The detection limit (LOD) of the chromatographic method is 10 µg.kg^{-1} and the quantification limit (LOQ) is 20 µg.kg^{-1}.

Days	Blanc reactor	Untreated effluent	Efficiency (%)	Treated effluent	Efficiency (%)
1	<LOD	94.5 ± 0.5		31.5 ± 4.6	
84	<LOD	37.3 ± 0.4	60.5	<LOD	100
168	<LOD	<LOQ	100	<LOQ	

The pre-treatment reactor starts (day 1) with a concentration of 31.5 µg.kg^{-1} (Table 2), lower than the reactor without pre-treatment (94.5 µg.kg^{-1}), due to the pre-oxidation in the photochemical reactor. And already in the 1st reaction stage 1 - 84 days (Figure 3), the system with pre-treatment managed to degrade the TB (≥ 68.2 % removal), and reached the LOQ. The reactor without pre-treatment, when receiving the highest concentration on day 1, managed to degrade 60.5 % at 84 days, reaching 100 % at 168 days. Therefore, this reactor showed a slower degradation, when compared to the reactor with pre-treatment.

Focusing only on the difference in initial concentration between the reactors, without taking into account the pre-treatment, different degradation results can be obtained in the Biobeds. Fogg et al. (2003) evaluated in a biomix of straw, soil and peat, the influence of the increase of the initial concentration of the pesticides isoproturon and chlorotalonil in the behavior of the Biobeds. Six different concentrations were tested, isoproturon (11 - 456 mg.kg^{-1}) and chlorothalonil (7 - 287 mg.kg^{-1}), and as a result they concluded that for both a.i. there was a significant effect between the increase in the initial concentration and the reduction of the respective degradation rates. And yet, Papadopoulou et al. (2016) evaluated in the laboratory the behavior of TB in the soil (x1, x2 and x10 the recommended dose) in 125 reaction days. As a result, they obtained a degradation dependent on the initial applied dose, with a decrease in their removal from the system (greater persistence) as the initial concentration increased. Thus, it can be understood that the difference in initial concentration between one reactor and another can lead to different degradation behaviors of pesticides. This indicates that the UV/H_2O_2 pre-treatment used made it possible to improve TB removal. Because of the inclusion of photochemical treatment, there was a reduction in the initial concentration of a.i. in Biobed

reactors, a reduction in the persistence of these substances in the system and, consequently, a decrease in reaction time to obtain the same result as in reactors fed with raw effluents.

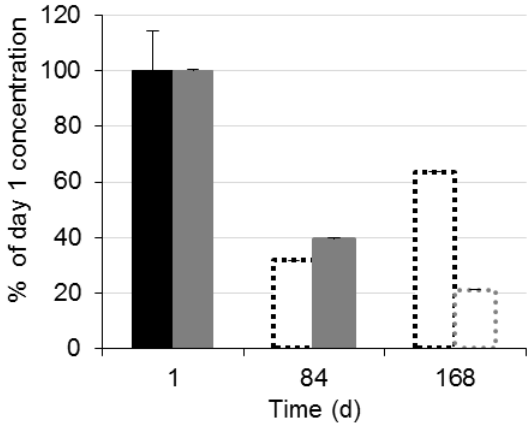

Figure 3: Removal of the tebuconazole fungicide in a Biobed with pre-treatment (black) and without pre-treatment (grey). Dotted lines represent equal or lower concentrations than LOD or LOQ of the chromatographic method. Bars represent mean values ± SD for triplicate samples.

The literature reports tebuconazole as a persistent substance in soils, with a half-life ranging from 49 to 610 days (EFSA, 2014; Strickland et al., 2004). And despite this difficulty in overcoming its toxicity and persistence, for the combination adopted in this work of the physical-chemical and biological processes, as well as for the treatment conditions tested, the fungicide TB could be degraded in Biobeds. So the pre-treatment by UV/H_2O_2 fulfilled its role in reducing the TB concentration in the effluent, without inhibiting the biological performance of the Biobed reactor. This physical-chemical treatment can then act as an ally in cases such as those of triazole fungicides, which have degradation difficulty in these biological systems based on lignolytic fungi. Or, the pre-treatment can be used in situations where the demand for space or reaction time for the Biobed is very high.

4. Conclusions

For the treatment scenarios tested, the Biobed reactors that had their effluents pre-oxidized achieved total removal of the tebuconazole contaminant in the 1st treatment period (84 days), while the reactors that did not have their pre-oxidized effluents achieved total removal only in the 2nd period (168 days). This indicates that the pre-treatment used enabled an improvement in the removal of tebuconazole.

The Advanced Oxidation Process by UV/H_2O_2 is a technology that shows good degradation results when coupled with Biobed bioremediation reactors and applied to pesticide effluents, increasing the overall efficiency of the system. This work then opens the door for the inclusion of physical-chemical treatments combined with the Biobed system.

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