Bioprocess and Biosystems Engineering (2020) 43:261–272
https://doi.org/10.1007/s00449-019-02222-6

RESEARCH PAPER

Advanced oxidation processes applied for color removal of textile effluent using a home-made peroxidase from rice bran

Natalia Klanovicz[1] · Aline Frumi Camargo[1] · Fábio Spitza Stefanski[1] · Jessica Zanivan[1] · Thamarys Scapini[1] · Rafaela Pollon[1] · Andressa Warken[1] · Letícia Paliga[1] · Karina Paula Preczeski[1] · Anderson André Genro Alves Ribeiro[1] · Jaqueline Garda-Buffon[2] · Gislaine Fongaro[3] · Helen Treichel[1]

Received: 17 July 2019 / Accepted: 22 September 2019 / Published online: 1 October 2019

Abstract
Enzymes are becoming tools in industrial processes because of several advantages, including activity in mild environmental conditions, and high specificity. Peroxidase, for one, stably oxidizes several substrates. The present study aimed to develop advanced oxidation processes (AOP), using non-commercial rice bran peroxidase to remove color and toxicity of synthetic textile wastewater. Using a microwave and shaker system, we obtained 38.9% and 100% of effluent color removal after peroxidase treatment, respectively. In addition, the shaker system decants residual dye particles through filtration, providing the textile industry with an economical and environmentally viable alternative to effluent treatment. In toxicity tests results, both treatment systems damaged the used genetic material. This damage occurs because of industrial discharge of wastewater into water bodies; effluent dilution reduced this damage. The data suggest that peroxidase as a textile effluent treatment has potential uses in industrial processes, because rice bran peroxidase has demonstrated affinity with dyes.

Keywords Enzymatic precipitation · Shaker system · Microwave radiation · Toxicity test

Introduction

The textile dyeing industry is one of the greatest threats to the environment. The sources of concern are the chemicals produced by this industry and the volume of water consumed, around 200 L to produce 1 kg of fabric. The process generates large amounts of effluent containing dissolved solids, chemicals, trace metals, odors, colors, and other pollutants, all of which are toxic to humans and the environment [1-4].

Dye concentration in textile industry wastewater may be as high as 250 mg/L, giving rise to the intense color of this effluent. When disposal is implemented incorrectly, dyes can block the penetration of sunlight, inhibiting biological processes, and raising biochemical oxygen demands. Furthermore, effluent chemicals harms humans and animals, causing nausea, dermatitis, and genetic mutations [3-8]. Azo dyes represent 70% in terms of production volume of all organic dyes worldwide. Compounding the problem of the aforementioned health and environmental problems, there is no international legislation determining discharge parameters for textile effluents. Many countries established their own legal limits, including the US, the European Union, Canada, and Australia; recommended values were set by India, Pakistan, and Malaysia [4, 9, 10]. This adds another concern regarding textile industry, especially when data were compiled from World Trade Organization (WTO) (2018) [11] showed that China, European Union, and India together represented 66.3% of world textile exports in 2017.

According to WTO reports (2018) [11], the top five exporters of textiles in 2017 were China, European Union, India, the US, and Turkey. In just these few countries, legislation produced varying levels of restrictions on textile effluents in water resources. In terms of chemical oxygen demand (COD), the US is more restrictive than India. In terms of color and temperature, legislation in the European Union and Turkey does not set limits as other countries report.

✉ Helen Treichel
helentreichel@gmail.com

[1] Laboratory of Microbiology and Bioprocess, Federal University of Fronteira Sul, Erechim, Brazil

[2] Escola de Química E Alimentos, Universidade Federal de Rio Grande, PO 474, Rio Grande, RS 96201-900, Brazil

[3] Laboratory of Virology, Federal University of Santa Catarina, Florianópolis, Brazil

Some countries are less restrictive than others; for example, in the European Union, it is possible to apply a simple treatment process in textile effluents, sometimes leaving the toxic chemicals behind [3, 12]. In this sense, the advanced oxidation processes (AOP) are increasingly used to treat wastewater, as they are able to remove around 79% of COD. In addition, AOP can occur at any temperature, and pressure does not produce secondary components [1, 3, 13, 14].

The AOP mechanism is to oxidize and destroy components through OH° radical production, processes that are accelerated using combined techniques such as H_2O_2, O_3, TiO_2, UV, electron beam irradiation, and ultrasound. The oxidation rate can be increased using process optimization, to identify the ideal concentration of the radical and the target pollutant [1, 3, 15]. The addition of heme peroxidases (PO) is a possibility in AOPs to treat textile effluents. POs oxidize and reduce various substrates, including dyes, phenolic components, aromatic structures, and lignin fractions, a characteristic that places them in the oxidoreductase group of enzymes [16].

In addition to the POs' ability to interact with several substrates, the enzyme has high stability and thermoresistance, giving them a broad range of applications. The main sources of this biomolecule are microorganisms, animals, and plants; however, the production and extraction costs often become high and unviable for environmental applications. An alternative is to extract them from agroindustry co-products, to obtain non-commercial POs [16, 17]. Marques et al. [18] demonstrated the properties of the enzyme extracted from rice bran in treatment of textile effluent discoloration. Although this process is simple and low cost, it could be improved by removing impurities with rice bran peroxidase (RBP), elevating its specific activity using a second reaction medium to attack the target pollutant.

The present study aimed to develop advanced oxidation processes in several configurations in the presence of rice bran peroxidase, to remove color and toxins from textile wastewater, and to propose to the textile dyeing industry an economically and environmentally viable alternative effluent treatment.

Materials and methods

Fig. 1 shows a schematic diagram of the methodology. Each step is described below.

Enzymatic acquisition and precipitation process

RBP enzymatic extract was donated by the Laboratory of Mycotoxin and Food Science (Federal University of Rio Grande, Carreiros, RS, Brazil), were the enzyme was extracted according to the method of Cardinali et al. [19].

Preliminary tests of the precipitation technique were developed according to Preczeski et al. [20], setting the sodium chloride concentration at 0.8 mol/L, the organic solvent concentration at 80% and the pump flow at 10 mL/min. The precipitates were collected and dissolved in 5 mM sodium phosphate buffer, pH 7.5 [21].

To improve RBP activity, a screening of organic solvents was made with acetone, ethanol, *N*-propyl, isopropyl, iso-butyl, and tert-butyl alcohol in the presence and absence of salt. The specific activity response was analyzed using the Tukey test.

In the sequence, RBP precipitation was improved using a central composite design (CCD) to evaluate the influence of a peristaltic pump flow, varying from 4.6 to 12.4 mL/min, of salt concentration from 0.36 to 0.84 mol/L, and of solvent concentration from 48.1% to 71.9%, in purification factor (PF) results.

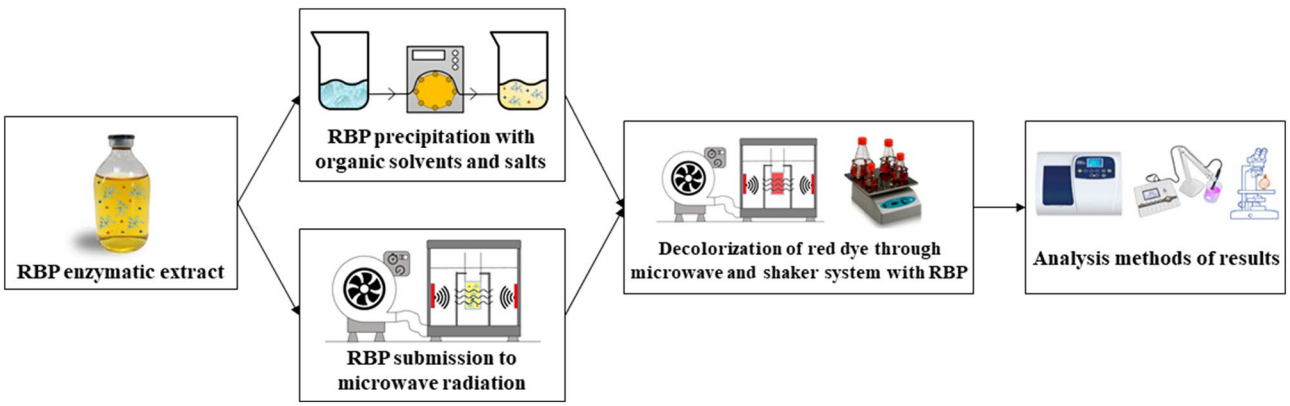


Fig. 1 Schematic diagram of methodology

Subjection of RBP to microwave radiation to improve activity

The first tests with non-commercial RBP began with the comparison between crude enzymatic extract activity (without treatment) and RBP treated in a microwave system. A central composite rotational design (CCRD) was developed to evaluate the influence of time and temperature variables in the microwave system on the enzymatic activity.

The minimum temperature evaluated was 35.8 °C and the maximum was 70 °C. The time of enzyme exposure in the microwaves was between 1 to 30 min. The choice of relatively short exposure times can be justified by the rapidity velocity with which the temperature is reached in this reaction system.

Dye decolorization using RBP

The synthetic effluent for all assays was prepared between 50 mg/L and 150 mg/L concentration, according to Marques et al. [18]. It was composed of various dyes diluted in distilled water.

Preliminary tests of the synthetic effluent color removal with known concentration were conducted in a microwave reaction system to evaluate possible process variants. Red, navy blue, yellow, and brown effluents were all analyzed at 100 mg/L.

The ratio of effluent/RBP/hydrogen peroxide 30% was set at 3:1:1 (mL), varying only the colors of the synthetic effluent, temperature, and microwave exposure time, according to the best results obtained during planning. Longer exposure times of the microwave samples were also evaluated than those conducted in the CCRD, justified by the inclusion of the effluent in the sample that had not yet been evaluated. The pH of the samples before and after microwave exposure was also measured.

After conducting the preliminary tests, only the red synthetic effluent generated some color removal result when exposed to microwaves. A Plackett–Burman (PB) design (Table 1) was elaborated to determine which variables had major influences in the color removal of the analyzed effluent.

For precipitated RBP, the assay chosen for dye decolorization was based on the best result found in CCD. The supernatants and the precipitates were subjected to shaker system separately, using 40 mg/L of hydrogen peroxide and red synthetic effluent 100 mg/L in a final volume of 100 mL, according to Marques et al. [18]. Color removal after treatment with RBP was quantified in a spectrophotometer at 455 nm.

Table 1 Plackett–Burman matrix of experimental design for process variable analysis in microwave system

Code	Process variable	Level		
		(− 1)	(0)	(+ 1)
X_1	RBP amount (mL)	1	1.5	2
X_2	Red dye concentration (mg/L)	50	100	150
X_3	Hydrogen peroxide amount 0.08% (mL)	1	1.5	2
X_4	Exposure time (min)	30	75	120
X_5	Temperature (°C)	30	50	70

Enzymatic activity and PF determination

Enzymatic activity was determined according to Devaiah and Shetty [22] and its units were defined as the mass of protein capable of causing an increase in absorbance at 0.001 per minute [23]. Protein concentrations were measured according to Bradford [24]. We calculated PF values using these parameters.

Toxicity tests

Treated effluents were evaluated for toxicity using meristems cells from *Allium cepa*, according to Levan [25] and Düsman et al. [26]. After 24 h in the dark at room temperature (25 ºC), the toxic effects on cells were evaluated by microscopic analysis using Panótico Rápido® kit in all cell colorations. The mitotic index (MI) was evaluated from the count of approximately 100 cells for each assay, considering raw effluent and dilutions 1:8 and 1:16. A negative control was performed in the presence of ultrapure water.

Data statistical treatment

The Tukey test and analysis of variance (ANOVA) were used to validate data using Statistica and Protimiza Experimental Design software, respectively [27].

Results and discussion

Activity of peroxidase precipitated with organic solvents

Through a screening developed with several solvents in the presence and absence of salt, with the aim of determining which condition had better affinity with RBP, we observed a specific activity of 10,385.5 U/mg when RBP was precipitated with acetone in the presence of salt. This

value was significantly different from the other conditions, according to the Tukey test with 95% of confidence.

The screening had relevant results to continue the study, because it allowed analysis of the influence of salt concentration, solvent concentration (only acetone) and pump flow in PF, through CCD (Table 2).

During the precipitation process, we obtained supernatant (S) and the precipitate (P). The S final volume was greater than the P final volume. Table 2 shows that, in different conditions, the enzyme can be concentrated in both phases of the system. An example of this phenomenon is shown in assay 1, where a PF of 1.9 was obtained in S phase, and assay 11, where a PF of 1.9 was obtained in P phase.

The improvement of PF value of an enzyme has advantages for its applications, because it improves catalytic efficiency, and thermal and pH stability, as well as the affinity for substrates. The method selection used concentrate and purify this biocatalyst must consider the process costs, scalability, yields and reproducibility [20, 28-33].

For precipitation method, the reproducibility is a process requirement that is difficult to achieve. Nevertheless, it has great cost benefits, because recent studies using expensive methods to purify RBP, including chromatography, did not achieve PF greater than 14.1 [21, 33, 34]. It is also important to highlight that applications in environmental areas, such as AOP, do not require high PF values, but rather require high affinity for the target substrate.

The treatment of CCD matrix results was made to obtain contour curves of PF behavior when variations occur in the precipitation process. The statistical analysis indicated that only in the supernatant phase was it possible to validate the model with 95% confidence. Table 3 shows the model equation and the contour curves, where can be observed that lower concentrations of acetone and sodium chloride resulted in better PF values. The minimum pump flow (around 4 mL/min) improved RBP concentration as well. On the large scale, this response would result in an economically viable purification process, because it requires low reagents concentrations and a slow process, because to achieve the best PF results, the flow has to be maintained between 4 to 6 mL/min.

Activity of peroxidase in the microwave

There are many studies of peroxidase inactivation caused by exposure to microwaves; for example, the studies of Soysal and Söylemez [35] and Matsui et al. [36]. There is research emphasizing that this clean, cheap, and convenient reaction system can be effective in increasing peroxidase activity, an extremely important feature in the environmental area, as for treatment of various types of effluents [18, 37-39].

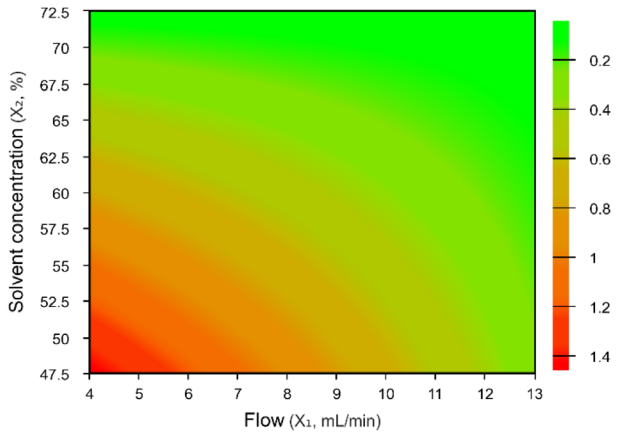
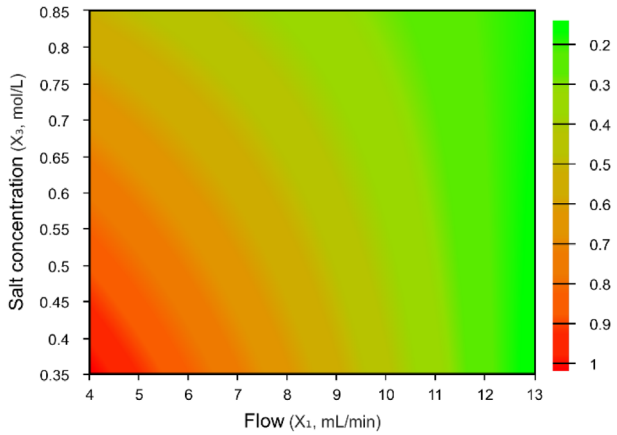
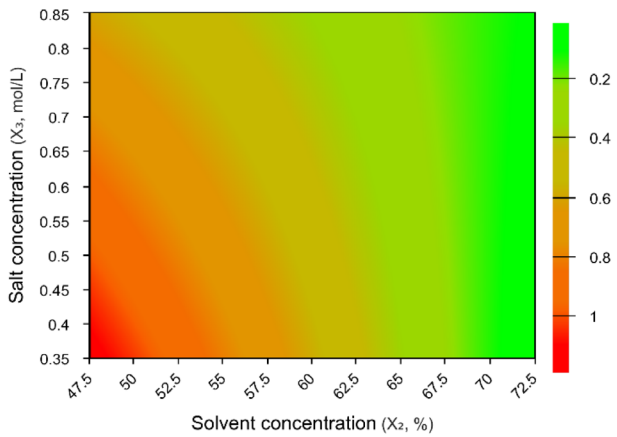
Table 4 presents the coded and real values for CCRD, the time, and temperature variables of the microwave reaction system and the responses in terms of RBP relative activity. The best increase result in enzyme activity was obtained when the exposure time was 15.5 min and the temperature was 50 °C (relative activity of 108.6%). The enzyme activity obtained in sample 1 (5.2 min and 35.8 °C) and in sample 5 (1 min and 50 °C) are also results that should be considered because of the significant increase that occurred in relation to RBP without microwave exposure, where there were activity increments of 102.2% and 88.4.4%, respectively.

The data shown in Table 4 were treated statistically, from which we obtained a coded empirical mathematical model (Eq. 1) that describes the behavior of RBP after treatment in the microwave reaction system:

Table 2 Matrix of central composite design (CCD) with coded and real values and respective answers in terms of purification factor (PF) in supernatant and in precipitate after RBP precipitation

Assay	Flow (mL/min)	Solvent concentration (%)	Salt concentration (mol/L)	PF supernatant	PF precipitate
Crude RBP extract	–	–	–	1.0	1.0
1	− 1 (4.6)	− 1 (48.1)	− 1 (0.36)	1.9	0.9
2	+ 1 (12.4)	− 1 (48.1)	− 1 (0.3s6)	0.4	1.4
3	− 1 (4.6)	+ 1 (71.9)	− 1 (0.36)	0.1	1.0
4	+ 1 (12.4)	+ 1 (71.9)	− 1 (0.36)	0.1	1.1
5	− 1 (4.6)	− 1 (48.1)	+ 1 (0.84)	0.9	0.2
6	+ 1 (12.4)	− 1 (48.1)	+ 1 (0.84)	0.4	0.4
7	− 1 (4.6)	+ 1 (71.9)	+ 1 (0.84)	0.2	1.0
8	+ 1 (12.4)	+ 1 (71.9)	+ 1 (0.84)	0.1	1.1
9	0 (8.5)	0 (60.0)	0 (0.60)	0.4	1.6
10	0 (8.5)	0 (60.0)	0 (0.60)	0.3	1.1
11	0 (8.5)	0 (60.0)	0 (0.60)	0.3	1.9

Table 3 Contour curves with the influence of process variables in PF results

Process variables	Supernatant phase
Solvent concentration versus flow	
Salt concentration versus flow	
Salt concentration versus solvent concentration	
Equation model	$Y_1 = 0.46 - 0.26\,X_1 - 0.39\,X_2 - 0.11\,X_3 + 0.24\,X_1\,X_2 + 0.11\,X_1\,X_3 + 0.14\,X_2\,X_3$
R^2	0.92

Y_1 purification factor (PF), X_1 flow, X_2 solvent concentration, X_3 salt concentration

Table 4 Matrix of experimental design central composite rotatable design (CCRD) 2^2 (coded and real values) and respective answers in terms of enzymatic and relative activity of rice bran peroxidase

Assay	Exposure time (min)	Temperature (°C)	Enzymatic activity (U/mL)	Relative activity (%)**
Control*	–	–	415	0
1	− 1 (5.2)	− 1(35.8)	839	102.2
2	+ 1 (25.8)	− 1(35.8)	603	45.4
3	− 1 (5.2)	+ 1 (64.2)	591	42.3
4	+ 1 (25.8)	+ 1 (64.2)	311	− 25.1
5	− 1.41 (1.0)	0 (50.0)	782	88.4
6	+ 1.41 (30.0)	0 (50.0)	459	10.7
7	0 (15.5)	− 1.41 (30.0)	632	52.4
8	0 (15.5)	+ 1.41 (70.0)	160	− 61.4
9	0 (15.5)	0 (50.0)	754	81.7
10	0 (15.5)	0 (50.0)	743	79.1
11	0 (15.5)	0 (50.0)	866	108.6

*Control refers to crude extract without treatment

**It was calculated taking enzymatic activity from control as a reference

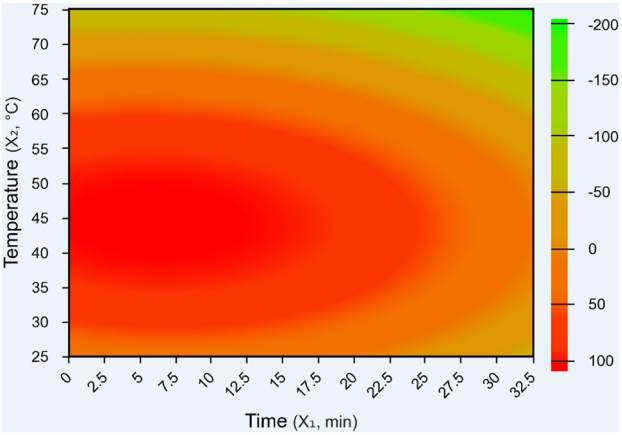


Fig. 2 Contour curves with the time and temperature influence of the microwave system on peroxidase activity

$$\text{Relative activity} = 89.80 - 29.26 \times \text{time} - 15.46 \times \text{time}^2 - 36.42 \times \text{temperature} - 42.48 \times \text{temperature}^2 - 2.65 \times \text{time} \times \text{temperature}. \quad (1)$$

Contour curves (Fig. 2) were constructed from the validation of the model through analysis of variance, in which the calculated f was greater than the f tabled and R^2 was 95.28%.

The greatest increase in enzymatic activity was reached when the enzyme exposure time to microwaves was short and the exposure temperatures were average. The low expenditure of time and energy shows promising results, because this makes the process economically viable.

Golunski et al. [40] obtained similar results when analyzing the enzymatic activity of non-commercial rice bran peroxidase in a domestic microwave. The authors reported relative activity of 107.5% in 10 s of irradiation, equivalent to a temperature of 50 °C and an activity decrease when microwave reaches approximately to 70 °C, arriving at 26% relative activity in relation to the best result. Lopes et al. [41] evaluated the enzymatic activity of peroxidase extracted from horseradish against microwave irradiation and their results were similar to the results obtained in the present study. They suggest that at low temperatures (30–45 °C), changes occur in the secondary structure of the enzyme, causing its activation, and at higher temperatures and longer microwave exposure times, there is a tendency for inactivation.

Synthetic effluent decolorization with microwave system in the presence of RBP

When using peroxidase extracted from rice bran to discolor a synthetic effluent in an orbital shaking system, Marques et al. [18] observed that color removal using peroxidase was influenced by effluent coloration. When analyzing the discoloration of the red and blue dye, they realized that only the red effluent was susceptible to removal, concluding that the RBP had affinity with only this dye. Nevertheless, removal analysis of other colors in the present reaction system was justified by the lack of knowledge of the RBP behavior using effluent as a substrate when subjected to microwave irradiation. The pH range of all the samples before and after the exposure to the microwaves was not altered (pH 5–6). Greater degradability of the red color in our preliminary tests was observed as the exposure times to the microwaves

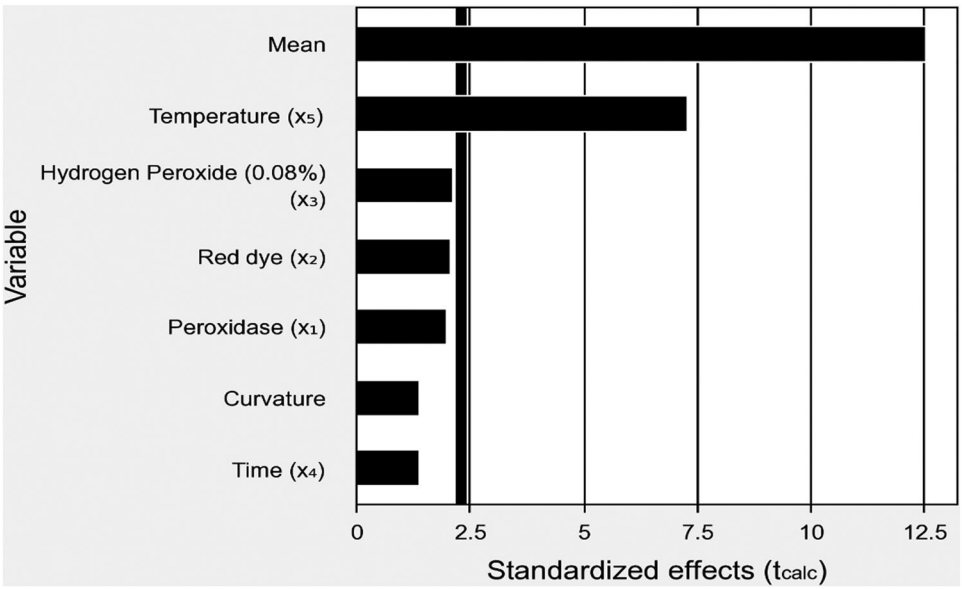


Fig. 3 Pareto graph showing the influence of five variables in red dye removal by RBP in the microwave system

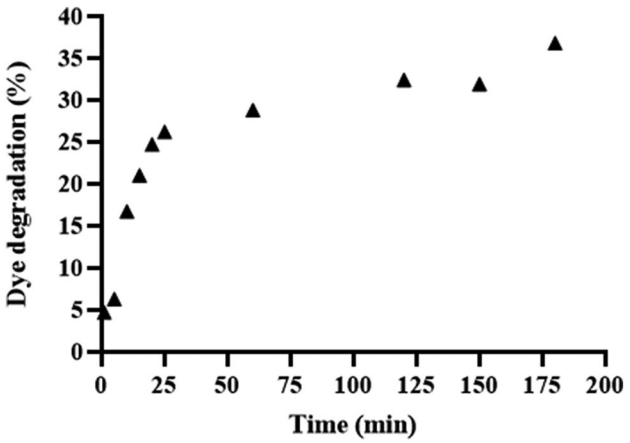


Fig. 4 Color removal kinetics in the microwave system

increased. Exposure times were evaluated up to 120 min, generating a satisfactory color removal result.

Based on the statistical analysis, we found that, among the analyzed variables, only the temperature has a significant influence on dye color removal (Fig. 3). Lopes et al. [41] studied horseradish peroxidase enzyme subjected to conventional and to microwave heating system and identified a temperature influence on enzymatic activity.

According to the PB design, the samples subjected to higher temperature (70 °C) resulted in greater red dye removal, reaching color removal quantities of 38.9%.

By determining that only temperature had a significant effect on color removal, a color removal kinetic (Fig. 4) was performed, setting 70 °C as the temperature, the minimum amounts of enzyme and hydrogen peroxide and the highest dye concentration (150 mg/L) analyzed from the PB, to create the most economically viable process.

The effluent removal kinetics in was microwave for up to 180 min was analyzed, because it is believed that subsequently the process may become economically infeasible in industrial application.

A very promising result found in this study was that the highest rates of removal occurred in the first 25 min, leading to 26.2% removal. After this period, the dye removal rates become lower, reaching 36.8% removal 180 min of analysis. This decrease in the color removal rate can be explained by the decreased availability of substrate. It is important to note that controls in the same temperature (70 °C) were carried out to evaluate the microwaves influence on an effluent sample containing only red dye and another on a sample containing red dye and hydrogen peroxide, both without RBP. In the two controls analyzed, no results were obtained in the dye removal, confirming that the enzyme was responsible for all the discoloration observed in the effluent.

In the literature, there are no studies using microwave systems allied to peroxidase enzyme from any source for effluent color removal; this may be an interesting study in the future. The use of microwave reaction system may further aid color removal, because irradiation may facilitate the access of enzymes to the substrate or may make the process more economically feasible, because the exposure time to reach the optimal temperature in this system is smaller [42].

Synthetic effluent decolorization with RBP precipitated in the shaker system

The results presented in Table 2 display the choice of assay to be used for color removal from effluents. The microwave

Table 5 Results of RBP application in AOP to remove color of red textile effluent in the shaker system at constant temperature (25 °C)

Time in shaker system (h)	Assay		pH	Color removal (%)	PF
5	Control		3.66	0	1
	RBP precipitated	Supernatant phase	4.32	10.59	0.3
		Precipitate phase	6.46	− 68.24	1.9
24	Control		3.04	0	1
	RBP precipitated	Supernatant phase	3.09	100	0.3
		Precipitate phase	3.28	61.06	1.9

system results reported here, as well as in the work of Marques et al. [18] support the choice of color, which was red dye. With this, the results of application of precipitated RBP to achieve effluent decolorization as an advanced oxidation process technique are presented in Table 5, using assay 11 because of its satisfactory PF value and the excellent process conditions, which is the part of central point of the CCD matrix (Table 2).

Color removal was not satisfactory at 5 h of reaction in the shaker system, the time, where best results occurred in Marques et al.'s [18] study. The supernatant phase presented color reduction of only 10.59% in relation of control. In the precipitate phase, there was a color increase (negative value in Table 5) of 68.24%.

Then, the reaction time was increased to 24 h, producing excellent results with 100% of color removal in the supernatant phase and 61.06% in the precipitate phase. Golunski et al. [40] and Marques et al. [18] only applied crude non-commercial peroxidase extracted from rice bran in red dye effluent, and obtained 40% and 48.51% of color removal, respectively. Because the best results of color removal were obtained with lower PF values (supernatant phase), as shown in Table 5, and commercial peroxidases with high purification remove less dye color then the non-commercial RBP precipitated, it is possible to assume a biochemical transformation in the enzyme properties. This causes the biocatalyst to have greater affinity for the target substance because of the precipitation process that can be explained by the presence of salt and organic solvent.

Although the control assay had color removal because of the presence of hydrogen peroxide, the assays with RBP had best color removal results. In the Erlenmeyer flask that contained supernatant phase, with 100% color removal, it was possible to observe dye particle decantation. On the large scale, this gives an advantage to the treatment process, because the effluent could be treated, decanted, and filtered to remove all dye particles.

Therefore, the RBP precipitation process and application in color removal demonstrate potential to be an AOP in textile dyeing industries, with low-cost and flexibility advantages, because both precipitate and supernatant phases could be used with satisfactory color removal results.

Effluent toxicity after RBP treatment in microwave and shaker systems

Based on the analysis of the Fig. 5(I), using the best synthetic effluent removal assay in the microwave reaction system, two cytotoxic events were observed: induction and inhibition of the mitotic index in relation to the control sample. Table 6 displays these data, demonstrating that, for both effluent conditions (treated and untreated), there were toxic effects. The analysis performed with precipitate phase removal tests was performed on a shaker system and presented similar toxicity characteristics. These similarities include induction of cell proliferation and damage to the genetic material.

Based on these aspects, effluent dilution tests were evaluated, simulating effluent discharge in water bodies. Using the microwave system, 1:8 dilutions were shown to be effective as they approached normal conditions tested by the control sample, whereas for the treatment using orbital shaking, larger dilution was required to regulate the deleterious effects of toxicity. For all assays, samples were examined for abnormalities perceived during mitotic division (Fig. 5).

Regarding the treatment process with microwave and sequence analysis (Fig. 5(I)), we found (1) the regular state of cell division through the chromosomes at the end of the anaphase, without any incoordination or delays, imitating normal developmental conditions; however (2) the raw (untreated) effluent samples presented higher frequency of division in relation to the control group (Table 6), suggesting that the synthetic effluent at 150 mg/L reproduced a cytotoxic effect on meristematic cells. According to Leme and Marín-Morales [43], mitotic indexes higher than the negative control may be harmful to cells, leading to the formation of tumors via disordered cell proliferation.

In the case of the effluent enzymatically treated in microwaves [Fig. 5(I) image (d)], a predominance of cellular abnormalities was observed, with the marked presence of micronuclei associated with delayed cell division. Here, the low mitotic index confirmed the retardation of cell division (Table 6). According to Fernandes et al. [44], changes such

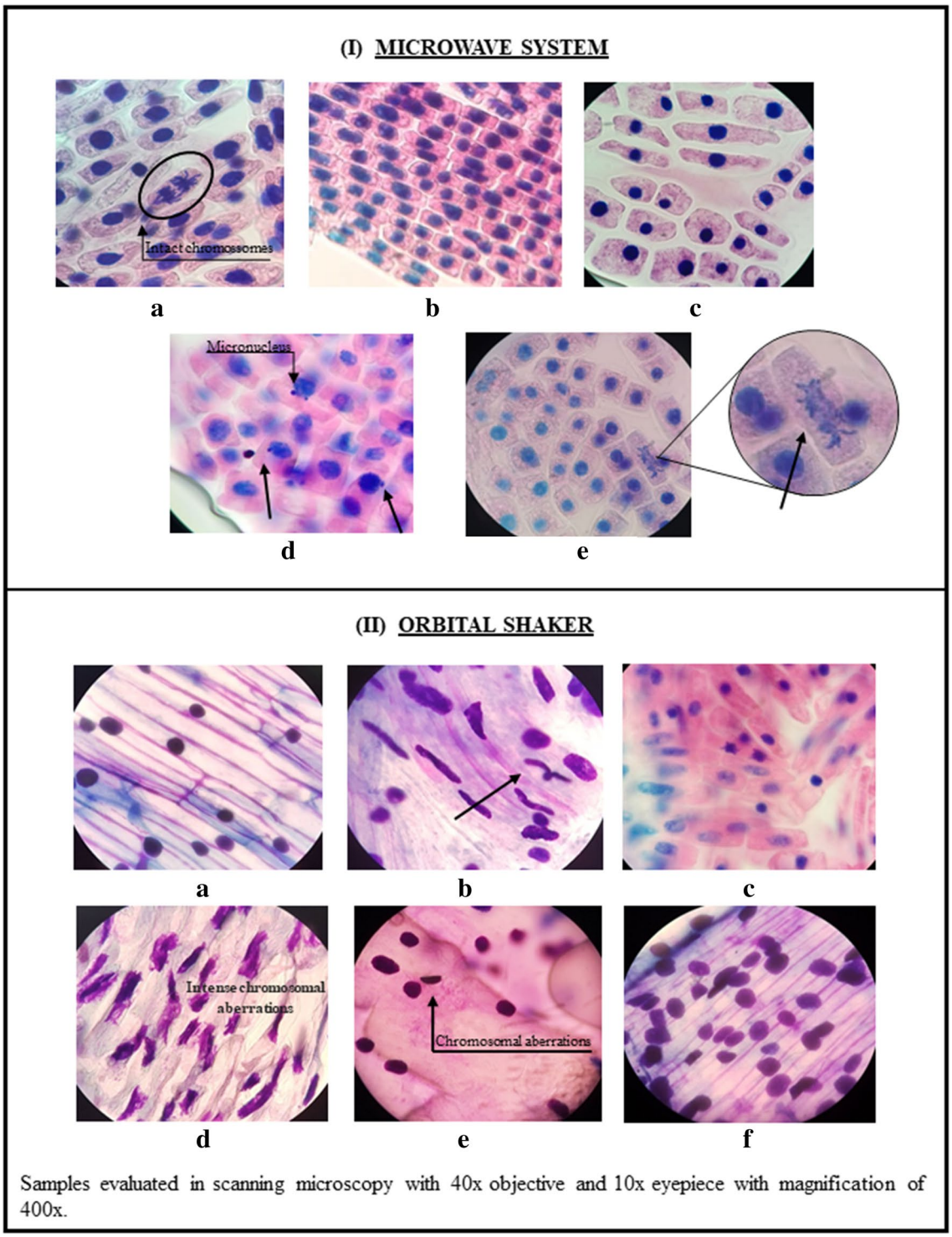


Fig. 5 Evaluation of cytotoxic and genotoxic effects in samples of synthetic textile effluent treated and not treated enzymatically in the microwave reaction system **(I),** where **a** control system in distilled water, **b** untreated raw effluent, **c** raw untreated effluent at 1:8 dilution, **d** effluent treated in microwave reaction system in RBP presence, **e** effluent treated in microwave reaction system in RBP presence at 1:8 dilution and in orbital agitation **(II),** where **a** control system in distilled water, **b** control of crude effluent with hydrogen peroxide, **c** untreated raw effluent, **d** effluent treated in shaker system in the presence of precipitated RBP with respective dilutions 1:8 (**e**) and 1:16 (**f**)

Table 6 Mitotic index (MI) data on apical meristems of *Allium cepa* in samples of effluent treated and not treated with RBP in microwave and orbital shaker systems

Microwave system		Orbital shaker	
Assay	MI (%)	Assay	MI (%)
Control	7.32 ± 2.49	Control	12.94 ± 2.68
Raw effluent	11.92 ± 0.02	Raw effluent	21.33 ± 2.31
Raw effluent (1:8)*	7.44 ± 0.65	Treated effluent	–
Treated effluent	3.82 ± 1.67	Treated effluent (1:8)*	29.04 ± 2.93
Treated effluent (1:8)*	6.49 ± 2.60	Treated effluent (1:16)*	15.86 ± 2.81
		Effluent with peroxide	14.00 ± 3.00

*Dilution factor

as micronuclei can originate spontaneously; however, their induction is often understood when genetic damages are detected from exposure to a mutagenic agent [45].

It is also interesting to note in this study that the treatment test performed on an orbital shaker [Fig. 5(II) image (d)] revealed no formation of micronuclei during division; however, there was intense aggressiveness in the genetic material, completely disrupting the nuclear structure and being impossible to calculate mitotic indices.

On this basis, we believe that the two processes involving different reaction systems differentially influence toxicity levels after removal due to the interaction with the enzymatic treatment process. Nevertheless, the validation of this hypothesis would require more in-depth studies on the interaction mechanisms, seeking ways to reduce post-treatment impacts. The results also suggest that metabolites produced by the enzyme during the removal process may have contributed to the genotoxic effects. Solís et al. [46] reported that metabolites produced during the removal of the dye are, in many cases, more toxic than the parent dye.

Tafurt-Cardona et al. [47] believe that genotoxic effects may be related to azo structure present in the dye composition, suggesting that such effluents pose dangers to human health. A number of studies have reported increased toxicity levels after effluent biodegradation in the presence of dyes [18, 48-50]. Punzi et al. [51] suggested the use of chemical processes complementary to the biological ones to reduce the levels of toxicity of textile industry effluents and to guarantee greater safety in the final disposal. According to Bilal et al. [52], it is expected that undetectable transformation products, called "micro-pollutants" of toxicity, and persistence that are as yet unknown exist in the final product of various types of removal reactions.

Considering the great toxic potential caused by removal, dilution factors were employed on similar discharge action in water resources. With the use of microwaves in the treatment process, 1:8 dilution contributed to decreased toxic effects, whereas for the orbital agitation system, an even greater factor was necessary to approach normal conditions, because in the 1:8 dilution, traces of toxicity and damage of genetic material were still observed.

Nevertheless, this assumption is lacking in studies, because it is not yet possible to ensure that small remnants of recalcitrant toxic substances remaining after enzymatic treatment do not impair the metabolism and way of life of aquatic organisms. Alternative treatments, such as AOP, as opposed to so-called traditional methods, using an enzyme originated from cheap sources as by-products, have been shown to be a compelling strategy for the treatment of synthetic effluents. In the present study, this enzyme catalyzed oxidative processes in the presence of toxic compounds and resulted in substantial color removal.

Conclusion

The advanced oxidation processes developed in this study showed great potential for the textile market as a low-cost, scalable, and efficient method to treat effluents.

In the shaker system, it was possible to remove 100% of effluent color after treatment with the non-commercial rice bran peroxidase that had been precipitated with acetone and salt. This condition can decant and remove residual dyes particles through filtration.

Despite the fact that, in both systems, effluent toxicity damaged genetic material of *Allium cepa*, dilution reduced these damages, simulating industrial discharge action in water resources. Therefore, both treatment systems appear to have potential to be exploited in industrial processes, because rice bran peroxidase demonstrated affinity with dyes.

Acknowledgements CAPES, CAPES-PNPD, CNPq, and FAPERGS for the financial support of this study.

Compliance with ethical standards

Conflict of interest The authors report no interest conflict.

References

1. Eswaramoorthi S, Dhanapal K, Chauhan D (2008) Advanced in textile waste water treatment: the case for UV-ozonation and membrane bioreactor for common effluent treatment plants in Tirupur, Tamil Nadu India. Environment with people's involvement & co-ordination in India, Coimbatore
2. Ntuli, F., D. Ikhu-Omoregbe, P. K. Kuipa, E. Muzenda, and M. Belaid (2009) Characterization of Effluent from Textile Wet Finishing Operations. *WCECS* v1.
3. Ghaly AE, Ananthashankar R, Alhattab M, Ramakrishnan VV (2014) Production, characterization and treatment of textile effluents: a critical review. J Chem Ecol 5:1000182
4. Yusuf M (2018) Handbook of textile effluent remediation, 1st edn. Routledge, Abingdon
5. Reife A, Freeman H (1996) Environmental chemistry of dyes and pigments, 1st edn. Wiley, Hoboken
6. Greijer H, Karlson L, Lindquist S, Hagfeldt A (2001) Environmental aspect of electricity generation from a nanocrystalline dye sensitized solar cell system. Renew Energ 23:27–39
7. Nese T, Sivri N, Toroz I (2007) Pollutants of textile industry wastewater and assessment of its discharge limits by water quality standards. Turk J Fish Aquat Sci 7:97–103
8. Wamburguh D, Chianelli RR (2008) Indigo dye waste recovery from blue denim textile effluent: a by-product synergy approach. New J Chem 32:2189–2194
9. Bafana A, Devi SS, Chakrabarti T (2011) Azo dyes: past, present and the future. Environ Rev 19:350–371
10. Yusuf M, Shabbir M, Mohammad F (2017) Natural colorants: historical, processing and sustainable prospects. Nat Prod Bioprospect 7:123–145
11. WTO (2018) World trade statistical review 2018. World Trade Organization, Geneva
12. ZDHC (2016) Textile Industry Wastewater Discharge Quality Standards: Literature Review. Zero Discharge of Hazardous Chemicals Programme, Leaders in Advancing Environmental Responsibility.
13. Rodríguez M (2003) Fenton and UV-vis Based Advanced Oxidation Processes in Wastewater Treatment: Degradation, Mineralization and Biodegradability Enhancement. University of Barcelona, Barcelona
14. Montaño JG (2007) Combination of advanced oxidation processes and biological treatment for commercial reactive azo dyes removal. University of Barcelona, Barcelona
15. Al-Kdasi A, Idris A, Saed K, Guan CT (2004) Treatment of textile wastewater by advanced oxidation processes—a review. Global Nest Int J 6:222–230
16. Medina JDC, Woiciechowski AL, Guimarães LRC, Karp SG, Soccol CR (2017) Peroxidases. In: Pandey A, Negi S, Soccol C (eds) Current developments in biotechnology and bioengineering: production isolation and purification of industrial products. Elsevier, Amsterdam, pp 217–232
17. Battistuzzi G, Bellei M, Bortolotti CA, Sola M (2010) Redox properties of heme peroxidases. Arch Biochem Biophys 500:21–36
18. Marques CT, Golunski SM, Venturin B, Camargo AF, Scapini T, Baldissarelli D, Modkovski TA, Preczeski KP, Dalla Rosa C, Vargas GDLP, Buffon JG, Rosin CK, Arenzon A, Mossi AJ, Treichel H (2017) Removal of a red dye using noncommercial peroxidase extracted from rice bran. EQM 27:25–31
19. Cardinali A, Tursi N, Ligoro A, Giuffrida MG, Napolitano L, Caliandro R, Sergio L, Di Venere D, Lattanzio V, Sonnante G (2011) Purification, biochemical characterization and cloning of a new cationic peroxidase isoenzyme from artichoke. Plant Physiol Biochem 3:395–403
20. Preczeski KP, Kamanski AB, Scapini T, Camargo AF, Modkovski TA, Rossetto V, Venturin B, Mulinari J, Golunski SM, Mossi AJ, Treichel H (2018) Efficient and low-cost alternative of lipase concentration aiming at the application in the treatment of waste cooking oils. Bioprocess Biosyst Eng 41:851–857
21. Feltrin ACP, Garcia SO, Caldas SS, Primel EG, Badiale-Furlong E, Garda-Buffon J (2017) Characterization and application of the enzyme peroxidase to the degradation of the mycotoxin DON. J Environ Sci Health B 52(10):777–783
22. Devaiah SP, Shetty HS (2009) Purification of an infection-related acidic peroxidase from pearl millet seedlings. Pest Biochem Physiol 94:119–126
23. Garda-Buffon J, Badiale-Furlong E (2010) Kinetics deoxynivalenol degradation by *Aspergillus oryzae* and *Rhizopus oryzae* in submerged fermentation. J Braz Chem Soc 21:710–714
24. Bradford MMA (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
25. Levan A (1938) The effect of colchicine on root mitoses in Allium. Hereditas 24:471–486
26. Düsman E, Luzza M, Savegnago L, Lauxen D (2014) *Allium cepa L.* as a bioindicator to measure cytotoxicity of surface water of the Quatorze River, located in Francisco Beltrão, Paraná Brazil. Environ Monit Assess 186:1793–1800
27. Rodrigues MI, Iemma AF (2014) Experimental design and process optimization, 1st edn. CRC Press, Florida
28. Rudrappa T, Lakshmanan V, Kaunain R, Singara NM, Neelwame B (2007) Purification and characterization of an intracellular peroxidase from genetically transformed roots of red beet (*Beta vulgaris* L.). Food Chem 105:1312–1320
29. Boucherit N, Abouseoud M, Adour L (2013) Degradation of direct azo dye by Cucurbita pepo free and immobilized peroxidase. J Environ Sci 25:1235–1244
30. Nadaroglu H, Celebi N, Demir N, Demir Y (2013) Purification and characterisation of a plant peroxidase from rocket (*Eruca vesicaria* sbsp. Sativa) (Mill.) (syn. E. sativa) and effects of some chemicals on peroxidase activity *in vitro*. Afr J Agric Res 8:2520–2528
31. Zeng G, Zhao M, Huang D, Lai C, Hurang C, Wei Z, Xu P, Li N, Zhang C, Li F, Cheng M (2013) Purification and biochemical characterization of two extracellular peroxidases from *Phanerochaete chrysosporium* responsible for lignin biodegradation. Int Biodeterior Biodegrad 86:166
32. Cunha AG, Besteti M, Manoel EA, Silva AAT, Almeida RV, Simas AB, Fernandez-Lafuente R, Pinto JC (2014) Preparation of core–shell polymer supports to immobilize lipase B from *Candida antarctica* effect of the support nature on catalytic properties. J Mol Catal B-Enzym 100:59–67
33. Gautério GV, Fernandes SS, Molon FO, Figueira FS, Buffon JG, Kalil SJ (2015) Purification of peroxidase from rice bran using expanded-bed ion-exchange chromatography. Adsorpt Sci Technol 33:153–164
34. Gautério GV, Malta DS, Reginatto L, Feltrin ACP, Garda-Buffon J, Kalil SJ (2017) Use of partially purified peroxidase of agricultural by-product rice bran in deoxynivalenol reduction. J Chem Technol Biotechnol 92:1998–2008
35. Soysal C, Söylemez Z (2005) Kinetics and inactivation of carrot peroxidase by heat treatment. J Food Eng 68:349–356
36. Matsui KN, Granado LM, de Oliveira PV, Tadini CC (2007) Peroxidase and polyphenol oxidase thermal inactivation by microwaves in green coconut water simulated solutions. LWT Food Sci Technol 40:852–859
37. Cooper VA, Nicell JA (1996) Removal of phenols from a foundry wastewater using horseradish peroxidase. Water Res 30:54–64

38. Wagner M, Nicell JA (2001) Peroxidase-catalyzed removal of phenols from a petroleum refinery wastewater. Water Sci Technol 43:253–260
39. Darwesh OM, Matter IA, Eida MF (2019) Development of peroxidase enzyme immobilized magnetic nanoparticles for bioremediation of textile wastewater dye. J Environ Chem Eng 7:1028–1035
40. Golunski SM, Scapini T, Modkovski TA, Marques CT, Camargo AF, Preczeski KP, Dalla Rosa C, Baldissarelli DP, Mulinari J, Venturin B, Vargas GDLP, Buffon JG, Mossi AJ, Treichel H (2017) Commercial and noncommercial peroxidases activity under ultrasound and microwave treatment: a pretreatment to improve wastewater treatment. J Braz Chem 28:1890–1895
41. Lopes LC, Barreto MTM, Gonçalves KC, Alvarez HM, Heredia MF, de Souza ROMA, Cordeiro Y, Dariva C, Fricks AT (2015) Stability and structural changes of horseradish peroxidase: microwave versus conventional heating treatment. Enzyme Microb Technol 69:10–18
42. Chiong T, Lau SY, Lek ZH, Koh BY, Danquah MK (2016) Enzymatic treatment of methyl orange dye in synthetic wastewater by plant-based peroxidase enzymes. J Environ Chem Eng 4:2500–2509
43. Leme DM, Marín-Morales MA (2009) *Allium cepa* test in environmental monitoring: a review on its application. Mutat Res 682:71–81
44. Fernandes TCC, Mazzeo DEC, Marin-Morales MA (2007) Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pest Biochem Physiol 88:252–259
45. Heddle JA, Hite M, Irkhart B, Macgregor JTE, Salamone MF (1983) The induction of micronuclei as a measure of genotoxicity a measure of the US environmental protection agency gene-tox program. Mutat Res 123:61–118
46. Solís M, Solís A, Pérez HI, Manjarrez N, Flores M (2012) Microbial decolouration of azo dyes: a review. Process Biochem 47:1723–1748
47. Tafurt-Cardona Y, Suares-Rocha P, Fernandes TCC, Marin-Morales MA (2015) Cytotoxic and genotoxic effects of two hair dyes used in the formulation of black color. Food Chem Toxicol 86:9–15
48. Wong PK, Yuen PY (1998) Decolourization and biodegradation of N N_-dimethylpphenylenediamine by Klebsiella pneumoniae RS-13 and Acetobacter liquefaciens S-1. J Appl Microbiol 85:79–87
49. Mansour HB, Ayed-Ajmi Y, Mosrati R, Corroler D, Ghedira K, Barillier D, Chekir-Ghedira L (2010) Acid violet 7 and its biodegradation products induce chromosome aberrations, lipid peroxidation and cholinesterase inhibition in mouse bone marrow. Environ Sci Pollut Res Int 17:1371–1378
50. Ayed L, Mahdhi A, Cheref A, Bakhrouf A (2011) Decolorization and degradation of azo dye Methyl Red by an isolated Sphingomonas paucimobilis: biotoxicity and metabolites characterization. Desalination 274:272–277
51. Punzi M, Nilsson F, Anbalagan A, Svensson B-M, Jönsson K, Mattiasson B, Jonstrup M (2015) Combined anaerobic–ozonation process for treatment of textile wastewater: Removal of acute toxicity and mutagenicity. J Hazard Mater 292:52–60
52. Bilal M, Rasheed T, Iqbal HMN, Yan Y (2018) Peroxidases-assisted removal of environmentally-related hazardous pollutants with reference to the reaction mechanisms of industrial dyes. Sci Total Environm 644:1–13