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Research Paper

Biological degradation of Reactive Black 5 dye by yeast *Trichosporon akiyoshidainum*

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ABSTRACT

The textile dyeing and other industries use an extensive amount of azo dyes. Their effluents are specifically colored and could cause severe damage to the environment. The anaerobic treatment of textile dying effluents could generate carcinogenic aromatic amines. For this reason, in the recent years yeasts have become a promising alternative, combining unicellular growth with oxidative mechanisms. This work reports the oxidative Reactive Black 5 (RB5) biodegradation mechanism by *Trichosporon akiyoshidainum* HP 2023, isolated from a non-contaminated environment and extensively studied for its exceptional decoloration abilities on azo dyes. Several analytical techniques (HPLC, FTIR, GC–MS, UV–vis) were used as to monitor the dye-decoloration process and the enzyme produced during biodecoloration. Starting with 200 mg L^{-1} of RB5, at 12 h, 89% color removal and a shift from dark blue to purple was observed, at 24 h no color was visible. Also, a decrease of aromatic amines and total aromacity (71 and 75%, respectively) was observed and biomass presented no color. The mechanism is driven by phenol oxidase and peroxidase enzymes, as they were no present in cultures without dye. During decoloration, at 15 h both enzymes reached it maximum activity levels, 353 UL^{-1} for phenol oxidase and 2750 UL^{-1} for peroxidase. The two-stages proposed mechanism involves the formation of a purple-colored quinone with an azo bond, which is subsequently degraded, finally the complete disappearance of color is achieved. These results make *Trichosporon akiyoshidainum* HP 2023 a promising tool for dye removal treatment of colored textile effluents.

1. Introduction

The textile industry is one of the largest consumers of water per kg of material produced and due to the nature of the process, contributes significantly to environment pollution. It is estimated that over 10,000 different dyes and pigments are used industrially and over 7×10^5 tons of synthetic dyes are annually produced worldwide [1]. During processing in these plants up to 200.000 tons of the applied dye may be lost per year, becoming a part of its wastewater and being finally released into the environment [2,3].

Many dyes are visible in water at concentrations as low as 5 mg L^{-1} [4]. Therefore, industrial effluents, with residual concentrations between 10 and 200 mg L^{-1} are highly colored and aesthetically unpleasant [5,6]. Dyes are designed to be stable to light; chemicals and washing conditions, therefore, if not properly treated can remain in the environment for long periods, with the consequent risk to bioaccumulate or biomagnify in the food chain. One example is dye Reactive Blue 19, whose half-life is 46 years at pH 7 and 25 °C [7]. Furthermore, the presence of color reduces the penetration of light into water bodies, changing or decreasing the photosynthetic activity [8]. Resuming, contamination by textile dyes not only affects the aesthetic quality and transparency of the water, but also the concentration of dissolved oxygen in lakes, rivers and other bodies of water, leading to the deterioration of the environment [6,9,10].

Methods for reducing the biochemical oxygen demand (BOD) of most effluents are well established. However, color removal from wastewater is more complicated since dyes may possess complex substituents or aromatic structures not found in naturally occurring compounds, thus being considered xenobiotics and extremely recalcitrant compounds [11].

Several physical, chemical and biological processes, with varying success, are employed in the removal of dyes from textile effluents [12]. Such processes depend on a variety of factors, including dye structure, effluent composition, concentration, and the cost of the chemical reagents used, operating costs (energy and material) and the disposal of the waste generated. As a rule, each technique has its limitations and implementing more than one for complete effluent decoloration is usually required [13].

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Despite the existence of physical or chemical processes for the removal of dyes, bioremediation is an eco-friendly and socially accepted process which remains as an attractive technique because of its low cost, [14]. Several purified enzymes (laccases, peroxidises, lipases, oxidases) usually degrade contaminants. However, whole microorganisms offer certain advantages over purified enzymes. Among these advantages is the reduction of costs by not having to purify the enzymes, the greater stability of involved enzymes and the possibility of using complete and complex enzymatic pathways, hardly reconstructed with purified enzymes [15].

Azo dye decolorization by bacteria usually involves azo bond reduction by unspecific or azo reductase-mediated mechanisms in anaerobic condition that lead to the production of aromatic amines. Subsequently, these intermediate metabolites are degraded in aerobic conditions [16].

White rot fungi (WRF) degrade lignin trough highly oxidative oxidases and peroxidases. Such enzymes have been employed to degrade azo dyes, without aromatic amines production. Thus, WRFs can be used in dyes bioremediation [17]. These fungi are sometimes poorly adapted for the treatment of effluents due to the generation of large amounts of sludge, the aging of mycelia and the risks of bacterial contamination in non-axenic cultures. Yeasts by contrast, have a faster unicellular growth and support more stringent environmental conditions, such as acidic pH and higher salt concentrations [18].

Yeast-mediated biodecoloration occurs by two processes, either individually or together depending on environmental conditions, these are: biosorption or biodegradation.

Biosorption involves the union of the solute to the cell biomass in a process that involves energy consumption. This may occur both in dead or living cells. The dye-biomass binding is primarily by union of nitrogen from the dye molecule to peptidoglycan or proteins groups in the cell wall. It may also be by union to other active groups in the cell surface, such as acidic polysaccharides, lipids, amino acids, and other cellular components. Biosorption depends primarily on environmental factors such as pH, initial dye concentration and temperature [19].

In the biodegradation process, there are several changes in the molecular structure of the dye until total mineralization of the dye molecule into simpler substances such as CO_2, H_2O or CH_4. When the compound is not completely mineralized, the process is called biotransformation. Yeast biotransformation or biodegradation of azo dyes is made by enzymatic mechanisms, either by reduction or oxidation mechanisms. As described for bacteria, reductive reactions lead to rupture of the azo dye link with consequent formation of aromatic amines that could be further metabolized by the yeast. The enzymes involved in these processes are NADH-dependent reductases and azoreductases, which depend on the redox activity of an extracellular plasma membrane system identified as a ferric reductase [20,21]. The oxidative cleavage of azo dyes by yeasts is comparatively poorly understood. Several works described the participation of ligninolytic enzymes such as, manganese peroxidases or lignin peroxidases, but the evidence remains circumstantial and up to date no MnP or LiP from yeast has been described.

The aim of this work was to study the mechanisms involved in azo dye Reactive Black 5 bioremediation mediated by yeast *Trichosporon akiyoshidainum* HP 2023, a fast dye-decolourizing yeast previously isolated from the Las Yungas rainforest (Tucumán, Argentina) [1,22–24]. Previous works with this dye decolorizing yeast were related with media and operation conditions optimization [25,26].

2. Materials and methods

2.1. Yeast

Yeast *T. akiyoshidainum* HP-2023, isolated from 'Las Yungas' and selected by its decolorization potential [23], is currently maintained in the American Type Culture Collection as accession number ATCC MYA-4129 and in the Central Bureau Voor Schimmelcultures as accession number CBS 10550. For routine work at the laboratory scale, the selected yeast is maintained on NDM agar slants [27] at 4 °C and subcultured at 15 days intervals.

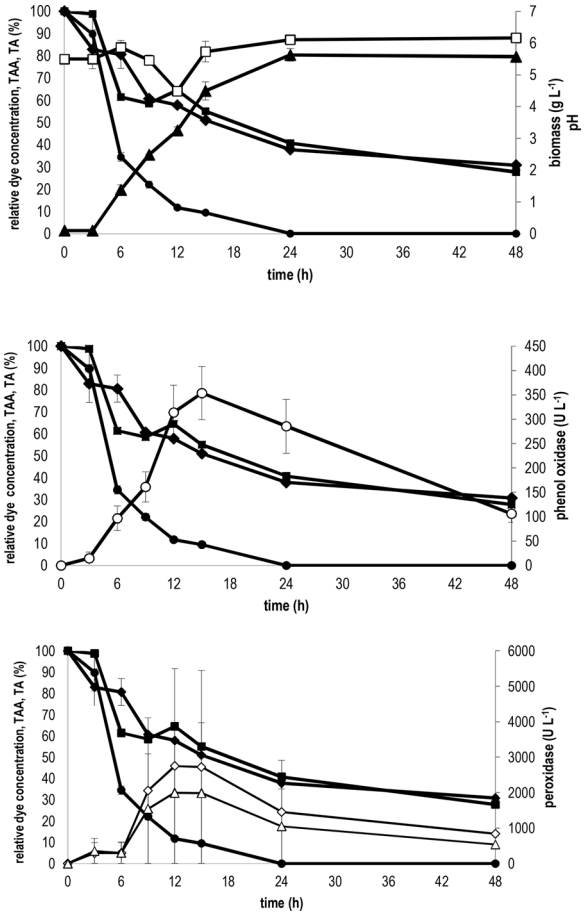

Fig 1. Biomass (▲), pH (□), dye removal (●), total aromaticity (■), total aromatic amines (♦), phenol oxidase (○), Mn-dependent peroxidase (◊) and Mn-independent peroxidase (Δ) during culture of *T. akiyoshidainum* HP 2023 in NDM_{opt} with 200 mg L^{-1} of Reactive Black 5 at 25 °C and 250 rpm.

2.2. Medium and dyestuff

Unless otherwise stated, Optimized Normal decolorization medium (NDMopt.; in g L^{-1}: lactose, 10; yeast extract, 1; urea, 0,5; KH_2PO_4, 1; and $MgSO_4{\cdot}7H_2O$, 1) was employed [26].

Azo dye Reactive Black 5 (Fig. 1) was kindly provided by Vilmax S.A. Stock solutions were prepared by dissolving powdered dyestuff, without prior purification, in distilled water up to a concentration of 2 g L^{-1} and filter-sterilized (Millipore filter, 0.22 mm; Millipore Corp., Bedford, USA).

2.3. Growth and decolorization in liquid cultures

Decolorization kinetics were evaluated in 500 mL-Erlenmeyer flasks containing 100 mL of $NDM_{opt.}$ medium plus Reactive Black 5 at a final concentration of 200 mg L^{-1}. 10-ml yeast suspension (OD_{550} = 0.8), prepared from a 24 h old $NDM_{opt.}$ broth culture were used to inoculate the flasks. Incubations were carried out at 25 °C and 250 rpm for 24 h. Biotic and abiotic controls were performed in all the experiments. Samples were aseptically collected at different time intervals and centrifuged for 10 min at 10000 rpm. Pellets were washed twice with sterile water and dried at 80 °C to constant weight for biomass dry weight and biomass color determination. Supernatants were kept for

estimating dye removal, pH, enzymatic activities and decolorization metabolites [25].

2.4. Dye monitoring, total aromatic amines and total aromaticity determinations

Dye decolorization was determined by using culture supernatants obtained as above described. Color removal was calculated at Reactive Black 5 λ_{opt} (595 nm) as percent decolorization, as it follows:

$$\% = (A_0 - A_t)/A_0 \times 100$$

where A_0 and A_t were the absorbance of dye-amended medium at the start point (0) and at a cultivation time (t), respectively [28].

Total aromatic amines were determined according to Pajot et al. [23] and total aromaticity at 310 nm, according to Lucas et al. [29].

2.5. Enzymes assays

Colorimetric enzymatic assays were performed at 25 °C. All enzymatic activities were determined by using a Beckman Coulter AD200 ELISA reader, following a miniaturized procedure that was setup with a 300-µl-microwell plate. Mn-dependent peroxidase (MnP) experiments were based on the oxidation of MBTH/DMAB (3-methyl-2-benzothiazolinone hydrazone/3-dimethylamino benzoic acid) followed by the increase in absorbance at 610 nm in a reaction mixture containing 0.07 MBTH, 1 mM DMAB, 0.3 mM $MnSO_4{\cdot}7H_2O$, 0.05 mM H_2O_2 in 100 mM succinate-lactate buffer (pH 4.5) [30].

Phenol oxidase (POX) measurements were based on several phenolic compounds oxidation followed by the increase in absorbance at 420 nm in a reaction mixture containing 10 mM of each compound individually (phenol, cathecol, guaiacol, pyrogalol, naphtol, phloroglucinol, tyrosine, resorcinol) and 0.07 nM MBTH in 50 mM phosphate buffer (pH 6.0) [31]. DCIP reductase was measured according to Salokhe and Govindwar [32] at 590 nm in a reaction mixture that contained DCIP (2,6-Dichlorophenolindophenol) 50 µM, NADH 50 µM in 50 mM phosphate buffer (pH 7.4). Azoreductase was evaluated according to Ramalho et al. [20] in both supernatants and cells suspensions, reaction mixture contained glucose 50 mM and Reactive Black 5 20 mg L^{-1} in 50 mM acetate buffer (pH 4.0). Dye removal was monitored at 595 nm.

2.6. Dye degradation analysis

Supernatants, previously filtered, were analyzed using several analytical techniques.

- *UV–vis analysis*

A scan in the UV–vis spectrum between 200 and 800 nm was made (Beckman DU640 UV-VIS).

- *HPLC-GPC analysis*

Separation was achieved in an Ultrahydrogel™ 250 column (exclusion limit 1–80 kDa) in series with an Ultrahydrogel™ 120 column (exclusion limit 0.2–5 kDa), both columns being packed with hydroxylated polymetacrylated-based gel. A guard column of the same material was also used. Deionised water (Millipore Q system) was used as mobile phase at a flow rate of 0.7 mL/min at 30 °C. Identification was achieved by using a Waters 996 Photodiode Array Detector, coupled in series with a refractive index detector [33].

- *FT-IR analysis*

Supernatants were also analyzed by FT-IR using a Perkin Elmer GX1, between 400 and 4000 cm^{-1}, with a 4 cm^{-1} resolution. Sixteen scans per sample were taken and an average was used for analysis.

- *GC–MS analysis*

For the GC–MS analysis, a 2-step ethyl acetate extractions was done in each supernatant. Briefly, in the first step samples were acidified to pH 1.5 using 0.5 N HCl before extraction, in the second step after the first extraction, the aqueous fraction was alkalinized to pH 9 with 6.5% NaOH and then the second extraction was performed. Both extractions, acid and basic, were treated with Na_2SO_4. Once dried, samples were analyzed using an Agilent Technologies 6890 GC with and Agilent Technologies 5973 MS detector. A 30 m long, 0.25 mm inner diameter and 0.25 mm film thickness Agilent JandW HP-5MS (phenyl methyl siloxane) column was used. Run conditions were: injector temperature: 280 °C, injection mode: splitless, injection volume: 1 mL, carrier gas: helium, run mode: constant flux at 1 mL min^{-1}, detector temperature: 280 °C, and mass scan: 40–500 amu.

3. Results and discussion

3.1. Growth and reactive black 5 decolorization in liquid media

Growth, RB5 decolorization, pH and reduction of total aromatic amines and total aromaticity are shown in Fig. 1a. It can be noted that decolorization happened during exponential growth, reaching complete removal of color at 24 h, as yeast growth entered stationary face. This reduction in color was coupled with a decrease in the concentration of aromatic amines and total aromacity (71 and 75%, respectively) .Also, pH values remained near neutrality and biomass presented no color.

DCIP-reductase and azo-reductase activities were not detected in any of the media and conditions assayed.

As previously reported [34], Mn (dependent or independent) peroxidase (MnP and MnInP) and phenol oxidase (Pox) (Fig. 1b, c) were detected during dye decolorization. Phenol oxidase activity against phenol, catechol, naphtol, resorcinol, tyrosine and guaiacol was detected after 12 h regardless MBTH presence. No Pyrogallol or phloroglusinol oxidative activity was detected during decolorization assays.

During decolorization of RB5, MnP activity showed a progressive augmentation after 6 h of culture, with a maximal between 12 and 15 h of culture, reaching values in the range 2722–2750 U L^{-1}. Surprisingly, Mn-independent peroxidase activity, presented a similar profile than MnP but with a minor maximal activity, 1988–1996 U L^{-1}. In both cases, from 15 h until the end of the culture, a decrease in activity was observed (Fig. 1c). Nevertheless, in absence of H_2O_2, no activity was observed (Data no shown). These two activities present in the supernatant, dependent and independent of Mn, could be related with the presence of a putative versatile peroxidase, which has been previously purified and identified in a related yeast species, *T. asahii* [35].

In the case of phenol oxidase, values augmented since 3 h of culture, with a maximal at 15 h (353 U L^{-1}) and then descending up to the end of the culture (Fig. 1b).

POx and peroxidase activities are inducible, as they could not be measured in control cultures without dyes.

3.2. Substrate specificity of phenol oxidase activity

In order to determine the type of phenolic substrate where the phenol oxidase previously detected has activity, various phenolic compounds such as catechol, guaiacol, phenol, pyrogallol, naphthol, phloroglucinol, resorcinol and tyrosine were evaluated.

As shown in Table 1, in some of these compounds phenol oxidase presented no activity (pyrogallol and phloroglucinol). In naphthol and tyrosine, phenol oxidase activity was very low. For diphenols, activity was higher in catechol (1,2-benzene diol or *ortho*-dihydroxybenzene) than resorcinol (1,3 diol benzene or *meta*-dihydroxybenzene). Guaiac

Table 1
Phenol oxidase activity of *T. akiyoshidainum* HP2023 supernatants from cultures with and without RB5 at 12 and 24 h using different phenolic compounds.

	Phenol oxidase activity(U L^{-1})							
	Phenol	Cathecol	Naphtol	Resorcinol	Phloroglucinol	Tyrosine	Guaiacol	Pyrogallol
12 h control culture	9,3	–	–	18,7	–	–	10,3	–
12 h dye culture	215,5	350,5	25	109,2	–	21,5	314,8	–
24 h control culture	–	–	–	–	–	–	–	–
24 h dye culture	52,3	43,8	–	42,9	–	19,8	–	–

activity was similar to that of catechol, which could be due to the similarity between these compounds as guaiacol (methylcatechol or *ortho*-methoxyphenol) is a methylated catechol. In phenol, lower activity values were observed, probably because in order to obtain the final product, *ortho*-benzoquinone, from phenol, two consecutive oxidations are required, whereas from catechol only one reaction is necessary.

Crude extract showed low activity against tyrosine and the ability of oxidize phenol, naphtol, catechol, resorcinol and guaiacol, pointing to the existence of a classical laccase and/or catecholase.

It should be noticed that previous works on similar conditions [28,36] were unable to detect laccase activity on *Trichosporon akiyoshidainum* cultures trough ABTS oxidation. Such negative results could be ascribed to the presence of organic acids of known antioxidant power like oxalic, malonic and glyoxalic, [37] or the concomitant production of Cellobiose dehydrogenase, an enzyme that could interfere with classical laccase detection assays [38].

3.3. UV–vis analysis

The absorbance spectrum (200–800 nm) of dye amended NDM_{opt} medium at initial, 12 and 24 h of culture is shown in Fig. 2, a closer look of the visible region of the spectrum is also shown in Fig. 3. Both figures shows a pronounced shift in the absorbance peak from 595 nm (Reactive Black V λopt), to 560 nm, which was accompanied with a change in supernatant color from the original dark blue to purple (Fig. 4). The rupture of the azo bonds in the molecule account for this color change of the culture supernatant before completely discoloration.

Enayatizamir et al. [39] observed a similar shift in *P. chrysosporium* cultures with Reactive Black 5. After 10 h, authors observed a purple color in the supernatant, which decreased up to total discoloration after 48 h. Supaka et al. [40] noted the same when working with a bacterial consortium, like Lucas et al. [29] in *C. oleophila*, Pearce et al. [15] in *Shewanella* sp., Chen [41] in *P. luteola*, Mohorcic et al. [42] in *Bjerkandera* sp., Mazmanci and Ünyayar [43] in *Funalia trogii* and Ramsay and Nguyen [44] in *T. versicolor*.

This two-step decoloration might occurred due to the different

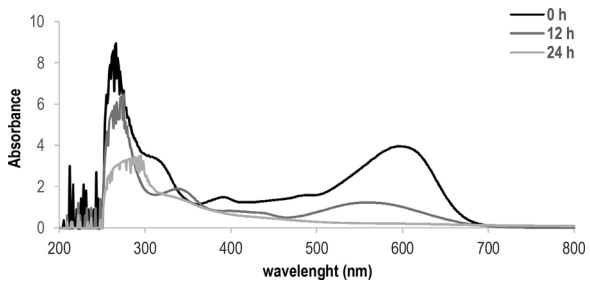

Fig. 2. UV–vis spectra of supernatants at initial, 12 and 24 h of culture of *T. akiyoshidainum* HP 2023 in NDM_{opt} with 200 mg $L^{-1}$of RB5.

reactivity of the two azo-bonds in RB5, in turn attributed to the different reactivity of the hydroxyl (–OH) and the amino ($–NH_2$) substituent, at the ortho position related to the azo bonds. Thus, discoloration, either through an oxidative or reductive mechanism, seems to occurs in two stages. In the first stage, an azo bond is broken, giving a purple intermediary. In a second stage, the breakdown of the second azo link causes the complete loss of color. These results were further confirmed by the other analytical techniques used.

The broad peak at 311 nm showed a marked decrease which was interpreted as a reduction in the concentration of the double ring in the culture supernatant by biodegradation, consistent with the decrease in total aromaticity in the culture supernatant (Fig. 1).

The stability of color in uninoculated controls, the disappearance of color, the shift in absorbance peaks in the visible spectrum, and the lack of color in the biomass, clearly points to the degradation of RB5 by *T. akiyoshidainum*.

3.4. HPLC-GPC analysis

As previously mentioned, Reactive Black 5 dye has three characteristic absorption bands, one at 595 nm corresponding to N=N double bond, the other at 311 nm corresponding to naphthalene double ring, and the last at 254 nm corresponding to benzene ring [45].

Online resource 1 shows the chromatograms extracted at 595 nm, at initial time (0 h). Two peaks were observed, at 12.782 and 12.794 min, both corresponding to the dye. The absorption spectra of these peaks are observed in online resource 2. These spectra are similar to those achieved in the previous assay (Fig. 2) and those reported in literature. Based on the obtained spectra for each of the peaks, it was concluded that the peak, at 12.782 min, corresponds to the dye Reactive Black 5, which shows the three peaks of maximum absorbance characteristic of this dye at 256, 311 and 595 nm, while the second peak, at 12.794 min, could be attributed to a compound of similar structure, possibly found as an impurity. Meanwhile, no peaks were observed at 595 nm after 12 and 24 h, in agreement with the UV–vis analysis. The decrease in the area of peaks at 595 nm together with the colorless biomass clearly points to the degradation of RB5 to a lower mass, colorless compound.

A similar trait was observed for chromatograms extracted at 310 nm (Online resource 1). At initial time two peaks were observed, corresponding to the dye and the putative compound of similar structure As in the above analysis, a decrease in the area of the peaks in the chromatograms of 24 and 48 h of culture was observed.

3.5. FT-IR analysis

Fig. 5, shows the IR spectrum analysis of culture supernatant at 0, 10 and 24 h. Most bands disappeared after 12 or 24 h cultivation, including bands corresponding to the amino group, with N–H bond stretching at 1610–1580 cm^{-1}, the N=N double bond stretching between 1550 and 1450 cm^{-1}; the R-SO_3 group with = S=O stretching at 1250 to 1140 cm^{-1} and 1070–1030 cm^{-1} and the R-SO_2-R group with S=O bond stretching at 1370–1290 cm^{-1} and 1170–1110 cm^{-1}.

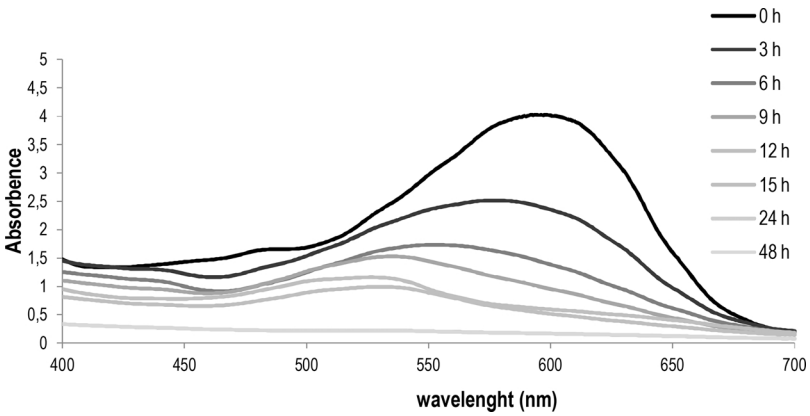

Fig. 3. Visible spectra between 400 and 700 nm of supernatants at different times of culture of *T. akiyoshidainum* HP 2023 in NDM_{opt} with 200 mg $L^{-1}$of RB5.

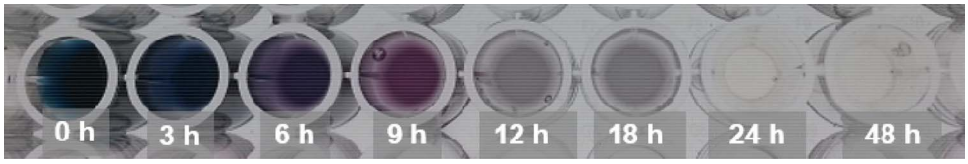

Fig. 4. Different colors observed in supernatants at different times of culture of *T. akiyoshidainum* HP 2023 in NDM_{opt} with 200 mg $L^{-1}$of RB5.

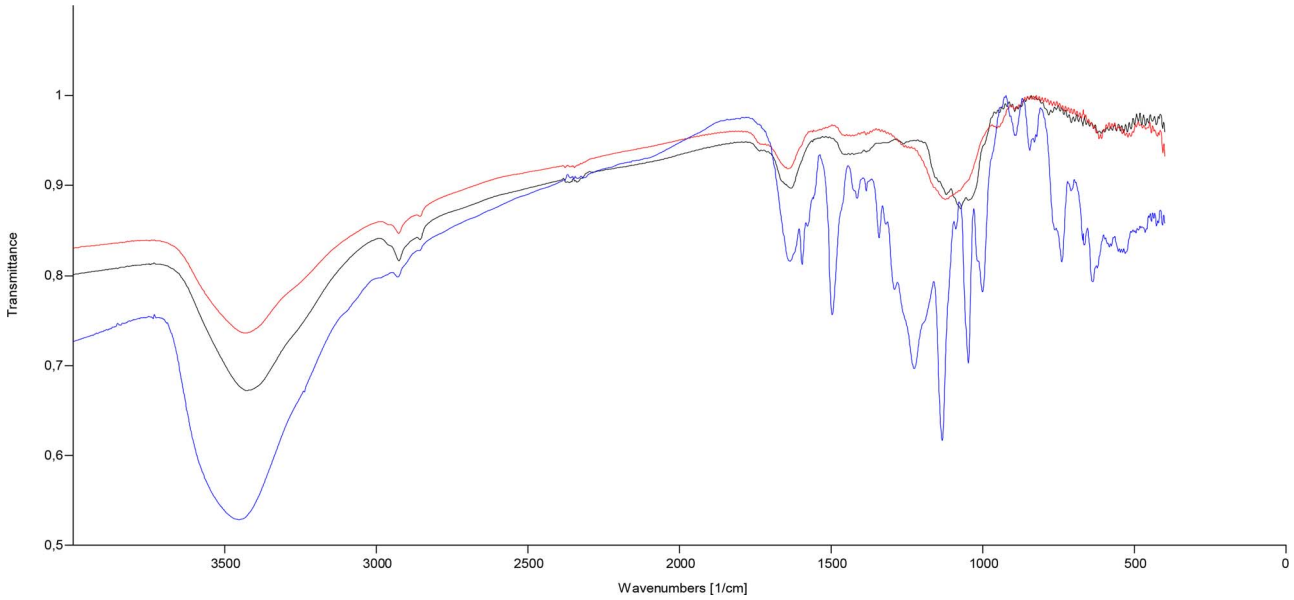

Fig. 5. FT-IR spectra of supernatants at initial (blue line), 12 (black line) and 24 h (red line) of culture of *T. akiyoshidainum* HP 2023 in NDM_{opt} with 200 mg $L^{-1}$of RB5.

The disappearing of Reactive black V characteristic bands and the colorless biomass were interpreted as a prove of dye degradation through the breaking of these bonds. Thus, azo (N=N) linkage (responsible for the color), aromatic amines, $-NH_2$, and $-RSO_3$ suffered a rupture during the first hours of cultivation, the same period in which a decrease in color was observed and even a change in color from dark blue to violet. These data are consistent with those observed in the UV–vis analysis and HPLC-GPC.

3.6. GC–MS analysis

Basic and acid extracts from culture supernatants of *T. akiyoshidainum* HP 2023 in NDM_{opt} medium with Reactive Black 5 were analyzed at initial time, 10 and 24 h of culture (Online resources 3, 4, and 5, respectively).

No peaks from alkaline extracts could be attributed to the parental dye or its degradation products. In acid extracts, however, several peaks appeared after 10 h and could be no longer detected after 24 h cultivation. A pronounced peak was detected at 13,613 min in the acid extract of 10 h culture supernatants (Online resource 6). Its mass analysis allowed to identify it as 4-((chlorodifluoromethyl)sulfonyl) aniline, with 43% of identity. This low percentage of identity is due to the limitations of the databases used, since the 4-((chlorodifluoromethyl) sulfonyl) aniline is not a part of the dye structure and no plausible mechanism to obtain it based on the composition and cultivation conditions used can be explained. Therefore, the mass analysis was compared with the theoretical masses of possible Reactive Black 5 degradation metabolites. The analysis was performed using the program ChemDraw® and based on the asymmetric rupture of the azo bonds to produce a diazene, 4- ((diazenilfenil) sulfonyl) methyl hydrogen sulfate. Ions structures of this theoretical compound and their accurate *m/z* ratio were obtained (data not shown). This analysis revealed that the theoretical metabolite had a molecular weight of 280.27, exact mass: 279.89 and ratio *m/z*: 279.98. The theoretical analysis indicated that the mass spectrum obtained from the peak at 13,613 min is consistent with the presence of 4 − ((diazenilfenil) sulfonyl) methyl hydrogen

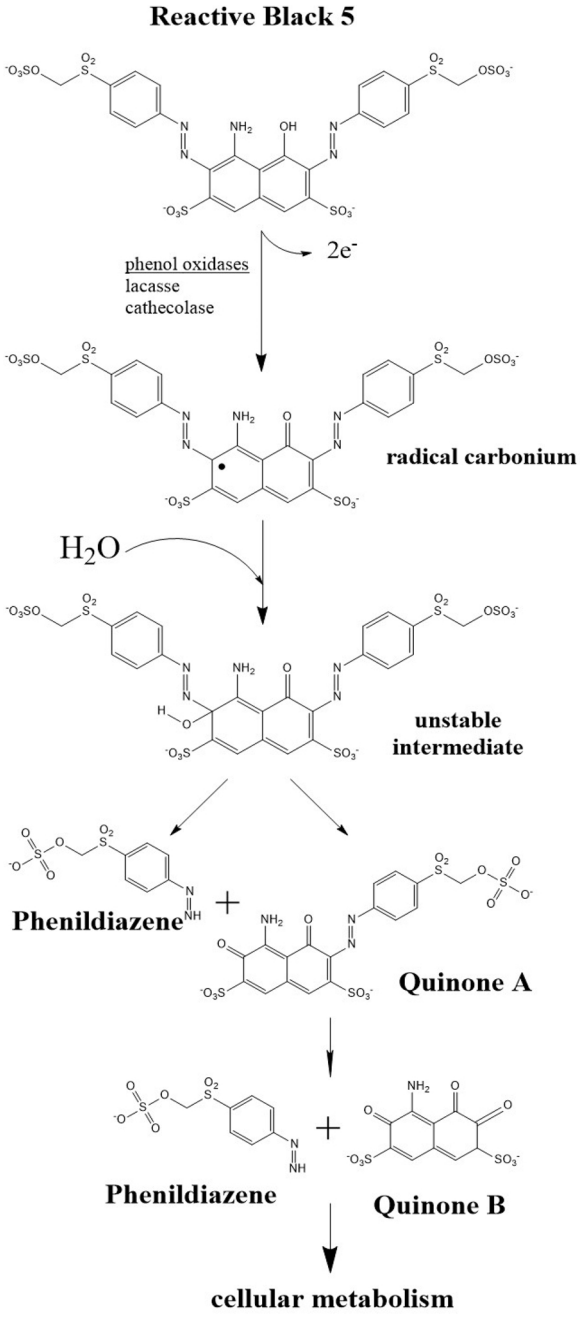

Fig. 6. Oxidative degradation mechanism proposed for Reactive Black 5 removal by *T. akiyoshidainum* HP2023.

sulfate.

Similar diazene compounds have been described as intermediate metabolites in the oxidative degradation of azo dyes [5,46–49].

The analysis of the mass spectra of peaks obtained times 8.17, 8.95 and 10.55 min in the acid extract at 10 h chromatogram permitted to identify them as 5,7-dimethoxy-1-naphthol with different values of identity (80, 90 and 83% respectively) (Online resource 7). These mass spectra are not identical to 5,7-dimethoxy-1-naphthol, but a great similarity between them was observed. Again, the limitations of the databases used may be responsible for this lack of identity. Thus, the obtained spectra showed characteristic peaks of 5,7-dimethoxy-1-naphthol, plus other peaks that may correspond to different aromatic ring substituents. Following an analysis similar to that described above it was concluded that these chromatograms were consistent with the presence of a naphthoquinone, part of the structure of Reactive Black 5, possibly 5-amino-4-hydroxy-3,6-dioxo-2,3, 5,6-tetrahydronaphthalene-2,7-sodium disulfonate.

All of the peaks analyzed disappeared and could no longer be detected in acid extracts from 24 h cultures, pointing to their degradation to simpler metabolites

4. Conclusion

The results here exposed present, the first description of a possible mechanism for Reactive Black 5 degradation by the basidiomycetous yeast, *T. akiyoshidainum* HP 2023 (Fig. 6). The putative mechanisms is driven by a phenol oxidase and peroxidase enzymes. However due to the presence of organic acids in the culture supernatants (oxalic acid, malic acid, etc.), the involvement of other mechanisms such as oxidative reactions Fenton-type mediated cannot be excluded. The proposed mechanism occurs in two stages, with the formation of a purple quinone (Quinone A, Fig. 6) with an azo bond, as an intermediate metabolite. Such quinone was subsequently degraded, which was accompanied by the complete disappearance of color. This process did not lead to the formation of aromatic amines, which confirms *Trichosporon akiyoshidainum* HP 2023 a promising tool for dye removal treatment of colored textile effluents.

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Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jece.2017.11.012.

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