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Oxidative degradation of Remazol Turquoise Blue G 133 by soybean peroxidase

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ABSTRACT

Reactive dyes are widely employed in textile industries and their removal from wastewaters is a relevant environmental problem. In addition to chemical and physical methods, several bioremediation approaches, involving intact micro-organisms or isolated enzymes, have been proposed to decolorize dye solutions. In this paper, we report the complete and fast decolourization of a Cu(II)-phthalocyanine based reactive dye (Remazol Turquoise Blue G 133) by means of the soybean peroxidase/H_2O_2 system. The oxidative degradation of the dye in aqueous solution at 25 °C was studied as function of pH, revealing a quantitative decolourization yield at acidic pH values with a maximum of activity at pH 3.3. The reaction products were identified and characterized by HPLC–diode array detector (DAD)–mass spectrometry (MS), ionic chromatography and EPR techniques. This analysis showed that the enzyme catalyses the breaking of the phthalocyanine ring producing sulfophthalimide as the main degradation product, and the release of stoichiometric amount of ammonium and Cu(II) ions.

1. Introduction

Reactive dyes are extensively used in dyeing activities and their use increases every year owing to capacity to bind on textile fibres by covalent bonds. This favourable characteristic facilitates their interaction with the fibre, enhancing the fixation rate and reducing the energy consumption [1]. On the other hand their use is problematic because 10–15% is released in the wastewaters and dispersed in the environment with toxic, mutagenic and carcinogenic effects [2].

Moreover, synthetic dyes are usually designed to be resistant to degradation by light, chemicals and micro-organisms, and therefore the development of efficient and economic technologies for the treatment of wastewaters is a priority for textile industries. Despite the existence of several chemical and physical methodologies [3,4], bioremediation of the effluents is an attractive solution and several studies have been already published about the use of intact micro-organisms or isolated enzymes. In particular some species of white-rot fungi have been demonstrated to be able to decolorize solutions of dyes by means of oxidative processes catalysed by their ligninolytic enzymes, laccases and peroxidases [2,5,6]. Since isolated enzymes are also able to perform decolourizing reactions without the problems generated from the use of living organisms, enzymatic processes have already replaced the traditional ones in some textile industries [7].

All these systems have advantages and disadvantages that must be carefully assessed in view of their practical use. Some of the factors to be considered are: cost, efficiency and environmental compatibility of the degradation products. Although white-rot fungi are generally inexpensive and often able to totally break down the pollutants, they are also rather slow in their action and the decolourization of dye solutions generally require some days of treatment [8–11]. On the other hand, isolated laccases and peroxidases are generally more expensive but allow to obtain high reaction rates and good yields [6,12,13]. In addition, these enzymes can be used in working conditions not suitable for living organisms, or immobilized on solid supports and reused for several reaction cycles [14–18].

Although the partial decolourization of industrial dyes solutions and textile effluents by means of horseradish peroxidase (HRP) [19–21] and soybean peroxidase (SBP) [20] has been recently reported, the application of plant peroxidases to the problem of reactive dyes degradation has been rather limited until now. On the other hand, it was also shown that SBP is able to oxidize a broad range of substrates [22–24] and is highly resistant to thermal and chemical denaturation [25–27], all features that make this enzyme really suitable for industrial applications. Therefore, we decided to use SBP to catalyse the degradation of Remazol Turquoise Blue G 133 (RTB), a reactive dye, based on a copper(II) tetrasulfonated phthalocyanine derivatized with N-(4-(2-sulfatoethylsulfonyl)phenyl)sulfonamide groups, taken here as an example of a widely used and commercially available reactive dye.

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In this work, dye solutions were degraded in the presence of catalytic amount of SBP and the reaction products were analysed by means of HPLC–diode array detector (DAD)–mass spectrometry (MS), ion analysis and EPR techniques.

2. Materials and methods

2.1. Enzymes and chemicals

Soybean peroxidase was purchased from Bio-Research Products Inc. (Iowa, USA) with RZ = 1.97 and used as received. Remazol Turquoise Blue G 133 was kindly supplied by DyStar Textilfarben (Frankfurt, D). The structure and percentage of purity of this commercial dye are not of public domain, but it is referable to Reactive Blue 21, a copper phthalocyanine with a sulfato ethyl sulfone as reactive group, from which the chemical formula $CuC_{40}H_{24}N_9O_{17}S_6Na_4$ was obtained.

Hydrogen peroxide (30% aqueous solution), phenol (99%), ammonium chloride, K_2CO_3 and $NaHCO_3$ were from Sigma-Aldrich. Acetonitrile was a Scharlau AC0331 Supergradient HPLC grade eluent, while ammonium acetate (98%) was from Fluka. Sodium hypochlorite solution (6–14%) and sodium nitroprusside (99%) were purchased from Riedel de Haen. 2-(4-Aminophenylsulfonyl)ethyl hydrogen sulphate (APSES) was from Maybridge (Fisher Scientific). Sulfophthalimide (SPI) was synthesized from 4-sulfophtalic acid (Aldrich, technical grade, with up to 25% of the 3-isomer), according to a method described elsewhere [10].

2.2. Remazol Turquoise Blue G 133 enzymatic degradation

The ability of SBP to decolourize aqueous solutions of RTB was initially investigated varying dye concentration from 20 to 200 mg L^{-1} (concentrations by weight referred to the weight of powder), hydrogen peroxide concentration from 6.4×10^{-7} to 2.4×10^{-3} M and pH values from 2.0 to 9.0. Solutions were prepared in a triple buffer: borate 50 mM, phosphate 50 mM and acetate 50 mM, and the decolourization isotherms were recorded at 621 nm and 25 °C, with an SBP concentration of 2.06×10^{-7} M and H_2O_2 9.98×10^{-5} M. The concentration of the enzyme solutions was determined by UV–visible (UV–VIS) spectroscopy at 403 nm, ($\varepsilon_{403\ nm} = 9.64\times10^4\ M^{-1}\ cm^{-1}$ [25]).

The initial rate of decolourization was also investigated as function of pH using the following concentrations: H_2O_2 6.4×10^{-5} M, SBP 2.06×10^{-8} M and RTB 70.8 mg L^{-1}. The same experiment was repeated replacing RTB with 2,4,6-trichlorophenol (2,4,6-TCP) at the concentration of 1.17×10^{-5} M. In this case we investigated the oxidative dechlorination of the 2,4,6-TCP following the formation of the final reaction product, 2,6-dichloro-1,4-benzoquinone, at 272 nm ($\varepsilon_{272\ nm} = 1.4\times10^4\ M^{-1}\ cm^{-1}$ [28]).

All UV–VIS measurements were acquired with a UNICAM UV300 Thermospectronic double beam spectrometer, equipped with a Peltier cell for temperature control.

2.3. HPLC–DAD–MS measurements

Thermofinnigan Surveyor MSQ equipped with a photodiode array detector and an electrospray ionization (ESI) probe with a single quadrupole Mass Spectrometer was employed to follow the RTB fate.

The separation was obtained with a C-18 reversed-phase encapped column [29]. The column was equilibrated with 30% of acetonitrile (A) and 70% of ammonium acetate 5 mM solution (B), and a 20 μL volume was injected and eluted with the following gradient: 0 min 30% A, 70% B and 30 min 100% B with 0.5 mL min^{-1} flow rate.

Photodiode array and Mass Spectrometer were used in parallel. Ion source conditions were optimized through direct infusion technique to maximize the signal: 400 °C of temperature, 3 kV of needle voltage and 50 V of cone voltage in negative-ion mode.

Only the samples representing the initial and the final point of degradation were taken into consideration. In the former case (t_0 sample), RTB solution (200 mg L^{-1} at pH = 5) was incubated with SBP for 30 s while, in the latter (t_{deg} sample), H_2O_2 was added to the same solution and left to react until maximum decolourization (~91%) was obtained. Before HPLC analysis every solution was eluted through a size exclusion PD-10 column in order to separate the enzyme from the mixture and to stop the reaction. The efficiency of the separation was verified by determining the SBP activity into eluted fractions and monitoring their UV–VIS spectra. Then, the more representative fractions, where the enzyme is not present anymore and the analyte and its products are eluted, were collected together in a final volume and the dilution factor was taken into account in later calculations.

The quantification of SPI and APSES was obtained by means of HPLC–MS analysis in selected ion monitoring (SIM) mode through standard addition calibration. The addition standard curves were prepared under four different added concentrations in the range 2–6×10^{-6} M for APSES and 2–6×10^{-5} M for SPI.

2.4. Detection of ions formation

The release in solution of heteroatoms as sulphate, nitrate, nitrite or ammonium ions, deriving from the breaking of the sulfophthalocyanine dye, has been investigated. The anions present in the solutions containing RTB and SBP were analysed with suppressed ion chromatography. A Dionex DX500 instrument equipped with Anion Self-Regenerating Suppressor-Ultra (ARSR-ULTRA, 4 mm, Dionex) a conductimeter detector (ED 40, Dionex) and GP40 pump (Dionex) was used. Injections of 20 μL were performed into AS4A-SC column Dionex IonPack (200 mm × 4 mm i.d.) with isocratic conditions: 90% of H_2O and 10% of K_2CO_3 12 mM and $NaHCO_3$ 5 mM (flow = 0.5 mL min^{-1}). The amount of sulphate, nitrate, and nitrite anions present in the samples was determined by standard addition calibration mode.

Ammonium cations were determined performing a colorimetric test [30] in which indophenol is produced by the reaction between ammonium ions, phenol and hypochlorite, catalysed by sodium nitroprusside. The tests were conducted onto 3 mL volumes of appropriately diluted samples adding 0.12 mL of phenol 4.3×10^{-2} M (in 95% ethanol), 0.12 mL of sodium nitroprusside (0.5% w/v), 0.24 mL of alkaline citrate solution (trisodium citrate 0.68 M, in 0.25 M of NaOH) and 0.06 mL of 6–14% hypochlorite solution. The colour was detected at 640 nm after 2 h from the beginning of the reaction. The quantification was obtained with a calibration curve (slope 0.72, intercept 0.04, $R^2 = 0.99$) realized with ammonium chloride standard solutions between 0.05 and 0.4 mg L^{-1} (each measure was repeated three times at least).

2.5. EPR spectroscopy

EPR spectra of RTB solutions were recorded at 77 K by an ESP300E Bruker X-band spectrometer equipped with a 4103TM cylindrical cavity. Samples were placed in quartz tubes and the experimental parameters were as follows: frequency 9.40 GHz, power 4 mW, modulation frequency 100 KHz, modulation amplitude 2 G, gain 4×10^4, time constant 8.091×10^{-2} s.

3. Results and discussion

3.1. Decolourization of the Remazol Turquoise Blue G 133 solutions

Aqueous solutions of RTB have an electronic spectrum characterized by the presence of a broad and intense band in the visible zone, which gives the characteristic blue colour. Since the composition of the dye mixture is not exactly known, we refer our concentration data to a weighed amount of sample to avoid any possible error due to an inaccurate estimate of the average molecular weight of the mixture. For

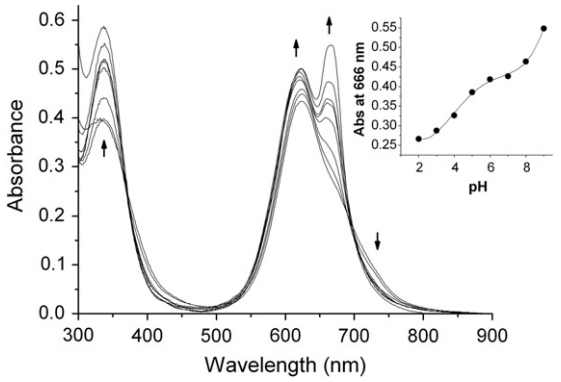


Fig. 1. UV–VIS spectra of a RTB solution (35 mg L^{-1}) in the pH range 2.0–9.0 (arrows indicate the trend of absorption bands as pH increases). Inset: absorbance at 666 nm as function of pH.

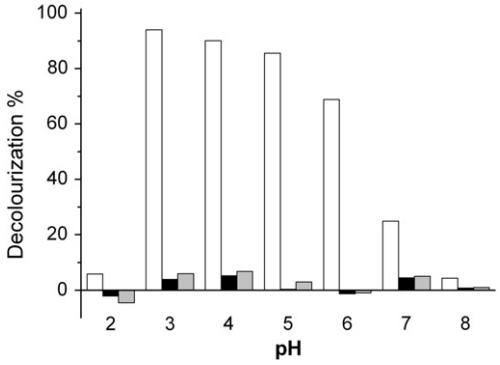


Fig. 3. Decolourization percentage registered after 4 h of treatment at 25 °C of the solution of RTB (200 mg L^{-1}) with SBP 2.06×10^{-7} M and H_2O_2 9.98×10^{-5} M (white columns), RTB only (black columns), RTB and H_2O_2 (grey columns).

the same reason the molar absorptivity was also referred to mg L^{-1} of RTB rather than a molar concentration. As we can see from Fig. 1, the structure of this band depends from the pH of the solution: at very low pH values an absorption with a maximum at 621 nm predominates ($\varepsilon_{621\ nm}=1.41\times10^{-2}$ mg^{-1} L cm^{-1} at pH 7.0; 1.28×10^{-2} mg^{-1} L cm^{-1} at pH 5.0); whereas, when the pH increases, a more intense absorption band appears at 666 nm.

Since the variations in molar absorptivity are greater at 666 nm than at 621 nm, we monitored for 4 h the decolourization trend induced by hydrogen peroxide in the presence of SBP through the decrease of absorbance at 621 nm. Moreover, as the pH of the batch of reaction resulted unaltered during all the degradation reaction, it was possible to calculate the percentage of decolourization as function of time, as reported in Fig. 2.

These experiments show that the decolourization process is strictly dependent from the pH of the solution: SBP activity increases drastically changing the pH from 2 to 3, and in the pH range of 3–5, it effectively and rapidly degrades about 2/3 of the RTB in just half an hour. After 4 h (Fig. 3), at pH 3 approximately 95–96% of the dye degradation occurred, and at pH 4 and 5 the results are slightly lower. At higher pH values the percentage of decolourization decreases and drops near to zero at pH 8. Since the stability of RTB solutions has been verified in presence of hydrogen peroxide as well, we can affirm that the dye degradation observed in these experiments is exclusively due to the enzymatic activity of SBP.

In order to better clarify the effect of pH on the enzymatic reaction, a kinetic study has been carried out measuring the pH dependence of the initial rate of the oxidation of RTB and 2,4,6-trichlorophenol, used as an alternative substrate with a known dissociation constant ($pK_a=6.0$ [31]). As well known, the catalytic cycle of peroxidases involves the formation of two intermediates, Compound I and Compound II, according to the following reactions (where SH indicates a generic substrate):

$$\text{Peroxidase} + H_2O_2 \rightarrow \text{Compound I} + H_2O$$

$$\text{Compound I} + SH \rightarrow \text{Compound II} + S^{\bullet}$$

$$\text{Compound II} + SH \rightarrow \text{Peroxidase} + S^{\bullet} + H_2O$$

Every step of the catalytic cycle is pH dependent: the Compound I generation is favoured by the presence of a network of hydrogen bonds between the Fe-heme/H_2O_2 adduct and the distal histidine and arginine side chains [23], whereas the substrate oxidation may depends on its protonation state [32,33].

The kinetic studies on the pH dependence of RTB degradation allowed to obtain the bell-shaped curve showed in Fig. 4 (solid line) with optimal pH = 3.3 ± 0.1. Two inflection points, at pH = 3.0 ± 0.1 and pH = 3.9 ± 0.1, can be extrapolated with a model derived from the Henderson–Hasselbach equation applied to a system with a double ionization process [32].

The proximity of the two inflection points, calculated for RTB degradation, gives to the profile a very narrow shape, rarely detected before with soybean peroxidase, and a optimum pH lower than those previously reported with other substrates like ABTS (pH = 5.5), guaiacol (pH = 5.5), *p*-cresol (pH = 5.0) [26], or phenol (pH = 6.4) [22]. Although a notable exception was observed for veratryl alcohol oxidation (pH = 2.4) [34].

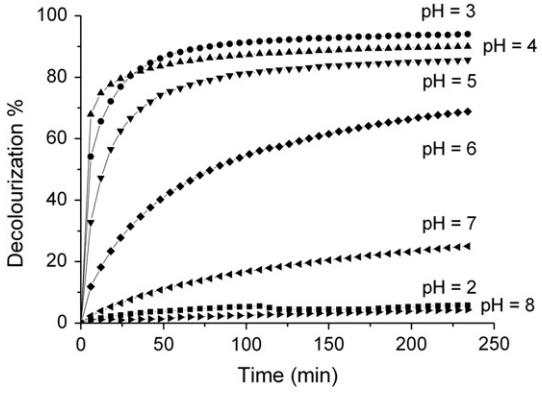


Fig. 2. Time course of the decolourization of Remazol Turquoise Blue G 133 solutions (200 mg L^{-1}) at different pH values in presence of SBP 2.06×10^{-7} M and H_2O_2 9.98×10^{-5} M.

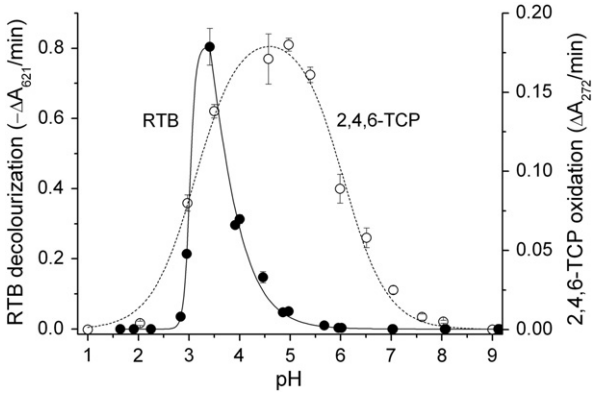


Fig. 4. Initial rate of RTB 70.8 mg L^{-1} decolourization (●, solid line) and 2,4,6-TCP 1.17×10^{-5} M oxidation (○, dotted line) obtained with H_2O_2 6.4×10^{-5} M and SBP 2.06×10^{-8} M as function of pH.

In the case of 2,4,6-trichlorophenol (Fig. 4, dotted line) two pK values can be calculated at pH 3.1 ± 0.1 and 6.0 ± 0.1 respectively. The former has essentially the same pK value registered for RTB degradation, whereas the latter was shifted at higher pH values, in good agreement with the 2,4,6-TCP ionization constant. The similarity in activity decrease for RTB and 2,4,6-TCP in the acidic range with pK value of about 3.0, presumably refers to an ionization process of the enzyme.

As previously demonstrated by Nissum et al. [23], in the case of SBP the Compound I formation shows the typical bell-shaped profile and is favoured between the two pK_a values of 3.2 ± 0.3 and 9.9 ± 0.2, therefore the absence of RTB decolourization at very low pH values and the presence of the first inflection point at pH 3.0 ± 0.1 are in agreement with such mechanism. On the other hand, the correspondence of 2,4,6-TCP ionization constant with the calculated pK for the descendant branch of the SBP catalysed oxidation curve suggests the presence of a second process, likely correlated with the substrate ionization also in the case of the rapid loss of activity observed above pH 4, for RTB oxidation. The ionic nature of the dye is confirmed by the variations observed in the shape of the UV–VIS spectrum acquired at different pH values (see Fig. 1). Since the dissociation constants for this molecule are not known, an extrapolation of the absorbance values at 666 nm is plotted against pH (inset of Fig. 1) and reveal a sigmoid trend characterized by an inflection point at pH = 4.3 ± 0.6. This profile can be explained by the establishment of an acid–base equilibrium in the surrounding of pH 4, essentially corresponding to the second inflection point of RTB decolourization profile observable at pH 3.9 ± 0.1.

3.2. HPLC–DAD–MS

A RTB aqueous solution (10 mg L^{-1}) was analysed by HPLC, before proceeding with the enzymatic reaction. The photodiode array (PDA) total scan chromatogram showed the presence of three main components, indicated in the Fig. 5 (upper part) as peaks A, B, and C respectively. The HPLC profile obtained at 240 and 621 nm revealed that the species corresponding to peaks A (t_R = 2.5 min) and C (t_R = 5.0 min) are the main responsible for the blue colour of RTB solution, whereas the species included in the broadband B (t_R = 3.1–3.6 min) are ascribable to less coloured or colourless components.

In order to identify the degradation products resulting from RTB decolourization, the enzyme must be separated from the batch of reaction before the injection in the HPLC system. The elution through a size exclusion chromatography column ensured the best results in terms of SBP separation from the batch of reaction and representativeness of gathered fractions, even inducing some changes in the composition of the mixture. Therefore a solution containing RTB and SBP but not hydrogen peroxide (t_0 sample) was also eluted through the column before the HPLC analysis (Fig. 6, upper left).

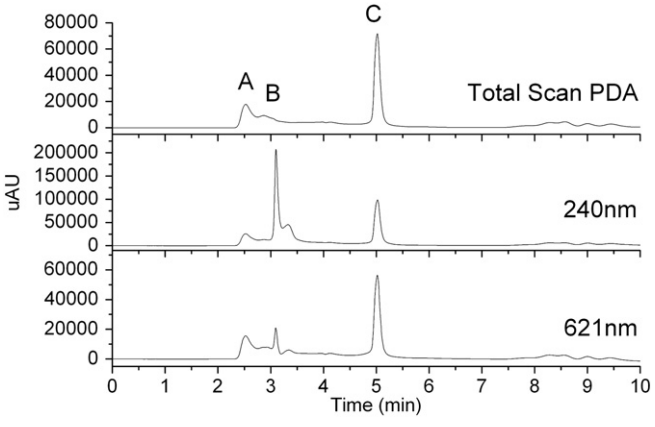


Fig. 5. HPLC–DAD chromatograms of the 10 mg L^{-1} aqueous solution of RTB, recorded in total scan PDA and at 240 or 621 nm.

As can be seen, A and C components were partially lost in the passage through the column, highlighting the presence of component B. On the other hand, as expected, the chromatogram of the final stage of the degradation (t_{deg} sample) representing the 91% of decolourization (Fig. 6, upper right), showed the almost complete disappearance of the blue-coloured components (peak A and C) and an additional growth of peak B, probably due to the coelution of the reaction products.

In order to identify the nature of the decolourization products, the same samples were also analysed with HPLC–ESI–MS technique. The MS spectra of the 10 mg L^{-1} aqueous solution of RTB, t_0 solution and of the 91% degraded sample of RTB, confirmed the trend observed with the PDA detector. In fact, in correspondence of the PDA chromatographic peak B (t_R = 3.6 min), all the analysed samples revealed the presence of one species with *m/z* 280, whereas in the case of the degraded sample, another signal with *m/z* 226 appeared at the same retention time, as highlighted by the SIM mode chromatogram (Fig. 6) and the corresponding MS spectrum (Fig. 7, upper right).

Based on these two *m/z* values, for *m/z* 280 APSES was hypothesized as possible precursor of the RTB in the commercial mixture and, for *m/z* 226, SPI as possible reaction product [10]. These *m/z* values corresponding to the anionic form $[M–H]^-$ of the parent molecules with calculated mass equal to 281.0 g mol^{-1} for APSES and 227.0 g mol^{-1} for SPI, respectively. The structural assignment was confirmed by means of HPLC–ESI–MS analysis of the corresponding authentic standards (Fig. 7), which have a very close retention time, similar to that of peak B.

Successively, we carried out the addition of the two compounds to the samples representing t_{deg}, t_0, and to a 10 mg L^{-1} aqueous solution of RTB; in all cases a significant enhancement of the peak B in the PDA acquired chromatogram, was observed.

The species with 280 *m/z* (Fig. 6, peak 1), present in all the samples analysed and identified as the reactive arm of the copper phthalocyanine, APSES, was quantified through a standard addition calibration method as 5–9% of the starting solution of Remazol Turquoise Blue G 133. Although the molecule was compatible with a degradation structure of the dye, the 280 *m/z* peak area showed no remarkable alteration imputable to SBP oxidation activity, therefore neither accumulation nor abatement was observed also in the decolourized sample. These results confirm that, even in little quantity, APSES is present like precursor of the RTB in the commercial mixture and that this compound remains substantially unaltered by the SBP/H_2O_2 system.

On the other hand, the SIM analysis of the *m/z* 226 (Fig. 6, peak 2) showed that this species is not present in the starting mixture, but that is produced during the RTB degradation. In agreement with the previously reported results about the degradation of sulfonated phthalocyanine dyes by the white-rot fungus *Bjerkandera adusta* [10], this compound is ascribable to a sulfophthalimide molecule deriving from the breaking of the phthalocyanine ring and the oxidation of the substituted indole groups. The assignation of the peak to the molecular anion of SPI was confirmed by standard addition of the reference synthesized as previously described [10].

3.3. Monitoring of ions formation

The identification of SPI as product of reaction presuppose the breaking of the conjugated ring of the phthalocyanine associated with the elimination of nitrogen atoms. Therefore we analysed the fate of nitrogen as nitrate, nitrite or ammonium ions release. The concentration of anions in initial and degraded solutions was investigated by means of ionic chromatography, while the presence of ammonium cations was detected with the phenate method [30].

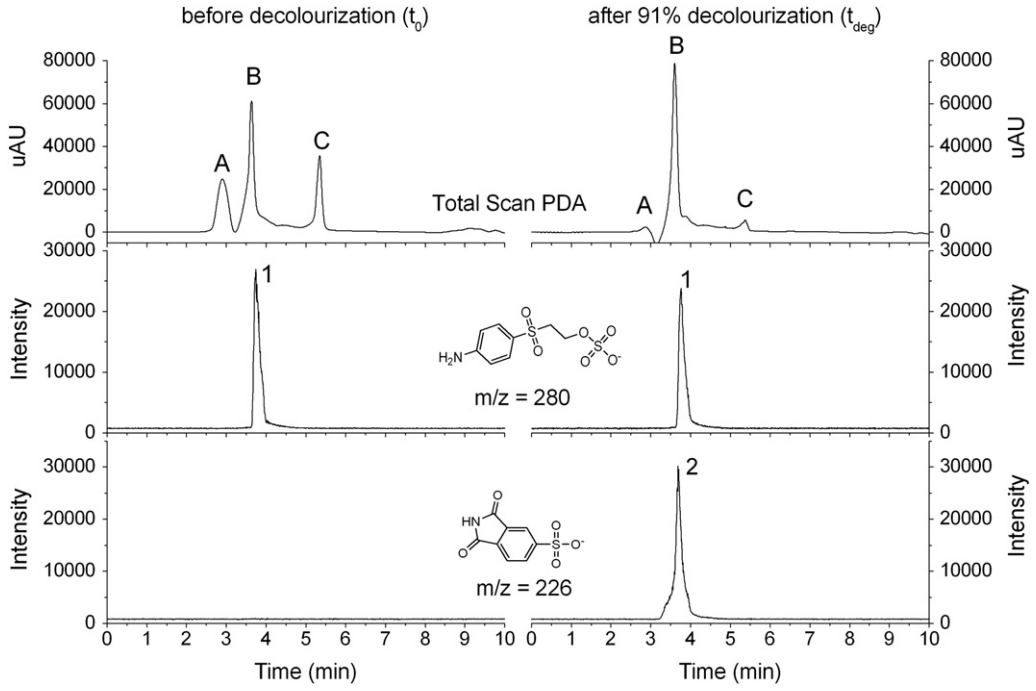


Fig. 6. HPLC–DAD–MS chromatograms recorded in total scan PDA and in SIM mode at 280 and 226 *m/z* before (left) and after 91% decolourization (right).

Three main peaks were found into ionic chromatograms of the samples, corresponding to nitrite, nitrate and sulphate ions, but no significant variation in the concentrations was revealed after the decolourization.

The determination of ammonium ions has been obtained by a colorimetric test, carried out onto the diluted samples corresponding to the starting and the final point of RTB degradation. The results showed the release of $8.92 \pm 0.33\ \mathrm{mg\,L^{-1}}$ of ammonium ions ($4.95 \times 10^{-4}\ \mathrm{M}$) into the sample with 91% of RTB degradation.

The concentration of ammonium ion obtained at the end of the reaction is consistent with the release of four ammonium cations from the breaking of each phthalocyanine ring, estimating the starting RTB

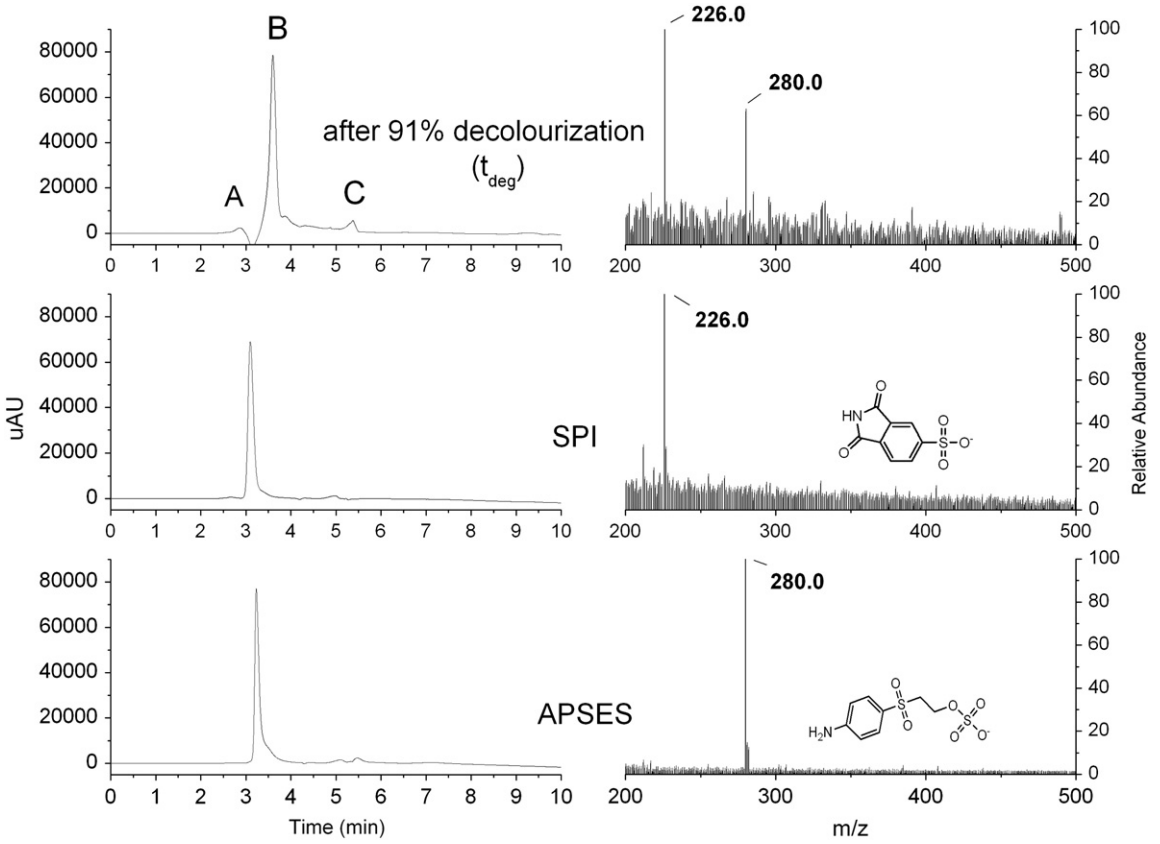
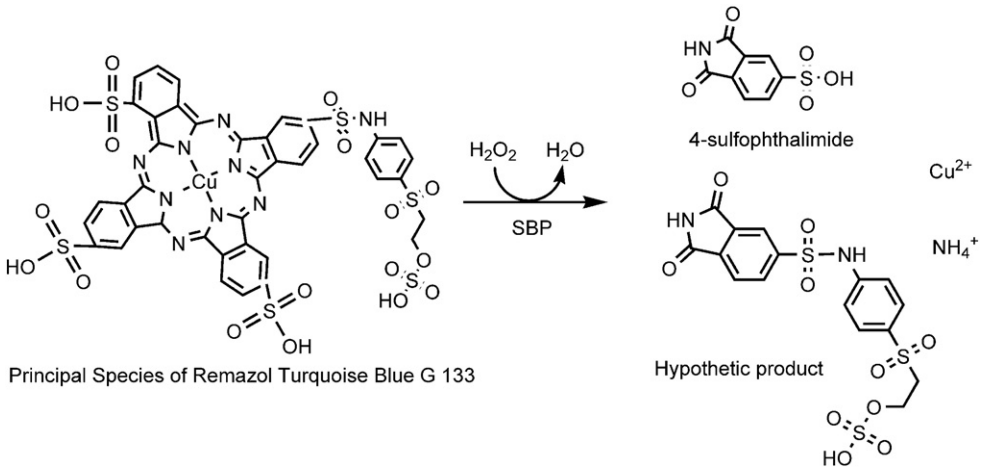


Fig. 7. HPLC–DAD–MS chromatograms recorded in total scan PDA (left) and MS spectra (right) of RTB sample after 91% of decolourization (peak B), and SPI or APSES standards.



Scheme 1. Hypothetical reaction scheme summarizing the degradation of RTB.

concentration as $1.46–1.52\times10^{-4}$ M, on the basis of a hypothetical molecular weight of RTB equal to 1246 g mol^{-1} (see Section 2.1), and of the presence of 5–9% w/w of APSES in the starting mixture. Thus, taking into account the uncertainty concerning the molecular weight of the parent dye molecule, and the purity of the furnished product, the results highlights that nitrogen atoms contained in the phthalocyanine ring are substantially released as ammonium ions.

3.4. EPR measurements

The breakdown of the phthalocyanine macrocycle could also involve the release of Cu(II) ions in solution. EPR measurements at 77 K confirmed this mechanism because, at the end of the reaction, we observed the presence of an axial spectrum (not shown) with $g_{||}=2.41$, $A_{||}=120$ G and $g_{\perp}=2.08$, quite different to the EPR spectrum of RTB ($g_{||}=2.27$, $g_{\perp}=2.05$) and clearly originated from the interactions of Cu(II) ions, released after RTB degradation, with N or O ligands present in solution.

4. Conclusions

Our study highlights that the Remazol Turquoise Blue G 133 degradation is due to the SBP enzymatic action. The formation of reactive intermediates at high potential, typical of the peroxidase reaction mechanism, allows the oxidation of isoindoles to sulphophtalimides with breaking of the macrocycles structure and release of ammonium and Cu(II) ions (Scheme 1). On the other hand, phtalimide derivatives were also observed as reaction products after the treatment of phthalocyanines in aqueous acid solution with strong chemical oxidants like nitric acid, potassium dichromate or permanganate [35,36].

The SBP has proved to be very effective in the degradation of RTB, its action is strongly influenced by the pH of the solution, but under optimal conditions can degrade the dye almost completely and very quickly. To the best of our knowledge, it was the first time that SBP was exploited for the degradation of a reactive dye and that an isolated plant peroxidase reached such a high yield of decolourization (96%) for this type of dyes, without the support of mediators. Only the bioremediation with micro-organisms, belonging to the family of white rot fungi, allowed to obtain the complete decolourization of copper phthalocyanines solutions within 7 days [8]. On the contrary, with the horseradish peroxidase isoenzyme C, which shows 57% amino acid sequence identity [37], only a maximum of 59% of decolourization in optimized conditions was obtained [21].

Finally, although further studies are needed to better understand the mechanism of action that will enable the SBP to break phthalocyanine ring, these results suggest that this enzyme can be successfully employed in the preparation of biocatalysts useful in the degradation of effluents of textile dyeing industries.

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