Journal of Molecular Catalysis A: Chemical 321 (2010) 27–33

Contents lists available at ScienceDirect

Journal of Molecular Catalysis A: Chemical

journal homepage: www.elsevier.com/locate/molcata

Is the bleaching of phenosafranine by hydrogen peroxide oxidation catalyzed by silica-supported 5,10,15,20-tetrakis-(sulfonatophenyl)porphine-Mn(III) really biomimetic?

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ARTICLE INFO

Article history:
Received 27 November 2009
Received in revised form 18 January 2010
Accepted 19 January 2010
Available online 1 February 2010

Keywords:
Phenosafranine
Metalloporphyrins
Mn(III)porphine
Biomimetic catalyst
Hydrogen peroxide
Lignin peroxidase

ABSTRACT

Phenosafranine is a toxic, polluting dye, belonging to the N-phenylphenazinium class, whose inexpensive and environmentally friendly elimination from industrial effluents could be of interest. In this study we report phenosafranine degradation and concomitant decolorization of the corresponding wastewaters by H_2O_2 oxidation in the presence of 5,10,15,20-tetrakis(4-sulfonatophenyl)-porphine-Mn(III) catalyst immobilized on a modified silica support bearing imidazolyl functionality. Some operational features of the catalytic process are described and a chemical mechanism for the bleaching process is hypothesized. The proposed method was compared to others, based on the use of the oxidizing enzymes such as laccase, horseradish peroxidase, and lignin peroxidase.

1. Introduction

Phenosafranine (C.I. 50200, 3,7-diamino-5-phenylphenazinium chloride, PNS, Fig. 1) is a synthetic dye, widely used as a redox probe [1,2], as a photosensitizer [3,4], as a histological dye [5]. PNS, which could well regarded as the virtually parent compound of other N-phenylphenazinium dyes, is a toxic [6] and recalcitrant compound, whose ability to intercalate within DNA double strands has been shown [3,7]. Although PNS and related compounds are conventionally regarded as amino-substituted phenylphenazinium cations, they could be well considered as tricyclic quinoneiminium cations, whose positive charge is substantially shared among the 5-nitrogen of the phenylphenazinium system and the two amine nitrogen atoms [8]. This delocalization accounts for the strong visible light absorption of the dye as well as for its chemical stability.

The redox properties of PNS are well known, and they are mainly related to the analytical use of this dye [1], which easily undergoes reversible two-electron reduction. By contrast, studies on the PNS *oxidation* are much less common, since relatively strong oxidants are required [8]. Consequently, PNS oxidative degradation under mild conditions is an interesting challenge, because the compound could be also regarded as the parent molecule of the N-phenylphenazinium dye family, this class of dyes being largely used in the textile, pharmaceutical, paper, cosmetic, etc., industries.

Ciric-Marjanovic et al. [8] have found that two consecutive one-electron oxidations of PNS by peroxodisulfate anion under drastic experimental conditions, passing through a very reactive, non-isolable dication radical, led to a nitrenium dication (Fig. 2). This latter – for being an extremely strong electrophile – in turn reacted with another PNS cation in the close proximity, leading to dimerization, and then to a polymerization process. An apparently quite similar result was reported for electrochemical oxidation, and was exploited to prepare redox-active surfaces for electric sensors [9]. Obviously, both processes are not suitable for industrial treatment of wastewaters containing PNS and/or related dyes.

As it is known, some conventional degradation processes used for the industrial treatment of pollutants involve chemical, physical and biological oxidation [10,11]; however, they are not always

Abbreviations: IPS, (3-[1-imidazolyl]-propylcarbamoyl)-3′-aminopropylsilica; PNS, phenosafranine; MnTSPP, 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine-Mn(III) chloride; APH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride; OH-TEMPO, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl; NHP, *N*-hydroxyphthalimide; NHS, *N*-hydroxysuccinimide; NHT, *N*-hydroxybenzotriazole; HRP, horseradish peroxidase; LiP, lignin peroxidase; LC, laccase.

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doi:10.1016/j.molcata.2010.01.015

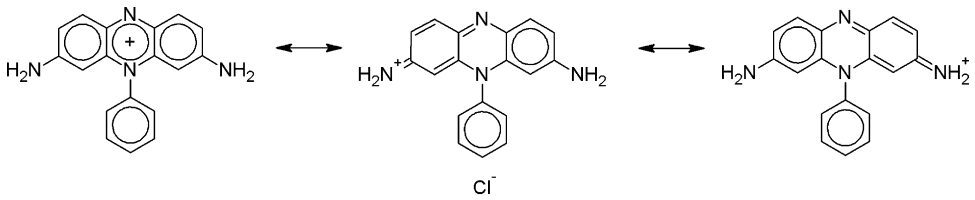

Fig. 1. The structure of phenosafranine. The positive charge is shared among the 5-nitrogen of the phenylphenazinium system and the two amine nitrogen atoms.

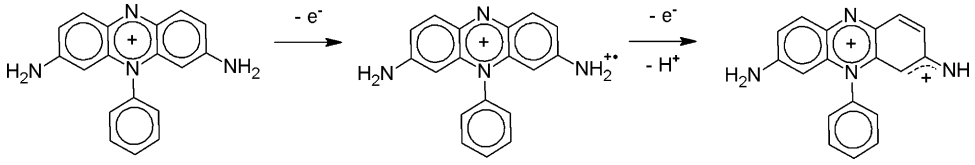

Fig. 2. Early steps in PNS oxidation by peroxodisulfate. The radical dication (left) and the nitrenium dication (right) arising from PNS upon treatment with ammonium peroxodisulfate in 0.2 M HCl.

efficient enough. Recently, new approaches based on photocatalytic [12–16], electrochemical [17,18] and microbiological [19,20] methods have been described; however they are only seldom fully satisfactory.

To our knowledge, no specific methods have been described until now for PNS degradation in wastewaters.

Therefore, starting from our recent work [21,22] on heterogeneous redox catalysts capable of promoting oxidative degradation of other aromatics, and among them the anthraquinone dye alizarin red S, in this study we have assessed the feasibility of a mild process to degrade PNS, that could be further extended to other N-phenylphenazinium salts. In particular, we propose a simple procedure for PNS degradation using diluted hydrogen peroxide and properly immobilized 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine-Mn(III) chloride (MnTSPP) as a biomimetic lignin peroxidase-like catalyst (Fig. 3) [21].

Various synthetic metalloporphines (whose structures somewhat resemble that of the commonest metalloporphyrin in hemoenzymes, i.e. ferriheme) have been deeply studied [23] for their biomimetic catalytic activity in the presence of hydrogen peroxide acting as an oxidant [24–28]. In particular, their efficient lignin peroxidase-like activity [29–31] has been shown.

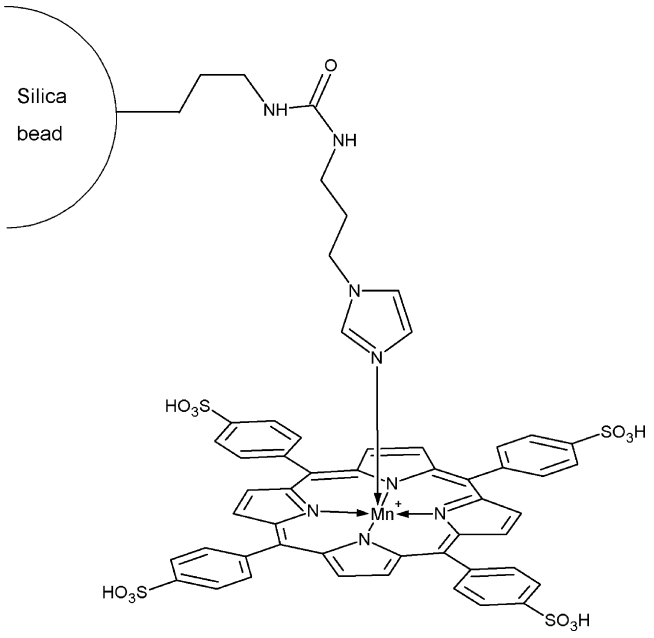

Fig. 3. The structure of the MnTSPP/IPS catalyst. MnTSPP is bound to IPS by means of an axial coordination bond between Mn(III) within the porphine ring and the imidazole nitrogen atom grafted on to the silica bead.

The wide range of potentially oxidizable substrates could enlarge the area of catalytic oxidative degradation of industrial dyes.

The advantages of MnTSPP over other redox-active metalloporphines are well known [32,33]; however, the use of this metalloporphine free in the reaction medium is limited by the need of efficient separation of the catalyst from products and unreacted substrates, and by side-reactions occurrence, such as metalloporphine dimerization through a μ-oxo bridge, and catalyst degradation [25].

Accordingly, in order to allow catalyst stabilization and facile recovery, MnTSPP immobilization on properly modified silica gel beads was carried out [21]: for a closer resemblance to hemoenzymes, the chosen immobilization strategy was based on grafting imidazole groups onto solid supports in order to coordinate metalloporphines. This is in agreement to the observation that the presence of imidazole in the reaction medium dramatically increases both catalytic activity and stability of such catalysts [26,34]. In this paper, we report the catalytic properties of immobilized MnTSPP towards PNS degradation by hydrogen peroxide.

Also, PNS degradation was investigated under a wide range of experimental conditions, either involving or not redox enzymes. The results obtained under various experimental conditions were compared, and a mechanism for the PNS degradation is proposed.

2. Experimental

2.1. Chemicals

All the reagents used were of the best grade availability, and were used without further purifications. In particular, Phenosafranine and Silica Gel 100 came from Fluka (cat. No. 199648 and 60746, respectively) and MnTSPP was purchased from Sigma–Aldrich (cat. No. 441813). Horseradish peroxidase (E.C. 1.11.1.7) and LiP (E.C. 1.11.1.14) were from Sigma–Aldrich (cat No. P-6782 and 42603, respectively). Fungal laccase (E.C. 1.10.3.2) was purified from *Pleurotus sajor-caju* liquid cultures as described elsewhere [35].

2.2. Methods

2.2.1. Instrumentation and analysis

Spectrophotometric measurements were carried out with an UltroSpec 2100pro, Amersham Bioscience.

PNS concentration was also measured with a Beckman System Gold apparatus equipped with an UV–Vis detector module. Before performing HPLC analysis, all samples were centrifuged at 10,000 × *g* for 10 min. A deproteinization step with syrupy phosphoric acid (0.05 M final concentration) was added prior to centrifugation for samples containing enzymes (*vide ultra*). The resulting supernatants were then immediately injected. The column used for chromatographic separations was an ODS-Hypersil (Hewlett-Packard), 250 mm × 4 mm i.d., 3.5 μm particle size. Runs were performed at room temperature with 0.085% phosphoric acid in water (solvent A) and 95% acetonitrile in 0.085% phosphoric acid (solvent B) as the mobile phase. Chromatographic conditions: initial 10% B, 10 → 90% B in 7′ at 1 mL min^{-1} flow rate.

2.2.2. Preparation of MnTSPP/IPS adduct and catalytic activity measurements

MnTSPP/IPS adduct was prepared as described elsewhere [21]. Briefly, silica gel was added to a pre-reacted mixture of (3-isocyanatopropyl)triethoxysilane and *N*-(3-aminopropyl)imidazole, and the obtained 3-(1-imidazolyl) propylcarbamoyl-3-aminopropylsilica (IPS) was added to a MnTSPP aqueous solution. The obtained MnTSPP/IPS adduct was washed, dried and stored in the dark until use.

PNS bleaching was quantified at 25 °C by a photometric measurement: 10 mg of MnTSPP/IPS catalyst (containing 0.14 mg MnTSPP) were added, in a final volume of 1 mL, to 25 mM buffer, 8.8 mM H_2O_2 and 0.3 mM PNS. A blank was prepared without hydrogen peroxide, and another one with hydrogen peroxide and without catalyst. The mixtures were kept under stirring in the dark at 25 °C for 30 min, then pH was adjusted to 7 with 0.1 mL of 1 M McIlvaine buffer (pH 7) and the absorbance decrease at 525 nm was recorded (measured ε_{525} = 32,000 M^{-1} cm^{-1}).

UV–Vis spectra of the reaction mixtures were also recorded at established times in the range of 230–700 nm.

In order to test MnTSPP/IPS ability in degrading PNS at various pH values some McIlvaine buffers were used: 3, 4, 5, 6, 7, and 8.

The catalyst was evaluated for multicyclic use by repeating the assays several times. Between cycles, the catalyst was regenerated by exhaustive washings with water and 2-propanol.

Where used, redox mediators (OH-TEMPO, NHP, NHS, and NHT) were present in the reaction medium at the final concentration of 1 mM. To evaluate hydroxyl radical formation and effect on PNS bleaching, control experiments were performed by adding 100 mM mannitol under the same conditions as above.

Additional experiments in the presence of the hydroxyl generator, APH, were also carried out, where 0.3 mM PNS was incubated with 20 mM APH, for 1 h at room temperature.

Michaelis and Menten kinetic parameters for both H_2O_2 and PNS were calculated by using R 2.5.1 software (*R Foundation for Statistical Computing, Vienna*). Results were averages of at least three independent determinations, and data were reported as mean value ± standard deviation (SD).

2.2.3. Degradation experiments with redox enzymes

When horseradish peroxidase was used, up to 20 Enzyme Units (E.U.) were present in a final volume of 1 mL of 25 mM buffer (pH 4, 5, 6, or 7) and 0.3 or 0.03 mM PNS. H_2O_2 concentration was 8.8 mM. One E.U. was defined as the enzyme amount capable of oxidizing 1 μmol of 2,2′-azino-bis(3-ethylbenzothiazolinone-6-sulfonic acid per minute at pH 6 and 25 °C.

The same conditions were adopted for the experiments with laccase; every experiment was repeated with and without added H_2O_2. One E.U. was defined as the laccase amount capable of oxidizing one micromole of syringaldazine per minute at pH 6 and 25 °C.

In the case of LiP, 0.2 E.U. were present in the final volume of 1 mL, and the H_2O_2 concentration was 0.176 mM. PNS concentration was set at 0.03 mM. For certain experiments, 0.1 mM veratryl alcohol (final concentration) was added to the reaction mixtures. One E.U. was defined as the LiP amount capable of oxidizing one micromole of veratryl alcohol per minute at pH 3 and 30 °C.

2.2.4. Uncatalyzed chemical oxidation of PNS

PNS was oxidized by ammonium peroxodisulfate in aqueous HCl, as already described [8]; also additional experiments were carried out with the same oxidant, but with 0.3 mM PNS concentration. In addition, different pH values, i.e. 4 and 7, were tested.

Moreover, other experiments were carried out using Mn(III) as the putative oxidizing agent. To this purpose, 1 mM manganese triacetate was dissolved in 50 mM sodium malonate buffer, pH 6.5 [36], and the final mixture contained this solution along with 0.3 mM PNS.

3. Results and discussion

3.1. Chemical oxidation of PNS

A complex mixture of oligomers and insoluble polymers arose [8], when 0.2 M PNS was oxidized by 0.5 M potassium peroxodisulfate dissolved in 0.2 M HCl (Fig. 4).

However, PNS concentrations as high as 0.2 M are quite unlikely in industrial wastewaters, and therefore a far lower concentration was tested in this study.

No any precipitate was seen during our experiments when 0.3 mM PNS was oxidized by peroxodisulfate. The dye was gradually bleached under all the tested pH values (Fig. 5). This is not surprising, taking into account the comparatively very low PNS concentration used; the formed nitrenium dication most probably underwent quick hydrolysis leading to a reactive quinone and further decomposition of the latter to colorless compounds. The efficacy of peroxodisulfate as an oxidizing agent towards PNS is also dependent on its concentration: below 10 mM it did not work at all.

It is also worth noting that concentrations of residual PNS obtained by HPLC analysis were in all experiments strictly proportional to absorbance decrease at 525 nm, so that decrease was assumed as a measure of PNS degradation.

Under the experimental conditions described here for the proposed method (H_2O_2 concentration as low as 8.8 mM in the presence of the MnTSPP/IPS catalyst), no oxidative polymerization became evident: on the contrary, a noticeable bleaching of the dye took place, leading to a clear and almost colorless solution (Fig. 6), where 87% PNS had been degraded within 20 min at pH 8. In particular, the observed spectral UV/Vis bleaching pattern noticeably differed from that observed with 0.5 M potassium peroxodisulfate at pH 7: a small absorption peak centered at about 295 nm was seen after peroxodisulfate action, whereas a small peak at 340 nm arose upon H_2O_2 treatment in the presence of our catalyst. As a point of fact, complete PNS inertness was found when 8.8 mM H_2O_2 was used in the absence of the MnTSPP/IPS catalyst. Moreover, control experiments with our catalyst in the absence of H_2O_2 showed that the dye remained perfectly unchanged. The same complete inertness of PNS was observed with plain silica gel and with IPS, both in the presence or in the absence of hydrogen peroxide.

The bleaching efficiency was dependent on pH (Fig. 7), in a different manner from that observed for alizarin red S under the same experimental conditions [33], therefore suggesting that the actual ionization state of the dyes must play a role at least as important as that of the catalyst itself. PNS exists as a monocation within the wide pH range studied here, and therefore in this case the varia-

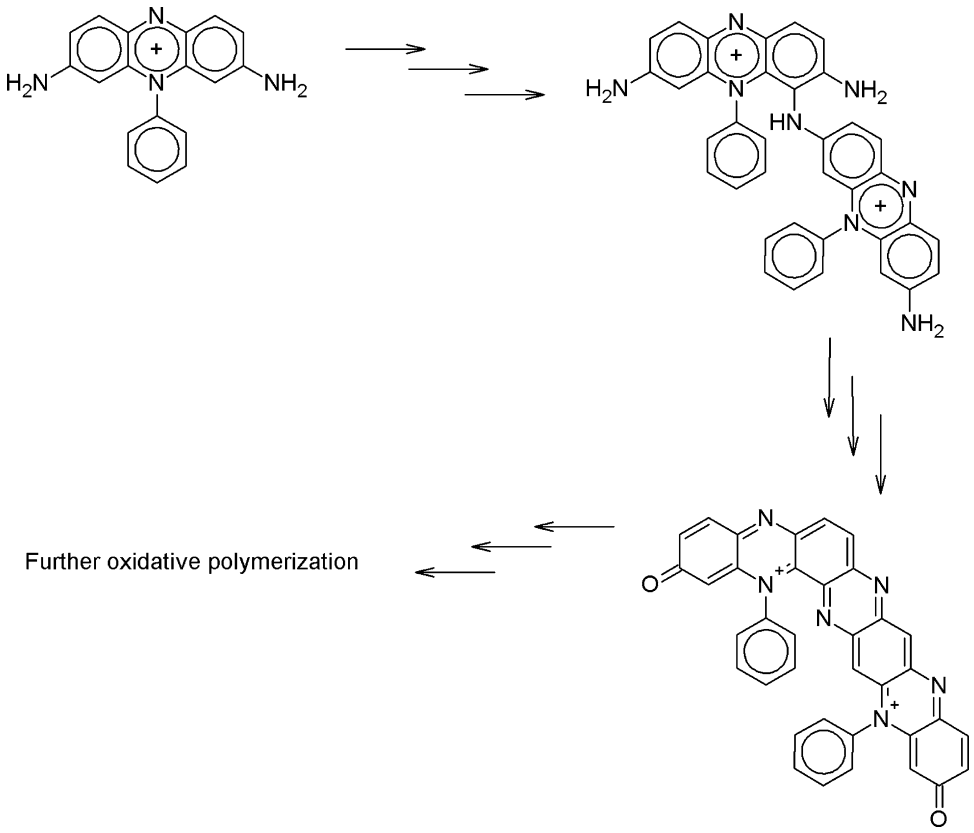

Fig. 4. The oxidative oligomerization of PNS upon treatment with ammonium peroxodisulfate in 0.2 M HCl. The very strong electrophile, nitrenium dication, arising from peroxodisulfate action, is the key intermediate for PNS dimerization and subsequent oligomerization.

tion in the catalytic efficiency should be ascribed exclusively to the catalyst.

The chosen pH range was 3–8, taking into account that the metalloporphine leaching was observed at pH values below 3 (as a consequence of imidazole protonation), and, on the other hand, slow solubilization of amorphous silica gel above pH 8 is a well-known phenomenon.

As recently confirmed [37] the first reaction product between a manganese porphine and hydrogen peroxide is a hydroperoxide derivative somewhat resembling the Compound 0 of peroxidases [38]. This could act directly as an oxygenating species, or further evolve to another product: in fact, the MnTSPP-derived high-valent species has been found to be a $Mn^{(V)}=O$ species, and this is presumably further stabilized by a coordinative bond with the imidazolyl moiety [39] on functionalized silica beads (the occurrence of such a coordination bond has been assessed previously [21]). This very reactive 'oxomanganyl' intermediate [40] somewhat paralleling the 'Compound I' in peroxidase catalytic cycle, could evolve further by following in principle two different routes: (a) the addition of one electron (from the oxidizable substrate) leading to a $Mn^{(IV)}=O$ intermediate [41] (and comparable to the 'Compound II' in the catalytic cycle of peroxidases), or (b) direct oxygenation of the oxidizable substrate leading to catalyst regeneration. By following this latter path, MnTSPP/IPS should be regarded as a P-450 emulator rather than a peroxidase-like catalyst, with two obvious differences: (i) the oxygen donor is H_2O_2 instead of the quite inexpensive O_2; (ii) on the other hand, no any sacrificial (and expensive) reductant is needed to obtain the hypervalent active intermediate.

Obviously, these two routes (a) and (b) could well co-exist, with relative importance depending on a number of conditions.

An alternative Fenton-like mechanism, involving the direct release of $OH^{\bullet}$ radicals upon interaction of Mn(III)TSPP with H_2O_2 and concomitant formation of a $Mn^{(IV)}$ hydroxospecies, has been

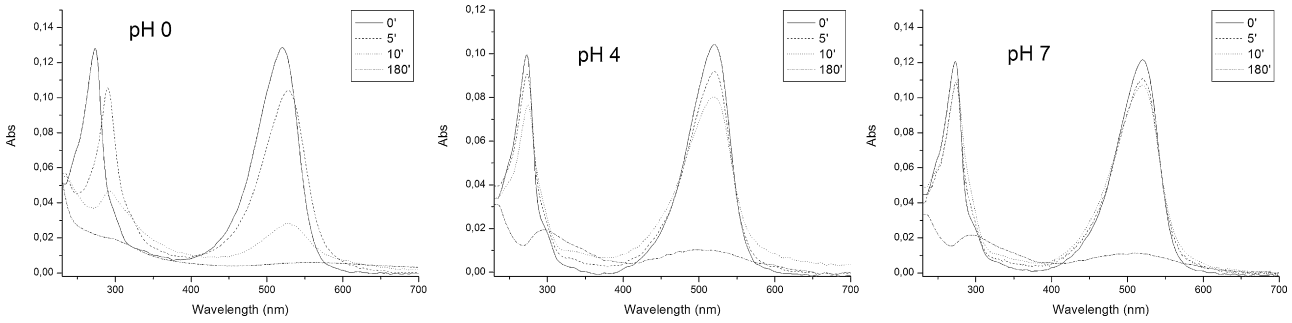

Fig. 5. PNS bleaching in the presence of 0.5 M ammonium peroxodisulfate at different pH values. 0.5 M peroxodisulfate was very effective as a bleaching agent for PNS along a wide pH range.

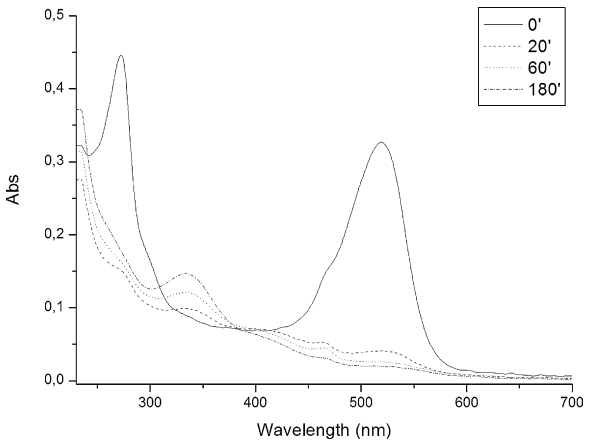

Fig. 6. PNS bleaching in the presence of 8.8 mM H_2O_2 and MnTSPP/IPS catalyst at pH 8. A nearly complete bleaching of PNS was seen under the described experimental conditions.

ruled out by earlier studies [32]. To corroborate this statement, some additional experiments were performed to gather information for depicting a reasonable reaction mechanism. The addition of the well-known hydroxyl radical scavenger, mannitol, to the reaction mixture containing the MnTSPP/IPS catalyst was void of any significant influence on the PNS oxidative bleaching; conversely, incubation of PNS with the well-known $OH^{\bullet}$ generator, APH, resulted in no alteration of the dye. The hydroxyl radical was therefore not involved in PNS bleaching, at least under the experimental conditions adopted here.

In another series of experiments, some well-known redox mediators were added to the reaction mixtures. Not surprisingly, a noticeable quenching of the catalytic action was observed with all the mediators, with share the feature of being oxygen-centered radicals, presumably less reactive than $OH^{\bullet}$ is. On the other hand, another dye, alizarin red S, tested under quite similar conditions, was bleached more rapidly in the presence of NHS and NHT [33]. The two catalytic routes (a) and (b) could therefore act at the same time, with a relative contribution depending on the particular substrate to be oxidized. In the case of PNS, its ionization energy of 7.98 eV [8] renders the abstraction of one electron to form the radical dication unlikely (although it could be possible), and the oxygen-centered radicals arising for the used redox mediators under the described experimental conditions are as seen unable to promote this process. Their action rather wasted the oxidizing power of the oxomanganyl reactive species arising from H_2O_2 action on the catalyst.

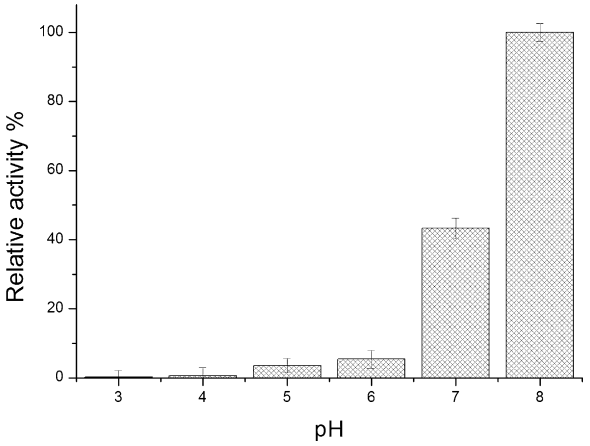

Fig. 7. Bleaching efficiency of PNS by H_2O_2 in the presence of MnTSPP/IPS catalyst as a function of pH. A sharp maximum at slightly alkaline pH made the method even more promising for future practical applications.

The direct, comparatively fast oxygenation (as alternative to oxidation) of the dye (or/and of any very reactive intermediate(s) along the degradation process) by the oxomanganyl reactive species could be therefore the main mechanism, triggering the bleaching process. The structure of the colorless species, absorbing at 340 nm and thus speaking of a deep destruction of the phenylphenazinium chromophore, gradually arising as the dye was bleached, is still waiting for a chemical characterization. It is worth noting that no intermediates were observed during the catalytic PNS bleaching. As a one-step bleaching of the dye seems quite unlikely, one could reasonably conclude that the formed putative intermediates should be much more sensitive to the oxidation than PNS is, such preventing their detection with the analytical techniques used here.

3.2. Enzymatic oxidation of PNS

As the MnTSPP/IPS adduct is a bio-inspired catalyst somewhat resembling hemoenzymes, it seemed reasonable to test the ability of commercial HRP to promote PNS bleaching by H_2O_2. However, no any catalytic activity was found within the tested pH range 3–8, even when a large excess (20 E.U. mL^{-1}) of the enzyme was present, and PNS remained quite unchanged even after several hours of incubation. This is not so surprising, taking into account the comparatively low redox potential of this enzyme.

The same inertness was found when a fungal LC was tested, either alone or in the presence of H_2O_2. This latter was added by following the idea that a putative radical species, arising from the enzyme action, could be oxidized by hydrogen peroxide, so enhancing the action of the enzyme on PNS. In fact, a synergistic action between LC and H_2O_2 has been described in the case of alizarin red S oxidative bleaching [33]. Such a synergistic action clearly did not take place in the case of PNS, where LC was quite unable to promote the first one-electron abstraction reaction, therefore rendering the presence of H_2O_2 useless.

Totally different was the case of LiP: here both H_2O_2 and PNS concentrations had been substantially reduced, to prevent inhibition by substrate (not shown here). As expected, the enzyme was inactive at pH 7 but active at pH 4.5, with a maximum activity at pH 3 (Fig. 8). The spectral pattern of PNS bleaching versus time was considerably different from that observed with the MnTSPP/IPS catalyst; on the contrary, the spectral pattern was similar to that observed when PNS was treated with potassium peroxodisulfate, therefore suggesting a similar mechanism. This conclusion is in agreement with the well-known LiP behavior, as this enzyme is a true peroxidase and cannot act as a mono-oxygenase. In fact, as for other similar peroxidases, LiP catalytic cycle consists in two consecutive one-electron abstractions from the substrate molecule, following enzyme oxidation by peroxide. Such a behavior could be compared (Figs. 5 and 8) to the action mechanism of peroxodisulfate, which reversibly dissociates in two sulfuryl radical anions $SO_4^{-\bullet}$. Such radical anions are the true active species, acting as one-electron oxidant.

At pH 3, LiP action was very fast, but it stopped within a few minutes while the PNS degradation was far from completion. This stop was not due to H_2O_2 exhaustion: addition of further hydrogen peroxide to the reaction mixture was void of any significant effect on the spectrum (not shown). The inspection of the spectral pattern of LiP action reveals that not even this enzyme was able to promote complete PNS degradation, as shown by the arising and persistence of a small absorption peak at about 295 nm, whereas the visible peak centered at 525 nm was replaced by a low peak at 534 nm. This corresponded to a new compound, much more polar than PNS. Only 58% PNS was bleached after 10 min, as confirmed by HPLC analysis and the reaction did not go further, thus

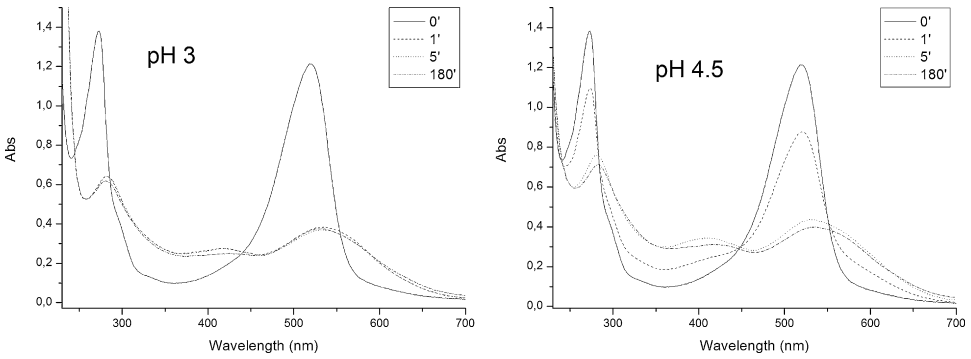

Fig. 8. PNS bleaching in the presence of 0.176 H_2O_2 and LiP at pH 3 (left) and pH 4.5 (right). The maximum efficiency in enzymatic bleaching was seen, as expected, at pH 3.

suggesting LiP inactivation by reaction product(s) such as the polar, colored compound absorbing at 534 nm. Therefore one could reasonably conclude that our catalyst and LiP differ fundamentally in their action mechanism, and H_2O_2 in the presence of immobilized MnTSPP is NOT an emulator of the H_2O_2/LiP system.

As the physiological intermediacy of veratryl alcohol (3,4-dimethoxybenzyl alcohol) in LiP catalysis is well known [42], further experiments were carried out where this redox mediator was present together with H_2O_2 and LiP. It adversely affected the bleaching process, which was slower and incomplete, and a new peak, most probably corresponding to veratraldehyde, arose at 310 nm (Fig. 9).

Only marginal changes in PNS spectra were observed in the presence of Mn(III)/malonate complex (not shown), therefore suggesting that manganese peroxidase should be substantially inactive towards the dye.

3.3. Kinetics and reuse

The catalyst showed saturation behavior with both the substrates H_2O_2 and PNS, and therefore a Michaelis-Menten-like treatment was applied. The obtained kinetic parameters are shown in Table 1. The K_M for PNS is quite lower than that obtained for alizarin red S [33]. This behavior could be explained by considering the anionic character of MnTSPP which could facilitate the approaching of the cationic PNS (alizarin red S is anionic).

The catalyst was also checked for a possible reuse. After PNS bleaching, the catalyst was recovered, washed, and reused. After six cycles, 50% of catalytic activity was still present (Table 2).

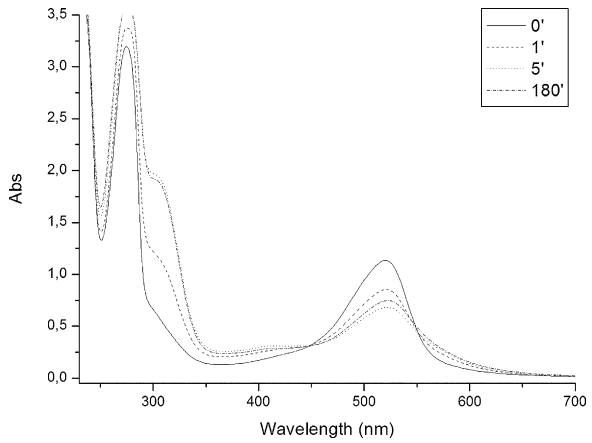

Fig. 9. PNS bleaching in the presence of 0.176 H_2O_2 plus 1 mM veratryl alcohol and LiP at pH 3. Veratryl alcohol prevented PNS degradation by LiP as it behaved rather as a competitive substrate than a redox mediator.

Table 1
Kinetic parameters for PNS bleaching by H_2O_2 in the presence of MnTSPP/IPS catalyst.

Substrate	Kinetic parameter	Value
PNS	K_M	0.32 ± 0.04 mM
	k_{cat}	2.1 ± 0.1 min^{-1}
	k_{cat}/K_M	7.0 ± 1.2 mM^{-1} min^{-1}
H_2O_2	K_M	3.0 ± 0.4 mM
	k_{cat}	1.9 ± 0.1 min^{-1}
	k_{cat}/K_M	0.63 ± 0.13 mM^{-1} min^{-1}

Table 2
Residual activity of the recycled MnTSPP/IPS catalyst.

Cycle	% residual activity
1	100
2	73
3	70
4	61
5	51
6	49
7	44

This could be explained if one considers that a fraction of original PNS remained tightly adsorbed on to the catalyst also when the supernatant was completely decolorized. This adsorbed dye most probably worsened the catalyst performance by a fouling effect whose occurrence is well known for heterogeneous catalysts. Also, the gradual oxidative destruction of the immobilized metalloporphine has to be taken into consideration, as underlined for high-valent oxometalloporphines by Rebelo et al. [37] and already observed for the same catalyst [33]. Inspection of FT-IR spectra of the exhausted catalyst corroborate this hypothesis whereas no any IPS alteration could be detected.

4. Conclusion

PNS (and probably other phenylphenazinium dyes) could be efficiently destroyed in aqueous environments under very mild conditions by oxidation with dilute hydrogen peroxide in the presence of the MnTSPP/IPS catalyst. Two catalytic mechanisms, which probably work at the same time, could be envisaged: the one being peroxidase-like, and the other one resembling the P-450 mode of action as a mono-oxygenase.

The proposed method is superior in comparison with alternative treatments involving peroxodisulfate (oxidant concentration far higher than stoichiometric) or LiP (incomplete bleaching, formation of a colored degradation product, poor efficiency due to inactivation by H_2O_2, expensive and rather unstable enzyme). Therefore

our system could be developed and scaled up in the view of future technological applications for the removal of PNS and/or chemically related dyes from industrial wastewaters.

Further studies are in progress to optimize the operative conditions, to explore new supports and methods to immobilize MnTSPP or also other metalloporphines, and to test other chemically related dyes.

Role of the funding source

Funding of this research came from the Italian Ministry of University and Research (FIRB Project RBAU01CJP9), and from Regione Autonoma Sardegna (PO Sardegna FSE 2007-2013, L.R.7/2007 "Promozione della ricerca scientifica e dell'innovazione tecnologica in Sardegna"). They had no any role for the conduct of the research and/or preparation of the article; in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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