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Degradation of Alizarin Red S under mild experimental conditions by immobilized 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine–Mn(III) as a biomimetic peroxidase-like catalyst

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ABSTRACT

Alizarin Red S, an industrially important anthraquinone dye coming from alizarin sulfonation, is a widespread polluting dye, whose inexpensive and environmental friendly elimination from effluents is a serious challenge. In this study we report its decoloration and partial mineralization by H_2O_2 in the presence of the heterogeneous catalyst obtained from 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine–Mn(III) immobilized on a modified silica support bearing imidazole functionality. The bleaching process was efficient and took place under very mild experimental conditions, where it mainly led to phthalic acid. Some operational features of the catalytic reaction are described and a chemical mechanism for the bleaching is proposed. Most efficient decoloration was seen at pH 7, the process most probably passing through an unstable anthradiquinone. Also some kinetic features of the catalyst have been studied, and a substrate saturation behavior for both hydrogen peroxide and the dye was observed and quantitatively determined.

1. Introduction

Alizarin Red S (1,2-dihydroxy-9,10-anthraquinonesulfonic acid sodium salt, ARS, alias Mordant Red 3, C.I. no. 58005, Fig. 1) is a water-soluble, widely used anthraquinone dye synthesized by sulfonation of alizarin, a natural dye obtained from madder (*Rubia tinctorum* L., Rubiaceae) and known since third millennium B.C. [1,2]. ARS is used by textile industry, and finds other applications such as for histochemical staining [3].

Anthraquinone dyes like ARS are recalcitrant and durable pollutants, released especially by textile industries in the aquatic ecosystems [4]. Removal of these from industrial wastewaters is a crucial process, from both economical and environmental points of view. Unfortunately, the methods developed to this purpose seem to be largely inadequate. The classical dye degradation processes are based on chemical, physical and biological oxidation [5–7]; however, they are not always enough efficient. Recently, new approaches have been described using photocatalytic [8–10] and electrochemical [11,12] means: but they are often affected by economical concerns.

To overcome some of the above-mentioned drawbacks, in this paper we propose a novel and inexpensive method for ARS degradation: diluted hydrogen peroxide and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine-manganese(III) chloride (MnTSPP) immobilized on imidazolyl-modified silica gel (IPS), acting as a biomimetic lignin-peroxidase-like catalyst (Fig. 2).

Both iron(III) and manganese(III) synthetic metalloporphines have been exhaustively studied for their biomimetic catalytic activity in the presence of hydrogen peroxide acting as an oxidant [13–15]. In particular, they showed efficient cytochrome P-450-like activity in catalyzing alkane hydroxylation [16–19] and alkene epoxidation [19–23]. A lignin-peroxidase-like activity has also been shown [13,14,24–26].

This wide range of potentially oxidizable substrates suggests a possible application of such metalloporphines also in catalytic oxidative degradation of industrial dyes.

Abbreviations: ARS, Alizarin Red S; IPS, (3-[1-imidazolyl]-propylcarbamoyl)-3′-aminopropylsilica; MnTSPP, 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine-manganese(III) chloride; ABTS, 2,2′-azino-bis(3-ethylbenzothiazolinone-6-sulfonic acid) diammonium salt; APH, 2,2′-azobis(2-methylpropionamidine) dihydrochloride.

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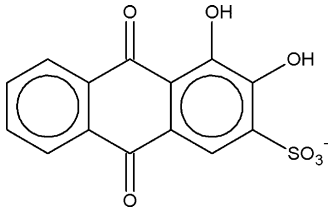

Fig. 1. Chemical structure of ARS.

We focused our attention on MnTSPP, because of its solubility in water, high redox potential and noticeable resistance against oxidative degradation by excess hydrogen peroxide (due to strong electron-withdrawing effect at the *meso* positions by 4-sulfonatophenyl substituents).

However, the use of metalloporphines free in the reaction bulk [20,21,25] is affected by economical matters and side reactions occurrence, such as μ-oxo porphine dimers formation [14].

Accordingly, in order to allow catalyst recovery, MnTSPP immobilization on a solid support was considered. Metalloporphine immobilization has already been achieved by using different approaches: covalent binding [27,28], adsorption [18,29] or ion exchange [30]. Unfortunately, only the covalent bond approach ensures adequate interaction strength. But, not even in this case, there is a real emulation of peroxidase or cytochrome P-450 active site, where the metal ion within the porphyrin ring is coordinated by imidazole-N of the proximal histidine, or by thiol-*S* of the proximal cysteine.

This is not a secondary aspect, as the presence of imidazole in the reaction bulk dramatically increases the catalytic activity of such catalysts [15,20]. Grafting imidazole groups onto solid supports in order to coordinate metalloporphines has been described earlier [23,31].

In this perspective, we have already immobilized MnTSPP on a silica support containing imidazole residues [32]: and in this paper we report the catalytic properties of this adduct on ARS degradation.

To our knowledge, this is the first report on the use of an immobilized metalloporphine as a catalyst for the oxidative degradation of an anthraquinone dye.

2. Experimental

All the reagents used were of the best grade available, and were used without further purification. In particular, Silica Gel 100 came from Fluka (cat. no. 60746) and MnTSPP was purchased from Sigma–Aldrich (cat. no. 441813).

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

MnTSPP/IPS adduct was prepared as described elsewhere [32].

ARS decoloration was quantified at 25 °C by spectrophotometric assay: 10 mg of catalyst were added, in a final volume of 1 mL, to 25 mM buffer, 8.8 mM H_2O_2 and 290 μM ARS. A blank was prepared without hydrogen peroxide. The mixtures were kept stirring in the dark at 25 °C for 30 min, then pH was corrected to 7 with 0.1 mL of McIlvaine buffer 1 M (pH 7) and the absorbance decrease at 520 nm was recorded (ε_{520} = 7200 M^{-1} cm^{-1}, [9]).

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

In order to test MnTSPP/IPS ability in degrading ARS at various pH some McIlvaine buffers were used: 3, 4.5, 6, 7, and 8.

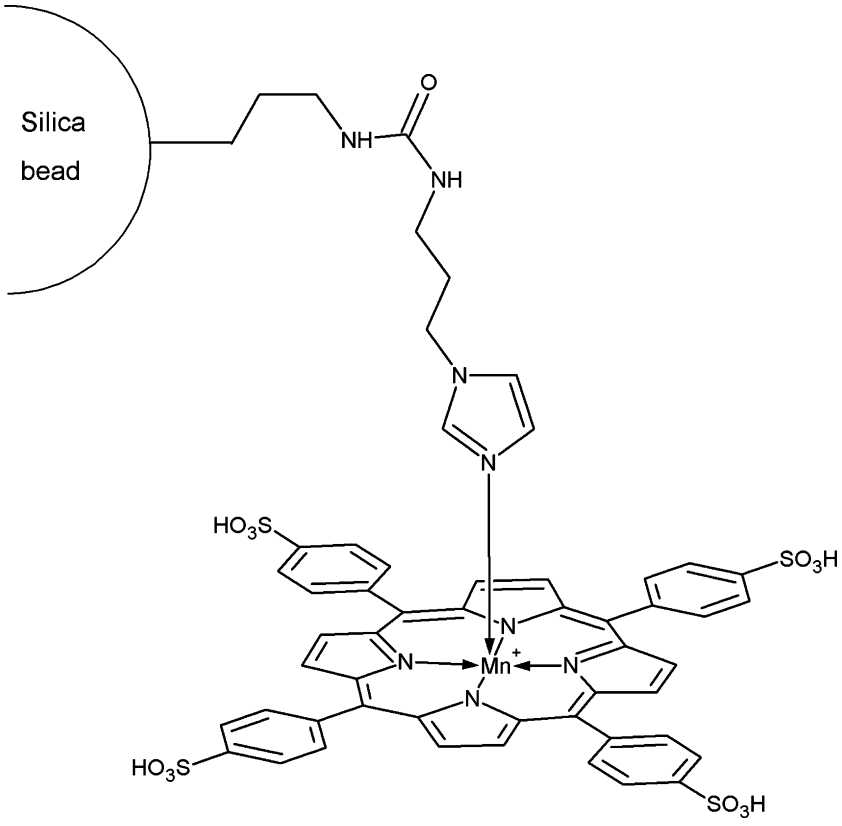

Fig. 2. The structure of MnTSPP/IPS catalyst.

Evaluation of catalyst multi-cyclic use was obtained by repeating the assays several times. Between cycles, the catalyst was regenerated through exhaustive washings with H_2O and 2-propanol.

Where used, redox mediators (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl, *N*-hydroxyphthalimide, 1-hydroxybenzotriazole and *N*-hydroxysuccinimide) were present in the reaction medium at the final concentration of 1 mM.

In order to evaluate hydroxyl radical formation and effect on ARS bleaching, control experiments were performed using, respectively, 100 mM mannitol and 20 mM APH, at the same conditions as above.

Michaelis and Menten kinetic parameters were calculated by using R 2.5.1 software (*R Foundation for Statistical Computing, Vienna)*.

2.2. HPLC analysis

Before performing HPLC analysis, all samples were centrifuged at 10,000 × *g* for 10 min. The resulting supernatants were then immediately injected.

Identification of the compounds was carried out with a Beckman System Gold apparatus equipped with an UV–vis detector module. The column used for chromatographic separations was an ODS-Hypersil (250 mm × 4 mm i.d., 3.5 μm particle size) purchased from Hewlett-Packard. Separations of the compounds were achieved 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 min at 1 mL min^{-1} flow rate.

2.3. Laccase effect on decoloration

Laccase was purified as described elsewhere [33].

ARS degradation by the enzyme was determined spectrophotometrically by monitoring the UV–vis spectral changes (in the range 230–700 nm), when 290 μM ARS was incubated in a final volume of 1 mL containing 23 E.U. laccase and 25 mM McIlvaine buffer, pH 6. For certain experiments, laccase acted in the presence of 8.8 mM H_2O_2. The same procedure as above was adopted to check ARS bleaching.

3. Results and discussion

The heterogeneous catalyst, whose preparation has been already described in detail [32], consisted of MnTSPP bound to IPS by means of coordinative bond between Mn(III) and the imidazole nitrogen of the solid silica matrix. In the presence of this catalyst, hydrogen peroxide became capable of efficiently bleaching ARS under very mild conditions (neutral pH, ambient temperature, low hydrogen peroxide concentration) (Fig. 3). As a point of fact, H_2O_2 alone was quite unable to bleach the dye under the same experimental conditions.

3.1. Dependence of catalytic activity on pH

The pH dependence of catalytic activity is reported in Fig. 4. Contrarily to that already seen in the case of the oxidation of veratryl alcohol by H_2O_2 in the presence of MnTSPP/IPS catalyst [32], where catalytic activity was almost pH-independent in the range 4.5–8, an optimum at pH 7 was found in ARS decoloration. ARS exists mainly as the monoanion (yellow) below pH 5.5, as the dianion (red) below pH 11, and is completely deprotonated under sharply alkaline conditions as the trianion (purple) [34]. The pH dependence of the oxidative bleaching strongly suggests that the process takes place on the dianion (Fig. 5).Over pH 7, the bleaching efficiency of the H_2O_2/catalyst system slightly decreased, most probably as the catalyst itself became less effective. Therefore pH 7 represents the best compromise between catalyst efficiency and substrate reactivity.

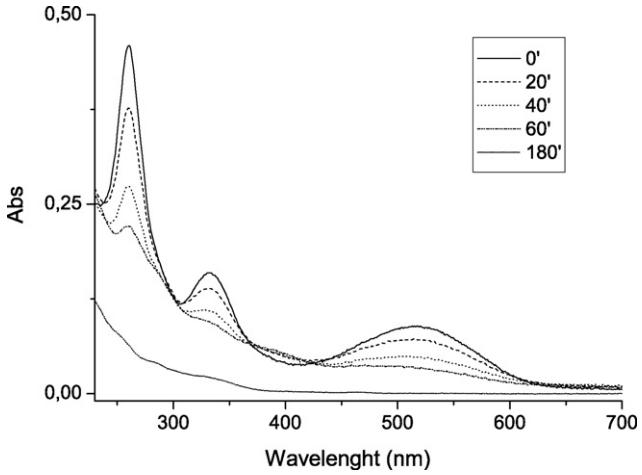

Fig. 3. Spectral changes during oxidative catalytic degradation of ARS by 8.8 mM H_2O_2 in the presence of MnTSPP/IPS catalyst in 25 mM McIlvaine buffer, pH 7.

3.2. Decoloration mechanism

Despite of the widespread occurrence of hydroxyanthraquinones in Nature and their use in several application fields, their oxidative degradation has been until now the target of a few studies, so the degradation mechanism(s) is largely unknown. Alizarin (1,2-dihydroxy-9,10-anthraquinone) bleaching by hydrogen peroxide in sharply alkaline environment has been studied in detail [35] and a mechanism involving double bond epoxidations as the key steps has been proposed.

As ARS could well represent a water-soluble, useful model for the oxidative degradation of anthraquinone derivatives, it has been the target of a number of studies. Electrochemical oxidation of ARS [11,12] and photoinduced oxidation over titania [8–10,36,37] have been described. All these studies proposed the involvement of reactive oxygen species – namely hydroxyl radicals – attacking the junction between the quinone and the catechol rings of the dye, and leading to a deep oxidative cleav-

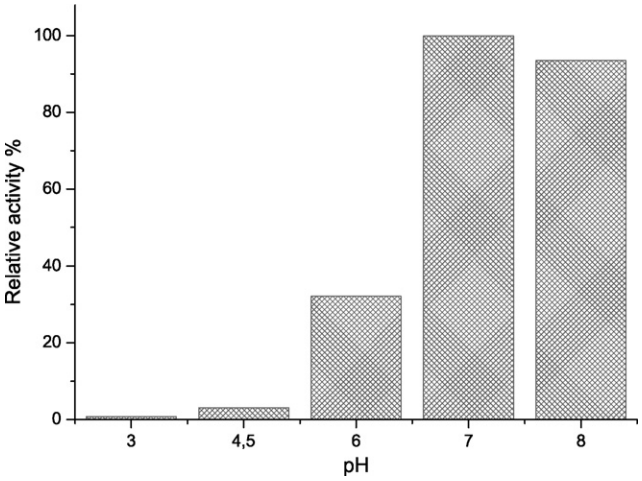

Fig. 4. Dependence on pH of the ARS degradation rate by H_2O_2 and MnTSPP/IPS. Data are obtained in the presence of 10 mg catalyst, 8.8 mM hydrogen peroxide, 290 μM ARS and 25 mM buffer (final volume 2 mL).

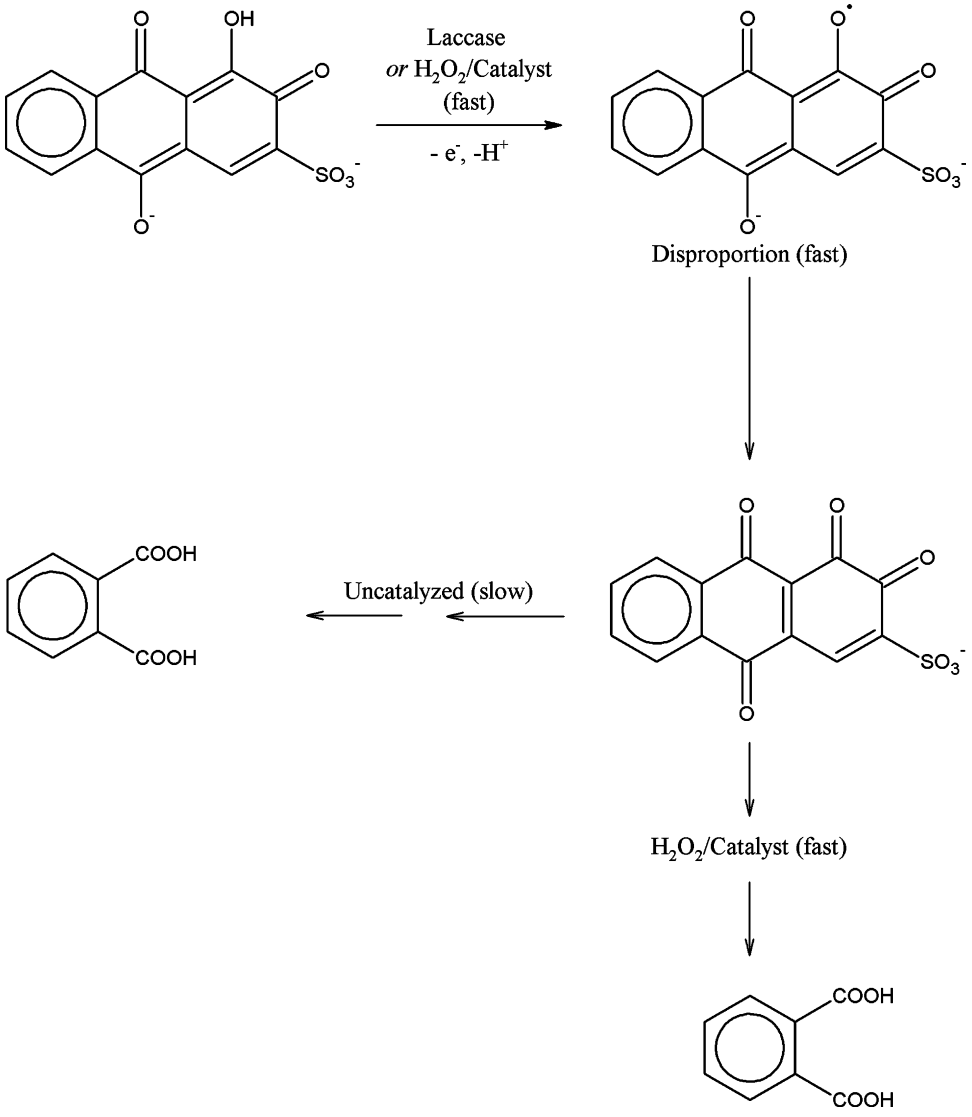

Fig. 5. The proposed route for ARS oxidative degradation.

age. The complete mineralization of the isolable intermediate phthalic acid under suitable experimental conditions has also been claimed.

With concern to our catalytic system, a mechanism involving hydroxyl radicals must be ruled out. In fact, treatment of ARS with the hydroxyl radical generator APH did not affect the dye under the described conditions. Moreover, addition of the hydroxyl radical scavenger mannitol could not influence ARS bleaching by hydrogen peroxide in the presence of MnTSPP/IPS.

Hydrogen peroxide reacted with MnTSPP/IPS leading to a PorphMn(V)=O intermediate, according to an earlier study [38] and to our finding that syringaldazine and ABTS, classical peroxidase substrates acting as one-electron donors, were rapidly oxidized by H_2O_2 in the presence of MnTSPP/IPS (not shown). By analogy, two consecutive monoelectronic reductions of PorphMn(V)=O to the resting state, leading to an ARS semiquinone radical, capable of evolving to the very reactive 1,2,9,10-anthradiquinone-3-sulfonic acid, could well be suggested. In other words, a reaction pathway strictly resembling that found with peroxidase or laccase should be considered. However, diquinones arising from hydroxyanthraquinones are – as expected – very prone to nucleophilic attack by water, so their isolation under the described experimental conditions is not possible. The formation of the same diquinone as above should have taken place also when ARS was treated with fungal laccase (ARS is so weak as a horseradish peroxidase substrate that the experiment involving this latter enzyme was useless). As laccase cannot catalyze reactions other than the extraction of one electron from phenolics leading to phenoxyl radicals (disproportion to quinones usually follows), the close similarity between the two spectral patterns strongly speaks in favor of the proposed formation of the diquinone as a key intermediate in ARS bleaching. However, MnTSPP/IPS in the presence of H_2O_2 was a much more efficient catalyst than laccase, perhaps because the former could act as an oxygen donor and therefore oxidize intermediates (as the above mentioned diquinone) that are simply nonsubstrates for laccase (Fig. 5). Accordingly, laccase plus H_2O_2 (the chosen concentration of the latter was unable to adversely affect laccase activity) was more efficient than laccase alone was, although not so efficient as seen with our biomimetic catalyst (Fig. 6).

However, despite of the speed and efficiency of the bleaching, no complete mineralization was achieved by H_2O_2/MnTSPP/IPS, and HPLC analysis after bleaching completion revealed the presence of phthalic acid as the main product of the process,

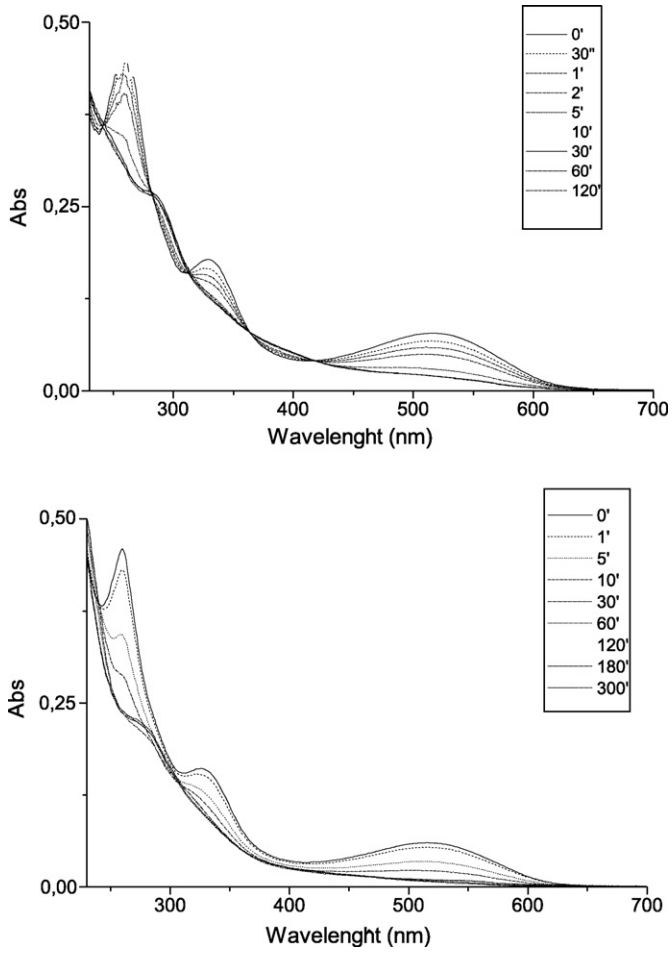

Fig. 6. ARS bleaching by laccase alone (top) and by laccase plus H_2O_2 (bottom). 23 E.U. laccase were present, dissolved in 25 mM McIlavine buffer, pH 6. When present H_2O_2 was 8.8 mM.

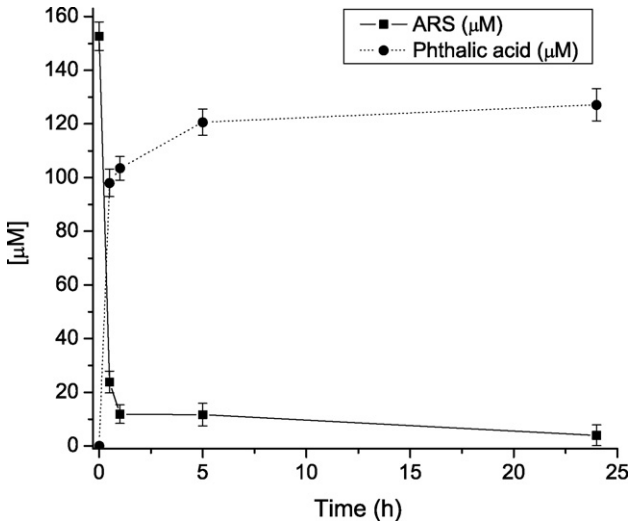

Fig. 7. Phthalic acid formation during ARS bleaching. Data obtained by HPLC analysis of the reaction mixture as described in the text.

Table 1
Kinetic parameters of the MnTSPP/IPS adduct in the presence of its substrates, H_2O_2 and ARS

Substrate	Kinetic parameter	Value
ARS	K_M (mM)	2.1 ± 0.7
ARS	k_{cat} (min^{-1})	0.14 ± 0.03
H_2O_2	K_M (mM)	3.2 ± 0.1
H_2O_2	k_{cat} (min^{-1})	0.13 ± 0.01

The assay mixture contained, in a final volume of 1 mL, 10 mg of catalyst, 8.8 mM hydrogen peroxide, 290 μM dye and 25 mM buffer pH 7. Absorbance decrease at 520 nm was then read after 30 min of dark stirring reaction at 25 °C ($n = 3$).

Table 2
Catalytic activity of the MnTSPP/IPS adduct upon multicyclic use: a final volume of 2 mL contained of 10 mg of catalyst, 8.8 mM hydrogen peroxide, 290 μM ARS and 25 mM buffer pH 7

Cycle number	% Catalytic activity
1	100
2	68
3	49
4	33
5	30
6	28
7	28
8	26

After 30 min of dark stirring reaction at 25 °C, absorbance decrease at 520 nm was detected. Between cycles, catalyst was regenerated through washings with H_2O and 2-propanol.

according to the relative chemical inertness of this compound (Fig. 7).

3.3. Kinetic behavior

The catalyst showed a saturation behavior with both the substrates H_2O_2 and ARS: so a Michaelis–Menten-like treatment could be well applied. The obtained kinetic parameters are shown in Table 1. Inspection of the table shows that the catalyst is semisaturated at relatively low ARS concentrations therefore enabling it to manage minute amounts of the dye in wastewaters. The k_{cat} values are in agreement with a reasonable bleaching speed.

3.4. Reuse of the catalyst

After the bleaching ended, the catalyst could be recovered by decantation/filtration, accurately washed and reused. After a noticeable drop in its catalytic efficiency, the activity tended to an almost stable value (Table 2). This behavior could be explained if one considers that a fraction of original ARS remained tightly adsorbed on to the catalyst also when the supernatant was completely decolorized. This adsorbed dye (it could be removed by 0.1 M

Table 3
The presence of some redox mediators seems to increase ARS decoloration rate

Redox mediator	% Catalytic activity
No redox mediator	100
4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl	98
N-Hydroxyphthalimide	103
1-Hydroxybenzotriazole	142
N-Hydroxysuccinimide	141

Reactions were performed in dark stirring at 25 °C in presence of 10 mg of catalyst, 8.8 mM hydrogen peroxide, 290 μM ARS, 1 mM redox mediator and 25 mM buffer pH 7 (final volume 2 mL). After 30 min, absorbance decrease at 520 nm was detected. Percentage of activity is referred to a control experiment in the absence of redox mediators.

NaOH washings) most probably worsened the catalyst performance by sterical hindrance or also by electrostatic repulsion.

3.5. Redox mediators

According to the hypothesis of a monoelectronic abstraction as the first step of the bleaching process, some redox mediators, capable of cycling between their 'resting' states and the oxidized, radical counterparts have been tested for their influence towards the decoloration process. Strong differences were observed, so that some compounds were quite ineffective, whereas others considerably enhanced the bleaching efficiency (Table 3). The reasons of these differences are still awaiting for a reasonable explanation.

4. Conclusions

In conclusion, the described catalytic bleaching process is characterized by a high efficiency combined with very mild conditions. The mineralization process stopped at the stage of phthalic acid, but this is anyway a substantial progress with respect to the parent anthraquinone dye. Dilute hydrogen peroxide is a relatively safe and inexpensive reagent, and the catalyst could be successfully recycled, rendering on the whole the bleaching process economically feasible. Further studies are in progress to optimize the features of the catalyst and the operational conditions.

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