Available online at www.sciencedirect.com
SCIENCE DIRECT
Dyes and Pigments 68 (2006) 227–234
DYES and PIGMENTS
www.elsevier.com/locate/dyepig

Degradation of pararosaniline (C.I. Basic Red 9 monohydrochloride) dye by ozonation and sonolysis

Aline de O. Martins [a], Vicente M. Canalli [b], Carla M.N. Azevedo [a], Marçal Pires [a,*]

[a] *Faculty of Chemistry, Pontifical Catholic University of Rio Grande do Sul, Av. Ipiranga 6681, Predio 12B, Porto Alegre-RS 90619-900, Brazil*
[b] *Faculty of Engineering, Pontifical Catholic University of Rio Grande do Sul, Av. Ipiranga 6681, 90619-900 Porto Alegre, Brazil*

Revised 28 January 2005; accepted 4 February 2005
Available online 11 April 2005

Abstract

Pararosaniline (C.I. Basic Red 9) is an important dye used in biological and chemical assays, suspected of being carcinogenic. Thus, the environmental and occupational issues related to it are very important. This study aims at investigating the chemical oxidation of pararosaniline dye by ozonation and sonolytic processes. Experimental results indicate that ozonation of pararosaniline solution is more efficient than ultrasonic irradiation alone or in combination with O_3. The detoxification was assessed by determining acute toxicity and mutagenicity of the ozonized dye solutions. Even for short-term ozonation (15 min) any mutagenic effect was verified although longer treatment periods (120 min) are necessary to obtain high toxicity removal. The results obtained indicate that ozonation is a powerful tool for the treatment of the pararosaniline wastes, even when compared to other advanced oxidation processes as photocatalytic and hydrogen peroxide dye degradation. The kinetics of different pararosaniline degradation techniques are also discussed such an understanding is necessary for the design and application of pararosaniline treatment processes.

Keywords: Ozone; Pararosaniline; Ultrasound

1. Introduction

Pararosaniline chloride, C.I. 42500, C.I. Basic Red 9 Monohydrochloride, also known as benzeneamine 4-(4-aminophenyl)(4-imino-2,5-cyclohexadian-1-ylidene)-methyl monohydrochloride, paramagenta, magenta or basic fuchsin is a triphenylmethane dye. It is used in the analysis of SO_2 and formaldehyde present in the air and water [1], as a pH indicator for the range of 1.0 (purple)–3.1 (red) and also as biological stain. In biological assays, the pararosaniline has been used for staining of bacteria, antibody or other organisms [2], in Feulgen reaction [3] and in Gomori's aldehyde-fuchsin method of staining elastic tissues [4].

Pararosaniline is suspected to be carcinogenic in animal experiments [5]. Its occupational and environmental issues are therefore of concern and especially the treatment and final disposition of pararosaniline wastes.

Ozonation is an effective way of degradation for a wide variety of dyes in aqueous solutions because it

* Corresponding author. Tel.: +55 51 33203500; fax: +55 51 3320 3612.
E-mail address: mpires@pucrs.br (M. Pires).

0143-7208/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2005.02.002

destroys conjugated double bonds often associated with color [6]. However, few studies have been reported about destruction of triphenylmethane dyes by ozonation [7,8]. None of these studies concerned the degradation of aqueous solution of pararosaniline or its wastes, although other techniques were used for the pararosaniline treatment [9–12].

The biodegradation of pararosaniline through the use of a laccase from *Trametes hirsuta* was reported [9]. Laccase catalizes the removal of the hydrogen from the hydroxyl group of the *ortho*- and *para*-substituted mono and polyphenolic substrates and from aromatic amines by one-electron abstraction, generating free radicals capable of undergoing further polymerization processes. Sani and Banerjee [12] used *Kurthia* sp. in decolorization test of seven triphenylmethane dyes. Among all the dyes, pararosaniline, was decolorized very rapidly (100% in 53 min).

Some authors [10,11] have reported studies about the photochemical stability of pararosaniline. Kosanic and Trickovic [11] present the process of photoassisted degradation of pararosaniline in aqueous solutions through treatment with visible light. The authors report that illumination of the pararosaniline solution in the presence of oxygen or argon leads to the decrease in dye absorption. Illumination of the dye solution in the presence of H_2O_2 promotes the degradation of the dye molecules. Additionally, the pararosaniline was easily decomposed in TiO_2 suspensions using oxygen and hydrogen peroxide electron acceptors.

The present study aims at investigating the oxidation of pararosaniline dye by ozonation and sonolysis processes on aqueous and acidic solutions. Since it has a high molar absorption coefficient ($\lambda_{max} = 540$ nm in visible region), the decrease in pararosaniline concentration during degradation processes can be monitored up to very small amounts. Besides color removal other parameters such as toxicity and mutagenicity, were used to assess treatment efficiency. GC–MS and ion chromatography analyses were performed to obtain information on by-products formation. The kinetics of different pararosaniline degradation techniques, from literature and this work, are also discussed. Such an understanding is necessary for the design and application of pararosaniline treatment processes.

2. Experimental

2.1. Materials

The pararosaniline chloride (88%, C.I. 42500) was obtained from Merck as a commercially available dye for microscopy (Certistain). The dye's chemical structure and main characteristics are shown in Fig. 1.

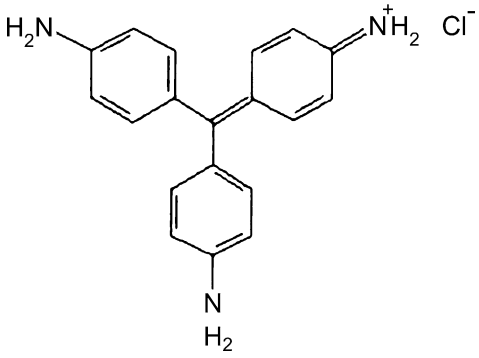

Fig. 1. Chemical structure and essential properties of pararosaniline (C.I. Basic Red 9): molecular formula: $C_{19}H_{18}ClN_3$, molecular weight: 323.834 g mol^{-1}; solubility: 0.26% in water, 5.9% in ethanol.

Since the efficiency of ozonation might be affected by the existence of impurities, the dye was purified following the recommended procedure in the SO_2 determination method [13]. Absorption spectra of the purified and not purified pararosaniline solutions (not shown) presented the same shape for the measured wavelength range (200–700 nm). All subsequent tests were performed with not purified pararosaniline solutions.

The aqueous solutions containing 1.2×10^{-5} mol L^{-1} pararosaniline were prepared with ultra pure water (MilliQPlus, Millipore). Considering that pararosaniline solution in HCl is used during the SO_2 and formaldehyde analysis (generating wastes that contain these compounds), experiments also were carried out using 1.2×10^{-5} mol L^{-1} pararosaniline in 0.01 M HCl. Additionally, as the solubility of the pararosaniline is more in acid than that in pure water, pararosaniline solutions in 0.005 M H_2SO_4 also were tested, as an alternative to HCl. The pH values of these solutions were 5.72–5.93 (H_2O) and 2.58–2.71 (HCl and H_2SO_4). All other reagents used were of analytical grade.

2.2. Ozonation

Ozone was generated from dried atmospheric air by a prototype corona discharge generator (OZ-Engenharia, Brazil), and driven by air pump with flow rate of 1.32 ± 0.04 L min^{-1}. Production rate was 35 mg O_3 h^{-1}, determined by iodometric (APHA) [14] and spectrophotometric [15] methods, which correspond to 0.4 mg O_3 L^{-1} on supplied gas. The on-line absorbance was measured simultaneously by UV–visible spectrophotometer (Micronal-B382) using a homemade quartz flow cuvette (path length 10 mm) at 258 nm. Pararosaniline solutions (0.15 L) were ozonized in a cylindrical glass reactor (volume 0.2 L) by bubbling ozone/air mixture into the solution through a sinterized glass filter (pore size 50–80 μm). Due to small reactor volume, 0.15 L fresh dye sample (stock solution) was used for each

reaction time period studied (2–120 min). After ozonation the samples were aerated for 5 min to remove residual ozone. Excess ozone leaving the reactor was trapped by two-sequential bubblers containing KI aqueous solution (2%). Then an iodometric titration procedure [14] was performed to measure the ozone concentration, trapped in the KI solutions, and this value was used to calculate the transferred ozone (produce ozone − trapped in KI). All experiments were conducted at room temperature (293 ± 1 K).

2.3. Ultrasound

Ultrasonic irradiations (US) were conducted in an ultrasonic bath (Unique USC 1450) at 25 kHz and 150 W, using the same glass reactor and sample volume used in ozonation tests. In the US/O_3 experiments, the glass reactor containing pararosaniline solution was appropriately positioned in the ultrasonic bath and the ozone/air mixture was constantly bubbled through the solution during the sonolysis. All ultrasound experiments were conducted in closed atmosphere at room temperature (293 ± 1 K) and no reactor cooling was provided.

2.4. Analysis

The absorption spectra (200–700 nm) of the dye solutions treated by ozonation and ultrasound were determined by a UV–visible spectrophotometer (Shimadzu UV-1601PC) through a 10 mm path length. In order to calculate the kinetics, the concentrations of the unconverted dye in solution were determined by the dye absorbance at maximum absorption wavelength (540 nm) in the visible region. Some kinetics calculations were also made using absorbance measured at UV region (206 nm).

The chemical oxygen demand (COD) was established using the closed reflux titrimetric method, according to the procedure described elsewhere [14]. Conductivity (Digimed DM-41) and pH (Digimed DM-21) were also determined in some tests.

2.4.1. Analysis of sub-products

Gas chromatograph (GC–MS) was used to identify some degradation products of pararosaniline. Firstly a screen analysis was made using 50 mL of aqueous solutions of the ozonized dye extracted with hexane and analysed using a gas chromatograph (QP5050A-Shimadzu) equipped with a fused-silica capillary column (30 m × 0.25 mm × 0.25 μm), detailed liquid–liquid extraction and chromatographic conditions are described in Ref. [14].

Determination of aldehydes and ketones present in ozonized solutions was carried out by derivatization with pentafluorobenzyldydroxilamine (PFBHA, Sigma Aldrich, 99.8%) according to a published procedure [16]. The oxime derivatives were analysed by GC–MS (Shimadzu QP 5050A) using a DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm). Carbonyl compounds were quantified by internal calibration using 4-bromofluorobenzene as internal standard.

Inorganic and organic anions were determined by ion chromatography (IC − Dionex DX500) operated in autosuppression recycle mode (ASRS-ULTRA II 4 mm, 50 mA) and equipped with a conductivity detector. Samples of 700 μL injected by an automatic sampler were eluted on a gradient mode (0.5–50 mM Na_2B4O_7) at a flow rate of 1.0 mL min^{-1} [17] through an IonPac AS11 analytical column (Dionex, 250 × 4 mm) and an IonPac AG11 guard column (Dionex, 50 × 4 mm). The analyses were carried out in triplicate and data analysis was made using software PeakNeat 5.0 (Dionex).

2.4.2. Toxicity measurement

The toxic effects of pararosaniline aqueous solutions before and after ozonation treatment were determined with *Daphnia similis* acute toxicity test, as described elsewhere [14]. The solutions ozonized during 0, 30, 60 and 120 min were tested and their pH was adjusted to a range 6.0–7.0 with NaOH prior to assay. The minimum dissolved oxygen was 6.7–6.8 mg L^{-1} and the microcrustacea were incubated in dark room at 293 ± 2 K for 48 h period. The samples were diluted at different rates ranging from 100% (no dilution) to 25% (1:4) using reconstituted dilution water. Results were evaluated on the basis of immobilization percentage obtained by dividing the number of immobilized animals by total animals ($n = 20$). The Effective Concentration 50% (EC_{50}) of the dye solution that causes toxicity was estimated by trimmed Spearman–Karber method at 95% confidence intervals (duration of exposure 48 h) [18].

2.4.3. Mutagenicity measurements

The Ames test was used to assess the mutagenic potency of the dye aqueous solution ozonized during 15 min. This test was performed with reverse induction of mutation for *locus his* in three strains of *Salmonella typhimurium* (TA98, TA100 and TA102) with the preincubation method in presence and absence of the metabolic activation of the post-mitochondrial fraction from the livers of rodents treated with Aroclor 1254 (S9). The tests were performed according to OECD − Guideline for Testing of Chemical [19].

The dose–response curves had been evaluated by statistical method SALANAL (EPA, software version 1.0, 1993 [19]. Analysis of variance (ANOVA) was used for statistical comparisons, with a value of $P \leq 0.05$ indicating a significant response. Dye solution was considered to be mutagenic when: (a) the number of revertant colonies in the assay was at least twice the

number of spontaneous revertants (MI $\geq$ 2) for strains tested, (b) analysis of variance (ANOVA) revealed a significant response ($P \leq 0.05$), and (c) a reproducible concentration–response curve ($P \leq 0.01$) was obtained.

3. Results and discussion

3.1. Ozonation and sonolysis applied to pararosaniline in acidic solutions

Fig. 2 shows the absorption spectra during the ozonation process of the pararosaniline solution (1.2×10^{-5} mol L^{-1}) prepared in 0.01 M HC1. A quick decrease in absorption band at 540 nm was observed, resulting in a decolorized solution, suggesting destruction of the dye chromophore structure by ozone. Under these conditions decolorization was completely by O_3 during 10 min reaction time. In these conditions the transferred ozone was calculated as 7 mg O_3 L^{-1} for the first 15 min of the reaction. For longer reaction times the difference (produce ozone − trapped in KI) became lower than the experimental errors.

Ozonation was more efficient than ozonation combined with ultrasonic irradiation, and showed a much better performance than the US process alone (Fig. 3). These results contrast with the ones reported in literature [20–22]. The reduction in the kinetic rate may be related to the temperature increase during US, causing greater decomposition of ozone and reduction of its solubility in water. These two factors probably contribute to the reduction of the O_3 concentration in solution.

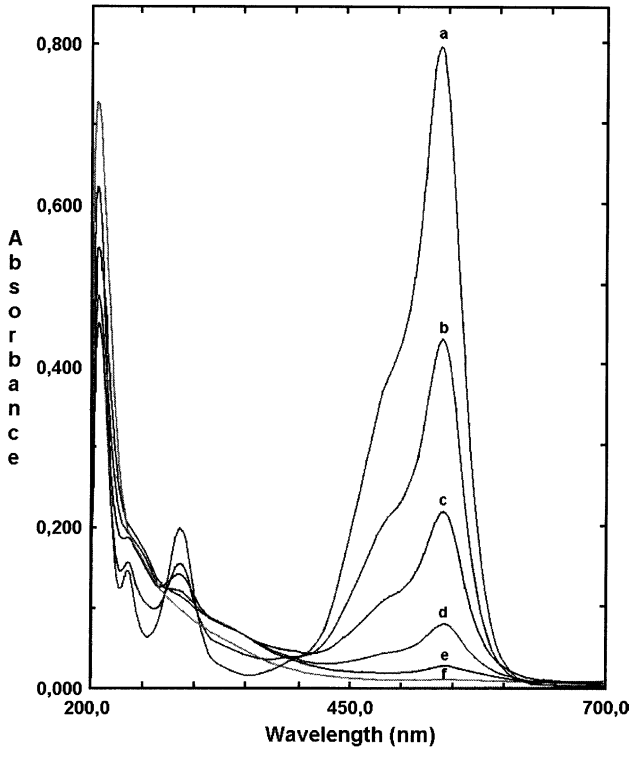

Fig. 2. Absorption spectra of the aqueous solution containing 1.2×10^{-5} mol L^{-1} pararosaniline ozonized during: (a) 0.0; (b) 2.0; (c) 4.0; (d) 6.0; (e) 8.0; and (f) 10.0 min at 293 K.

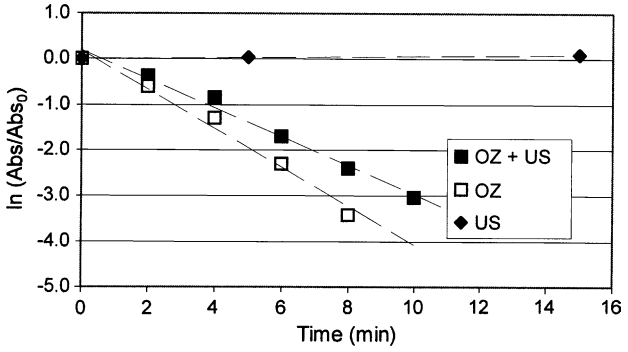

Fig. 3. First-order degradation of pararosaniline due to ozonation (OZ), sonolysis (US) and sonolytic ozonation (US + OZ), measured by absorbance (540 nm) at 293 K.

As observed in Fig. 3, dye degradation under ozonation and/or sonolysis processes follows pseudo first-order kinetics. Langlais et al. [23] also reported pseudo first-order kinetics for reactions between different triphenylmethane dyes and ozone. The rate constants and reaction half-times obtained for pararosaniline solutions, in different degradation systems, are presented in Table 1. No significant difference in rate constants was observed between aqueous and acidic dye solutions under ozone degradation (mean $t_{1/2} = 1.5$ min). A more detailed discussion about acidity influence is made in Section 3.1.1.

The poor effects of US alone on the rate constant may be attributed to the fact that low ultrasound frequencies hinder the development of OH radicals [21]. In addition, US treatment generally demands a high time of contact for a significant degradation efficiency [22]. These facts may partially explain the very low rate constant (0.006 min^{-1}) found for the US system (Table 1). In addition, as previously discussed, the combination of ultrasound with ozonation reduces the rate constant (~30%) of HCl dye solution degradation.

Table 1
Rate constant and reaction half-times of pararosaniline (1.2×10^{-5} mol L^{-1}) in different degradation systems (at max. absorption $\lambda = 540$ nm) at room temperature (293 K)

Systems	Solvent	k (min^{-1})	$t_{1/2}$ (min)	n	R^2
OZ (ozone)	H_2O	0.473	1.5	5	1.000
OZ	0.005 M H_2SO_4	0.491	1.4	5	0.988
OZ	0.01 M HCl	0.427	1.6	5	0.999
US (ultrasound)	0.01 M HCl	0.006	114	3	0.997
US + OZ	0.01 M HCl	0.316	2.2	6	0.985

n − Number of samples used in linear regression calculation.

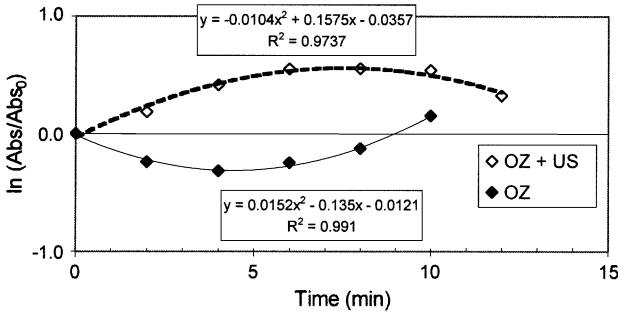

Fig. 4. Second-order reactions of pararosaniline and/or degradation by-products due to ozonation (OZ) and sonolytic ozonation (US + OZ) measured by absorbance (206 nm) at 293 K.

Fig. 4 shows the second-order reactions for pararosaniline and/or degradation by-products due to ozonation and sonolytic ozonation, measured by absorbance at 206 nm. The second-order kinetics at this wavelength, when compared to first order at 540 nm (Fig. 3), suggests a different mechanism of attack. In addition, sonolytic ozonation seems to produce different intermediates than those of ozonation alone, indicating that degradation processes under the previous conditions are more complex. Due to the low efficiency of sonolysis all subsequent tests will be performed with ozonation system.

To achieve detailed information on the reaction processes during ozonation, complementary studies were performed. COD of the pararosaniline acidic solutions (in HCl 0.01 M) was estimated before and after ozonation (15 min), however these experiments did not present conclusive results. Even with the addition of mercuric sulfate to remove interferences of chloride [14] present in the solutions, an apparent increase in COD results was observed (not presented). Similar behavior was observed in COD tests made with aqueous dye solution, which also contains chloride ions in lower concentration (the reagent used was pararosaniline chloride). These results could be explained by the fact of the ozonation, in the operating conditions, to generate recalcitrant by-products. Probably, a longer time ozonation is necessary to reduce the COD levels, promoting the mineralization of remaining recalcitrant substances.

3.1.1. Influence of acidity on ozonation process

Due to the reported variations of the pararosaniline absorbance with pH [1,11] this parameter was monitored during the ozonation process. No significant pH variations were noticed for the HCl or H_2SO_4 solutions (ΔpH lower than 0.02). On the other hand, pH varied from 5.93 to 3.58 during dye ozonation in water system. However, no significant variation of pararosaniline absorbance in the visible light region was noticed in this pH range, corroborating the results reported by Kosanic and Trickovic [11]. Thus, the rate constant determined for the unbuffered water system (at 540 nm) is due to pararosaniline degradation and not due to artificial change in absorbance. A changing pH in water system was expected, due to the formation of inorganic and organic acids during ozonation, confirmed by the conductivity increase from 3.5 to 25 $\mu S\,cm^{-1}$.

In addition, as shown in Table 1, the rate constants found are similar for water, hydrochloric acid and sulfuric acid solutions that present initial pH range from 5.93 to 2.58. So the ozonation kinetics seems not to be influenced by acidity, at least in low concentrated acid solutions (0.01 M HCl or 0.005 M H_2SO_4). Based on these results all subsequent tests were made in aqueous solutions.

3.2. Ozonation of aqueous pararosaniline solutions

3.2.1. Analysis of sub-products

The compounds formed by degradation process of the pararosaniline by action of the ozone were extracted by hexane and analysed by GC–MS. The identified compounds in this screening test are mainly oxygenated by-products, such as long-chain carboxylic acids and alcohols, usually found in this kind of oxidation process [24,26]. To obtain detailed information on the ozonization by-products (carbonyl compounds and nitrate) further analyses were made on dye solution ozonized during 15 min, and the identified compounds are shown in Table 2.

Formic acid is the main organic compound formed, corresponding 75% of total short chain acids identified and with concentration being greater than the all other carbonyls. Oxalic and acetic acids were the second most abundant carboxylic acids (10 and 8%, respectively), with pyruvic and glycolic acids presenting lower concentrations ($<0.5\,\mu M$). The total aldehyde/ketones observed level (1.7 μM) is about 10 times smaller than total carboxylic acid concentration (12.2 μM). In addition, formaldehyde (0.94 μM), acetone (0.45 μM) and acetaldehyde (0.26 μM) were the only short chain aldehyde/ketones identified in this experiment. The identified by-products molar percentage correspond to $\sim$9% of the original dye concentration (expressed in carbon basis) with net predominance of carboxylic acids (8.2%) as expected.

The molecule of pararosaniline also contains three nitrogen atoms in the form of amino group (Fig. 1). The molar percentage ratio of nitrate and dye concentration after 15 min of ozonation was 73%, indicating a partial transformation of nitrogen atoms into nitrate. The remaining nitrogen atoms could be still in amino form or transformed into nitrite [26]. However, nitrite ion was not detected by IC (LOD $< 0.04\,\mu M$) suggesting that, if formed, was completely oxidised into nitrate. This result and the presence of the small organic fragments

Table 2
Comparison of the by-products generated from ozonized aqueous solutions of the two different dyes

Dye	Pararosaniline[a]		Reactive Red 120[b]	
References	This work	%[c]	[24]	%[c]
$[Dye]_0$	1.2×10^{-5} M	(19 mg L^{-1})	1.4×10^{-4}	(200 mg L^{-1})
Aldehyde/ketone				
Formaldehyde (μM)	0.94	57	n.i.	
Acetaldehyde (μM)	0.26	16	n.i.	
Acetone (μM)	0.45	27	n.i.	
Others (μM)	n.d.		n.i.	
Total (μM)	1.7	100		
$[Carbon]_0$ (%)	0.9			
Carboxylic acids				
Formic (μM)	9.11	75	20	14
Acetic (μM)	1.00	8	0.0	0
Glycolic (μM)	0.28	2	n.i.	
Pyruvic (μM)	0.54	4	n.i.	
Oxalic (μM)	1.25	10	125	86
Total (μM)	12.2	100	145	100
$[Carbon]_0$ (%)	8.2		2.4	
Nitrate (μM)	97		200	
$[N]_0$ (%)	73		10	

n.d. – Not detected; n.i. – not informed or not measured.
[a] Dye ozonized (7 mg O_3/L) during 15 min.
[b] Data from unpurified dye ozonized (8.9 mg O_3/L) during 150 min.
[c] Percentage contribution of individual compound to each specie's classes.

indicated that the dye structure was not completely mineralized under the prevailing oxidation conditions.

In Table 2 are also presented the literature data of the by-products generated during ozonation of C.I. Reactive Red 120 (M_w 1469.98 g/mol), a commercial azo dye [24]. Unfortunately, other studies with detailed chemical composition of by-products generated from triphenylmethane dyes ozonation were not found. However, the work of Zhang et al. [24] addressed an important issue about the influence of short- and long-term ozonation on the formation of toxic and recalcitrant by-products. Sulfate, nitrate, formic and oxalic acids were identified as the main oxidation by-products of this dye with increasing ion concentrations with ozonation time. A direct comparison between these two dyes is difficult due to the different chemical structures and ozonation times used. However, the higher levels of oxalic acid, 86% compared to formic acid, and low nitrogen transformation into nitrate (only 10%) indicate a less effective oxidation of Reactive Red 120 when compared to pararosaniline.

3.2.2. Toxicity and mutagenicity tests

The acute toxicity of the pararosaniline solution was determined prior to ozonation process and monitored in different ozone contact times. As can be seen in Fig. 5 the acute toxicity tests with *D. similis* showed decreased immobility values with long-term ozonation. However, ozone treatment was more effective in reducing dye toxicity with exposure time of 120 min. These tests gave toxicity responses compatible with the degree of ozonation treatment.

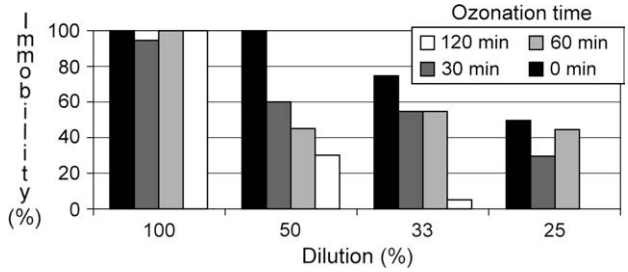

Fig. 5. The results obtained in the acute toxicity tests with *Daphnia similis* using ozonized and not ozonized aqueous pararosaniline solution.

A better comparison between toxicity results could be made using EC_{50} values. Similar toxicity levels were obtained for the dye solutions ozonized during 30–60 min (EC_{50} 37% dilution ratio) and not ozonized (39%). The slight toxicity increase in ozonized solutions, could be explained by the formation of some intermediate by-products, probably more toxic than original dye. On the other hand, the increase in ozonation time up to 120 min decreases significantly the toxicity (EC_{50} 59%). Laglais et al. [23] reported similar behaviour for water treatment with ozone.

Pararosaniline has been shown to cause cancer in animals and mutagenic effects in laboratory studies [5]. Under the conditions of these studies, pararosaniline was mutagenic in strains TA98 and TA100 of *S. typhimurium* by the preincubational protocol with or without metabolic activation. In our work the Ames test was used to evaluate possible mutagenic effects of the ozonized pararosaniline solution (treated during 15 min). Fortunately, the treated dye solution was not capable to induce reverse mutations in the three strains tested until the concentration of 500 μL/plate in presence as in the absence of metabolic activation of livers of rats. It seems that expected mutagenicity associated with the pararosaniline was removed by ozonation, even using relative short-term treatment and in the presence of some potentially toxic by-products (Section 3.2.1).

3.2.3. Comparison with literature

A comparison of the sparse literature data about pararosaniline degradation is presented in Table 3. Reaction half-time for different processes (visible light, H_2O_2, TiO_2-assisted photodegradation, sonolysis, biodegradation and ozonation) are shown. It becomes

Table 3
Kinetic data of aqueous solutions of pararosaniline in different degradation systems

Systems	Milieu	k	$t_{1/2}$ (min)	n	R^2	References
Visible light (VL)[a]	H_2O	6.3×10^3 (L mol^{-1}min^{-1})	19.84	6	0.992	[11]
VL + H_2O_2	H_2O	0.072 (min^{-1})	9.6	6	0.987	[11]
TiO_2 + VL	H_2O	0.143 (min^{-1})	4.8	6	0.994	[11]
TiO_2 + VL + H_2O_2	H_2O	0.127 (min^{-1})	5.5	6	0.994	[11]
Ozone (OZ)	H_2O	0.473 (min^{-1})	1.5	5	1.000	This work
Biological[b]	*Laccase*	2.0×10^{-4} (mol min^{-1} L^{-1})	0.03	n.i.	n.i.	[9]
	T. hirsute	2.7×10^{-6} (mol min^{-1} L^{-1})	1.8	n.i.	n.i.	[9]

All tests were performed using aqueous solution $1.0 \pm 0.2 \times 10^{-5}$ mol L^{-1} pararosaniline at pH 5.92 except biological systems with 2.5×10^{-6} mol L^{-1} pararosaniline buffered at pH 5.0. n.i.: Not informed; n: number of samples used in linear regression calculation.

[a] Visible light process following second-order kinetics.

[b] Biological processes from Ref. [9] following a zero-order kinetics, $t_{1/2}$ calculated for $[\text{dye}]_0 = 1.0 \times 10^{-3}$ mol L^{-1}.

evident that the dye decomposition by ozone is faster than the majority of non-biological systems. The photocatalytic treatment (TiO_2 + VL) presented the second faster kinetics for non-biological systems. However, that process is three times slower than ozonation and produces a solid waste that needs further treatment.

Biological treatments present zero-order kinetics and very fast pararosaniline degradation rates. The enzymatic system presents the fastest reported kinetics, showing a half-time of 0.03 min calculated for an initial pararosaniline concentration of 1.2×10^{-5} mol L^{-1}. The degradation with *T. hirsuta* presents similar half-time to ozone degradation. However, biological systems are more complex processes than ozonation. Finally, the results suggested that a combination of the biological and ozone treatment could be cost effective than using any tested pararosaniline degradation system alone.

4. Conclusions

Experimental results show that ozonation of pararosaniline solutions is more efficient in the destruction of the dye chromophore structure than ultrasonic irradiation alone or combined with O_3. In addition, the reaction mechanisms of these two processes are possibly different as suggested by the data obtained from monitoring the 206 nm absorbance.

The results obtained indicate that ozonation is a powerful tool for the treatment of pararosaniline dye solutions with complete decolorization in few minutes (lower than 30 min). However, in order to obtain high toxicity removal, longer ozonization periods with high ozone doses have to be applied. In these conditions ozone could promote a complete degradation of toxic sub-products. Fortunately, even for the most toxic dye solution (ozonized during 30 min) no mutagenic effect was verified although evidence of acute toxicity still remains. Ozonation can be applied as a very effective treatment of pararosaniline solution, even when compared to other processes as ultrasound, photodegradation and peroxide dye degradation.

Complementary tests are under consideration to identify other oxidation by-products by HPLC and to determinate organic carbon contents in different pararosaniline ozonation times.

Acknowledgements

The authors wish to thank OZ Industrial de Equipmentos Geradores de Ozônio Ltda, CNPq and FAPERGS for the financial support.

References

[1] Scaringelli FP, Saltzman BE, Frey SA. Spectrophotometric determination of atmospheric sulfur dioxide. Analytical Chemistry 1967;39:1709–19.

[2] Schrijver IA, Melief M, Meurs M, Companjen AR, Laman JD. Pararosaniline fixation for detection of co-stimulatory molecules, cytokines, and specific antibody. Journal of Histochemistry and Cytochemistry 2000;48:95–104.

[3] Chieco P, Derenzini M. The Feulgen reaction 75 years on. Histochemistry and Cell Biology 1999;111:345–58.

[4] Epple A, Hilliard RW, Potter IC. Histological demonstration of elastin-like fibres in the blood vessels of lampreys. Comparative Biochemistry and Physiology Part A: Physiology 1984; 79(1):105–7.

[5] National Toxicology Program. Technical Report Series No. 285. Toxicology and carcinogenesis studies of C.I. Basic Red 9 Monohydrochloride (pararosaniline) (CAS No. 56961–9) in F344/N rats and B6C3F1 mice (feed studies); Report Date: January 1986. <http://www.ntp-server.niehs.nih.gov, 28/09/2003>.

[6] Tosik R, Wiktorowski S. In: Proceedings of the IOA annual conference, August 17–20, Lake Tahoe, Nevada, USA; 1997. p. 363–8.

[7] Matsul M, Nakabayashi H, Shibata K, Takase Y. Ozonization of triphenylmethane dyes. Bulletin of the Chemical Society of Japan 1984;57(11):3312–6.

[8] Grosjean D, Whitmore PM, Cass GR. Ozone fading of triphenylmethane colorants: reaction products and mechanisms. Environmental Science and Technology 1989;23:1164–7.

[9] Abadulla E, Tzanov Y, Costa S, Robra K-H, Cavaco-Paulo A, Gubitz GM. Decolorization and detoxification of textile dyes with a Laccase from *Trametes hirsute*. Applied and Environmental Microbiology 2000;66(8):3357–62.
[10] Ball MY, Morjaria B, Motara AI, O'Boyle PM, Russel EJ, Sugden JK. A study of ageing of Gomori aldehyde fuschin stain. Dyes and Pigments 1998;36(3):223–9.
[11] Kosanic MM, Trickovic JS. Degradation of pararosaniline dye photoassisted by visible light. Photochemistry and Photobiology A: Chemistry 2002;149:247–51.
[12] Sani RK, Banerjee UC. Decolorization of triphenylmethane dyes and textile and dye-stuff effluent by *Kurthia* sp. Enzyme and Microbial Technology 1999;24:433–7.
[13] Standard test methods for sulfur dioxide content of the atmosphere (West–Gaeke Method), ASTM D 2914-95, vol. 11.03. West Conshohocken, PA: American Society for Testing and Materials; 1996.
[14] Standard methods for the examination of water and wastewater. 18th ed. Washington: APHA; 1992.
[15] Kunz A, Freire RS, Rohwedder JR, Duran N, Mansilla H, Rodriguez J. Construção e otimização de um sistema para produção e aplicação em escala de laboratório. Química Nova 1999;22(3):425–8.
[16] Le Lacheur RM, Sonnenberg LB, Singer PB, Christman RF, Charles MJ. Identification of carbonyl compounds in environmental samples. Environmental Science and Technology 1993;27:2745–53.
[17] Souza, IA. Master thesis, PUCRS; 2003.
[18] Hamilton MA, Russo RC, Thurston RV. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science and Technology 1977;11(7):714–9.
[19] OECD – guideline for testing of chemical. Method 471 "Bacterial Reverse Mutation Test"; Adapted: 21st July 1992.
[20] Weavers LK, Ling FH, Hoffmann MR. Aromatic compound degradation in water using a combination of sonolysis and ozonolysis. Environmental Science and Technology 1998; 32:2727–33.
[21] Vinodgopal K, Peller J, Makogon O, Kamata PV. Ultrasonic mineralization of a reactive textile azo dye, remazol black B. Water Research 1998;32(12):3646–50.
[22] Ince NH, Tezcanlí G. Reactive dye stuff degradation by combined sonolysis and ozonation. Dyes and Pigments 2001;49: 145–53.
[23] Langlais B, Reckhow DA, Brink DR. Ozone in water treatment, Applications and engineering. Denver: AWWARF and Lewis Publishers, Inc; 1991.
[24] Zhang F, Yediler A, Liang X, Kettrup A. Effects of dye additives on the ozonation process and oxidation by-products: a comparative study using hydrolyzed C.I. Reactive Red 120. Dyes and Pigments 2004;60:1–7.
[26] Zhao W, Shi H, Wang D. Ozonation of cationic Red X-GRL in aqueous solution: degradation and mechanism. Chemosphere 2004;57:1189–99.