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Monitoring ecotoxicity of disperse red 1 dye during photo-Fenton degradation

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HIGHLIGHTS

- Acute toxicity to *Daphnia similis* increased after 10 min degradation of disperse red 1.
- Maximum concentration of intermediates coincides with highest toxicity.
- Most intermediates were formed after addition of •OH to benzenic ring
- Mineralization achieved 55% with non-toxic levels after 45 min.

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ABSTRACT

The present work assessed the ecotoxicity of the commercially available form of the azo dye Disperse Red 1 (DR1) and the main degradation products generated during photo-Fenton degradation. The acute toxicity tests with the microcrustacean *Daphnia similis* showed that toxicity increased after 10 min of treatment, when 35% of the original concentration of the dye has been degraded but without decrease in total organic carbon concentration (TOC). The increase of toxicity was a consequence of generation of degradation products of higher toxicity than DR1, which achieved maximum concentration after 10 min reaction. The structures identified using LC/MS indicated that most of the intermediates were formed after addition of hydroxyl radical to benzenic ring but the cleavage of azo bond was also observed. The intermediates were further degraded and toxicity was then reduced to non toxic levels after 45 min experiment, when 98% of the initial concentration of DR1 was degraded and mineralization achieved 55%. The results of this study showed that the textile dye DR1 can be degraded by photo-Fenton process with removal of acute toxicity to *D. similis* even with incomplete mineralization.

1. Introduction

Azo compounds correspond to an important class of dyes used in various industrial sectors such as textiles, food, cosmetics and paints. However, the industry of greatest environmental concern in relation to dyes is the textile sector due to the use of high amounts of different classes of dyes, high water demand and especially due to the great losses of dyes during the dyeing process, which may achieve 20% in some cases (Azbar et al., 2005; Zaharia and Suteu, 2012). As a consequence, wastewaters from textile industry can contain a variety of toxic compounds such as dyes as well as degradation products such as aromatic amines (Umbuzeiro et al., 2004). Furthermore, conventional processes applied for the treatment of wastewaters from textile industries, aerobic lagoons or activated sludge, are not efficient in the biological degradation of dyes resulting in contamination of surface water (Robinson et al., 2001). The inefficiency of wastewater treatment processes applied in textile industries for the complete removal of dyes is also demonstrated by the detection of dyes and their transformation products in surface waters (Umbuzeiro et al., 2005; Kummrow and Umbuzeiro, 2008). Furthermore, it was also demonstrated that chlorination process applied for water disinfection generates chlorinated products, which can be even more toxic than the parent

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dye (Oliveira et al., 2010; Vacchi et al., 2013; Yang et al., 2015).

Therefore alternative wastewater treatment processes, capable of promote removal of dyes and toxicity are necessary to prevent water contamination. Advanced oxidation processes are very interesting for this application since the high oxidizing hydroxyl radicals generated may degrade complex structures non selectively (Huang et al., 1993; Nogueira et al., 2007; Wang and Xu, 2012). Photo-Fenton process combines iron salts, H_2O_2 and irradiation (solar or ultraviolet) for the generation of hydroxyl radical and presents as advantages the low cost and high efficiency and simplicity, which has been demonstrated by the removal of color, chemical oxygen demand and total organic carbon (Neamtu et al., 2004; Lucas and Peres, 2006; Liu et al., 2007; Blanco et al., 2014; Torrades and García-Montaño, 2014). However, few works have demonstrated the efficiency of treatment process for the removal of toxicity (Luna et al., 2014; Punzi et al., 2015).

Azo dyes include a class of highly hydrophobic substances known as disperse dyes, which are often commercialized containing dispersing agents to improve their solubility in water and color transfer (Reife and Freeman, 1995). The Color Index Disperse Red 1 dye (DR1) is an azo textile dye extensively used for dyeing polyester fabrics in industrial plants in Brazil. It presents high ecotoxicity before and after chlorination (Ferraz et al., 2011; Vacchi et al., 2013). The aim of the present study was to evaluate the efficiency of the photo-Fenton process for removal of ecotoxicity of the commercial dye DR1 and to identify the main degradation products.

2. Experimental

2.1. Chemicals

Commercial textile dye DR1 (N-ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo)aniline) was purchased from PCIL© Dyes, Brazil. This dye product was chemically characterized by Vacchi et al. (2013) and contains 60% of the main dye disperse red 1, 20% of other azo dye products, and 20% of a surfactant. Iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) (Mallinkrodt), hydrogen peroxide 30% (Synth) were used in degradation experiments. Bovine liver catalase (Aldrich) was used to consume residual hydrogen peroxide. Ammonium metavanadate (Vetec) 0.06 mol L^{-1} prepared in 0.36 mol L^{-1} H_2SO_4 (Merck) was used for H_2O_2 monitoring. Methanol (HPLC grade) and formic acid, purchased from JT Baker, and acetonitrile (HPLC grade) from Panreac were used in HPLC analysis. Sulfuric acid and sodium hydroxide (Chemis) were used for pH adjustment.

2.2. Photo-Fenton treatment

Photo-Fenton degradation experiments were carried out using an upflow photoreactor previously described (Nogueira and Guimarães, 2000). It consists of a 3.8 cm diameter glass cylinder with a 15 W blacklight lamp in the center. The irradiated volume was 280 mL and a total volume of 500 mL was pumped with a peristaltic pump (Masterflex 7518-12) at 90 mL min^{-1} flow rate. DR1 solutions were prepared in distilled water at 20 mg L^{-1} concentration of commercial dye and pH was adjusted to 2.5 ± 0.1, optimum pH value according to previous works (Pignatello, 1992; Nogueira and Guimarães, 2000). Adequate volumes of $Fe(NO_3)_3$ and hydrogen peroxide solutions were then added to result in concentrations of 0.2 mM and 5 mM, respectively. The resulting solution was immediately pumped to the reactor while magnetically stirred. The lamp was turned on when the reactor was completely filled and the time started to be monitored.

2.3. Chemical analysis

Before chromatographic analysis, 10 mL samples were solid phase extracted using Sep-Pack C 18 (360 mg) cartridges, previously conditioned with methanol and water. The retained compounds were eluted with 2.5 mL methanol and then diluted with ultrapure water (Milli Q Millipore) to result in a 60:40% methanol:water solution and a 2.4 times concentration factor. Under these conditions, detection limit of DR1 is 0.2 mg L^{-1}. Recovery tests indicated recovery percentages between 75 and 115% for dye concentrations between 1.6 and 21 mg L^{-1}. All samples were then filtered using 0.45 μm regenerated cellulose filters (Chromafil).

The decay of dye concentration during irradiation was monitored by HPLC analysis using a Shimadzu LC 20AT Prominence equipment coupled to a photodiode detector SPD-M20A. A Hyperclone C8-BDS (250 × 4.6 mm, 5 μm) (Phenomenex) column was maintained at 40 °C and at 1.0 mL min^{-1} flow rate. The mobile phase was methanol:0.1% formic acid (60:40, v/v).

Mineralization of the dye, total degradation to CO_2, was followed by the decay of the total organic carbon concentration (TOC) during degradation measured in a Shimadzu equipment (TOC 5000A). These analyses were carried out without any pretreatment immediately after sample withdrawn to avoid further dark Fenton reaction.

The residual hydrogen peroxide concentration was determined using spectrophotometry (Shimadzu UV mini-1240) by measuring the absorption at 450 nm after reaction with ammonium metavanadate (Nogueira et al., 2005).

Liquid chromatography coupled to mass spectrometry (LC-MS/MS) analysis was performed using the same chromatographic conditions as described for HPLC-DAD analysis, however using a Liquid Chromatograph (1200 Agilent Technologies) coupled to a 3200 QTRAP Mass Spectrometer (Linear Ion Trap Quadrupole, AB Sciex Instruments) operating in a positive mode and TurboIonSpray ionization. The parameters used were: curtain gas: 10 psi; Corona: 4.0 V; Gas 1: 40 psi; temperature: 700 °C; declustering potential: 51.00 V; entrance potential: 4.00 V; collision energy potential: 24.0 V and interface heater: ON. Enhanced fullscan experiments were performed in the range 100–600 Da.

2.4. Ecotoxicity tests

For the toxicity tests, the pH of the samples after degradation experiments was adjusted to 6–7 with NaOH. The volume of 3 mL of catalase solution (0.1 g L^{-1}) was added to 500 mL sample, which was magnetically stirred during 10 min for total consumption of residual hydrogen peroxide. Under these conditions iron hydroxide precipitation occurs. The samples were then allowed to settle down for iron precipitation, stored under refrigeration for a maximum of 35 h and the supernatant was then taken for toxicity tests.

Acute toxicity tests were performed with the microcrustacean *Daphnia similis* using four replicates per concentration along with negative control (OECD, 2004; ABNT, 2009). Five neonates (less than 24 h old) were exposed in the dark and without food at 20 ± 2 °C to 10 mL solutions of DR1 and of samples from its photo-Fenton degradation with dilutions between 0 and 100%. Reconstituted water was used for dilutions as well as for the negative controls. Sensitivity of the culture was monitored every month performing tests with NaCl (Sigma; purity 99%) at 0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 g L^{-1}. After 48 h, the number of immobile organisms in each tested concentration was reported and the EC_{50} was calculated according to the "Trimmed Spearman-Kraber" method using the software JSPEAR (version GW basic 3.10) (Hamilton et al., 1977) and expressed as percentage (% v/v). The tests were considered valid when the immobile organisms in negative controls were below

Table 1
Concentration of DR1 during photo-Fenton treatment before and after neutralization and acute toxicity of samples to *Daphnia similis* (48 h).

Reaction time (min)	pH 2.5			pH 6.8		
	Concentration of DR1 (mg L^{-1})	EC_{50} (%)	TU (%)[b]	Concentration of DR1 (mg L^{-1})	EC_{50} (%)	TU (%)[b]
0	23 ± 4[a]	1	100	1.2 ± 0.3	22	4.5
10	15 ± 4	nd	nd	3 ± 2	4.4	23
30	4 ± 3	nd	nd	1.9 ± 0.7	28	3.6
45	0.4 ± 0.3	nd	nd	0.3 ± 0.2	100	NT[c]

[a] Measured before iron addition.
[b] Toxicity units.
[c] NT – non-toxic; nd: not determined.

10%. EC_{50} values were transformed to toxicity units (TU), TU = 100/EC_{50} (Costa et al., 2008).

3. Results and discussion

3.1. Ecotoxicity monitoring during photo-Fenton degradation of DR1

Firstly, a blank experiment was carried out to evaluate the toxic effect of the reagents used in the photo-Fenton process in the absence of DR1. The solution contained initially $Fe(NO_3)_3$ and H_2O_2, and the pH was then adjusted to 2.5 with H_2SO_4 and neutralized with NaOH to pH 7. Bovine catalase used for H_2O_2 consumption was added following the same procedure applied for the evaluation of toxicity of the samples containing the dye. No toxic effect was observed in these blank experiments, indicating that any toxicity observed in the samples during DR1 degradation was related to the dye and its products generated during the photo-Fenton treatment.

The EC_{50} of the untreated dye and in the absence of iron was determined to be 1.0% (Table 1) of a solution containing 23 mg L^{-1}, which is equivalent to 230 μg L^{-1} of the commercial dye. Considering that the purity of the commercial dye is 60%, this EC_{50} value is very close to the previously reported for the 95% purity DR1 (127 μg L^{-1}) (Ferraz et al., 2011). The photo-Fenton degradation experiments were carried out at pH 2.5 ± 0.1, aiming highest degradation efficiency (Pignatello, 1992; Nogueira and Guimarães, 2000). Considering that this low pH value is not biologically compatible to perform toxicity tests, neutralization of the samples taken after different treatment times to pH 6.8 ± 0.6 was necessary before performing toxicity tests. However, the increase of pH resulted in precipitation of iron hydroxides, which adsorbed the remaining dye, decreasing its concentration in solution by about 95%, from 23 mg L^{-1} to 1.2 mg L^{-1}, while sample toxicity was significantly reduced to an EC_{50} = 22% due to decrease of dye concentration in bulk solution (Table 1). This implies that the EC_{50} expressed as dye concentration in the presence of iron salt is 264 μg L^{-1}.

The toxicity of the sample after 10 min reaction increased considerably in relation to the initial sample containing iron achieving an EC_{50} of 4.4% of the treated sample. At this time, the concentration of the dye decreased to 15 mg L^{-1} in the bulk solution at pH 2.5, indicating that 35% of the initial dye concentration has been converted to degradation products, without any TOC removal (Fig. 1). On the other hand, after neutralization, the measured concentration of the dye in solution was 3 mg L^{-1}, very close to the initial concentration. However, increase of dye concentration after neutralization may occur due to different species and amount of iron precipitated since under irradiation photoreduction of Fe^{3+} to $-Fe^{2+}$ occurs, which is more soluble than Fe^{3+} ($K_{PS}\ Fe(OH)_3 = 2 \times 10^{-39}$; $K_{PS}\ Fe(OH)_2 = 4.1 \times 10^{-15}$) resulting in lower precipitation and consequently, lower adsorption. Calculations based on solubility products of the iron ions indicate that at 0.2 mM, 27% of the total Fe(III) concentration is present as free Fe^{3+} and 69% as $Fe(OH)^{2+}$ at pH 2.5, while 100% of Fe(II) is present as Fe^{2+}, thus totally soluble. When pH is increased to 6.8, 99% of Fe(III) is present as $Fe(OH)_2^+$ and precipitation of $Fe(OH)_3$ occurs (0.3%), while 99.8% of Fe(II) is present as Fe^{2+}. This implies that during irradiation, when reduction of Fe(III) occurs, most of iron is soluble

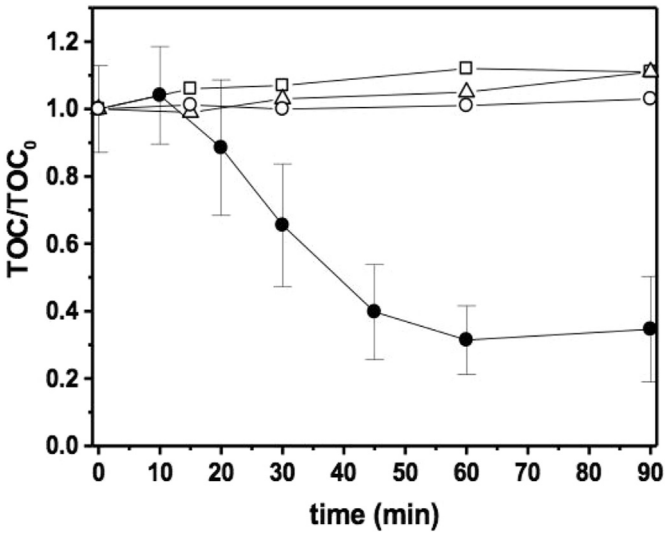


Fig. 1. Mineralization of DR1 under different conditions: Δ UV only; ○ H_2O_2 only; □ UV/H_2O_2; ● UV/$Fe(NO_3)_3$/H_2O_2. Initial conditions: TOC_0 = 11 mg L^{-1}; $Fe(NO_3)_3$ = 0.2 mM; H_2O_2 = 5 mM.

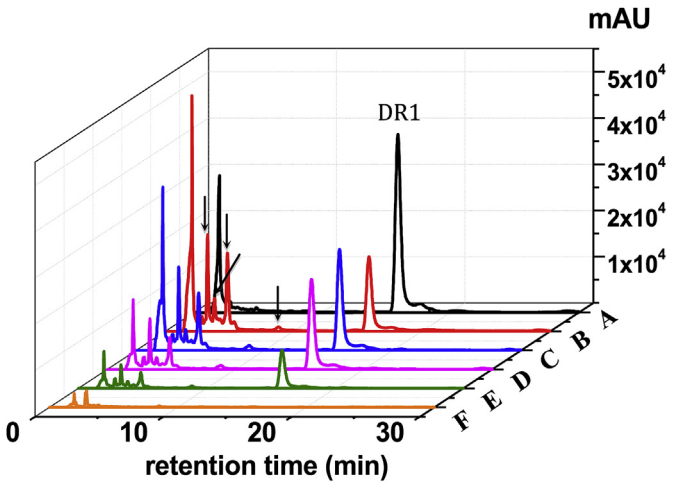


Fig. 2. HPLC-DAD chromatograms (detection at 254 nm) of the samples taken from DR1 photo-Fenton degradation after 0 min (A); 3 min (B); 10 min (C); 20 min (D); 30 min (E); 90 min (F). Arrows indicate the main intermediates detected after 3 min.

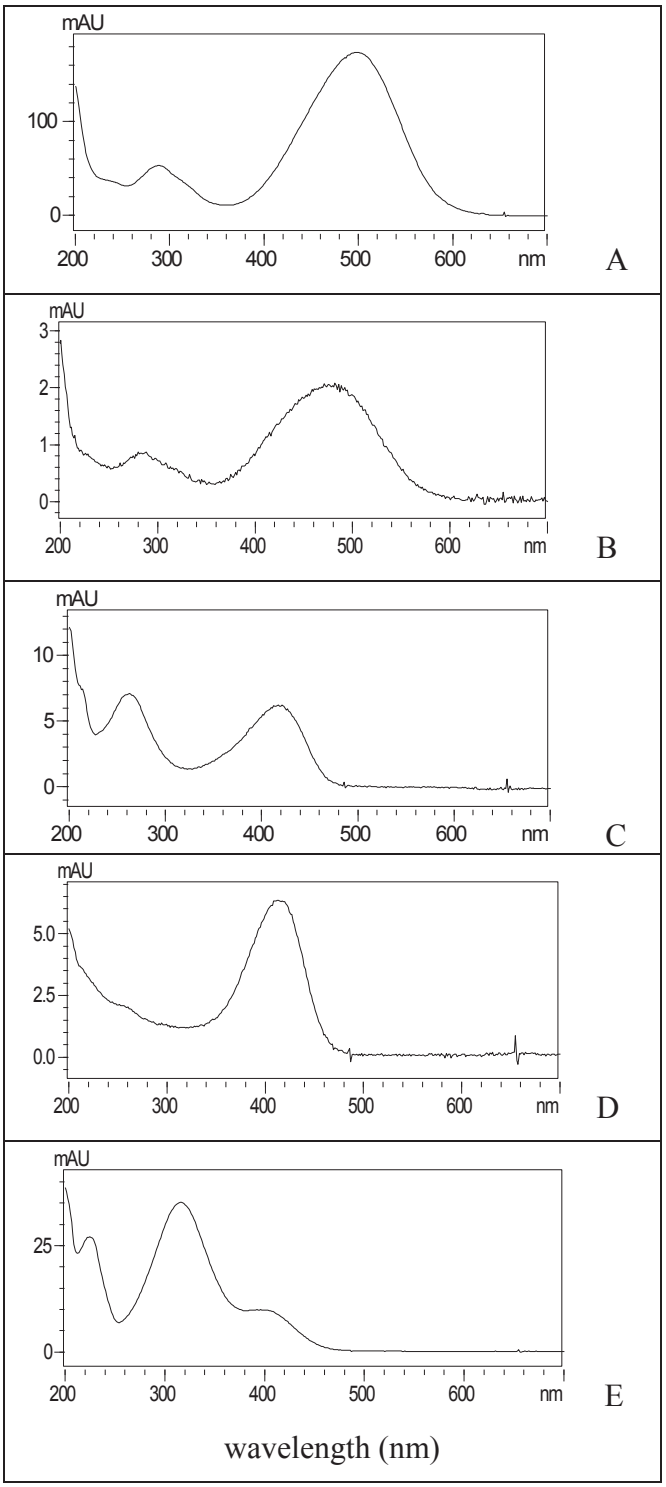


Fig. 3. UV–vis spectra of DR1 and initial intermediates detected after 3 and 7 min degradation obtained with diode array detection (HPLC-DAD) under hydrodynamic conditions. Retention times: DR1, 16 min (A); 8.5 min (B); 4.8 min (C); 5.5 min (D); 3.8 min (E). HPLC/DAD conditions: methanol/0.1% (v/v) formic acid 60:40 as mobile-phase, C8 column as stationary-phase, oven temperature 40 °C and 1.0 mL min^{-1} flow rate.

even at pH 6.8 (Calculations using Visual MINTEQ 3.0).

The increase in toxicity after 10 min indicated the formation of more toxic products than the original dye despite the significant decrease of DR1 initial concentration. Considering that 3 mg L^{-1}

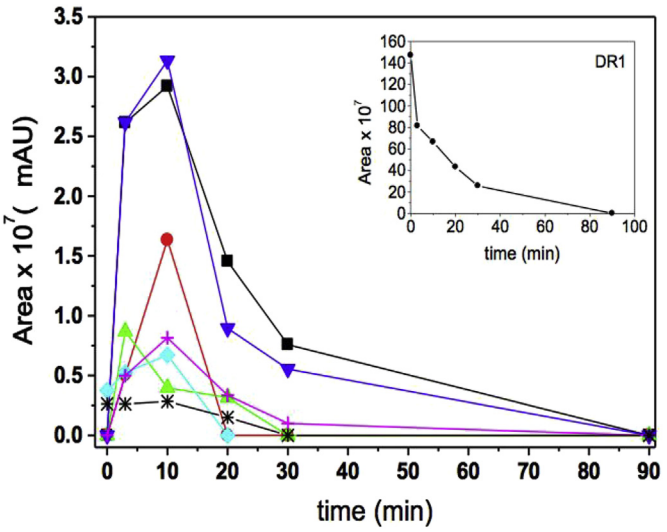


Fig. 4. Evolution of the intermediates (C1–C7) detected during DR1 degradation. ▼(C7, m/z 287); ■ (C1, m/z 194); ● (C4, m/z 357); + (C5, m/z 347); ▲ (C2, m/z 273); ◆ (C6, m/z 331); ∗ (C3, m/z 331). Insert: area of DR1 measured by LC/MS analysis (m/z 315).

dye concentration resulted in the EC_{50} 4.4%, the toxicity equivalent in dye concentration corresponds to 132 μg L^{-1}, which is 50% of the initial toxicity equivalent in dye concentration (264 μg L^{-1}), indicating that half of the toxicity observed for this sample is due to the degradation products. Increase in toxicity during initial stages of Fenton or photo-Fenton treatment was also previously observed such as in the degradation of azo dye 3BS and reactive black 5, indicating the generation of products of higher toxicity depending on the Fenton reagent ratio (Zhang et al., 2014; Luna et al., 2014). As degradation continued, the toxicity of the sample decreased to an EC_{50} of 28% after 30 min reaction, when dye concentration in the neutralized solution was 1.9 mg L^{-1}. Considering that 1.9 mg L^{-1} dye concentration resulted in the EC_{50} 28%, the toxicity equivalent in dye concentration corresponds to 530 μg L^{-1}, which is higher than the EC_{50} of DR1 denoting that after 30 min, there is no contribution of degradation products to the overall toxicity of the sample, which is due only to the residual DR1 concentration. In relation to the bulk sample at pH 2.5, the concentration of the dye decreased to 4 mg L^{-1}, which means that 83% of the dye was converted to degradation products.

Further degradation resulted in non-toxic levels after 45 min reaction, when DR1 concentration in neutral sample and in bulk solution at pH 2.5 was approximately the same, 0.3 and 0.4 mg L^{-1}, respectively. Degradation reaction during 45 min resulted only in partial mineralization (55% of the initial TOC concentration), with a residual organic carbon concentration of about 4 mg L^{-1} decreasing to 3 mg L^{-1} after 60 min without further improvement up to 90 min (Fig 1). As the H_2O_2 concentration during photo-Fenton degradation decreased linearly to 0.70 mM during the first 45 min and to concentrations below detection limit of the method (147 μM) after 90 min (not shown), it is possible that it has limited further degradation of residual carbon concentration. However, considering the non-toxicity of the sample after this treatment time, further additions of peroxide would not be necessary.

3.2. Degradation intermediates

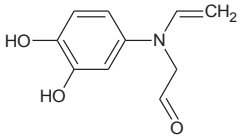
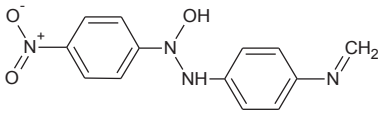
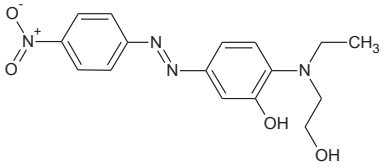
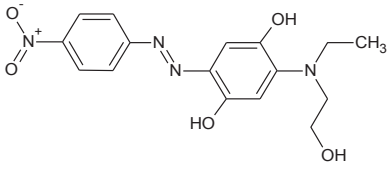
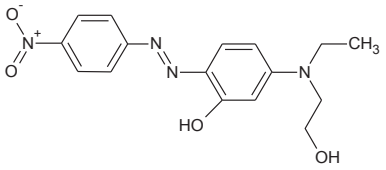
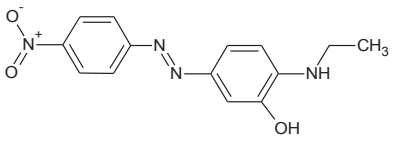
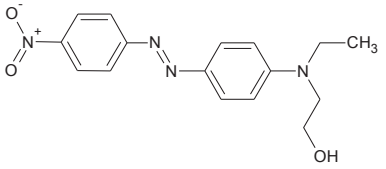
The HPLC-DAD chromatograms of DR1 photo-Fenton degradation revealed the formation of four main degradation products detected at 254 nm after already 3 min reaction (Fig. 2). The

corresponding absorption spectra registered under hydrodynamic conditions in HPLC-DAD analysis of the 3 and 10 min degradation sample of each detected intermediate indicated the presence of the azo chromophore group (−N=N−), with strong absorption bands between 400 and 500 nm (Fig. 3). However, a slight hypsochromic shift (shifting of the maximum absorption to lower wavelength values) was observed for the more polar products (with lower retention times). The only intermediate detected in this analysis, which showed maximum absorption at much shorter wavelength (320 nm), was the one with 3.8 min retention time, suggesting the cleavage of the azo bond.

The samples taken from DR1 photo-Fenton degradation were also analyzed by liquid chromatography coupled to mass spectrometry aiming the detection and identification of the main degradation products generated. Seven initial intermediates could be detected with mass to charge ratio (*m/z*) of 194 (C1), 273 (C2), 287 (C7), 347 (C5), 357 (C4) and 331, two isomers (C3 and C6). It was possible to monitor the increase of concentration of these intermediates, which achieved a maximum after 10 min degradation (Fig. 4), that is in accordance with the steady TOC concentration in the first 10 min and maximum toxicity as discussed before. The concentration of these compounds decreased significantly after 20 min reaction achieving levels below detection limit between 30 and 90 min when no toxicity was observed.

The proposition of the chemical structure of the intermediates was mainly based on the mass to charge ratio (*m/z*) of $[M+H]^+$

Table 2
LC/MS data of intermediates detected during DR1 degradation, m/z ratio, main fragments, proposed structure and corresponding Log K_{OW} and solubility predicted using Estimation Programs Interface Suite™, K_{ow}win version 1.68 [US EPA, 2012].

Intermediate	t_R (min)	$[M+H]^+$	Main fragments (m/z)	Proposed structure	log K_{OW}	Solubility 25° (mg L^{-1})
C1	3.05	194	216 194 166		0.22	31,000
C2	3.99	273	165		2.58	111
C3	4.79	331	283		3.72	1.99
C4	4.68	357	297/147	[a]		
C5	6.10	347	329/179		3.94	1.04
C6	8.28	331	283		4.07	1.01
C7	9.83	287	241		4.1	1.56
DR1	18.1	315	255/147/134		4.3	0.796

[a] Structure not attributed.

product and MS/MS fragmentation. It was also taken into account the characteristics of the reaction medium containing hydroxyl radicals generated from Fenton reaction that can attack DR1 molecule in an electrophilic addition reaction, especially in the first steps of degradation (Garcia-Montaño et al., 2008). The estimated values of octanol–water partition coefficients (log K_{OW}) were also considered to certify the consistency of the proposed structures and their polarity. The retention times of the compounds detected, the main fragments detected in LC/MS analysis and the proposed structures, as well as the estimated values of log K_{OW} and solubility are shown in Table 2.

The isomeric compounds C3 and C6 with *m/z* 331 were detected with retention times 4.8 min and 8.3 min, respectively, which could be ascribed based on the extracted ion chromatogram of *m/z* 331 (Fig. S1). These two isomers were already detected before start of degradation indicating their presence as contaminants in commercial DR1. However, the concentration of intermediate C6 increased during reaction time up to 10 min, indicating that it was also a degradation product generated as a consequence of electrophilic addition of hydroxyl radical to the more activated aromatic ring of DR1, the ethyl and ethanol (N–(CH_2CH_3) (CH_2CH_2OH)) substituted ring. On the other hand, the concentration of C3 did not increase, but started to decrease after 10 min indicating its degradation. The large difference of their retention times can be explained by the position of OH group in the ring. In the case of C6 (8.3 min), the attack of OH radical to carbon 2 of the benzene ring (forming a 2-hydroxy benzene group) favors the formation of a hydrogen bond between H of OH group and N from azo group as previously reported (Brode et al., 1952) explaining the lower polarity and longer retention time of this isomer in relation to C3. Its lower polarity is also denoted by the estimated log K_{OW} = 4.1 (Table 2). The fragmentation of this intermediate resulted in the ion with *m/z* 283, a product of the loss of water followed by the loss of ethane group. On the other hand, 3-hydroxy benzene group of C3 (t_r = 4.8 min) does not favor the interaction of H and N from azo bond and the OH group imposes higher polarity to the molecule.

The extracted ion chromatogram of *m/z* 357 indicated that intermediate C4 was also detected at 4.8 min retention time due possibly to a coelution with product C3, which can be verified in MS/MS spectrum between 4.5 and 5.1 min (Fig. S2). However, no structure could be attributed so far. The structure of the intermediate C5, with *m/z* 347, was proposed to be a product of the addition of two hydroxyl radicals to the same aromatic ring (Table 2). Its fragmentation led to the loss of water resulting in an ion with *m/z* 329, followed by the cleavage of the bond between the aromatic rings and azo group (*m/z* 179) (Fig. S3). The intermediate C7, with *m/z* 287 is a result of loss of ethanol group from product C3. The fragment with *m/z* 241 detected in MS^2 spectrum was formed with the loss of water molecule and another ethane group (Fig. S4).

The intermediate C1, with *m/z* 194 presented very similar spectrum as reported previously for a product of DR1 degradation by a consortium of aerobic and anaerobic bacteria (Franciscon et al., 2015). It was formed from the cleavage of the bond between nitrogen from azo group and the ethyl and ethanol (N-(CH_2CH_3) (CH_2CH_2OH)) substituted ring, addition of two hydroxyl radicals, to carbon 2 and 3 and oxidation of ethanol and ethyl group. This intermediate was previously detected during DR1 degradation by a consortium of aerobic and anaerobic bacteria (Franciscon et al., 2015). The fragmentation of this intermediate led to the formation of the ion with *m/z* 166 due to the loss of CO from the aldehyde group. The fragment with *m/z* 216 corresponding to [M+Na] was also detected confirming the ion [M+H] (Fig. S5).

Finally, the intermediate C2, with m/z 273 is a product of the cleavage of azo bond due to the addition of hydroxyl radical after

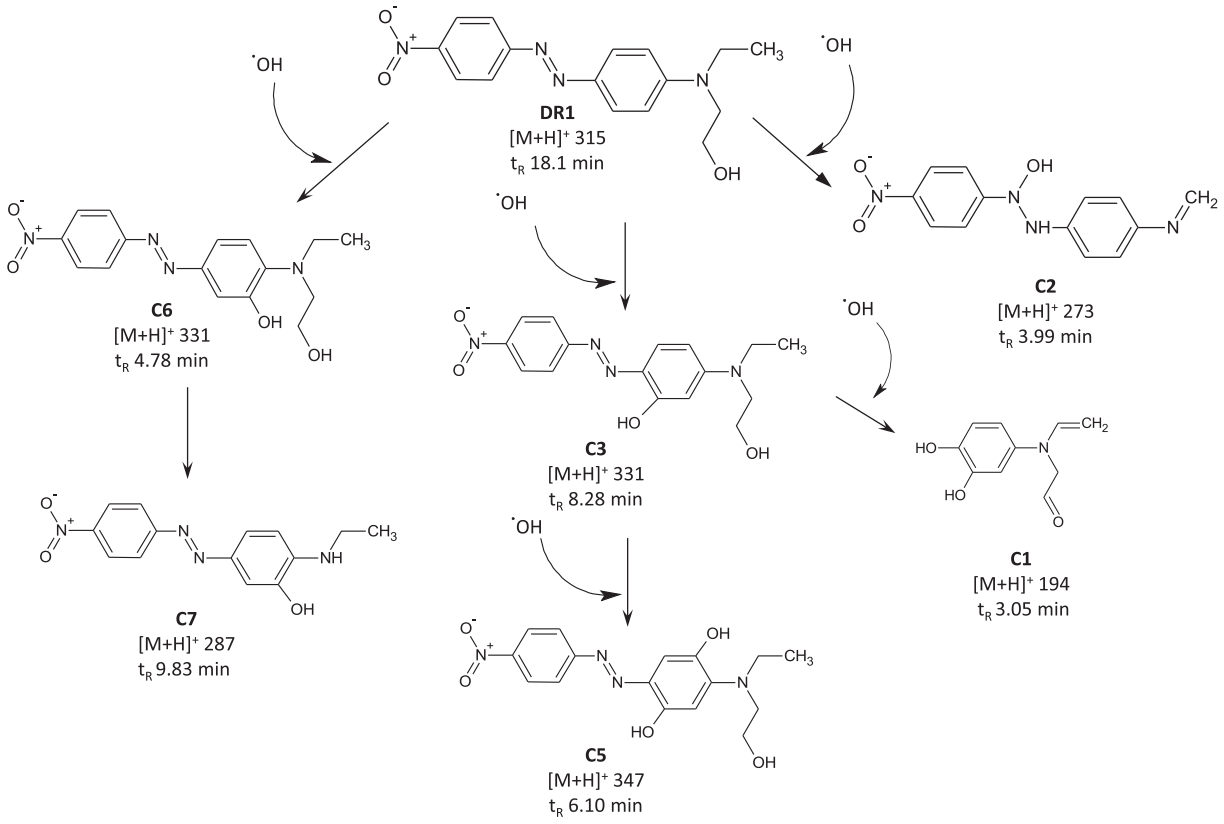


Fig. 5. Proposed initial pathway for degradation of commercial DR1 dye.

loss of ethane group and further decarboxylation (Fig. S6). This is the only intermediate detected resulting from azo bond cleavage, what is in accordance with the DAD absorption spectrum of the intermediate with 3.8 min retention time. The identification of degradation products of DR1 allowed proposing a degradation rout, which occurred by the attack of hydroxyl radicals both to benzenic ring and to the azo bond (Fig. 5).

4. Conclusions

The photo-Fenton process was effective for complete removal of acute toxicity to *D. similis* of DR1 azo dye after 45 min degradation reaction. However, the initial 10 min reaction showed the highest concentration of degradation products and considerable increase of toxicity due equally to the remaining concentration of original dye and intermediates formed. Most of the intermediates detected maintained the azo group since degradation was initiated mainly from hydroxyl radical addition to aromatic ring. However attack of hydroxyl radical to nitrogen of azo bond was also verified in one intermediate. The results demonstrated that total mineralization must not be the main goal of the photo-Fenton treatment considering the non-toxicity of residual organic carbon concentration, but toxicity monitoring is essential for adjusting the reaction time and conditions to avoid the release of intermediates of higher toxicity than the original dye.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.01.053.

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