Decolorization of azo dye C.I. Reactive Black 5 by ozonation in aqueous solution: influencing factors, degradation products, reaction pathway and toxicity assessment
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ABSTRACT
In this study, ozonation treatment of C.I. Reactive Black 5 (RB5) was investigated at various operating parameters. The results showed that the aqueous solution initially containing 200 mg/L RB5 was quickly decolorized at pH 8.0 with an ozone dose of 3.2 g/h. Reaction intermediates with m/z 281, 546, 201, 350, 286 and 222 were elucidated using liquid chromatography-mass spectrometry, while sulfate ion, nitrate ion and three carboxylic acids (i.e., oxalic acid, formic acid, and acetic acid) were identified by ion exchange chromatography. Thus, the cleavage of the azo bond and the introduction of OH groups in the corresponding positions were proposed as the predominant reaction pathway. The detachment of sulfonic groups was also commonly observed during the ozonation treatment. The proposed degradation mechanism was confirmed by frontier electron density calculations, suggesting the feasibility of predicting the major events in the whole ozonation process with the computational method. Compared with RB5 degradation, the reduction of total organic carbon (TOC) proceeded much more slowly, and approximately 54% TOC was removed after 4 h of ozonation. Acute toxicity tests with Photobacterium phosphoreum showed that the toxicity of reaction solution was firstly increased and then decreased to a negligible level after 160 min.

Key words | acute toxicity, degradation products, frontier electron density, ozonation, reaction pathway

INTRODUCTION
Large quantities of wastewaters containing synthetic dyes are produced worldwide due to their extensive use in industry (Ozcan et al. 2007). Among the most widely used commercial reactive dyes, azo dyes, which are characterized by nitrogen double bonds (–N = N–), account for approximately 70% of the world dye production (Martinez-Huitle & Brillas 2009). Only 40–90% of dyestuffs are fixed to the fabric during the dyeing process, while the rest tend to pass through conventional biological treatment systems unaffected before finally entering into the aquatic environment (Panswad & Luangdilok 2000; Pearce et al. 2003; Baban et al. 2010). The strongly colored wastewaters not only cause aesthetic problems but also threaten the environment and human health. Therefore, it is imperative to reduce or eliminate the discharge of colored wastewater.

The advanced oxidation processes that usually generate hydroxyl radicals (•OH) to strongly oxidize the organics have been widely employed in treating industrial wastewater and groundwater (Lamsal et al. 2011). Specifically, as one of the most common and powerful oxidizing agents, ozone can degrade a wide variety of dyes in aqueous solutions by destroying most of the double bonds, such as C = C, C = N, and N = N bonds (Martins et al. 2006; Nakada et al. 2007; Beltran et al. 2008). Moreover, ozonation is considered one of the potential methods to be used in combined treatments, e.g., oxidative and biological processing of wastewater from the textile dyeing industry (Wang et al. 2003), and the implementation of an ozonation treatment setup is quite feasible in terms of energy and cost (Joss et al. 2008).
The azo dye C.I. Reactive Black 5 (RB5) is one of the most commonly used dyestuffs in textile industries that is widely present in the aquatic environment. Until now, several techniques have been applied for RB5 degradation, including Fenton/Fenton-like processes (Lucas & Peres 2006), photocatalytic degradation (Song et al. 2007b), electrochemical processes (Wang et al. 2008), biological treatments (Vijayaraghavan & Yun 2007), ozonation (Chen et al. 2005), UV/H₂O₂ oxidation (El-Dein et al. 2003), and resin adsorption (Elwakeel & Rekaby 2011). However, limited information is available on the reaction intermediates and products formed during the ozonation of RB5. This deficiency prompted us to conduct the current research, trying to provide some reference for the potential application of this technique in RB5 treatment.

In this work, the removal efficiency of RB5 by ozonation was examined at various operating conditions. The degradation products generated from the ozonation treatment were identified by liquid chromatography-mass spectrometry and ion exchange chromatography analyses. On the basis of the reaction intermediates, the possible oxidation degradation mechanisms and reaction pathways of RB5 in aqueous solution were proposed and further interpreted using molecular orbital calculations of the frontier electron densities (FEDs). Moreover, to assess the extent of mineralization, the reduction of total organic carbon (TOC) was investigated during the oxidation process of RB5. According to previous studies (Wang et al. 2002, 2003), the initial intermediates after short-term ozonation may have higher toxicity than the starting compound. Thus, luminescence inhibition in Photobacterium phosphoreum was also measured to evaluate the toxicity change of dye solution after ozonation treatment.

**EXPERIMENTAL**

**Chemicals**

RB5 (C₂₆H₂₁N₅O₁₉S₆Na₄) with a purity >95% was obtained from Tianjin Shengda Ruitai Chemical Co., Ltd (Tianjin, China). The dye stock solution was prepared by dissolving RB5 in ultrapure water from a Millipore Milli-Q system (Joffrey, USA). The high performance liquid chromatography (HPLC)-grade methanol and tert-butanol were supplied by Merck (Darmstadt, Germany) and Sigma–Aldrich (Steinheim, Germany), respectively. Ammonium acetate (purity >99%) was purchased from J&K (China).

**Ozonation procedures**

The experimental setup (Figure 1) consisted of a 500 mL three-necked, round-bottomed flask as the reaction vessel, a thermostat bath, an electrolysis-type ozone generator (DJ-Q2020A, Yichang, China), a vacuum pump, a motor stirrer, and an effluent ozone exhaust system. During a typical experimental run, ozone gas was continuously bubbled into the reaction solution through a glass tube with a sintered end, and the excess ozone was introduced into a washing bottle containing 2% (w/v) KI solution. The decolorization efficiency of RB5 by ozonation was investigated under different experimental conditions by varying pH values (2.0, 4.0, 6.0, 8.0, 10.0 and 12.0), initial dye concentrations (100, 200, 300 and 500 mg/L), and ozone dosages (0.8, 1.6, 2.4 and 3.2 g/h). The effect of the hydroxyl radical scavenger t-butanol (100 mM) on the ozonation of RB5 was also evaluated. The pH of the solution was adjusted to the desired value with phosphate buffers. During ozonation, aliquots of 5 mL were withdrawn at various reaction times and...
immediately bubbled with N₂ to remove residual ozone. These samples were diluted five times and passed through a 0.22 μm membrane filter before the analysis. To ensure the accuracy of the results, each experiment was replicated three times.

**Analytical methods**

The ozone concentration was determined using an iodometric method. Decolorization was evaluated by measuring the absorbance at 600 nm with a TU-1810 spectrophotometer. The TOC content was measured by a TOC analyzer (TOC-5000A, Shimadzu, Japan).

The degradation products of RB5 during ozonation were detected by a Thermo Finnigan Surveyor Modular HPLC system tandem a Finnigan LCQ Advantage MAX ion trap mass spectrometer (liquid chromatography-mass spectrometry (LC-MS), Thermo, USA). A Syncronis aQ column (250 × 4.6 mm i.d., 5 μm particle size, Thermo) was used for the HPLC separation. The mobile phase was a mixture of water (20 mM ammonium acetate, phase A) and methanol (phase B) eluted at 1.0 mL min⁻¹ in a gradient mode (98% A at 0–6 min, 98–10% A at 6–8 min, 10% A at 8–25 min). The column temperature was set to 30 °C. The mass spectrometer was operated with electrospray ionization (ESI) source in negative mode for the MS and MS/MS measurement. Capillary voltage and cone voltage were 4.5 kV and 25 V, respectively. Desolvation and source temperature were 300 °C and 120 °C, respectively. Nitrogen was used as nebulizer and sheath gas at a flow rate of 35 arbitrary units, and argon was used as a collision gas at 0.25 MPa.

A Dionex ion chromatograph (IC, model ICS 1000, Dionex, Sunnyvale, CA, USA) equipped with a dual-piston pump, a Dionex IonPac AS11-HC analytical column (4 × 250 mm) and a Dionex DS6 conductivity detector were used to detect carboxylic acids and nitrate and sulfate ions. The flow rate of the eluent (20 mM ammonium acetate, phase A) and methanol (phase B) eluted at 1.0 mL min⁻¹ in a gradient mode (98% A at 0–6 min, 98–10% A at 6–8 min, 10% A at 8–25 min). The column temperature was set to 30 °C. The mass spectrometer was operated with electrospray ionization (ESI) source in negative mode for the MS and MS/MS measurement. Capillary voltage and cone voltage were 4.5 kV and 25 V, respectively. Desolvation and source temperature were 300 °C and 120 °C, respectively. Nitrogen was used as nebulizer and sheath gas at a flow rate of 35 arbitrary units, and argon was used as a collision gas at 0.25 MPa.

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**Calculations**

The molecular orbital (MO) calculations of the FEDs were performed via the density functional theory method at the B3LYP/6-31G° level using Gaussian 09 program package (Frisch et al. 2009). The geometrical configurations were optimized, and frequency calculations were performed to ensure convergence upon a true energy minimum. The FEDs of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were determined from Gaussian output files. Values of \( \text{FED}_{\text{HOMO}} \) and \( \text{FED}^2 \_{\text{LUMO}} \) were then obtained to predict the reaction sites for hydroxyl radical attack (Fukui et al. 1952, 1954; Lee et al. 2001). The solvent effect of H₂O was also considered in the computational process with the keyword ‘scrf = (Dipole)’.

**Acute toxicity tests**

The luminescence inhibition in the marine photobacteria *P. phosphoreum* was measured according to the ISO standard method. Before toxicity tests, sample pH for the reaction solution was readjusted to 8.0 using 0.1 M HCl or 0.1 M NaOH solution. The sample was applied in its original state without dilution, and the bioluminescence was measured using a Tecan Infinite 200® PRO multimode microplate reader (Tecan, Switzerland) after a 15 min incubation time. Then, inhibition ratio in luminescent intensity against the blank control was calculated for each sample. To ensure the accuracy, all sample tests and control experiments were performed in duplicate.

**RESULTS AND DISCUSSION**

**Effect of operation parameters**

The reaction of ozone with an organic compound has been reported to be of first order with respect to both ozone and the organic compound (Langlais et al. 1990). In the current work, influent ozone gas was continuously sparged into the solution, and therefore, the ozone concentration can be assumed to be constant during the course of ozonation. Thus, degradation of RB5 by ozonation was evaluated by fitting with the pseudo-first-order kinetic model. The degradation kinetics of RB5 at various operating conditions is shown in Figure 2, and the apparent rate constants obtained are listed in Table S1 of the Supporting Information (available with the online version of this paper).

**Effect of pH**

As is shown in Figure 2(a), the decolorization efficiency after 5 min of ozonation increased from 11.85 to 46.84%, with the initial pH of the dye solution increasing from 2.0 to 8.0. Meanwhile, the apparent pseudo-first-order rate coefficients increased from 0.0544 to 0.1252 min⁻¹. However, a further increase of the initial pH of the solution led to a slight decrease in the decolorization efficiency. For instance, the apparent pseudo-first-order rate coefficients decreased to 0.0558 min⁻¹ at pH 12.0.
The pseudo-first-order rate coefficient at pH 8.0 was larger than the others. This is related to the different reactive species involved in the degradation process. Ozonation of dyes predominantly proceeds by a direct oxidation mechanism in neutral-acidic conditions (Sevimli & Sarikaya 2002; Colindres et al. 2010), while alkaline conditions facilitate the rapid decomposition of ozone to hydroxyl radicals, resulting in a higher reaction rate because ·OH (oxidation potential, 2.80 V) is a stronger oxidant than molecular ozone (oxidation potential, 2.07 V). At pH > 8.0, the recombination reaction among hydroxyl radicals may predominate over the reaction between hydroxyl radicals and RB5, therefore decreasing the decolorization efficiency (Kusvuran & Yildirim 2013). Considering the high decolorization efficiency of RB5, pH 8.0 was selected for subsequent experiments.

**Effect of initial dye concentration**

Figure 2(b) shows the effect of the initial dye concentration on the decolorization of RB5 at pH 8.0 with an ozone dose of 3.2 g/h. When the initial RB5 concentration increased from 100 to 500 mg/L, the apparent pseudo-first-order rate constant decreased from 0.1252 to 0.0422 min⁻¹. Wu et al. (1998) also reported that in the treatment of reactive-dye wastewater, the decolorization rate decreased with increasing initial dye concentration. This result can be explained by the following: first, because the ozone inlet concentration and the flow rate are maintained constant throughout the experiment, the amount of O₃ and -OH radicals generated in solution is assumed to be in a steady state, resulting in a better decolorization efficiency at a lower initial dye concentration. Furthermore, a higher concentration of oxidation by-products was produced with increasing initial RB5 concentration, and the competition between the by-products and RB5 for the oxidants will reduce the rate of RB5 degradation.

**Effect of ozone dosage**

The decolorization efficiency increased with increasing ozone dosage (Figure 2(c)). For example, after 10 min of ozonation, the RB5 removal efficiency was 25.61%, 41.93%, 60.07% and 70.21% at O₃ dosages of 0.8 g/h, 1.6 g/h, 2.4 g/h and 3.2 g/h, respectively. As seen in Table S1, the apparent pseudo-first-order rate constant increased from 0.0467 min⁻¹ (O₃: 0.8 g/h) to 0.1252 min⁻¹
This result was not unexpected, because the O₃ concentration in the solution and the generation of •OH radicals are increased with increasing ozone dosage (Song et al. 2007b).

Ozonation of RB5 in the presence of t-butanol

In order to determine the role of hydroxyl radicals in RB5 removal, t-butanol was pre-spiked into the reaction solution as the OH scavenger. t-Butanol selectively reacts with OH (6 × 10⁸ M⁻¹s⁻¹) to form stable intermediates, thus terminating any radical-induced reactions (Guzman-Perez et al. 2014). The effect of t-butanol on RB5 removal in the ozonation process is shown in Figure 2(d). It was found that the presence of t-butanol significantly inhibited the decolorization of RB5. Compared with the treatment without TBA at pH 8.0 (100% removal after 60 min), the decolorization rate was 47.0% after the addition of TBA. This result suggests that the OH radical-induced indirect mechanism dominates the reaction process at pH 8.0.

Identification of organic intermediates by ESI mass spectroscopy

The HPLC/UV chromatogram of the reaction solution was monitored at 254 nm in the time course of RB5 degradation (Figure 3). Before the ozonation treatment, the original dye showed a peak with a retention time of 11.08 min. During the ozone oxidation process, the RB5 peak was decreased in intensity, and some new peaks appeared, which is indicative of the generation of degradation intermediates. As the reaction continued, the intermediate products formed in the initial reaction stage also underwent a process of further degradation, leading to the formation of other compounds. Moreover, after treatment for 120 min, two peaks with retention times of 1.71 and 1.91 min still existed, suggesting they are relatively resistant to ozonation degradation.
In line with the appearance of the new peaks in the HPLC/UV chromatogram, a total of eight degradation intermediates were identified by LC-MS analysis (Table 1). The MS and MS/MS spectra of the initial dye and the ozonation products are listed in Figures S1 and S2 of the Supporting Information for reference (available with the online version of this paper). For the negative-ion ESI analysis, the presence of sulfonic groups generally causes reduced stability of quasi-molecular ions, lowering the relative abundances of the peaks of deprotonated molecules \([M-H]^-\). Moreover, due to the high concentrations of sodium salts in the dye samples, the mass spectra always show abundant adducts with sodium ions in a general formula \([M-(x+y)H+yNa]^x-\). In addition, the presence of sulfuric acid \((-SO_2H)\) and sulfate ester \((-O-SO_3H)\) functional groups often leads to a considerable amount of fragmentation with typical neutral losses of \(H_2SO_4\) and \(SO_3\) in the mass spectra. The title compound, RB5, is a typical polysulfated compound, and its mass spectrum was characterized by an increased level of background noise and a surprisingly high extent of fragmentation. As seen in Figure S1, singly (\(m/z\) 902) and doubly (\(m/z\) 450.5) charged fragment ions and the sodium adduct \([M-2H + Na]^+\) (\(m/z\) 924) were observed in the mass spectrum of RB5. The hydrolysis of the \(-O-SO_3H\) groups in RB5 occurred during the treatment, yielding products P2 (11.53 min, MW = 823) and P5 (11.64 min, MW = 743). The detection of P2, P5, and other degradation products (P4, P7 and P8) indicates that the detachment of sulfonic groups is common during the ozonation treatment of RB5. The peaks with retention times of 7.43 min, 10.84 min and 2.22 min were found to have base ions at \(m/z\) 281, 546 and 350, which were identified as P1, P3 and P6, respectively. The formation of these reaction intermediates suggests that the cleavage of the azo bond and the introduction of OH groups in the corresponding positions occurred in the ozone oxidation process. Likewise, some degradation products (e.g., P1) have also been observed in the electrochemical degradation of sulphonated azo dyes in a previous study (Vanerkova et al. 2006). Obviously, the naphthalene skeleton remained intact in all of these.

### Table 1 | Molecular weights (MW), retention times (\(t_R\), min), product ions and proposed structures of degradation products of C.I. Reactive Black 5 dye by ozonation*

<table>
<thead>
<tr>
<th>Compound, MW</th>
<th>(t_R)</th>
<th>Product ions observed in MS</th>
<th>Product ions observed in MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 MW=282</td>
<td>7.43</td>
<td>281[M-H]^-</td>
<td>217[M-H-SO_3]^-</td>
</tr>
<tr>
<td>P5 MW=547</td>
<td>10.84</td>
<td>546[M-H]^-</td>
<td>466[M-H-SO_3]^</td>
</tr>
<tr>
<td>P4 MW=202</td>
<td>7.09</td>
<td>201[M-H]^-</td>
<td>201[M-H], 95[C_6H_5O]^-</td>
</tr>
<tr>
<td>P8 MW=223</td>
<td>1.91</td>
<td>222[M-H]^-</td>
<td>222[M-H]^-</td>
</tr>
</tbody>
</table>

*See Figure 4 for proposed structures.

1For P2 and P5, M represents initial dye molecule (MW=903).
compounds, possibly indicating that the naphthalene ring structure was stable toward oxidative degradation (Mendez-Martinez et al. 2012).

Identification of inorganic ions and carboxylic acids

In the present study, the sulfate ion, nitrate ion and carboxylic acids formed during RB5 ozonation were identified via ion exchange chromatography by comparison with authentic standards. If the sulfur atoms in the RB5 molecule were completely transformed into sulfate ions, the $\text{SO}_4^{2-}$ concentration should be 116.15 mg/L at an initial dye concentration of 200 mg/L. However, the concentration of sulfate ions was measured as 77.35 mg/L, corresponding to a conversion rate of 66%. The fact that some relatively stable sulfur-containing intermediates limit the release of sulfur atoms may account for the partial conversion to $\text{SO}_4^{2-}$ (Wu & Chang 2006). Similarly, the theoretical maximum concentration of nitrate is 62.51 mg/L, but the measured value was very low, only 7.22 mg/L. The low transformation rate was also reported by Gao et al. (2012) in the ozonation treatment of azo dye Acid Red 14.

![Proposed degradation pathways of RB5 during ozonation treatment based on products identified by HPLC/MS. * means that the degradation product was not detected but postulated from the experimental results. [RB5] = 200 mg/L, pH = 8.0, [O₃] = 3.2 g/h, T = 25.0 °C.](image)
reason for the loss of nitrogen may be that most nitrogen atoms were converted into N₂, NO, or NO₂ during the ozonation treatment, and some might still exist in the resistant by-products. In addition, three carboxylic acids (oxalic acid, formic acid, and acetic acid) were identified as the ozonation products of RB5. This is consistent with previous reports that the ozonation of azo reactive dyes usually generates a series of carboxylic acids with lower molecular weights (MW) (Lopez et al. 1998; Koch et al. 2002; Wang et al. 2003).

Degradation mechanism

Based on the identified degradation products, the reaction pathways for the ozonation degradation of RB5 were tentatively proposed (Figure 4). As seen, two major reactions, namely, the detachment of sulfonic groups and the cleavage of azo bonds, along with the introduction of OH groups were involved in the oxidation of RB5. Specifically, the detachment of the sulfonic moiety from the mother compound led to the formation of P2 (when one -O-SO₃H group was cleaved) and P5 (when both -O-SO₃H groups were eliminated). Meanwhile, the cleavage of the azo group in the initial dye molecule yielded the compounds S1 (MW = 627) and P1. This is similar to the observation that hydroxyl substituted naphthalene compounds are produced from the cleavage of the azo bond in the azo dye Acid Red 14 when treated by ozonation (Gao et al. 2012). The compound S1 can also be generated as a result of azo bond breakage in P2. Then, P3 was formed either from the compound S1 via the elimination of the sulfonic group or from P5 via the breaking of the azo bond and subsequent hydroxylation reaction. A similar azo bond cleavage reaction can also occur on P3, with the formation of the hydroxylated naphthalene compound (P6) and the alkylsulfonyl phenolic compound (P4). Then, the de-sulfonic and subsequent hydroxyl addition reactions on the P6 molecule gave rise to the formation of P7, which can be further transformed into P8. Moreover, P1 was converted into P4 through the detachment of sulfonic groups. Finally, further oxidation of P8 may generate low-molecular-weight organic acids (e.g., formic, acetic and oxalic acids), carbon dioxide and water.

Interpretation of electronic density calculation

As mentioned above, the hydroxyl radicals were the major reactive species for RB5 degradation under the experimental conditions (pH 8.0). To predict the reaction site for hydroxyl radical attack, the FEDs of RB5 and the ozonation products were calculated. According to the frontier orbital theory, the addition of hydroxyl radicals usually occurs at positions with the highest FED HOMO + FED LUMO values (Lee et al. 2001; Zhang et al. 2008).

As shown in Figure 5, the highest FED HOMO + FED LUMO values for the initial dye molecule were found to be distributed at the N(18) and N(17) atoms, indicating that azo linkage (N 18 = N 17) was the most reasonable site at which the attack of a hydroxyl radical could occur. Thus, the formation of the degradation intermediates P1 and S1 can be attributed to the attack of hydroxyl radicals on the azo bond. The FED HOMO + FED LUMO values for the degradation intermediates are listed in Table S2 of the Supporting Information (available with the online version of this paper). From the FED calculation result for the P1 molecule, we can see that the S(14) atom possessed the highest FED HOMO + FED LUMO value. Thus, the hydroxyl radical attacked this site, leading to the cleavage of the -SO₃H group with the formation of P4. Similarly, the highest FED HOMO + FED LUMO value was found at the S(36) atom for the tentative product S1, suggesting that the hydroxyl radical may preferentially attack the S(36) site. The detection of P5 by HPLC/MS analysis confirms this prediction. In the P3 molecule, where the sulfate ester group was

![Figure 5](https://iwaponline.com/wst/article-pdf/73/7/1500/183136/wst073071500.pdf)
replaced by a hydroxyl group, the N(19) and N(18) atoms were found to have higher $\text{FED}_{\text{HOMO}}^{2} + \text{FED}_{\text{LUMO}}^{2}$ values (0.587 and 0.351) than other atoms, showing that the azo bond was the prior preferred position for hydroxyl radical attack. This was confirmed by the observation of P6 in mass spectra. As for the P6 molecule, the S(11) atom had the highest $\text{FED}_{\text{HOMO}}^{2} + \text{FED}_{\text{LUMO}}^{2}$ value, demonstrating that this position was more likely to be attacked by hydroxyl radicals. With the elimination of the $-\text{SO}_3\text{H}$ group and subsequent addition of the $-\text{OH}$ group, P6 was transformed into P7. In a similar way, the generation of P8 can be well interpreted by the FED calculations, because the highest $\text{FED}_{\text{HOMO}}^{2} + \text{FED}_{\text{LUMO}}^{2}$ value was located at the S(16) atom in the precursor compound P7.

On the other hand, the O-S bond in the $-\text{O}-\text{SO}_3\text{H}$ group always has the smallest Wiberg bond order (Figure S3 in the Supporting Information, available with the online version of this paper), implying that this bond was susceptible to bond cleavage and the hydrolysis reaction may occur. Thus, the initial dye was transformed to P2 and finally P5. Compounds S1 and P1 can also be hydrolyzed to form P3 and P4, respectively. According to the FED calculations, the N(17) and N(18) atoms in the P2 molecule were found to have the highest $\text{FED}_{\text{HOMO}}^{2} + \text{FED}_{\text{LUMO}}^{2}$ values. Therefore, the hydroxyl radical tended to attack the $-\text{N}_1\text{=N}_1\text{=N}_1$ bond to form the tentative product S1. Similarly, the initial position for hydroxyl radical attack should be on the $-\text{N}_1\text{=N}_1\text{=N}_1$ azo linkage for P5 molecule. As a result, P5 was oxidized to P3.

Mineralization

To assess the extent of mineralization, the variation of TOC content was measured during the ozonation treatment.

![Figure 6](https://iwaponline.com/wst/article-pdf/73/7/1500/183136/wst073071500.pdf)

**Figure 6** | The variation of TOC content during the ozonation process. [RB5] = 200 mg/L, pH = 8.0, $[\text{O}_3]$ = 3.2 g/h, $T$ = 25.0 °C.

It was observed that the decolorization of RB5 proceeded much faster than the decay of TOC. When the rate of decolorization reached nearly 100% after the 60 min reaction, the corresponding mineralization rate was 20%. The high decolorization rate can be attributed to the ease of chromophore destruction, while the low mineralization rate may indicate the generation of more stable intermediates during ozone oxidation (He et al. 2008). As indicated by the HPLC-UV chromatograms, P7 and P8 are still present in the reaction solution after ozonation for 120 min, from which we may infer that hydroxyl-substituted naphthalene compounds are the primary components of TOC during the earlier stage of ozonation, while various carboxylic acids generated by the opening of aromatic rings mainly constitute the TOC of the later ozonation treatment. Thus, ozonation can lead to the decomposition of structure but rarely completely mineralizes organic compounds to CO₂ and H₂O (Zhao et al. 2004). After a 4 h reaction, approximately 54% TOC was removed, suggesting that a longer treatment time or combined treatment is necessary for higher mineralization.

**Toxicity assessment of reaction solution**

Aqueous solution with an initial dye concentration of 200 mg/L was treated by ozonation for different times, and the reaction solution was then measured for luminescence inhibition using *P. phosphoreum* as the test organism. As shown in Figure 7, the toxicity of the reaction solution to luminescent bacteria was increased in the early stage of ozonation. After 5 min of ozonation, inhibition rate in luminescence reached the maximum, followed by a slight decrease in 10 min. Then, the toxicity of ozonated samples after 15 min decreased to be lower than the original dye solution. As the ozonation reaction proceeded, the toxicity was continuously decreased until no detectable inhibition to *P. phosphoreum* after 160 min. Taking into account the LC-MS analysis results, we may conclude that degradation products such as product 1, product 2 and product 3 are main contributors to the increased toxicity after ozonation.

**CONCLUSIONS**

Overall, the current work suggests that ozonation treatment can effectively decolorize the azo dye in aqueous solution. An aqueous solution initially containing 200 mg/L RB5 was almost completely decolorized after 60 min of ozonation at pH 8.0 with an ozone dose of 3.2 g/h. Eight
oxidation products were identified by LC-MS, and the detachment of sulfonic groups and the cleavage of azo bonds with the subsequent introduction of OH groups in the corresponding positions were proposed as the major reaction pathways. The ion chromatography analysis showed that most of the sulfur atoms in RB5 were converted into sulfate ions, while the transformation rate of nitrogen atoms to nitrate ions was very low (~1.55%). The FEDs calculation-based prediction of the possible reaction sites for hydroxyl radical attack is in satisfactory agreement with the experimental results, indicating that it is feasible and operable to predict the major events during the whole ozonation process of RB5, not only the initial oxidation stage, with this computational method. Compared with the rate of RB5 decolorization, TOC decreased much more slowly, and the TOC removal was only 54% after a 4 h ozonation treatment.

Acute toxicity tests using Photobacterium phosphoreum suggested that the toxicity of the reaction solution was increased to be higher than the initial RB5 solution in the first 10 min and then decreased to a negligible level after 160 min.

REFERENCES


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Figure 7 | Luminescence inhibition in Photobacterium phosphoreum as a function of ozonation time. [RB5] = 200 mg/L, pH = 8.0, [O3] = 3.2 g/h, T = 25.0 °C.
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