CHAPTER 3

Decolouration and degradation of Reactive Red-120 dye
3.1. Radiolysis of aqueous solution of Reactive Red - 120

Dye is an organic compound that absorbs light in the visible range (i.e., 400 nm to 700 nm) of the electromagnetic spectrum. The physical colour of the dye is characterized by the complementary colour of the absorption band. The chromophore and auxochrome shift the absorption band in the visible region and intensify the colour. The reactive group helps to bind the dye molecule with the fibers and the sulphonic acid groups help in the solubility of dye in water. Destruction of the chromophore group of the dye causes the decolouration of the dye solution, whereas mineralization happens from the complete oxidation of the dye molecule.

3.1.1. Structure of Reactive Red - 120

The molecular structure of Reactive Red – 120 (RR-120) is shown in Figure 3.1. RR-120 bears two azo groups as the chromophoric moiety and two chlorotriazine groups as reactive groups. The hydroxyl groups present in the cellulose fibres forms covalent bond with RR-120 by the nucleophilic substitution of the chlorine atom of the chlorotriazine group. The phenyl and naphthol rings provide the extended \( \pi \)-conjugation through the azo group.

The UV-Visible absorbance spectrum of 40 \( \mu \)M aqueous RR-120 solution at pH 7 is shown in Figure 3.2 (a). The peaks at (1) 235 nm and (2) 293 nm correspond to \( \pi-\pi^* \) transition of the benzene and naphthalene units of RR-120. The small hump (3) at 373 nm represents n- \( \pi^* \) transition from the nitrogen atoms of the azo group to the naphthalene ring [68]. The peaks at (4) 510 nm and (4') 538 nm represent two energetically closed \( \pi-\pi^* \) transition between the molecular orbitals formed by extended \( \pi \) conjugation including the benzene ring, azo linkage and naphthol ring [68].
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Figure 3.1 Azo and Hydrazone tautomeric forms of Reactive Red – 120.

Figure 3.2 UV-Visible spectra of aqueous solution of 40 µM RR-120 at (a) pH 7 and (b) pH 13.8.
Upon increasing the pH of the solution to 13.8, the colour of the dye solution becomes orange, which is also evidenced from the shift of the visible band (4, 4') to lower wavelength (*Figure 3.2(b)*). The splitting peak (4 and 4') disappeared and a single peak appeared at 482 nm.

RR-120 has two tautomers viz. azo and hydrazone (*Figure 3.1*). The H-atom of the naphtholic unit (in azo form) is positioned at the β-N of azo linkage in the hydrazone form. The H-atom on the β-N of the azo group is shared by the oxo group of the naphthalene ring forming a six member ring in hydrazone tautomer. Therefore, long extended π-electron conjugation is evidenced in the hydrazone tautomer (*Figure 3.2(a)*). The tautomeric H-atom of the naphthol unit is abstracted by the HO⁻ ions at pH 13.8. This stops the formation of hydrazone tautomer, which is evidenced from the shift of the visible band of the spectrum to the lower wavelength (*Figure 3.2(b)*). The variation of absorbance with pH was more prominent at 450 and 550 nm.

*Figure 3.3* shows the variation of optical density (OD) at 450 and 550 nm of aqueous solutions of 40 µM RR-120 as a function of pH. Therefore, pKₐ of RR-120 corresponding to the dissociation of the naphtholic hydrogen was calculated as 12.5 from the point of intersection of the ODs at 450 and 550 nm as a function of pH (*Figure 3.3*). The higher pKₐ value of RR-120 compared to other H-acids (i.e. phenol etc.) is attributed to the strength of the six member ring formed in the hydrazone tautomer involving the dissociating H-atom of RR-120.
3.1.2. Radiolysis of RR-120 by Oxidising Radicals

3.1.2.1. Reaction of RR-120 with Hydroxyl Radical (\(\cdot OH\))

Aqueous solution of 40 µM RR-120 solution (at pH 7) was irradiated for different doses in \(^{60}\)Co gamma chamber having a dose rate of 3 kGy h\(^{-1}\) (measured using Fricke dosimetry). During the radiolysis of water, hydroxyl radicals (yield of \(\cdot OH = 0.28 \mu\text{mol J}^{-1}\)), hydrated electrons (yield of \(e^-_{aq} = 0.28 \mu\text{mol J}^{-1}\)) and hydrogen atoms (yield of \(\cdot H = 0.06 \mu\text{mol J}^{-1}\)) are the three main reactive inter-mediates produced in the pH range between 3 and 11. The reaction between \(\cdot OH\) and RR-120 was investigated in \(\text{N}_2\text{O}\) saturated solution, because \(e^-_{aq}\) is converted to \(\cdot OH\) by \(\text{N}_2\text{O}\) as shown in Eq. 1.29. Under this reaction condition, the yield of \(\cdot OH\) becomes 0.56 \(\mu\text{mol J}^{-1}\).

Figure 3.4(a) shows the spectra of \(\text{N}_2\text{O}\) saturated 40 µM RR-120 solutions (at pH 7) (i) before radiolysis and after steady state radiolysis at (ii) 0.5, (iii) 1.0 and (iv) 2.0 kGy. The intensities of the peaks at 235, 293, 510 and 538 nm decreased with increasing dose. It is attributed to the destruction of the benzene, naphthalene and
extended π-conjugation of RR-120 by the oxidising •OH radical. Further, the doublet peaks at 510 and 538 nm merged during radiolysis to a single peak and developed significant absorption at higher wavelength than 600 nm (It should be noted that RR-120 has no absorption at λ > 600 nm). It suggests a possibility of the formation of more extended π-conjugated system compared to RR-120 by the addition of •OH to either benzene or naphthalene ring. However, the overall absorbance of RR-120 significantly decreased at 2.0 kGy.

Figure 3.4 (a) Spectra of N₂O saturated 40 µM RR-120 solutions (at pH 7) (i) before radiolysis and after steady state radiolysis at (ii) 0.5 kGy, (iii) 1.0 kGy and (iv) 2.0 kGy. (b) ΔODₜ and (c) transient absorption as a function of λ for N₂O saturated 95 µM RR-120 solution (at pH 7) during pulse radiolysis at pulse dose of 0.01 kGy.
The reaction between the \( \cdot \text{OH} \) radical and RR-120 was investigated by pulse radiolysis experiment employing 0.01 kGy/pulse in 95 µM RR-120 solution (at pH 7) saturated with N\(_2\)O gas. The change in absorption at any wavelength \( \lambda \) (\( \Delta \text{OD}_\lambda \) of \( \Delta A \)) due to the formation of the transient species is shown in Figure 3.4b. The absorption decreased significantly in the wavelength range 460-570 nm, while this increased at 650 nm and in the region of 340-390 nm. The increase or decrease in \( \Delta \text{OD}_\lambda \) depends on the values of the molar extinction coefficients of the transient (\( \epsilon_t \)) and parent (\( \epsilon_p \)), respectively (Eq. 2.3). Therefore, for the same concentration of the transient formed, \( \Delta \text{OD}_\lambda \) increases when \( \epsilon_t > \epsilon_p \) and it decreases when \( \epsilon_p > \epsilon_t \). Thus, the observed spectrum was corrected for parent absorption by Eq. 2.5 and the corrected absorption spectrum of the species is given in Figure 3.4c, which shows a doublet peak at 510-540 nm and a weak band at 660 nm. [69] The transient spectrum shows a peak at 660 nm (Figure 3.4c), where parent dye (RR-120) has no absorption.

The formation signal of the transient species recorded at 660 nm fitted well to the second order kinetics indicating a bimolecular type of formation of the transient. The pseudo first order rate constant (\( k_\phi \)) for the reaction of \( \cdot \text{OH} \) with RR-120 was determined at 660 nm in the presence of different concentrations (50 – 100 µM) of RR-120. The bimolecular rate constant of \( \cdot \text{OH} \) with RR-120 was calculated as 7.9×10\(^9\) M\(^{-1}\) s\(^{-1}\) from the slope of the plot of \( k_\phi \) versus concentrations of RR-120.

The addition of \( \cdot \text{OH} \) to the aromatic ring of RR-120 forms cyclohexadienyl radicals, which react through disproportionation producing the dye molecule having an extra OH-group (Figure 3.5) [70]. The regenerated dye molecules having an extra OH-group should have more extended \( \pi \)-conjugation as compared to the parent dye molecule and thus it justifies the development of absorption at longer wavelength beyond 600 nm (Figures 3.4a&c). The addition \( \cdot \text{OH} \) to triazine ring is a slow reaction.
[71]. It should be noted that the decrease in absorbance at 535 nm could not be used to determine G(-Dye) in N₂O saturated solution because of the interference of the products’ absorption at the same wavelength.

Figure 3.5 Addition of *OH to the aromatic ring of RR-120 forms cyclohexadienyl radicals, which react through disproportionation producing the dye molecule having an extra OH-group. R represents the residual part of RR-120.

3.1.2.2. Reaction of RR-120 with Azide Radical (N₃*)

Aqueous solution of 40 µM RR-120 solution (at pH 7) containing 20 mM NaN₃ and saturated with N₂O was irradiated for different doses in ⁶⁰Co gamma chamber having a dose rate of 2.5 kGy h⁻¹ (measured using Fricke dosimetry). N₂O converts e⁻aq to *OH, which in turn converts N₃⁻ to N₃* as shown in Eqs. 1.29 & 1.30. Figure 3.6(a) shows the spectra of N₂O saturated 40 µM RR-120 solutions (at pH 7) containing 20 mM NaN₃ (i) before radiolysis and after steady state radiolysis at (ii) 0.5, (iii) 1.0 and (iv) 2.0 kGy. The intensities of the peaks at 235, 293, 510 and 538 nm decreased with increasing dose. It is attributed to the destruction of the benzene, naphthalene and extended π-conjugation of RR-120 by the oxidising N₃* radical. In contrast to the
reaction of RR-120 with \( \cdot \)OH, no absorbance was developed at \( \lambda > 600 \) nm during the radiolysis of RR-120 by \( N_3^\cdot \) (Figure 3.6a).

![Diagram](image)

**Figure 3.6** (a) Spectra of \( N_2O \) saturated 40 \( \mu M \) RR-120 solutions (at pH 7) containing 20 mM \( NaN_3 \) (i) before radiolysis and after steady state radiolysis at (ii) 0.5 kGy, (iii) 1.0 kGy and (iv) 2.0 kGy. (b) \( \Delta OD_\lambda \) and (c) transient absorption as a function of \( \lambda \) for \( N_2O \) saturated 100 \( \mu M \) RR-120 solution (at pH 7) containing 20 mM \( NaN_3 \) during pulse radiolysis at pulse dose of 0.01 kGy.

The reaction between the \( N_3^\cdot \) radical and RR-120 was investigated by pulse radiolysis experiment employing 0.01 kGy/pulse in \( N_2O \) saturated 100 \( \mu M \) RR-120 solution (at pH 7) containing 20 mM \( NaN_3 \). The \( \Delta OD_\lambda \) for the reaction between RR-
120 and $N_3^\cdot$ increased at 400 nm and 570 nm (**Figure 3.6b**). The observed spectrum was corrected for parent absorption by Eq. 2.5 and the corrected absorption spectrum of the species is given in **Figure 3.6c**, which shows a broad peak at 510-540 nm and a band at 395 nm. $N_3^\cdot$ radical causes one electron oxidation of RR-120 and the products formed in the reaction of RR-120 with $N_3^\cdot$ radical differs from the products formed in the reaction of RR-120 with $^\cdot$OH radical (**Figures 3.4c & 3.6c**). Hence the mechanisms of the reaction of $^\cdot$OH and $N_3^\cdot$ radical with RR-120 are different.

It is to be noted that the transient shows little absorption at $\lambda > 600$ nm. Therefore, $k_o$ for the reaction of $N_3^\cdot$ with RR-120 was determined from the formation signal of the transient species at 620 nm in the presence of different concentrations (50 – 100 $\mu$M) of RR-120. The bimolecular rate constant of $N_3^\cdot$ with RR-120 was calculated as $1.1 \times 10^9$ M$^{-1}$ s$^{-1}$ from the slope of the plot of $k_o$ versus concentrations of RR-120. The decay kinetics of the transient species was studied at 610 nm, where contribution of parent absorption was absent. It was observed that the transient decays through first order reaction with a rate constant of $3.6 \times 10^3$ s$^{-1}$.

3.1.2.3. Reaction of RR-120 with Chlorine Radical ($Cl_2^\cdot$)

Aqueous solution of 40 $\mu$M RR-120 solution (at pH 1) containing 50 mM NaCl and saturated with O$_2$ was irradiated for different doses in $^{60}$Co gamma chamber having a dose rate of 2.5 kGy h$^{-1}$ (measured using Fricke dosimetry). $^\cdot$OH converts Cl$^-$ to Cl$_2^\cdot$ by the reactions shown in **Eqs. 1.31-1.33**. **Figure 3.7(a)** shows the spectra of O$_2$ saturated 40 $\mu$M RR-120 solutions (at pH 1) containing 50 mM NaCl (i) before radiolysis and after steady state radiolysis at (ii) 0.5, (iii) 1.0 and (iv) 2.0 kGy. The intensities of the peaks at 235, 293, 510 and 538 nm decreased with increasing dose. However, a small build up of absorbance with increasing dose was observed at 420 nm.
Therefore, the benzene, naphthalene and extended $\pi$-conjugation of RR-120 are destroyed by Cl$_2^\bullet$ during radiolysis resulting into smaller molecular fragments.

**Figure 3.7** (a) Spectra of O$_2$ saturated 40 µM RR-120 solutions (at pH 1) containing 50 mM NaCl (i) before radiolysis and after steady state radiolysis at (ii) 0.5 kGy, (iii) 1.0 kGy and (iv) 2.0 kGy. (b) $\Delta$OD$_\lambda$ and (c) transient absorption as a function of $\lambda$ for O$_2$ saturated 100 µM RR-120 solution (at pH 1) containing 50 mM NaCl during pulse radiolysis at pulse dose of 0.01 kGy.

The reaction between the Cl$_2^\bullet$ radical and RR-120 was investigated by pulse radiolysis experiment employing 0.01 kGy/pulse in O$_2$ saturated 100 µM RR-120 solution (at pH 1) containing 50 mM NaCl. The $\Delta$OD$_\lambda$ for the reaction between RR-120
and Cl$_2^*$ increased in the range 340-390 and at 590 nm (Figure 3.7b). The observed spectrum was corrected for parent absorption (at pH 1) by Eq. 2.5 and the corrected absorption spectrum of the species is given in Figure 3.7c, which shows a broad peak at 510-540 nm and a band at 385 nm. The transient shows sufficient absorption at $\lambda > 600$ nm. The $k_\phi$ for the reaction between Cl$_2^*$ and RR-120 was determined from the formation signal of the transient species at 610 nm in the presence of different concentrations of RR-120. The bimolecular rate constant of the oxidation of RR-120 by Cl$_2^*$ radicals was calculated as $1.0 \times 10^8$ M$^{-1}$ s$^{-1}$.

Figure 3.8 shows the spectra of the transients produced at pH 1 from the reaction of RR-120 with (i) Cl$_2^*$ and (ii) *OH. It is evidenced that different transients are produced from these two reactions. Cl$_2^*$ radical causes one electron oxidation of RR-120 producing semi-oxidised species, which subsequently got fragmented. The transient produced by the reaction of RR-120 with Cl$_2^*$ transient decays (monitored at 610 nm) through first order reaction with a rate constant of $4.8 \times 10^3$ s$^{-1}$.

\[
\text{Figure 3.8} \quad \text{Transient absorption as a function of } \lambda \text{ for (i) } O_2 \text{ saturated 100 } \mu M \text{ RR-120 solution (at pH 1) containing 50 mM NaCl and (ii) } N_2O \text{ saturated 100 } \mu M \text{ RR-120 solution (at pH 1). Pulse dose } = 0.01 \text{ kGy.}
\]
The %decolouration (at 538 nm) of RR-120 for steady state gamma radiolysis in presence of (i) \(^{1}\text{OH}\) (at pH 7), (ii) \(^{1}\text{OH}\) (at pH 1), (iii) \(\text{N}_3\) \(^{1}\) (at pH 7) and (iv) \(\text{Cl}_2\) \(^{1}\) (at pH 1) was calculated by Eq. 3.1.

\[
\text{% decolouration} = \left(\frac{A_0 - A_D}{A_0}\right) \times 100
\]

(3.1)

where, \(A_0\) and \(A_D\) are the absorbance measured at 538 nm for unirradiated solution and irradiated solution with dose \(D\), respectively. Figure 3.9 shows the extent of %decolouration of RR-120 during gamma radiolysis in presence of (i) \(^{1}\text{OH}\) (at pH 7), (ii) \(^{1}\text{OH}\) (at pH 1), (iii) \(\text{N}_3\) \(^{1}\) (at pH 7) and (iv) \(\text{Cl}_2\) \(^{1}\) (at pH 1).

![Figure 3.9 %decolouration of RR-120 during gamma radiolysis in presence of (i) \(^{1}\text{OH}\) (at pH 7), (ii) \(^{1}\text{OH}\) (at pH 1), (iii) \(\text{N}_3\) \(^{1}\) (at pH 7) and (iv) \(\text{Cl}_2\) \(^{1}\) (at pH 1).](image)

The %decoloration efficiency of \(\text{N}_3\) \(^{1}\) was found to be highest among the studied oxidising radicals. The G value of \(^{1}\text{OH}\) (0.28 \(\mu\text{mol J}^{-1}\)) and \(\text{Cl}_2\) \(^{1}\) (0.28 \(\mu\text{mol J}^{-1}\)) at pH 1 is lower than that of \(^{1}\text{OH}\) (0.58 \(\mu\text{mol J}^{-1}\)) at pH 7. However, no appreciable difference was observed in the %decolouration efficiency of (i) \(^{1}\text{OH}\) (at pH 7), (ii) \(^{1}\text{OH}\) (at pH 1) and (iv) \(\text{Cl}_2\) \(^{1}\) (at pH 1).
3.1.3. Radiolysis of RR-120 by Reducing Radicals

3.1.3.1. Reaction of RR-120 with Hydrated Electron (\(e_{aq}^-\))

Aqueous solution of 40 µM RR-120 solution (at pH 7) containing 0.2 M tert-butanol (\(^1\)Bu-OH) and saturated with N\(_2\) was irradiated for different doses in \(^{60}\)Co gamma chamber having a dose rate of 3 kGy h\(^{-1}\) (measured using Fricke dosimetry). The dissolved oxygen is removed from the solution by purging with N\(_2\) gas and \(^1\)Bu-OH converts \(^{\bullet}\)OH to lesser reactive radical \(^{\bullet}\)CH\(_2\)(CH\(_3\))\(_2\)COH by reaction shown Eq. 1.35. Under this reaction condition, the yield of \(e_{aq}^-\) becomes 0.28 µmol J\(^{-1}\).

Figure 3.10a shows the spectra of N\(_2\) saturated 40 µM RR-120 solutions (at pH 7) containing 0.2 M \(^1\)Bu-OH (i) before radiolysis and after steady state radiolysis at (ii) 0.05, (iii) 0.2 and (iv) 0.33 kGy. The intensities of the peaks at 235 and 293 nm did not appreciably change with increasing dose. Therefore the benzene and naphthalene units of RR-120 did not change during the radiolysis by \(e_{aq}^-\). However, the intensities of the peaks at 510 and 538 nm decreased rapidly with increasing dose. The absorption of the doublet peak decreases with the dose, but any change in the overall shape of the absorption spectra is rarely observed. Further, a build up of absorbance was observed at wavelength \(\sim\) 400 nm during the radiolysis by \(e_{aq}^-\). It is attributed to the reductive cleavage of the azo group by \(e_{aq}^-\) resulting into destruction of the extended \(\pi\)-conjugation of RR-120 (Figure 3.11). The amines produced by the reductive cleavage of azo bond shows \(n-\pi^*\) transition at \(\sim\)400 nm. The dye solution completely decolourized at 0.33 kGy.

\(G\) (-Dye) was estimated by following the decrease in absorbance of N\(_2\) saturated 40 µM RR-120 solutions (at pH 7) containing 0.2 M \(^1\)Bu-OH at 535 nm with the increase in dose (inset of Figure 3.10a). \(G\) (-Dye) was calculated as 0.14 µmol J\(^{-1}\)
using an extinction coefficient value of RR-120 as 35110 M\(^{-1}\) cm\(^{-1}\). Therefore, G (\(-\text{Dye}\)) is just half of the yield of \(e_{\text{aq}}^-\) (0.28 µmol J\(^{-1}\)) up to 0.15 kGy dose. Hence, RR-120 undergoes a two-electron reduction process producing secondary amine having characteristic absorption at ~ 400 nm (Step 1 of Figure 3.11). At doses higher than 0.15 kGy, G (\(-\text{Dye}\)) steadily decreased. This is attributed to the interference of the competitive reduction of the secondary amino product by \(e_{\text{aq}}^-\) in parallel with the reaction of RR-120 with \(e_{\text{aq}}^-\) (Step 2 of Figure 3.11).

**Figure 3.10** (a) Spectra of N\(_2\) saturated 40 µM RR-120 solutions (at pH 7) containing 0.2 M \(\text{tBu-OH}\) (i) before radiolysis and after steady state radiolysis at (ii) 0.05 kGy, (iii) 0.2 kGy and (iv) 0.33 kGy. (b) \(\Delta OD_\lambda\) and (c) transient absorption as a function of \(\lambda\) for N\(_2\) saturated 100 µM RR-120 solution (at pH 7) containing 0.2 M \(\text{tBu-OH}\) during pulse radiolysis at pulse dose of 0.01 kGy.
Figure 3.11 The mechanism of radiolysis of RR-120 by $e_{aq}^-$. $R$ represents the residual part of RR-120.

The reaction between the $e_{aq}^-$ and RR-120 was investigated by pulse radiolysis experiment employing 0.01 kGy/pulse in N₂ saturated 100 μM RR-120 solution (at pH 7) containing 0.2 M tert-butanol (tBu-OH). The absorption at any wavelength $\lambda$ ($\Delta OD_\lambda$) changes due to the formation of the transient species is shown in Figure 3.10b. The spectrum shows a strong absorption in the region 350-410 nm and weak absorption at 660 nm. The decay kinetics of the transient species was studied at 660 nm, where contribution of parent absorption was absent. The decay trace of the transient fitted well to the second order kinetics indicating a bimolecular type of decay of the transient. No bleaching recovery at 535 and 560 nm was observed indicating the absence of regeneration of the parent dye in the decay step. The $k_\phi$ for the reaction between $e_{aq}^-$
and RR-120 was determined from the decay of $e^{-}_{aq}$ absorption at 610 nm in the presence of different concentrations of RR-120. The bimolecular rate constant of the reduction of RR-120 by $e^{-}_{aq}$ radicals was calculated as $1.2 \times 10^{10}$ M$^{-1}$ s$^{-1}$.

3.1.3.2. Reaction of RR-120 with Isopropyl Radical ((CH$_3$)$_2$C$^\bullet$OH)

Aqueous solution of 40 µM RR-120 solution (at pH 7) containing 0.2 M 2-propanol ((CH$_3$)$_2$CHOH) and saturated with N$_2$O was irradiated for different doses in $^{60}$Co gamma chamber having a dose rate of 2.5 kGy h$^{-1}$ (measured using Fricke dosimetry). N$_2$O converts $e^{-}_{aq}$ to $^\bullet$OH. Further, (CH$_3$)$_2$CHOH converts $^\bullet$OH and $^\bullet$H to (CH$_3$)$_2$C$^\bullet$OH by the reactions shown in Eqs. 1.36 & 1.37. Figure 3.12a shows the spectra of N$_2$O saturated 40 µM RR-120 solution (at pH 7) containing 0.2 M (CH$_3$)$_2$CHOH (i) before radiolysis and after steady state radiolysis at (ii) 0.05, (iii) 0.2 and (iv) 0.33 kGy. The intensities of the peaks at 510 and 538 nm decreased with increasing dose. It is attributed to the reductive cleavage of the azo group by (CH$_3$)$_2$C$^\bullet$OH resulting into destruction of the extended $\pi$-conjugation of RR-120. However, the absorbance at 250 and 350 nm increased with increasing dose indicating the production of smaller amino benzene fragments from RR-120 by (CH$_3$)$_2$C$^\bullet$OH.

The reaction between RR-120 and (CH$_3$)$_2$C$^\bullet$OH radicals was studied in N$_2$O purged aqueous solution of 200 µM RR-120 and 0.65 M (CH$_3$)$_2$CHOH at pH 7 (pulse dose = 0.01 kGy). The spectrum shows a strong absorption at 350, 410 nm and weak absorption at 590 nm (Figure 3.12b). The corrected absorption spectrum of the transient species formed in the reaction RR-120 with (CH$_3$)$_2$C$^\bullet$OH shows strong shows strong absorption in the region of 530–550 nm (Figure 3.12c). The rate constant of the reaction of RR-120 with (CH$_3$)$_2$C$^\bullet$OH radicals was observed as $1.8 \times 10^9$ M$^{-1}$ s$^{-1}$. 
Figure 3.12 (a) Spectra of N$_2$O saturated 40 µM RR-120 solution (at pH 7) containing 0.2 M (CH$_3$)$_2$CHOH (i) before radiolysis and after steady state radiolysis at (ii) 0.05 kGy, (iii) 0.2 kGy and (iv) 0.33 kGy. (b) ΔOD$_\lambda$ and (c) transient absorption as a function of $\lambda$ for N$_2$O saturated 200 µM RR-120 and 0.65 M (CH$_3$)$_2$CHOH at pH 7 during pulse radiolysis at pulse dose of 0.01 kGy.

Figure 3.13a shows the corrected absorption spectra of the transient species formed in the reaction RR-120 with (i) $e^-_{aq}$ and (ii) (CH$_3$)$_2$C$^\bullet$OH. The decay traces of the transient species produced in the reaction of $e^-_{aq}$ and (CH$_3$)$_2$C$^\bullet$OH radicals with RR-120 were studied at 400, 560 and 660 nm. At 400 nm, transient formed between the reaction of RR-120 and (CH$_3$)$_2$C$^\bullet$OH decayed in 1 ms time whereas such decay was absent for the transient produced in the reaction of $e^-_{aq}$ with dye (Figure 3.13b). It indicates that the transients produced from RR-120 by $e^-_{aq}$ and (CH$_3$)$_2$C$^\bullet$OH are
different. The bleaching traces obtained for $e^-_{\text{aq}}$ and $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$ reactions with RR-120 at 560 nm were totally different (Figure 3.13c). After initial fast bleaching, light level remained steady without any recovery for the reaction of $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$ with RR-120. However, after initial fast bleaching, further slow bleaching takes place for the reaction of $e^-_{\text{aq}}$ with RR-120.

![Figure 3.13](image)

**Figure 3.13** (a) Transient absorption as a function of $\lambda$ and decay traces recorded at (b) 400 nm, (c) 560 nm and (d) 660 nm for the reaction of RR-120 with (i) $e^-_{\text{aq}}$ and (ii) $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$.

The $\varepsilon$ values of RR-120 and that of the transients produced in the reaction of RR-120 with $e^-_{\text{aq}}$ and $(\text{CH}_3)_2\text{C}^\cdot\text{OH}$ at 560 nm are calculated as about 19000 and 12000 M$^{-1}$ cm$^{-1}$, respectively. In the reaction of RR-120 with $e^-_{\text{aq}}$, initial bleaching at 560 nm
was observed due to higher $\varepsilon$ value of parent molecule compared to that of transient species. The semi-reduced species formed in the reaction of $e^{-}_{aq}$ and RR-120 may react with tert-butanol radicals. No parent dye molecule was regenerated during the decay of the semi-reduced species. Therefore, the bleaching further increased with time. In the reaction of $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$ with RR-120, decay of semi-reduced species was accompanied by regeneration of parent molecules. At 660 nm, the transient species formed in the reaction of $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$ with RR-120 decayed slowly with a rate constant of $2.1\times10^8 \text{ M}^{-1}\text{ s}^{-1}$ as compared to the reaction of $e^{-}_{aq}$ with RR-120 ($2\times10^9 \text{ M}^{-1}\text{ s}^{-1}$). Therefore, both $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$ and $e^{-}_{aq}$ reduce RR-120. In the reaction of $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$ with RR-120, the semi-reduced species decayed bi-molecularly regenerating one parent dye molecule; whereas, in the reaction of $e^{-}_{aq}$ with RR-120, the semi-reduced species reacted with either $e^{-}_{aq}$ or tert-butanol radical resulting to reduced product which absorbs at 400 nm.

3.1.3.3. Reaction of RR-120 with Formate Radical ($\text{CO}_2^\bullet^-$)

Aqueous solution of 40 $\mu$M RR-120 solution (at pH 7) containing 50 mM sodium formate (HCOONa) and saturated with $\text{N}_2\text{O}$ was irradiated for different doses in $^{60}\text{Co}$ gamma chamber having a dose rate of 2.5 kGy h$^{-1}$ (measured using Fricke dosimetry). The $\text{^\bullet OH}$ and $\text{^\bullet H}$ were converted to $\text{CO}_2^\bullet^-$ by the reactions shown in Eq. 1.38. Figure 3.14a shows the spectra of $\text{N}_2\text{O}$ saturated 40 $\mu$M RR-120 solution (at pH 7) containing 50 mM HCOONa (i) before radiolysis and after steady state radiolysis at (ii) 0.05, (iii) 0.2 and (iv) 0.33 kGy. The intensities of the peaks at 510 and 538 nm decreased with increasing dose. It is attributed to the reductive cleavage of the azo group by $\text{CO}_2^\bullet^-$ resulting into destruction of the extended $\pi$-conjugation of RR-120. The change in absorbance spectra with dose for the reaction of RR-120 with $\text{CO}_2^\bullet^-$ shows similar trends as observed for the reaction of RR-120 with $(\text{CH}_3)_2\text{C}^\bullet\text{OH}$.
Figure 3.14 (a) Spectra of N$_2$O saturated 100 µM RR-120 solution (at pH 7) containing 50 mM HCOONa (i) before radiolysis and after steady state radiolysis at (ii) 0.05 kGy, (iii) 0.2 kGy and (iv) 0.33 kGy. (b) ΔOD$_{λ}$ and (c) transient absorption as a function of $λ$ for N$_2$O saturated 100 µM RR-120 and 50 mM (CH$_3$)$_2$CHOH at pH 7 during pulse radiolysis at pulse dose of 0.01 kGy.

The reaction between RR-120 and CO$_2$•− radicals was studied in N$_2$O purged aqueous solution of 100 µM RR-120 and 50 mM HCOONa at pH 7 (pulse dose = 0.01 kGy). The spectrum shows strong absorption in the region of 350-400 nm and a small band at 590 nm (Figure 3.14b). The corrected absorption spectrum of the transient species formed in the reaction RR-120 with CO$_2$•− shows strong peak at 520 nm (Figure 3.14c). The $k_φ$ for the reaction between CO$_2$•− and RR-120 was determined from the formation signal of the transient species at 620 nm in the presence
of different concentrations of RR-120. The bimolecular rate constant of the reduction of RR-120 by \( \text{CO}_2^- \) was calculated as \( 1.4 \times 10^8 \text{ M}^{-1} \text{ s}^{-1} \), which is lesser compared to the bimolecular rate constant of the reaction of RR-120 with (CH\(_3\))\(_2\)C*OH and (CH\(_3\))HC*OH. The bimolecular rate constant of the reaction of RR-120 with CO\(_2\)* increased to \( 2.2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1} \) by increasing the ionic strength of the solution by adding 0.2 M Na\(_2\)SO\(_4\) in the dye solution. Therefore, small value of the rate constant of the reaction of RR-120 with CO\(_2\)* may be attributed due to the coulombic repulsion between anionic RR-120 (due to the presence of six -SO\(_3\)\(^-\) groups in the molecular structure of RR-120) and CO\(_2\)*. The coulombic repulsion decreased upon increasing the ionic strength of the solution and subsequently the rate constant of the reaction of RR-120 with CO\(_2\)* increased. Figure 3.15 shows the % decolouration (at 538 nm), as shown in Eq. 3.3, as a function of dose during the radiolysis of 40 µM RR-120 by (i) \( \text{e}^-_{\text{aq}} \), (ii) (CH\(_3\))\(_2\)C*OH and (iii) CO\(_2\)*. The efficiency of % decolouration of RR-120 by these radicals is almost similar, though radiolytic yields of \( \text{e}^-_{\text{aq}} \) (0.28 µM J\(^{-1}\)), (CH\(_3\))\(_2\)C*OH (0.58 µM J\(^{-1}\)) and CO\(_2\)* (0.58 µM J\(^{-1}\)) are different.

![Figure 3.15](image)

**Figure 3.15** % decolouration of 40 µM RR-120 during gamma radiolysis in presence of (i) \( \text{e}^-_{\text{aq}} \), (ii) (CH\(_3\))\(_2\)C*OH and (iii) CO\(_2\)* at pH 1.
3.1.4. Radiolysis of Aerated RR-120 Solution

In the Sections 3.1.2 and 3.1.3, it was discussed that the selective oxidising radicals (viz. \( \cdot \text{OH}, \text{N}_3\cdot \) and \( \text{Cl}_2\cdot \)) and reducing radicals (viz. \( \text{e}^-_{\text{aq}}, (\text{CH}_3)_2\text{C}^\cdot \text{OH} \) and \( \text{CO}_2\cdot \)) react with RR-120 through different mechanisms and finally influence the extent of %decolouration of RR-120. However, the in-situ preparation of these radicals during the irradiation process needs some special additives or reaction condition. Therefore, it is of interest to study the radiolysis of aqueous solution of RR-120 (at pH 7) under ambient aerated solution. Aqueous solution of 40 µM RR-120 was irradiated with gamma radiation at pH 7 for different dose. Under this reaction condition, \( \text{e}^-_{\text{aq}} \) and \( \cdot \text{H} \) are scavenged by the dissolved \( \text{O}_2 \) producing perhydroxyl radicals (\( \text{HO}_2\cdot \)) and superoxide radical anions (\( \text{O}_2\cdot^- \)) as shown by Eqs. 1.27 & 1.28. The reduction potential of azo group of RR-120 at pH 7 is about -0.35 V vs. NHE \([72]\); whereas the reduction potentials of \( \text{HO}_2\cdot \) and \( \text{O}_2\cdot^- \) at pH 7 are -0.037 and -0.33 V vs. NHE. Therefore, reduction of azo group (i.e. decolouration of RR-120) at pH 7 is thermodynamically impossible by \( \text{HO}_2\cdot \) and \( \text{O}_2\cdot^- \). Hence, under the reaction condition, only \( \cdot \text{OH} \) becomes responsible for the decolouration of RR-120.

Figure 3.16a shows the spectra of 40 µM RR-120 solution at pH 7 irradiated at (i) 0 kGy, (ii) 0.5 kGy, (iii) 1.0 kGy and (iv) 2.0 kGy doses. The spectra shows similar trends, except lesser pronounced shift in absorbance maxima around 530 nm, as observed for the radiolysis of \( \text{N}_2\text{O} \) saturated RR-120 solution (Figure 3.4a). Therefore, the transient produced by the reaction of RR-120 with \( \cdot \text{OH} \) reacts with the dissolved oxygen forming an adduct, which has slightly different spectral feature. Figure 3.16b shows the spectra of \( \text{O}_2 \) saturated 40 µM RR-120 solution at pH 7 irradiated at (i) 0, (ii) 0.5, (iii) 1.0 and (iv) 2.0 kGy doses. Under this reaction conditions, the main reacting radicals are \( \cdot \text{OH} \) and \( \text{O}_2\cdot^- \). It can be seen that the decolouration behaviour of dye in
aerated and O₂ saturated RR-120 solutions are quite similar.

![Figure 3.16](image)

**Figure 3.16** (a) Spectra of (a) aerated (b) O₂ saturated and (c) N₂ saturated 40 µM RR-120 solutions (at pH 7) (i) before radiolysis and after steady state radiolysis at (ii) 0.5 kGy, (iii) 1.0 kGy and (iv) 2.0 kGy.

Figure 3.16c shows the spectra of N₂ saturated 40 µM RR-120 solution at pH 7 irradiated at (i) 0, (ii) 0.5, (iii) 1.0 and (iv) 2.0 kGy doses. Since, the dissolved O₂ is purged off from the solution by the N₂, thus Figure 3.16c represents the decolouration behaviour of RR-120 by the combined effect of *OH and e⁻ₐq. In contrast to the observation from Figure 3.10a, no building up of absorbance was observed at wavelength ~ 400 nm. One of the possible reasons is that the reduced species formed in this reaction condition may react with the transient species formed by *OH and finally, the resultant products may not have any absorption at 400 nm.
Figure 3.17 shows the % decolouration of 40 µM RR-120 solution at different doses for air, O₂, N₂, tBu-OH contained N₂ purged and N₂O saturated systems. The rate of decolouration was found to be very slow for *OH dominating system (in N₂O saturated) as compared to the e⁻aq dominating system (tBu-OH contained N₂ purged). It was also observed from Figure 3.17 that the decolouration efficiency of O₂, N₂, air and N₂O saturated solution was almost same upto 0.5 kGy, whereas at higher doses than 0.5 kGy, decolouration efficiency in O₂ and air saturated systems is distinctly more than that in N₂ and N₂O saturated systems. Since, the products also start absorbing at higher doses, so at this point it is difficult to elucidate the actual mechanism.

Figure 3.17 % decolouration of 40 µM RR-120 solution at different doses for air, O₂, N₂, tBu-OH contained N₂ purged and N₂O saturated systems.

The total organic carbon (TOC) represents the amount of organic carbon (irrespective of the oxidation state) present in the aqueous solution of organic compounds. The TOC of 20 µM RR-120 dye (Molecular weight = 1469 g mol⁻¹) solution was calculated as 10.56 mg mL⁻¹. Figure 3.18 shows the % TOC removal of
(a) aerated and (b) oxygen saturated 20 µM RR-120 solutions (at pH 7) during the steady state gamma radiolysis. It should be noted that only ~20% TOC was removed at 2 kGy, though the solution was almost decolourised at that time. On further irradiation, 38% and 48% TOC removal was observed at 3 kGy in aerated and O2 saturated RR-120 solution, respectively. Therefore, the reduction efficiency of %TOC was found to be more for O2 saturated solution than aerated solution. This suggests that oxygen helps in mineralization of the transient species produced from RR-120 during radiolysis.

![Graph showing % TOC removal vs dose for aerated and oxygen saturated solutions.](image)

**Figure 3.18** % TOC removal of (a) aerated and (b) oxygen saturated 20 µM RR-120 solutions (at pH 7) during the steady state gamma radiolysis.

### 3.1.5. Electron Beam Irradiation of Aerated RR-120 Solution

Figure 3.19a shows the absorbance spectra of 68 µM RR-120 at pH 7 (i) before radiolysis and after electron beam irradiation with 2 MeV beam energy for (ii) 1.5 kGy, (iii) 2.5 kGy, (iv) 5.0 kGy and (v) 10.0 kGy dose. The doublet peaks at 512 nm and 535 nm appreciably decreased with increasing doses. The % decolouration of the dye solution was calculated by monitoring the decrease in the absorbance at 535 nm. About 94% decoloration of 68 µM RR-120 solution was observed at the dose of 1.5 kGy,
whereas the solution was about 99% decolorized at 10 kGy (Figure 3.19b(i)). At 1.5 kGy dose, about 94%, 89% and 83% decolouration was observed for (i) 68 µM, (ii) 100 µM and (iii) 130 µM of RR-120 solution at pH 7, respectively (Figure 3.19b). Therefore, the extent of % decolouration of the dye solution was decreased with increasing dye concentration because of the higher extent of radical–radical recombination at concentrated dye solution.

Figure 3.19 (a) Absorbance spectra of 68 µM RR-120 at pH 7 (i) before radiolysis and after electron beam irradiation with 2 MeV beam energy for (ii) 1.5 kGy, (iii) 2.5 kGy, (iv) 5.0 kGy and (v) 10.0 kGy dose. (b) % decolouration of (i) 68 µM, (ii) 100 µM and (iii) 130 µM of RR-120 solution at pH 7, respectively.

COD determines the amount of oxygen required to fully oxidize organic compounds into carbon dioxide using a strong oxidizing agent such as K$_2$Cr$_2$O$_7$. Thus COD depends on the oxidation states of carbon atoms of the organic constituents present in the solution. However, COD indirectly determines the amount of organic compounds present in the aqueous solution. However, TOC represents the total amount of the organic carbon (irrespective of the oxidation state) present in the solution.

The COD of unirradiated 100 µM and 130 µM RR-120 aqueous solution (at pH
7) of RR-120 was calculated as 83 and 106 mg L\(^{-1}\), respectively. Figure 3.20 shows the %COD removal (hollow circles) of (i) 100 µM and (ii) 130 µM of RR-120 aqueous solution (at pH 7) as a function of the applied electron beam irradiation dose.

![Image](image.png)

**Figure 3.20** %COD removal (hollow circles) of (i) 100 µM and (ii) 130 µM of RR-120 aqueous solution (at pH 7) as a function of the applied electron beam irradiation dose. %TOC removal (hollow stars) of (a) 100 µM and (b) 130 µM of RR-120 aqueous solution (at pH 7) as a function of the applied electron beam irradiation dose.

The RR-120 gets fragmented into the smaller organic units along with the destruction of its chromophore group (azo linkage) during the irradiation process. Upon further irradiation, the fragmentation process sometimes leads to complete mineralization of RR-120 to CO\(_2\) and H\(_2\)O. Therefore, the %COD removal of the aqueous dye solution increased smoothly with increasing the dose (Figure 3.20(i,ii)). However, a significant decolouration (89%) but minor mineralization (19% COD removal) was observed for irradiating 100 µM dye solution at 1.5 kGy dose. Therefore, it can be understood that the destruction of the chromophoric group of the dye
molecules is only responsible for decolouration, whereas % COD removal solely depends on the complete mineralization of the dye molecules. Lower irradiation dose is sufficient to destroy the chromophoric group of the dye molecules, but it would partially mineralize RR-120 resulting into smaller organic compounds. Hence the rate of % COD removal with respect to the applied dose (Figure 3.20(i)) was relatively lower compared to the rate of % decolouration with respect to the applied dose (Figure 3.19b(ii)).

The % COD removal of RR-120 is also influenced by the concentration of dye. 45% and 25% COD removal was observed for the radiolysis at 10 kGy of (i) 100 µM and (ii) 130 µM dye solutions, respectively. Therefore, the extent of % COD removal of RR-120 decreased with increasing dye concentration because of the higher extent of radical–radical recombination at concentrated dye solution and thus reducing the degradation of dye (Figure 3.20(ii)). Figure 3.20 shows the %TOC removal (hollow stars) of (a) 100 µM and (b) 130 µM of RR-120 aqueous solution (at pH 7) as a function of the applied electron beam irradiation dose. The %TOC removal of the aqueous dye solution also increased with increasing the dose (Figure 3.20(a,b)). The % TOC removal also decreased with increasing the concentration of dye. It is important to mention that %TOC and %COD removal do not overlap as they do not follow the same trend. The higher the amount of intermediate oxidized species (of higher oxidation state) generated after radiolysis, the lesser the number of moles of oxygen (or oxidant like K₂Cr₂O₇) was necessary to oxidize the intermediate oxidized products to CO₂. However, some of those oxidized species (of higher oxidation state) could still remain in the irradiated solution resulting into no change in the total organic carbon content of the solution. Therefore, the extent %TOC removal is lesser compared to the %COD removal of RR-120 under identical operational conditions (Figure 3.20).
The pH of the unirradiated 100 µM and 130 µM dye solutions was measured as 6.2. The pH of both the solutions abruptly decreased by 40% during the radiolysis of the dye solutions at 1.5 kGy. However, only 5% decrease in pH was observed upon prolonged irradiation of the dye solutions up to 10 kGy. The initial drastic decrease of pH at lower dose is attributed to the fragmentation of large dye molecule into smaller organic acids such as dicarboxylic acids or acetic acid components and other benzoic compounds [74]. Upon prolonged irradiation, these organic acids completely mineralize to carbonic acid. The change of is less susceptible at higher concentration of dye in solution.

3.2. Enhancement of the Biodegradability of RR-120 Solution by Radiolysis

The microbial or enzymatic decolouration and degradation of dye solution is an eco-friendly and cost-competitive process [75, 76]. However, the biodegradation of synthetic dye molecules sometimes becomes very slow and even many synthetic dyestuffs are toxic to the micro-organisms [33, 34]. The five-day biochemical oxygen demand (BOD₅) is the amount of oxygen consumed by microorganism to degrade organic compounds for a period of five-days. On the other hand, COD represents the amount of oxygen (in terms of equivalent amount of K₂Cr₂O₇) required for chemical degradation of organic compounds. The BOD₅/COD ratio is represented as biodegradability index for the aqueous dye solution. Nevertheless, waste waters having BOD₅/COD ratio ≥ 0.3-0.4 are generally accepted as biodegradable [77]. The BOD₅/COD ratio of the unirradiated 100 µM and 130 µM dye solutions was calculated as 0.20 and 0.17, respectively. Therefore, accordingly to the biodegradability index, these dye solutions are non-biodegradable. However, %BOD₅ increased, but COD decreased for both the solutions with increasing radiation dose because of the formation of more biodegradable intermediates during radiolysis (Table 3.1) [33]. As a result,
BOD₅/COD ratio increased during the radiolysis process. However, the extent of biodegradability depends on the concentration of dye in solution. The concentrated dye solution exhibited lower biodegradability due to the higher extent of radical–radical recombination. The BOD₅/COD ratio of 100 µM dye solution increased from 0.20 to 0.30 upon irradiating the dye solution at 1.5 kGy and finally at 10 kGy, it became 0.56. It suggested that the non-biodegradable dye solution became biodegradable only upon irradiating with 1.5 kGy dose and its biodegradability enhanced with increasing the applied dose. This results encouraged exploring the combination of radiation and biological treatment for the treatment of textile wastewater.

**Table 3.1** % BOD₅ and BOD₅/COD ratio of 100 µM and 130 µM aqueous RR-120 solutions at pH 7 for different radiation doses.

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Concentration of RR-120</th>
<th>100 µM</th>
<th>130 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase of BOD₅ (%)</td>
<td>BOD₅/COD</td>
<td>Increase of BOD₅ (%)</td>
</tr>
<tr>
<td>0.0</td>
<td>0.00</td>
<td>0.20</td>
<td>0.00</td>
</tr>
<tr>
<td>1.5</td>
<td>16.18</td>
<td>0.30</td>
<td>15.38</td>
</tr>
<tr>
<td>2.5</td>
<td>35.26</td>
<td>0.34</td>
<td>41.21</td>
</tr>
<tr>
<td>5.0</td>
<td>42.77</td>
<td>0.37</td>
<td>56.60</td>
</tr>
<tr>
<td>10.0</td>
<td>50.23</td>
<td>0.56</td>
<td>60.23</td>
</tr>
</tbody>
</table>

**3.3. Combined Radiation and Microbial Treatment of aqueous RR-120 Solution**

Steady state gamma radiolysis of 102 µM aqueous RR-120 solution was carried out at pH 7 for 0.5 and 1.0 kGy by using ⁶⁰Co gamma radiation with a dose rate of 2.5 kGy h⁻¹. Henceforth, the 102 µM unirradiated and irradiated RR-120 solutions at doses
of 0.5 kGy and 1 kGy will be designated as RR-120-0, RR-120-0.5 and RR-120-1, respectively. The % decolouration of (i) RR-120-0, (ii) RR-120-0.5 and (iii) RR-120-1 after 24 h of microbial treatment was calculated as 27, 56 and 66, respectively (Figure 3.21). The % decolouration of (i) RR-120-0, (ii) RR-120-0.5 and (iii) RR-120-1 increased after 96 h of biological treatment to 87, 94 and 98, respectively. Henceforth, 96 h biologically treated RR-120-0, RR-120-0.5 and RR-120-1 will be designated as RR-120-0-B, RR-120-0.5-B and RR-120-1-B, respectively.

Figure 3.21 % decolouration of (i) RR-120-0, (ii) RR-120-0.5 and (iii) RR-120-1 during the microbial treatment.

Figure 3.21 indicates that the introduction of radiation pretreatment increased the efficiency and throughput of the microbial decolouration of RR-120 solution in shorter time scale. It is important to note that the amount of enzymes present in the microbial degradation process is very small compared to the amount of the substrate. At higher substrate concentration, the biodegradation process follows zero-order reaction kinetic with respect to the substrate concentration. Therefore, faster decolouration was evidenced at the initial stage of the microbial treatment. However, the reaction kinetic
switches over at lower substrate concentration from zero-order to pseudo-first order with respect to the substrate concentration. It is well known that the rate of a pseudo-first order reaction becomes more sluggish as the substrate concentration decreases. Therefore, a smaller variation in the extent of % decolouration (87 and 98 % decolouration for RR-120-0-B and RR-120-1-B, respectively) was evidenced at later stage of microbial treatment (Figure 3.21).

![Figure 3.22 % TOC removal of RR-120-0.5, RR-120-1, RR-120-0-B, RR-120-0.5-B and RR-120-1-B.](image)

At the initial stage of the radiolysis process, the dye molecules break down into smaller fragments. The % TOC removal of RR-120-0.5, RR-120-1, RR-120-0-B, RR-120-0.5-B and RR-120-1-B was calculated as 46, 52, 78, 88 and 90, respectively (Figure 3.22). The higher TOC removal in case of combined treatment of RR-120 might be due to the acceleration of the fragmentation of the dye molecules upon irradiating with high energy gamma-rays. A similar type of enhancement in the extent of mineralization was also observed in combined electron beam-biological treatment for dyeing wastewater [78, 79].

The treated wastewater from the textile industries is sometime used in the
agricultural fields for irrigation purpose \[80\]. Therefore, the assessment of the toxicity level of the treated textile effluent is one of the important factors for the seed germination and plant growth \[80\]. The toxic effect of the treated dye solution was studied on Indian agricultural seeds viz. Phaseolus mungo at room temperature. Germination (%) as well as the length of plumule and radical were recorded after 7 days. The results were averaged over the 10 seeds under the same experimental condition. The control set was carried out by using distilled water under the same time scale. Although the other experiments were carried out at 102 µM of initial dye concentration, no appreciable differences were observed in seed germination and plant growth between control (distilled water) and treated dye solution at that initial dye concentration. Our preliminary studies showed that Pseudomonas sp. SUK1 was able to effectively decolourise RR-120 up to 410 µM of initial dye concentration and no effective decolouration was observed above that concentration. Therefore, the seed germination and plant growth studies were carried out at 410 µM for both control and treated dye.

The seed germination was reduced to 30% with respect to the control, when the seeds were treated with 410 µM RR-120-0 (Table 3.2). About 80%, 90% and 90% germination was recovered after treating the seeds with 410 µM degraded metabolites of RR-120-0-B, RR-120-0.5-B and RR-120-1-B, respectively. The growth of the plumule and radical length of the seeds was found to be reduced to 48% and 32% in RR-120-0 as compared to the growth of the same in the distilled water, respectively. The growth of the plumule was significantly improved to 89% of the normal growth whereas for radical growth, it was 98% in RR-120-0-B. Again, 92%, 94% and 98%, 99% of the normal plumule and radical growth were recovered for RR-120-0.5-B and RR-120-1-B, respectively. Therefore, biodegradation as well as the combined radiation-
microbial treatment on RR-120 solution showed better reduced toxicity than the unirradiated dye solution.

**Table 3.2** Toxicity studies of 410 µM RR-120-0 and metabolites produced from 410 µM RR-120-0-B, RR-120-0.5-B and RR-120-1-B using *Pseudomonas* sp. SUK1.

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Water</th>
<th>RR-120-0</th>
<th>Metabolites produced by <em>Pseudomonas</em> sp. SUK1 from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RR-120-0-B</td>
</tr>
<tr>
<td>Germination (%)</td>
<td>100</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>Plumule (cm)</td>
<td>14.8 ± 0.6</td>
<td>7.2 ± 0.6***</td>
<td>13.2 ± 0.5</td>
</tr>
<tr>
<td>Radical (cm)</td>
<td>5.0 ± 0.3</td>
<td>1.6 ± 0.3***</td>
<td>4.9 ± 0.2</td>
</tr>
</tbody>
</table>

*Values are mean of three experiments ± SEM, significantly different from control (seed germination in distilled water) at *P* < 0.05, **P** < 0.01 and ***P** < 0.001 by one-way analysis of variance (ANOVA) with Turkey–Kramer comparison test.*

3.3.1. Investigation on the mechanism of the decolouration and degradation in combined radiation and microbial treatment of aqueous RR-120 Solution

3.3.1.1. FTIR analysis

The broad peak at 3433 cm\(^{-1}\) in the FTIR spectrum of RR-120-0 represents N-H stretching frequency of 2º aromatic amine (Figure 3.23a). The peak at 1739 cm\(^{-1}\) corresponds to the C=O stretching frequency of the hydrazone tautomer and the peaks at 1017 and 1042 cm\(^{-1}\) are attributed to the phenolic -OH groups present in the azo tautomer of the dye. The peaks at 1201 and 1624 cm\(^{-1}\) represent the aromatic C-O and −N=N- stretching of the azo group, respectively. The small band at 1443 cm\(^{-1}\) is attributed to the in-plane vibrations of the s-triazine ring. The peak at 1114 cm\(^{-1}\)
represents the signature of the C-N-C of triazine moiety. The characteristic peak of C-Cl could not be identified due to the overlapping peaks of the other groups in the region of 700-800 cm\(^{-1}\).

**Figure 3.23** FTIR spectra of (a) RR-120-0, (b) RR-120-0.5, (c) RR-120-1 and (d) RR-120-0-B, (e) RR-120-0.5-B, (f) RR-120-1-B.

The FTIR spectra of RR-120-0.5 and RR-120-1 revealed the increase in the peak intensities at 1198, 1049, 1020 cm\(^{-1}\) (Figure 3.23b) and 1197, 1046, 1019 cm\(^{-1}\) (Figure 3.23c), respectively. The increase in the intensities of these peaks might be attributed to the increase in the population of the phenolic -OH groups, which were formed by the addition of \(^{1}\)OH radicals to the benzene ring during the irradiation of the dye solution. The peak at 1650 cm\(^{-1}\) (primary aromatic amines) which could not be observed in both Figure 3.23b&c might be overlapped with the peak of azo bond (1624 cm\(^{-1}\)). The appearance of the peaks at 1650, 1338 and 1407 cm\(^{-1}\) (Figure 3.23b&c) might be
ascribed due to the C-N stretching of primary aromatic amines and -CH₂ scissoring bend of cyclohexadiene, respectively, formed upon irradiating RR-120 dye solutions as discussed later in the sub-section 3.3.1.3.. The intensity of the peak at 1114 cm⁻¹, which is the characteristic peak of the C-N-C of triazine moiety, remained constant upon irradiation up to 1 kGy.

The FTIR spectrum of the extracted metabolites from RR-120-0-B solution is shown in Figure 3.23d. The disappearance of the peak at 1624 cm⁻¹ is attributed to the destruction of the azo (-N=N-) group. The new pair of peaks at 1650 and 1338 cm⁻¹ correspond to the primary aromatic amines (C-N stretching) formed during the biodegradation process. The intensities of the characteristic peaks of the phenolic groups in the range between 1198 and 1020 cm⁻¹ significantly changed during the biological treatment process. The relative intensity of the peak at 1650 cm⁻¹, which is the characteristics of the C-N stretching, increased in RR-120-0.5-B and RR-120-1-B (Figure 3.23e&f).

3.3.1.2. HPLC analysis

Two characteristic retention times of RR-120-0 were observed at 1.52 and 1.74 min (Figure 3.24a). However, no remarkable change in the intensity and retention time were observed for RR-120-0.5 and RR-120-1 (Figures 3.24b&c). At this point, we can presume that either (i) the fragmented products were of similar polarities or (ii) the amount of the fragmented products was very low to produce significant response at 280 nm or (iii) the fragmented products did not absorb at the monitoring wavelength or (iv) total concentration of the 280 nm absorbing species remains same. The significant changes in the retention time as well as in the intensity were observed in the extracted metabolites from RR-120-0-B (Figures 3.24d). In this case, the characteristic retention times of the metabolites were observed at 1.22 and 1.34 min compared to 1.52 and 1.74
min for RR-120-0. Biological degradation of RR-120 resulted heavy fragmentation producing metabolites having absorption at 280 nm. HPLC analysis of RR-120-0.5-B showed prominent peaks at retention times 1.25 and 1.36 min (Figures 3.24e), which are quite similar to the extracted metabolites of RR-120-0-B. HPLC analysis of the extracted products from RR-120-1-B showed a remarkable increase in the intensity at retention time 1.22 min (Figures 3.24f), which indicates a better fragmentation of RR-120. The results indicate that degradation products obtained by radiation, biological and combined radiation-microbial treatment of RR-120 may not be similar.

**Figure 3.24** Chromatograms of (a) RR-120-0, (b) RR-120-0.5, (c) RR-120-1 and (d) RR-120-0-B, (e) RR-120-0.5-B, (f) RR-120-1-B.
3.3.1.3. ESI-MS analysis

A closer inspection of Figure 3.21 revealed that in the combined radiation-microbial process ~25% (for 0.5 kGy) and ~30% (for 1 kGy) decolouration of 102 µM RR-120 solution occurred at the onset of the biological treatment. Therefore, negative ion ESI-MS analysis was performed to study the compositions of (a) RR-120-0, (b) RR-120-0.5 and (c) RR-120-1 solutions just prior to microbial treatment. The peaks corresponding to the parent dye molecule (RR-120; M = 1337) can be assigned for the ion series of [M - xH]x⁻ ions or their sodiated adducts [M - (x + y)H + yNa]x⁻ (where the maximum value of x or (x + y) is equal to the total number of acidic protons) or their in-source fragmented units. The mass spectrum of RR-120-0 exhibited the [M - 6H + Na]⁵⁻, [M - 6H + 2Na]⁴⁺ and [M - 6H + 3Na]³⁻ ion peaks from ion series at m/z 271, 344 and 467, respectively and in-source fragmentation peaks at m/z 171 and 337.6 (Figure 3.25a). The most probable structures of the in-source fragments at m/z 171 and 337.6 are shown in Figure 3.26. Five additional peaks at m/z 172, 173, 189, 274.7 and 348 were observed in RR-120-0.5 (Figure 3.25b). The peaks at m/z 172, 173 and 189 correspond to the radiolysis fragments of RR-120 viz. aminobenzosulphonic acid, hydroxybenzosulphonic acid and di-hydroxybenzosulphonic acid, respectively (Figure 3.27). The peaks at m/z 274.7 and 348 correspond to the sodiated adduct of the hydroxylated dye molecule produced during radiolysis from the aromatic electrophilic addition of •OH to benzene or naphthalene ring, e.g. [M - 6H + O + Na]⁵⁻ and [M - 6H + O + 2Na]⁴⁺, respectively. The aromatic electrophilic addition of •OH to benzene or naphthalene ring of RR-120 was also supported by the UV–visible spectra of (a) RR-120-0, (b) RR-120-0.5 and (c) RR-120-1 (Figure 3.28), where the increase in the absorbance at longer wavelengths beyond 600 nm due to extra conjugation was evidenced for RR-120-0.5 and RR-120-1.
Figure 3.25 Negative ion ESI-MS spectra of (a) RR-120-0, (b) RR-120-0.5 and (c) RR-120-1.
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**Figure 3.26** In-source fragments of RR-120-0 in negative ion ESI-MS.

**Figure 3.27** Radiolytic fragments of RR-120 found ESI-MS.
The relative intensities of the peaks at m/z 172, 173, 189, 274.7 and 348 increased significantly in the RR-120-1 solution (Figure 3.25c). Therefore, the relative concentration of RR-120 decreased and the relative concentrations of aminobenzosulphonic acid, hydroxybenzosulphonic acid, dihydroxybenzosulphonic acid, [M - 6H + O + Na]^5- and [M - 6H + O + 2Na]^4- increased significantly for irradiating the dye solution with 1 kGy dose.

3.3.1.4. Enzyme analysis

The major mechanism behind the biodegradation of azo dyes in static condition is the synchronised action of several oxidative and reductive enzymes viz. laccase, tyrosinase, azoreductase and NADH-DCIP reductase leading to the degradation of dye molecules and their fragmented products [81, 82]. The activity of laccase, tyrosinase, azoreductase and NADH-DCIP reductase was studied in the cell free extracts obtained from control, RR-120-0-B and RR-120-1-B. The enzyme activity was defined in µM of
ABTS oxidised min⁻¹ mL⁻¹ (for laccase), units min⁻¹ mL⁻¹ (for tyrosinase), µM of MR reduced min⁻¹ mL⁻¹ (for azoreductase), µM of DCIP reduced min⁻¹ mL⁻¹ (for NADH-DCIP reductase) and the % variation of each enzyme activity in the dye solution with respect to the control. The activity of the tyrosinase was decreased to 58% and increased to 107% in RR-120-0-B and RR-120-1-B, respectively (Table 3.3). The activity of the azoreductase was increased to 122% and 116% in RR-120-0-B and RR-120-1-B, respectively. The laccase activity was increased to 231% and 200% in RR-120-0-B and RR-120-1-B, respectively. The activity of NADH-DCIP reductase increased to 541% and 154% in RR-120-0-B and RR-120-1-B, respectively (Table 3.3).

Table 3.3 Activities of laccase, tyrosinase, azoreductase and NADH-DCIP reductase in control Pseudomonas sp. SUK1 cells and cells obtained from RR-120-0-B and RR-120-1-B.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Cells obtained from RR-120-0-B</th>
<th>Cells obtained from RR-120-1-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laccase&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9 ± 0.2</td>
<td>4.4 ± 0.5*</td>
<td>3.8 ± 0.8*</td>
</tr>
<tr>
<td>Tyrosinase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.0*</td>
<td>0.7 ± 0.1*</td>
</tr>
<tr>
<td>Azoreductase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.8 ± 0.1</td>
<td>2.2 ± 0.1*</td>
<td>2.0 ± 0.1*</td>
</tr>
<tr>
<td>NADH-DCIP reductase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.1 ± 0.5</td>
<td>65.2 ± 1.7*</td>
<td>18.6 ± 0.3*</td>
</tr>
</tbody>
</table>

[Values are mean of three experiments ± standard error of measurement, significantly different from control cells *P<0.001 by one way analysis of variance (ANOVA) with Turkey-Kramer multiple comparison test. aµM of ABTS oxidized min⁻¹ mL⁻¹; bUnits min⁻¹ mL⁻¹; cµM of Methyl Red reduced min⁻¹ mL⁻¹; dµM of DCIP reduced min⁻¹ mL⁻¹.]
3.3.1.5. GC–MS analysis

GC–MS analysis was carried out with the metabolites formed from RR-120-0-B, RR-120-0.5-B and RR-120-1-B solutions. The metabolites of m/z 154 and 170 obtained from RR-120-0-B are attributed to the biodegraded products viz. N-(4-aminophenyl)-1,3,5-triazine-2,4-diamine and 2-aminobenzenesulfonic acid, respectively (Table 3.4).

Table 3.4 GC–MS analysis of the metabolites formed from RR-120-0-B, RR-120-0.5-B and RR-120-1-B solutions.

<table>
<thead>
<tr>
<th>Identified product name</th>
<th>Molecular weight of product</th>
<th>m/z obtained of product</th>
<th>GC–MS Peaks (y-axis in relative % intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-(4-aminophenyl)-1,3,5-triazine-2,4-diamine (III)</td>
<td>157</td>
<td>154</td>
<td></td>
</tr>
<tr>
<td>2-aminobenzenesulfonic acid (I)</td>
<td>172</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>N-(7-amino-8-hydroxy-5,6-dioxo-5,6-dihydrornaphthalen-1-yl)-guanidine (IX)</td>
<td>246</td>
<td>244</td>
<td></td>
</tr>
</tbody>
</table>
An additional peak at m/z 246 was obtained in RR-120-0.5-B. That new peak at m/z 246 is attributed to the N-(7-amino-8-hydroxy-5,6-dioxo-5,6-dihydronaphthalen-1-yl)-guanidine, which is formed upon the biological treatment of the irradiated solution (Table 3.4). No good quality mass spectrum was observed for RR-120-1-B because of the higher extent of mineralization in that solution.

3.3.2. Discussion on the decolouration and degradation mechanisms of RR-120 for combined radiation-microbial treatment

The probable reason behind the improvement in the process efficiency in case of the combined radiation-microbial treatment in comparison with the general microbial treatment can be proposed by correlating the results obtained from the FTIR, HPLC, ESI-MS, enzyme assay and GC-MS analysis. It is evidenced from Figure 3.27 that primary amines are produced by the reaction of RR-120 with $e_{aq}$ and $\cdot H$ (Figure 3.11). On the other hand, three parallel reactions are possible between RR-120 and $\cdot OH$ viz. (a) adduct formation to the organic $\pi$-system, (b) addition to the chromophoric group and (c) one electron oxidation [83]. Therefore, the cleavage of the C-N bond of RR-120 by $\cdot OH$ forms hydroxybenzosulphonic, di-hydroxybenzosulphonic acid, etc. (Figure 3.27). The cleavage of active azo bond of RR-120 forms aminobenzosulphonic acid (Figure 3.27) [84, 85]. The aromatic electrophilic substitution of $\cdot OH$ to phenyl ring and one electron oxidation of RR-120 also competes with the bond cleavage reaction (Figure 3.27). Aromatic electrophilic substitution of $\cdot OH$ to the phenyl produces cyclohexadienyl type of radical, which produces the cyclohexadiene ring (confirmed by -CH$_2$ scissoring bend in FTIR analysis) and regenerates the aromatic ring with an extra OH group via disproportionation reaction.

Laccase is an oxido-reductase, which is able to catalyze the oxidation of various aromatic compounds particularly phenols, aromatic amines, benzenethiols etc. [86].
The activity of laccase is increased in RR-120-0-B solution compared to control because of the presence of the dye molecule containing phenolic group and its amino metabolites. However, 30% decolouration and 52% TOC removal of RR-120-1 was observed. Therefore, the laccase activity is expected to decrease in the irradiated solution; but almost similar laccase activity was observed in RR-120-1-B (Table 3.3). Therefore, it can be assumed that the radiation induced fragmented products like hydroxybenzosulphonic, di-hydroxybenzosulphonic acid, aminobenzosulphonic acid, etc. helped to recover the laccase activity in RR-120-1-B solution.

Tyrosinase catalyzes the o-hydroxylation of monophenols to yield o-diphenols (cresolase activity) and subsequently oxidation of o-diphenols to o-quinones (catecholase activity) in the presence of oxygen [87]. RR-120 and its amino metabolites are supposed to inhibit the first step of the reaction and that is why the activity of tyrosinase is decreased in RR-120-0-B with respect to the control. On the other hand, hydroxybenzosulphonic acid and di-hydroxybenzosulphonic acid were formed in RR-120-1 solution. These irradiated fragments are supposed to be the specific substrates for tyrosinase. Therefore, the activity of tyrosinase increased in RR-120-1-B solution as compared to the RR-120-0-B (Table 3.3).

Azoreductase is the key enzyme for the reductive cleavage of the azo bond (-N=N-) of RR-120 resulting to aromatic amines. The activity of azoreductase was increased in RR-120-0-B solution as compared to the control because of the presence of the azo group in RR-120 [81]. The number of azo groups was decreased in the irradiated dye solution and therefore the activity of the azoreductase decreased in RR-120-1-B (Table 3.3). From the overall enzyme activity results, it can be concluded that the radiation induced fragmented products of RR-120 showed diverse enzymatic activities; for some enzymes the activity increased whereas it decreased for other
enzymes. The type of enzymes as well as their rate of secretion and activity towards a particular substrate varies with microbial strain to strain \cite{88}. Therefore, selection of the microbial strain may be one of the crucial aspects in this type of experiments.

On the basis of various enzyme inductions and GC-MS analysis, the possible biodegradation pathways of unirradiated and irradiated RR-120 dye solution adapted by Pseudomonas sp. SUK1 are illustrated in Figures 3.29 and 3.30. Initial cleavage of RR-120 may proceed through the cleavage of azo bond by azoreductase leading to the formation of 2-aminobenzenesulfonic acid [I] of m/z 170 and reactive intermediate [II], which undergoes subsequent oxidative and reductive cleavages by various oxidative and reductive enzymes viz. laccase, tyrosinase and DCIP reductase followed by the dechlorination resulting the formation of N-(4-aminophenyl)-1,3,5-triazine-2,4-diamine[III] of m/z 154.

The azo group of the product [IV] formed in the irradiated dye solution (as observed in Figure 3.27) may be reduced by azoreductase to 2-aminobenzenesulfonic acid [I] of m/z 170 and reactive intermediate [V]. Asymmetric cleavage of the reactive intermediate [V] by laccase and subsequent dechlorination formed another reactive intermediate [VI] and one stable product N-(4-aminophenyl)-1,3,5-triazine-2,4-diamine [III] of m/z 154. Subsequent desulphonation of [VI] led to the formation of [VII], which produces N-(7-amino-8-hydroxy-5,6-dioxo-5,6-dihydronaphthalen-1-yl)-guanidine[IX] having m/z at 246 by further attack of reductase and tyrosinase, subsequently.

The above results demonstrate that irradiating the RR-120 solution with a lower dose (≤ 1 kGy) and then microbial treatment of the irradiated solution with Pseudomonas sp. SUK1 under static condition increased the performance of the degradation and decolouration of RR-120 dye solution. As compared to the chemical
oxidation processes, radiation treatment does not produce any toxic byproducts, needs simple technical operation and most importantly, it is not perturbed by the highly coloured or turbid effluent, which makes it as a potential alternative compared to the other AOPs. This study explores a reliable and promising way to use industrially viable dose and microbial strain for transformation of carcinogenic dyes to non-toxic compounds.

Figure 3.29 Biodegradation pathways of RR-120-0 dye solution adapted by Pseudomonas sp. SUK1.
Figure 3.30 Biodegradation pathways of RR-120-0.5 dye solution adapted by Pseudomonas sp. SUK1.
3.4. Radiolysis of simulated textile dye waste water (STDWW) containing RR-120

A detailed discussion is presented in the previous sections on the radiolytic mineralization of RR-120. The combined radiation-microbial treatment was also explored to increase the efficiency of the biodegradation process. Further, real textile dye bath contains the auxiliary chemicals (i.e., surfactants, sequestering agent, pH-adjusting acids, inorganic salts etc.) along with the bio-resistant synthetic dye molecules. These auxiliary chemicals of the dye bath contribute to ~83% of the organic load of the effluent [89, 90]. The heavy organic load of the textile effluent causes a negative impact to the aquatic lives owing to the decrease in the dissolved oxygen concentration in the water stream [91]. The COD of real textile effluents varies in the ranges 2900-3000 ppm, which is well above the permissible discharge limit (COD ≤ 250 ppm) set by the Central Pollution Control Board under the Ministry of Environment and Forest, Government of India.

In this section, the process efficiency of radiolysis is validated on simulated textile dye waste water (STDWW), mimicking the compositions used in dye industries. The process efficiency of the radiolytic mineralization of STDWW was also compared with a couple of AOPs, viz. photocatalysis, ozonolysis. The constituents of the simulated textile dye bath and the role of each constituent in the dyeing process are given in Table 3.5. The hydrolyzed dye effluent was prepared by boiling the constituents with 1 M NaOH at 80-90°C for 3 h under reflux [11]. The pH, conductivity, COD and dissolved oxygen of STDWW were measured as 10, 28.9 mS, 3128 mg L⁻¹, 3.7 mg L⁻¹, respectively.
Table 3.5 Composition of STDWW.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mg L(^{-1}))</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.I. Reactive Red 120</td>
<td>70</td>
<td>Colouring agent</td>
</tr>
<tr>
<td>Sodium dodecylbenzenesulfonate (SDBS)</td>
<td>375</td>
<td>Detergent used for washing excess dye</td>
</tr>
<tr>
<td>Ethylenediaminetetraacetic acid (EDTA)</td>
<td>150</td>
<td>Removal of unwanted metal ions in the dye bath</td>
</tr>
<tr>
<td>Na(_2)CO(_3)</td>
<td>4876</td>
<td>Adjustment of the starting pH (8.8–9.3)</td>
</tr>
<tr>
<td>NaOH</td>
<td>188</td>
<td>Adjustment of the Final dyeing pH (10.5–11.0)</td>
</tr>
<tr>
<td>NaCl</td>
<td>(1.5\times10^4)</td>
<td>Promotes dye binding onto cotton</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>As required</td>
<td>For pH (10) adjustment</td>
</tr>
</tbody>
</table>

3.4.1 Decolouration and mineralization of STDWW by photocatalysis and ozonolysis

The \(\text{OH}\) radical is produced from the surface of TiO\(_2\) nanoparticles (Degussa (P25), particle size ~ 30 nm) upon irradiating with UV light. The photocatalytic treatment of STDWW was carried out for different time intervals. It took about 500 min of this process to completely decolourise STDWW. However, at the same time, it could mineralise only 21% of the total organic loads of STDWW (Figure 3.31a). The rate of photocatalytic mineralization decreased with time. Therefore, only 25% mineralization was observed at 720 min and no significant change in the extent of mineralization was observed on prolonged irradiation.

The TiO\(_2\) nanoparticles made very stable suspensions with the aqueous solution of the individual components of STDWW. However, it settled down rapidly in STDWW. It can be speculated that the presence of high salts concentration (~1.5\times10^4 ppm of NaCl) in the STDWW might be responsible for changing the surface properties.
of TiO$_2$ particles and it finally led to the easy settlement of the catalyst in STDWW. On the other hand, the coulombic repulsion between the negatively charged surface of TiO$_2$ (pH$_{pzc} = 6.0 \pm 0.2$) and OH$^-$ (at pH 10) also prevents the production of *OH resulting into the poor mineralization of STDWW [92, 93].

Figure 3.31 Mineralization of STDWW by (a) photocatalysis (b) ozonolysis.

Ozone (O$_3$) produces *OH via decomposition in alkaline pH (pH 10) [46]. O$_3$ is also a strong chemical oxidant and it can directly react with the unsaturations present in the organic molecules [94]. Therefore, O$_3$ and *OH both could react with the components of STDWW. However, the direct reaction of O$_3$ is selective and slow. The ozonolysis of STDWW was carried out for different time intervals. The complete decolouration and negligible mineralization of STDWW were observed at ~3 min of ozonolysis. About 25% mineralization of STDWW was observed after 120 min and remained almost constant at prolonged ozonolysis (Figure 3.31b). The process efficiency (in terms of the % mineralization of STDWW) of ozonolysis was tried to increase even by adding some additives such as H$_2$O$_2$, Al$_2$O$_3$, K$_2$S$_2$O$_8$ etc., but the
extent of % mineralization did not increase. The continuous flow of oxygen during ozonolysis also did not improve the extent of % mineralization of STDWW.

3.4.2. Decolouration and mineralization of STDWW by gamma radiolysis

The radiolysis of STDWW was carried out for different doses. The complete decolouration and negligible mineralization of STDWW were observed at ~3 kGy dose. The extent of mineralization of STDWW was increased only up to 16% at 50 kGy dose and no further enhancement in the mineralization was observed at higher doses (Figure 3.32a). The above discussed results in conjugation with the results discussed in Section 1.4.1. suggested that OH cannot effectively mineralize the organic load of STDWW. It was observed that the radiolytic mineralization of ibuprofen was enhanced by gamma radiolysis in presence of K₂S₂O₈ [95]. The eaq⁻ and H preferentially reacts with S₂O₈²⁻ during radiolysis resulting into sulphate radical (SO₄•⁻) (Eq. 1.34) [96].

The SO₄•⁻ is itself an oxidising radical. In addition, OH radical also can react independently with organic compounds [97]. The gamma radiolysis of STDWW was carried out for different doses in presence of 40 mM K₂S₂O₈. The % mineralization of STDWW was enhanced to 50% at 50 kGy dose, though it remained almost constant at higher doses than 50 kGy (Figure 3.32b). It is important to mention that K₂S₂O₈ itself can produce SO₄•⁻ by thermal decomposition at 38-40°C [98], which is the usual temperature of the solution during gamma radiolysis. Therefore, the extent of mineralization of STDWW in presence of 40 mM K₂S₂O₈ was studied at 40°C in absence of gamma source and no mineralization of STDWW was obtained.

In this context, it is important to mention that the COD of STDWW was 3128 ppm, which was much higher than the COD of pure dye solution (70 ppm) and it indirectly represented the amount of other organic compounds (viz. SDBS, EDTA,
CH₃COOH etc.) present in STDWW. After some preliminary experiments, it was found that the high value of COD was mainly contributed by SDBS, EDTA and CH₃COOH. The concentration of CH₃COOH varies from case to case as it is used to neutralize the pH of the dye bath and thus the radiolysis of pure CH₃COOH component was not investigated. The aqueous solutions of EDTA (150 ppm) and SDBS (375 ppm) were irradiated at pH 10 individually at a dose of 50 kGy both in the absence and presence of K₂S₂O₈. About 80% and 95% mineralization of EDTA were observed in the absence and presence of K₂S₂O₈. It suggests that EDTA can be mineralized easily by irradiation unlike SDBS. However, more interestingly, about 16% and 62% mineralization of SDBS was observed in the absence and presence of K₂S₂O₈. Therefore, the extent of mineralization of SDBS was observed to be enhanced by approximately 4 times in case of gamma radiolysis in the presence of K₂S₂O₈.

Figure 3.32 Mineralization of STDWW on gamma radiolysis (a) without and (b) with K₂S₂O₈.

In presence of HCO₃⁻ and CO₃²⁻, the extent of mineralization of the organic components present in STDWW was expected to be decreased because of the expected
scavenging of \( \cdot \text{OH} \) radical by the HCO\(_3\)\(^-\) and CO\(_3\)\(^2-\) [99]. However, no appreciable enhancement in the extent of mineralization was observed during the radiolysis in absence of Na\(_2\)CO\(_3\). It indicates that CO\(_3\)\(^2-\) might not have interfered during the radiolysis of STDWW.

3.4.3. Comparison of the process efficiencies of photocatalysis, ozonolysis and gamma radiolysis for the mineralization of STDWW.

The process efficiencies of different AOPs were compared in terms of oxygen-equivalent chemical-oxidation capacity (OCC), which is defined as the kg of O\(_2\) that are equivalent to the quantity of oxidant reagents used in an AOP to treat 1 m\(^3\) of wastewater [100]. It gives an index of the chemical efficiency of the oxidants used in an AOP by quantifying the amount of the oxidants (kg O\(_2\)) added per m\(^3\) of the wastewater. The OCC of photocatalysis, ozonolysis and gamma radiolysis are calculated by the following Eq. 3.2-3.4:

\[
1 \text{OCC}_{\text{Photo}} (\text{kg O}_2 \text{ m}^{-3}) = \frac{[l_0 (\text{cm}^{-2} \text{s}^{-1}) \times A (\text{cm}^2) \times t (\text{s}) \times 10^6 (\text{cm}^3 \text{m}^{-3})]}{[6.023 \times 10^{26} (\text{kmol}^{-1}) \times V (\text{cm}^2)]} \times \frac{1 \text{ kmol} \text{O}_2}{4 \text{ kmol}} \times \frac{32 \text{ kg} \text{O}_2}{1 \text{ kmol} \text{O}_2} \tag{3.2}
\]

\[
1 \text{OCC}_{\text{Ozo}} (\text{kg O}_2 \text{ m}^{-3}) = O_3 (\text{kg O}_3 \text{ m}^{-3}) \times \frac{1 \text{ kmol} \text{O}_2}{48 \text{ kg} \text{O}_3} \times \frac{6 \text{ kmol e}^-}{1 \text{ kmol} \text{O}_3} \times \frac{1 \text{ kmol} \text{O}_2}{4 \text{ kmol} \text{e}^-} \times \frac{32 \text{ kg} \text{O}_2}{1 \text{ kmol} \text{O}_2} \tag{3.3}
\]

\[
1 \text{OCC}_{\text{Radio}} (\text{kg O}_2 \text{ m}^{-3}) = D (\text{J kg}^{-1}) \times \rho (\text{kg m}^{-3}) \times G (\text{kmol J}^{-1}) \times \frac{1 \text{ kmol} \text{O}_2}{4 \text{ kmol}} \times \frac{32 \text{ kg} \text{O}_2}{1 \text{ kmol} \text{O}_2} \tag{3.4}
\]

where, D is the dose, \( \rho \) is the density of water, \( G(\text{SO}_4^{\cdot}) = 3.4 \times 10^{-10} \text{ kmol J}^{-1} \) or 3.3/100 eV; \( G(\text{\textbullet{OH}}) = 2.8 \times 10^{-10} \text{ kmol J}^{-1} \) or 2.7/100 eV.

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The lowest degree of mineralization (16%) of STDWW was observed for gamma radiolysis (Figure 3.33). Thus the OCCs of different AOPs were compared for only 16% mineralization of STDWW and they were calculated as 4.02, 16.19, 0.13, 0.05 kg equiv. O\textsuperscript{2} m\textsuperscript{3} for photocatalysis, ozonolysis and gamma radiolysis in the absence and presence of K\textsubscript{2}S\textsubscript{2}O\textsubscript{8}, respectively. Therefore, for the same extent of mineralization, least amount of oxidant was required for the gamma radiolysis in presence of K\textsubscript{2}S\textsubscript{2}O\textsubscript{8}. To the best of our knowledge, this is the first report on the calculation of OCC for photocatalysis and gamma radiolysis.

**Figure 3.33** Variation in mineralization extent with OCC of (a) radiolysis (b) photocatalysis (c) ozonolysis (d) radiolysis in presence of K\textsubscript{2}S\textsubscript{2}O\textsubscript{8}. Inset: mineralization extent at lower OCC (a) radiolysis (b) photocatalysis (c) ozonolysis (d) radiolysis in presence of K\textsubscript{2}S\textsubscript{2}O\textsubscript{8}. 
Gamma radiolysis in the presence of K\(_2\)S\(_2\)O\(_8\) showed better chemical efficiency (i.e. 47% mineralization) of the oxidants at OCC of 0.23 kg equiv. O\(_2\) m\(^{-3}\), which was equivalent to 16% mineralization for the radiation treatment in absence of K\(_2\)S\(_2\)O\(_8\). Moreover, no mineralization was observed at that OCC value for both ozonolysis and photocatalysis. The overall results suggest that the gamma radiolysis in the presence of K\(_2\)S\(_2\)O\(_8\) is the most efficient process for the treatment of STDWW compared to the general gamma radiolysis, photocatalysis and ozonolysis.

3.4.4. Mechanism of mineralization of the components present in STDWW

Mendez-Diaz et al. speculated that the conjugative action of \(\bullet\)OH and SO\(_4\)\(^{\bullet-}\) might be led to the higher extent of mineralisation of SDBS during the photooxidation of SDBS in presence of K\(_2\)S\(_2\)O\(_8\) at pH 7 \([101]\). We want to note that the yield of oxidizing radicals in gamma radiolysis in presence of K\(_2\)S\(_2\)O\(_8\) is 6 \([ = G(\bullet\)OH\) + \(G(\text{SO}_4\bullet\)\(^{\bullet-}\) + \(G(\bullet\)H)\]), whereas, that in absence of K\(_2\)S\(_2\)O\(_8\) is 2.7 \(= G(\bullet\)OH\)). Therefore, about 2.2 times enhancement in the extent of mineralization of SDBS could be expected during gamma radiolysis in the presence of K\(_2\)S\(_2\)O\(_8\) compared to the same in the absence of K\(_2\)S\(_2\)O\(_8\). However, in the Section 3.4.2, we showed that the extent of mineralization of SDBS was enhanced by approximately 4 times due to the presence of K\(_2\)S\(_2\)O\(_8\) during gamma radiolysis.

Therefore, the reactions between SDBS with \(\bullet\)OH and SO\(_4\)\(^{\bullet-}\) were investigated by pulse radiolysis experiments in 0.5 mM aqueous solution of SDBS saturated with (i) N\(_2\)O and (ii) N\(_2\), respectively, by employing 14 Gy per pulse at pH 10. The 20 mM K\(_2\)S\(_2\)O\(_8\) and 20 mM t-Bu-OH were added additionally to the solution for the second reaction. Figure 3.34 shows the absorbance per unit G value (\(\Delta\)OD/G) of the transient species formed by \(\bullet\)OH and SO\(_4\)\(^{\bullet-}\) as a function of wavelength. In Figure 3.34a, a strong absorption at 290 nm, a weak absorption peak at 320 and a weak hump in the range of
325-340 nm was observed for the reaction between SDBS and \( ^\cdot \text{OH} \). The peak around at 270-290 nm is attributed to the benzyl type radical and broad peak at 300-350 nm correspond to the formation of OH-adduct with the benzene ring \([95, 102]\). All of the transient species absorbing at 290, 320 and 325-340 nm decayed faster in presence of 4:1 (v/v) mixture of \( \text{N}_2\text{O} \) and \( \text{O}_2 \) and it suggests that the produced transient species had carbon centered radicals. The decay constant of the transient reacting with \( \text{O}_2 \) could not be determined because of the interference of the formation signal of the peroxy type radical with the decay signal of the reacting transient.

![Figure 3.34](image)

**Figure 3.34** Transient absorption spectra for the reaction of SDBS with (a) \( ^\cdot \text{OH} \), (b) \( \text{SO}_4^\cdot \) and (c) \( \text{O}^\cdot \) at dose of 14 Gy per pulse.

Figure 3.35a represents the probable routes of formation of the OH-adducts and benzyl type radicals from the reaction between SDBS and \( ^\cdot \text{OH} \). The \( ^\cdot \text{OH} \) first conjugates with the benzene ring of SDBS forming the OH-adduct, which reacts with the water molecules leading to the formation of the benzene radical cation. The benzene radical cation is unstable and it forms a benzyl type radical by dissociation of the weak
benzylic Cα-hydrogen \[95, 103\]. Both the intermediates viz. OH-adduct and benzyl type radicals are observed for the reaction of \(^{•}\)OH with SDBS. Apart from these above phenomena direct H-atom abstraction may also take place from the alkyl chain of SDBS by \(^{•}\)OH \[102\]. The bi-molecular rate constant of the reaction between \(^{•}\)OH and SDBS was calculated as \(1.8 \times 10^9 \text{ M}^{-1} \text{s}^{-1}\) (at pH 10), which is similar to the value reported \(~10^{10} \text{ M}^{-1} \text{s}^{-1}\) (at pH 7) by Mendez-Diaz et al. \[101\].

The similar types of transient absorption peaks were observed at 290, 320 and 330 nm in the reaction of SDBS with \(\text{SO}_4^{•-}\) (Figure 3.34b). However, the intensity of the peak at 290 nm significantly increased by 1.5 times in case of \(\text{SO}_4^{•-}\) as compared to the \(^{•}\)OH. The \(\text{SO}_4^{•-}\) is more selective electrophile compared to \(^{•}\)OH \[101\]. Figure 3.35b represents the probable routes of reaction between \(\text{SO}_4^{•-}\) and SDBS. The \(\text{SO}_4^{•-}\) does not directly add to the aromatic ring. Instead it produces a very short lived (< 0.1 µs) radical cation followed by benzyl type radical and hydroxycyclohexadienyl radical by

---

**Figure 3.35 Reactions of SDBS with \(^{•}\)OH and \(\text{SO}_4^{•-}\).**
the reactions with water molecules [95, 104]. SO₄• can also produce benzyl radical by H-abstraction reaction from the alkyl chain of the SDBS [101, 102, 105]. The bimolecular rate constants for the reaction of SO₄• with SDBS was calculated as 3.8×10⁸ M⁻¹ s⁻¹ from the decay of SO₄• at 450 nm (at pH 10) and it is similar to the value reported about 3.6×10⁸ M⁻¹ s⁻¹[101].

The H-abstraction reaction of SO₄• from the alkyl chain was verified by monitoring the decay of SO₄• with the reaction of SDS in N₂ purged 0.5 mM SDS containing 20 mM K₂S₂O₈ and 20 mM t-Bu-OH at pH 10 by employing 14 Gy per pulse. The decay constant of SO₄• for the reaction with SDS was calculated as 4.7× 10⁵ s⁻¹ at 440 nm and it was found to be about 1.5 times higher compared to the decay of SO₄• in a solution containing no SDS (spectra is not shown). It supports that the H-abstraction reaction of SO₄• from the alkyl chain of SDS and SDBS are of similar nature.

The absorption peaks of the transients formed by the reaction between SDBS and O• were also monitored in N₂O saturated 0.5 mM aqueous SDBS solution at pH 13.5 by applying 14 Gy per pulse (Figure 3.34c). The O•, being a nucleophilic species cannot add to the benzene ring, rather it is known to react via one electron oxidation producing benzene radical cation followed by benzyl type radicals by the dissociation of Cα-hydrogen [106]. Therefore, it showed ΔOD/G features similar to the reaction between SBDS and SO₄•. The higher peak intensity at 290 nm observed for the reaction between SO₄• and SDBS is attributed to more favourable formation of benzyl radicals by SO₄•. Therefore, the higher extent of mineralization of SDBS is not because of the conjugative effect of both •OH and SO₄•, but because of the preferential formation of benzyl type of radicals via the formation of benzene radical cation.
3.4.5. The influence of the nature of neutralizing acid on the radiolysis of STDWW

The pH of the STDWW solution did not appreciably change even after 50 kGy dose of radiolysis (Figure 3.36a) because of the lesser extent of formation of the organic acids from the mineralization of the organic components of STDWW. On the other hand, the pH of the STDWW decreased to 6.3 on irradiating the solution in presence of K$_2$S$_2$O$_8$ for the same dose (Figure 3.36b) because of the higher extent of formation of the organic acids from the mineralization of the organic components. The SO$_4^{\bullet^-}$ efficiently oxidises the organic compounds and itself converts into SO$_4^{2-}$, which is a conjugate base of strong acids (HSO$_4^-$, H$_2$SO$_4$). Therefore, the pH of the solution significantly decreased during radiolysis in presence of K$_2$S$_2$O$_8$ [107]. This phenomenon led to the existence of the organic acids in their protonated form and therefore, the rate constants of these acids with SO$_4^{\bullet^-}$ decreased leading to almost negligible extent of mineralization at higher doses than 50 kGy [107]. Moreover, the HCO$_3^-$ ion concentration in equilibrium with CO$_3^{2-}$ increases with decreasing the pH and it minimizes the scavenging effect as HCO$_3^-$ [108]. However, the minimum COD achieved in the irradiated solution (50 kGy) in presence of K$_2$S$_2$O$_8$ was 1558 ppm, which was much above the permissible discharge limit ($\leq$ 250 ppm).

The acetic acid, which was used to adjust the pH of the simulated dye solution, is an organic acid and it contributes significantly in the residual COD of the irradiated solution. Therefore, we did the radiolysis of STDWW in presence of K$_2$S$_2$O$_8$ by replacing the acetic acid (organic acid) by H$_2$SO$_4$ (mineral acid) in the pH adjustment step. The STDWW solution, where the pH was adjusted by diluted H$_2$SO$_4$ will be henceforth designated as modified simulated textile dye waste water (MSTDWW). The initial CODs of the STDWW and MSTDWW were calculated as 3128 and 1544 ppm,
respectively. It could itself give an idea about the contribution of the acetic acid in the total COD of the simulated solution.

Moreover, the extent of mineralization of MSTDWW increased to 84% upon gamma irradiation for 50 kGy in presence of K₂S₂O₈ and the pH of the solution drastically decreased to about 2.1. Despite the incomplete mineralization, The COD of MSTDWW could bring down to 245 ppm (which is below the recommended discharge limit) upon irradiation for about 60 kGy in presence of K₂S₂O₈. The pH of the irradiated solution was remained constant in the range 1.5–2.0 (Figure 3.36c).

![Figure 3.36](image)

**Figure 3.36** Variation of pH as a function of dose during gamma radiolysis of STDWW in the (a) absence and (b) presence of K₂S₂O₈ and (c) MSTDWW in presence of K₂S₂O₈.

Therefore, the use of H₂SO₄ in place of CH₃COOH in the pH adjustment step followed by the gamma radiolysis of STDWW in presence of K₂S₂O₈ is recommended for an effective effluent treatment process. Thus, further investigations on the process efficiency of radiolysis in comparison to other AOPs were performed on MSTDWW.
3.4.6. Radiolysis of MSTDWW in the presence of $K_2S_2O_8$

The mineralization of MSTDWW irradiated in the presence of 40 mM $K_2S_2O_8$ at different doses is shown in Figure 3.37a. It shows 20% and 75% mineralization at doses of 11 kGy and 60 kGy, respectively. It could be noted that, at the same time, only 16% and 54% mineralization was observed for the STDWW (pH adjusted with CH$_3$COOH). Furthermore, about 80% mineralization was observed for the gamma radiolysis of MSTDWW at the 60 kGy dose, while only 60% mineralization was observed for the gamma radiolysis of STDWW at 100 kGy (Figures 3.32b & 3.37a). Therefore, the nature of the pH adjusting acid influences the extent of the mineralization of MSTDWW with that of the STDWW. As discussed earlier, $K_2S_2O_8$ itself can produce SO$_4^{–}$ by thermal decomposition at 38-40 °C, which is the usual temperature of the solution during gamma radiolysis. Therefore, the extent of mineralization of MSTDWW in the presence of 40 mM $K_2S_2O_8$ was studied at 40 °C under room conditions (no irradiation), and here, no appreciable mineralization of MSTDWW was observed.

![Figure 3.37 Mineralization of MSTDWW in the presence of 40 mM $K_2S_2O_8$ during (a) gamma radiolysis and (b) EB radiolysis.](image)
The MSTDWW solution was irradiated at different doses by electron beam (EB) at pH 10 in the presence of 40 mM K$_2$S$_2$O$_8$. The extent of mineralization of MSTDWW increased with each dose by about 20% (Figure 3.37b). The high intensity electron beam rapidly deposits energy to the aqueous solution and elevates the temperature of the aqueous solution from ambient temperature [109, 110]. Therefore, it can be speculated that the high yield of *OH and SO$_4$•− (by the conjugated effects of radiolytic and thermal decompositions) enhances the % mineralization of MSTDWW to about 34% and 96% at 11 kGy and 60 kGy doses, respectively. At 60 kGy, the COD of the final solution was brought down to below 100 ppm, which is below the recommended limit of discharge (≤ 250 ppm).

3.4.7. Photocatalysis of MSTDWW

The photocatalysis of MSTDWW was carried out over different time intervals. However, only 30% mineralization of MSTDWW was observed after 10 hours of photocatalytic treatment (Figure 3.38a), and no appreciable change in the extent of mineralization was observed over longer times. It could be noted that only 24% mineralization of STDWW was observed under the same photocatalytic conditions (Figure 3.31a). It is important to mention that the TiO$_2$ nanoparticles made very stable suspensions with the aqueous solution of the individual components of MSTDWW. However, TiO$_2$ nanoparticles settled down rapidly in MSTDWW. The reason is quite similar as discussed in Section 3.4.1. Instead of molecular oxygen, S$_2$O$_8^{2−}$ can also take the CB electron from TiO$_2$ nanoparticles, thereby producing SO$_4$•− (Eq. 3.5)

\[
S_2O_8^{2−} + e^{−}_{CB} \rightarrow 2 SO_4^{•−}
\] (3.5)

Therefore, the effect of K$_2$S$_2$O$_8$ on the photocatalytic degradation of MSTDWW was also investigated in the presence of 40 mM K$_2$S$_2$O$_8$. The extent of mineralization increased by about 10-12% during the photocatalysis of MSTDWW in the presence of
K$_2$S$_2$O$_8$ (Figure 3.38b). The increase in the %mineralization of MSTDWW during the photocatalysis in the presence of K$_2$S$_2$O$_8$ is attributed to: (i) the decrease in the probability of recombination of the photogenerated electrons and holes, and (ii) the formed SO$_4^{\bullet^-}$ having higher mineralization efficiency. However, the application of this process is limited by the coulombic repulsion between the negatively charged surface of TiO$_2$ (pH$_{pzc} = 6.0 \pm 0.2$) and S$_2$O$_8^{2-}$ (at pH 10) and the rapid settlement of the catalyst in MSTDWW.

The photolysis (photochemical decomposition of K$_2$S$_2$O$_8$ in the absence of TiO$_2$) did not impart any enhancement in the extent of mineralization of MSTDWW (Figure 3.38c). This is speculated by the lower yield of SO$_4^{\bullet^-}$ from the photolysis of S$_2$O$_8^{2-}$ with about 350 nm UV light [111].

![Figure 3.38 Mineralization of MSTDWW over different durations in: (a) photocatalysis, (b) photocatalysis in the presence of 40 mM K$_2$S$_2$O$_8$, (c) photolysis in presence of 40 mM K$_2$S$_2$O$_8$, (d) ozonolysis, and (e) ozonolysis in the presence of 40 mM K$_2$S$_2$O$_8$.](image-url)
3.4.8. Ozonolysis of MSTDWW

The extent of mineralization of MSTDWW was studied at different durations of ozonolysis at pH 10. About 30% and 60% extent of mineralization of MSTDWW was achieved after 0.5 hours and 4 hours of ozonolysis, respectively. However, after 4 hours, no significant increase in the extent of mineralization was observed (Figure 3.38d). It should be noted that only 13% and 25% mineralization of STDWW was observed under the same ozonolytic conditions (Figure 3.31b). The effect of K₂S₂O₈ on the ozonolysis of MSTDWW was investigated in the presence of 40 mM K₂S₂O₈ (Figure 3.38e). The extent of mineralization decreased drastically in case of ozonolysis in the presence of K₂S₂O₈. The K₂S₂O₈ does not produce SO₄⁻ during ozonolysis (in the absence of any radiation or thermal activation); instead, some of the •OH radicals formed during ozonolysis will react with K₂S₂O₈ giving some products (Eq. 3.6) which may not react with components of MSTDWW.

\[
•OH + K₂S₂O₈ \rightarrow \text{Products} \quad (3.6)
\]

Therefore, the extent of %mineralization decreased during ozonolysis in presence of K₂S₂O₈.

3.4.9. Comparison of the process efficiencies of radiolysis, photocatalysis, and ozonolysis for the mineralization of MSTDWW

3.4.9.1. In terms of the OCC

The OCC of radiolysis (gamma and electron beam) (Eq. 3.4), photocatalysis/photolysis (Eq. 3.2) and ozonolysis (Eq. 3.3) were calculated to compare the process efficiencies of different AOPs. It can be seen from Figure 3.39 that the lowest degree of mineralization of MSTDWW (to an extent of 28%) was observed in the photocatalysis and photolysis of K₂S₂O₈. Thus OCC values and the cost of the energy source and other
ancillary inputs of different AOPs were compared only for 28% mineralization of MSTDWW. It could be noted that the OCCs of radiolysis, photocatalysis, ozonolysis, and radiolysis (+K₂S₂O₈) of STDWW could be calculated only for 16% mineralization, which was the lowest observed degree of mineralization of STDWW (Figure 3.33). The OCC values of photocatalysis, photocatalysis (+K₂S₂O₈), photolysis in presence of K₂S₂O₈, ozonolysis, ozonolysis (+K₂S₂O₈), and gamma (+K₂S₂O₈) and EB (+K₂S₂O₈) radiolysis for 28% mineralization were calculated to be 6.29, 2.46, 7.63, 9.29, 38.83, 0.08, and 0.04 kg equivalent O₂ m⁻³, respectively. EB radiolysis in the presence of K₂S₂O₈ showed a maximum chemical efficiency (about 96% mineralization) of the oxidants at 0.3 kg equivalent of O₂ m⁻³ OCC. About 78% mineralization was observed in gamma radiolysis, and less than 10% mineralizations were observed for others at 0.3 kg equivalent of O₂ m⁻³ OCC. It could be noted that 0.3 kg equivalent of O₂ m⁻³ OCC could mineralize only 54% of STDWW by gamma radiolysis in the presence of K₂S₂O₈ (Figure 3.33). Therefore, it could be safely concluded that the amount of oxidant required achieving the same extent of mineralization of MSTDWW by EB radiolysis was the least, compared to other processes studied here. Therefore, the OCC for a 28% mineralization of MSTDWW follows the order: EB (+K₂S₂O₈) radiolysis < gamma (+K₂S₂O₈) radiolysis < photocatalysis (+K₂S₂O₈) < photocatalysis ≈ photolysis in presence of K₂S₂O₈ < ozonolysis < ozonolysis (+K₂S₂O₈). The mechanism of enhancement in the extent of mineralization of STDWW (Section 3.4.4.), ibuprofen (Section 4.2) during radiolysis in the presence of K₂S₂O₈ has been studied in details. Since, only the nature of the pH adjusting acid changes, we speculate that the mechanism of mineralization of the components of MSTDWW was quite similar to that of STDWW.
3.4.9.2. In terms of the cost of the energy source and other ancillary inputs

The efficiencies of EB (+K₂S₂O₈) and gamma (+K₂S₂O₈) radiolysis, photocatalysis (+K₂S₂O₈), and ozonolysis were evaluated in terms of the cost of energy and other ancillary inputs. The cost of the electrical energy required for EB (+K₂S₂O₈) radiolysis, photocatalysis (+K₂S₂O₈), and ozonolysis can be calculated using Eq. 3.7.

\[
EEC = P \times \frac{t}{60} \times \frac{1000}{v}
\]  

(3.7)

where EEC (in kWh m⁻³) is the electric energy consumed (in kWh) to degrade a contaminant in unit volume (in m³), P is the rated power (in kW) of the AOP system, t is the duration (in min) of treatment, and v is the volume (in L) of MSTDWW treated in
time \( t \). The duration of treatment for 28\% mineralization of MSTDWW by EB (+K\(_2\)S\(_2\)O\(_8\)) radiolysis, photocatalysis (+K\(_2\)S\(_2\)O\(_8\)), and ozonolysis were observed to be 0.6, 180, and 30 min, respectively. The cost of the electrical energy, along with the ancillary chemicals (if any), for these AOPs are summarized in Table 3.6. Among these processes, the costs involved in EB (+K\(_2\)S\(_2\)O\(_8\)) treatment were the lowest.

**Table 3.6** Comparison of the cost of energy and ancillary chemicals for different AOPs.

<table>
<thead>
<tr>
<th></th>
<th>Ozonolysis</th>
<th>Photocatalysis (+K(_2)S(_2)O(_8))</th>
<th>Electron beam radiolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power employed in the process (kW)</td>
<td>0.08</td>
<td>0.128</td>
<td>1</td>
</tr>
<tr>
<td>Treatment time (min)</td>
<td>30</td>
<td>180</td>
<td>0.6</td>
</tr>
<tr>
<td>Volume of MSTDWW treated (L)(^a)</td>
<td>0.04</td>
<td>0.26</td>
<td>1.9</td>
</tr>
<tr>
<td>EEC (kWh m(^{-3})) of MSTDWW</td>
<td>1000</td>
<td>1477</td>
<td>5.3</td>
</tr>
<tr>
<td>Electrical energy cost (INR m(^{-3})) of MSTDWW @INR 8.5 (kWh(^{-1}))</td>
<td>8500</td>
<td>12554</td>
<td>45</td>
</tr>
<tr>
<td>Cost of additional chemicals or gas (INR m(^{-3})) of MSTDWW</td>
<td>246000 (O(_2) cylinder cost @ INR 164 m(^{-3}))</td>
<td>3000 (TiO(_2) cost @ INR 3 g(^{-1})) + 12975 (K(_2)S(_2)O(_8) cost @ INR 1.2 g(^{-1}))</td>
<td>12975 (K(_2)S(_2)O(_8) cost @ INR 1.2 g(^{-1}))</td>
</tr>
<tr>
<td>Total cost m(^{-3}) of MSTDWW</td>
<td>254500</td>
<td>28529</td>
<td>13020</td>
</tr>
</tbody>
</table>

\(^a\) Guided by the maximum volume capacity of the instrument to treat the MSTDWW under the same treatment condition.

In the gamma radiolysis of MSTDWW (which did not involve electrical energy), the cost of the energy source could be estimated by accounting for five effective half-lives of \(^{60}\)Co source using Eq. 3.8

\[
CTP = I \times R \times \frac{t}{5 \times 365 \times 24 \times \frac{1}{t \times \frac{1000}{v_{max}}} (3.8)}
\]

where CTP (INR m\(^{-3}\)) is the average cost of the treatment process in Indian rupee (INR), \( I \) is the initial activity in Curie (Ci) of the \(^{60}\)Co source, \( R \) is the price (in INR) of
$^{60}$Co source Ci$^{-1}$, $t$ is the treatment time (in hours), $t_{1/2}$ is the half-life (hour) of $^{60}$Co, and $v_{max}$ is the maximum volume capacity (in L) of the gamma chamber that can be treated in time $t$. In our study, the initial activity of $^{60}$Co was 10000 Ci, involving a cost of INR 70 Ci$^{-1}$, and volume of the gamma chamber was 5 L. Therefore, the cost for 28% mineralization using gamma radiolysis was calculated as INR 7931 m$^{-3}$. The total cost of gamma radiolysis (+$K_2S_2O_8$) for the treatment of MSTDWW was found to be INR 20906 m$^{-3}$. This is the first approach to calculate the equivalent cost of gamma radiolysis in comparison to other AOPs consuming electrical energy.

The above results showed that the cost involved in EB (+$K_2S_2O_8$) treatment was the lowest one among the studied AOPs for the mineralization of MSTDWW. It is important to note that the AOPs are emerging technologies currently being commercialized worldwide. A few UV/H$_2$O$_2$-based AOPs have been internationally commercialized for the treatment of drinking water and industrial water, using the advantage of both chemical and energy inputs [112-115]. There are few companies, such as AST clean water technologies, China; Trojan Technologies, Canada; Calgon Carbon Corporation and Xylem Global, US, who have brought some of the AOPs to international markets. In parallel, the radiation technology is internationally emerging for waste-water treatment [116–118]. Radiation-based pilot sludge treatment plants have been established in New Mexico, USA (Gamma); Weldel, Germany (EB); Verginia Key, USA (EB); Takasaki, Japan (EB); Sao Paulo, Brazil (EB); Tucuman, Argentina (Gamma); and Daejeon, Korea (EB) [116-118]. In addition, radiation-based commercial sludge treatment plants have also been established in Vadodara, India (Gamma), and Munich, Germany [116-118]. A pilot plant for treating 1000 m$^3$ day$^{-1}$ of dyeing waste-water with EB has been constructed and operated since 1998 in Daegu, Korea, together with a biological treatment facility [78, 79]. Therefore, we understand
that the studies presented in this paper have a lot of scope to advance radiation-based technologies for the treatment of textile effluents. Furthermore, the EB has the ability to simultaneously disinfect the water during the degradation process [119]. At this stage, the used EB (+K$_2$S$_2$O$_8$) treatment process does not produce water suitable for reuse or for drinking. Hence, a multi-step treatment system would need to be designed in the near future.