CHAPTER 2

Experimental

Waste water before irradiation  
Waste water after irradiation

Spectrophotometer
COD Analyser
ESI-MS Analyser
The following experimental techniques or procedures were employed during the course of the study.

2.1. Linear Electron Accelerator (LINAC)

2.1.1. 7 MeV LINAC

A 7 MeV nanosecond LINAC pulse radiolysis technique was used to determine fast reaction kinetics. A highly short intense pulse of beam current of electrons is given to a system to achieve a non-equilibrium situation in which significant concentration of transient species is produced in a short time interval and these transient species are monitored following the changes in their optical absorption [59]. The LINAC assembly available at the institution is from M/s Viritech, U.K. (formerly M/s Radiation Dynamics, U.K.). The essential components of a 7 MeV LINAC are (i) electron gun and (ii) wave guide. A 2 μs electron pulse of energy 43 keV is generated in electron gun and these electrons are allowed to enter into the wave guide in a phase synchronous with the radio frequency (R.F.) field (generated by magnetron). The electrons are accelerated in vacuum (10⁻⁸ mbar) into the wave guide to the final energy of 7 MeV. The parameters for 7 MeV LINAC during the experiment are summarized in Table 2.1.

Table 2.1 Specifications of 7 MeV LINAC

<table>
<thead>
<tr>
<th>Electron energy</th>
<th>Pulse Width (ns)</th>
<th>Peak current (mA)</th>
<th>RF Frequency (MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 MeV</td>
<td>500</td>
<td>90</td>
<td>3000</td>
</tr>
</tbody>
</table>
2.1.1.1. Kinetic spectrophotometer

The transient behaviour in pulse radiolysis is monitored by the most popular technique of absorption spectroscopy. The different units of the analyzing set-up consists of (i) 450 W Xenon arc lamp as the analyzing light source (wavelength range 250-850 nm), (ii) Optical components like electromechanical shutter (to prevent photodecomposition of the samples during the passive mode), light filters, lenses and mirrors, monochromator (with usable range of 180-850 nm with a dispersion of 3 nm mm\(^{-1}\)) and photomultiplier detector (with spectral response in the working range of 200-900 nm and rise time of detection of 75 ns) and (iii) recorder i.e., oscilloscope, computer and printer.

2.1.1.2. Analysis of the recorded signal

The transient signal obtained is plot of signal (mV), which is proportional to intensity of the absorbed light (I\(_{\text{abs}}\)), versus time. These signals are converted into optical density (O.D.) using Eq. 2.1.

\[
O.D. = \log \frac{I_1}{I_2}
\]  
(2.1)

where, I\(_1\) is the intensity of the light passing to the solution before pulse and I\(_2\) is the intensity of the light transmitted to the solution after pulse. After substituting I\(_0\) by DC (mV), which is the difference of the PMT output under identical conditions with and without the analyzing light and I\(_i\) by [DC(mV) - Signal(mV)], Eq. 2.1 turns to Eq. 2.2.

\[
O.D. = \log \left( \frac{\text{DC(mV)}}{\text{[DC(mV) - Signal(mV)]}} \right)
\]  
(2.2)

To obtain the absorption spectrum of a transient species, the O.D. value is plotted as a function of the wavelength of the analyzing light at a selected time.
2.1.1.3. Corrected transient absorption spectra and molar absorptivity

A fraction of the parent molecules is lost in a radiation chemical reaction. The transient absorption is given by Eq. 2.3

$$\Delta A = (\varepsilon_t - \varepsilon_p) C_t l$$  \hspace{1cm} (2.3)

where, $\Delta A$ is observed absorbance, $\varepsilon_t$ and $\varepsilon_p$ are the extinction coefficients of the transient and the parent molecule at a particular wavelength, $C_t$ is the concentration of the transient produced and $l$ is the optical path length. When the absorptions due to the parent molecule and the transient formed overlap, the observed absorbance does not represent the true characteristics of the transient. Thus, a correction term for the amount of parent molecules depleted needs to be included in the calculation.

The molar absorptivity of a transient can be estimated as follows. The concentration of the transient of interest is obtained from the absorbed dose, measured by thiocyanate dosimetry. Under identical condition, the two absorbances are related by Eq. 2.4.

$$\frac{A_{\lambda}^{\text{sample}}}{(G \cdot \varepsilon)^{\text{sample}}} = \frac{A_{500nm}^{\text{dosimeter}}}{(G \cdot \varepsilon)^{500nm \text{ dosimeter}}}$$  \hspace{1cm} (2.4)

In case the parent molecule also has absorption at wavelength $\lambda$, then Eq. 2.3 is modified as Eq. 2.5.

$$\varepsilon_{\lambda}^{\text{sample}} = \varepsilon_{\lambda}^{\text{parent}} + \frac{A_{\lambda}^{\text{sample}}}{G_{\text{sample}}} \cdot \frac{(G \varepsilon)^{500nm \text{ dosimeter}}}{A_{500nm}^{\text{dosimeter}}}$$  \hspace{1cm} (2.5)
2.1.1.4. Dosimetry for Pulse Radiolysis

An aerated aqueous solution of 20 mM potassium thiocyanate (KSCN) is used to measure the absorbed dose per pulse. Amongst the primary radicals produced, \( ^{\cdot}H \) and \( e_{aq}^{-} \) are scavenged by the dissolved oxygen. The \( \cdot \text{OH} \) oxidizes \( \text{CNS}^{-} \) ions producing \( (\text{SCN})_{2}^{-} \) according to Eqs. 2.6 & 2.7.

\[
\text{CNS}^{-} + \cdot \text{OH} \rightarrow \text{CNS}^{\cdot} + \text{OH}^{-} \quad (2.6)
\]

\[
\text{CNS}^{\cdot} + \text{CNS}^{-} \rightarrow (\text{CNS})_{2}^{\cdot} \quad (2.7)
\]

The G.\( \varepsilon \) value for this species is reported to be \( 2.15 \times 10^{4} \text{ M}^{-1} \text{ cm}^{-1} \) or \( 2.23 \times 10^{-4} \text{ m}^{2} \text{ J}^{-1} \) with \( G = 2.9 \) at 500 nm for 100 eV energy absorbed \( [60] \). From the measured absorbance (A), the absorbed dose per pulse (D) is computed from Eq. 2.8.

\[
D = \frac{A \cdot N \cdot 100}{G \cdot \varepsilon \cdot 1000} \text{ eV cm}^{-3} \quad (2.8)
\]

The above equation is simplified by substituting appropriate numerical values of G, \( \varepsilon \) and N (Avogadro number), one can get

\[
D = 2.8 \cdot A \cdot 10^{18} \text{ eV cm}^{-3} \quad (2.9)
\]

The absorbed dose in the units of Gy is obtained by multiplying the value of A by a factor 448.6. Generally the maximum dose obtained from a 25 ns pulse is 10 Gy and that for a 2 \( \mu \text{s} \) pulse is about 120 Gy.
2.1.2. 2 MeV Linear Electron Accelerator

The pulse linear electron beam accelerator of ILU-6 type is obtained from Budker Institute of Nuclear Physics, Russia. The accelerator is based on resonator cavity inductively coupled to a self-excited RF generator (120 MHz) and can be operated up to energy 2 MeV and average 20 kW power with a repetition rate of 50 Hz [61]. The machine is designed to deliver an average dose of about 33 kGy s\(^{-1}\) at the centre. The machine is housed in a shielded cell having a labyrinth with separate entry and exit ports where power roller conveyor system has been installed for the material transport in & out of the irradiation zone. The electrons are injected in the cavity and accelerated in a single step to attain the energy of 2 MeV. A vacuum of 10\(^{-6}\) torr is maintained in the cavity. The output beam is extracted in to the atmosphere in pulse form with 500 μs and each pulse is scanned over a length of 100 cm.

2.1.2.1. Dosimetry of electron beam accelerator

Dosimetry was performed using a FWT60 radiochromic film dosimeter calibrated using graphite calorimeter [62]. ILU-6 pulse accelerator parameters during the experiment were as follows:

Table 2.2 Specifications of 2 MeV LINAC

<table>
<thead>
<tr>
<th>Electron energy (Mev)</th>
<th>Pulse Repetition frequency (Hz)</th>
<th>Average beam current current (mA)</th>
<th>Beam power (kW)</th>
<th>conveyor speed (cm s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>50</td>
<td>1.2</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>
### 2.1.3. 10 MeV Linear Electron Accelerator

A 10 MeV RF electron LINAC was also used in the present study. The electron beam at 50 keV in electron gun with LaB₆ cathode is injected into the on-axis coupled cavity LINAC which accelerates the electron to a maximum energy to 10 MeV. Dose rate was maintained at 10 kGy/pass. 10 MeV accelerator parameters during the experiment were as follows:

**Table 2.3 Specifications of 10 MeV LINAC.**

<table>
<thead>
<tr>
<th>Electron energy (MeV)</th>
<th>Pulse Repetition frequency (Hz)</th>
<th>Average beam current (mA)</th>
<th>Beam power (kW)</th>
<th>conveyor speed (cm s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>300</td>
<td>33</td>
<td>1</td>
<td>1.67</td>
</tr>
</tbody>
</table>

### 2.2. Cobalt-60 Gamma Source [GC-5000]

γ-ray emitting ⁶⁰Co radioisotope is produced by irradiating natural cobalt (⁵⁹Co) in the form of pellets or small slugs or thin disks in nuclear reactor by ⁵⁹Co(n,β)⁶⁰Co reaction. It gives uniformly active material which emits β particle followed by two γ-rays of energy 1.33 MeV and 1.17 MeV. The walls of the metal container filters out β radiation emitted by ⁶⁰Co. Gamma irradiation of the samples were carried at room temperature using Gamma Chambers GC-5000, which were supplied by the Board of Radiation & Isotope Technology, Mumbai, India. Figure 2.1 shows one of the gamma chambers used for our studies. The gamma chamber mainly consists of a set of stationary ⁶⁰Co sources placed in a cylindrical cage surrounded by a lead shield provided around the source to keep external radiation field well within the permissible limits. The material for irradiation is placed in
an irradiation chamber located in the vertical drawer inside the lead flask. The particular gamma source used in the experiments produces a dose rate of 3 or 2.5 kGy hr\(^{-1}\).

**Figure 2.1 Gamma Chamber 5000.**

2.2.1 *Dosimetry for \(^{60}\text{Co}\) Gamma Source*

In general, Fricke (ferrous sulfate) dosimeter is used for the dose measurement of \(^{60}\text{Co}\) \(\gamma\) source [63]. The principle involved is the radiation induced oxidation of ferrous ion to ferric ion at low pH and in presence of oxygen. The standard dosimetric solution
contains 1 mM ferrous sulfate, 1 mM NaCl in 0.4 M H₂SO₄ (pH 0.46). The solution is saturated with oxygen. The reactions involved are given in Eqs. 2.10-2.16.

\[ e_{aq}^- + H^+ \rightarrow \cdot H \quad (2.10) \]

\[ \cdot H + O_2 \rightarrow HO_2^- \quad (2.11) \]

\[ \cdot OH + Fe^{2+} \rightarrow OH^- + Fe^{3+} \quad (2.12) \]

\[ HO_2^- + Fe^{2+} \rightarrow HO_2^- + Fe^{3+} \quad (2.13) \]

\[ HO_2^- + H^+ \rightarrow H_2O_2 \quad (2.14) \]

\[ H_2O_2 + Fe^{2+} \rightarrow OH^- + \cdot OH + Fe^{3+} \quad (2.15) \]

\[ G(Fe^{3+}) = 2 G(H_2O_2) + 3 [G(e_{aq}^-) + G(\cdot H) + G(HO_2^-)] + G(\cdot OH) \quad (2.16) \]

The G(Fe³⁺) is calculated as 15.5 by substituting the G values of the primary radicals.

The spectrophotometric method is mostly used to measure the concentration of ferric ion formed by comparing the absorbance of the irradiated and non-irradiated dosimeter solutions at the wavelength at which ferric ion shows maximum absorption (about 304 nm). The optical readings should be taken soon after irradiation so that adventitious oxidation of the solutions is minimized. The mean absorbed dose (D) for the volume occupied by dosimeter solution is given by Eq. 2.17.

\[ D = \frac{9.684 \times 10^6 \Delta A_D}{\Delta \varepsilon \rho G(P)} Gy \quad (2.17) \]

where, \( \Delta A_D \) is the change in absorbance of dosimeter solution before and after irradiation. G(P) is yield (number of molecules per 100 eV) of ferric ions due to irradiation, \( \Delta \varepsilon \) is
extinction coefficient \( \left( \text{M}^{-1} \text{ cm}^{-1} \right) \) of ferric ions at the measuring wavelength, \( \rho \) is the density \( \left( \text{g cm}^{-3} \right) \) of dosimeter solution, \( l \) is the path length (cm).

### 2.3. Chemical Oxygen Demand

The COD test is commonly used to measure the amount of organic compounds in water. It is expressed in milligrams of oxygen consumed per liter (mg L\(^{-1}\)) of solution. Sometimes, it is also expressed in ppm (parts per million). The organic compound is fully oxidized to carbon dioxide and water by a strong oxidizing agent under high temperature. In this method, the solution containing organic compound is digested at 120°C in presence of \( \text{K}_2\text{Cr}_2\text{O}_7 \) and \( \text{H}_2\text{SO}_4 \) in a closed reactor called “thermoreactor” and then the amount of consumed \( \text{K}_2\text{Cr}_2\text{O}_7 \) (which is equivalent to the amount of \( \text{O}_2 \) consumed for the oxidation) is determined by spectrophotometrically (Figure 2.2).

![Figure 2.2](image)

*Figure 2.2* (a) Thermoreactor and (b) spectrophotometer for COD measurement.

The amount of equivalent oxygen required for oxidising an organic compound \( (\text{C}_n\text{H}_a\text{O}_b\text{N}_c) \) to carbon dioxide, ammonia, and water is given by Eq. 2.18.

\[
\text{C}_n\text{H}_a\text{O}_b\text{N}_c + \left( n + \frac{a}{4} - \frac{b}{2} - \frac{3}{4}c \right) \text{O}_2 \rightarrow n\text{CO}_2 + \left( \frac{a}{2} - \frac{b}{2} - \frac{3}{2}c \right) \text{H}_2\text{O} + c\text{NH}_3 \quad (2.18)
\]
In the present studies, the COD of the solutions were measured by using Spectroquant® Pharo 300 COD analyser from M/s. Merck, Germany.

### 2.4. Total Organic Carbon

Total organic carbon (TOC) represents the amount of carbon bound in an organic compound. The TOC analysis of a solution has three main steps viz. (i) acidification and purging with inert gas (removes inorganic carbon), (ii) photocatalytic oxidation of carbon content to CO$_2$ and (iii) detection and quantification of CO$_2$ by non-destructive infrared method. The TOCs of the solutions were measured in this study by using ANATOC II TOC analyser from SGE Australia (Figure 2.3).

![Figure 2.3 Total organic carbon analyser.](image)

### 2.5. Biological Oxygen Demand (BOD)

Biochemical oxygen demand is the amount of dissolved oxygen needed by aerobic biological organisms in water to degrade organic molecules present in water at certain temperature over a specific time period. The BOD value is most commonly expressed in milligrams of oxygen consumed per litre of sample water during 5 days of incubation at 20 °C. BOD measures the amount of the biodegradable fraction of the organic molecules
present in the polluted water. BOD was measured by using Eutech DO meter (Cyberscan DO6000) from M/s. Merck, Germany. The BOD bottles were incubated at 20°C for 5 days and difference in dissolve oxygen (DO) was used to calculate BOD₅ (APHA1998). The BOD values are expressed in mg L⁻¹ according to Eq. 2.19.

\[
\text{BOD (mg L}^{-1}\text{)} = \frac{(D_1-D_5)}{p}
\]  \hspace{1cm} (2.19)

where, D₁ and D₅ are the initial and final DO of sample dilution (in mg L⁻¹); P is the decimal volumetric function of sample used.

2.6. UV-Visible spectrophotometer and pH meter

The spectrophotometric measurement was carried out by Hitachi U-2800 UV-Vis spectrophotometer. The pH of the solution was measured by using pH meter 600.

2.7. Gas chromatography mass spectrometry

Gas chromatography mass spectrometry (GC-MS) is used for separating and analyzing compounds that can be vaporized without decomposition. The GC-MS analysis was carried out by using a Shimadzu 2010 MS, equipped with integrated gas chromatograph. In GC, the components are separated based on their distribution coefficient between non polar, nonvolatile liquid stationary phase (100% dimethyl-polysiloxane (60 mm long, 0.25 mm inner diameter)) and gaseous mobile phase (Helium, flow rate: 13 mL min⁻¹). The column is held in an oven that can be programmed to increase the temperature gradually. As the temperature increases, that compounds which have low boiling points elute from the column sooner than those that have higher boiling points and show lesser retention time. Then the components are detected by respective m/z ratio by mass.
spectrometer which consists of an ion source (EI), an analyzer (quadrupole) and a detector (microchannel plate detector). The injector temperature in the study was kept at 280°C which was programmed as follows: 80°C kept constant for 2 min, increased up to 200°C with 10°C min⁻¹, and raised up to 280°C with 20°C min⁻¹ rate. The compounds were identified on the basis of m/z values by using the NIST library.

2.8. High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is an analytical technique used to separate and identify the components of a mixture. The reversed phase HPLC experiment was performed by using Waters (Model 2690). The important components of HPLC are the solvent delivery system, sample injector, column, detector and data processor. The preparation of samples for HPLC analysis follows the same procedure as described in for the FTIR analysis (Section 2.10). The components are separated by the distribution coefficient between non polar stationary phase (C-18 column (symmetry, 4.6 mm × 250 mm) and polar liquid mobile phase (HPLC grade methanol with an isocratic flow rate 0.50 mL min⁻¹). More polar compound will elute earlier due to lesser extent of interaction with stationary phase and show lesser retention time. The eluted components were detected by UV detector with a fixed wavelength at 280 nm in the present study.

2.9. Electrospray Ionization Mass Spectrometry (ESI-MS)

In the study electrospray ionization (ESI) technique was used as the ion source in mass spectrometry (MS) to produce ions. MS is an analytical chemistry technique that helps to identify the amount and type of ions generated by measuring the mass-to-charge ratio and abundance of gas-phase ions. MS consists of a time of flight mass analyzer and a
detector (electron multiplier). A negative ion mode electrospray mass spectrometer with Quadruple-Time-of-Flight analyzer (model micrOToFQ-II, Bruker Daltonik GmbH) was used in a part of the present study where aqueous dye solutions were directly infused with an automatic syringe pump (NEMESYS from cetrol GmbH). A high voltage is applied to the liquid to create a spray of charged droplets called aerosol. The charged droplets are migrated under the influence of the potential where a flow of heated nitrogen dries the droplets and carries away uncharged mobile phase molecules. This process results in the continuous shrinkage of the droplet size until the repulsive electrostatic forces exceed the surface tension, leading to droplet explosions as indicated in the inset of the Figure 2.4. Other parameters for negative mode ESI were: end plate offset: -1000 V; dry heater temperature: 100 °C; capillary voltage: 3000 V; dry gas flow rate: 4.0 L min⁻¹; nebulizer gas: 30 kPa and collision energy was kept at 1 eV to avoid heavy in-source fragmentation.

**Figure 2.4** Schematic of electrospray ionisation system.
2.10 Fourier transformed infrared spectroscopy

Fourier transformed infrared (FTIR) spectroscopy is an important analysis technique that detects various characteristic functional groups present in the sample. In this study the FTIR spectra of radiation, microbiological and combined radiation-microbiological treated dye solutions were recorded by using Perkin-Elmer 783 Spectrophotometer. In the first set of samples, the irradiated solutions were evaporated to dryness in rotary evaporator and employed for FTIR analysis. For the other two sets, after complete decolouration, the decolourised media was centrifuged at 5000 rpm for 20 min and the metabolites were extracted from the supernatant by equal volume of ethyl acetate. The extract was dried over anhydrous Na₂SO₄ and was evaporated to dryness in rotary evaporator. The crystals obtained were mixed with anhydrous KBr in the 5:95 weight percentages and pressed into pellets under high pressure and used for FTIR analysis. The % transmittance of the samples was measured at different wavenumbers in the range between 400-4000 cm⁻¹ by averaging 16 scans for each spectrum.

2.11 Photocatalytic reactor

The photocatalytic experiments were carried out by using Rayonet Photochemical Reactor. The light source contains 16 mercury lamps of 8 W power each and emitting in the near-UV (mainly around 350 nm) having incident radiation intensity (I₀) of 5.0×10¹⁵ photons cm⁻² s⁻¹. The volume and area of the photoreactor chamber are V (250 cm³) and Aₜₐ (32 cm²), respectively.
2.12. Ozone reactor

Ozone was continuously produced from pure oxygen by UOS04 model ozone generator and bubbled into the aqueous solution. The ozone generator can produce 4 g h\(^{-1}\) ozone from pure oxygen feed of 2 L min\(^{-1}\). The input rate of ozone from the ozone generator into the aqueous solution was determined as 7.3\times 10^{-3} \text{ mol m}^{-3} \text{ min}^{-1} by the standard iodometric method.

2.13. Chemicals and Reagents

Reactive Red 120 (RR-120), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), sodium dodecylbenzenesulfonate (SDBS), sodium dodecylsulphate (SDS), potassium persulfate (K_2S_2O_8), tert-butanol (tBu-OH) were purchased from Sigma-Aldrich, India. L-tyrosine, Methyl Red (MR) and microbiological media (such as nutrient broth) were obtained from Hi-Media Laboratory, India. 2,6-dichlorophenol indophenol (DCIP) and NADH were purchased from Sisco Research Laboratory, India. All chemicals used were of the highest purity and were used as such. Millipore water was used for the pulse radiolysis, COD, TOC, HPLC, GC-MS, ESI-MS measurements.

2.14. Enzyme assay

The activity of laccase, tyrosinase, azoreductase and NADH-DCIP reductase was studied in the cell free extracts obtained from the test solutions.

2.14.1. Determination of laccase activity

Laccase activity was determined in a 2.0 mL reaction mixture containing 10% ABTS in 20 mM potassium phosphate buffer (pH 4.0). The reaction was started by adding 0.2 mL
of enzyme solution and the increase in the optical density was measured at 420 nm [64]. One unit of enzyme activity was defined as µM of ABTS oxidized min⁻¹ mL⁻¹ of enzyme. The molar extinction coefficient of ABTS is 34450 M⁻¹ cm⁻¹ at 420 nm.

2.14.2. Determination of tyrosinase activity

Tyrosinase activity was determined in a 3.0 mL reaction mixture containing 2.5 mL of sodium acetate buffer (20 mM, pH 4.0) and 100 µM of L-tyrosine. The reaction was started by adding 0.2 mL of enzyme solution and increase in absorbance was measured at 280 nm [65]. One unit of enzyme activity was defined as a change in absorbance units min⁻¹ mL⁻¹ of enzyme. Molar extinction coefficient of L-Tyrosine is 1420 M⁻¹ cm⁻¹ at 280 nm.

2.14.3. Determination of azoreductase activity

Azoreductase assay mixture (2.0 mL) contained 4.45 µM MR, 50 mM potassium phosphate buffer (pH 7.5) and 0.2 mL of enzyme solution. The reaction was started by adding 100 µM of NADH and then the absorbance was monitored at 430 nm. The molar extinction coefficient of MR is 2.34×10⁴ M⁻¹ cm⁻¹ at 430 nm [66]. One unit of enzyme activity was defined as µM of MR reduced min⁻¹ mL⁻¹ of enzyme.

2.14.4. Determination of NADH-DCIP activity

NADH-DCIP reductase assay mixture (5.0 mL) contained 25 µM DCIP, 50 mM potassium phosphate buffer (pH 7.5) and 0.2 mL of enzyme solution. The reaction was started by adding 100 µM of NADH into the assay mixture. The absorbance was measured at 595 nm. The molar extinction coefficient of DCIP is 1.9×10⁴ M⁻¹ cm⁻¹ at 595 nm [67].
One unit of NADH-DCIP reductase activity was defined as µM of DCIP reduced min\(^{-1}\) mL\(^{-1}\) of enzyme.

2.15. Method of sample preparation for the toxicity study of the treated dye solution on plants

The metabolites produced in the test solution were extracted from the supernatant by equal volume of ethyl acetate and the extract was dried over anhydrous Na\(_2\)SO\(_4\) and was finally evaporated to dryness in a rotary evaporator. The extracted products were dissolved in sterile distilled water to make a final concentration of 600 ppm. Toxicity study was carried out by watering the seeds with 10 mL of 600 ppm sample solution. The toxicity of the original dye solution was studied with 10 mL of 600 ppm dye solution in the same time interval.

2.16. Micro-organisms and culture conditions

The stock culture of Pseudomonas sp. SUK1 was maintained on nutrient agar slants which was stored in sealed tubes at 4 °C and sub-cultured monthly to maintain viability. The pure culture of Pseudomonas sp. SUK1 was grown for 24 h in 250 mL Erlenmeyer flask containing 100 mL nutrient broth having 10, 5, 2 and 1 g L\(^{-1}\) peptone, NaCl, yeast extract and beef extract, respectively, by using a static temperature controlled incubator at 30 ± 2 °C (which is the optimum growth temperature of Pseudomonas sp. SUK1).

2.17. Microbial treatment

The grown bacterial cells having optical density (OD) 1.0 at 620 nm were inoculated in 250 mL Erlenmeyer flasks containing 100 mL nutrient broth followed by incubation
with dye solution at 30 ± 2 °C under static condition up to 96 h as show in the Figure 2.5. Aliquots of 3 mL of the reaction medium were withdrawn at different time intervals and were centrifuged at 5000 rpm for 15 min. Then the absorbance of the supernatant liquid was measured at 535 nm, which is the characteristic wavelength of RR-120. The absorbance was measured in triplicate for each aliquot. The percentage decolouration at any time was calculated from the initial and final absorbances.

![Image of unirradiated and 1 kGy samples](image-url)

**Figure 2.5** Incubated dye solution at 30 ± 2 °C under static condition up to 96 h.

### 2.18. Preparation of the cell free extract

The grown cells of Pseudomonas sp. SUK1 were centrifuged at 10000 rpm for 20 min. These cells (75 g L⁻¹) were suspended in 50 mM potassium phosphate buffer (pH 7.4) and sonicated at 4 °C for 6.5 min. Then the homogenate was centrifuged and supernatant was used as a source of enzyme for control.