IN THE NAME OF
ALLAH
THE MOST BENEFICENT,
THE MOST MERCIFUL
Treatment of Industrial Wastewater by Advanced Oxidation Processes

By
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M. Phil. (UAF)

A thesis submitted for partial fulfillment of the requirements for the degree of
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CHEMISTRY

DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY
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PAKISTAN

2014
DECLARATION

I hereby declare that the contents of the thesis, "Treatment of Industrial Wastewater by Advanced Oxidation Processes" is product of my own research and no part has been copied from any published source (expect the references, standard mathematical and geometrical models/ equations/ formulate/ protocols etc.). I further declare that this work has not been submitted for the award of any other diploma/ degree. The university may take actions if the information provided is found inaccurate at any stage.

Munawar Iqbal
2005-ag-222
To

The Controller of Examination
University of Agriculture,
Faisalabad.

We, the Supervisory Committee, certify that the contents and form of the thesis submitted by Mr. Munawar Iqbal, Regd. No. 2005-ag-222, have been found satisfactory and recommend that it be processed for evaluation, by the External Examiner for the award of Ph.D. degree.

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To

My loving Parents
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ABSTRACT

The industrial wastewater is the leading source of water pollution due to diverse nature of pollutants present in the effluents. The goal of this work was to establish methods for the treatment of petroleum refinery, soap & detergent and pulp & paper effluents as well as simulated solution of nonylphenol ethoxylates (NPEOs) using gamma and UV radiation in the presence of H₂O₂ and TiO₂. The degradation of pollutants was monitored through UV/Vis, FTIR, GC-MS and HPLC techniques. The efficiency of advanced oxidation processes (AOPs) was evaluated on the basis of extent of degradation, water quality parameters and detoxification. The toxicities were determined through various bioassays such as allium cepa, haemolytic, shrimp and Ames tests. The independent variables such as radiation dose, catalysts concentration, exposure time, pH, temperature etc. were optimized using response surface methodology for maximum degradation of pollutant. Through advanced characterization techniques, it was found that the AOPs were able to oxidize recalcitrant, toxic and non-biodegradable compounds to various intermediates and eventually to inert end products by generating intermediacy hydroxyl radicals. The gamma ray/H₂O₂ treatment degrade the toxic residues, improve water quality and reduce the toxicity significantly, however, UV/TiO₂/H₂O₂ furnished better response. From the results, it is concluded that the AOPs (UV/TiO₂/H₂O₂ and gamma ray/H₂O₂) could be successfully used for the treatment of petroleum refinery, soap & detergent and pulp & paper effluents as well as NPEOs since the water quality fall within the permissible limits recommended by the environmental agencies.
Chapter # 1

INTRODUCTION

From last few decades, there is a growing concern of pollution due to its harmful effect on living organisms. The water bodies serve as temporary or final receptors of diverse variety of contaminants, which consequently are contaminating the watersheds and adjoining territories (Bianchi et al., 2011; Sponza, 2003). The rapid stride in industrial sector is a major concern and a severe threat to the survival of living organisms which is jeopardizing the ecological balance (Tabrez et al., 2011). As a result of exposure to contaminated water containing toxic agent, negative impact ranging from cytotoxicity to mutagenicity in various model (plants and animal) have been documented well (Grisolia et al., 2004; Hoshina and Marin-Morales, 2009; Leme and Marin-Morales, 2008b, 2009).

Among the damages caused by chemical agents to exposed organisms, genotoxic and mutagenic effects have shown to be worrying, which can lead to several health problems and also can affect future generations due to inheritable alterations in genetic material (Leme and Marin-Morales, 2008b, 2009). Genetic toxicology involved in detecting compounds capable of causing genetic damage with the aim of understanding potential biological penalty. Toxicants screening in environmental samples using method in vitro are reliable to measure the extant of pollution load and this biological assessment is compulsory for effluent monitoring, discharged from industries before being mixed with water bodies (Margot et al., 2013; Rodrigues et al., 2010).

The major threat is chemical pollution from industrial sector that enter into the aquatic medium in several different ways, either dumped directly, such as industrial effluents, or from wastewater treatment plants that do not fulfill their obligations. Due to universal solubility of water, the substances can be transported and distributed more easily in the water cycle (Bai et al., 2012; Malmqvist and Rundle, 2002; Oller et al., 2011a) and the persistent substances are being detected at long distances from their sources of discharge (Meyer and Wania, 2007; Prevedouros et al., 2006; Shakir et al., 2012).

At present, the safe disposal of industrial wastewater is one of the major ecological challenges. Therefore, environmental degradation has now become a global problem and
maintaining the ecosystem health is a serious issue being confronted by the environmentalist. Due to lack of effluent treatment facilities and proper disposal system of wastewater, water bodies are getting polluted day by day and are responsible for very adverse effects on soil, aquatic life, agriculture as well as all types of flora and fauna in biosphere because of toxic and persistence nature of pollutants. Disposal of industrial effluents into fresh water bodies disturbs water quality parameters (BOD, COD, DO, color and pH), which are necessary to sustain aquatic life, primary productivity and food chain (Auriol et al., 2006; Rao et al., 2001). If the safety of wastewater discharged from industries is assured by industrialists or by pollution control boards, then treated industrial wastewater may be potentially used for fish production, irrigation for non-edible cash crop, aquaculture and for many other such types of multifarious uses (Molinos-Senante et al., 2011; Singh and Singh, 2006; Wong, 2003).

In this regard, there are several physicochemical and biological methods being in practice for the treatment of industrial wastewater (Chan et al., 2009; Chandra et al., 2011; Fu and Wang, 2011; Judd and Judd, 2011; Mahmoodi et al., 2011; Muneer et al., 2012; Oller et al., 2011a; Schwarzenbeck et al., 2005; Thompson et al., 2001). Among the physical wastewater treatment methods, radiation treatment has been emerged as very effective technology for the degradation and mineralization of pollutants, which can be employed as; O₃, O₃-UV, H₂O₂-UV, O₃-H₂O₂, TiO₂-UV, Fe²⁺-H₂O₂, gamma and electron beam irradiation which are named as advanced oxidation processes (Comninellis et al., 2008; Han et al., 2002; Muneer et al., 2012).

1.1 Advanced oxidation process (AOP)

First of all the concept of “advanced oxidation processes” was established by Glaze (Glaze et al., 1987). The ultimate aim of oxidation of pollutants in water and wastewaters is to “mineralize” that is to convert the constituents of an organic pollutant into simple, relatively harmless and inorganic molecules. The major conversions could be visualized as being carbon to carbon dioxide, hydrogen to water, phosphorous to phosphates or phosphoric acids, sulfur to sulfate, nitrogen to nitrates, and halogens to halogen acids. Oxidation can be generalized as for inorganic compounds to the removal of electrons to produce higher oxidation state and for organic compounds to the production of oxygenated compounds (Belgiorno et al., 2011; Martinez et al., 2003). To achieve this goal, oxidants i.e. compound
or species that are electron donors are utilized. The main aim of AOP is to produce hydroxyl radicals (HO’) which degrade the organic compound efficiently. Some other characteristic features of the HO’ are as follows; very powerful oxidant, highly reactive, short lived and relatively non selective electrophilic oxidizing agent. Moreover, under natural water conditions HO’ is very easy to produce, therefore, ubiquitous in nature having very high oxidation potential (Table 1.1) in comparison to various other oxidant. The HO’ has the second highest thermodynamic oxidation potential following fluorine (Belgiorno et al., 2011; Rodríguez, 2002).

Table 1.1: Comparison of the various oxidant thermodynamic oxidation potential (Rizzo et al., 2009).

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>F₂</th>
<th>OH</th>
<th>O</th>
<th>O₃</th>
<th>HO’₂</th>
<th>H₂O₂</th>
<th>HOCl</th>
<th>Cl₂</th>
</tr>
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<td>Redox potential (V)</td>
<td>3.03</td>
<td>2.8</td>
<td>2.42</td>
<td>2.07</td>
<td>1.7</td>
<td>1.78</td>
<td>1.49</td>
<td>1.36</td>
</tr>
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The HO’ can be generated by photochemical and non photochemical processes e.g., UV radiation in combination with O₃, H₂O₂ or a photo–sensitizer natural organic matter and non photochemical processes such as electron beam irradiation, O₃, in combination with H₂O₂ or Fenton’s reagent are used. The AOPs can be broadly divided into the following groups: (1) UV/oxidation processes (2), Fenton and photo-Fenton process, (3) sensitized AOPs, photo–catalysis and (4) sonolysis. Moreover, radiation induced processes, wet air oxidation, supercritical water oxidation and electrochemical processes could also be counted as AOPs for water and wastewater treatment (Naddeo et al., 2011; Rizzo et al., 2009). On the basis of techniques through which OH⁺ are produced, they can be divided into two categories, 1) photochemical and 2) non-photochemical process (Table 1.2) (Mota et al., 2009).
Table 1.2: Classification of advanced oxidation processes

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<th>Non-photochemical processes</th>
<th>Photochemical processes</th>
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</tr>
<tr>
<td>O₃/H₂O₂</td>
<td>Water photolysis in vacuum UV</td>
</tr>
<tr>
<td>Fe²⁺/H₂O₂</td>
<td>UV/H₂O₂</td>
</tr>
<tr>
<td>Electrochemical oxidation</td>
<td>UV/O₃</td>
</tr>
<tr>
<td>Gamma</td>
<td>UV/O₃/H₂O₂</td>
</tr>
<tr>
<td>E-beam</td>
<td>Photo-Fenton</td>
</tr>
<tr>
<td>Non-thermal plasma</td>
<td></td>
</tr>
<tr>
<td>Electrohydraulic</td>
<td></td>
</tr>
<tr>
<td>Ultrasound</td>
<td></td>
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<tr>
<td><strong>Heterogeneous photo-catalysis</strong></td>
<td></td>
</tr>
<tr>
<td>Catalytic wet air oxidation</td>
<td>ZnO/UV, SnO₂/UV, TiO₂/UV, TiO₂/H₂O₂/UV</td>
</tr>
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</table>

The AOPs have been employed for wastewater treatment; especially for the degradation of organic compounds and mineralization. During AOPs treatment a strong oxidizing species like OH⁻ produced in situ, which break down the complex organic molecule into less harmful smaller substances such as CO₂, H₂O and inorganic ions through a chain reactions (Rauf and Ashraf, 2009a, b; Rauf et al., 2008). These processes are easy to handle, produces significantly less residual effects as compared to classical treatment approaches and are being employed for color removal, mineralization of toxic chemicals and industrial wastewater treatment (Alvares et al., 2001; Uygur, 2001; Uygur and Kargı, 2004).

Currently, the trend in the development of new innovative technologies is focused on those processes that eventually cause destruction of the organic contaminants rather than transferring into another form. The application of high energy radiation has been accepted efficient in this regard (Cooper et al., 2002; Kurucz et al., 2002; Spinks and Woods, 1990). The radiation induced degradation of wastewater by gamma radiation, electron beam and UV radiation is drawing more attention around the world. The treatment of wastewater effluents by radiation has advantages such as it degrade the non biodegradable organic systems, do not cause secondary pollution, are cost effective and eco-friendly (Hu and Wang, 2007; Melo et al., 2008; Muneer et al., 2012).
Radiolytic techniques have been found to be convenient in elucidating radical reaction mechanisms. The production of $e^-_{aq}$, H$^*$ and OH$^*$ upon irradiation in water makes it an effective process for the removal of pollutants. The initial radiolysis products are formed in isolated volume elements called “spurs”. Only few eV per molecule energy is required in bringing about the chemical changes. The molecular and radical production during radiolysis is determined by the primary yield or “G” value. The most reactive species are the oxidizing OH$^*$, the reducing species such as $e^-_{aq}$ and H$^*$ also interact with pollutants present in wastewater (Basfar et al., 2005; Cooper et al., 2002; Shukla et al., 2010; Xue and Wang, 2008). Different studies have shown that the degradation of biologically resistant pollutant as well as the killing of pathogenic micro-organisms and sewage sludge management can be achieved by using ionizing radiation. The degradation of pollutants depends on different factors, such as types of radiation, absorbed dose, dose rate, pollutant concentration, oxidant concentration and synergistic effects of radiation (Getoff, 2002; Han et al., 2002; Shin et al., 2002).

The OH$^*$ radical produced as a result of AOPs attack target molecule in different ways such as hydrogen abstraction (Eq.1.1), electrophilic addition (Eq. 1.2) and electron transfer (Eq. 1.3). This technique has also several advantages over the other treatments such as no sludge formation, operative at ambient temperature, oxygen formed during process and the final products are H$_2$O, CO$_2$ and low molecular weight aliphatic carboxylic acids etc. (Behnajady et al., 2006; Muneer et al., 2012; Sánchez-Polo et al., 2002).

\[
\begin{align*}
R-CH_3 + OH^* & \rightarrow R-CH_2^* + H_2O & (1.1) \\
CH_2=CH_2 + OH^* & \rightarrow HOCH_2CH_2^* & (1.2) \\
Y + OH^* & \rightarrow Y'' + OH^- & (1.3)
\end{align*}
\]

1.2 Chemistry of radiation induced AOPs

Photolysis is defined as the use of radiant energy without presence of any catalyst to irradiate a polluted aqueous solution, thereby to produce a chemical change, whereas photochemical reaction is a chemical change initiated by light or other electromagnetic radiation. The primary step in photolysis is the electronic excitation of the substrate (Eq. 1.4),
followed by the electron transfer from the excited state (Eq. 1.5) to ground state molecular oxygen (O2) to form O2•- (Belgiorno et al., 2011; Naddeo et al., 2011).

\[
S + hv \rightarrow S^* \quad (1.4) \\
S^* + O_2 \rightarrow S^{*+} + O_2^{-} \quad (1.5)
\]

Through hemolytic scission, free radicals generate and further reaction of these species with dissolved oxygen generates peroxide radicals. The formation of reactive oxygen species and radicals lead to the degradation of the targeted compound (Naddeo et al., 2009).

### 1.2.1 UV Photolysis mechanism

Generation of HO' by UV photolysis from H2O2, can be described by the following equations (Eq. 1.7-1.9) (Belgiorno et al., 2011).

\[
\begin{align*}
H_2O_2 + \lambda (254 \text{ nm}) & \rightarrow 2HO' \quad (1.6) \\
H_2O_2 + HO' & \rightarrow HO_2' + H_2O \quad (1.7) \\
H_2O_2 + HO_2' & \rightarrow HO' + H_2O + O_2 \quad (1.8) \\
HO_2' + HO_2' & \rightarrow H_2O_2 + O_2 \quad (1.9)
\end{align*}
\]

Low-pressure mercury UV lamps with a 254 nm peak emission are typically used to produce UV radiation. If low pressure mercury vapor lamps are used, a high concentration of H2O2 is needed in the medium to generate sufficient HO' because of the low molar absorption coefficient. However, high concentrations of H2O2 may scavenge the HO' make the process less effective (Naddeo et al., 2011), So, for effective treatment, there is a need to optimize the conditions.

### 1.2.2 Radiolysis of water

When pure water or aqueous solutions are irradiated by gamma radiation, a number of chemical species are produced (Eq. 1.10 and scheme 1.1). The reactive primary species produced per 100 eV absorbed energy are HO', hydrated eaq− and H' species. The participation of H2O2 and H2 might be neglected in the decomposition reaction of the water pollutants because of their low reactivity and yields (Chu et al., 2011).
Radiation process such as electron beam irradiation and gamma irradiation are considered as a promising for the treatment of wastewater or sludge. This technology is regarded as a promising alternative due to its high efficiency in pathogen inactivation, organic pollutants oxidation and odor nuisance elimination, which will facilitate the downstream process of sludge treatment and disposal (Wang, 2007). However, this promising technology has attracted little interest until now and has not yet been widely accepted mainly due to the conservative attitude of governments regarding safety concerns resulting from little knowledge on this technology (Chu et al., 2011; Rizzo et al., 2009).

**Scheme 1.1:** Showing the formation of gamma radiation induced product form water (Spinks and Woods, 1990)

### 1.2.3 Chemistry of hydrogen peroxide

The direct photolysis of hydrogen peroxide leads to the formation of HO• radicals (Eq. 1.11) (Gottschalk et al., 2000) and HO2− which is in acid–base equilibrium with H2O2, absorbs the UV radiation of the wavelength 254 nm (Eq. 1.12 and 1.13).

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{hv} & \rightarrow 2\text{HO}^\bullet & (1.11) \\
\text{H}_2\text{O}_2 & \leftrightarrow \text{HO}_2^\bullet + \text{H}^+ & (1.12) \\
\text{HO}_2^\bullet + \text{hv} & \rightarrow \text{HO}^\bullet + \text{O}^- & (1.13)
\end{align*}
\]

The H2O2/UV process has been successfully used for the destruction of organic compounds to carbon dioxide within reasonable irradiation times and can be used for water
treatment (Naddeo et al., 2011; Oppenländer, 2003). However, more pilot and field scale demonstration sites under a variety of water quality matrices are needed prior to generalize the regulatory acceptance (Naddeo et al., 2011).

1.2.4 Chemistry of titanium dioxide assisted AOPs

The photo–excitation of TiO$_2$ particles promotes an electron from the valence band to the conduction band, generating an electron/hole pair. Both reductive and oxidative processes can occur at or near the surface of the photo–excited TiO$_2$ particle (Gomes de Moraes et al., 2000). In general, O$_2$ is used to scavenge the conduction band electron, producing a superoxide anion radical, effectively preventing electron/hole recombination, and prolonging the lifetime of the hole. The photo–generated hole has the potential to oxidize several substrates by electron transfer. In aqueous solutions, oxidation of water to hydroxyl radical by the photo–generated hole appears to be the predominant pathway. Hydroxyl radicals and, to a lesser extent, superoxide anion can act as oxidants, ultimately leading to the mineralization of organic compounds (Pérez et al., 2001). In aerated aqueous suspensions, the photo–generated electrons promote the reduction of dissolved oxygen. Whereas the photo–generated holes oxidize adsorbed water molecules to give hydroxyl radicals and overall, reaction takes place are shown in equations (Eq. 1.14 – 1.19).

\[
\begin{align*}
\text{TiO}_2 + h\nu \text{ (UV)} & \rightarrow \text{TiO}_2 (e^- , h^+) \quad (1.14) \\
\text{TiO}_2 (h^+) + \text{H}_2\text{O}_{\text{ads}} & \rightarrow \text{TiO}_2 + \text{H}^+ + \text{HO}^-_{\text{ads}} \quad (1.15) \\
\text{TiO}_2 (h^+) + \text{HO}^-_{\text{ads}} & \rightarrow \text{TiO}_2 + \text{HO}^+_{\text{ads}} \quad (1.16) \\
\text{TiO}_2 (e^-) + \text{O}_2 & \rightarrow \text{TiO}_2 + \text{O}_2^- \quad (1.17) \\
\text{HO}^+_{\text{ads}} + \text{R} & \rightarrow \text{Degradation} \quad (1.18) \\
h^+ + \text{R}^+ + \text{R} & \rightarrow \text{Oxidation} \quad (1.19)
\end{align*}
\]

Literature survey indicates that the first step of the photo–catalytic degradation corresponds to the formation of hydroxylated derivatives. The product distribution may be modified when a HO$^+$ scavenger is added to the solution. This suggests that two types of oxidizing species are involved in the photo–catalytic process: HO$^+$ generated by oxidation of
adsorbed water molecules (Eq. 1.15) and positive holes. The efficiency of the degradation depends on the oxygen concentration since O₂ scavenges the conduction band electron, preventing (e⁻, h⁺) recombination.

The O₂⁻ formed from reaction (Eq. 1.17) can lead after several steps, to the generation of H₂O₂ as shown in equations (1.20 – 1.21) (Brillas et al., 1998). The formation of H₂O₂ in equation 1.22 lead to the formation of HO' (Eq. 1.11, Fig. 1.2).

\[
\begin{align*}
O_2^- + H^+ + 2e^- & \rightarrow \text{HO}'_2 \\
\text{HO}'_2 + e^- & \rightarrow \text{HO}^-_2 \\
\text{HO}^-_2 + H^+ & \rightarrow \text{H}_2\text{O}_2
\end{align*}
\] (1.20)

Fig. 1.2: Action mechanism of titanium dioxide showing e⁻ and h⁺ pair (Brillas et al., 1998).

### 1.3 Advantages of AOPs versus conventional wastewater treatment methods

The AOPs efficiency depend on the generation of OH⁻ to facilitate the oxidation of organic compounds. Thus, their applications in water and wastewater treatment should be very similar, i.e., to detoxify and degrade the toxic and persistent compounds. From laboratory testing and very limited industrial applications, it is believed that AOPs offer several distinct advantages over conventional treatment processes because (i) they are very effective to remove biologically resistant organic compounds (Naddeo et al., 2011), (ii) they are capable of complete mineralization of organic contaminant into carbon dioxide and water (Muneer et al., 2012), (iii) they are less susceptible to the presence of toxic chemicals (Belgiorno et al., 2011), and (iv) they produce less harmful by-products (Sanches et al.,
Since OH* is very unstable in water, therefore, the use of AOPs can lower the effective disinfectant concentration and offer benefits for microbial disinfection also. Along with biological oxidation, for example, AOPs can be used as a pretreatment process for the partial oxidation of organic compounds that are too toxic to biodegrade. Despite these similarities, different AOPs require different radical initiators to generate OH*. Because of this difference, the cost to generate OH* varies greatly for different AOPs (Al-Kdasi et al., 2004; Alaton et al., 2002; Gogate and Pandit, 2004; Petrović et al., 2003; Rizzo et al., 2009; Zhou and Smith, 2002). At present, the AOPs can be divided into established and emerging technologies for wastewater treatment. Emerging technologies are defined as technologies that have very limited on full-scale applications for water treatment (Table 1.2).

During the past three decades that UV–based technologies emerged as an important class of treatment processes for water treatment. They include advanced oxidation in the homogeneous and heterogeneous phase (UV oxidation, vacuum-UV (VUV) oxidation, photo–catalysis) (Oppenländer, 2003).

1.4 Effect of AOPs on toxicity reduction of wastewater

The AOPs have been widely used in water and wastewater treatment for the removal of organic and inorganic contaminants as well as to improve biodegradability of industrial wastewater. In the recent past, different AOPs have been investigated for the removal of emerging contaminants from urban wastewater effluents (Naddeo et al., 2011) and drinking
water (Sanches et al., 2010). Unfortunately, the partial oxidation of organic contaminants may result in the formation of more toxic intermediates than parent compounds. In order to avoid this drawback, AOPs are expected to be carefully operated and monitored, and toxicity tests should be used to evaluate toxicity of effluent (Rizzo et al., 2009). The water quality parameters such as pH, DO, BOD, COD, TDS, and TSS are generally used for quality evaluation of wastewater. These quality parameters can not be used for the evaluation of toxic effect on receiving waters. The best way to evaluate effluent toxicity effect is to use bioassay tests (Leme and Marin-Morales, 2009; Movahedian et al., 2005; Tchobanoglous et al., 2003). Different organisms such as culture cells, fish, algae, bacteria, shrimp, higher plant and other microorganisms may be used in this regard. It is reported that the effluent meets all physicochemical requirements, regarding its toxicity, it may cause considerable negative effects on living organisms (Leme and Marin-Morales, 2009; Movahedian et al., 2005; Oliveira-Martins and Grisolia, 2009; Radix et al., 2000). In this regard, few attempts have been carried out to study the toxicity of industrial wastewater after AOPs applications (Andreozzi et al., 2004; Gomes de Moraes et al., 2000; Mahmoodi and Arami, 2009; Pérez et al., 2006; Selcuk, 2005; Tezcanli-Guyer and Ince, 2003). Although AOPs display different reacting systems, they are almost all characterized by the OH* via a nonselective oxidation pathway. Therefore, AOPs are considered as attractive alternatives to non-destructive physical wastewater treatment processes and the state of knowledge of AOPs is still incomplete regarding the complex and interrelated photo, physical, chemical, and radical mechanisms. So, in view of importance of AOPs, the present project was designed to meet the following aims and objectives.

1. Determination of water quality parameter before and after application of AOPs.
2. Comparison of AOPs with conventional wastewater treatment methods and international water quality parameters.
3. To evaluate the biological efficiency (toxicity) before and after application of AOPs on wastewater as well as model system.
4. To determine the radiolytic end products.
Chapter #2

REVIEW OF LITERATURE

1.5 Nature of pulp and paper mill wastewater (PPMWW)

The pulp and paper industry is one of the most important industries in any country and accounts for a major portion of the country’s economy in terms of value of production and total wages paid. Urban industrial activity has long been identified as a major source of contaminants for aquatic environments, via wastewater discharge (Ahmad et al., 2008). The pulp and paper industry is the sixth largest polluter (after oil, cement, leather, textile, and steel industries) discharging a variety of gaseous, liquid, and solid wastes into the environment (Calvo et al., 2007). Potential pollutants from a pulp and paper mill are effluents, particulates and solid wastes responsible for pollution of watersheds (Ali and Sreekrishnan, 2001). However, the effluents are major concern because large volumes of wastewater are generated for each metric ton of paper produced, depending upon the nature of the raw material, finished product and extent of water reuse (Pokhrel and Viraraghavan, 2004a). Since the pulp produced corresponds to only approximately 40–45% of the original weight of the wood, the effluents are heavily loaded with organic matter. These effluents cause considerable damage to the receiving waters if discharged untreated since they have a high BOD, COD, (suspended solid) SS, chlorinated compounds, fatty acids, tannins, resin acids, lignin and its derivatives, sulfur and sulfur compounds, etc (Pokhrel and Viraraghavan, 2004a). Some of these pollutants are naturally occurring wood extractives (tannins, resin acids, stilbenes, lignin), while others are xenobiotic compounds that are formed during the process of pulping and paper making (chlorinated lignins, resin acids and phenols, dioxins, furans) (Peck and Daley, 1994; Pokhrel and Viraraghavan, 2004a). Some of the pollutants such as polychlorinated dibenzodioxins and dibenzofurans (dioxins and furans), are recalcitrant to degradation and tend to persist in nature. They are thus known as persistent organic pollutants and have been classified as ‘priority pollutants’ by the US Environmental Protection Agency (USEPA) (Sun et al., 1998), Canadian Environmental Protection Agency (CANEPA) (Armstrong and Newhook, 1992) and United Nation Environmental Program (UNEP) (Heywood, 1995).
1.6 PPMWW Sources

Each pulping process utilizes large amounts of water, which reappear in the form of an effluent. The most significant sources of pollution among various stages of process are wood preparation, pulping, pulp washing, screening, washing, bleaching, and paper machine and coating operations (Biermann, 1996; Rodrigues et al., 2008). Among the processes, pulping generates a high-strength wastewater especially by chemical pulping. Pulp bleaching generates most toxic substances as it utilizes chlorine for brightening the pulp (Pokhrel and Viraraghavan, 2004a). Pulp fibers can be prepared from a vast majority of plants in nature such as woods, straws and grasses, bamboos, or canes and reeds. Wood is the most abundant source of paper making fiber. Wood consists of various compounds (lignin, carbohydrate, and extractives) which are hard to biodegrade, and these derivatives are isolated from the fibers by washing, dewatering, and screening processes which generate a huge amount of wastewater (Ali and Sreekrishnan, 2001; Pokhrel and Viraraghavan, 2004a).

1.7 Fate and effects on the environment of PPMWW

The pollutants discharged from the pulp and paper industry affect all aspects of the environment such as water, air and land (Owens, 2009). Various authors reported the fate and effect of pollutant on environment such as resin acid (Makris and Banerjee, 2002), wood extractives (Kostamo et al., 2004; Leiviskä et al., 2009), organic halides (Bryant et al., 1987), polychlorinated dibenzodioxins and polychlorinated dibenzofurans (Amendola et al., 1989) and alkylphenol ethoxylate surfactants (Hawrelak et al., 1999). The toxic effect on PPMWW has also been well documented such as on fish species (respiratory stress, mixed function oxygenase activity, toxicity and mutagenicity, liver damage, or genotoxic effects, and lethal effects) (Lindström-Seppä et al., 1998; Pokhrel and Viraraghavan, 2004a; Schnell et al., 2000), Mn accumulation in the Crayfish (King et al., 1999) and toxicity to rainbow (O'connor et al., 2000). A significant change in surface plankton population in Elengabeel's wetland ecosystem in India due to untreated paper mill effluent discharge into the system has been reported (Baruah and Das, 1997). The sub-lethal effects to the aquatic organisms in the Dong Nai River in Vietnam due to PPMWW has also been highlighted (Yen et al., 1996). However, there are also some contradictory reports available which states that neither significant evidence of depressed plasma steroids nor increase in mixed function oxygenase
activity in fish occur (Kovacs et al., 2002), no significant adverse effect in sediments, and river biota or on fish attributable to the treated mill effluent (D'surney et al., 2000) and no clear evidence of mutagens in most of aquatic animals (Stepanova et al., 2000) and no effect on the tree shallow appeared, which feed on the insects downstream of the pulp mill (Wayland et al., 1998). Some authors also reported the effect of PPMWW on soil and vegetation such as a serious change in soil chemistry change (Howe and Wagner, 1999), paddy field damage (Dutta, 1999), trace metals, high loads of organic pollutants, coliform bacteria (Gupta, 1997; Singh et al., 1996) and trace metals (Skipperud et al., 1998) as a result of PPMWW irrigation. While studying the impact of PPMWW on human, diarrhea, vomiting, headaches, nausea and eye irritation in children and workers has also been reported (Mandal, 1996).

1.8 Toxic potential of PPMWW

It is well established that many of PPMWW contaminants are acute or even chronic toxins. Chlorinated organic compounds, which include dioxins and furans, have the ability to induce genetic changes in exposed organisms. In particular, DNA-damaging agents have been shown to induce inherited genetic defects and cancer (Ali and Sreekrishnan, 2001), with dioxins being named as ‘known human carcinogens’ by the WHO (McGregor et al., 1998). This has resulted in a growing concern about the potential adverse effects of mutagens on aquatic biota and public health through the contamination of drinking water supplies, recreational waters, or edible organic species. It has been noticed that the toxicity is not restricted to the aquatic ecosystem alone; rather, some of the residual toxicity from PPMWW also ultimately makes an appearance in paper products such as coffee filters, paper cups, plates and facial tissues (Ali and Sreekrishnan, 2001; Freire et al., 2001; Nielsen and Rank, 1994b; Pokhrel and Viraraghavan, 2004a). Various authors reported the toxicity of PPMWW with reference to the aquatic life, higher plant and microorganisms (Ali and Sreekrishnan, 2001; Baruah and Das, 1997; Calvo et al., 2007; Chaparro et al., 2010; Chen et al., 2001; D'surney et al., 2000; Dutta, 1999; Freire et al., 2001; Gana et al., 2008b; Gupta, 1997; Härdig et al., 1988; Howe and Wagner, 1999; King et al., 1999; Kovacs et al., 2002; Landman et al., 2006; Lindström-Seppä et al., 1998; Owens, 2009; Pokhrel and
Viraraghavan, 2004a; Roa et al., 2012; Sponza, 2003; Stepanova et al., 2000; Ukagwu et al., 2012).

The PPMWW affect the water bodies adversely and the presence of toxic polluting agent in the wastewater has been already reported (Pokhrel and Viraraghavan, 2004b). In view of toxic nature of pulp and paper mill wastewater, various authors evaluated the toxicity by *A. cepa* test system and positive results have been reported. The micronuclei (MN) and anaphase aberration were recorded in *A. cepa* roots when exposed to PPMWW (Grover and Kaur, 1999). In another study, *A. cepa* root tip cells were analyzed for CA’s after 24 h of exposure to PPMWW and results showed a considerable induction of chromosomal aberrations (CA’s) and genotoxicity (Nielsen and Rank, 1994a) and Kraft mill effluent also showed signs of toxicity in *A. cepa* (Tipirdamaz et al., 2003). Other than the root inhibition (55.72%), the CA’s abnormalities were also observed which include C-mitosis, chromosome fragment, laggard chromosome, chromosome stickiness and bridges in root tip cells. The frequency of C-mitosis observed up to 47.7% in root tip cells treated with paper mill (Kraft) effluent, while chromosomal fragment and bridge were recorded up to 26.6% and 33.3%, respectively. Moreover, the chromosome stickiness were found to be 12.2%. The banded chromosome was observed at the frequency of 13.3% of aberrant cells. The genotoxicity of bleach plant effluents (paper mill) by applying the CA, MN and MI in *A. cepa* root cells has also been evaluated. The largest CA and MN incidence in the MC of *A. cepa* were recorded after exposure to the untreated bleach plant effluent, however, anaerobically biodegraded sample showed lower CA’s and MN formation (Chaparro et al., 2010). In another study, the genotoxic effects on *A. cepa* roots exposed to PPMWW have also been reported (Gana et al., 2008a). Recently, the cytotoxicity and genotoxicity tests were performed on root cells of *A. cepa* in order to evaluate wastewater quality of cellulose bleaching process (Roa et al., 2012). The results revealed a toxic effect of the effluent such as inhibition of meristem cell growth and generally lower values of metaphase, anaphase and telophase.

There are few studies on the evaluation of the hazards of PPMWW on bacterial count; however no study is available highlighting the effect in vitro on RBC using haemolytic bioassay. While studying the haematological changes in juvenile catfish exposed to PPMWW under field condition, toxic effect has been revealed (Ukagwu et al., 2012). The haematological investigation revealed that fish exposed to effluent discharge site had lower
haemoglobin count, red blood cell count, packed cell volume and higher concentrations of mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and white blood cell indicating that test fish suffered haemolytic anaemia and leucocytosis. These results indicate a toxic nature of effluents of PPMWW on the haematological functions of fish. In another study, (McLeay, 1973) also reported the RBC counts and hematocrts of juvenile *coho salmon* which were found to unaltered after 12 h exposure to neutralized kraft pulp mill effluent, although hematocrts were decreased by exposure for 25 days. The number of circulating immature erythrocytes increased in effluent exposed fish in both the 12 h and 25 day exposures. The heamotological studies in *fourhorn sculpin* as a result of PPMWW exposure were also performed by (Härdig et al., 1988). Author exposed organism for 5–9 months to WW and found that bleached kraft mill effluent affected the hematology. Decreased values for hematocrit and hemoglobin and a reduced RBC count as well as increased levels of methemoglobin, indicated disturbances in the RBC status were observed. In another study, the RBC counts were increased slightly at 12 and 48 h exposure in response of PPMWW (McLeay, 1975). Author reported that the mean RBC counts were decreased over 24–96 h, but differences were significant at 24 h only. While studying the hematology of juvenile *rainbow* trout exposed to PPMWW, (Landman et al., 2006) reported positive results when trout were exposed for 4 weeks to 10%, 30%, and 70% effluents.

Brine shrimp (*Artemia salina* L.) is considered a standard organism in toxicological assays, despite the recognition that is too robust an organism to be a sensitive indicator species. In pollution research, the brine shrimp has had extensive use as a test organism and in some circumstances is an acceptable alternative to the toxicity testing of mammals in the laboratory (Krishnaraju et al., 2005). The fact that millions of brine shrimp are so easily raise up and helpful in assessing the effects of a large number of environmental pollutants on the shrimps under well controlled experimental conditions (Carballo et al., 2002; Harwig and Scott, 1971). To best of our knowledge, there is lack of literature regarding the toxicity of PPMWW on brine shrimp. However, some studies highlighted the toxicity of PPMWW to other aquatic organisms are available. e. g., while studying the toxicity of secondary effluent from sewage treatment plant, raw sewage water sample and effluents samples from secondary treatment, ultra–filtration treatment, micro–filtration treatment, activated carbon
column adsorption, as well as reverse osmosis treatment from PPMWW, (Zha and Wang, 2005) reported the toxicity to embryo of *Japanese medaka* (*Oryzias latipes*) and concluded that the pulp and paper mill wastewater was toxic. Another study also highlighted the toxicity of PPMWW toxicity to the *Japanese medaka* and in comparison to other industrial wastewater, the PPMWW was found to more toxic (Chen *et al.*, 2001).

**1.9 Nature of petroleum effluents**

Petroleum refinery effluents (PRE) are wastes originating from industries primarily engaged in refining of crude oil and manufacturing fuels, lubricants and petrochemical intermediates and these effluents are a major source of aquatic environmental pollution (Wake, 2005). The process of refining the crude oil consumes large amounts of water. Consequently, significant volumes of wastewater are generated (Diya'uddeen *et al.*, 2011). It is reported that the volume of PRE generated during processing is 0.4–1.6 times the amount of the crude oil processed (Coelho *et al.*, 2006). Thus, based on the current yield of 84 million barrels per day (mbpd) of crude oil, a total of 33.6 mbpd of effluent is generated globally (Doggett and Rascoe, 2010). World oil demand is expected to rise to 107 mbpd over the next two decades, and oil will account for 32% of the world's energy supply by 2030. This situation clearly indicate that effluents from the oil industry will continually be produced and discharged into the world's main watersheds significantly in higher amounts (Diya'uddeen *et al.*, 2011).

The PRE are priority pollutants due to their high polycyclic aromatics contents, which are toxic and tend to be more persistent in the environment (Mrayyan and Battikhi, 2005; Wake, 2005). Other than oil and grease, the PRE also contain phenolic compounds (Kavitha and Palanivelu, 2004; Kušić *et al.*, 2006), nitrogen and sulphur components (Altaş and Büyükgüngör, 2008; Zhang *et al.*, 2009) and bisulphide (HS⁻) and sulphide (S²⁻) (Doggett and Rascoe, 2010; Poulton *et al.*, 2002).

**1.10 Petroleum wastewater sources**

There are numerous possible refinery configurations, and each is designed to achieve the specific target of transforming crude oil into useful products such as dual-purpose kerosene, gasoline and petrochemical feed stock (Diya'uddeen *et al.*, 2011). Based on WW generation refineries are classified into either a hydro-skimming unit comprised of three sub-
units (a crude distillation unit, which fractionates crude oil into various components, a desulphurising unit, which reduces the sulphur content of some fractions such as kerosene and naphtha and a reforming unit for producing reformate) or a complex unit, which incorporates an additional catalytic cracking unit into the hydro-skimming refinery (Al Zarooni and Elshorbagy, 2006). The final waste stream generated is the contribution of the units involved with crude oil processing, e.g., hydro-skimming, hydro-skimmer flare, hydro-cracking, hydro-cracker flare, sour water, condensate, condensate flare and the desalter. Other units do not directly involve in processing, e.g., sanitary, crude tank and laboratory water, also contribute significantly to the total volume of the effluent (Al Zarooni and Elshorbagy, 2006; Diya'uddeen et al., 2011).

1.11 Fate and effects on the environment of petroleum effluents

PRE are priority pollutants due to their high polycyclic aromatics contents, which are toxic and tend to be more persistent in the environment (Mrayyan and Battikhi, 2005; Wake, 2005). They encompass a wide range of contaminants at varied concentrations that are generally harmful. Decreased productivity of algae (a very important link in the food chain) observed for PRE-receiving water bodies (El-Naas et al., 2010a, b; Pardeshi and Patil, 2008). The minimum amount of DO necessary for normal life in an aquatic environment is about 2 mg/L, and the discharge of high organic matter containing waste waters into watersheds results in the excess consumption of oxygen by the bacteria. This is in an attempt to oxidize the effluent, thus depleting oxygen from the water faster than it dissolves back into the water from the air (Attiogbe et al., 2009). This problem leads to the inadequate maintenance of higher life forms. In addition, oxygen availability is important because the end products of chemical and biochemical reactions in anaerobic systems often produce aesthetically displeasing color, taste and odour in water (Attiogbe et al., 2009; Diya'uddeen et al., 2011). The oil and grease content are sticky in nature; they tend to aggregate, clogging drain pipes and sewer lines, causing unpleasant odour and corroding sewer lines under anaerobic conditions. They also interfere with unit operations in municipal wastewater treatment plants because they float as a layer on top of the water. They also stick onto pipes and walls consequently blocking strainers and filters (Xu and Zhu, 2004).
Phenolic compounds pose a significant threat to the environment due to their extreme toxicity (Kavitha and Palanivelu, 2004), stability, bioaccumulation and ability to remain in the environment for longer periods. They generally are carcinogenic, causing considerable damage and threaten the eco-system in water bodies (Abdelwahab et al., 2009; Lathasree et al., 2004; Pardeshi and Patil, 2008; Yang et al., 2008). The nitrogen and sulphur components of the effluent are highly toxic and are represented in the form of ammonia and hydrogen sulphide, respectively (Altaş and Büyükgüngör, 2008). Sulphide has a high oxygen demand of 2 mol O$_2$/L mol S$^{2-}$ thus contributing significantly to oxygen depletion (Poulton et al., 2002). This results in mass fish mortality when the threshold limit exceeds 0.5 mg/L for freshwater or saltwater fish (Diya'uddeen et al., 2011). Various authors also reported the toxic effect of PRE on aquatic life, higher plant and microorganisms (Campagna and da Motta, 2001; Gupta and Ahmad, 2012b; Hoshina and Marin-Morales, 2009; Leme and Marin-Morales, 2008b; Mazzeo et al., 2011a; Oliveira-Martins and Grisolia, 2009; Souza et al., 2009a; Türkmen et al., 2009; Wake, 2005).

1.12 Petroleum wastewater toxicity

The PRE consist of compounds from original crude oil, metals and non-metallic compounds. Moreover, among the hydrocarbons (HCs) present in crude oil, polycyclic aromatic hydrocarbons (PAHs) are among the most dangerous environmental contaminants due to their toxic, carcinogenic and mutagenic nature (Gupta and Ahmad, 2012a). Aromatic hydrocarbons are considered hazardous environmental pollutants due to the harmful effects, they have on different effects of living organisms (Leme and Marin-Morales, 2009). Here are few studies highlighted the cytotoxicity (CT), and genotoxicity (GT) and mutagenicity (MT) of PRE.

The toxicity of river water impacted by an oil pipeline leak showed clastogenic and aneugenic effects in *A. cepa* meristematic cell (MC) and the CA’s inductions which were attributed with the presence of HCs (Leme and Marin-Morales, 2008a). In another study, same authors also reported the CA’s and MN induction in *A. cepa* roots when exposed to river water contaminated with petroleum HC and PAHs. The larger CA’s and MN incidence in the MC of *A. cepa* was recorded when roots were exposed to wastewater samples where
the oil leak was high. The F₁ cells also showed significant higher MN inductions, however, MN induction were lower in F₁ cell as compared to F₀ cells (Leme et al., 2008).

In order to assess the toxicity before and after landfarming bioremediation of PRE, (Souza et al., 2009b) observed the MN, NA and CA in the A. cepa. Authors investigated the landfarming with rice hulls before and after HC biodegradation. Landfarming showed 13.5 g/kg of petroleum HCs and strong clastogenic and mutagenic induced effects were observed in A. cepa root MC in response of exposure. After 108 days of biodegradation, HC contents decreased by 27% and resultantly considerable reduction in mitotic abnormalities, CA, MN and nuclear bud have been observed. Landfarming treated with rice hulls decreased the CA, MN and nuclear bud, which were found to be correlated with the landfarming biodegradation efficiency. Finally, authors concluded that the A. cepa test is suitable method to assess biodegradation efficiency of petroleum products which contaminate soils. In another study, WW collected from PRE, showed negative effect on the growth of A. cepa roots (CA’s) for 24 h of exposure only (Nielsen and Rank, 1994a) and (Turkmen et al., 2009) applied the MI, MN and CA’s as indicators of toxicity in A. cepa exposed to PRE and results showed that there was a significant alteration in MI and MN and CA’s induction.

In another study, wastewater from Mathura refinery, Mathura, India showed the toxicity by applying the A. cepa test organism and in response a significant formation of MN and CA’s were observed (bridges) in A. cepa MC (Gupta and Ahmad, 2012a). Similarly, (Odeigah et al., 1997) reported the toxicity (root growth inhibition, malformation and CA’s formation) of an oil field wastewater in the A. cepa root cells. Ten bulbs of A. cepa were cultivated in different concentrations for 48 h and then root tips were subjected to cytological analysis, while root length was measured after 96 h, which resulted in significant dose dependent root growth inhibition. The group of plant exposed to wastewater showed significantly increased frequency of CA’s such as sticky chromosomes, spindle multipolarity, fragments, C-mitosis and bridges.

Some authors reported the toxicity by exposing the A. cepa roots to aqueous solution of aromatic hydrocarbons and reported positive results (Rank and Nielsen, 1994). In another study, the GT and MT potential of benzene, toluene, ethylbenzene and xylene has also been reported by applying CA and MN by A. cepa test system (Mazzeo et al., 2011b).
1.13 Soap and detergent wastewater

Over the last decade there has been a rapid growth in the use of detergents. Despite this increased usage, relatively little work has been focused on the environmental effects, especially the surfactant which are known endocrine disruptor due to weak ability to mimic estrogen and in turn disrupt the natural balance of hormones in affected organisms (Jaworska et al., 2002). Surfactants are one of the most common organic pollutants characterized with very high potential to enter the environment, since their widespread use, primarily in aqueous solutions, leads to mixing into the environment via wastewater discharges. After use, consumer detergents are usually discarded down the drain into municipal sewer systems and resultantly mixed with water sheds (Petrovic et al., 2006).

The release estimation of chemicals used in toilet products, fabric washing, dish cleaning, surface cleaning and shampoos is rather simple since the products are subject to wide dispersive use with ‘down-the-drain’ disposal. Overall, most of the losses to the environment occur at the use phase and for the purposes of exposure modeling it is assumed that 100% emission to sewer occurs at the post consumer stage (Franke et al., 1995). The annual production of detergents in the USA, Western Europe and Japan is 6 x10^6 tones. The wastewaters from the manufacture of detergents, which contain residual product material, can have a very variable polluting load and, if left untreated, can have an appreciable impact on the environment (Modler and Ishikawa, 2001). The soap and detergent wastewater generally contain surfactants (nonylphenol ethoxylates) along with other components. So, the literature survey regarding fate, environmental impact, distribution, toxicity and degradation of NPs is discussed in detail in following sections.

1.14 Nonylphenol ethoxylates (NPEOs)

Nonylphenol ethoxylates contain an ethoxylate chain with a length that specify the recalcitrance nature (Soares et al., 2008). The NPEO are highly cost effective surfactants with excellent performance and consequently used widely in industrial, institutional, commercial and household applications such as detergents, emulsifiers, wetting and dispersing agents, antistatic agents, demulsifiers and solubilisers (Langford and Lester, 2003). Due to the extensive use of NPEO, they reach sewage treatment works in significant amounts where they are incompletely degraded to nonylphenol shorter units (Johnson et al.,
2005; Koh et al., 2005; Nakada et al., 2006; Shao et al., 2003). Millions of kilograms are produced annually. The nonyl group, which is highly branched, attaches to the phenol ring via the 4- and, to lesser extent, the 2-positions. This mixture of isomers is usually available as a pale yellow liquid, although the pure compounds are colorless (Soares et al., 2008).

### 1.14.1 Fate and environmental impact of NPEOs

The NPnEOs are present in municipal WW and naturally undergo a rapid transformation into short-chain NPEOs such as NP1EO, NP2EO, NP, nonylphenol ethoxylates carboxylate (NPECs) and carboxylated NPnEOs (CAPECs) (Soares et al., 2008). The NP’s and short-chain NPnEOs are lipophilic compound with the long-chain EO and are classified as EDCs (Scrimshaw and Lester, 2002). Moreover, NP1EC and NP2EC are also proved to have the estrogenic properties (Harries et al., 2009). So far, the fate and occurrence of NPnEOs and their metabolites in wastewater treatment plants have been well documented in several studies around the world (Körner et al., 2000; Soares et al., 2008; Stasinakis et al., 2008). However, most of the authors reported limited number of analytes of NP and NPnEOs, while only a few reports revealed the presence of NPECs and CAPECs formation (Di Corcia et al., 2000; Loyo-Rosales et al., 2007a). A number of studies revealed the presence of NPs compounds in wastewater treatment plants, most of them focused exclusively on concentrations in influents and effluents (Shao et al., 2003). Only a few reports have made mass balance of NPs compounds in wastewater treatment plants to obtain the output pathways of NPs compounds during treatment (Bruno et al., 2002; Loyo-Rosales et al., 2007b).

### 1.14.2 Distribution of NPEOs in the environment

#### 1.14.2.1 Water sample

Water samples from agricultural drains were tested for the presence of NP, NP1EO and NP2EO. The analytes belong to biodegradation products of long-chained NPEO, which are used in pesticide formulations and resultanty a significant NP contents were detected in water samples (Zgoła-Grześkowiak et al., 2009). In another study in China, the NPEOs residual with \( n > 2 \) in the Chongqing area of the Changjiang river were investigated and NPEOs with \( n \) ranging from 1 to 22 were found to vary between 0.1 and 2900 ng/L with a
distribution depending on the depth of water samples (Shao et al., 2002). While studying the behavior of NPnEOs and their metabolites at wastewater treatment plants (Lian et al., 2009) showed that the total concentrations of NP compounds in the effluents of the four wastewater treatment plants ranged from 0.115 to 0.347 μmol/L and removal efficiencies recorded were 75.7% to 90.8%. The analysis revealed that 21.8–47.6% of NPnEOs and their metabolites entering wastewater treatment plants were released via effluents and into sludge. In order to assess the NP in river water (Valsecchi et al., 2001), detected 85 - 87% for 4-NP and NP4EO. The river receiving wastewater treatment plants effluents in Belgium and Italy were analyzed for NPs. The nonylphenol monoethoxylate (NPE1C) and nonylphenol diethoxylate (NPE2C) exhibited the highest concentrations in the water samples, up to 4.5 μg/L NPE1C in a wastewater treatment plant effluent and 3.6 μg/L NPE2O in a river and author concluded that potential adverse effects to the aquatic environment may occur (Loos et al., 2007).

1.14.2.2 Sediments

The profiles of NP and NPEOs like water bodies have also been investigated in sediments samples. In a study from the Beipaiming Channel, North China, the NP and NPEOs occurred in the surface sediment (> 40 cm), with the maximum value of NP and NPEOs reaching 3539 and 12735 ng/g, respectively. NPEOs with short ethoxy chains were dominant in the NPEO mixture with percentages from 54% to 78%, which were similar to the distribution of homolog NPEO in effluents from nearby STPs, indicating that the channel received the effluents from these sludge treatment plants (Jin et al., 2007). In another study in the Dutch coastal zone of the North Sea, the NP were detected up to 0.63 μg/L (Jonkers et al., 2005b). Same author also revealed the presence of NP up to 8.1 μg/L in Dutch estuaries Western Scheldt and the Rhine estuary (Jonkers et al., 2003). The concentration of NP up to 1100 ng/g d.w. has also been reported in the Rhine and Scheldt estuaries (The Netherlands) (Jonkers et al., 2005a).

1.14.2.3 Sludge

The sewage sludge has been also studied by researcher and a significant concentration NPs is detected. In a study, sewage sludge samples from the sewage treatment plants of three Greek cities: Athens, Patras and Heraklion and were evaluated and 12.8–233.5 mg/kg for NPEO and 3.6–93 mg/kg for NP have been detected (Fountoulakis et al., 2005). A sludge
sample from wastewater treatment plants in Seville (South Spain) also revealed significant contents of NP, NP1EO and NP2EO (Aparicio et al., 2007). Primary, secondary, mixed, and digested-dehydrated sludge samples from two wastewater treatment plants based on aerobic and on anaerobic biological stabilization showed a NPEs 136–2357 mg kg$^{-1}$ dry mass (d.m.) Overall, the sum of NPEs were higher than the limits fixed in the third draft of the future Sludge Directive for land application of sludge (Santos et al., 2007). A similar study, three year later revealed a considerable concentration of NP, NP1EO and NP2EO in primary, secondary, mixed, aerobically-digested, anaerobically-digested, dehydrated, compost and lagoon sludge samples from different sludge treatment plants in Andalusia (south of Spain). The sum of NP, NP1EO and NP2EO concentrations has been evaluated in relation to the limit value of 50 mg/kg set by the European Union Sludge Directive draft published in April, 2000, were at higher concentration levels. The most contaminated samples were compost, anaerobically-digested sludge, lagoon sludge and aerobically-digested sludge samples, which contained NPE concentrations in the ranges 44–962 mg/kg d.m., 8–669 mg/kg d.m., 27–319 mg/kg d.m. and 61–282 mg/kg d.m., respectively (González et al., 2010).

1.14.2.4 Toxicity

The toxicity of NPnEO and NP has been studied in recent years (Correa-Reyes et al., 2007; Soares et al., 2008; Vazquez-Duhalt et al., 2005). The toxicity of NPnEOs is relatively low for mammals, whereas short chain NPnEOs and NP4EO exert a highly toxic effect towards several freshwater and marine organisms; NPs may interfere with regulation mechanisms controlled by oestrogens in birds, mammals and fishes by competing for the binding sites of the oestrogen receptors (Planas et al., 2002; Soares et al., 2008). Nonylphenols are also capable of interfering with regulatory systems of different types of cells and organs by several mechanisms (Soares et al., 2008). However, there are few studies concerning the genotoxicity of NPnEOs and 4-NP. It has been reported that NP cause DNA damage (Frassinetti et al., 2011; Tayama et al., 2008). A significant increase in MN in turbot kidney after exposure to NP has also been reported (Baršienė et al., 2006).

A 3-day exposure to NP in concentrations of 1.0, 10.0, and 16.0 μl/L of water increased the frequency of reproductive stages in males and females (Oreochromis niloticus). The histology of the reproductive tract of the exposed fish was significantly altered in
females and results showed that the *O. niloticus* reproductive system is sensitive to NP estrogenicity (Rivero *et al.*, 2008).

Other than aquatic life, the toxicity of NPs has also been reported in higher organisms. In a study the effects of 4-NP on the mitotic and meiotic division of *V. faba* (broad beam) root cells has been reported (Adam and El-Ashry, 2010). Root-tips of *V. faba* were treated with a series of NP concentrations (25, 50 and 100 ppm) for 3 and 6 h and resultantly, meto-depressive effect on MI inhibition for all concentrations at 3 and 6 h except 25 ppm for 3 h were seen. The NP (100 ppm) caused 40% reduction in flower buds. Several types of abnormalities were observed after all treatments in both mitotic and meiotic divisions and CA’s were of stickiness, disturbed chromosomes, ana-telophases bridges and lagging chromosomes type. In another study, it is revealed that NP was the most toxic compound to mice, *A. cepa*, *Drosophila melanogaster*, and *Biomphalaria tenagophila* (Grisolia *et al.*, 2004).

While evaluating the comparative toxicity of six detergents (3 pre-manufacture detergents and 3 commercial), (Pedrazzani *et al.*, 2012) found that the EC$_{50}$ values varied for all products and were higher than the more stringent value considered for aquatic toxicity assessment (*V. fischeri* 10–60 mg/L; *D. magna* 25–300 mg/L; *A. cepa* 250–2000 mg/L). One commercial product induced an increase in MN frequency in *A. cepa* root cells. All pre-manufacture detergents and one commercial one, which gave negative results in the Ames and *A. cepa* tests, induced DNA damage in human leukocytes and author suggested battery of tests to describe toxicological and mutagenic features of NPs. In another study, NP genotoxicity and toxicity studies in different organisms have been reported. The toxicological evaluations showed that NP was the most toxic compound to mice, *A. cepa*, *Drosophila melanogaster*, and *Biomphalaria tenagophila* (Grisolia *et al.*, 2004). In the aquatic environment NPE breakdown to shorter chains naturally which are considered more stable and persistent and can act as an endocrine disruptor in fish, avian, and mammals (Rivero *et al.*, 2008).

### 1.15 Wastewater treatment methods

At present, there are several methods such as physicochemical treatment (sedimentation/flotation) (Thompson *et al.*, 2001), coagulation and precipitation (Fu and
Wang, 2011), adsorption (Mahmoodi et al., 2011), oxidation (Oller et al., 2011b), membrane filtration (Judd and Judd, 2011) and biological treatment- aerobic treatment (Chan et al., 2009), aerated lagoons (Chandra et al., 2011), aerobic biological reactors (Schwarzenbeck et al., 2005), anaerobic treatment (Demirel et al., 2005), fungal treatment (Novotný et al., 2000) and integrated treatment processes (Pokhrel and Viraraghavan, 2004a) used for the wastewater treatment. Among oxidation treatment methods, the advance oxidation process (AOP) has emerged as very effective for the degradation and mineralization of pollutants (Muneer et al., 2012; Naddeo et al., 2011), which can be employed as; O₃, O₃-UV, H₂O₂-UV, O₃-H₂O₂, TiO₂-UV, US, Fe²⁺-H₂O₂, gamma and electron beam irradiation (Comninellis et al., 2008; Han et al., 2002). Here, the AOPs (UV/H₂O₂/TiO₂ and gamma/H₂O₂) were used for the degradation of industrial wastewater as well as model compound. For maximum degradation of contaminants present in wastewater, various independent variables such as radiation dose, UV exposure time, pH, concentrations of TiO₂ & H₂O₂ and shaking were optimized. Other than degradation, the water quality parameters such as BOD, COD, DO, TSS, TDS and pH were measured before and after treatment to evaluated the efficiency of selected AOPs. For the evaluation of biological efficacy, various toxicity assays such as Allium cepa, shrimp, haemolytic and Ames test were performed before and after the application of AOPs.
Chapter # 3

MATERIAL AND METHODS

The present research work was carried out in the Radiation Chemistry Lab, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad in collaboration with Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad and Pakistan Council for Scientific and Industrial Research (PCSIR) laboratories complex Lahore, Pakistan.

1.16 Industrial wastewater samples and model compounds

Petroleum industry, pulp & paper mills and soap & detergent industries were selected to study their wastewater and NP6EO, NP9EO and NP30EO were selected as model compounds to represent these industries.

1.17 Instrumentation

Digital pH meter & DO meter (Lovibond Senso Direct 150, Germany), BOD & COD meter (Lovibond Oxi Direct, Germany), UV/Vis double beam spectrophotometer (CE Cecil 7200, Germany), FTIR (U-2001 Schimadzu, Japan), HPLC (SCL-10A Schimadzu, Japan- C-18 ODS column coupled with SPD-10 AV UV/Vis detector and DGV-12A) and GC-MS (6890 N Agilent, Japan- DB-5 column) were used for the analysis of samples.

1.18 UV/Vis spectrophotometer

Double beam spectrophotometer (CE Cecil 7200, Germany) was calibrated using K2Cr2O7 standard solutions. Five increasing concentrations of potassium dichromate of 20, 40, 60, and 100 mg/L were prepared along with blank 0.001M perchloric acid. The potassium dichromate solutions were scanned and four characteristic peaks at 257, 235, 350 and 313 nm were observed. Finally, the absorbance’s of four solutions were recorded at four wavelengths and plotted (Fig. 3.1) versus concentrations. A linear relationship between the absorbance and the concentration was observed.
Fig. 3.1: (A) Absorbance of potassium dichromate 20, 40, 60, 80 and 100 mg/L solution at 235, 257, 313 and 350 nm (linearity curves used for the calibration of spectrophotometer) (B) UV–Vis spectra of 20, 40, 60, 80 and 100 mg/L solutions showing characteristics peaks at 235, 257, 313 and 350 nm.

1.19 Gamma Radiation Source

Cesium-137 (Cs-137) gamma radiation source was used for irradiation of wastewater samples at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The dose rate at the time sample irradiation 1.25 kGy h⁻¹ (15th March, 2011). The gamma radiation source was calibrated using Fricke dosimeter (Eq. 3.4).

\[
Dose = \frac{N \times \Delta A \times 100}{\epsilon \times \rho \times G(Fe^{3+})}
\]  
(Eq. 3.4)

Where \(Dose\) = gamma radiation absorbed dose, \(N\) = Avogadro’s number (6.02x10²³), \(\Delta A\) is the difference in the absorbance of the irradiated and un–irradiated samples, \(\rho\) is the density of dosimetric solution (1.024 g/cm³ for 0.4 M H₂SO₄), \(\epsilon\) is the molar extinction coefficient of ferric ion (0.2205 M⁻¹ cm⁻¹ at 304 and 25°C) and \(G(Fe^{3+})\) is the number of Fe³⁺ ions produced/100 eV of absorbed energy (15.6) for Fricke solution.

1.20 Collection of wastewater samples

The wastewater samples were collected from three industries namely petroleum, pulp & paper and soap and detergent by standard methods (Eaton et al., 2005). Briefly, dried plastic gallon (washed with distilled water and drenched in 1% HNO₃ for 24 h) was used to collect the water samples. Sampling was performed for three days for each industry and one
sample was collected three times per day. In this way total 27 (10 L each) samples were collected from 9 industries. After filling of the gallon, they were immediately sealed and transported to the water toxicity and radiation chemistry laboratories, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan. The petroleum industry wastewater samples were collected from Karachi, Kot Addu and Rawalpindi, while pulp and paper mill from Faisalabad, Gujranwala and Shaikhupura and soap industries were selected from Gujranwala, Faisalabad and Sargodha, Pakistan. The collected wastewater samples were stored at -4 °C to avoid any type of change in wastewater.

1.21 Statistical design

Commonly used experimental design techniques for optimization and modeling are the full factorial, partial factorial and central composite rotatable designs. A full factorial design requires at least three levels per variable to estimate the coefficients of the quadratic terms in the response model (Box and Wilson, 1951). A partial factorial design requires fewer experiments than the full factorial. However, the former is particularly useful if certain variables are already known to show no interaction (Box and Hunter, 1961). An effective alternative to the factorial design is the rotatable central composite design (RCCD), originally developed by (Box and Wilson, 1951) and later on updated by (Box and Hunter, 1957). The RCCD gives almost as much information as a three-level factorial, requires much fewer tests than the full factorial and has been shown to be sufficient to describe the majority of steady-state process responses (Obeng et al., 2005). In present study, the experimental design was constructed using coded values for each variable, maximum (+1) and minimum (−1) and \( n \) factors using minimum number of run 2\( n \). The model allows the construction of response surface plots and the prediction of values at any point in the region of interest (Box and Hunter, 1957). The proposed equation obtained from the model is expressed by a second order polynomial equation (Eq. 3.1).

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_1 X_1^2 + \beta_2 X_2^2 + \varepsilon
\]  

(Eq. 3.1)

Where, \( \beta_0, \beta_1 \) and \( \beta_2 \) are coefficients, while \( X_1 \) and \( X_2 \) are explanatory variables. An experimental design, rotatable central composite design (RCCD) was developed using the
statistical program R (version 10.1) for the determination of optimal experimental variables. Table 3.6 show the experimental matrix obtained from the model with the coded and un–coded values and the order in which the experiments were carried out. Analysis of variance (ANOVA) and response surfaces were generated with respect to variables. A given mathematical model was only considered acceptable when ANOVA reached high statistical significance (favorable adjustment values and no apparent lack of fit), with $F$ values at a level of confidence of 95% and $P$ values $<0.05$. The quality of the fit of the polynomial model equation was evaluated by the coefficient of determination $R^2$. The optimum values of the selected variables were obtained by analyzing the response surfaces and solving the regression equation (Yeber and Fernández, 2010).

Table 3.6: Selected statistical design showing the coded and uncoded levels of variables

<table>
<thead>
<tr>
<th>Runs</th>
<th>Coded</th>
<th>Uncoded</th>
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<td>Factor X₁</td>
<td>Factor X₂</td>
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<td>0</td>
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<td>4</td>
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<td>7</td>
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</table>

Where, $X_1$ and $X_2$ are representing solution concentration and TiO₂ amount, respectively in case of UV treatment, whereas in case of gamma radiation treatment, the $X_1$ and $X_2$ are representing solution concentration and gamma radiation absorbed dose, respectively, otherwise explained where changed.

### 1.22 UV treatment

The UV radiation source was a low pressure UV lamp (44 Watt, emission at 253.7 nm, manufactured by Galvano Scientific, Pakistan) which was protected by a quartz tube and was maintained at a temperature of 25±2°C. The solution was projected at the bottom of the reactor at a distance of 6 cm. All independent variables were optimized by analyzing the data through response surface methodology (RSM). For each batch 12 sample (10 mL) were run
whose level and design can be seen in Table 3.6. Samples were withdrawn after specific time intervals and analyzed for absorbance and other water quality parameters. The titanium dioxide used was degussa p25 and hydrogen peroxide was 35% pure. Throughout UV treatment, the TiO$_2$ concentration used was mg/10 mL of solution.

### 1.23 Gamma radiation treatment

The industrial wastewater samples and solutions of model compounds were irradiated by gamma radiation to the absorbed doses range of 5 to 15 kGy under RCCD using Cs-137 source (dose rate 1.25 kGy h$^{-1}$) at NIAB, Faisalabad. Freshly prepared solution (10 mL) were taken in Pyrex glass vials in triplicate and irradiated for predetermined interval of times at a fixed position in radiation chamber of radiation source. To ensure reproducible, the geometry and positions of the tubes were kept uniform.

### 1.24 Measurement of G-values

The NPEOs aqueous solutions were irradiated at different absorbed doses such as 5, 10 and 15 kGy. The G values (removal efficiency) were calculated at each absorbed dose using equation 3.9a (Spinks and Woods, 1990).

\[
G = \frac{[R]N_A}{D(6.02 \times 10^{17})} \quad \text{(Eq. 3.9a)}
\]

Where $R$ is the change in concentration of NPEOs in µmol dm$^{-3}$ at given dose, $D$ is the absorbed dose in Gy, $6.02 \times 10^{17}$ is conversion factor from Gy to 100 eV L$^{-1}$ and $N_A$ is Avogadro's number. The dose constant $k$ value was calculated as the slope of the plot between ln [R] and dose (kGy), while $R$ represents the concentration (µmol dm$^{-3}$) of NPEOs. The $D_{0.5}$ ($D_{50}$) and $D_{0.9}$ ($D_{90}$) were calculated using equations 3.9b and 3.9c.

\[
D_{0.5} = \frac{\ln 2}{k} \quad \text{(Eq. 3.9b)}
\]

\[
D_{0.9} = \frac{\ln 10}{k} \quad \text{(Eq. 3.9c)}
\]
1.25 Analysis

1.25.1 Spectrophotometric analysis

Before treatment of solution the $\lambda_{\text{max}}$ of each compound and wastewater was recorded and after treatment the absorbance was measured at $\lambda_{\text{max}}$ and the percentage degradation was measured using relation given below. For the determination of $\lambda_{\text{max}}$ the solution was scanned from 190 to 900 nm. In case of UV treatment in the presence of TiO$_2$, the TiO$_2$ was separated by centrifugation at 1400 rpm for 5 min. The percentage degradation was measured using the following relationship, where $A_i$ and $A_f$ are representing the absorbance before and after treatment, respectively.

$$D(\%) = \left[ \frac{A_i - A_f}{A_f} \right] \times 100$$

1.25.2 Analysis of water quality parameters

Before use all instruments were properly calibrated. The water quality parameters of industrial effluents such as pH, DO, BOD and COD were analyzed by pH, DO, BOD (Method 5210 B, Aqualytic BOD-OxiDirect) and COD meters, while TSS and TDS were measured by standard methods before and after treatments (Eaton et al., 2005). Briefly, a well-mixed sample was filtered by filter paper; the filtrate was evaporated to dryness in a pre-weighed china dish and dried to constant weight at 180°C. The increase in dish weight represents the total dissolved solids. The amount of TDS was calculated as;

$$TDS (mg \ / \ L) = \left[ \frac{A - B}{V (mL)} \right] \times 1000$$

Where $A =$ weight of dried residue + dish (mg), $B =$ weight of dish (mg) and $V$ is the volume of the sample.

For the measurement of TSS, a well-mixed sample was filtered by pre weighed filter paper. The filter paper was kept for one hour at 103 to 105°C in an oven, cooled in desiccators, weighed and TSS was measured by the relation used for TDS measurement. All water quality parameter were reported as percentage decrease and increase and were calculated with following formulae.
\[ WQP(\%) = \left[ \frac{A_i - A_f}{A_f} \right] \times 100 \]

Where \( A_i \) and \( A_f \) are representing the initial and final values of the measured parameters.

1.25.3 Fourier transform infrared spectrophotometer FTIR

Fourier transform infrared spectrometry (FTIR) was used before and after treatments in order to identify their degraded end products. Before treatment the NPEOs sample was subjected to analysis using FTIR spectrophotometer (U-2001, Schimadzu, Japan) at PCSIR Laboratories complex Lahore. Prior to analysis of irradiated samples, the aqueous irradiated samples were extracted with acetone. For this, treated sample (up to 5 mL) was mixed with acetone and stirred for 15 min and mixture was transferred to separatory funnel and aqueous phase was decanted and the organic phase was mixed with MgSO\(_4\) for 24 hours, filtered, concentrated and subjected to FTIR analysis (Zhang et al., 2005).

1.25.4 Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometric (GC-MS) analyses of model compounds were carried out after treatment in order to identify the degraded products. Before analysis, the aqueous irradiated samples were extracted with acetone. The organic phase were decanted and then dehydrated by MgSO\(_4\) for 24 hours. The organic phases were then concentrated and stored for analysis. The GC system (Agilent Technologies 6890N) with DB-5 capillary column coupled to MS detector through EI interface was used for this study (Zhang et al., 2005). The identified compounds were compared with GC-MS system software library to identify the probable degradation products.

1.25.5 High performance liquid chromatography (HPLC)

The HPLC analysis was performed for the NPEOs solution before and after treatment using the following method reported elsewhere (Alnaizy and Akgerman, 2000). The NPEOs sample before and after treatment were run and compared to evaluate the degradation effect.
1.26 Toxicity determination of wastewater and model compounds

1.26.1 Toxicity evaluation

The industrial wastewater and aqueous solutions of NPEOs were tested for their toxic potential before and after AOPs treatment to evaluate the effect on toxicity reduction.

1.26.2 H₂O₂ removal from treated sample

To avoid the H₂O₂ toxic effect, it was important to eliminate it from irradiated solutions. To remove H₂O₂, small amounts of MnO₂ (< 1 mg /mL) were added to the solutions (Weihua et al., 2002). After a reaction time of 1 h, the solution was filtered and subjected to the toxicity tests such as A. cepa, haemolytic, brine shrimp and Ames bioassays. The A. cepa, brine shrimp and haemolytic tests are used for cytotoxicity, while Ames test is used to evaluate the mutagenicity of tested samples.

1.27 Bioassays

1.27.1 Allium cepa test

The root initiation and growth assessment was done using the procedure of modified A. test (Abu and Mba, 2011). Onion bulbs of equal size of same species were purchased from local market, Faisalabad, Pakistan. The bases of the bulbs were gently scrapped and root primordia were exposed to wastewater. Before transformation of bulbs in tested solution, bulbs were germinated in tap water seven for each treatment and finally, best five were transferred in wastewater and aqueous solution of model compound along with negative and positive control under same condition. The control and germinated bulbs were kept in solution for 48 h and the tested samples were replaced with the gap of 6 h. Ultra-pure water was used as a negative control and methyl methanesulfonate (MMS) (Sigma-Aldrich) was used as a positive control. The roots were harvested after 28 h period and transferred in aceto-alcohol (1:3). The root tips were hydrolyzed in 1N HCl at 60°C until they become soft. Aceto-orcein squash technique was used in preparing the root tip for cytological examination. Slides were examined and counts were made for normal and abnormal cells. The mitotic index (MI) was calculated as the number of aberrant cells over the number of dividing cells.
1.27.2 Haemolytic assay

Three mL of young and healthy voluntaries human blood cells and animal (sheep) blood cells were gently mixed, poured into a sterilized 15 mL polystyrene screw-cap tube and centrifuged at room temperature for 5 min, 850 x g. The supernatant was poured off and the viscous pellet were washed three additional times with 5 mL of chilled (4°C) sterile isotonic phosphate buffered saline (PBS) solution (NaCl, 8 g/L; KH₂PO₄, 0·2 g/L; Na₂HPO₄, 1·2 g/L; and KCl, 0·2 g/L, adjusted to pH 7·4, using 1M NaOH and 1% HCl solution and mixed for 60 min to stabilize pH). The washed cells were suspended in a final volume of 20 mL chilled, sterile PBS and the cells counted on a haemacytometer. The blood cell suspension was maintained on wet ice and diluted with sterile PBS to 7·068 × 10⁸ cells mL for each assay. 20 µL of sample were aseptically placed into 1·5 mL appendrof tubes. For each assay, 0·1% Triton X-100 was the positive, 100% lytic control and PBS was the negative, background (0% lysis) control. A 180 µL diluted blood cell suspension were aseptically placed into each 1·5 mL tube and gently mixed three times with a wide mouth pipette tip. Finally, the samples were mixed and incubated for 35 min at 37°C with agitation (80 rev/min) and then immediately, the tubes were placed on ice for 5 min followed by centrifugation for 5 min at 1310 x g. A 100 µL of supernatant were carefully collected, placed into a sterile 1·5 mL appendrof tube, and diluted with 900 µL chilled and sterile PBS. All tubes were maintained on wet ice after dilution. Absorbance at 576 nm was measured on μ quant (BioTek, Winooski, VT, USA) (Powell et al., 2000). All samples were run in triplicate and results were averaged. The percentage lysis of RBC was measured using the following relationship; where Aₛ and Aₑ are representing the absorbance of sample and absorbance of triton X-100 (standard).

\[
Lysis(\%) = \left[ \frac{Aₛ}{Aₑ} \right] \times 100
\]

1.27.3 Brine shrimp assay

Brine shrimp eggs, Artemia salina L. were hatched in artificial seawater prepared by dissolving 38 g of sea salt in 1 L of DW and pH of the solution was adjusted to 8.5. After 48 h incubation at 26-30°C under constant aeration, the larvae (napoli) were attracted to one side
of the vessel with a light source and collected with a pipette. Napoli were separated from eggs by aliquoting them three times in small beakers containing seawater.

Wastewater samples and model compound aqueous solution were dissolved in dimethyl sulfoxide (DMSO) and diluted with artificial sea salt water so that final DMSO concentration did not exceed 0.05% and 50 mL of sample was placed in one sample tube and a two-fold dilution was carried out down the column of sample tubes. The total volume was adjusted to 5 mL with sea salt water and 100 mL of suspension of napoli containing 10 larvae was added into each tube and incubated for 24 h. The tubes were then examined under a magnifying glass and the numbers of dead napoli in each tube were counted. All the experiments were conducted with control (vehicle treated), and different concentrations of the test sample in a set of three tubes per dose. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes (Moshafi et al., 2010).

1.27.4 Ames assay (mutagenicity assay)

The Ames test was performed in agar plate as precisely reported elsewhere (Maron and Ames, 1983). The plates were sealed in plastic bags and incubated at 37°C for 4 days. The blank plate was observed first and the rest of plates were read only when all wells in the blank plate were colored purple indicating that the assay was not contaminated. The background, standard and test plates were scored visually and all yellow, partial yellow or turbid wells were scored as positive wells, while purple wells were scored as negative. The samples were considered toxic to the test strain if all wells in the test plate showed purple coloration and if the sample showed the intense purple color more than twice the background that was considered mutagenic in nature.
Chapter # 4

RESULTS AND DISCUSSION

1.28 Pulp and paper mills wastewater

The WQPs of pulp and paper mills wastewater samples, before treatment are given in Table 4.1. The measured pH, DO, COD, TDS and BOD were found to be beyond the permissible limits. The wastewater form pulp and paper mill was subjected to UV and gamma radiation treatment and various operating variable were optimized for maximum degradation of pollutant as well as for the improvement of WQPs. Finally, the effect of AOPs on toxicity reduction of effluents was evaluated on the basis of bioassays such as microbial load, haemolytic, shrimp and allium cepa tests.

Table 4.1: Water quality parameters of pulp and paper mills wastewater before treatment

<table>
<thead>
<tr>
<th>Wastewater</th>
<th>pH</th>
<th>BOD* (mg/L)</th>
<th>COD (mg/L)</th>
<th>TDS (mg/L)</th>
<th>TSS (mg/L)</th>
<th>DO (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industry I</td>
<td>10.50±0.53</td>
<td>1600±70</td>
<td>3600±175</td>
<td>2100±100</td>
<td>1300±60</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industry II</td>
<td>10.30±0.49</td>
<td>1570±63</td>
<td>3300±155</td>
<td>2050±110</td>
<td>1190±54</td>
<td>1.6±0.14</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industry III</td>
<td>10.40±0.60</td>
<td>1480±76</td>
<td>3900±185</td>
<td>2036±95</td>
<td>1280±68</td>
<td>2.2±0.17</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*BOD = Biological oxygen demand, COD = Chemical oxygen demand, TDS = Total dissolve solids, TSS = total suspended solids, DO = dissolve oxygen

1.28.1 Gamma ray treatment of pulp and paper effluents

Treatment of pulp and paper wastewater was carried out by gamma radiation at absorbed doses of 5, 10 and 15 kGy in the presence of H₂O₂. The data shows that the wastewater samples were highly polluted due to the elevated values of water quality parameters. The treatment efficiency was evaluated by analyzing the degradation, change in WQPs such as COD, BOD, DO, TDS, TSS, pH and toxicity reduction.

The degradation of contaminants in wastewater is directly related to the WQPs before treatment which were found to be beyond the permissible limit. The gamma radiation treatment was performed in the presence of 3, 5 and 7% H₂O₂ for the absorbed doses of 5, 10
and 15 kGy and resultantly a significantly enhanced degradation rate was observed. A degradation of 36%, 42% and 53% was recorded for absorbed doses of 5, 10 and 15 kGy in the presence of 3% H₂O₂, respectively. By increasing the H₂O₂ concentration to 5%, the degradation increased up to 55%, 87% and 97% for the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively and by increasing H₂O₂ further to the level of 7%, the degradation of pulp and paper contaminants was not enhanced which is attributed to formation of HO₂⁻ which compete with hydroxyl radicals. Through RSM analysis, it was also observed that 5.6% H₂O₂ furnished the maximum degradation of contaminants present in pulp and paper wastewater (Fig. 4.1.1) and the polynomial relationship between gamma radiation absorbed dose and H₂O₂ concentration is given in Eq. 4.1.1. As expected from the degradation of pulp and paper mills waste by gamma ray treatment in the presence of H₂O₂, the WQPs also improved significantly. The reduction in COD is considered as an important parameter to analyze the degree of pollution in term of organic contents present in wastewater as well as treatment efficiency. Upon gamma irradiation, considerable improvement was observed because the COD value decreased sharply with the radiation absorbed dose. The decrease in COD values were 14%, 21% and 31% when samples were exposed to the radiation absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively in the presence of 3% H₂O₂. By increasing the H₂O₂ up to 5%, the reduction in COD was recorded to be 36%, 51% and 77% for the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively (Fig. 4.2.1a). The COD reduction data was also analyzed through RSM analysis and it was found that the maximum reduction in COD can be achieved using 5.43% of H₂O₂ concentration and this reduction in COD was found to be related with degradation. It has been reported that when complex organic molecules break down into small molecules upon treatment then COD reduces significantly (Fang and Wu, 1999). Similar to the COD reduction, other WQPs such as BOD, TDS, TSS (Fig. 4.1.1a) and dissolve oxygen (DO) (Fig. 4.1.1b) were also improved. The percentage reduction in BOD of pulp and paper wastewater samples subjected to gamma ray absorbed doses of 5 kGy, 10 kGy and 15 kGy in the presence of 3% H₂O₂ were 23%, 29% and 37% and by increasing the H₂O₂ concentration to the level of 5%, the BOD value decreased up to 41%, 59% and 83% for the absorbed dose of 5 kGy, 10 kGy and 15 kGy, respectively. The reduction in TDS, TSS and increase in DO of treated sample were also found maximum for 5% H₂O₂. The decrease in TDS was recorded to be 39%, 61% and 81%, while reduction in
TSS was 44%, 65% and 83% for the absorbed dose of 5 kGy, 10 kGy and 15 kGy in the presence of 5% H₂O₂ concentration, respectively. Under the same conditions, the increase in DO was recorded to be 34%, 36% and 59%. After treatment, the pH of the treated sample reduced significantly. Before treatment, the pH of pulp and paper wastewater was highly basic and after treatment that was decreased and recorded to be in the range of 5-6 which is indication of degradation of organic pollutant present in wastewater, which converted into low molecular mass organic acids and resultantly the pH decreased (Fig. 4.1.1b).

\[ D(\%) = -148.84(\pm 4.75) + 75.68(\pm 13.88)X_2 - 7.21(\pm 1.30)X_2^2 + \epsilon \] (Eq. 4.1.1)

Where \( X_2 \) is representing the gamma radiation absorbed dose.

---

Fig. 4.1.1: Degradation of pulp and paper wastewater subjected to the absorbed doses of 5 kGy, 10 kGy and 15 kGy in the presence of H₂O₂
Figs. 4.1.1a: Reduction of COD, BOD, TDS and TSS of pulp and paper wastewater using gamma ray treatment in the presence of $\text{H}_2\text{O}_2$: A-COD, B-BOD, C-TDS and D-TSS

Fig. 4.1.1b: Effect of gamma ray treatment on DO and pH of pulp and paper wastewater subjected to the absorbed doses of 5 kGy, 10 kGy and 15 kGy in the presence of $\text{H}_2\text{O}_2$: A-DO and B-pH
1.28.2 Effect of gamma ray treatment on toxicity of pulp and paper wastewater

The toxicity of pulp and paper wastewater was evaluated on the basis of toxicological and biological assays before and after application of AOPs. It was found that the wastewater samples were highly toxic before treatment and as a result of treatment, the toxicity reduced significantly. The toxicity of wastewater samples subjected to an absorbed dose of 15 kGy in the presence of 5.6% H$_2$O$_2$ was evaluated and results are shown in Table 4.1.2. The total bacterial count and total coliform were recorded as $>1 \times 10^6$ and $>1 \times 10^5$ before treatment and after treatment both the microbes eliminated and were not detected. The A. cepa test also revealed the efficiency of treatment, before treatment the number of root and root lengths were recorded to be in the range from 8-10 and 2.5-3.1 cm, respectively and after treatment, the increase in number of roots and root lengths were recorded to be 23-40% and 26-41%, respectively. The pulp and paper wastewater also showed 79% red blood cell lysis and 87% shrimp napoli death, however, after treatment death rate reduced significantly. The reduction in red blood cell lysis and shrimp napoli death were found to be 70.14% and 92.18%, respectively (Table 4.1.2). Overall, toxicological tests showed that the gamma radiation treatment in the presence of H$_2$O$_2$ exerted promising effect for the reduction of toxicity of pulp and paper wastewater.
Table 4.1.2: Effect of gamma ray treatment (15 kGy) on toxicity reduction evaluated by total bacterial count, total coliform, *A. cepa*, *haemolytic* and *shrimp* tests

<table>
<thead>
<tr>
<th>Before treatment</th>
<th>Microbial</th>
<th>ACT</th>
<th>Haemolytic</th>
<th>Shrimp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBC</td>
<td>T. coliform</td>
<td>RC</td>
<td>RL</td>
</tr>
<tr>
<td></td>
<td>CFU</td>
<td>CFU</td>
<td>cm</td>
<td>%</td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>&gt;1 x 10^6</td>
<td>&gt;1 x 10^5</td>
<td>09±0.3</td>
<td>2.9±0.15</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>&gt;1 x 10^6</td>
<td>&gt;1 x 10^5</td>
<td>08±0.21</td>
<td>3.1±0.17</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>&gt;1 x 10^6</td>
<td>&gt;1 x 10^5</td>
<td>10±0.39</td>
<td>2.5±0.10</td>
</tr>
<tr>
<td>PC</td>
<td>---</td>
<td>---</td>
<td>14±0.48</td>
<td>6±0.25</td>
</tr>
<tr>
<td>NC</td>
<td>---</td>
<td>---</td>
<td>6±0.50</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>After treatment</td>
<td>ND</td>
<td>ND</td>
<td>16±0.59</td>
<td>6.7±0.25</td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>18±0.65</td>
<td>8.9±0.41</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>19±0.81</td>
<td>5.4±0.19</td>
</tr>
<tr>
<td>% reduction</td>
<td>Industry 1 (n = 3)</td>
<td>100</td>
<td>40.25</td>
<td>36.58</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>100</td>
<td>37.56</td>
<td>25.93</td>
<td>48.57</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>100</td>
<td>23.11</td>
<td>40.9</td>
<td>70.14</td>
</tr>
</tbody>
</table>

PC-positive control, NC-negative control, TBC-total bacterial count, ACT-*Allium cepa* test, RC-Root count, RL-Root length, ND-not detected.

For *haemolytic* test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively.

For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively.

For *shrimp* test, PC and NC were cyclophosphamide (10 µg/mL) and sea water, respectively.

### 1.28.3 Pulp and paper wastewater treatment by UV/H_2O_2/TiO_2

In this study, the treatment of pulp and paper wastewater was done by UV irradiation and different independent variable such as H_2O_2 concentration, UV exposure time, pH, shaking speed and TiO_2 concentration were optimized for maximum degradation of pollutants. The treatment efficiency was evaluated on the basis of degradation of toxic residue, improvement of WQPs (COD, BOD, DO, TDS, TSS, pH) and evaluation of the toxicity level. The UV treatment was carried out in the presence of 3-7% H_2O_2, 3-9 mg TiO_2/10 mL solution, in the pH range of 5-11, 30-90 min UV exposure time at 50-150 rpm shaking speed using RCCD (Figs. 4.1.3a and 4.1.3b). The polynomial relation between TiO_2 concentration and nature of wastewater can be seen in Eq. 4.1.3, where X_2 is representing the TiO_2 concentration. By optimizing all independent parameters, a maximum degradation of 98% was observed. The RSM analysis revealed that H_2O_2 (5.26% v/v), UV irradiation time...
(90 min), pH (7.6), shaking speed (105 rpm) and TiO$_2$ (8 mg) were effective for maximum degradation.

$$D(\%) = 49.17(\pm 2.15) + 12.83(\pm 0.5)X_2 - 8.33(\pm 0.04)X_2^2 + \epsilon$$  \hspace{1cm} (Eq. 4.1.3)

Fig. 4.1.3a: Percent degradation of pulp and paper wastewater by UV-irradiation (44 Watt) in the presence of H$_2$O$_2$ and TiO$_2$. A-Effect of H$_2$O$_2$, B-Effect of UV exposure Time, C-Effect of pH, D-Effect of shaking speed
1.28.4 Effect of UV treatment on water quality parameters (WQPs)

The pulp and paper wastewater sample were subjected to UV treatment (44 Watt) using 3-9 mg TiO$_2$, shaking speed 105 rpm, adjusted at pH 7.6, exposing 90 min for reaction in the presence of 5.26% H$_2$O$_2$. The results regarding WQPs are shown in Fig. 4.1.4ab. The reduction in COD and BOD were observed up to 48% and 54% for 3 mg TiO$_2$ and for 6 mg TiO$_2$, the treatment efficiency increased and COD and BOD values of pulp and paper wastewater further decreased to 77% and 79%, respectively. By increasing TiO$_2$ up to 9 mg, no more considerable reduction in COD and BOD was observed. Similarly, the TDS and TSS were also reduced significantly i.e 84% TDS was reduced using 9 mg TiO$_2$ and 84% TSS using 6 mg TiO$_2$. The variations in pH and DO were also significant that could be correlated with degradation of the pollutant. Although the radiation treatment in the presence of H$_2$O$_2$ has significant effect on degradation and improvement of WQPs, however, addition of TiO$_2$ furnished promising effect due to its catalytic power.
1.28.5 Effect of UV/H$_2$O$_2$/TiO$_2$ on pulp and paper wastewater toxicity

The toxicity of pulp and paper wastewater was determined before and after the application of AOPs (UV/H$_2$O$_2$/TiO$_2$) and the results are shown in Table 4.1.5. The total bacterial count and total coliform were recorded as $> 1 \times 10^6$ and $>1 \times 10^5$ before treatment and after treatment both the microbes were not detected. In *A. cepa*; the number of root were
recorded in the ranges from 8-10 and root lengths 2.5-3.1 cm, respectively and after treatment, the increase in number of roots and root lengths were recorded to be 52-60% and 58-60%, respectively. The red blood cell lysis and shrimp napoli death reduced up to 91.42% and 95.95%, respectively (Table 4.1.5). The toxicity results showed that the UV treatment in the presence of H₂O₂ and TiO₂ could be used successfully for the reduction of toxicity of pulp and paper mills wastewater and UV/H₂O₂/TiO₂ treatment system was more effective as compared to gamma radiation treatment for the reduction of wastewater toxicity.

![Table 4.1.5: Toxin content reduction of pulp and paper mills wastewater before and after treatment by total bacterial count, total coliform, A. cepa, haemolytic and shrimp tests](image)

For haemolytic test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively. For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively. For shrimp test, PC and NC were cyclophosphamide (10 µg/mL) and sea water, respectively.

Similar to present study various researchers used the AOPs to evaluate their efficiency for the treatment of PPMWW such as by ozonation (Mao and Smith, 1995; Rodrigues et al., 2008), O₃/UV and through photo-assisted catalysis (Yeber et al., 1998) and revealed promising efficiency of AOPs for the treatment of PPMWW. It was demonstrated that the photo-catalysis in the presence of O₂ was faster in degradation of bleaching effluents.
than O₃, O₃/UV, O₂/ZnO/UV, O₂/TiO₂/UV, O₃ and O₃/UV. Authors concluded that O₂/ZnO/UV and O₂/TiO₂/UV were the best systems to oxidize the effluent in a short period of time (Pokhrel and Viraraghavan, 2004a). In another study, the degradation of PPMWW using different AOPs such as O₃/UV, O₃/UV/ZnO, O₃/UV/TiO₂, O₂/UV/ZnO, O₂/UV/TiO₂ have been reported (Yeber et al., 1999). However, O₃/UV, O₃/UV/TiO₂ and O₂/UV/TiO₂ systems are considered as the most efficient. The COD, TOC and toxicity reduction correlated well with the degradability enhancement after AOPs treatments. Our findings regarding degradation of PPMWW by AOPs were found consistent with previous studies. Similar to the reported studies, the applied AOPs improved the WQPs significantly. To evaluate the biological efficacy of selected AOPs, the toxicity profile of wastewater was also measured using different bioassays and resultantly it was found that the toxicity of treated sample was also reduced significantly. The reduction in toxicity and improvement of WQPs were found to be correlated with degradation of pollutant present in wastewater. To improve the WQPs and for the reduction of toxicity of PPMWW, different independent variables were optimized and it was found that at specific set of independent variables the degradation was found to be maximum. Similar to this study, while studying the efficiency of H₂O₂ (Ahmed et al., 2009), UV/H₂O₂ was applied to treat the PPMWW as a function of initial pH, H₂O₂ concentration and temperature. The results showed that the UV/H₂O₂ system performed well for almost complete elimination of COD and TOC from an actual PPMWW at pH 11.25 using 2.1 g of H₂O₂/L at 28°C. In another study, the mixed raw, coagulated and biologically pretreated PPMWW were investigated on the basis of COD, TOC, BOD₅ and color removal by heterogeneous photo–catalytic oxidation (Balcioglu and Arslan, 1998). Results showed that photo–catalytic oxidation process with 15 mM H₂O₂ was more efficient for the removal of pollutants.

Other than degradation, the AOPs applied also enhanced the WQPs and similar effect of AOPs on WQPs has also been reported previously for industrial wastewater. In a study, the AOPs consisting of treatments by H₂O₂, H₂O₂/Fe²⁺, UV, UV/H₂O₂, UV/ H₂O₂/Fe²⁺ and O₃/H₂O₂ in laboratory-scale reactors for color, TOC and AOX removals were compared for PPMWW treatment (Catalkaya and Kargi, 2007). Almost every method used resulted in some degree of color removal. However, the Fenton’s reagent (H₂O₂/Fe²⁺) resulted in the highest color, TOC and AOX removal under acidic conditions when compared with the other
AOPs tested. Approximately, TOC (88%), color (85%) and AOX (89%) removals were obtained by the Fenton’s reagent at pH 5 within 30 min. The PF process yielded comparable TOC (85%), color (82%) and AOX (93%) removals within 5 min due to oxidations by UV light in addition to the Fenton’s reagent. Fast oxidation reactions by the FP treatment makes this approach more favorable as compared to the others used. In present study, the use of UV/H₂O₂/TiO₂ showed superior efficiency as compared to reported literature which might be due the high efficiency of TiO₂ as compared to Fenton reagent. However, without the use of TiO₂ the efficiency of AOP tested was lower then the Fenton reagent reported previously. While evaluating the efficiency of the TiO₂, (Rodrigues et al., 2008) investigated the combined treatment of post-bleaching effluent from PPMWW on the basis of WQPs. The PPMWW were submitted to the coagulation–flocculation treatment applying FeCl₃ as the coagulating agent and chitosan as an auxiliary. The aqueous soluble phase obtained from the first treatment was submitted to a UV/TiO₂/H₂O₂ system using mercury lamps. The optimized photo–catalysis conditions were: pH 3.0 in 0.50 g/L of TiO₂ and 10 mmol/L of H₂O₂ and reduction in COD by UV/H₂O₂, UV/TiO₂ and UV/TiO₂/H₂O₂ were recorded to be 344 mg/L, 326 mg/L and 246 mg/L, respectively (Muñoz et al., 2006) and these results were in line to our study regarding COD reduction of PPMWW wastewater. In another study, the use of TiO₂/UV/O₂ and TiO₂/UV/Cu (II) has been reported (Yeber et al., 2007). Results indicate that color removal was 94% at pH (3.0) in the presence of Cu(II) as an electron acceptor and up to 70% of COD was removed.

In order to evaluate the efficiency of TiO₂-photocatalytic degradation of PPMWW, (Pérez et al., 2001) the effect on TOC, COD, color and acute toxicity removal was checked. The pH, concentration of catalyst and H₂O₂ were optimized. The pH showed large effect in the AOX degradation at values below 5 and over 10, while COD removal was improved at low pH. The presence of H₂O₂ in the photo–catalytic reaction has a small effect in degrading the AOX and COD reduction. The optimal concentration of TiO₂ was found to be around 1 g/L. After 30 min of reaction more than 95 and 50% AOX and TOC removals, respectively were recorded. A significant improvement in WQPs such as TS, TSS, TDS, pH, COD, BOD and Cl⁻ has also been reported by treating the PPMWW with TiO₂ and ZnO under UV/solar irradiation. Author suggested the use of heterogeneous photo–catalytic process at industrial scale for wastewater detoxification and to improve the WQPs (Kansal et al., 2008).
In few studies the use of gamma radiation for wastewater treatment which is an emerging technology has also been reported and effect of various factor on the degradation and mineralization of PPMWW was optimized. In order to assess the efficiency of $^{60}$Co gamma radiation source for the treatment of PPMWW, (Wang et al., 1994) used 800 krads absorbed dose for the reduction of BOD, AOX and COD. Results showed that COD was reduced by 13.5% and BOD$_5$ up to 58.6%. These changes altered the value of COD/BOD$_5$ from 14 to 5. For the same dose, the AOX was reduced to 76.2% and author suggested the use of radiation process to treat PPMWW. Another study also showed a promising efficiency for the treatment of PPMWW (Bunnag, 1995). In this study the effect of gamma radiation on color, pH, absorbance, turbidity and COD have been evaluated. Color of wastewater decreased sharply at 1-3 kGy, but not decreased at higher radiation levels. It was observed that the radiation could not completely fade the color, but it can be decreased to a satisfactory level.

Some author reported the AOPs applications without radiation exposure and were found not to be very effective for the treatment of PPMWW such as O$_3$ (Ko et al., 2009; Kreetachat et al., 2007; Medeiros et al., 2008; Rodriguez et al., 1998), heterogeneous catalytic O$_3$ processes (Balcioğlu et al., 2007; Fontanier et al., 2005) and Fenton's treatment (Pirkanniemi et al., 2007). From literature survey, it is concluded that the AOP are efficient for the treatment of wastewater however, photo-catalytic treatment has been found superior for wastewater treatment, WQPs improvement and toxicity reduction.

### 1.29 Soap and detergent mills wastewater treatment

The water quality parameters of soap and detergent wastewater, before treatment, are given in Table 4.2. The measured pH, DO, COD, TDS and BOD were beyond the permissible limits which were found to be in the following range; pH 11.0-11.6, DO 1.7-2.01 mg/L, COD 1350-1500 mg/L, TDS 1289-1370 mg/L, TSS 700-790 mg/L and BOD 550-700 mg/L. The wastewater samples collected from different soap and detergent units were subjected to UV and gamma radiation treatment and various independent variables were optimized for maximum degradation of the pollutants as well as for the improvement of water quality parameters. Finally, the effect of AOPs on toxicity reduction of effluents under
investigation was evaluated on the basis of biological and toxicological tests such as microbial load, *haemolytic, shrimp* and *allium cepa*.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ph</th>
<th>DO mg/L</th>
<th>COD mg/L</th>
<th>TDS mg/L</th>
<th>TSS mg/L</th>
<th>BOD mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>11.6±0.63</td>
<td>1.8±0.6</td>
<td>1500±71.5</td>
<td>1165±58.25</td>
<td>700±82.7</td>
<td>600±35</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11.5±0.53</td>
<td>1.7±0.15</td>
<td>1700±76.5</td>
<td>1370±68.50</td>
<td>750±62.62</td>
<td>700±40</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11.0±0.58</td>
<td>2.1±0.7</td>
<td>1350±64.25</td>
<td>1289±64.45</td>
<td>790±37.00</td>
<td>550±41</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Industry 1, 2 and 3 wastewater was collected from Faisalabad, Sargodha and Gujranwala, respectively, COD-chemical oxygen demand, BOD-biological oxygen demand, TDS-total dissolve solids and TSS-total suspended solids.

1.29.1 UV radiation treatment of soap and detergent wastewater

The soap and detergent wastewater was treated by UV radiation and different independent variable (H₂O₂ concentration, UV exposure time, pH, shaking and TiO₂ concentration) were optimized for maximum degradation of the pollutants. The treatment efficiency was evaluated on the basis of percentage degradation, improvement of water quality parameters and toxicity reduction of treated wastewater. For the assessment of water quality, parameters such as BOD, COD, DO, pH, TDS and TSS were recorded, while toxicity was measured using different biological and toxicological tests like total bacterial count, total coliform count, *allium cepa, haemolytic* and *shrimp* tests. The UV treatment was carried out in the presence of 1.5-3.5% H₂O₂ for 40-120 min UV exposure (22-44 Watt) at 6-12 pH and shaking speed 50-150 rpm using 3-7 mg TiO₂. Maximum degradation (97%) of wastewater collected from soap and detergent units was recorded. The RSM analysis also revealed that UV power, 44 Watt for 100 min exposure, concentration of TiO₂ (5.93 mg) at 4.39% v/v of H₂O₂, pH (6.50) and shaking speed (110 rpm) were the most effective for maximum degradation of pollutant present in soap and detergent wastewater (Figs. 4.2.1ab) and the polynomial relation between TiO₂ concentration and wastewater is shown in Eq. 4.2.1, where X₂ is representing the TiO₂ concentration which showed that the TiO₂ concentration affected the degradation significantly.
D(%) soap & detergent = 54.47(±4.13) + 13.73(±0.5)X2 + \epsilon \quad \text{(Eq. 4.2.1)}

Fig. 4.2.1a: Response surface and contour plots showing percentage degradation of pulp and paper wastewater subjected to the UV irradiation in the presence of hydrogen peroxide.

Fig. 4.2.1b: Response surfaces and contour plots showing percentage degradation of pulp and paper wastewater treated by UV radiation in the presence of H₂O₂. (A) Effect of pH, (B) effect of shaking speed, (C) effect of UV exposure time and (D) effect of TiO₂.
1.29.2 Effect of UV/H₂O₂/TiO₂ on water quality parameters

The water quality parameter such as BOD, COD, DO, pH, TSS and TDS of soap and detergent wastewater were determined in order to evaluate the efficiency of treatment. The samples were treated by UV radiation (UV power 44 Watt) for 100 min using TiO₂ (3-7 mg) and H₂O₂ (4.39%) at shaking speed of 150 rpm. The results regarding wastewater quality parameters are shown in Figs. 4.2.2ab. The reduction in COD and BOD were observed up to 48 and 52% for 3 mg TiO₂, by increasing the TiO₂ concentration to 5 mg, the reduction in COD and BOD were observed 61% and 68%, respectively and no considerable reduction in COD and BOD was observed on further increasing concentration of TiO₂ to 7 mg. Through RSM analysis, it was observed that maximum reduction in COD and BOD took place for TiO₂ concentration of 3.66 mg. The pH of the solution reduced significantly after UV/TiO₂/H₂O₂ treatment which is considered as a good efficiency of the treatment. Before treatment the pH of soap and detergent wastewater was in the alkaline range (11.0-11.6) and after UV/TiO₂/H₂O₂ treatment, it was reduced and become acidic (5.4-6.3). Similarly, the TDS and TSS values of soap and detergent wastewater were also reduced significantly, 45% and 46% after treatment using 3 mg TiO₂. However, TDS and TSS reduction was enhanced to 64% and 77% using 5 mg TiO₂. The radiation treatment in the presence of H₂O₂ and TiO₂ increased the DO values (66%) of soap and detergent wastewater. Although the radiation treatment in the presence of H₂O₂ has significant effect on degradation of the pollutants and to improve water quality, however, addition of TiO₂ furnished promising efficiency.
Fig. 4.2.2a: Response surfaces and contour plots showing the COD, BOD, pH and TDS of soap and detergent wastewater after treatment by UV radiation in the presence of TiO$_2$ and H$_2$O$_2$.

Fig. 4.2.2b: Response surfaces and contour plots showing the TSS and DO of soap and detergent wastewater treated by UV radiation in the presence of TiO$_2$ and H$_2$O$_2$. 

### 1.29.3 Effect of UV/TiO₂/H₂O₂ on soap and detergent wastewater toxicity

The toxicity of soap and detergent wastewater was evaluated before and after the application of UV/H₂O₂/TiO₂ and the results are shown in Table 4.1.3.4. The wastewater were treated by UV radiation (UV power 44 Watt) having concentration of TiO₂ (5.93 mg) and H₂O₂ (4.39%) at shaking speed of 150 rpm and UV exposure time 100 min. The total bacterial and total coliform population was recorded to be >1 x 10⁶ and >1 x 10⁵ before treatment and after treatment both the microbes were not detected. The *A. cepa* tests also showed the treatment efficiency, before treatment the number of roots and root lengths were recorded to be in the range 10-11 and 3.2-4.1 cm, respectively and after treatment, the increase in number of roots and root lengths were observed 44% and 51%, respectively. Treatment also showed reduction in lysis of red blood cell and shrimp death up to 80% and 85%, respectively (Table 4.2.3). However, toxicological tests showed that the UV/H₂O₂/TiO₂ treatment system was very effective and efficient for the reduction of microbial load and toxicity as well which were evaluated through *A. cepa*, haemolytic and shrimp tests.

#### Table 4.2.3: Toxicity of soap and detergent wastewater before and after treatment evaluated through biological and toxicological tests

<table>
<thead>
<tr>
<th>Microbial test</th>
<th>Before treatment</th>
<th>Allium cepa</th>
<th>Haemolytic</th>
<th>Shrimp test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBC CFU</td>
<td>T coliform CFU</td>
<td>RC cm</td>
<td>RL cm</td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>&gt;1 x 10⁶</td>
<td>1 x 10⁵</td>
<td>10±0.20</td>
<td>3.2±0.04</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>&gt;1 x 10⁶</td>
<td>1 x 10⁵</td>
<td>11±0.19</td>
<td>3.7±0.03</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>&gt;1 x 10⁶</td>
<td>1 x 10⁵</td>
<td>10±0.22</td>
<td>4.1±0.03</td>
</tr>
<tr>
<td>PC</td>
<td>----</td>
<td>----</td>
<td>15±0.26</td>
<td>7.0±0.11</td>
</tr>
<tr>
<td>NC</td>
<td>----</td>
<td>----</td>
<td>11±0.20</td>
<td>3.0±0.02</td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>17±0.40</td>
<td>6.5±0.12</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>16±0.32</td>
<td>7.3±0.25</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>18±0.51</td>
<td>6.9±0.09</td>
</tr>
</tbody>
</table>

| % reduction        | Industry 1 (n = 3)| 100          | 41.00 | 50.76 | 79.71 | 85.24 |
|                    | Industry 2 (n = 3)| 100          | 31.25 | 49.31 | 75.00 | 83.33 |
|                    | Industry 3 (n = 3)| 100          | 44.44 | 40.57 | 76.11 | ---- |

| TBC-total bacterial count, TC-total coliform, ACT-*Allium cepa* test, MI-mitotic index, RC-root count, RL-root length, NR-not recorded, ND-not detected, PC-positive control, NC-negative control, n-sample seeded in triplicate |
| For *haemolytic* test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively |
| For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively |
| For *shrimp* test, PC and NC were cyclophosphamide (10 µg/mL) and sea water, respectively |
1.29.4 Gamma ray treatment of soap and detergent wastewater

The soap and detergent wastewater was treated by gamma radiation for the absorbed doses of 5, 10 and 15 kGy in the presence of H\textsubscript{2}O\textsubscript{2}. The efficiency of gamma radiation treatment was evaluated on the basis of WQPs like COD, BOD, DO, TDS, TSS and pH as well as toxicity reduction efficiency of soap and detergent wastewater on the basis of microbial load, *A. cepa*, *haemolytic* and *brine shrimp* tests. It has been observed that the degradation of contaminants present in wastewater is directly related to the WQPs before treatment which were found to be much higher than permissible limits. The gamma radiation treatment was carried out in the presence of H\textsubscript{2}O\textsubscript{2} (1.5-5.5\%) for the absorbed doses of 5, 10 and 15 kGy and resultanty a significantly enhanced degradation of pollutants was recorded. A degradation of 41\%, 57\% and 69\% was observed for absorbed doses of 5, 10 and 15 kGy in the presence of 1.5\% H\textsubscript{2}O\textsubscript{2}, respectively. By increasing the H\textsubscript{2}O\textsubscript{2} percentage to 3.5\%, the degradation increased up to 65\%, 97.7\% and 98.5\% for the absorbed doses of 5, 10 and 15 kGy, respectively and by increasing H\textsubscript{2}O\textsubscript{2} further to 5.5\%, the degradation of soap and detergent wastewater was not enhanced which indicate that more than the optimized concentration of H\textsubscript{2}O\textsubscript{2} was not effective for the degradation of contaminants (Fig. 4.2.4a). It is also considered that the degradable contaminants are degraded at 10 kGy of absorbed dose and further degradation of the remaining residues became slow. Through RSM analysis, it was observed that 12.69 kGy absorbed dose and 4.65\% H\textsubscript{2}O\textsubscript{2} were found to be better for maximum degradation of contaminants present in soap and detergent wastewater. The polynomial equation (Eq. 4.2.4) showed that gamma radiation absorbed dose and H\textsubscript{2}O\textsubscript{2} concentration both affected the degradation significantly, however, the second degree term was non significant in case of H\textsubscript{2}O\textsubscript{2}. As a result of gamma radiation treatment in the presence of H\textsubscript{2}O\textsubscript{2}, the WQPs improved significantly. The reduction in COD is considered an important parameter to analyze the degree of pollution in term of organic contents present in the wastewater. After gamma ray treatment, a considerable improvement was observed since the COD value decreased sharply with increasing the radiation absorbed dose. The decreases in COD values were 26\%, 36\% and 43\% when samples were exposed to the absorbed doses of 5, 10 and 15 kGy in the presence of 3\% H\textsubscript{2}O\textsubscript{2}. By increasing the H\textsubscript{2}O\textsubscript{2} up to 5\% for the same absorbed doses, the reduction in COD were recorded up to 46\%, 70\% and 71\% and by increasing the H\textsubscript{2}O\textsubscript{2} up to 5.5\%, there was no considerable reduction in COD values (Fig. 4.2.4b).
4.2.4b). It is reported that COD reduction is directly related to degradation because when the complex organic molecules break down into small molecules upon treatment, then COD reduced significantly (Fang and Wu, 1999). Similar to the COD reduction, other WQPs such as BOD and TDS were improved and pH of the treated sample also reduced significantly which is also a good indication of pollutants degradation (Figs. 4.2.4b). The percentage reduction in BOD of soap and detergent wastewater sample subjected to the absorbed doses of 5, 10 and 15 kGy in the presence of 1.5% H₂O₂ were 31%, 39% and 49% and by increasing the H₂O₂ concentration to 3.5%, the BOD value decreased to 52%, 72% and 75% for the absorbed dose of 5, 10 and 15 kGy. The reduction in TDS, TSS and increase in DO of treated sample was also found maximum for 3.5% H₂O₂. The decrease in TDS was recorded to be 21%, 28% and 34%, while reduction in TSS was 26%, 37% and 44% for the absorbed dose of 5, 10 and 15 kGy in the presence of 3.5% H₂O₂ concentration, respectively (Fig. 4.2.4c). Under same conditions, the increase in DO was recorded to be 26%, 37% and 44% for 1.5% H₂O₂ and 53%, 70% and 76% for 3.5% H₂O₂ concentration (Figs. 4.2.4c). After treatment, the pH of treated samples reduced significantly which was a good indication of degradation because the organic compound after degradation are converted into low molecular weight organic acids and ultimately, the pH of treated sample decreases. Before treatment, the pH of samples was highly basic and after treatment that was decreased and recorded to be in the range of 5.9-7.9.

\[
D(\%) \text{ soap & detergent} = -43.18(\pm12.97) + 6.90(\pm2.25)X_1 + 41.35(\pm5.14)X_2 - 0.60(\pm0.21)X_1 X_2 - 3.56(\pm0.65)X_2^2 + \epsilon
\]  
(Eq. 4.2.4)

Where \(X_1\) and \(X_2\) are representing H₂O₂ concentration and gamma radiation absorbed dose, respectively.
Fig. 4.2.4a: Response surface and contour diagram showing percentage degradation of soap and detergent wastewater treated by gamma ray for the absorbed doses of 5, 10 and 15 kGy in the presence of H₂O₂.

Fig. 4.2.4b: Response surfaces and contour plots showing BOD, COD, pH and TDS of soap and detergent wastewater irradiated to the absorbed doses of 5, 10 and 15 kGy in the presence of H₂O₂.
1.29.5 Effect of gamma ray treatment on soap and detergent wastewater toxicity

The biological safety of soap and detergent wastewater was evaluated on the basis of various biological and toxicological assays before and after the application of gamma radiation treatment in the presence of H$_2$O$_2$. It was found that the wastewater samples were highly toxic before treatment and the total bacterial and coliform counts were detected more than 1 x 10$^6$ and >1 x 10$^5$ CFU (Table 4.2.5). After gamma radiation treatment, a significant reduction in toxicity was recorded. The toxicity of wastewater samples subjected to the absorbed dose of 15 kGy in the presence of 3.5% H$_2$O$_2$ was evaluated and results are shown in Table 4.2.5. The total bacterial and total coliform counts were not detected after treatment. The *A. cepa* tests also showed a significant reduction in cytotoxicity and before treatment the number of root and root lengths were recorded to be ranges from 10-11 and 3.2-4.1 cm, respectively and after treatment, the increase in number of roots and root lengths were recorded to be 23.07-28.57% and 30.50-41.26%, respectively. The soap and detergent wastewater also showed red blood cell lysis and *shrimp* napoli death up to 66% and 59% before gamma radiation treatment. However, after treatment, the lysis of red blood cell and *shrimp* death rate reduced considerably. The reduction in red blood cell lysis and *shrimp* napoli death were reduced up to 70.76% and 82.75%, respectively. Overall, toxicological tests showed that the gamma radiation treatment in the presence of H$_2$O$_2$ showed a significant
efficiency for toxicity reduction and this technique can be used successfully for the treatment of soap and detergent wastewater to make it eco-friendly.

Table 4.2.5: Toxicity of soap and detergent wastewater before and after treatment of gamma radiation in the presence of H₂O₂ evaluated through microbial analysis, *Allium cepa*, *haemolytic* and *brine shrimp* assays

<table>
<thead>
<tr>
<th>Microbial test</th>
<th>Allium cepa</th>
<th>Haemolytic</th>
<th>Shrimp test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>TBC</td>
<td>T coliform</td>
<td>RC</td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>&gt;1 x 10⁶</td>
<td>1 x 10⁵</td>
<td>10±0.20</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>&gt;1 x 10⁶</td>
<td>1 x 10⁴</td>
<td>11±0.19</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>&gt;1 x 10⁶</td>
<td>1 x 10⁵</td>
<td>10±0.22</td>
</tr>
<tr>
<td>PC</td>
<td>----</td>
<td>----</td>
<td>15±0.26</td>
</tr>
<tr>
<td>NC</td>
<td>----</td>
<td>----</td>
<td>11±0.20</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>16±0.78</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>17.0±89</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>14±0.65</td>
</tr>
<tr>
<td>% reduction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>100</td>
<td>100</td>
<td>37.50</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>100</td>
<td>100</td>
<td>35.29</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>100</td>
<td>100</td>
<td>28.57</td>
</tr>
</tbody>
</table>

PC-positive control, NC-negative control, TBC-total bacterial count, TC-total coliform, ACT-*Allium cepa* test, MI-mitotic index, RC-root count, RL-root length, NR-not recorded, ND-not detected, n-sample seeded in triplicate
For *haemolytic* test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively
For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively
For *shrimp* test, PC and NC were cyclophosphamide (10µg/mL) and sea water, respectively

Soap and detergent wastewater contained surfactant, so the literature survey regarding degradation, improvement of water quality parameter and toxicity reduction is discussed in nonylphenol ethoxylate section (surfactant) below.

1.30 Treatment of petroleum wastewater by UV and gamma ray treatment

1.30.1 Water quality parameters of petroleum wastewater before treatment

The water quality parameters of petroleum refinery wastewater collected from Karachi, Rawalpindi and Kot Addu, before treatment are given in Table 4.3.1. The recorded pH, DO, COD, TDS, TSS and BOD values were found to be very high and beyond the permissible limit. After measuring the WQPs and toxicity, the selected AOPs were applied for the treatment of petroleum refinery wastewater. Various independent variables were optimized through RSM analysis under RCCD for maximum degradation of pollutant and for the improvement of WQPs. Finally, the effect of AOPs on toxicity reduction of effluents was
evaluated on the basis of cytotoxicity and mutagenicity such as *haemolytic, shrimp, A. cepa* and *Ames* tests. The microbial population of wastewater was also measured before and after treatment. Before treatment, the petroleum refinery wastewater was found to be highly cytotoxic and mutagenic in nature and direct discharge of petroleum wastewater into the environment might be harmful for the living organisms.

Table 4.3.1: The water quality parameters of petroleum wastewater before treatment

<table>
<thead>
<tr>
<th>Wastewater</th>
<th>pH</th>
<th>DO</th>
<th>COD</th>
<th>BOD5</th>
<th>TDS</th>
<th>TSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum refinery I(^a)</td>
<td>09±0.2</td>
<td>0.9±0.04</td>
<td>1800±70</td>
<td>620±21</td>
<td>3200±120</td>
<td>420±14</td>
</tr>
<tr>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petroleum refinery II(^b)</td>
<td>09±0.2</td>
<td>1.1±0.05</td>
<td>2150±95</td>
<td>580±26</td>
<td>4000±141</td>
<td>510±24</td>
</tr>
<tr>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petroleum refinery III(^c)</td>
<td>10±0.2</td>
<td>1.2±0.04</td>
<td>1300±25</td>
<td>598±16</td>
<td>3650±129</td>
<td>485±19</td>
</tr>
<tr>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
\(^a\)Karachi, \(^b\)Rawalpindi and \(^c\)Kot Addu

1.30.2 Treatment of petroleum refinery wastewater by UV/H\(_2\)O\(_2\)/TiO\(_2\)

The petroleum refinery wastewater was treated by UV radiation and different independent variables such as H\(_2\)O\(_2\) concentration, UV exposure time, pH, shaking speed and TiO\(_2\) concentration were optimized for maximum degradation of the toxic compounds. The treatment efficiency was evaluated on the basis of degradation, WQPs improvement as well as cytotoxicity and mutagenicity reduction of treated wastewater. The UV treatment was applied and data thus obtained was analyzed through RSM for the optimization of independent parameters for maximum degradation of pollutant present in the wastewater. The UV treatment was carried out in the presence of 3.5-7.5% H\(_2\)O\(_2\) and 4-10 mg TiO\(_2\) for 50-130 min UV exposure time (55 Watt) at 50-150 rpm shaking speed and at 4-10 pH. Degradation data analysis showed that the maximum degradation of pollutant present in petroleum wastewater took place in the presence of 6.96% H\(_2\)O\(_2\) concentration and 7.54 mg TiO\(_2\) for 128 min of UV exposure time at 140 rpm shaking speed and 5.5 pH. By using these optimized levels of independent variables, up to 96% degradation of pollutant was achieved (Fig. 4.3.2) and polynomial relation of TiO\(_2\) based degradation can be seen in Eq.4.3.2, Where X\(_2\) is the concentration of TiO\(_2\).
D(%) petroleum = 52.08(±2.93) + 12.36(±0.73)X2 − 8.19(±0.49)X2 2 + ϵ  (Eq.4.3.2)

Fig. 4.3.2: Response surfaces and contour plots showing degradation of pollutant in petroleum wastewater treated by UV in the presence of H2O2 and TiO2: A-effect of H2O2, B-effect of UV exposure time, C-effect of shaking speed, D-effect of TiO2 concentration and E-effect of pH on degradation

1.30.3 Water quality of petroleum wastewater treated by UV/H2O2/TiO2

The water quality parameter such as BOD, COD, DO, pH, TSS and TDS of petroleum refinery wastewater were considered to evaluate the efficiency of UV treatment.
The WQPs of petroleum wastewater was measured for those samples which were treated at optimized conditions such as 6.96% H₂O₂ concentration, 128 min. of UV exposure, 140 rpm shaking speed and 4-10 mg TiO₂ concentration. Before treatment, the values of water quality parameters were much higher than the permissible limit and as expected from degradation behavior, the WQPs improved significantly and results are shown in Fig. 4.3.3. The reduction in COD and BOD were observed up to 68% and 81% for 4 mg TiO₂, by increasing the TiO₂ concentration more than 4 mg the efficiency of degradation was reduced and reduction in COD and BOD were 77% and 86%, respectively for 7 mg TiO₂ concentration. The pH of the petroleum wastewater reduced significantly after UV/TiO₂/H₂O₂ treatment which was considered as a good sign of the treatment because as a result of degradation of organic pollutants, low molecular mass acid were produced and resultantly, the pH of the treated solution decreased significantly. Before treatment the pH of petroleum refinery wastewater was in the range of 9.0-10.0 and after photo-catalytic (UV/TiO₂/H₂O₂) treatment that reduced to 4.8-5.9. The TDS and TSS values were also decreased significantly after UV/TiO₂/H₂O₂ application, the reduction were 52% and 66% when 4 mg TiO₂ and 63% and 71% reductions in TDS and TSS values were observed by using 7 mg TiO₂, respectively. The radiation treatment in the presence of H₂O₂ and TiO₂ increased the DO value of treated samples and the increase in DO was recorded up to 53% when treatments were performed at optimum conditions.
**1.30.4 Toxicity of UV irradiated petroleum wastewater**

The microbial load, cytotoxicity and mutagenicity assays of petroleum refinery wastewater were performed before as well as after the application of UV/H₂O₂/TiO₂ in order to evaluate the efficiency of AOP for toxicity reduction; the results are shown in table 4.3.4. The total bacterial and total coliform count were recorded as $> 1 \times 10^5$ and $>1 \times 10^4$ before treatment and after treatment both the microbes were not detected in any of the sample. The cytotoxicity evaluated through *A. cepa*, haemolytic and shrimp assays showed that the petroleum refinery wastewater was cytotoxic in nature and after treatment the cytotoxicity reduced significantly. The root count, root length and mitotic index increased up to 70%, 64% and 89%, respectively (Table 4.3.4). The *Ames* test (TA98 and TA100) was applied to evaluate the mutagenicity of refinery wastewater. The results show that the refinery effluents were mutagenic in nature. After treatment, the mutagenicity reduced up to 84.09% and 86.36% for TA98 and TA100, respectively. The toxicity results showed that the UV
treatment in the presence of H₂O₂ and TiO₂ could be used successfully for the reduction of microbial load, cytotoxicity and mutagenicity of petroleum refinery wastewater.

The studies evaluating the detoxification effect of AOPs from treated petroleum refinery effluents (PRE), few reports have revealed the efficiency of AOPs for toxicity reduction of PRE’s, the effect of UV/TiO₂/O₃ has been evaluated by (Corrêa et al., 2009). Authors showed that this treatment reduces the PRE toxicity tested by Vibrio fischeri and the fish Poecilia vivipara. The treatment of 60 min showed lower acute toxicity toward bacteria and remained high for Poecilia vivipara. Thus, the results showed that a combination of O₃/UV/TiO₂ seems to be a good option for cost effective treatment of water streams, especially for toxicity reduction.

Table 4.3.4: Microbial load, cytotoxicity and mutagenicity of petroleum refinery wastewater before and after treatment by UV radiation in the presence of H₂O₂ and TiO₂:

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>ACT</th>
<th>Haemolytic</th>
<th>Shrimp</th>
<th>Ames</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBC CFU</td>
<td>TC CFU</td>
<td>RC</td>
<td>RL</td>
<td>MI</td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>&gt;1 x 10⁸</td>
<td>&gt;1 x 10⁹</td>
<td>8±±0.33</td>
<td>2.7±0.11</td>
<td>9.0±0.40</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>&gt;1 x 10⁸</td>
<td>&gt;1 x 10⁹</td>
<td>6±±0.20</td>
<td>3.1±0.15</td>
<td>8.0±0.31</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>&gt;1 x 10⁸</td>
<td>&gt;1 x 10⁹</td>
<td>9±±0.39</td>
<td>2.9±0.09</td>
<td>10.0±4±4</td>
</tr>
<tr>
<td></td>
<td>PC ---</td>
<td>---</td>
<td>18±±0.80</td>
<td>7.0±±0.38</td>
<td>23±±0.98</td>
</tr>
<tr>
<td></td>
<td>NC 0</td>
<td>0</td>
<td>12±±0.53</td>
<td>3.0±±0.15</td>
<td>11.3±±0.3</td>
</tr>
<tr>
<td></td>
<td>Industry 1 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>21±±1.1</td>
<td>7.5±±0.37</td>
</tr>
<tr>
<td></td>
<td>Industry 2 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>20±±1.0</td>
<td>8.0±±0.40</td>
</tr>
<tr>
<td></td>
<td>Industry 3 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>22±±1.2</td>
<td>7.0±±0.38</td>
</tr>
<tr>
<td>% reduction</td>
<td>aIndustry 1 (n = 3)</td>
<td>100</td>
<td>100</td>
<td>61.9</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>aIndustry 2 (n = 3)</td>
<td>100</td>
<td>100</td>
<td>70</td>
<td>61.25</td>
</tr>
<tr>
<td></td>
<td>aIndustry 3 (n = 3)</td>
<td>100</td>
<td>100</td>
<td>59.09</td>
<td>58.57</td>
</tr>
</tbody>
</table>

aKarachi, bRawalpindi and cKot Addu

TBC—total bacterial count, TC—total coliform, ACT—Allium cepa test, MI-mitotic index, RC-root count, RL-root length, ND—not detected, PC-positive control, NC-negative control, n-sample seeded in triplicate

For haemolytic test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively

For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively

For shrimp test, PC and NC were cyclophosphamide (10 µg/mL) and sea water, respectively

For Ames test, PC for TA98 and TA100 were K₂Cr₂O₇ (0.01 g/L) and NaN₃ (0.5 µg/100 µL), respectively and background (without standard and tested compound) was used as NC
1.30.5 Petroleum wastewater gamma ray/H\textsubscript{2}O\textsubscript{2} treatment and WQPs

The optimum H\textsubscript{2}O\textsubscript{2} concentration was used during gamma ray treatment; the wastewater samples from different petroleum industries (Karachi, Rawalpindi and Kot Addu) were used as variables under RCCD and data thus obtained was analyzed through RSM for the detection of optimum radiation absorbed dose. The gamma radiation treatment efficiency in the presence of H\textsubscript{2}O\textsubscript{2} was evaluated on the basis of extent of degradation of pollutants, improvement in WQPs and toxicity reduction. The petroleum refinery wastewater was treated to the absorbed doses of 5 kGy, 10 kGy and 15 kGy in the presence of 6.96% H\textsubscript{2}O\textsubscript{2} concentration (optimized for the UV treatment). The maximum degradation of 64%, 82% and 94% was achieved for the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively (Fig. 4.3.5). Through RSM analysis, it was observed that the maximum degradation of petroleum refinery wastewater can be achieved for the absorbed dose of 14.98 kGy under this experimental set up. As a result of gamma radiation treatment in the presence of H\textsubscript{2}O\textsubscript{2}, the water quality parameter of petroleum refinery wastewater also improved significantly. The reduction in COD has been considered an important parameter to analyze the degree of degradation in term of organic contents present in wastewater. The COD removal was 51%, 61% and 77% when samples were exposed to the absorbed doses of 5 kGy, 10 kGy and 15 kGy in the presence of 6.96% H\textsubscript{2}O\textsubscript{2}. The BOD removal was 64%, 81% and 86% for the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively. The TDS removed up to 38%, 53% and 56%, while TSS values reduced to 48%, 61% and 70% and increase in DO was recorded to be 38%, 41% and 47% for the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively. The pH of treated samples were also reduced significantly and came within the mild acidic range (Fig. 4.3.5) and the polynomial equation representing the effect of TiO\textsubscript{2} and wastewater nature on degradation is shown in equation 4.3.5, where X\textsubscript{2} is the gamma radiation absorbed dose. Polynomial equation showed that TiO\textsubscript{2} concentration played a significant role for the degradation of pollutant irrespective of wastewater nature.
D(%) Petroleum = 25.79(±1.67) + 12.36(±0.73)X2 – 5.45(±1.20)X22 + ε  \hspace{1cm} (Eq. 4.3.5)

Fig. 4.3.5: Response surfaces and contour plots showing degradation of petroleum refinery wastewater treated by gamma radiation in the presence of H₂O₂ and its effect on water quality parameters: (A) % degradation, (B) COD, (C) BOD, (D) pH, (E) TDS, (F) TSS and (G) DO, WW = wastewater

1.30.6 Toxicity of petroleum wastewater treated by gamma ray/H₂O₂

The main aim of wastewater treatment is the biological efficacy that the treated wastewater are fit to discharge into the environment or its re-utilization. So, the efficiency of gamma ray treatment in the presence of H₂O₂ was evaluated by measuring the microbial load, cytotoxicity and mutagenicity of petroleum refinery wastewater before and after
treatment. It was found that the wastewater samples of petroleum refinery were cytotoxic and mutagenic in nature. The microbial population was also high enough before treatment (Table 4.3.6). As expected for the effect of gamma ray, the microbial population (bacterial and coliform) was not detected even for 5 kGy treatment. After gamma ray treatment, the cytotoxicity of wastewater sample was also reduced significantly evaluated through *A. cepa*, *haemolytic* and *shrimp* tests and the results are shown in Table 4.3.6. In case of *A. cepa* test, the root count, root length and mitotic index increased to 53.84%, 54.41% and 52.94%, respectively. The reduction in red blood cell lysis was recorded to be 80.24% and 85.29% for human and bovine cells, respectively. In case of *shrimp* test, the percentage death of napoli was also reduced significantly. Before treatment, the napoli died 100% within the period of 24 h and after treatment, 75% napoli death reduced. For mutagenicity evaluation, *Ames* test was used and results are shown in Table 4.3.6. Results indicated that gamma ray treatment in the presence of H2O2 reduced the mutagenicity considerably. The reduction in mutagenicity was recorded up to 72% and 74% using TA98 and TA100 strains, respectively (Table 4.3.6).

Table 4.3.6: Microbial load, cytotoxicity and mutagenicity of petroleum wastewater before and after gamma ray treatment in the presence of H2O2

<table>
<thead>
<tr>
<th>Microbial</th>
<th>ACT</th>
<th>Haemolytic</th>
<th>Shrimp</th>
<th>Ames</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFU</td>
<td>CFU</td>
<td>cm</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>&gt;1 x 10^4</td>
<td>&gt;1 x 10^4</td>
<td>8±0.33</td>
<td>2.7±0.11</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>&gt;1 x 10^4</td>
<td>&gt;1 x 10^4</td>
<td>6±0.20</td>
<td>3.4±0.15</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>&gt;1 x 10^4</td>
<td>&gt;1 x 10^4</td>
<td>9±0.39</td>
<td>2.9±0.09</td>
</tr>
<tr>
<td>PC</td>
<td>18±1.0</td>
<td>7±0.41</td>
<td>18±0.80</td>
<td>7±0.38</td>
</tr>
<tr>
<td>NC</td>
<td>5±0.23</td>
<td>2±0.11</td>
<td>12±0.53</td>
<td>3±0.15</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industry 1 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>13±0.48</td>
<td>5.1±0.20</td>
</tr>
<tr>
<td>Industry 2 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>13±0.65</td>
<td>6.8±0.31</td>
</tr>
<tr>
<td>Industry 3 (n = 3)</td>
<td>ND</td>
<td>ND</td>
<td>14±0.88</td>
<td>5.6±0.26</td>
</tr>
</tbody>
</table>

% reduction

| Industry 1 (n = 3) | 100 | 100 | 38.46 | 47.05 | 43.75 | 80.24 | 83.58 | 75 | 70.11 | 74.11 |
| Industry 2 (n = 3) | 100 | 100 | 53.84 | 54.41 | 52.94 | 78.75 | 85.29 | 70 | 71.59 | 71.08 |
| Industry 3 (n = 3) | 100 | 100 | 35.71 | 48.21 | 37.5 | 78.04 | 83.82 | 75 | 70 | 74.39 |

TBC-total bacterial count, TC-total coliform, ACT-*Allium cepa* test, MI-mitotic index, RC-root count, RL-root length, ND-not detected, PC-positive control, NC-negative control, n-sample seeded in triplicate

For *haemolytic* test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively

For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively

For *shrimp* test, PC and NC were cyclophosphamide (10 µg/mL) and sea water, respectively

For *Ames* test, PC for TA98 and TA100 were K2Cr2O7 (0.01 g/L) and NaN3 (0.5 μg/100 μL), respectively and background (without standard and tested compound) was used as NC

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It has been reported that petroleum refineries wastewater has very complex nature (Al Zarooni and Elshorbagy, 2006; Diyauddeen et al., 2011; Wake, 2005) and for the degradation of petroleum wastewater different methods and technologies have been used and reported such as (sedimentation/flotation) (Thompson et al., 2001), coagulation and precipitation (Fu and Wang, 2011), adsorption (Mahmoodi et al., 2011), oxidation (Oller et al., 2011b) and membrane filtration (Judd and Judd, 2011), biological treatment- aerobic treatment (Chan et al., 2009) and integrated treatment processes (Pokhrel and Viraraghavan, 2004a). However, the most widely applied technique is bioremediation and the complete degradation of pollutants present in petroleum wastewater prove difficult (Fratila-Apachitei et al., 2001).

The application of photo-catalytic treatment of industrial wastewater is an emerging technology and was found very promising for the improvement of water quality parameters and toxicity reduction. The photo–catalytic oxidation and mineralization of PRE in aqueous catalyst suspensions of TiO₂ were carried out (Shahrezaei et al., 2012). The treatment effect was evaluated on the basis of COD reduction as function of pH, catalyst, temperature and reaction time and resultanty considerable COD removal was achieved. A maximum reduction in COD of more than 83% was achieved at the optimum conditions (pH of 4, catalyst concentration of 100 mg/L, temperature of 45°C and reaction time of 120 min). In another study, the application of TiO₂/UV irradiation for the treatment of PRE has been reported (Saien and Nejati, 2007). The optimum catalyst concentration, pH and temperature were found to be 100 mg/L, 3 and 318 K, respectively. A maximum reduction in COD was > 90% for 4 h treatment and 73% for 90 min irradiation.

While studying the photo Fenton process to treat wastewater contaminated with HC’s, (Moraes et al., 2004) evaluated the effect of H₂O₂, (100–200 mM), iron ions (0.5–1 mM), and sodium chloride (200–2000 ppm) on TOC variation. Results showed that the PF process was feasible for the treatment of wastewaters containing HC’s in the presence of high concentration of salt. While comparing the efficiency of TiO₂ and ZnO, (Santos et al., 2006) found the optimized levels 3.0 g/L TiO₂ and pH 6.3 for the treatment PRE having high COD and BOD values and resultantly improved the quality of the treated wastewater significantly. In another study, (Saien and Shahrezaei, 2012) reported the use of UV/TiO₂ for PRE treatment. The light source was mercury UV lamp (400 W, 200–550 nm). Optimal catalyst
concentration, fluid pH, and temperature were obtained at amounts of near 100 mg/L, 3 and 45°C, respectively. A maximum reduction in COD of more than 78% was achieved after about 120 min and 72% after 90 1.5 h treatments. Similar to this study, (Corrêa et al., 2009) also used UV/TiO₂ in addition to O₃. The results showed that for 5 min of treatment, the O₃/UV/TiO₂ combination was very effective and phenol concentration decreased by 99.9%, sulfide by 53.0%, COD by 37.7%, O & G by 5.2%, and NH₃ by 1.9%. The following reductions in contaminants were obtained after 60 min of oxidation treatment: phenols 99.9%, O&G 98.2%, sulfide 97.2%, COD 89.2% and NH₃ 15%. Thus, author concluded that the combination of O₃/UV/TiO₂ for 10 min seems to be a good option for cost effective treatment of PRE.

Another AOPs treatment, the Fe(III)/H₂O₂/Solar-UV process has also been applied for the treatment of PRE (Parili, 2010). Organic matter removal was monitored throughout the operation period. The maximum reduction in the TOC was 49% with the addition of 2.6 g/L H₂O₂ and 0.5mM Fe(III) at a 10 L/h flow rate after 8 h of exposure to solar irradiation. In another study, ZnO/SnO₂/UV was also used to remove organic pollutants from PRE. The ZnO/SnO₂ (2:1) catalyst gave the optimum photo–degradation of 23.38 % (Ali et al., 2010).

Metal oxides such as TiO₂ and ZnO have been used for the treatment of PRE treatment by the UV radiation. The optimized conditions obtained with an experimental design were 3.0 (g/L) TiO₂ and pH = 6.3 for maximum degradation (Soltanian and Bebbahani, 2011). In another study, the application of AOPs for the treatment of effluent contaminated with HC and oils was performed (Tony et al., 2009a). The AOPs conducted were Fe²⁺/H₂O₂, Fe²⁺/H₂O₂/UV and UV-photolysis. The experiments utilizing the photo Fenton treatment method with an artificial UV source, coupled with Fenton’s reagent, suggest that the oil is readily degradable. The results showed that the COD removal rate was affected by the PF significantly. Under these conditions, more than 70% the COD removal was achieved. Similar to this study, (Tony et al., 2009b) also applied homogenous PF and Fenton/TiO₂/UV, Fenton/ZnO/UV and Fenton/TiO₂/UV/Air) for the treatment of a diesel-oil wastewater emulsion. The augmentation of the photo Fenton process by heterogeneous TiO₂ increased the reaction rate, in terms of COD reduction efficiency from 61%-71%.

The studies evaluating the detoxification effect of AOPs from PRE are not so common, however, the reported studies revealed the efficiency of AOPs for toxicity reduction of PREs.
Using *Vibrio fischeri* as a test organism, the effect of UV/TiO₂/O₃ has been evaluated. Author showed that this treatment reduced the PRE toxicity tested by *Vibrio fischeri* and the fish *Poecilia vivipara* (Corrêa *et al.*, 2009). The treatment of 60 min showed lower acute toxicity toward bacteria and remained high *Poecilia vivipara*. Thus, the results showed that a combination of O₃/UV/TiO₂ seems to be a good option for cost effective treatment of water streams, especially for toxicity reduction. In another study, O₃/UV/TiO₂ and biological remediation by macroalgae were performed in a laboratory-scale reactor to evaluate the efficiency of these processes from ecotoxicological point of view. The results showed that after 5 min of treatment the O₃/UV/TiO₂ combination was very effective and the acute toxicity tests with the bacterium *Vibrio fischeri* (Lumistox) and the fish *Poecilia vivipara* showed a high toxicity of the raw effluents, while after 60 min of treatment effluents showed significantly less acute toxicity toward bacteria as compared to fish. Thus, author concluded that the O₃/UV/TiO₂ treatment for 10 min followed by biological treatment seems to be a good option for cost effective treatment PRE for the reduction of toxicity (Corrêa *et al.*, 2009).

### 1.31 Nonylphenol ethoxylates (NPEOs)

The NPEOs have extensive use in soap and detergent, pulp & paper and many other formulations due to their cost effectiveness and efficiency. Due to environmental concerns, they were banned by EPA and their substitute alcohol ethoxylates are being used. Alcohol ethoxylates are environmental friendly but very costly and are also less efficient than NPEOs. Several biological methods have been tried for the degradation of NPEOs, but their degradable intermediate and end products were found to be more toxic than the parent compounds. In present study NPEOs were selected and used as model compound for the photo-catalytic treatment. The simulated solution of NP6EO, NP9EO and NP30EO were treated by UV/H₂O₂/TiO₂ and γ ray/H₂O₂. The efficiency of AOPs using selected compound was evaluated on the basis of degradation, COD, TOC reduction and change in pH. The COD, TOC and pH of NPEOs were in the range of 338-321 mg/L, 208-216 mg/L and 7.1-7.3. The effect of AOPs on toxicity reduction of same selected samples of NPEOs treated at
optimized doses, were also subjected to toxicity tests such as *A. cepa, haemolytic, shrimp* and *Ames* tests which are considered as standard cytotoxic and mutagenic tests.

### 1.31.1 Degradation of NPEOs by UV/H$_2$O$_2$/TiO$_2$ system

The degradation of NPEO solution was carried out by UV irradiation in the presence of TiO$_2$ and H$_2$O$_2$. Various independent parameters such as H$_2$O$_2$ concentration, UV exposure time, pH, shaking speed, TiO$_2$ concentration and temperature were optimized for maximum degradation of NPEOs. For the treatment of NP6EO, the UV intensity of 55 Watt, H$_2$O$_2$ 1-5%, UV exposure time 30-80 min, pH 5-9, shaking speed 50-150 rpm, TiO$_2$ 1-5 mg and temperature 25-45°C were selected under rotatable central composite design. All independent variables were optimized such as UV exposure time, TiO$_2$ concentration, H$_2$O$_2$ percentage, pH, temperature and shaking speed and at optimum levels of all independent parameters, maximum degradation of 99% was achieved for 60 min UV irradiation time (55 Watt UV intensity) at 3.30% H$_2$O$_2$ and 3.95 mg% (per 10 mL of solution) TiO$_2$, 5.31 pH, shaking speed 110 rpm shaking speed and temperature 42°C (Figs. 4.4.1a and 4.4.1b). UV irradiation without TiO$_2$ & H$_2$O$_2$ and shaking did not affect the degradation of NPEO, while all other independent parameters such as H$_2$O$_2$, TiO$_2$, pH, UV exposure time and temperature significantly enhanced the degradation of NPEOs.
Fig. 4.4.1a: Response surfaces and contour plots showing degradation of NP6EO by UV radiation in the presence of H₂O₂ and TiO₂

Fig. 4.4.1b: Response surfaces and contour plots showing the effect of temperature and pH on NP6EO degradation using UV irradiation (60 min, 55 Watt) in the presence of H₂O₂ and TiO₂
Similar to NP6EO, NP9EO solution was also treated by UV radiation in the presence of H₂O₂ and TiO₂ and independent parameters were optimized for maximum degradation of NP9EO. The UV radiation was fixed 55 Watt for exposure of NP9EO solution for the period of 30-80 min using H₂O₂ 1-5%, TiO₂ 1-5 mg at 5-9 pH and shaking speed of 50-150 rpm under RCCD. At optimized levels of all selected independent parameters (H₂O₂ concentration, TiO₂ concentration, UV exposure time, pH, shaking and temperature), maximum degradation of 99.6% was achieved using UV lamp having 55 Watt intensity, 4.65% H₂O₂, 55 min UV exposure time, 5.10 pH, shaking speed 92 rpm, TiO₂ 4.45 mg and at 42°C temperature (Fig. 4.4.1c and 4.4.1d). The degradation behavior of NP30EO was also found to be similar to NP6EO and NP9EO. However, the H₂O₂ and TiO₂ concentrations were consumed more as compared to NP6EO and NP9EO and resultantly, the degradation of NP30EO were also recorded high as compared to NP6EO and NP9EO. A degradation up to 99.8% was achieved at optimized levels of all selected independent variables. The maximum degradation of NP30EO was observed for 5.70% H₂O₂, 4.85 mg TiO₂, UV exposure time 64 min (55 Watt), pH 5.35, shaking speed of 140 rpm and at temperature of 45°C (Fig. 4.4.1e and 4.4.1f). The UV–Vis spectrum of UV treated and untreated NP30EO and NP9EO are shown in Fig. 4.4.1i and Fig. 4.4.1j, respectively. The UV intensity without TiO₂ and H₂O₂ and shaking did not affect the degradation of NPEOs considerably, while all other independent parameters like H₂O₂, TiO₂, pH, UV exposure time and temperature significantly affected the degradation of NPEOs. The polynomial relationships between NPEOs concentrations and TiO₂ concentration for the degradation of NP6EO, NP9EO and NP30EO can be seen in Eqs.4.4.1abc, where X₁ and X₂ are representing the NPEOs and TiO₂ concentration, respectively.

\[
\begin{align*}
D(\%) \text{ NP6EO} & = 63.32(\pm2.12) + 19.14(\pm0.82)X₂ - 2.46(\pm0.11)X₂^2 + \epsilon \quad \text{ (Eqs.4.4.1a)} \\
D(\%) \text{ NP9EO} & = 70.88(\pm5.28) + 11.10(\pm2.04)X₂ - 1.40(\pm0.29)X₂^2 + \epsilon \quad \text{ (Eqs.4.4.1b)} \\
D(\%) \text{ NP30EO} & = 67.16(\pm5.63) - 0.1(\pm0.0009)X₁ + 15.49(\pm2.23)X₂ + 0.07(\pm0.009)X₁X₂ - 0.03(\pm0.0004)X₁^2 - 1.84(\pm0.02)X₂^2 + \epsilon \quad \text{ (Eqs.4.4.1c)}
\end{align*}
\]
Fig. 4.4.1c: Response surfaces and contour plots showing the degradation of NP9EO by UV radiation in the presence of H₂O₂ and TiO₂

Fig. 4.4.1d: Response surfaces and contour plots showing the effect of temperature and pH on NP9EO degradation by UV treatment (55 min, 55 Watt) in the presence of H₂O₂ and TiO₂
Fig. 4.4.1e: Response surfaces and contour plots showing degradation of NP30EO by UV radiation (55 Watt) in the presence of H$_2$O$_2$ and TiO$_2$

Fig. 4.4.1f: Response surfaces and contour plots showing the effect of temperature and pH on NP30EO degradation by UV irradiation (64 min, 55 Watt) in the presence of H$_2$O$_2$ and TiO$_2$
The degradation efficiency of UV/TiO$_2$/H$_2$O$_2$ for NPEOs was evaluated on the basis of pH change, TOC and COD removal. The effects of UV/TiO$_2$/H$_2$O$_2$ on COD and TOC removal are shown in Fig. 4.4.1g. The COD, TOC and pH were measured for those samples which were treated at optimized conditions. As expected from degradation, the COD and TOC removal take place effectively by UV/TiO$_2$/H$_2$O$_2$ treatment. By UV/TiO$_2$/H$_2$O$_2$ treatment, the reduction in COD was recorded up to 78%, 79% and 90% of NP6EO, NP6EO and NP30EO, while reduction in TOC was 62%, 70% and 68%, respectively (Fig. 4.4.1h). The pH of treated solution was also reduced significantly. After treatment, pH reduced to the level of 4.3, 4.8 and 5.1 for 50 mg/L solution of NP6EO. Similar trend in pH reduction was observed for the degradation of NP9EO and NP30EO. The reduction in COD, TOC and pH are considered good precursors of degradation.

Fig. 4.4.1g: Reduction in chemical oxygen demand of NP6EO, NP6EO and NP30EO treated by UV irradiation in the presence of TiO$_2$ and H$_2$O$_2$
Fig. 4.4.1h: Reduction in TOC of NP6EO, NP6EO and NP30EO treated by UV irradiation in the presence of TiO$_2$ and H$_2$O$_2$.

Fig. 4.4.1i: UV–Vis spectra of NP30EO treated by UV radiation in the presence of TiO$_2$ and H$_2$O$_2$ at optimized conditions.
1.31.2 Effect of UV/TiO₂/H₂O₂ on toxicity reduction of NPEOs

Standard cytotoxicity and mutagenicity tests were performed to evaluate the toxic potential of NPEOs as well as the efficiency of AOPs for the reduction of toxicity. For cytotoxicity evaluation, *A. cepa*, *haemolytic* (human and bovine) and *shrimp* tests were performed and to measure the mutagenic potential of NPEOs, *Ames* test was used. The NPEOs solution were found to be cytotoxic and mutagenic before treatment (Table 4.4.2). After UV/TiO₂/H₂O₂ treatment at optimum conditions, a significant reduction in cytotoxicity and mutagenicity was observed. The *A. cepa* test showed that after treatment root count, root length and mitotic index were increased up to 64.28%, 70.83% and 33.33% for NP6EO, 58.82%, 62.82% and 42.85% for NP9EO and 61.90%, 60.46% and 31.25% in case of NP30EO, respectively. The *haemolytic* test has also indicated that there was a significant reduction in toxicity i.e. 73.56%, 81.57% and 86.36% reduction in RBC lysis for human cell and 75.57%, 84.05% and 88.52% against bovine cells for NP6EO, NP9EO and NP30EO, respectively were observed. *Shrimp* test showed that after treatment, the *shrimp* napoli death reduced significantly to 95%, 95.71% and 96.36% for NP6EO, NP9EO and NP30EO,
respectively. Similarly after treatment, the mutagenicity of NPEOs was also reduced. The *Ames* test is a standard test for mutagenicity evaluation and treatment of NPEOs solution by UV/TiO₂/H₂O₂ (AOPs) indicated that this technique was suitable for the reduction of mutagenicity. The NPEOs showed mutagenicity before treatment and however, after treatment no mutagenicity was detected. The mutagenicity was evaluated using well plate method and out of 96 wells, 83, 72 and 63 wells were found affected in TA98 test, while 85, 75 and 66 wells in case of TA100 for NP6EO, NP9EO and NP30EO, respectively. After treatment the mutagenic effects of NPEOs reduced significantly and came within the permissible limit (recommended < 40 plate out of 96). By using TA98 test, the mutagenicity reduced up to 68.67%, 81.94% and 90.47%, while 77.64%, 88% and 93.93% in case of TA100 for NP6EO, NP9EO and NP30EO, respectively (Table 4.4.2). It was found that the NPEOs were potential candidates to induce cytotoxicity and mutagenicity, after treatment using UV/TiO₂/H₂O₂ system NPEOs were found safe. Radiation treatment showed promising efficiency for the reduction of toxicity and could be used successfully for the treatment of industrial wastewater polluted with NPEOs to save aquatic life from the harmful effects of these toxic compounds.
Table 4.4.2: Toxicity profile of NPEOs before and after UV/TiO₂/H₂O₂ treatment evaluated through *A. cepa*, haemolytic, shrimp and Ames tests

<table>
<thead>
<tr>
<th></th>
<th>ACT</th>
<th>Haemolytic</th>
<th>Shrimp</th>
<th>Ames</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>RC</td>
<td>RL</td>
<td>MI</td>
<td>Human</td>
</tr>
<tr>
<td>Before</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>24 h</td>
<td>24 h</td>
<td>72h</td>
</tr>
<tr>
<td>NP6EO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 3)</td>
<td>05±0.25</td>
<td>2.1±0.10</td>
<td>6±0.26</td>
<td>0</td>
</tr>
<tr>
<td>NP9EO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 3)</td>
<td>07±0.31</td>
<td>2.9±0.15</td>
<td>8±0.41</td>
<td>0</td>
</tr>
<tr>
<td>NP30EO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 3)</td>
<td>08±0.40</td>
<td>3.4±0.21</td>
<td>11±0.65</td>
<td>0</td>
</tr>
<tr>
<td>PC</td>
<td>18±0.9</td>
<td>7±0.41</td>
<td>23±1.26</td>
<td>---</td>
</tr>
<tr>
<td>NC</td>
<td>5±0.20</td>
<td>2±0.13</td>
<td>6.5±0.25</td>
<td>---</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NP6EO</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(n = 3)</td>
<td>14±0.80</td>
<td>7.2±0.25</td>
<td>9±0.45</td>
<td>---</td>
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<tr>
<td>NP9EO</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(n = 3)</td>
<td>17±0.96</td>
<td>7.8±0.31</td>
<td>14±0.75</td>
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</tr>
<tr>
<td>NP30EO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 3)</td>
<td>21±1.25</td>
<td>8.6±0.37</td>
<td>16±0.91</td>
<td>---</td>
</tr>
</tbody>
</table>

% reduction

<table>
<thead>
<tr>
<th></th>
<th>NP6EO</th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 3)</td>
<td></td>
<td>64.28</td>
<td>70.83</td>
<td>33.33</td>
<td>---</td>
<td>73.56</td>
<td>75.67</td>
<td>95</td>
</tr>
<tr>
<td>NP9EO</td>
<td></td>
<td>58.82</td>
<td>62.82</td>
<td>42.85</td>
<td>---</td>
<td>81.57</td>
<td>84.05</td>
<td>95.71</td>
</tr>
<tr>
<td>NP30EO</td>
<td></td>
<td>61.9</td>
<td>60.46</td>
<td>31.25</td>
<td>---</td>
<td>86.36</td>
<td>88.52</td>
<td>96.36</td>
</tr>
</tbody>
</table>

ACT-Allium cepa test, RC-root count, RL-root length, MI-mitotic index, PC-positive control, NC-negative control
For haemolytic test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively
For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively
For shrimp test, PC and NC were cyclophosphamide (10 µg/mL) and sea water, respectively
For Ames test, PC for TA98 and TA100 were K₂Cr₂O₇ (0.01 g/L) and NaN₃ (0.5 µg/100 µL), respectively and background (without standard and tested compound) was used as NC

1.31.3 Gamma ray treatment of NPEOs in the presence of H₂O₂

The gamma radiation treatment of NPEOs was carried out in the presence of H₂O₂; the optimum concentrations 3.30%, 4.65% and 4.58% of H₂O₂, optimized for UV treatment, were used during gamma radiation treatment of NP6EO, NP9EO and NP30EO, respectively. The gamma ray treatment was also applied under RCCD using initial concentration of NPEOs and absorbed dose as a variable factors. The initial concentrations of 50 mg/L, 100 mg/L and 150 mg/L were used for the absorbed doses of 5 kGy, 10 kGy and 15 kGy. In case of NP6EO, a degradation of 63%, 92% and 95% for initial concentration of 50 mg/L, while 55%, 88% and 92% for initial concentration of 100 mg/L and 44%, 84% and 88% for 150 mg/L irradiation to the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively were observed (Fig. 4.4.3a). Through RSM analysis it was observed that the maximum degradation of NP6EO can be achieved for the absorbed dose of 14.70 kGy for initial
concentration of 115 mg/L NE6EO solution. The degradation of NP9EO was found to be slightly higher at same absorbed doses and initial concentration as compared to NP6EO. For 50 mg/L solution of NP9EO, a degradation of 69%, 94% and 97% was achieved for the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively. For the initial concentration of 100 mg/L of NP9EO, the degradation was 62%, 89% and 92% and for 150 mg/L solution, the degradation of 59%, 88% and 91% was achieved for the absorbed doses of 5 kGy, 10 kGy and 15 kGy, respectively. The RSM analysis of data showed that the maximum degradation of NP9EO can be achieved at absorbed dose of 12.90 kGy (Fig. 4.4.3a). The degradation of NP30EO was recorded to be 81%, 96 and 98.5% for 50 mg/L solution, 73%, 92% and 95% for 100 mg/L and 68%, 91% and 94% for 150 mg/L initial concentration, respectively for the absorbed doses of 5 kGy, 10 kGy and 15 kGy (Fig. 4.4.3a). The UV–Vis spectrum of gamma radiation treated and untreated of NP30EO and NP9EO are shown in Figs. 4.4.3bc, respectively. The optimum dose of gamma radiation was found to be 12.5 kGy for maximum degradation of NP30EO. The polynomial relationships between gamma radiation absorbed dose and H2O2 concentration are shown in Eqs.4.4.3abc for the degradation of NP6EO, NP9EO and NP30EO, respectively.

\[
D(\%) \text{ NP6EO } = 14.12(\pm 0.90) + 8.90(\pm 0.16)X_1 - 0.46(\pm 0.03)X_2 + 0.006(\pm 0.0002) X_1X_2 - 0.33(\pm 0.03)X_1^2 + 0.007(\pm 0.0001)X_2^2 + \epsilon \quad (\text{Eq. 4.4.3a})
\]

\[
D(\%) \text{ NP9EO } = 28.55(\pm 1.49) + 13.12(\pm 0.23)X_1 - 0.30(\pm 0.02)X_2 + 0.003(\pm 0.0008) X_1X_2 - 0.52(\pm 0.48)X_1^2 + 0.009(\pm 0.0001)X_2^2 + \epsilon \quad (\text{Eq. 4.4.3b})
\]

\[
D(\%) \text{ NP30EO } = 63.38(\pm 2.53) + 6.53(\pm 0.3)X_1 - 0.1(\pm 0.03)X_2 - 0.26(\pm 0.001)X_1^2 + \epsilon \quad (\text{Eq. 4.4.3c})
\]

Where X_1 and X_2 are representing gamma radiation absorbed dose and NPEOs concentration, respectively.

The degradation of NPEOs using γ ray/H2O2 was also confirmed by measuring COD, TOC reduction as well as change in pH. The effects of γ ray/H2O2 on COD and TOC removal of NPEOs are shown in Figs. 4.4.3d and 4.4.3e, respectively. The COD and TOC were also reduced significantly by gamma ray treatment in the presence of H2O2. The γ ray/H2O2 treatment reduced the COD up to 76%, 72% and 68%, while reduction in TOC was recorded to be 76%, 72% and 68% for NP6EO solution having 50 mg/L, 100 mg/L and 150 mg/L initial concentration subjected to the absorbed dose of 15 kGy. Similar trend in COD and
TOC reduction was observed for NP9EO and NP30EO, however, the reduction in COD and TOC was slightly higher for NP9EO and NP30EO as compared to NP6EO. Maximum reduction in TOC and COD for NP9EO was recorded to be 58% and 77%, while 78% and 59% in case of NP30EO for initial concentration of 50 mg/L for the absorbed dose of 15 kGy. The pH of treated solution was also reduced significantly and final pH was observed in the range of 4.0-4.5. Similar trend in pH reduction was also observed for NP9EO and NP30EO. The reduction in TOC and COD was found to be significant for the samples treated by γ ray/H₂O₂, however, UV/TiO₂/H₂O₂ system was found to be superior in comparison to γ ray/H₂O₂.

Fig. 4.4.3a: Response surfaces and contour plots showing the degradation of NPEOs solutions (50, 100 and 150 mg/L) for the absorbed doses of 5 kGy, 10 kGy and 15 kGy: a) NP6EO, b) NP9EO, and c) NP30EO
Fig. 4.4.3b: UV–Vis spectra of NP30EO degradation treated by gamma ray in the presence of H₂O₂ at optimized conditions.
Fig. 4.4.3c: UV–Vis spectra of NP9EO degradation treated by gamma ray in the presence of H₂O₂ at optimized conditions.

Fig. 4.4.3d: COD reduction of gamma ray treated NP6EO, NP6EO and NP30EO in the presence of H₂O₂.
1.31.4 Effect of gamma radiation/H$_2$O$_2$ on toxicity reduction of NPEOs

The cytotoxicity and mutagenicity of NPEOs samples were evaluated after gamma ray treatment in the presence of H$_2$O$_2$. Authentic bioassays which are commonly used for the determination of cytotoxicity and mutagenicity were used. The toxicity results before and after treatments using $\gamma$ ray/H$_2$O$_2$ are shown in table 4.4.4. The A. cepa test showed that after treatment root count, root length and mitotic index were increased up to 58.33%, 68.65% and 37.5% for NP6EO, while 53.33%, 57.97% and 46.15% increase was observed in case of NP9EO and 46.66%, 50.00% and 46.66% for NP30EO having initial concentration of 150 mg/L initial concentration treated to an absorbed dose of 15 kGy. The haemolytic test showed reduction in cytotoxicity of 68.96% (NP6EO), 69.73% (NP9EO) and 73% (NP30EO). The decrease in RBC lysis was observed 72% for human cell and 77% in case of bovine for NP9EO and NP30EO, respectively. Shrimp test showed that after treatment, the shrimp napoli death reduced up to 92%, 92.85% and 92.72% for NP6EO, NP9EO and NP30EO, respectively. Before treatment the samples of NPEOs were found mutagenic and after treatment < 30 plates were affected both for TA98 and TA100 and it was found that NPEOs solution exerted no mutagenicity. The mutagenicity of gamma ray treated sample was also evaluated similar to UV treated sample. In case of TA98, the mutagenicity reduced up to 65.06%, 76.83% and 85.71%, while 72.94%, 87.50% and 89.39% in case of TA100 for NP6EO, NP9EO and NP30EO, respectively (Table 4.4.4). Keeping in view the degradation.
response and effect on toxicity reduction, the treatment $\gamma$ ray/H$_2$O$_2$ showed the promising effect; therefore, it can be used for the treatment of NPEOs.

Table 4.4.4: Toxicity profile of NPEOs before and after gamma radiation/H$_2$O$_2$ treatment evaluated through $A$. cepa, haemolytic, shrimp and Ames tests

<table>
<thead>
<tr>
<th></th>
<th>ACT</th>
<th>Haemolytic</th>
<th>Shrimp</th>
<th>Ames</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBT</td>
<td>Human</td>
<td>% lysis</td>
<td>72 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bovine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP6EO (n=3)</td>
<td>5±0.30</td>
<td>2.1±0.12</td>
<td>5±0.21</td>
<td>0</td>
</tr>
<tr>
<td>NP9EO (n=3)</td>
<td>7±0.39</td>
<td>2.9±0.17</td>
<td>7±0.33</td>
<td>0</td>
</tr>
<tr>
<td>NP30EO (n=3)</td>
<td>8±0.4</td>
<td>3.4±0.18</td>
<td>8±0.44</td>
<td>0</td>
</tr>
<tr>
<td>PC</td>
<td>18±1.0</td>
<td>7±0.41</td>
<td>23±1.26</td>
<td>---</td>
</tr>
<tr>
<td>NC</td>
<td>5±0.23</td>
<td>2±0.11</td>
<td>6.5±0.25</td>
<td>---</td>
</tr>
<tr>
<td>TAT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP6EO (n=3)</td>
<td>12±0.60</td>
<td>6.7±0.39</td>
<td>08±0.3</td>
<td>---</td>
</tr>
<tr>
<td>NP9EO (n=3)</td>
<td>15±0.70</td>
<td>6.9±0.45</td>
<td>13±0.5</td>
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</tr>
<tr>
<td>NP30EO (n=3)</td>
<td>15±0.61</td>
<td>6.8±0.41</td>
<td>15±0.6</td>
<td>---</td>
</tr>
<tr>
<td>% reduction</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>NP6EO (n=3)</td>
<td>58.33</td>
<td>68.65</td>
<td>37.5</td>
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</tr>
<tr>
<td>NP9EO (n=3)</td>
<td>53.33</td>
<td>57.97</td>
<td>46.15</td>
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</tr>
<tr>
<td>NP30EO (n=3)</td>
<td>46.66</td>
<td>50</td>
<td>46.66</td>
<td>---</td>
</tr>
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</table>

TBT-toxicity before treatment, TAT-toxicity after treatment, ACT-$Allium$ cepa test, RC-root count, RL-root length, MI-mitotic index, PC-positive control, NC-negative control
For haemolytic test, PC and NC were TritonX-100 (0.1%) and phosphate buffer saline, respectively
For ACT, PC and NC were distilled water and methyl methanesulfonate (MMS) (10 mg/L), respectively
For shrimp test, PC and NC were cyclophosphamide (10 $\mu$g/mL) and sea water, respectively
For Ames test, PC for TA98 and TA100 were K$_2$Cr$_2$O$_7$ (0.01 g/L) and NaN$_3$ (0.5 $\mu$g/100 $\mu$L), respectively and background (without standard and tested compound) was used as NC

Literature survey indicated that numerous studies have been focused on the biodegradation of NPEOs and as a result of biological treatment the intermediates were found to be more recalcitrant than the parent compounds (Mann and Boddy, 2000). These products include NP2EO (major product) and NP1EO as well as the dicarboxylated NPNEOs. The NPs was not identified as a product of aerobic degradation of NPEO in any of the bench-scale studies referenced but, in field studies, it was observed to occur in well-aerated environments originally contaminated with NPEO (Corsi et al., 2006; Koh et al., 2005) indicating possible difficulties in simulating the degradation of NPEO in bench-scale experiments (Manzano et al., 1999). Reports about the degradation of NPEOs in anaerobic conditions are scarce. However, it appears that NP is the major product of degradation; it
does not undergo further transformation and is strongly adsorbed onto the solids surfaces (de la Fuente et al., 2010; Scrimshaw and Lester, 2002; Soares et al., 2005).

From present study, it has been observed that the degradation efficiency of AOPs (UV/H₂O₂/TiO₂ and gamma radiation in the presence of H₂O₂) was found significantly higher than biodegradation of NPEOs used for the treatment of surfactant. Different photo–catalytic advanced oxidation treatments (PAOTs) such as direct UV-C photolysis, UV-C/H₂O₂ and UV-A/TiO₂, UV-A/H₂O₂/Fe²⁺ and photo Fenton for the degradation of NP9EO was investigated (de la Fuente et al., 2010). The UV-A processes showed better performance than UV-C. In another study, UVA and simulated sunlight were tested for the degradation of NPEOs in the presence of humic acid, H₂O₂, and Fe(III). The 96 h degradation efficiencies of NPEOs in pure water solution were found to be 36.6 and 22.6% under UVA and simulated sunlight irradiation, respectively. The presence of humic acid and Fe(III) in solution increased the photo–degradation efficiency (Wang et al., 2009). In another comparative study on the degradation of anionic, cationic and nonionic surfactants by H₂O₂/UV-C on the basis of COD and TOC removal efficiencies has been evaluated (Olmez-Hanci et al., 2011). Results have indicated that treatment time and H₂O₂ concentration played more significant roles in surfactant photo–degradation. The oxidative degradation of non-ionic surfactant (mixture) by the photo Fenton process has also been reported (Ono et al., 2012). Effects of operating parameters such as Fenton reagents dose and UV intensity on the degradation of surfactants were studied. Although the optimum dosages of the Fenton reagents showed degradation, further addition of the reagents did not enhance the rate of degradation. In another study, a comparative degradation of anionic, cationic and nonionic surfactants by H₂O₂/UV-C on the basis of COD and TOC removal efficiencies has been reported (Olmez-Hanci et al., 2011). Results indicates that treatment time and initial COD content played more significant roles in surfactant photo–degradation than the process variable “initial H₂O₂ concentration.

The electron beam efficiency for the decomposition along with radiolytic degradation mechanism of NP has been investigated (Gang et al., 2012). The study illustrated that the decomposition of NP in a methanol/water reduction system was higher than in an acetonitrile/water oxidation system and in an acetone/water system that appeared to be due to both •OH and e⁻aq playing a role in the NP degradation, although the latter is more effective.
The degradation rate of NP enhanced by low initial concentration at a constant absorbed dose in a methanol/water solution. The preliminary investigation of the reaction mechanism under reduction system suggested that the initial products of the reaction were ethyl benzene and along with other low molecular weight organic acids.

1.3.1.5 Gamma ray treatment efficiency for NPEOs degradation

The aqueous solutions of NPEOs having concentrations 50, 100 and 150 mg/L were subjected to 5, 10 and 15 kGy gamma radiation absorbed doses. The data showed that 50% removal ($D_{0.5}$) of NP6EO was achieved at 3.25 kGy, 4.35 kGy and 4.37 kGy for the initial concentration of 50, 100 and 150 mg/L, while 90% removal ($D_{0.90}$) can be achieved for the absorbed doses of 10.81 kGy, 14.46 kGy and 14.53 kGy, respectively (Table 4.4.5). In case of NP9EO, the $D_{0.5}$ was recorded to be 2.8 kGy, 4.09 kGy and 4.26 kGy for the initial concentrations of 50, 100 and 150 mg/L, while $D_{0.90}$ values were 9.3 kGy, 13.93 kGy and 14.15 kGy, respectively (Table 4.4.5). Similar trend was observed of G-values for NP30EO, the $D_{0.50}$ values were 2.59 kGy, 3.65 kGy and 4.32 kGy, while 8.62 kGy, 12.13 kGy and 14.37 kGy were deduced as $D_{0.90}$ for the degradation of NP30EO (Table 4.4.5). Results showed that degradation efficiency was related to absorbed doses, i.e. the degradation efficiency enhanced as the absorbed dose increased and similar trend has been reported elsewhere (Yang et al., 2007). It was observed that as initial concentration of NPEOs increases, the G-value also decreases. This is attributed to fact that the radical–radical recombination competes with solute decomposition (Michałowicz et al., 2011).
### Table 4.4.5: NPEOs removal efficiencies as a function of gamma radiation absorbed dose and dose rate constants at different concentrations

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Conc. mg/L</th>
<th>Dose kGy</th>
<th>D50</th>
<th>D90</th>
<th>G-value</th>
<th>k</th>
<th>R²</th>
</tr>
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<tbody>
<tr>
<td>NP6EO</td>
<td>50</td>
<td>5</td>
<td>3.25</td>
<td>10.81</td>
<td>0.12</td>
<td>0.213</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td>0.02</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5</td>
<td>4.35</td>
<td>14.46</td>
<td>0.29</td>
<td>0.159</td>
<td>0.91</td>
</tr>
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<td></td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>5</td>
<td>4.37</td>
<td>14.53</td>
<td>0.26</td>
<td>0.158</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
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<td>0.11</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NP9EO</td>
<td>50</td>
<td>5</td>
<td>2.8</td>
<td>9.3</td>
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<td>0.247</td>
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<td>5</td>
<td>4.09</td>
<td>13.93</td>
<td>0.19</td>
<td>0.169</td>
<td>0.93</td>
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<td>0.003</td>
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<tr>
<td>NP30EO</td>
<td>50</td>
<td>5</td>
<td>2.59</td>
<td>8.62</td>
<td>0.05</td>
<td>0.266</td>
<td>0.93</td>
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<td>0.002</td>
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<td>15</td>
<td></td>
<td></td>
<td>0.003</td>
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<td>5</td>
<td>3.65</td>
<td>12.13</td>
<td>0.1</td>
<td>0.189</td>
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<td>0.004</td>
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<tr>
<td></td>
<td>150</td>
<td>5</td>
<td>4.32</td>
<td>14.37</td>
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<td>0.169</td>
<td>0.86</td>
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<td>0.0006</td>
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</tr>
</tbody>
</table>

D50 = dose required for 50% removal of NPEOs, D90 = dose required for 90% removal of NPEOs, G-value = removal efficiency and k = rate constant

### 1.31.6 Adsorption behavior of NPEOs on TiO₂ surface

The adsorption isotherms is the study of solute concentration in the adsorbed state as a function of its concentration in the solution at constant temperature and it gives a valuable information that is useful for the selection of an adsorbent. It also facilitates the evaluation of the feasibility of the adsorption process for a given application (Javed et al., 2007; Manzoor et al., 2013; Nadeem et al., 2009). The adsorption isotherms are characterized by definite
parameters, whose values express the surface properties and affinity of adsorbent. In present study, two isotherm models (Langmuir and Freundlich isotherm models) were selected to fit the experimental data. The mathematical expressions of linear form of Langmuir equation and log form of Freundlich isotherm are represented in Eq. 4.4.6a and 4.4.6b, respectively.

\[
\begin{align*}
\frac{C_e}{q_e} &= \frac{1}{q_{\text{max}}K_L} + \frac{C_e}{q_{\text{max}}} \quad \text{(Eq. 4.4.6a)} \\
\log q_e &= \frac{1}{n(\log C_e)} + \log k \quad \text{(Eq. 4.4.6b)}
\end{align*}
\]

Where “\(q_e\)” is the concentration removed (mg/g), “\(C_e\)” the concentration at time \(t\), and “\(K_L\)”, is the Langmuir adsorption constant, while “\(k\)” and “\(n\)” are Freundlich constants.

Data fitted with Langmuir and Freundlich isotherm models is given in Table 4.4.6 for NPEOs. The higher \(R^2\) and \(q_{\text{max}}\) values suggested that the Langmuir isotherm describes the adsorption process well as compared to Freundlich model. The Langmuir isotherm model indicates the formation of monolayer coverage on the outer surface of TiO\(_2\). The Freundlich isotherm model was employed to describe the adsorption on heterogeneous surface and is not restricted to the formation of monolayer and the fractional, Freundlich isotherm model describe the heterogeneity of adsorbent surface. However, the values of regression coefficients (\(R^2\)) and \(q_e\) (mg/g) obtained in case of Freundlich isotherm model were lower than those obtained in case of Langmuir model which indicate that the Freundlich model is not sufficient to describe the adsorption process of NPEOs, respectively onto TiO\(_2\) surface.

Table 4.4.6: Comparison between Langmuir and Freundlich isotherm for NPEOs adsorption onto TiO\(_2\) surface

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Freundlich isotherm</th>
<th>Langmuir isotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(k) (1/n) (R^2)</td>
<td>(q_{\text{max}}) (K_L) (R^2)</td>
</tr>
<tr>
<td>NP6EO</td>
<td>1.08 0.726 0.98</td>
<td>111.1 0.16 0.99</td>
</tr>
<tr>
<td>NP9EO</td>
<td>1.02 0.487 0.97</td>
<td>58.82 0.18 0.99</td>
</tr>
<tr>
<td>NP30EO</td>
<td>1.09 0.419 0.98</td>
<td>55.86 0.37 0.99</td>
</tr>
</tbody>
</table>
1.31.7 Determination of radiolytic end products

Gas chromatographic-mass spectrometric study was performed in order to study the degradation of NPEOs and to identify the radiolytic end products in treated samples. The results clearly indicate that both the AOPs (UV/H₂O₂/TiO₂ and gamma/H₂O₂) used for the treatment of NPEOs completely degraded into low molecular mass carboxylic acids and finally into carbon dioxide and water. The NPEO solution treated at optimized conditions showed no characteristics peak, which indicate that the NPEOs are highly susceptible to advanced oxidation treatment. The data obtained after GC-MS analysis showed that the NPEO degradation adopted a complex pathway and the degradation intermediate identified are given in Table 4.4.7. The GC-MS chromatograms of treated NPEOs solution are shown in Figs. 4.4.5a-f (UV treated) and Figs. 4.4.5g-L (gamma treated). The NPEOs solutions treated by UV radiation in the presence of H₂O₂ only, showed the presence of 2-propanone, n-hexadecanoic acid, tetradecanoic acid, pentanoic acid, benzaldehyde, hexanoic acid, 1-hexadecanol, hexadecanoic acid, 2-Benzyl-[1,4]benzoquinone and 14-Octadecanoic acid, which indicated that by UV radiation treatment in the presence of H₂O₂ the NPEOs did not degraded completely and the intermediate radiolytic product of high molecular weight were detected. On the other hand, same NPEOs solution when treated by UV radiation in the presence of H₂O₂ and TiO₂, the radiolytic end product were acidic units (acetic acid and other lower molecular carboxylic acids), which indicated that the NPEOs were completely degraded in the presence of TiO₂ in addition to H₂O₂ (Figs. 4.4.5d-f). The NPEOs solution subjected to gamma ray treatment also showed similar degradation pattern. The NPEOs solution treated to the absorbed doses of 5 kGy and 15 kGy were subjected to GC-MS analysis and the chromatograms are shown in Figs. 4.4.5g-l. The samples treated to an absorbed dose of 5 kGy showed the presence of high molecular weight alcohols, aldehydes and ketones (Figs. 4.4.5g-i), while sample treated to the absorbed dose of 15 kGy showed the presence of low molecular mass carboxylic acids and acetic acids similar to UV radiation treatment (Figs. 4.4.5j-l) which indicated that the end product of gamma radiation treatment were also low molecular mass carboxylic acids. Based on the detection of radiolytic intermediates and end products, the degradation pathway of NPEOs was proposed (Fig. 4.4.5m) which showed that NPEOs adopted a complex degradation pathway. During radiolysis, the hydroxyl radicals attack the ethoxylate unit of nonylphenol and then aliphatic
side chain followed by benzene ring opening. In send step, the ethoxylate units are converted into alcohols, while aliphatic side chain and the benzene ring after destruction are converted into acidic units. The alcohol and acidic units are finally converted into carbon dioxide and water through different intermediate steps.

Table 4.4.7: Radiolytic intermediates of NPEOs indentified by GC–MS analysis

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Retention Time (min)</th>
<th>Probable Match</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.622</td>
<td>2-Propanone</td>
</tr>
<tr>
<td>2</td>
<td>19.644</td>
<td>Tetradecanoic acid</td>
</tr>
<tr>
<td></td>
<td>19.633</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21.126</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>4</td>
<td>22.253</td>
<td>n-Hexadecanoic acid</td>
</tr>
<tr>
<td>5</td>
<td>15.970</td>
<td>Pentanoic acid</td>
</tr>
<tr>
<td>6</td>
<td>16.399</td>
<td>Carboxylic acid</td>
</tr>
<tr>
<td>7</td>
<td>21.057</td>
<td>Benzaldehyde</td>
</tr>
<tr>
<td>8</td>
<td>21.601</td>
<td>Hexadecanoic acid</td>
</tr>
<tr>
<td>9</td>
<td>22.991</td>
<td>1-Hexadecanol</td>
</tr>
<tr>
<td>10</td>
<td>22.431</td>
<td>Hexadecanoic acid</td>
</tr>
<tr>
<td>11</td>
<td>20.508</td>
<td>2-Benzyl-[1,4]benzoquinone</td>
</tr>
<tr>
<td>12</td>
<td>21.498</td>
<td>Hexadecanoic acid</td>
</tr>
<tr>
<td>13</td>
<td>22.585</td>
<td>14-Octadecanoic acid</td>
</tr>
</tbody>
</table>
Fig. 4.4.5a: GCMS chromatogram of NP30EO (50 mg/L) treated by UV radiation in the presence of H$_2$O$_2$.

Fig. 4.4.5b: GC-MS profile of NP9EO (50 mg/L) treated by UV radiation in the presence of H$_2$O$_2$. 
Fig. 4.4.5c: GC-MS profile of NP6EO (50 mg/L) treated by UV radiation in the presence of \( \text{H}_2\text{O}_2 \)

Fig. 4.4.5d: GC-MS profile of NP30EO (50 mg/L) treated by UV radiation in the presence of \( \text{H}_2\text{O}_2 \) and \( \text{TiO}_2 \)
Fig. 4.4.5e: GC-MS profile of NP9EO (50 mg/L) treated by UV radiation in the presence of H₂O₂ and TiO₂

Fig. 4.4.5f: GC-MS profile of NP6EO (50 mg/L) treated by UV radiation in the presence of H₂O₂ and TiO₂
Fig. 4.4.5g: GC-MS profile of NP30EO (50 mg/L) irradiated to the absorbed dose of 5 kGy in the presence of H₂O₂.

Fig. 4.4.5h: GC-MS profile of NP9EO (50 mg/L) irradiated to the absorbed dose of 5 kGy in the presence of H₂O₂.
Fig. 4.4.5i: GC-MS profile of NP6EO (50 mg/L) irradiated to the absorbed dose of 5 kGy in the presence of H$_2$O$_2$

Fig. 4.4.5j: GC-MS profile of NP30EO (50 mg/L) irradiated to the absorbed dose of 15 kGy in the presence of H$_2$O$_2$
Fig. 4.4.5k: GC-MS profile of NP9EO (50 mg/L) irradiated to the absorbed dose of 15 kGy in the presence of H₂O₂

Fig. 4.4.5L: GC-MS profile of NP6EO (50 mg/L) irradiated to the absorbed dose of 15 kGy in the presence of H₂O₂
Fig. 4.4.5m: Proposed degradation pathway NPEOs treated by UV and gamma radiation in the presence of H₂O₂ and TiO₂ (n = 6, 9 and 30 in case of NP6EO, NP9EO and NP30EO, respectively)

1.31.8 FTIR analysis

The degradation of NPEOs by advanced oxidation treatments (UV/H₂O₂/TiO₂ and gamma/H₂O₂) was confirmed by FTIR analysis. The FTIR profile of NPEO before treatment showed specific vibration peaks at 3800 cm⁻¹ (background peaks), 3500-3700 cm⁻¹ (OH str,
free), 3200-3600 cm\(^{-1}\) (OH str), 2915.16 cm\(^{-1}\) (–CH stretch vibration), 1638.15 cm\(^{-1}\) (vibration for alkenes with C=C stretching), 1460.39 cm\(^{-1}\) (for alkanes with C–H deformation), 1381.08 cm\(^{-1}\) (OH in plane), 1248.49 cm\(^{-1}\) (C-C-OH str), 1099.80 cm\(^{-1}\) (C-O Str vibration), 947.75 cm\(^{-1}\) (for benzene ring with three adjacent H atoms, C–H deformation) (Figs. 4.4.6a-c). FTIR spectrum of irradiated solution by UV and gamma ray showed disappearance of peaks as shown in Figs. 4.4.6d-f for NP9EO, NE6EO and NE30EO, respectively treated by UV/H\(_2\)O\(_2\)/TiO\(_2\) as well as treated by gamma radiation in the presence of H\(_2\)O\(_2\) at optimized conditions (Figs. 4.4.6g-i). The major peaks at 3000-3100 cm\(^{-1}\) (=C-H str), 2100 cm\(^{-1}\) (C-C triple bond), 1600 cm\(^{-1}\) (C=C) and 1100 cm\(^{-1}\) (C-O-C) cm\(^{-1}\) were observed which correspond to the end product detected through GC-MS analysis. Results showed that complete destruction of NPEOs occurred after radiation treatment because most of the peak detected before treatment were disappeared after treatment. The FTIR spectrum of treated samples clearly indicates that NPEOs molecules have been degraded, which support the assumption that OH\(^{•}\) bring about the chain oxidation reaction to break down complex molecule into simpler compounds. Some of the other peaks correspond to the functional groups present in intermediate and end products were also observed. The results revealed that the large NPEOs structure was destroyed yielding smaller acidic units. The results are comparable with previous studies which showed that the oxidative degradation of organic compound occurred through breakdown of functional group. Therefore, our practical findings e.g. more than 90% reduction in COD and decrease of pH of the solution revealed the degradation of the NPEOs.
Fig. 4.4.6a: The FTIR spectrum of NP30EO (50 mg/L) before treatment

Fig. 4.4.6b: The FTIR spectrum NP9EO (50 mg/L) before treatment
Fig. 4.4.6c: The FTIR spectrum NP6EO (50 mg/L) analysis before treatment

Fig. 4.4.6d: The FTIR spectrum of NP9EO (50 mg/L) after UV radiation/H₂O₂/TiO₂ treatment
Fig. 4.4.6e: The FTIR spectrum of NP6EO (50 mg/L) after UV radiation/H₂O₂/TiO₂ treatment

Fig. 4.4.6f: The FTIR spectrum of NP30EO (50 mg/L) after UV radiation/H₂O₂/TiO₂ treatment
Fig. 4.4.6g: The FTIR spectrum of NP9EO (50 mg/L) after gamma/H₂O₂ treatment

Fig. 4.4.6h: The FTIR spectrum of NP6EO (50 mg/L) after gamma/H₂O₂ treatment
1.31.9 HPLC analysis

After GC-MS and FTIR analysis, the degradation of NPEOs samples were also confirmed by HPLC analysis. The HPLC analysis was carried out for the samples treated at optimized conditions by using both for UV and gamma ray treatments (Figs. 4.4.7a-i). The HPLC analysis of NPEOs control sample showed peaks at 26.0 min, 9.2 min and 5.3 min for NP30EO, NP9EO and NP6EO, respectively (Figs. 4.4.7a-c). After treatment, the peaks were completely shifted and appeared at retention time of 2.64-2.66 min (figs. 4.4.7d-f). From HPLC analysis, it was observed that the NPEOs degraded completely and single peaks was recorded at lower retention time as compared to the untreated samples. The peaks with earlier retention time ranging from 2.64 to 2.66 min are shown, suggesting that intermediate compounds were not of diverse nature.

Through GC-MS analysis, NPEOs samples treated by UV/H₂O₂ and gamma ray treatment to an absorbed dose of 5 kGy, it was observed that different types of intermediate were produced which indicated that those compounds were further degraded while prolonging of degradation variables and finally single type of radiolytic end product was observed in both UV/H₂O₂/TiO₂ and gamma ray to an absorbed dose of 15 kGy (Figs. 4.4.7g-i). Photo–degradation in different pathways occurred in the present study became recognizable via the resultant products. The degradation took place in combination with the
shortening of ethoxylate (EO) chain, benzene ring opening as well as the degradation of side aliphatic chain. These intermediates were not reported before in either biodegradation or photo–catalyzed degradation. Although some reports have described photo–catalysis degradation, there was lack of the examination of intermediates. Degradation products with shorter EO side chain, including NP without EO unit, have been reported to cause greater toxic and estrogenic effects to biota than their parent compounds. On the influence of the radiation, the destruction of the benzene ring would possibly reduce the persistent or/and toxic effect against organisms (Chen et al., 2007). Similar trend has also been reported regarding degradation of NP by UV radiation treatment previously (Neamțu and Frimmel, 2006).

The summary of characterization techniques revealed that NPEOs degradation took place by cleavage of benzene ring and shortening of EO units. It has been reported that the degradation mechanism of NPEOs depend on the number of EO units and the photo–degradation conditions. For example, the degradation of NPEO (n= 40) started from the benzene ring under UV exposure, while degradation NPEO (n < 40) began from the EO side chain (Goto et al., 2004) because destruction of the benzene ring need higher energy than shortening the EO chain (Peiitzetl, 1989). The destruction of the benzene ring, shortening of EO units and degradation of aliphatic side chain caused by UV and gamma irradiation in the presence of H₂O₂ and TiO₂ were clearly observed from the UV spectrum in the present study. The benzene ring of NP10EO had a maximum absorbance at 270 nm in the UV spectrum. UV irradiation brought about a lower peak at 270 nm, indicating the break down of the benzene ring.
Fig. 4.4.7a: HPLC analysis of NP30EO (50 mg/L) before treatment

Fig. 4.4.7b: HPLC analysis of NP9EO (50 mg/L) before treatment

Fig. 4.4.7c: HPLC analysis of NP6EO (50 mg/L) before treatment
Fig. 4.4.7d: HPLC analysis of NP6EO (50 mg/L) after treatment to the absorbed dose of 15 kGy

Fig. 4.4.7e: HPLC analysis of NP9EO (50 mg/L) after treatment to the absorbed dose of 15 kGy

Fig. 4.4.7f: HPLC analysis of NP30EO (50 mg/L) after treatment to the absorbed dose of 15 kGy
Fig. 4.4.7g: HPLC analysis of NP6EO (50 mg/L) after UV/H$_2$O$_2$/TiO$_2$ treatment

Fig. 4.4.7h: HPLC analysis of NP9EO (50 mg/L) after UV/H$_2$O$_2$/TiO$_2$ treatment

Fig. 4.4.7i: HPLC analysis of NP30EO (50 mg/L) after UV/H$_2$O$_2$/TiO$_2$ treatment
1.32 Effect of H\textsubscript{2}O\textsubscript{2} concentration on degradation

The degradation data obtained shows that the removal efficiency of pollutants increased with H\textsubscript{2}O\textsubscript{2} concentrations to a certain limit. Further increase in H\textsubscript{2}O\textsubscript{2}, however, reduced both the rate and extent of pollutant removal. A similar pattern of H\textsubscript{2}O\textsubscript{2} dependent removal was observed for the degradation of organic pollutants and was also observed by other researchers (Cater et al., 2000; Hu et al., 2008). The non selective oxidation of organic compounds by HO' free radicals produced by UV radiation treatment in the presence of H\textsubscript{2}O\textsubscript{2} is well established. During irradiation H\textsubscript{2}O\textsubscript{2} absorbs light of specific energy in UV range and O–O bond is ruptured leading to the production of activated HO’ radicals and the atomic oxygen. By increasing H\textsubscript{2}O\textsubscript{2} concentration in the presence of UV irradiation, production of HO’ radical is enhanced with the absorption of that much energy required for the cleavage of O–O bond. The production of HO’ radicals and the oxidative destruction of organic pollutants is shown in Eqs. 5.1-5.2 below;

\begin{align*}
\text{H}_2\text{O}_2 + h\nu & \rightarrow 2 \text{HO'} & \text{Eq. (5.1)} \\
\text{RH} + \text{HO'} & \rightarrow \text{H}_2\text{O} + '\text{R} \rightarrow \text{CO}_2 + \text{H}_2\text{O} & \text{Eq. (5.2)}
\end{align*}

When excessive H\textsubscript{2}O\textsubscript{2} is present, it is possible that the H\textsubscript{2}O\textsubscript{2} itself becomes an active competitor for HO’ (Cater et al., 2000). It is also possible that a less reactive HO\textsubscript{2}’ is formed as a result of the reactions between H\textsubscript{2}O\textsubscript{2} and HO’ (Pérez et al., 2002), as shown in Eq. 5.3. The competition for HO’ and the formation of less reactive HO\textsubscript{2}’ may explain the reduced reaction rate at a higher concentration of H\textsubscript{2}O\textsubscript{2}, since HO\textsubscript{2}’ has less oxidizing potential (1.7) as compared to HO’ (2.8) (Kritikos et al., 2007; Ragaini et al., 2001; Zhang et al., 2013)

\begin{align*}
\text{H}_2\text{O}_2 + \text{HO}' & \rightarrow \text{HO}_2' + \text{H}_2\text{O} & \text{Eq. (5.3)}
\end{align*}

The amount of H\textsubscript{2}O\textsubscript{2} is the primary factor contributing to the chemical costs of the photo–catalytic process (Glaze et al., 1987). Therefore, it is important to minimize the amount of H\textsubscript{2}O\textsubscript{2} required through optimization process. The higher H\textsubscript{2}O\textsubscript{2} dosage level more than optimized would only make a slight increase of reaction rate which is not economical.
1.33 Effect of TiO$_2$ dose on degradation

It is well known that photo–generated holes (h$^+$) are produced when TiO$_2$ particles are irradiated by UV light. Hydroxyl radicals are formed mainly by the oxidation of OH$^-$ or H$_2$O via photo–generated holes and HO' are principally responsible for degradation of organic compound. Oxygen acts primarily as an efficient electron trap which prevents the recombination of electrons and photo–generated holes. The effects of TiO$_2$ on the photo–catalytic degradation of selected model compound and wastewater were tested from 0.1 to 10 g TiO$_2$/L. The results indicated that the degradation of pollutants increased with increasing concentration of TiO$_2$ and beyond the optimum dose, the rate of reaction declined. At either lower or higher doses of TiO$_2$, the degradation was recorded to be low. It has been reported that when there was an excessive amount of TiO$_2$, the degradation of pollutant declined. This might be presumably due to two opposing factors regarding the effects of TiO$_2$ on degradation. When insufficient TiO$_2$ is present, there might be a limited production of HO' due to the inadequate conversion from light energy to chemical energy. However, excessive amount of TiO$_2$ is converted into slurries which also adversely affect the reaction rate due to light scattering that precludes the UV light permeation in the reaction system (Hu et al., 2008). Furthermore, it is also reported that the light penetration may decrease with the increase of light scattering (Kansal et al., 2007), agglomeration, and sedimentation of TiO$_2$ under high catalyst concentration may also occur (So et al., 2002). The reduction in the rate constant may be due to the reduction in the penetration of light with surplus amount of TiO$_2$. The surplus addition of the catalyst makes the solution more turbid and light penetration is retarded. The addition of surplus catalyst also results in the deactivation of activated molecules by collision with ground state molecules (Gupta, 2012). Our results agree well with previous studies on degradation of bisphenol (Kaneco et al., 2004) and others (Elmolla and Chaudhuri, 2010). A number of experiments during this study were carried out by adding different amounts of TiO$_2$ (0.1-10 g/L NPEOs and industrial wastewater), in order to obtain an optimum concentration of catalyst to achieve the maximum photo–degradation efficiency and save unnecessary use of excess photo-catalyst. Generally, it was observed that maximum degradation of the pollutant occurred by using TiO$_2$ catalyst up to 6 g/L, more amounts than 6 g/L, however, showed gradual reduction in the rate constant and would not be cost effective.
The optimal pH for photo-catalytic systems is of practical importance due to its ease in process control. It was observed that the degradation rate was fast and higher in acidic conditions, while at neutral pH rate decreased and again under alkaline condition increased. It is evident from the results that pH affected the removal of pollutant very differently. In UV/H₂O₂ system, the pollutant removal was the highest in the acidic pH range and decreased when pH was increased. One major factor responsible for this behavior might be the instability of H₂O₂ at higher pH since H₂O₂ decomposes rapidly in an alkaline condition. Under alkaline condition, the scavenging of HO• by HCO₃⁻ and CO₃²⁻ may take place that could be formed from CO₂ as the mineralization product of organic pollutant. At an alkaline pH, CO₂ is present predominantly in the form of HCO₃⁻ (pH > pKₐ₁ = 6.35) or CO₃²⁻ (pH > pKₐ₂ = 10.33), furthermore, in alkaline medium the HCO₃⁻ and CO₃²⁻ scavenge the HO• and preclude them from reaction (Safarzadeh-Amiri, 2001). The effect of UV/TiO₂/H₂O₂ on degradation was found slightly different as compared to UV/H₂O₂. Here, degradation under acidic condition was higher and then decreased by increasing the pH to 7. In alkaline condition, again the degradation of pollutant increases. Available data from literature pointed out several factors, including the effects of pH on the surface charge of the semiconductor TiO₂ particles, the dispersion of TiO₂ suspensions, and the dissolution of TiO₂ at extremely acidic pH (<1.0) (Chiang et al., 2004; Mu et al., 2004; Vamathevan et al., 2002; Xu et al., 2006). The dispersion of TiO₂ is more favorable when solution pH is at its isoelectric point (zeta potential is zero), which was reported to be near pH 7 (Fernandez-Ibanez et al., 2003). Under a well-mixed condition employed in this study, however, the dispersion should not be an important contributing factor. It then appears that change in surface charge of TiO₂ is essential for the observed pH effect. When the pH was lower (acidic), the surface of TiO₂ is protonated and became positively charged, which is more favorable for the e⁻ to move from the valance band to the conduction band of the semiconductor particle, a process essential for the photo–catalytic activity (Schwarz et al., 1997). Under a very alkaline pH, the surface of TiO₂ was negatively charged due to the abundance of OH⁻, which also favor the positively charged electron “hole” (hₒᵇ⁺) to move.
from the inside to the catalyst surface and resultanty the degradation increased under alkaline condition in UV/TiO$_2$/H$_2$O$_2$ as compared to UV/ H$_2$O$_2$ (Hu et al., 2008).

**1.35 Effect of initial concentration on degradation**

The effects of initial concentrations on the removal efficiency of pollutant in two selected treatment systems (gamma/H$_2$O$_2$ and UV/TiO$_2$/H$_2$O$_2$) showed initial concentration of the solution is also an important parameter for effective degradation (Deeb et al., 2003; Hu et al., 2008). The concentration dependent pollutant removal has also been reported by several earlier studies under similar conditions (Cater et al., 2000; Bertelli, 2004). The reduced reaction rate at higher concentrations were attributed to the competition between various molecules and/or the intermediates formed during the oxidative reaction process. Both initial concentration and its transformation products can compete for HO$^\cdot$ radicals generated from gamma/H$_2$O$_2$ or UV/TiO$_2$/H$_2$O$_2$. The removal efficiency was, therefore, decreased as a result of increasing competition with the HO$^\cdot$ radicals when initial concentration were higher.

**1.36 Effect of temperature on degradation**

Temperature of the reaction also affected the degradation in the UV/H$_2$O$_2$/TiO$_2$ system. The $k$ values of NP6EO treated by UV/H$_2$O$_2$/TiO$_2$ system at different temperature and pH are shown in Table 5.5. The rate of degradation increased with temperature evaluated through first order kinetics. The effect of temperature was investigated in the range of 25–45$^\circ$C and rate constant ‘$k$’ was determined from the first order plots. Over the examined range degradation rate increased with the temperature. An increase in temperature helps the reaction to compete more efficiently with $e^-$/h$^+$ recombination, the increased collision frequency of molecules; leading to an enhancement in the degradation. It is reported that during UV/H$_2$O$_2$/TiO$_2$ treatment, the rate of reaction also depends upon activation energy which is decreased in the presence of TiO$_2$ catalyst and molecule easily participate in the reaction. The higher temperature that has also optimized, also affect the collision frequency of the reacting molecule, therefore, facilitating the formation of activated complex and resultanty the degradation product are formed. The reaction rate reflects the rate at which molecules migrate from a bulk solution to the interfacial regions at a temperature where
maximum HO’ concentration is available and organic compounds are transferred from solution to the surface of the catalysts by diffusion process at higher temperature (Kim et al., 2001; Laidler, 1987). Therefore, the higher temperature as well as the addition of TiO₂ both facilitates the degradation of the pollutants. The degradation can be enhanced at higher temperature which is generally available from May-September in Pakistan, whereas the degradation also occur at low temperature but relatively at small rate which is almost naturally available in rest of the spam of the year. So, this treatment process for the degradation of the industrial wastewater is viable for treatment

Table 5.5:  Pseudo-first-order degradation rate constants (k) of UV/H₂O₂/TiO₂ system at different temperature (100 mg/L)

<table>
<thead>
<tr>
<th>NPEOs</th>
<th>Temperature</th>
<th>k min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP6EO</td>
<td>25 °C</td>
<td>0.0155</td>
</tr>
<tr>
<td></td>
<td>35 °C</td>
<td>0.0221</td>
</tr>
<tr>
<td></td>
<td>45 °C</td>
<td>0.0246</td>
</tr>
<tr>
<td>NP6EO</td>
<td>25 °C</td>
<td>0.0155</td>
</tr>
<tr>
<td></td>
<td>35 °C</td>
<td>0.0214</td>
</tr>
<tr>
<td></td>
<td>45 °C</td>
<td>0.0246</td>
</tr>
<tr>
<td>NP6EO</td>
<td>25 °C</td>
<td>0.0229</td>
</tr>
<tr>
<td></td>
<td>35 °C</td>
<td>0.0438</td>
</tr>
<tr>
<td></td>
<td>45 °C</td>
<td>0.0489</td>
</tr>
</tbody>
</table>

1.37 Effect of advanced oxidation process on WQPs and toxicity reduction

As expected after degradation, the WQPs are likely to improve because the water quality depends upon the concentration of pollutant present in wastewater. It has been observed that the pH of treated solution decrease linearly in response of degradation. It is reported that after treatment of wastewater and simulated solutions of model compounds the pH of solution is likely to decrease. As a result of degradation of organic pollutant, the intermediates such as low molecular mass carboxylic acids are produced and consequently pH would drop (Feki et al., 2009; Zhang et al., 2012). The COD and BOD values depend upon the organic content in media and their reduction reflects the degradation process. It is reported that in response of partial degradation of pollutant, a significant reduction in pH of the medium can not be achieved. A number of studies highlighted that when intermediate are further degraded in to inorganic ions then reduction in COD was recorded to be significant
(Lucas and Peres, 2009; Mantzavinos and Kalogerakis, 2005). The factor like pH of medium, catalysts concentration, exposure time, temperature and level of pollutant in treated solution influenced the COD reduction (Farhadi et al., 2012). Actually, the change in variables and COD reduction depend upon the extent of degradation. The condition at which maximum degradation was obtained, the reduction in COD and BOD should also be higher. The toxicity evaluated also showed that UV/H₂O₂ process was not effective for toxicity reduction which might be due incomplete degradation of pollutants present in the medium, while gamma/H₂O₂ and UV/H₂O₂/TiO₂ reduced the toxicity of treated samples significantly and UV/H₂O₂/TiO₂ was found more effective for the reduction of toxicity as compared to the gamma/H₂O₂ process. It is concluded from present investigation that if the wastewater contains NPEOs is treated by AOPs before being discharged into the water bodies. Its toxicity is reduced significantly and the degradable end products are low molecular aliphatic carboxylic acids which may decrease slightly pH of the effluents. In this way the use of NPEOs will become viable and the cost and efficiency of the process will be improved significantly.
Chapter # 5

SUMMARY

The industrial wastewater is the leading source of water pollution due to diverse nature of pollutant present in effluents at elevated concentrations. The goal of this work was to establish treatment methods for wastewater effluents of petroleum refinery, soap & detergent and pulp & paper mill wastewater as well as selected model compounds by AOPs such as gamma ray/H₂O₂ and UV/H₂O₂/TiO₂. The AOPs showed a promising efficiency for the degradation of pollutants present in wastewater of industrial origin as well as simulated aqueous solutions of model compounds. The degradation was monitored through UV/Vis spectrometry and radiolytic end products were evaluated using advanced characterization techniques such as FTIR, GCMS and HPLC. The effect of selected AOPs was evaluated on the basis of degradation, water quality parameters and toxicity. The toxicity was determined through various biological tests such as *A. cepa*, haemolytic and shrimp tests. *Ames* test was also performed for mutagenicity measurement before and after application of AOPs. The water quality parameters and toxicity of industrial effluents as well as simulated solutions of model compound were checked before and after treatment. The independent variables such as radiation dose, catalysts concentration, exposure time, pH, temperature etc. were optimized through response surface methodology and finally, the degradation of pollutants present in the wastewater and model compounds were measured at optimized conditions. It was found that through response surface methodology that completes degradation of pollutants was possible by optimizing the independent variables. There was a significant reduction in cytotoxicity and mutagenicity of sample treated at optimized conditions. The water quality parameters like BOD, COD, DO, TDS and TSS were improved significantly after treatment. It has observed that the water quality parameters values could be achieved within permissible limits recommended by the international agencies such as USEPA and Canadian EPA. Among industrial wastewater, the petroleum wastewater was found more toxic and resistive to treat as compared to pulp & paper and soap & detergent wastewater, while among model compounds, the NP6EO was found more toxic. While comparing the treatments, it was observed that UV/TiO₂/H₂O₂ was more efficient regarding degradation,
improvement in water quality parameters and toxicity reduction as compared to gamma ray/H₂O₂ treatment. However, the treated wastewater in both the cases seemed suitable for industrial reuse, irrigation for non edible crops and aquatic life since water quality parameters fall within permissible limits. From results, it is also concluded that the AOPs such as UV/TiO₂/H₂O₂ and gamma/H₂O₂ could successfully used for the treatment of industrial wastewater at commercial scale. However, it should also be mentioned that there remains a significant amount of uncertainty concerning the technical and economic effectiveness of AOPs. It could also be stated that besides fundamental studies, more pilot and field scale studies should be performed to determine the pollutants removal efficiencies economically that could be considered as dependent on both wastewater quality and operational parameters.
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