Degradation of Textile Azo dye Acid Orange 7 by Advanced Oxidation Process

A project dissertation submitted in partial fulfillment for the award of degree of

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CERTIFICATE

This is to certify that the dissertation / project report entitled "**Degradation of Textile Azo dye Acid Orange 7 by Advanced Oxidation Process**" done by Ashish Sharma *(2K13/ENE/03)* is an authentic work carried out by him at the Department of Environmental Engineering, Delhi Technological University, under my guidance. The matter embodied in this project has not been submitted earlier for the award of any degree or diploma in this or any other university or institute to the best of my knowledge and belief.

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DISSERTATION APPROVAL SHEET

The dissertation **"Degradation of Textile Azo dye Acid Orange 7 by Advanced Oxidation Process"** submitted by Ashish Sharma (2K13/ENE/03) is approved for the award of the degree of Master of Engineering in Environmental Engineering, Delhi Technological University (formerly, Delhi College of Engineering), Delhi.

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Abstract

The present study was undertaken to determine the feasibility of Advanced Oxidation Processes (AOPs) in treatment of textile dyes in wastewater. Acid Orange 7 (AO7) is an azo dye with potential ecotoxicity to the exposed organisms. Natural attenuation, photo –catalytic degradation (with TiO_2), and Fenton's treatment were studied as AOPs for effective degradation of AO7. The Experimental results confirmed that natural attenuation under sunlight and shade is not an effective process for degradation of AO7. It was observed that a fraction of dye is degraded/ transformed during the day time with exposure to sunlight but the colour regenerated during night time. Photo – catalytic degradation with TiO_2 was found to be higher than without TiO_2 but the rate of degradation was quite low. The dose of photo – catalyst regulates dye degradation and the concentration 1.0 g/l of TiO_2 was observed to have maximum degradation of 76% in a period of about 32 hours. Complete decolourisation was observed in a period of about 127 hours. The recovery of photo – catalyst was found to be 62% for its reuse. The decolourisation of Acid Orange 7 dye by Fenton's oxidation process was observed to be dependent on several process variables. Concentration of Hydrogen peroxide, Ferrous sulphate, pH, and contact time were considered in this study. Preliminary experimental runs were carried out using univariate approach. It was found that minimum contact time for complete decolourisation by varying H_2O_2 concentration was obtained at 5 mmol/l. Increase in $FeSO_4$ (mg/l) concentration resulted in decrease of time for complete decolourisation; but an increase above 38 mg/l resulted in precipitation of Fe^{3+}, thereby reducing decolourisation. Maximum decolourisation was observed at pH 3.0. The experimental Box – Behnken Design was used to validate and optimize the process variables. Maximum and minimum levels of pH (3 – 5), H_2O_2 (4 – 6 mmol/l), $FeSO_4$ (30 – 46 mg/l) and contact time (5 – 15 minutes) were used. The statistical analysis revealed a value of 0.88 for coefficient of regression (R^2) indicating a good fit of model. Calculated F – value was found to be higher than the tabulated value confirming to significance of the model. Based on student's t – test, Ferrous sulphate, pH, and contact time have a positive effect on the percent decolourisation of Acid Orange 7. The optimised values were H_2O_2 concentration = 4.97 mmol/l, $FeSO_4$ = 40.83 mg/l, pH = 3.1 and contact time = 13.6 minutes. Comparison of different AOPs revealed that Fenton's process is the most efficient treatment method for degradation of AO7.

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List of Abbreviations

ANOVA	Analysis of Variance
AO7	Acid Orange 7
AOPs	Advanced Oxidation Processes
BBD	Box – Behnken Design
BOD	Biological Oxidation Demand
BR14	Basic Red 14
CI	Colour Index
COD	Chemical Oxidation Demand
CR	Congo Red
DB3	Disperse Blue 3
DCPC	Department of Chemical and Petrochemicals
DNA	Deoxyribose Nucleic Acid
E. Coli.	*Escherichia coliform*
EC_{20}	Effective Concentration for effect on 20% population
EC_{50}	Effective Concentration for effect on 50% population
ED_{50}	Effective Dose for effect on 50% population
eV	electron volt
f.w.	Fresh weight
HCO1	HC Orange No. 1
LC_{50}	Lethal Concentration for effect on 50% population
LD_{50}	Lethal Dose for effect on 50% population
LiP	lignin peroxidases

mg/L	milligram per litre
MnP	manganese peroxidases
MT	Metric Tonne
MTCC	Microbial Type Culture Collection
NHE	Neutral Hydrogen Electrode
OD_{600}	Optical Density at 600 nm
PAC	Polyaluminium chloride
PDMDAAC	polydimethyldiallylammonium chloride
PFC	Polyferric chloride
RB2	Reactive blue 2
RB5	Reactive black 5
RBBR	Remazol Brilliant Blue R
RO16	Reactive Orange 16
rpm	rounds per minute
RR141	Reactive Red 141
RR2	Reactive red 2
RSM	Response Surface Methodology
RY2	Reactive yellow 2
U/mL	units per millilitre
UV	Ultra Violet
λ_{max}	wavelength at which maximum absorbance takes place

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1. Introduction

Water is most important resource of the world. All organism have water as their major component. Humans contain about 60% of water. It helps in transportation of minerals and salts in the body of organisms. It is also a reactant in photosynthesis which is the basic source of generating food on earth. In developing countries about 90% of water withdrawn is used for irrigation while that percentage is about 30% in developed countries. Earth is covered by about 71% of water, but most of it is found as saline water in the seas and oceans (about 96.5%). Freshwater which can be used by humans has major percentage present as permafrost and in groundwater. Only about 0.3% is available in lakes, rivers and the atmosphere.

Wastewater is the water which has been adversely affected in quality by some human activity. Sources of wastewater can be domestic, commercial, industrial or agricultural runoff. Domestic and commercial wastewater is mainly organic in nature and can be easily and efficiently treated in municipal wastewater treatment plants using biological treatment methods. Agricultural runoff is difficult to collect, hence not feasible to treat. Industrial wastewater is the most important wastewater as industries are lifeline of a nation, and they require large quantities of water for different process. Industrial wastewater is difficult to treat as its composition varies from industry to industry and also within a type of industry it varies according to different processes being used. Industrial wastewater is generally toxic and harmful to organisms if disposed without any treatment. It contains recalcitrant compounds which cannot be treated by conventional biological treatment process used for domestic wastewater. The discharge from various industries adversely affect soil fertility, water resources, aquatic organisms and ecosystem integrity. There are different types of industries in India like, dairy industry, pharmaceutical, leather processing, chemical, textile etc.

India's textile industry is one of the major sector. It contributes about 11 percent to the country's total exports basket. Approximately 40,000 different dyes and pigments are used in industries. Out of it more than 2,000 are different azo dyes. Annually over 7 X 10^5 tons of dyes are produced worldwide. During the year 2013 – 14 about 200.54 MT of dyes and pigments were produced, 2.33 MT of azo dyes (DCPC Annual Report 2013-14). Dyes are substances which impart its colours to the substrate on which it is applied. While a pigment is a material that changes the colour of reflected or transmitted light as the result of wavelength – selective absorption. It should have high limiting strength relative to the materials it colours. In contrast with a dye, a pigment generally is insoluble, and has no affinity for the substrate. Some dyes can be precipitated with an inert salt to produce a lake pigment, and based on the salt used they

could be aluminium lake, Calcium Lake or barium lake pigments. Both dyes and pigments appear to be coloured because they absorb some wavelengths of light more than others. Chromophore is the part of dye chemical structure which imparts colour to it. Dyes are classified on the basis of origin, chromophore, application and dyeing process. Azo dyes are represented by the presence of –N=N– bond. Based on dyeing process the dyes are classified as acid dyes, basic dyes, direct dyes, disperse dyes, vat dyes, reactive dyes, azo dyes, sulphur dyes etc. The history of textile dying dates back to prehistoric times as evidenced from dyed flax fibres found in the Republic of Georgia dating back to a prehistoric cave. Archaeological evidence shows that, particularly in India and Phoenicia, dyeing has been carried out for over 5,000 years. The dyes were obtained from animal, vegetable or mineral origin, with none to very little processing. By far the greatest source of dyes has been from the plant kingdom, notably roots, berries, bark, leaves and wood, but only a few have ever been used on a commercial scale.

Wastewater from textile industry generally contains high COD (in the range of about 1400 mg/l or more), moderate BOD (in the range of about 700 – 1000 mg/l), high suspended solids (in the range of 6000 mg/l or more), pH can be highly alkaline or acidic depending upon the process used and it may also contain heavy metals depending upon the dyes used. About 10% of dye stuff used in dyeing processes do not bind to textile fibres and are released into the environment (Maguire, 1992). When this wastewater is released in water bodies, it adds colour to it. These dyes and their intermediate degradation products (like aromatic amines) are toxic to the fauna and are also carcinogenic and mutagenic. Azo Dyes constitute more than 50% of total dyes produced worldwide (Neamtu *et. al.*, 2002; Sun *et. al.*, 2009). These are widely used in a number industries like textile, food, cosmetics and paper printing. These posses lethal toxicity, mutagenicity, genotoxicity and carcinogenicity to aquatic organsms as well as animals. Azo dyes are identified to cause urinary bladder tumors. Acute toxicity (EC_{50}) was observed at 87 mg/l of Acid Orange 7; and 0.13 mg/l of Disperse Red 1 when tested against *Daphnia similis* (de Luna *et. al.*, 2014; Vacchi *et. al.*, 2013). These dyes escape conventional wastewater treatment processes and persist in the environment as a result of their high stability to light, temperature, water, detergents, chemicals, soap and other parameters such as bleach and perspiration. Conventional treatment methods like Activated sludge process, adsorption onto activated carbon, coagulation by a chemical agent, ozone oxidation, hypochlorite oxidation, electrochemical method etc. were found to be ineffective and complete decolourisation/ degradation was not achieved (Tanaka *et. al.*, 2000; Yoshida *et. al.*, 1999; Shen *et. al.*, 2001; Sun *et. al.*, 2007). Flocculation causes generation of large amount of sludge;

and activated carbon adsorption poses the problem of adsorbent regeneration (Xu *et. al.*, 2004). Advanced Oxidation processes are used to treat the wastewater containing dyes. These generate hydroxyl radicals using different mechanisms. Hydroxyl radicals generated attack the organic compounds (dyes) and cause the degradation of the same. Photo – catalysis process uses light photons to generate the hydroxyl radicals. The light also helps in degradation of simple organic compounds. For Photo – catalysis method titanium dioxide was used as photo –catalyst. Fenton's oxidation process generates hydroxyl radicals by reaction between ferrous ions and hydrogen peroxide. Ferrous ions were regenerated from ferric ions by hydrogen peroxide. Fenton process is divided into 2 stages. First stage is called Fe^{2+}/H_2O_2. Reaction rate of this stage is very fast. This stage occurs for first few minutes. Second stage is called Fe^{3+}/H_2O_2. Reaction rate of this stage is slow compared to first stage. Fenton's reagents, i.e. ferrous sulphate and hydrogen peroxide were used for Fenton's oxidation process.

Azo dyes are highly hazardous to humans and environment and are resistant to degradation using conventional treatment methods. Hence, treatment technology needs to be developed for removal of azo dyes from the environment. Keeping in mind above facts, the present study was undertaken with the following objectives:

- To study the Photo – catalytic degradation of AO7 in sunlight and UV light.
- To study the chemical degradation of AO7 using Fenton oxidation method.
- To optimize and validate the oxidation process using response surface methodology.

2. Review of Literature

A **dye** is a coloured substance that has an affinity to the substrate on which it is being applied. The dye is generally applied in an aqueous solution, and requires a mordant to improve the fastness of the dye on the fibre.

Dyes are ionising and aromatic organic compounds. The colour of dyes is due to the chromophores present in them and these are applied to numerous substrates like textiles, leather, plastic, paper, food etc. Their structures have aryl rings that has delocalised electron systems. This helps in absorbing radiation within the visible range of spectrum, and responds to the colours. Generally they are represented as carbon, nitrogen, oxygen and sulphur. Some dyes and chromophores are shown in the Table 1.

2.1. Classification of Dyes

Dyes can be classified based on origin, dyeing process, chromophore and application as shown in Table 1.

2.2. Textile Dyes

Synthetic textile dyes constitute an important segment of business of specialty chemicals in the 21st century. They are mostly derived from two sources namely, coal tar and petroleum-based intermediates. These dyes are marketed as powders, granules, pastes or liquid dispersions. The concentration of active ingredient typically ranges from 20 to 80 percent. These are now characterised as new dyes and are regularly developed for meeting the demands of new technology, new kinds of fabrics, detergents, advances in dyeing machineries, along with overcoming the serious environmental concerns posed by some existing dyes. Another important factor is the fact that almost all the products are subjected to seasonal demand and variation. Industrial textiles Dyes must rise up to meet all these new and specific technical requirements. With the fast changing of the product profile of the textile industry, from high-cost cotton textiles to the durable and versatile synthetic fibres, the pattern of consumption of these dyes is also going through rapid changes.

Table 1 Classification of Dyes

<table>
<tr><th colspan="4">Based on Origin of dyes</th></tr>
<tr><td colspan="4">Natural dyes and Synthetic dyes</td></tr>
<tr><td colspan="4">Organic dyes and Inorganic dyes</td></tr>
<tr><th colspan="4">Based on Chromophore</th></tr>
<tr><td colspan="2">• Azo dyes - N = N –</td><td colspan="2">• Nitro - NO_2</td></tr>
<tr><td colspan="2">• Nitroso - NO (or = N – OH)</td><td colspan="2">• Carbon nitrogen group $\gtrdot C = NH$
- CH = N -</td></tr>
<tr><td colspan="2">• Ethylene $\gtrdot C = C \lessdot$</td><td colspan="2" rowspan="2">• Carbon sulphur group $\gtrdot C = S$
$\gtreqless C – S – S – C \lesseqgtr$</td></tr>
<tr><td colspan="2">• Carbonyl group $\gtrdot C = O$</td></tr>
<tr><th colspan="4">Based on Application</th></tr>
<tr><td colspan="2">• Food, Cosmetics and Drug dyes
• Laser dyes
• Leather dyes</td><td colspan="2">• Solvent dyes
• Contrast dyes
• Carbine dyes</td></tr>
<tr><th colspan="4">Based on Dyeing process</th></tr>
<tr><td colspan="4">Acid dyes are water soluble anionic dyes and insoluble in acid bath. Acid dyes are used for dying of wool, silk, nylon, acrylic fibre, paper, leather</td></tr>
<tr><td colspan="4">Basic dyes are water soluble cationic dyes. Basic dyes are mostly amino and substituted amino compounds. Basic dyes are used for dyeing acrylic fibre, cotton, wool, paper</td></tr>
<tr><td colspan="4">Direct dyes are used in a neutral or slightly alkaline dye bath without addition of mordant. Direct dyes are used for dyeing cotton, wool, silk, paper, nylon</td></tr>
<tr><td colspan="4">Disperse dyes are used for dyeing synthetic fibre like cellulose acetate, polyesters, nylon and acrylic fibres. These dyes are applied as finely divided materials in presence of dispersing agents.</td></tr>
<tr><td colspan="4">Vat dyes have highly complex structures and are insoluble in water and are used after reduction in alkaline liquor which produces water soluble alkali salt.</td></tr>
<tr><td colspan="4">Reactive dyes react to form covalent bond which directly react with the fibre and provide excellent wash resistance e.g. Procin MX, Cibacron F and Drimarene K</td></tr>
<tr><td colspan="4">Azoic dyeing, colours are made on the fibre by coupling diazotized materials while on contact with fibre. Final colour is controlled by the choice of diazoic and coupling components</td></tr>
<tr><td colspan="4">Sulphur dyes colour is produced by reacting sulphide and polysulphides with chlorinated aromatics. It is used for cotton cloth. Sulphur black dye is most commonly used.</td></tr>
</table>

(Source: Austin, 1985 and CPCB, 2004)

2.3. Azo Dyes

Dyes which contain R – N = N – R` functional group are called azo dyes. R and R` can be either aryl or alkyl, and an azo dye can contain one azo (mono azo dye), two (diazo) or more azo bonds. The Azo bond is the main reason for the colour developed in these dyes, and as per reports azo dyes constitute more than 50% of total dyes produced worldwide (Neamtu *et. al.*, 2002; Sun *et. al.*, 2009). Some common examples of azo dyes are Congo Red, Crystal Violet, Acid Yellow 23, Acid Red 14, Acid Orange 7, Reactive Black 5 etc. Among those most of the research work is concentrated on azo dyes. The studies on azo dyes have been comparatively more since these are widely used and their environmental apprehensions are extensively studied. Because of the reasons as mentioned, the present study stresses upon the various environmental, chemical and toxicological aspects of azo dyes.

2.4. Toxicity of Azo Dyes

Azo dyes are highly toxic and their degradation products like aromatic amines are also observed to be toxic, carcinogenic and mutagenic. The toxicity is usually measured as acute toxicity and chronic toxicity. Acute toxicity is caused immediately following a short term exposure to a chemical and chronic toxicity is due to prolonged exposure to a chemical in small quantities. Mortality or morbidity are the effects caused by acute toxicity. These are expressed as LC_{50}, EC_{50}, LD_{50} or ED_{50}. The concentration of the material to which the organisms were exposed that causes mortality or effect in 50% of the exposed population is called LC_{50} or EC_{50}. Owing to toxic azo dyes the effects are produced over a wide range if exposed organisms and micro – organisms are no exception.

2.4.1. Effect on micro – organisms

Azo dyes are observed to be toxic with EC_{20} value as low as 0.1 mg/l of Crystal violet for *E. coli.* (Lee *et. al.*, 2003). Mutagenicity is studied using Ames test, salmonella/ microsome assay test, stress inducible bioluminescent bacteria etc. Bioluminescent bacteria emits high levels of bioluminescence under non – toxic conditions but under toxic conditions the bioluminescence intensity decreases, hence effect of toxicity can be quantified. It is observed that azo dyes are mutagenic towards bacteria. Crystal violet was observed to affect the cellular membrane, proteins and DNA (Lee *et, al.*, 2003).

Microbial cultures of *Bacillus cereus* and *Escherichia coli* were used to study the toxicity of five different azo dyes (Michaels and Lewis, 1985). Survival was observed to be 74.2 ± 17.2%

at 0.5 mg/l and 27.9 ± 11.74% at 5.0 mg/l for Basic Violet 1. Basic Violet 2 showed survival of 83.2 ± 12.73% at 0.5 mg/l and 67.1 ± 15.83% at 5.0 mg/l. Basic Violet 3 showed survival of 69.8 ± 18.64% at 0.5 mg/l and 20.7 ± 6.57 at 5.0 mg/l. Basic Green 4 showed survival of 71.3 ± 25.82 % at 0.5 mg/l and 36.5 ± 22.39 at 5.0 mg/l. While least toxicity was observed for Trapaeolin 0 with survival of 92.0 ± 9.37 at 5.0 mg/l. It is now well established that azo dyes are toxic and mutagenic. Owing to the chemical structure and its activity, the degree of toxicity varies. There have been several studies on lower concentration of dyes as present in effluents and azo dyes are toxic even at lower concentrations.

Most of the textile dyeing and finishing process effluents containing reactive dyes were observed to be toxic to bioluminescent bacteria *Vibrio fischeri* (Wang *et al.*, 2002). Textile effluents were observed to contain dyes like Procion yellow HEXL, Procion blue HERD, Procion yellow H – E4R, Ambifix navy HER, Ambifix black BFGR, Procion navy HEXL, Remozal Black B, Procion deep red HEXL Procion red H – E3G, Procion red HE3B and Ambifix yellow VRNL and auxiliaries like Setaglesin A, Mollan 129, Detergent X, Perizym red, Breviol A69, Hydroxy clean, Dispersan PNG and Peristal BFL. The 15 minute EC_{20} values of bioluminescent bacteria due to dyes were observed in the range of 5 – 600 mg/l while that of due to auxiliaries were observed in the range of 9 – 6930 mg/l. It was observed that most toxic auxiliary was Peristal BFL with 15 minute EC_{50} of 45mg/l and lowest toxic was Setaglesin A with 15 minute EC_{50} of 22910 mg/l. Most toxic dyes among the tested dyes was observed to be Ambifix yellow VRNL with 15 minute EC_{20} of 5 mg/l and lowest toxic dye was Procion blue HERD with 15 minute EC_{20} of more than 610 mg/l. Different groups of dyes have an effect over the exposed micro – organisms and the solubility of dyes in water is the prime factor responsible for varying toxicity levels. In a study, bioluminescent bacteria *Photobacterium phosphoreum* and *E. coli.* GC2 were used to study effect of Basic dyes (Crystal Violet, Basic Violet 1, Basic Violet 2); Direct dyes (Direct Blue 71, Congo Red, Thiazol yellow G); Acid dyes (Orange II, Acid Green 25); Reactive dyes (Reactive Black 5, Reactive Blue 15); Disperse dyes (Disperse Yellow 5, Disperse Orange 11) and Domestic dyes (Navy Blue, Yellow Brown, Blue, Black) (Lee *et. al.*, 2003). It was observed that all 16 dyes caused cellular toxicity to both bioluminescent bacteria. Based on EC_{20} values the cellular toxicity decreased in the order: basic dyes, direct dyes, disperse dyes, domestic dyes, acid dyes and reactive dyes, due to addition of azo dyes. Crystal violet was observed to be most toxic among the dyes tested with EC_{20} value of 0.1 mg/l for *E. coli* GC2. It was observed that toxicity increased with increase in hydrophobicity. Mutagenic effects of crystal violet were studied using four stress –

inducible bioluminescent bacteria namely: DPD2794 (*recA::luxCDABE*) a DNA – damage sensitive strain, DPD2540 (*fabA::luxCDABE*) a membrane – damage sensitive strain, DPD2511 (*katG::luxCDABE*) an oxidative – damage sensitive strain and TV1061 (*grpE::lux CDABE*) a protein damage sensitive strain. It was observed that Crystal violet showed a dose – dependent bioluminescent response for DPD2794, DPD2540 and TV1061. At 2.5 mg (of Crystal violet)/l for DPD2794 and DPD2540 and mg (of Crystal violet)/l for TV1061 the maximum bioluminescent response was observed. This confirmed that Crystal violet caused damage to the cellular membrane, protein and DNA.

It has been reported that dyes are toxic and mutagenic even after the chemical structure is changed during biodegradation or chemical oxidation. The toxicity persists in metabolites of biodegradation and it may be higher for some of the dyes. A study on comparison of toxicity of bacterial decolourisation (by *Enterococcus faecalis* and *Clostridium butyricum*) product and parent dyes using *Vibrio fischeri* (Gottlieb *et. al.*, 2003). Dyes used were Reactive Black 5, Procion Yellow H – EXL, Procion Navy H – EXL, Procion Crimson H – EXL, Acid Orange 7 and Food Yellow 3 before and after biological decolourisation. It was observed that toxicity of Reactive Black 5 after hydrolysis was more than that of parent dye as shown by the EC_{50} of hydrolysed was 11.4 ± 3.68 mg/l while that of parent dye was 27.5 ± 4.01 mg/l. After decolourisation by bacteria, the toxicity of Reactive Black was increased with EC_{50} value of 0.7 ± 0.09 mg/l (with decolourisation by *E. faecalis*) and 2.2 ± 0.71 mg/l (with decolourisation by *C. butyricum*). Toxicity of Procion Navy, Procion Yellow and Procion Crimson was observed to increase after decolourisation by *C. butyricum* with EC_{50} values of 9.1 ± 0.53, 9.1 ± 1.04 and 8.1 ± 0.66 mg/l respectively (after decolourisation) from 18.9 ± 5.65, 71.0 ± 6.00 and 34.7 ± 0.27 mg/l respectively (of parent dye). Genotoxicity of parent Reactive Black 5 was not observed but after decolourisation with *C. butyricum* it was observed to be weakly genotoxic. It was observed that toxicity of reactive Black 5 in the effluent of anaerobic baffle bioreactor was increased as shown by EC_{50} value to 7.0 ± 4.5 mg/l. The toxicity of Food Yellow decreased slightly as observed by EC_{50} decrease from 22.1 ± 2.47 mg/l to 32.5 ± 1.53 mg/l when it was decolourised using *E. faecalis*. Acid Orange 7 toxicity was observed to increase about 100 times as observed by EC_{50} value was increased from 15.7 ± 2.68 mg/l to 0.2 ± 0.0 mg/l when it was decolourised using *E. faecalis*.

Azo dyes are not only toxic to bacterial strains, there inhibitory and toxic effects are established over other classes of micro – organisms like algae, protozoa etc. In a study by Novotný *et. al.* (2006) bacterium *Vibrio fischeri*, microalga *Selenastrum capricornutum* and ciliate

Tetrahymena pyriformis were used to study the toxicity of azo dyes [Reactive Orange 16 (RO16); Congo Red (CR)] and anthroquinone dyes [Remazol Brilliant Blue R (RBBR); Disperse Blue 3 (DB3)]. Acute toxicity was measured as bioluminescence inhibition of bacteria, growth inhibition of algae and viability, inhibition of growth, grazing effect and morphometric effects of protozoa (ciliate). Ames test was used to determine the mutagenicity of the dyes. EC_{50} for *Vibrio fischeri* after 30 minutes of exposure time for azo dyes was 1375 and 1623 mg/l for RO16 and CR, respectively. For anthroquinone dyes EC_{50} values were 813 and 359 mg/l for RBBR and DB3 respectively for 30 seconds of exposure time. EC_{50} for algal toxicity was observed to be 7.8, 4.8, 81.1 and 0.5 mg/l for RO16, CR, RBBR and DB3 respectively. For exposure time of 96 hours. DB3 was observed to be most toxic towards algae. The mean generation time for protozoa was increased by about 4 hours due to DB3 whereas other tested dyes exhibited no measureable effect. Significant change in W/L ratio and cell area were observed due to exposure to DB3 and CR respectively. All dyes except RO16 showed a decrease in ingestion capability (grazing). Maximum reduction in ingestion capability was observed to be 70% compared to control due to DB3 exposure. RO16 showed mutagenicity, in case of TA98 strain without metabolic activation and in case of TA98 and TA100 with metabolic activation. Whereas metabolic activation was required for DB3 to exhibit mutagenicity. At 50 µg dye of DB3 per plate showed a two fold increase of revertant number per plate for TA98. For strains YG1041 and YG1042, a low concentration of 15µg per plate respectively. RBBR also exhibited potential mutagenic effect.

2.4.2. Effect on aquatic organisms

Apart from microbes azo dyes are observed to be toxic to aquatic organisms. These are observed to have mutagenic potential and are also observed to be teratogenic too. Azo Dye HC Orange No 1 was observed to be 100% lethal to zebrafish at 8 mg/l and at 10 mg/l towards Goldfish with 48 hours of exposure in a study by Liu *et. al.* (2007).

In another study (Walsh *et. al.*, 1980) the toxicity of textile mill effluent to freshwater (*Selenastrum capricornutum*, *Daphnia pulex*, *Pimephales promelus*) and estuarine (*Skeletonema costatum*, *Palaemonetues pugio*, *Cyprinodon variegatus*) organisms was determined using effluents of 23 textile mills. The effluent at low concentration was observed to be stimulatory and at higher concentration to be inhibitory to *S. costatum*. Hence the concentration which had stimulated growth of *S. costatum* by 20% compared with controls is termed as SC_{20}. For freshwater alga *S. capricornutum* it was observed that 8 samples were

inhibitory and 15 were stimulatory when concentration of sample tested were 20%. For estuarine alga *S. costatum*, 14 samples were tested and it resulted in EC_{50} range of 1.5% to 93% while SC_{20} range was 0.5% to 21.8%. It was reported that freshwater organisms were more sensitive than the estuarine organism. It was observed that out of samples tested 61% were toxic to *D. pulex* and 40% were to *P. promelus* and 53% were toxic to *P. pugio* and *C. variegatus*. EC_{50} was observed to be in the range of 6.3% to 81.7% for *D. pulex* while LC_{50} for *P. promelus* was in the range of 16.5% and 64.7%, for *P. pugio* it was in the range of 12.8% to 34.5% and for *C. variegatus* it was in the range of 37.5% to 100%. Studies have confirmed that textile dyes have inhibitory effects over a wide range of aquatic organisms from microbes to zooplanktons to fishes. Toxicity of HC Orange No. 1 (HCO1) was studied by measuring immobilisation *Daphnia magna*, inhibition of embryo development of Zebrafish (*Brachydanio rerio*) and acute lethality to Zebrafish and Goldfish (*Carassius auratus*). *Daphnia magna* was immobilised when exposed to HCO1 with EC_{50} of 1.54 mg/l at 48 hours exposure period and 4.47 mg/l at 24 hours exposure period. HCO1 was lethal and caused deformity of Zebrafish embryos. It was observed that at a concentration of 4.93 mg of HCO1/l, 50% of eggs were coagulated after 4 hours of exposure. At EC_{50} of 0.66 mg HCO1/l, it was observed that there was lack of melanocyte development after 48 hours of exposure. It was observed that failure to hatch occurred at EC_{50} 0.19 mg HCO1/l. Abnormalities in embryos especially deformity of tail flexures was observed in 50% of embryos at concentration of 0.43 mg HCO1/l, which suggests that HCO1 is a potential teratogen. For adult Zebrafish LC_{50} was observed to be 4.38 mg/l for 48 hours of exposure and 4.04 mg/l for 96 hours of exposure. For Goldfish, at 48 hours of exposure LC_{50} was 7.52 mg/l, at 72 hours of exposure LC_{50} was 6.08 mg/l and at 96 hours of exposure LC_{50} was 5.37 mg/l. It was observed that at concentration 8.0 mg/l 100% of Zebrafish died within 48 hours of exposure while at concentration 10.0 mg/l, 100% of Goldfish died within 48 hours of exposure (Liu *et. al.*, 2007).

Toxicity of dyes has been reported on exposed organisms, and it is irrespective of its nature classified on the basis of functional group. Toxicity of Reactive Red 141 (RR141), which is an anionic dye with large molecule and Basic Red 14 (BR14), which is a cationic dye with small molecule, was studied by using green algae (*Chlorella sp.*) and waterfleas (*Moina macrocopa*). It was observed that the dry weight analysis of algae decreased with increase in initial dye concentration after 4 days of exposure. BR14, when dye concentration was increased from 10 mg/l to 50 mg/l, the dry weight decreased from 63.2% to 16.2%, while for RR141, when dye concentration was increased from 50 mg/l to 250 mg/l, dry weight decreased from 73.6% to

16.6%. For *Chlorella sp.* 96 hour EC_{50} was 10.88 mg/l and 95.55 mg/l for BR14 and RR141 respectively. For water fleas the 48 hour LC_{50} was 4.91 mg/l and 18.26 mg/l for BR14 and RR141 respectively. Combination of Humic acid and dyes decreased the toxicity of dyes to *Chlorella sp.* (Vinitnantharat *et. al.*, 2008). Azo dyes are not only toxic, some of the dyes are even mutagenic, it is reported that the mutagenicity of dye may be attributed to its chemical structure, position of substituents on aromatic rings and electron density. In a study (Ferraz *et. al.*, 2011) mutagenicity towards *Salmonella*, cell viability by *Hep*G2 and aquatic toxicity by *Daphnia similis* were studied due to Disperse Red 1 and Disperse Red 13. Disperse Red 13 was chlorine substituted Disperse Red 1. In the absence of mutagenic activation Disperse Red 1 and Disperse Red 13 exhibited mutagenic activity for all the strains except for Disperse Red 13 which tested negative for TA100. It was observed that mutagenicity of these dyes was due to frame – shift mutations. It was reported that Disperse Red 1 showed 43 times higher mutagenic potency of YG1041 compared to TA98 strain, while Disperse Red 13 showed 100 times higher mutagenic potency of YG1041 compared to TA98. The mutagenicity of Disperse Red 13 decreased by 32 times with the strain TA98 and 14 times with the strain YG1041 when compared to Disperse Red 1. This may be due to increase in electron density on the azo group of Disperse Red 1. Cell viability of *Hep*G2 was not affected by the dyes. It was observed that for Disperse Red 1 EC_{50} was 127 µg/l and NOEC was 10 µg/l for *Daphnia similis*, while Disperse Red 13 showed EC_{50} of 18.7 µg/l and NOEC of 1 µg/l.

Treatment of dyes by chlorination is reported to increase the mutagenicity of azo dyes. Ecotoxicity and mutagenicity of Disperse Red 1 was evaluated before and after treatment by chlorine using *Daphnia similis, Hydra attenuate* and *Salmonella*/ microsome assay (Vacchi *et. al.*, 2013). The EC_{50} of Disperse Red 1 was 0.13 mg/l and 1.9 mg/l for *D. similis* and *H. attenuata* respectively. The toxicity, after 30 minutes of chlorine treatment, had Ecc_{50} values of 4.3 mg/l and 0.7 mg/l for *D. similis* and *H. attenuate*. Mutagenicity of Disperse Red 1 was measured using *Salmonella*/ microsome assay with TA98 strain and YG1041 strain. In the absence of metabolic activation, Disperse Red 1 was non – mutagenic to ta98, while gave positive responses to TA98 in presence of metabolic activation and in YG1041 with and without metabolic activation. The mutagenicity was observed in chlorination products in both strains and the potencies were higher than the parent dye.

2.4.3. Effect on plants

Effect of textile effluent on plants has been observed to be stimulatory at low concentration and inhibitory at high concentrations. The effects are also dependent on the plants used for the study. It was observed that germination and growth of Bengal gram (*Cicer arietinum*) were inhibited by concentration as low as 5% of textile effluent (Dayama, 1987), while many other plants showed stimulatory effect upto 50% of textile effluent concentration (Mohammad and Khan, 1985; Swaminathan and Vaidheeswaran, 1991; Chhonkar *et. al.*, 2000).

In a study the effect of textile effluent on germination and growth of Kidney bean (*Phaseolus aureus*) and Lady's finger (*Abelmoschus esculentus*) was studied. It was observed that average time taken for germination was 5 and 7 days for Kidney beans and Lady's finger respectively when irrigated with water which was delayed by 9 and 12 days when irrigated with 100% effluent, and 7 and 9 days when irrigated with 75% effluent. Dilution of 50% and 25% had no effect on germination time. It was observed that percent germination decreased to 75% and 80% for Kidney beans and Lady's finger when irrigated with 100% effluent while it decreased to 90% for both Kidney beans and Lady's finger when irrigated with 75% effluent. 50% and 25% dilution of effluent had no effect on percent germination. When 50% effluent dilution was used to irrigate the plants the maximum increase in shoot and root length, size of leaves and number of leaves, and dry weight was observed compared to water. It was observed that shoot length increased from 13.5 cm (Kidney beans) and 10.3 cm (Lady's finger) to 15.2 cm and 16.8 cm when irrigated with 50% effluent dilution. Root length was observed to increase from 6.7 cm and 6.0 cm for Kidney beans and Lady's finger when irrigated with water to 7.7 cm and 7.8 cm when irrigated with 50% effluent dilution. Dry weight of plants was observed to increase from 1.173 g and 1.325 g for Kidney beans and Lady's finger when irrigated by water to 1.732 g and 1.893 g when irrigated by 50% effluent dilution (Ajmal and Khan, 1985).

In some of the studies, stimulatory effects have been observed on crops at lower concentration of effluent. The effect of textile effluent on two varieties of peanut (*Arachis hypgaea L.*) (TMV – 10 & JL – 24) were studied (Saravanamoorthy & Kumari, 2007). Parameters like increased germination, chlorophyll a, chlorophyll b, total chlorophyll, growth parameters, yield and yield contributing characteristics were studied. For 100% textile effluent lowest percent germination of 38% and 42% for TMV – 10 and JL – 24 respectively was observed, while for 50% textile effluent highest percent germination of 95% and 92% for TMV – 10 and JL – 24. After 50 days, chlorophyll content was observed to increase with increase in effluent percentage, from

0 to 100% of effluent with chlorophyll a increased from 0.45 ± 0.02 and 0.49 ± 0.01 mg/g fresh weight (f. w.) (for JL – 24 and TMV – 10) to 0.69 ± 0.02 and 0.78 ± 0.01 mg/l f. w. Chlorophyll b was observed to increase from 0.16 ± 0.02 and 0.20 ± 0.01 mg/g f. w. (for JL – 24 and TMV – 10) to 0.34 ± 0.002 and 0.42 ± 0.01 mg/g f. w. Total chlorophyll was observed to increase from 0.61 ± 0.2 and 0.69 ± 0.02 mg/g f. w. (for JL – 24 and TMV – 10) to 0.96 ± 0.03 and 1.20 ± 0.1 mg/g f. w. Similarly root and shoot length were observed to increase in effluent concentration from 0 to 100% after 50 days of irrigation. Shoot length was observed to increase 21.4 ± 0.3577 cm and 17.6 ± 0.4560 cm (for TMV – 10 and JL – 24) to 28.4 ± 0.4560 cm and 19.4 ± 0.8294 cm. Root length was observed to increase from 12.36 ± 0.2102 cm and 11.45 ± 0.3081 cm (for TMV – 10 and JL – 24) to 14.11 ± 0.2323 cm and 12.44 ± 0.1340 cm. Number of pod yield per plants were observed to increase upto 50% of effluent concentration and it decreased for 75% and 100% of effluent concentration.

In another similar study (Malaviya *et. al.*, 2012) the effect of dyeing effluent was studied on germination and growth of Pea (*Pisum* sativum). Parameters like percent germination, root and shoot length were studied. It was observed that speed of germination was increased for 20% effluent dilution but decreased as effluent concentration increased to 100%. Germination period for 20% effluent dilution was observed to be 14 days while that of 100% effluent it was 22 days. Percent inhibition was increased above 20% effluent dilution with, maximum percent inhibition of 19.44% for 100% effluent. Root length was observed to increase for 20% effluent dilution at 9.38 ± 0.53 cm compared to control with root length of 8.56 ± 0.20 cm, whereas it decreases for higher concentrations with minimum at 100% effluent with value of 6.44 ± 0.70 cm. Shoot length was observed to increase for 20% effluent dilution at 9.22 ± 1.81 cm compared to control with shoot length of 8.44 ± 1.53 cm, it decreases as concentration of effluent increases with minimum at 100% effluent with value of 6.81 ± 0.62 cm.

Since other nutrients/ pollutants present in textile effluent may affect the germination and growth of plant a study was carried out to understand the effect of wastewater generated from different unit processes in a textile industry. Effect on germination of country Beans (*Labbab niger* variety *typicus*) due to textile dyeing effluent was studied by Hassan *et. al.* (2013). Seven types off dyeing effluents were used comprising tube well water (D_1), 2nd wash after scouring and bleaching (D_2), Enzyme treatment (D_3), 2nd wash after block design (D_4), neutralization treatment (D_5), 2nd wash after soaping (D_6), Fixing treatment (D_7) and mixed effluent from ETP (D_8). It was observed that percentage of germination was highest for seeds irrigated with D_2 sample, while lowest for D_4 and D_8 with values of 33.33% and 13.33% after 3 days. After 5

days, highest percent germination was observed for D_5 irrigated samples with value at 93.33% and lowest percent germination was observed for D_4 irrigated samples with values at 53.33%. D_5 irrigated samples showed maximum value of shoot length of 23.47 cm while D_7 irrigated samples showed minimum value of shoot length of 17.26 cm. The maximum value of root length was observed for D_6 irrigated samples while minimum value was observed for D_1 irrigated samples. It can be confirmed that effluents that exceeded the standard limits have a negative effect on germination and growth of plants whereas treated effluent showed no negative impacts. It can be concluded that treated effluent can be used for irrigation of these crops.

2.4.4. Effect on animals

Azo dyes have been linked to human bladder cancer, splenic sarcomas, hepatocarcinomas and nuclear anomalies in experimental animals (Bragger *et. al.*, 1997). There have been high number of cases of bladder cancer in dye workers exposed to large quantities of azo dye. Azo dye gets reduced by enzymes present in intestines of animals to form aromatic amines which are mutagenic and carcinogenic (Umbuzeiro *et. al.*, 2005; de Lima *et. al.*, 2007). Benzidine based azo dyes are observed to cause human urinary bladder cancer (Haley, 1975). These dyes pass through food chain and reach human and cause hypertension, sporadic fever, renal damage, cramps etc. Myslak *et. al.*, (1991) showed that there was a high risk of bladder tumor for persons with employment as a painter using azo dyes, the relative risk was estimated to about 2.76. During that time the paints were prepared by themselves by grinding and mixing the dyes and colouring mixture was prepared by addition of solvents, this caused the inhalation of dyes by the workers and affecting them.

The occurrence of cancer on exposure of azo dyes may be a result of alteration in DNA or DNA adduct formation. Tsuda *et. al.*, (2000) used 24 azo compounds: Amaranth (CI Food Red 9), *p* – Aminoazobenzene, *o* – Aminoazobenzene, Azobenzene, CI Acid Red 114, CI Pigment Red 3, Dacarzine, *p* – Dimethylaminoazobenzene, Direct Black 38, Direct Blue 6, Direct Blue 15, Disperse Yellow 3, Evans Blue (CI Direct Blue 53), Oil Orange SS, Orange G, Orange I, Phenaopyridine HCl, Ponceau 3R, Ponceau SX, Sudan I, Sudan II, Sudan III, Sudan Red 7B and Trypan Blue (CI Direct Blue 14) to study the comet assay in eight mouse organs and understand the DNA damage due to the azo compounds. DNA damage was observed in stomach by *p* – Aminoazobenzene, Azobenzene, Disperse Yellow 3, *p* – Dimethylaminoazobenzene, Sudan I, Ponceau SX, Amaranth, Orange I, Sudan III, Direct Black

38, Direct Blue 6 and Phenazopyridine; in colon by *p* – Aminoazobenzene, *o* – Aminoazobenzene, Azobenzene, *p* – Dimethylaminoazobenzene, Pigment Red 3, Sudan I, Ponceau SX, Ponceau 3R, Amaranth, Orange I, Sudan III, Direct Black 38, Direct Blue 6 and Phenazopyridine; in liver by *p* – Aminoazobenzene, *o* – Aminoazobenzene, Disperse Yellow 3, *p* – Dimethylaminoazobenzene, Ponceau SX, Ponceau 3R, Amaranth, Direct Black 38, Direct Blue 6, Phenazopyridine, Dacarbazine and CI Direct Blue 15; in urinary bladder by *p* – Aminoazobenzene, Azobenzene, *p* – Dimethylaminoazobenzene, Ponceau 3R, Amaranth, Sudan III, Direct Black 38 and Direct Blue 6; in lung by *p* – Aminoazobenzene, *o* – Aminoazobenzene, Azobenzene, *p* – Dimethylaminoazobenzene, Ponceau SX, Ponceau 3R and Direct Blue 6; in brain by *p* – Aminoazobenzene, Disperse Yellow 3, Amaranth, Direct Black 38 and Direct Blue 6; in bone marrow by *p* – Dimethylaminoazobenzene, Ponceau 3R, Amaranth and Direct Black 38; and in kidney by Ponceau 3R. Oil Orange SS, Orange G, Sudan II, Sudan Red 7B, CI Acid Red 114, Evans Blue and Trypan Blue showed no significant DNA damage in any of the organs.

In a similar study the carcinogenic and mutagenic potential of azo dye textile processing plant effluent using Wistar rats and *Salmonella*/ microsome assay with the strains TA98 and YG1041 were studied. The rats were exposed to effluent through drinking water with concentrations of 0.1%, 1% and 10%. It was observed that when rats were exposed to concentrations 1% and 10% of the effluent caused increased number of preneoplastic lesions in the colon of rats. Mutagenicity by *Salmonella*/ microsome assay showed positive potential (de Lima *et. al.*, 2007).

The studies on toxicity, mutagenicity and carcinogenicity have confirmed that azo dyes, its metabolites and other azo compounds have associated effects, it can affect a wide range of plants, animals and microbes exposed to them and thus pose a serious eco – toxicological hazard if proper treatment and disposal is not attempted. The treatment of dyeing effluent is, therefore, an important environmental concern.

2.5. Treatment of dyes

There are different treatment methods used for treatment of dyes. Among them most commonly used methods are coagulation, flocculation, adsorption, air floatation etc. Microbial degradation is also used to treat dyeing wastewater. During conventional treatments Activated sludge process is observed to be less efficient in degradation of dyes.

2.5.1. Coagulation/ flocculation

Coagulation is a complex process having different mechanisms like charge neutralisation, complex formation, adsorption, bridge formation and surface precipitation. Charge neutralisation is major process in most coagulation process. When traditional aluminium salts are used, aluminium hydrolysis products are formed *in situ* after dosing induces the charge neutralisation. Amorphous hydroxide precipitates are formed when aluminium hydrolysis products aggregate, rearrange and further hydrolyse. These amorphous solids further neutralise, adsorb colloidal matters and forms bridge among fine particles. This mechanism is called sweep – flocculation. Electrostatic patch coagulation is the process in which the charge on colloids is neutralised by charge on coagulant, hence the particles are settled due to coagulation of the particles as they agglomerate to form larger settleable particles.

Comparison between different types of Aluminium coagulants on removal of textile dyes are studied. Removal of Direct black 19, Direct red 28 and Direct blue 86 was studied by coagulation using Aluminium chloride ($AlCl_3$), Polyaluminium chloride (PAC) and purified Al_{13} (Shi et. al., 2007). It was observed that the removal increased with increase in coagulant dosage. The efficiency of coagulant was in the order Al_{13} > PAC > $AlCl_3$. For the removal of Direct red 28, when the dosage was low Al_{13} performed better than other two coagulants but with increase in dosage most efficient coagulant was $AlCl_3$. Highest removal was achieved by Al_{13} and poorest by $AlCl_3$ in case of Direct blue 86. About 90% removal of Direct red 28 was achieved at 6×10^{-5} mol/l dosage of all the coagulants, while it was observed that 60%, 80% and 70%, for $AlCl_3$, Al_{13} and PAC respectively, was achieved for Direct blue 86 at the same dosage. With decrease in pH the coagulation efficiencies of PAC and Al_{13} were observed to increase with maximum efficiency at pH<6. With decrease in pH efficiency of $AlCl_3$ increases first then decreased rapidly with maximum at pH around 6. At pH 5.8 $AlCl_3$ was able to remove 100% dye at high dosage but at pH 3.5 it was able to remove less than 60% of dye at all dosages. Al_{13} was observed to be most efficient while PAC showed higher removals than $AlCl_3$.

Effect of addition of flocculant to improve the colour removal from textile wastewater has been studied. Synthetic wastewater containing Reactive black 5 (RB5), Reactive blue 2 (RB2), Reactive red 2 (RR2) and Reactive yellow 2 (RY2) and also real wastewater was studied using alum or ferric salt and a polymer flocculant, synthesized from cyanoguanidine and formaldehyde (Joo *et. al.*, 2007). For synthetic wastewater, 1 g/l of inorganic coagulant and 0.25 g/l of synthetic polymer were used to study the effect of pH. Over the pH range of 4 – 7,

there was no significant difference in removal for RY2, RB5 and RR2, while removal of RB2 decreased as pH was increased when ferric salt/ polymer were used. When alum/ polymer were used removal of RR2 decreased with increase of pH in alkaline condition and optimum pH was 5, while removal of RY2, RB 5 and RB2 remained independent of pH. When inorganic coagulation was used without polymer, removal of 20% or less was observed. With increase in polymer dosage, dye removal increased, with 99% decolourisation above 0.15 g/l (for RY2, RB5 and RB2) and 0.25 g/l (for RR2) dosage of polymer and 1 g/l dosage of alum/ ferric salt. The removal efficiency was not changed significantly, when dosage of inorganic coagulants was varied from 0.4 g/l to 1.0 g/l. Without polymer only 12% and 5% of decolourisation of real wastewater was observed for ferric salt and alum, respectively when pH was 3.5 for ferric salt and 5.0 for alum at dosage of 2 g/l. With polymer dosage of 0.25 g/l, when alum dosage was increased from 2g/l to 5 g/l, colour removal increased from 39% to 54% and maximum removal of 48% was observed for ferric salt dosage of 3 g/l. About 40% and 44% of COD removal was observed when 5 g/l dosage of alum and 4 g/l dosage of ferric salt were used with 0.25 g/l of polymer dosage. When polymer dosage was increased from 0 to 0.5 g/l for fixed dosage of inorganic coagulant 2.0 g/l, colour removal increased up to 62% for alum/ polymer treatment with polymer dosage of 0.5 g/l, while for ferric salt/ polymer treatment maximum colour removal was observed for 0.15 g/l dosage of polymer.

Effect of inorganic and organic coagulants on removal of colour is also studied and their combination was also prepared to study the effect of inorganic – organic composite coagulant. Decolourisation of simulated dye wastewater containing 0.1 g/l of Disperse blue HGL and Reactive blue STE and actual textile wastewater (Gao *et. al.*, 2007) using Polyferric chloride (PFC), polydimethyldiallylammonium chloride (PDMDAAC) and a composite coagulant produced by premixing PFC and PDMDAAC. Initial dye concentration was 0.1 g/l for the study. Optimum colour removal of 74% at PDMDAAC dosage of 18 mg/l and 88% at PFC dosage of 30 mg/l was observed for Disperse blue HGL wastewater. While PFC – PDMDAAC achieved 95% removal at 16 mg/l dosage and optimum colour removal of 98% at 30 mg/l for Disperse blue HGL wastewater. For Reactive blue STE wastewater, 68% colour removal was observed for 45 mg/l of PDMDAAC while 86% colour removal was observed for 45 mg/l of PFC – PDMDAAC. For PFC dosage of 30 mg/l, Disperse blue HGL showed maximum colour removal of 98% at pH 6, while for PFC dosage of 60 mg/l Reactive blue STE showed maximum colour removal of 77% at pH 6. For PFC – PDMDAAC dosage of 60 mg/l, Disperse blue HGL showed small change in colour removal which was higher than 96% for different pH values

(3.0 to 10.5). For PFC – PDMDAAC dosage of 16 mg/l, it was observed that for Reactive blue STE colour removal increased from 89% to 99% when pH increased from 3.0 to 7.5 and appreciable decrease was observed above pH 7.5. For actual wastewater, colour removal of 88.9%, 52.7% and 43.1% was observed for PFC – PDMDAAC, PFC ad PDMDAAC for dosage of 150 mg/l.

2.5.2. Adsorption

Adsorption is a surface – based process and it can be physical or chemical in nature. In case of physical adsorption the adsorbate is stuck physically in the pores present in adsorbent surface and van der walls forces are involved in interaction between adsorbate and adsorbent. While in case of chemical adsorption the adsorbate and adsorbent interact by covalent bonding. There have been several studies on removal of dyes by adsorbents, and the process is regarded as a popular method for treatment of dyes. Adsorption of dyes is dependent on parameters like agitation time, dye concentration, adsorbent dose, pH and temperature. Namasivayam and Kavitha (2002) reported that when dye concentrations of 20, 40, 60 and 80 mg/l were used, amount of dye adsorbed (mg/g) on the activated carbon prepared from coir pith increased as agitation time was increased and equilibrium was reached after 10 minutes. As the dye concentration increased, the percent decolourisation decreased from 66.5 to 30.5%. It was observed that adsorption followed second – order kinetics. Percent decolourisation increased with increase in adsorbent dosage and the process was observed to follow Langmuir isotherm. As pH was increased from 2 to 4, percent decolourisation for 20 mg/l initial dye concentration was observed to decrease from 70 to 57%, while for 40 mg/l initial dye concentration it was observed to decrease from 50 to 42% and remained almost same up to pH 10. Percent decolourisation percent increased as temperature was increased. Regeneration and reuse of adsorbent in the process is an important aspect for waste minimization and resource conservation. Regeneration of adsorbent is also not easy as shown by in desorption studies that percent desorption only increased from 0.5 to 9.0 % when pH was increased from 2 to 10 when initial dye concentration was 20 mg/l and from 1.2 to 11% when pH was increased from 2 to 10 when initial dye concentration was 40 mg/l. Surface area of an adsorbent and free active binding sites play a major role in adsorption to dyes. Adsorption of Methylene blue using activated carbon produced from oil palm fibre was studied (Tan *et. al.*, 2007). Parameters like contact time, initial Methylene blue concentration and temperature were studied at pH of 6.5. It was observed that for the prepared activated carbon BET surface area was 1354 m^2/g, with

total pore volume of 0.778 cm^3/g and average pore diameter of 2.3 nm. For initial concentration of Methylene blue in the range of 50 to 200 mg/l, contact time to reach equilibrium was 1 to 6 hours, while for initial concentration of 300 to 500 mg/l the contact time was observed to be 24 hours. Adsorption process was observed to follow Langmuir isotherm with adsorption capacity of 277.78 mg/g. It was observed that adsorption process follow pseudo – second order kinetics. When temperature was increased from 30 to 50°C adsorption capacity of Methylene blue increased from 277.78 to 384.62 mg/l.

In another study (Gomez *et. al.*, 2007) decolourisation of Acid red 97, Acid orange 61 and Acid brown 425 by using activated carbon at room temperature (25°C) was studied. It was observed that when equilibrium is reached the maximum amount of adsorption of each dye decreases. It was observed that adsorption process followed second – order kinetics and the rate constants were similar for individual dyes and in mixture. Adsorption capacities were similar for individual dyes and mixtures at equilibrium for Acid red 97 and Acid orange 61 but for Acid brown 425 adsorption capacity decreased to more than half when studied individually and it followed Freundlich isotherm. It has been confirmed in the studies that the dyes compete for adsorption if present in a mixture and some of the dyes may fail to adsorb.

2.5.3. Microbial Degradation

Degradation of azo dyes has been reported by many micro – organisms. The degree of degradation of dyes depends on microbial population and the activity of enzymes facilitating degradation. The type of enzymes is further determined by the prevalent environmental conditions. It has been observed that under anaerobic conditions, azo dyes are degraded to aromatic amines by azoreductase enzymes (Plumb *et. al.*, 2001; Yoo *et. al.*, 2001). The intermediate products of aromatic amines are observed to be carcinogenic, mutagenic and toxic (Bell *et. al.*, 2000) and are degraded under aerobic conditions for complete detoxification. Microbial degradation is influenced by pH, salinity, cations, anions, temperature, BOD, COD and oxygen as important regulatory factors (Ganesh *et. al.*, 1994).

2.5.3.1 Bacteria

Bacteria has been used to degrade organic dyes from wastewater. A number of species of bacteria like *Pseudomonas* sp., *Bascillus* sp., *Acinetobactor* sp., *Legionella* sp., *Staphylococcus* sp., *Proteus* sp., *Enterococcus* sp., *Salmonella* sp. and *Klebsiella* sp. Adapted bacteria can be isolated from discharge and drainage pipes and soil samples of drains of textile industries. It is

reported that adapted bacteria was able to degrade a mixture of dyes by about 40.62% with COD removal of about 40.95% (Olukanni *et. al.*, 2006), whereas in case of individual dyes decolourisation of 82% and 75% for Crystal Violet 51 and Tatrazine respectively is reported (Chaube *et. al.*, 2010). The decolourisation is affected by the agitation conditions of the reactor vessel. Decolourisation is more when the reactor is in stationary condition. In shaking conditions only 16% decolourisation is reported for Direct Blue 6 and 30% of Acid Blue 113 while in stationary conditions it was 92% and 90%, respectively (Kalme *et. al.*, 2007; Gurulakshmi *et. al.*, 2008). COD reduction also is more in static condition 88.95%, while in stationary condition it is only 22.16% (Kalme *et. al.*, 2007). The reason for stationary condition to be more effective may be due to competition of oxygen and the azo compounds for the reduced electron carriers under aerobic condition.

pH affects the growth rate of bacteria. Generally optimum pH for growth of bacteria is in the range of 6.5 to 7.0. Optimum pH of 7.0 and 6.5 is reported for Orange II and Direct Blue 71, respectively, when degraded with *Pseudomonas* sp. (Oranusi and Ogugbue 2005). *Bacillus subtilis* showed maximum decolourisation at pH 7 for degradation of Acid Blue 113 (Gurulakshmi *et. al.*, 2008). Maximum degradation of Orange 3R is reported for *Bacillus* sp. at pH 7, *Klebsiella* sp. at pH 9 and *Pseudomonas* sp. at pH 6 (Ponraj *et. al.*, 2011). It has been reported that degradation of azo dyes by bacteria results in rise in pH of medium by 0.8 – 1.0 due to formation of aromatic amines (Knapp and Newby 1995; Hu 1994; Bhatt *et. al.*, 2005). Increase in temperature caused increase in degradation of dyes upto a limit above which viability of bacteria decreases and hence the degradation is negligible. Maximum decolourisation of 79% and 66.6% for Direct Violet 51 and Tatrazine, respectively was observed at 40°C after 72 hours of incubation period (Chaube *et. al.*, 2010).

Initial concentration of dye affects the degradation rate. As initial concentration of dye increases, the rate of degradation decreases. It was reported that after 72 hours of incubation degradation of Direct Blue 6 by *P. desmolyticum* for 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l and 250 mg/l was 100%, 92%, 80%, 40% and 15% respectively (Kalme *et. al.*, 2007). While in case of mix culture of bacteria was used for degradation of Direct Violet 51 and Tatrazine, as initial concentration was increased from 20 to 100 mg/l, the degradation decreased from 66.6% to 43.58% for Direct Violet 51 and from 81.1% to 23% for Tatrazine dye for 72 hours of incubation (Chaube *et. al.*, 2010).

Amount of initial inoculum used also affects the degradation rate of dyes. It was reported by Gurulakshmi *et. al.* (2008) that when inoculum was increased from 5% to 20% (v/v) the decolourisation rate increased from 1.246 mg/l/h to 1.984 mg/l/h, but further increase in inoculum size did not affect the decolourisation significantly. Different species of bacteria show effect of inoculum size on decolourisation differently. *Salmonella sp.* and *Klebsiella sp.* showed maximum decolourisation of 67.19% and 53.91%, respectively at 6% of inoculum, while *Bacillus sp. and Pseudomonas sp.* showed maximum decolourisation of 86.72% at 4% inoculum and of 50% at 10% inoculum respectively (Ponraj *et. al.* 2011).

Textile wastewater contains large amount of salts which can be inhibitory to bacteria, hence halophilic and halotolerant bacteria can be used to decolourise it. It is reported that when salt concentration is increased from 10 to 150 g/l *Halomonas aquamarina* showed increase in degradation from 70% to 100% of Remazol Black B, while decolourisation decreased from 80% to 40% when *Halomonas meridian* and *Halomonas salina* are used (Asad *et. al.*, 2007).

Carbon and nitrogen are the basic nutrients required by the bacteria for growth. Effect of changing the source of carbon like Glucose, sucrose, mannitol etc. is also reported in the literature. Different species of bacteria shows maximum degradation of dye using different carbon source. It is reported that for decolourisation of Orange 3R by *Bacillus* sp. glucose showed maximum decolourisation of 87.80%; *Klebsiella* sp. and *Pseudomonas* sp. showed maximum decolourisation of 72.36% and 80.49% respectively when sucrose is used; while mannitol showed maximum decolourisation of 86.18% for *Salmonella* sp. (Ponraj *et. al.*, 2011). Mix culture showed maximum degradation using glucose and lactose as carbon source of 65% and 63% for Direct Violet 51 and of 59% and 56% for Tatrazine dye (Chaube *et. al.*, 2010). Effect of changing the source of nitrogen like beef extract, peptone, yeast extract, sodium nitrate etc. is reported in the literature. When sodium nitrate concentration is increased from 0.01 g/l to 1.0 g/l degradation decreased from 74.84% to 48.88% for Orange II while for Direct Blue 71 degradation decreased from 71.54% to 23.75% when *Pseudomonas* sp. was used (Oranusi and Ogugbue, 2005). The reason can be attributed to the fact that in absence of any inorganic nitrate salts, azo bonds of dye act as source of nitrogen for microbes and decolourisation increases, but when there is an inorganic nitrate salt, microbe prefer nitrate salt over azo bond for nitrogen source. Different species of bacteria shows maximum degradation with different nitrogen source. It is reported that *Bacillus* sp. showed maximum degradation of 85.29% for Orange 3R when peptone is used as nitrogen source; *Salmonella* sp. and *Pseudomonas* sp. showed 70.67% and 87.33% when beef extract is used; while *Klebsiella* sp.

showed maximum degradation of 70.17% when yeast extract is used as nitrogen source (Ponraj *et. al.*, 2011). Sulphate is also a nutrient required for growth of bacteria. It is reported that when Magnesium sulphate concentration is increased from 0.02 g/l to 0.08 g/l degradation of Orange II decreased from 64.44% to 41.45%, while that of Direct Blue 71 decreased from 76.25% to 6.25% by *Pseudomonas* sp. The sulphur requirements of microbes were provided by desulphonation of the sulphonate group of the dye in the absence of sulphate in medium (Oranusi and Ogugbue, 2005).

It has been reported in the literature that degradation of dyes is associated with the enzyme activity. Lacasse and tyrosinase activity were induced upto 72 hours of incubation with 411% and 240% respectively compared with 0 hour activity for the decolourisation of Direct Blue 6 by *Pseudomonas desmolyticum*. After complete decolourisation these enzymes were decreased in the batch culture. Lignin peroxidase activity was 260% upto 96 hours of incubation. Activity of aminopyrine N – demethylase, DCIP reductase and malachite green were observed to be 187%, 134% and 206% after 96 hours compared to 0 hour activity (Kalme *et. al.*, 2007).

2.5.3.2 Fungi

White rot fungi has the ability to degrade the natural polymers such as lignin and cellulose. These have also been reported to degrade synthetic chemicals which are recalcitrant compounds (Field *et. al.*, 1993; Knapp *et. al.*, 1995; Martins *et. al.*, 2003). Different white rot fungi generally used for dye degradation are *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Irpex lacteus* etc. Some other fungi are also used for degradation of dye are *Aspergillus niger grp.*, *Streptomyces krainskii* SUK – 5, *Sclerotium rolfsii*, *Funalia trogii* etc. White rot fungi produces extracellular enzymes like manganese peroxidases (MnP), lignin peroxidases (LiP) and laccases has been reported to degrade the recalcitrant compounds.

Shaking conditions add oxygen to the solution. This affects the degradation of dyes by fungi. Different fungal species are affected differently. *Phanerochaete chrysosporium* favours static conditions, whereas *Trametes villosa* showed maximum degradation under shaking conditions (Machado *et. al.*, 2006). It is reported that degradation by *Aspergillus niger grp.* decreases from 87.8% and 80% for Reactive Red 195 and Reactive Green 11, respectively under static conditions to 26.9% and 30.7% under shaking conditions (Zope *et. al.*, 2007). Degradation of Reactive Blue 59 by *Streptomyces krainskii* SUK – 5 showed degradation of 95.33% under shaking conditions (Mane *et. al.*, 2008).

pH affects the degradation of dyes by fungus. pH affects the growth of fungus which in turn the degradation of dyes. Fungi prefers slightly acidic conditions for growth. It is reported decolourisation by *P. chrysosporium* of Orange II increased from 39.84% to 86.34% as pH is increased from 3 to 5, as pH is further increased to 7 decolourisation decreases to 51.42% (Sharma *et. al.*, 2009). When a mix consortia of fungi is used for degradation of Levafix blue E – RA, it is reported that optimum pH of 5 to 6 resulted in decolourisation of more than 80% (Kalpana *et. al.*, 2012).

Initial dye concentration affects the degradation of dye by fungi. The main reason may be due to the toxicity of dye at higher concentration of textile dyes. It is reported that complete degradation of Navy Blue Rx for concentration of 30mg/l and 50 mg/l is observed in 24 and 48 hours, while concentrations above that were toxic to fungal strain (Mane *et. al.*, 2008).

Temperature helps in the growth of fungi, but high temperature causes decrease in viability of fungal strains hence optimal condition of temperature is reported for the degradation of dyes by fungi. It is reported that for *A. niger* optimum temperature is 30°C for the degradation of Reactive Red 195 and Reactive Green 11 (Zope *et. al.*, 2007). Similarly for *P. chrysosporium* optimum temperature is 30°C for degradation of Orange II (Sharma *et. al.*, 2009). When mix consortia of fungi is used at 20°C only 10% decolourisation is observed while, optimum temperature is reported as 35°C (Kalpana *et. al.*, 2012).

As the concentration of inoculum is increased the degradation of dyes by fungi is increased. It is reported that the decolourisation increased from 34% to 70% when amount of *T. villosa* is increased from 0.002 g/l to 0.006 g/l, but after 21 days of incubation there was no significant difference in decolourisation rates (Machado *et. al.*, 2006). Decolourisation of Reactive Red 195 increased from 60% to 87.8% while that of Reactive Green 11 it increased from 55% to 76.1% when *A. niger* amount is increased from 0.2 g/l to 1.0 g/l (Zope *et. al.*, 2007).

When a different carbon source or nitrogen source, which is easily catabolisable, is used decolourisation increases. When Glucose is used as carbon source maximum decolourisation of 93% and 80% for Reactive Red 195 and Reactive Green 11 respectively, is reported at 10 g/l of glucose concentration. Similarly maximum decolourisation of 87.8% and 70.3% for Reactive Red 195 and Reactive Green 11 respectively, is reported for 2 g/l for Reactive Red 195 and 3 g/l for Reactive Green 11 when ammonium sulphate is used as nitrogen source (Zope *et. al.*, 2007).

Extra cellular enzymes play an important role in degradation of dyes by Fungi. Lacasse and Manganese peroxidase activities are detected from 8 hours to 48 hours for the decolourisation of Reactive blue 19, Reactive black 5, Acid violet 43, Reactive orange 16 and Acid black 52. In case of decolourisation of Acid black 52 maximum activity of lacasse and manganese peroxidase with the value of 35.9 U/ml and 559.0 U/ml respectively after 40 hours of incubation are observed (Park *et. al.*, 2007). When real textile wastewater is used to be degraded by *P. chrysosporium* after 5 days of incubation at optimal conditions a 79.0% of decolourisation is reported with Manganese peroxidase activity of 43 U/l, 137 U/l, 249 U/l and 265 U/l after 1, 3, 5 and 7 days of incubation is observed (Sharma *et. al.*, 2009).

2.5.4. Advanced Oxidation Processes

Advanced Oxidation Processes (AOPs) are the process which generate Hydroxyl radicals using different mechanisms. Hydroxyl radicals are highly non selective oxidising species. They degrade complex organic compounds to carbon dioxide and water. AOPs are known as destructive treatment methods as unlike other methods they don't convert harmful compounds from liquid to solid state, these methods completely oxidise the harmful organic compounds which are recalcitrant in nature. Some of AOPs are photo – catalytic degradation, Fenton's oxidation process are discussed below.

2.5.4.1. Photo – catalytic degradation

Photo – catalytic degradation requires a photo catalyst and a source of light. The photo catalyst absorbs the light photons and gets electron in an exited state which in turn helps in exciting the molecules and reaching the energy required for the reaction. As a result, Hydroxyl radicals ($OH^{\bullet}$) are generated which helps in degradation of pollutants. These $OH^{\bullet}$ radicals have high oxidative power (2.80 V vs NHE) second only to Fluorine. This method is one of the Advanced Oxidation Processes (AOPs), which helps in complete mineralisation of organic pollutants.

Photo – catalytic degradation involves following steps (Houas *et. al.*, 2001)

1) Absorption of efficient photons ($h\nu \geq E_G = 3.2$ eV) by Titanium dioxide causes valence band electrons are promoted to the conduction band leaving behind a hole.

$$(TiO_2) + h\nu \rightarrow e_{CB}^{-} + h_{VB}^{+} \quad (1)$$

2) Electron in the conduction band on the catalyst surface can reduce molecular oxygen to superoxide anion. Hence, oxidation state of oxygen passes from 0 to -1/2.

$$(O_2)_{ads} + e_{CB}^- \rightarrow O_2^{\cdot -} \quad (2)$$

3) The holes in valence band can oxidise adsorbed water or hydroxide ions to produce $OH^\bullet$ radicals.

$$(H_2O \leftrightarrow H^+ + OH^-)_{ads} + h_{VB}^+ \rightarrow H^+ + OH^\bullet \quad (3)$$

4) Superoxide anion may form organic peroxides.

$$O_2^{\cdot -} + H^+ \rightarrow HO_2^\cdot \quad (4)$$

5) Transient hydrogen peroxide formation and dismutation of oxygen.

$$2HO_2^\cdot \rightarrow H_2O_2 + O_2 \quad (5)$$

6) Decomposition of H_2O_2.

$$H_2O_2 + e^- \rightarrow OH^\cdot + OH^- \quad (6)$$

7) Oxidation of the organic reactant via successive attacks by $OH^\bullet$ radicals.

$$R + OH^\bullet \rightarrow R^{/\bullet} \quad (7)$$

8) Direct oxidation by reaction with holes.

$$R + h^+ \rightarrow R^{+\bullet} \rightarrow \text{degradation products} \quad (8)$$

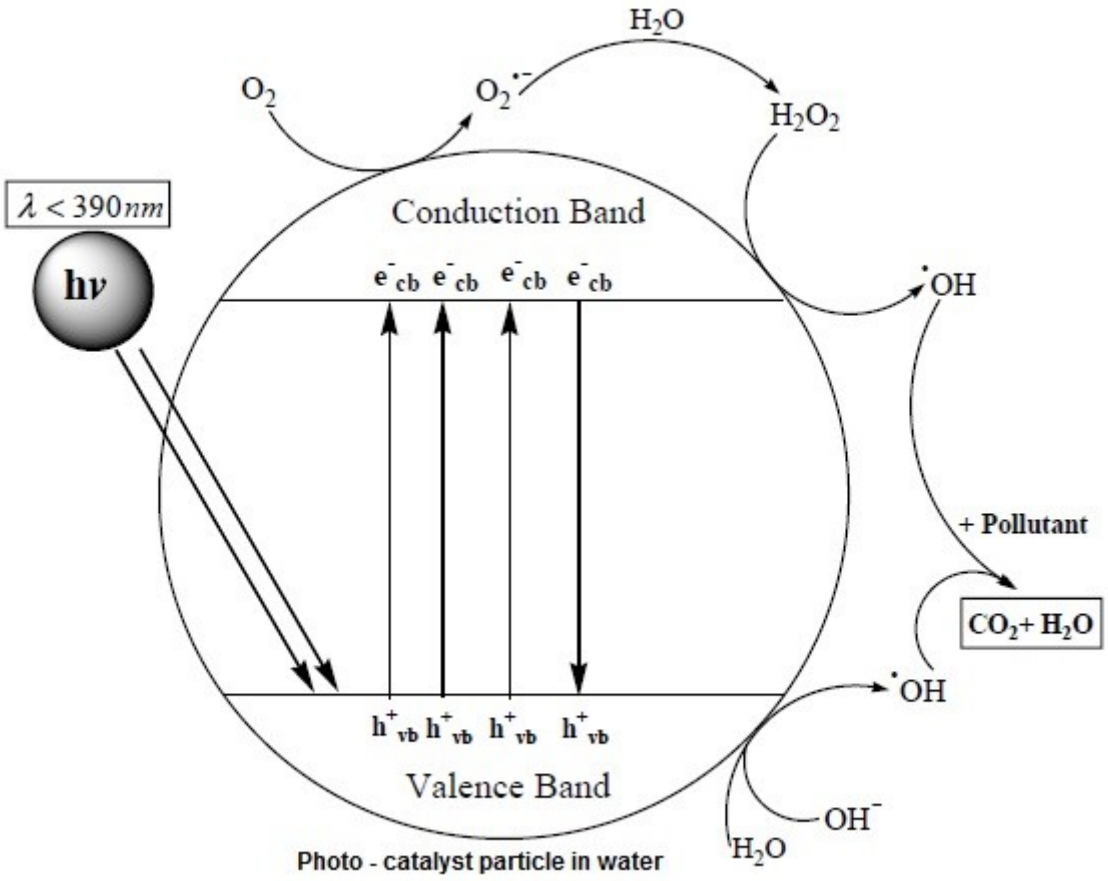

Fig. 1. General mechanism of Photo – catalysis (Daneshwar et. al. 2004)

There are different types of photo – catalyst available like Titanium dioxide (TiO_2) and Zinc Oxide (ZnO). The main difference among the two is that ZnO can absorb light from 470 to 350 nm wavelength while TiO_2 can absorb only in the limited range of 420 to 400 nm. This results in difference between degradation efficiencies of the two photo – catalysts. The band gap energy for TiO_2 is 3.10 eV and that of ZnO is 3.17 eV. Complete decolourisation of acid brown 14 was observed in 120 minutes with ZnO and in 300 minutes with TiO_2, while complete degradation was observed in 360 minutes with ZnO and in 420 minutes with TiO_2 (Sakthivel *et. al.*, 2003).

Photo – catalysis depends upon the pH of the solution. The surface charge of photo – catalyst is affected by the pH. In acidic conditions TiO_2 and ZnO becomes positively charged and attracts negatively charged dyes hence improve in degradation at lower pH, while for positively charged dyes basic pH is more suitable. Maximum degradation of Methylene blue is observed at pH 5 and for methyl orange at pH 3 when TiO_2 is used (Akbal, 2005). When ZnO is used pH 9.7 showed maximum decolourisation for methylene Blue (Chakrabarti and Dutta, 2004).

When TiO_2 is used maximum degradation is reported at pH 2, pH 11.4, pH 4.4, pH 11 and pH 9 for Acid Red 14, Safranin – T, Fast Green FCF, Patent Blue VF and Amido Black 10B respectively (Daneshvar *et. al.*, 2003; Gupta *et. al.*, 2007; Saquib *et. al.*, 2008; Qamar *et. al.*, 2005). When ZnO is used maximum decolourisation is reported at pH 10, pH 4 and pH 10.9 for Acid Brown 14, Acid Red 14 and Acid Yellow 23 respectively (Sakthivel *et. al.*, 2003; Daneshvar *et. al.*, 2004; Benajady *et. al.*, 2006).

Initial dye concentration effects the decolourisation efficiency by photo – catalyst. As concentration increased the degradation decreases. The reason for this can be due to the reduction of photons adsorbed by the photo – catalyst as they are intercepted before they can reach the catalyst surface. When ZnO is used as photo – catalyst the degradation reduced from 87% to 40% for increase in concentration from 25 to 100 mg/l of Methylene Blue, while for Eosin Y it decreased from 93% to 63% for increase in concentration of 25 to 50 mg/l (Chakrabarti and Dutta, 2004). When TiO_2 is used as photo – catalyst the decolourisation decreased from 99 to 92% as concentration of Methylene Blue is increased from 50 to 150 μM, while for Methyl Orange it decreased from 99% to 85% for increase in concentration of 25 to 100 μM (Akbal, 2005).

Catalyst concentration also affect the decolourisation efficiency of dyes. Increase in catalyst dosage first increases reaches the optimum dose and then decreases. The reason behind this may be as the concentration of catalyst increases it results in more adsorption of light photons which causes more generation of $OH^{\bullet}$ radicals, while above the optimized dose the turbidity causes reduction in the amount of photons (Daneshvar *et. al.*, 2003; Qamar *et. al.*, 2005; Saquib and Muneer 2003; Gupta *et. al.*, 2007; Saquib *et. al.*, 2008). Optimum dose of catalyst for degradation of Acid Brown 14 when TiO_2 and ZnO are used as catalyst optimum dose is 2.5 g/l (Sakthivel *et. al.*, 2003). When TiO_2 concentration is increased from 0.2 to 0.5 g/l the degradation of Methylene Blue increased from 93% to 99% while that of Methyl Orange increased from 86% to 94% (Akbal, 2005).

Irradiation intensity of light affects the degradation efficiency of dyes by photo – catalytic degradation. As the intensity of light increases, it adds more photons to the reactor, which aid in increase in degradation rate. It is reported that for Acid Brown 14 that the light intensity in the range of $1.32 – 1.37 \times 10^5$ lux gives maximum degradation with TiO_2 and ZnO as photo – catalyst (Sakthivel *et. al.*, 2003). In case of Eosin Y as light intensity increased from 0.1048 to 0.1919 $\mu W/cm^2$ the degradation increased from 82% to 93% (Chakrabarti and Dutta, 2004).

There are some inhibitory effects due to some compounds for the degradation of dyes by photo – catalysis. Carbonates and bicarbonates are commonly used in dye industry. It is reported that as concentration of HCO_3^- and CO_3^{2-} is increased the percentage degradation decreases (Behnajady *et. al.*, 2006). Ethanol also has been reported to be inhibitory at 8% (v/v) towards photo – degradation (Daneshvar *et. al.*, 2004).

The mineralisation of dyes is observed using photo – catalytic degradation process. It has been reported that the sulphur from S – link in Methylene Blue or sulphonic groups in Alizarin S, Orange G and Congo Red is released as sulphate. Nitrogen, present n dyes in -3 oxidation state, is released as ammonia and nitrates, while nitrogen present as azo link, it evolves as N_2 gas. Hence, the resulting water is completely mineralised by photo – catalysis (Lachheb *et. al.*, 2002). Formation of nitrogen to ammonia is affected by the substituent present in the dyes. Sulphonate groups are present in Indigo Carbine which are not in the Indigo. Sulphonate groups of Indigo Carmine helped in simultaneous evolution of ammonia and nitrates from the initial steps of the reaction as degradation products because of electrophilic attack on nitrogen atoms in the dye molecule may be favoured by the sulphonate as electro – attractor group. Degradation of Indigo, on the other hand, showed that due to absence of sulphonate groups the ammonia and nitrate are not formed in the initial steps of degradation (Vautier *et. al.*, 2001).

2.5.4.2. Fenton Oxidation Process

Fenton oxidation process was developed by H. J. H Fenton in 1894 (Fenton, 1894) for oxidation of tartaric acid. Since then this process has been used for oxidation of wide range of compounds that are otherwise difficult to degrade (Wang, 2008) like various chlorinated phenols, benzene, toluene, xylene, chlorobenzene, nitrophenols, nitrobenzene, formaldehyde, polyaromatic hydrocarbons etc. (Sudoh *et. al.*, 1986; Potter and Roth 1993; Watts *et. al.*, 1990; Lou and Lee 1995; Sedlak and Andren 1991; Lipcynska – Kochany 1991; Murphy *et. al.*, 1989; Srivastava *et. al.*, 1991; Malik and Saha 2003). Kuo (1992) demonstrated that Fenton's reagent (Hydrogen peroxide and ferrous salt) can decolourize both soluble and insoluble dyes (Hsueh *et. al.*, 2005).

Fenton's oxidation process involves production of hydroxyl radicals ($OH^{\bullet}$). Hydrogen peroxide reacts with ferrous salts in acidic environment to produce $OH^{\bullet}$ radicals as shown in equation (9) (Walling, 1975; Walling 1998; Neyens and Baeyens 2003; Lucas and Peres 2006; Sun *et. al.*, 2007; Sun *et. al.*, 2007; Torrades and Garcia – Montano 2014).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad k_1 = 76\ M^{-1}s^{-1} \qquad (9)$$

Ferrous ions are regenerated from Ferric ions by further reacting with H_2O_2 through following reactions:

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}OOH + H^+ \qquad k_2 = 0.01 - 0.02\ M^{-1}s^{-1} \qquad (10)$$

$$Fe^{3+} + {}^{\bullet}OOH \rightarrow Fe^{2+} + O_2 + H^+ \qquad k_3 = 3.1 \times 10^5\ M^{-1}s^{-1} \qquad (11)$$

Consumption of ferrous ions is quick as compared to regeneration from ferric ions as can be seen from reaction rate constants of equation (9) and (10). Hence the reaction rate is quicker in first few minutes and decreases as reaction progresses and ferrous ions are consumed.

In presence of excess of H_2O_2, $OH^{\bullet}$ radicals reacts with H_2O_2 (as shown in equation 12 and 13) resulting in decrease in efficiency of degradation by Fenton's reagent.

$$^{\bullet}OH + H_2O_2 \rightarrow {}^{\bullet}OOH + H_2O \qquad (12)$$

$$^{\bullet}OH + {}^{\bullet}OOH \rightarrow O_2 + H_2O \qquad (13)$$

When Fe^{2+} ions are present in excess, $^{\bullet}OH$ radicals reacts with Fe^{2+} ions (as shown in equation 14), and results in decrease in $^{\bullet}OH$ radicals present for degradation.

$$^{\bullet}OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \qquad (14)$$

$OH^{\bullet}$ radicals generated attacks the unsaturated dye molecules and azo bonds (N = N) through hydroxylation of azo ring, hydroxylation of aromatic rings, opening of naphthalene ring, desulfonation etc. to complete mineralisation of dyes (Meetani *et. al.*, 2010; Bansal *et. al.*, 2010; de Luna *et. al.*, 2014).

Fenton oxidation process has many process parameters like concentration of H_2O_2, concentration of $FeSO_4$, pH, initial dye concentration etc.

Effect of pH on degradation of dyes by Fenton's oxidation process has been studied in the literature. Generally optimal pH range is 2 – 4 for Fenton's oxidation process. It has been reported that when pH was varied between 2.5 and 4.0 for decolourisation of Reactive Black 5 the maximum COD removal of 87% was at pH 3.5 (Meric *et. al.* 2004). Optimum dosage of pH for degradation of Red MX – 5B is found to be 2.5 for Fenton and Fenton – like process, maximum decolourisation of 96% and 95.1% were reported for Fenton and Fenton – like process after 60 minutes of reaction time (Hsueh *et. al.* 2005). Degradation of Direct Blue 54

and Direct Red 31 decreased when pH increased more than 3 (Malik and Saha, 2003). When pH of 1.8 is used decolourisation of 86% is reported and at the other extreme of pH decolourisation of only 22% while at pH 2.5 maximum decolourisation of 99% is reported for Methylene Blue (Dutta *et. al.*, 2001). The reason behind this may be due to at alkaline pH H_2O_2 becomes unstable and loses its oxidising potential, also Fe^{3+} can get hydrolysed at higher pH; while at lower pH the hydrogen ions (H^+) act as scavengers for hydroxyl radicals.

H_2O_2 concentration has major effect on the degradation of dyes by Fenton's oxidation process. $OH^{\bullet}$ radicals are generated by the reaction from H_2O_2. It is reported that as concentration of H_2O_2 increased from 5.88×10^{-5} to 2.94×10^{-2} mol dm^{-3} the degradation increases from 40% to 98%, further increase in H_2O_2 concentration caused decreased in degradation of Methylene Blue (Dutta *et. al.*, 2001). For degradation of Direct Red 31 and Direct Blue 54 optimal initial concentration of H_2O_2 was reported as 2.94 mM and 1.47 mM respectively, for concentrations more than it resulted in same value extent of degradation after 30 minutes (Malik and Saha, 2003). It is reported that as initial concentration of dye increases, the optimal concentration of H_2O_2 also increases. For initial concentration of 100 mg/l of Reactive Black 5, when H_2O_2 concentration increased from 100 mg/l to 400 mg/l decolourisation increased from 68% to 98%, COD removal at optimum concentration is 76% and when initial dye concentration of Reactive Black 5 increased to 200 mg/l, optimal H_2O_2 concentration is reported to be 1000 mg/l with decolourisation of 99% and COD removal of 82% (Meric *et. al.*, 2004). Optimal concentration of H_2O_2 for Red MX – 5B (0.1 mM), Reactive Black 5 (0.1 mM) and Direct blue 15 (4.7×10^{-5} mol/l) is 200 mg/l, 7.3×10^{-4} mol/l and 2.8×10^{-3} mol/l (Hseuh *et. al.*, 2005; Lucas and Peres, 2006; Sun *et. al.*, 2007). H_2O_2 added to Fenton's process is the source of $OH^{\bullet}$ radicals. As initial concentration of H_2O_2 increases, there is an increase in the generation $OH^{\bullet}$ radicals which increase the degradation rate until optimum concentration is reached. Above the optimal concentration due to scavenging effect i.e. instead of reacting to organic dye $OH^{\bullet}$ radicals start reacting with excess H_2O_2 molecules resulting in stabilisation of degradation of dye.

Ferrous ions concentration affect the degradation of dye. When initial ferrous ions concentration is increased from 7.16×10^{-6} mol dm^{-3} to 3.58×10^{-5} mol dm^{-3} the degradation of Methylene Blue increased to 98.5%, with further increase in ferrous ions degradation remains constant (Dutta *et. al.*, 2001). For degradation of Direct Blue 54 and Direct Red 31 there was steady increase in degradation till 8.93×10^{-2} mM above that concentration the degradation remains constant (Malik and Saha, 2003). This can be explained as the $OH^{\bullet}$

radicals are generated by reaction between H_2O_2 and ferrous ions, without increase in H_2O_2 the total amount of $OH^{\bullet}$ radicals generated is fixed, only the time for $OH^{\bullet}$ radicals generation is reduced with increase in Ferrous ions hence the degradation remains same after a fixed concentration of ferrous ions. Optimum concentration of ferrous ions is affected by the initial concentration of dye used. Maximum COD removal of 79% is obtained for the degradation of 100 mg/l of Reactive Black 5 when 200 mg/l of $FeSO_4$ concentration is used, while 84% of COD removal is reported for 200 mg/l of Reactive Black 5 by 225 mg/l of $FeSO_4$ concentration (Meric *et. al.*, 2004). As $FeSO_4$ concentration is increased further the COD removal efficiency decreased, this may be explained by the scavenging of $OH^{\bullet}$ radicals by excess ferrous ions. Optimum concentration of Ferrous ions for Red MX – 5B, Reactive Black 5, Amido Black 10B, Reactive Red 141, Reactive Red 238, Reactive Black B, Acid Black 1 and Reactive Yellow 84 is 1 mg/l, 1.5×10^{-4} mol/l, 0.025 mM, 10 mg/l, 10 mg/l, 200 mg/l, 3.4×10^{-5} M and 25 mg/l (Hsueh *et. al.*, 2005; Lucas and Peres, 2006; Sun *et. al.*, 2007; Nunez *et. al.*, 2007; Huang *et. al.*, 2008; Wang, 2008; El Haddad *et. al.*, 2014).

Temperature affects the rate of reaction of Fenton's process. As temperature is increased the degradation is increased due to increase in rate of reaction. Maximum degradation of Methylene Blue is observed at 299 K (Dutta *et. al.*, 2001), as temperature is increased the degradation is reduced due to thermal degradation of Hydrogen peroxide. Optimum temperature is not affected by increase in initial concentration, when Reactive Black 5 concentration increased from 100 mg/l to 200 mg/l, optimum temperature is reported to be 40°C with COD removal of 71% and 84% respectively (Meric *et. al.*, 2004). Optimum temperature for Amido Black 10B, Procion Red H – E7B, Cibacron Red FN – R and Acid Black 1 is 25°C, 298 ± 1K, 298 ± 1K and 30°C (Sun *et. al.*, 2007; Nunez *et. al.*, 2007; Wang, 2008).

Initial concentration of dye affects the degradation of dye by Fenton's oxidation process. Percent degradation decreases as initial concentration of dye increases. It has been reported that when initial concentration of methylene blue was 3.31×10^{-5} mol dm^{-3} degradation was 90% in 10 minutes while when it was 6.26×10^{-5} mol dm^{-3} and 9.39×10^{-5} mol dm^{-3} degradation was 80% and 68% respectively in 2 hours (Dutta *et. al.*, 2001).

The effect of concentration of Fe^{2+}, H_2O_2 is interdependent and also depends on dye concentration. The optimum ratio between them has been reported in the literature. Molar ratio Fe^{2+}:H_2O_2 value of 1:15 is reported to be best for $OH^{\bullet}$ radical formation by Fenton's process.

It is reported that optimum ratio of [Dye]:[Fe^{2+}]:[H_2O_2] is 1:1.15:14.1 for Methylene Blue (Dutta *et. al.*, 2001). For Direct Blue 54 and Direct Red 31 the optimum ratio of [Fe^{2+}]:[H_2O_2]:[Dye] is 1:16.5:1.8 and 1:32.9:2.4 respectively (Malik and Saha, 2003).

There are some modifications over Fenton's process by improving the slowest step in the cycle of Fenton's process, i.e., the regeneration of Fe^{2+} ions from Fe^{3+} ions (equation 10). This step is improved in photo – Fenton process by the regeneration using photons (equation 15); in Electro – Fenton process by using electrode to regenerate the Fe^{2+} etc.

$$Fe^{3+} + H_2O + UV \rightarrow OH^{\bullet} + Fe^{2+} + H^{+} \quad (15)$$

Photo – Fenton process improves efficiency of degradation of dye by increasing the formation of $OH^{\bullet}$ radicals. It is reported that photo – Fenton and Fenton process follow first order kinetics and rate constant of photo – Fenton process is 1.234 min^{-1} while that of Fenton is 1.225 min^{-1}, indicating faster rate of reaction for photo – Fenton process (Lucas and Peres, 2006). COD removal of 92.7% by electro – Fenton process is reported to be much improved when compared with that by Fenton's process of 68.5% (Huang *et. al.*, 2008). The reason may be due to the degradation of barely oxidizable intermediates by electro – Fenton process.

Azo dyes are toxic to microbes, aquatic organisms and are mutagenic and carcinogenic to mammals. These dyes also effect the germination and growth of plants. Hence these dyes are required to be removed before discharge into aquatic environment or for land disposal of wastewater or for irrigation of crops.

It has been observed from the literature that treatment methods like coagulation/ flocculation and adsorption were not efficient and when the efficiency is more, the amount of required chemicals is more or time required for treatment is more (12 hours or more). Sludge is generated in coagulation process which requires special disposal. In adsorption the adsorbent gets consumed regularly, which requires replacement and regeneration of consumed adsorbent is difficult. Hence these methods only transfer dyes from liquid to solid phase and creates the problem of hazardous solid waste management.

On the other hand, Advanced Oxidation Processes allow the complete degradation of dyes and they are not transferred from liquid to solid but are mineralised to carbon dioxide and water. The time required for the treatment was generally less than 30 minutes. These methods do not require skill labourers as the treatment method is simple and handling of chemicals is easy. Hence these methods are better than the general methods used.

3. Materials and Methods

This study is carried out to determine the efficiency of different methods and to select the best suited method for decolourisation of azo dye. Acid Orange 7 was used as the model azo dye. Photo – catalysis and Fenton's oxidation process were performed in this study. Response surface methodology (RSM) was used to optimise and validate the univariate results obtained in Fenton's oxidation process. RSM is a multivariate approach and helps in interpreting the interdependencies between different parameters of the process. It uses lower order polynomial which is the fit of empirical models to the experimental data obtained in relation to experimental design.

3.1. Chemicals

Dye Acid Orange 7 (min. assay 85%) was purchased from Vishnu Chemicals, Ankleshwar, Gujrat and was used without further purification. Titanium dioxide (analytical grade 99% assay) was obtained from High Purity Laboratory Chemicals Pvt. Ltd., Mumbai. Ferrous sulphate (99%), Hydrogen peroxide solution (30% w/v), Sulphuric acid (97%) and Sodium hydroxide (97%) were obtained from CDH, India and were of analytical grade. Stock solution of dye Acid Orange 7 was prepared by dissolving dye powder in Grade I ultrapure water. Sulphuric acid (0.1 M) and Sodium hydroxide (1.0 M) were used to adjust the pH.

3.2. Experimental Set – up

3.2.1. Generation of absorption spectrum and determination of λ_{max}

Absorption spectrum was generated to know the λ_{max} for the Acid Orange 7. Absorption spectrum was generated using 50 mg/l of Acid Orange 7. Spectrum was generated by noting the absorbance in the range of 400 to 700 nm. The spectrum graph was plotted between wavelength and absorbance as shown in Fig. 2. λ_{max} was taken for the wavelength having the maximum absorbance.

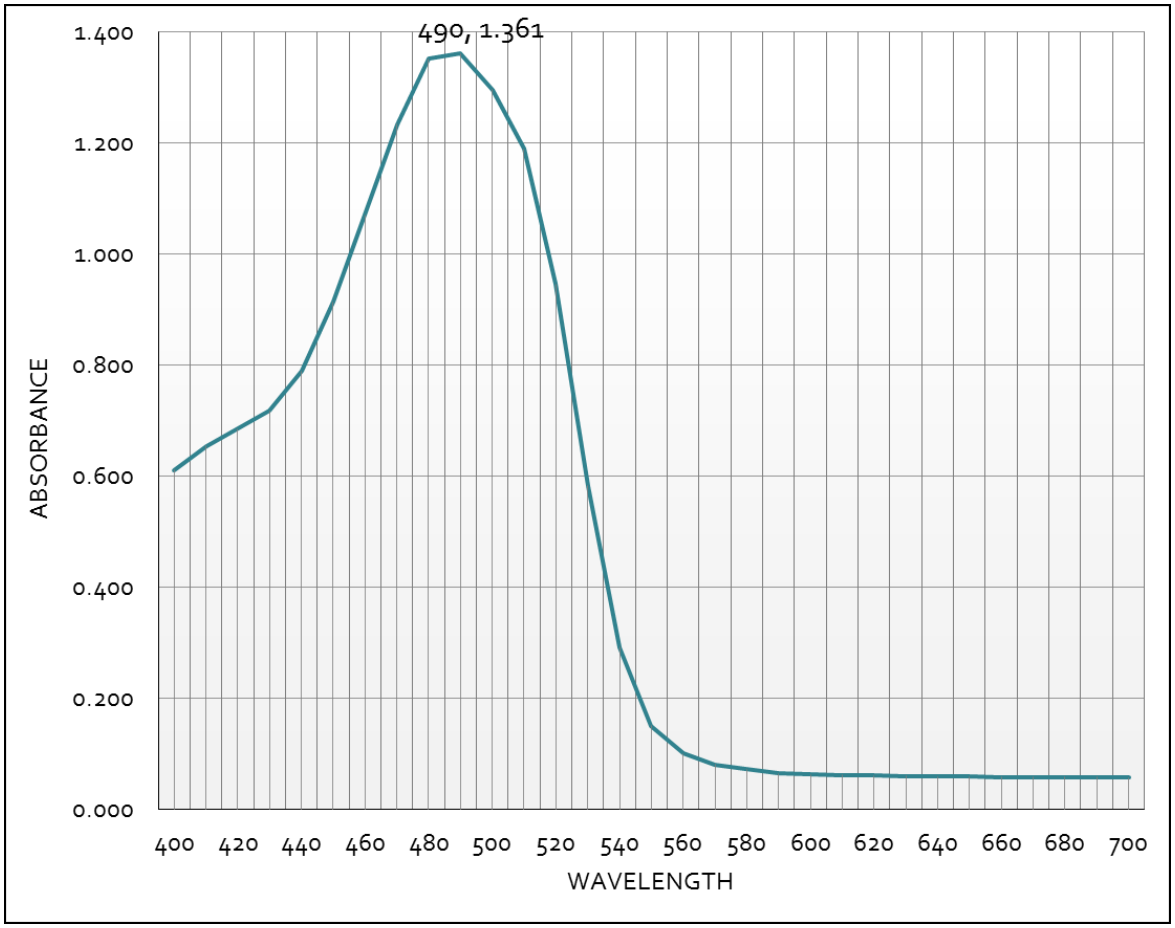

Fig. 2. Absorption spectrum of AO7

It was observed that λ_{max} was 490 nm (Fig. 2).

3.2.2. Calibration curve of Acid Orange 7

Calibration curve of Acid Orange 7 was plotted to determine the concentration of Acid Orange 7 during the experiments. Standards were prepared of 10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l, 50 mg/l, 60 mg/l, 70 mg/l, 80 mg/l, 90 mg/l and 100 mg/l of Acid Orange 7. Absorbance was noted that at 490 nm. Best fit curve was plotted and equation was determined.

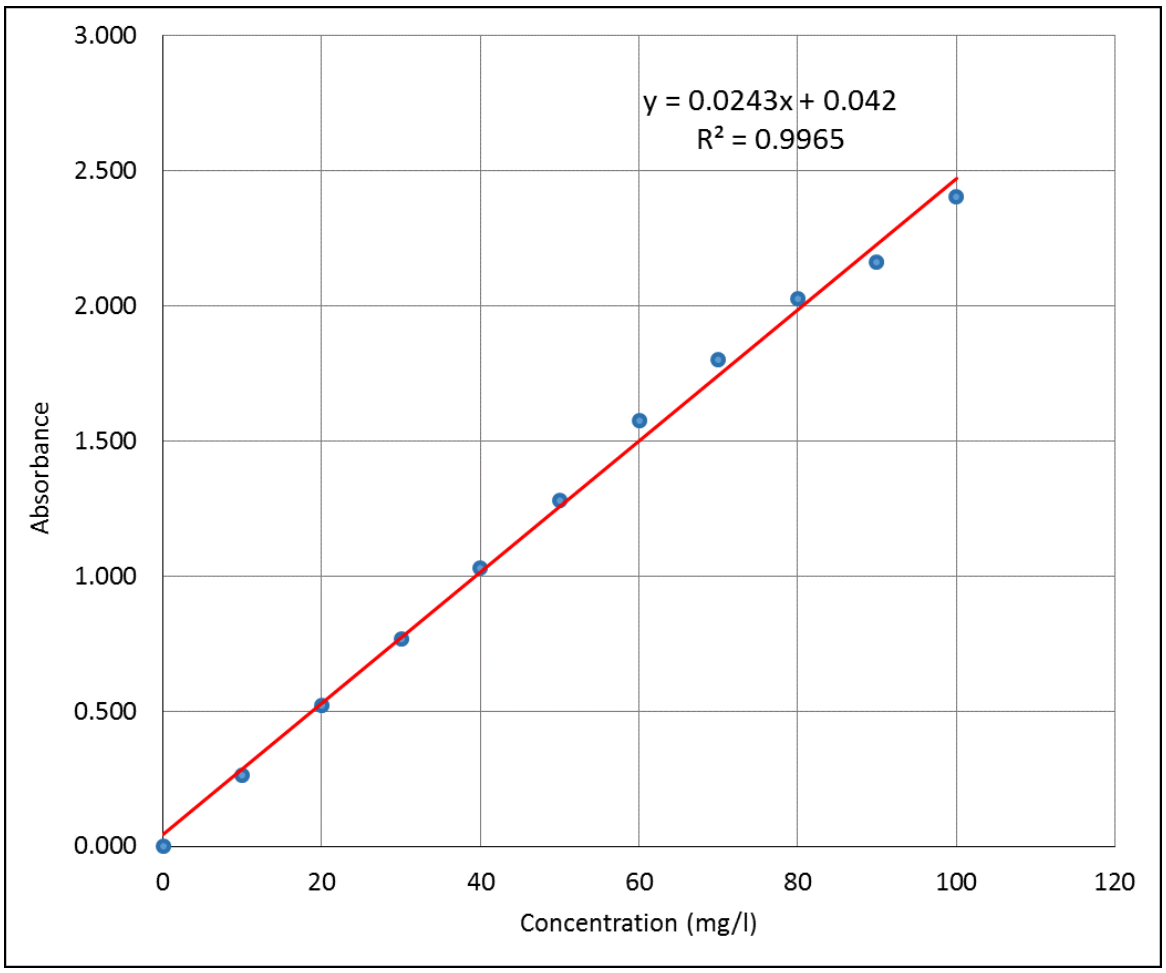

Fig. 3. Calibration curve for AO7

3.3. Design of Experiments

3.3.1. To study the natural attenuation of Acid Orange 7

Initial concentration of dye was taken as 500 mg/l. 500 ml of this solution was placed in sunlight and 500 ml in dark. Exposure given to sunlight was around 7 to 8 hours. Samples were taken every morning and after exposure to sunlight in the evening for analysis. Dye concentration was measured in samples by measuring the absorbance at 490 nm using spectrophotometer (Labtronics Model LT 290). The calibration curve plotted was used to determine the dye concentration in the solution. All experiments were carried out in triplicates mean of results is presented here.

The degradation percentage was calculated using following formula:

$$Degradation\ (\%) = \frac{(initial\ concentration - observed\ concentration) \times 100}{initial\ concentration}$$

Fig. 4. Effect of natural attenuation on AO7

3.3.2. Experimental set up for Photo – catalytic degradation

Required amount of sample was taken in a flask. The samples were stirred using magnetic stirrer to mix the solution and to keep the catalyst in suspension. UV light was provided using Osram 30W UV lamp with flux of 3.17×10^{19} photons/s. Time was started when UV light was switched on.

3.3.2.1. To study the effect of photo – catalyst (TiO_2) on degradation of dye under UV light

Initial concentration of 500 mg/l of Acid Orange 7 was taken. Photo – catalyst, Titanium dioxide, was added @ 2.0 g/l in 250 ml of prepared sample. Another 250 ml of prepared solution was also taken without photo – catalyst. Both were placed in UV radiation. Samples were taken hourly for a period of 32 hours. The samples taken were centrifuged to separate the photo – catalyst from the solution. Dye concentration was measured in samples by measuring the absorbance at 490 nm using spectrophotometer (Labtronics Model LT 290). The calibration curve plotted was used to determine the dye concentration in the solution. All experiments were carried out in triplicates mean of results is presented here.

The degradation percentage was calculated using following formula:

$$Degradation\ (\%) = \frac{(initial\ concentration - observed\ concentration) \times 100}{initial\ concentration}$$

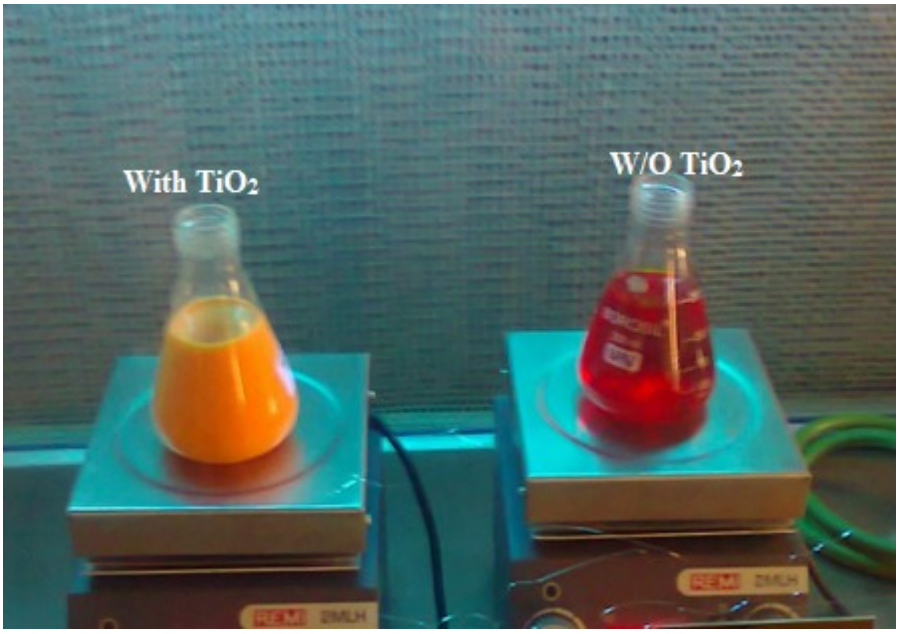

Fig. 5. Effect of photo - catalyst (TiO_2) on degradation of AO7 under UV light

3.3.2.2. To study the effect of amount of photo – catalyst (TiO2) for degradation of dye

Dye sample of initial concentration of 50 ppm of Acid Orange 7 was prepared. Photo – catalyst was added @ 0.5 g/l, 1.0 g/l and 2.0 g/l in 500 ml of prepared sample. These were placed in UV radiation. Samples were taken hourly for analysis for a period of 32 hours. The samples taken were centrifuged to separate the photo – catalyst from the solution. Dye concentration was measured in samples by measuring the absorbance at 490 nm using spectrophotometer (Labtronics Model LT 290). The calibration curve plotted was used to determine the dye concentration in the solution. All experiments were carried out in triplicates mean of results is presented here.

The degradation percentage was calculated using following formula:

$$Degradation\ (\%) = \frac{(initial\ concentration - observed\ concentration) \times 100}{initial\ concentration}$$

3.3.2.3. To study complete degradation of Acid Orange 7

Initial concentration of 50 mg/l of Acid Orange 7 was taken. Photo – catalyst was added @ 1.0 g/l in 500 ml of prepared solution. This was placed in UV radiation. Samples were taken hourly for analysis till complete decolourisation was observed. The samples taken were centrifuged to separate the photo – catalyst from the solution. Dye concentration was measured in samples by measuring the absorbance at 490 nm using spectrophotometer (Labtronics Model LT 290). The calibration curve plotted was used to determine the dye concentration in the solution. All experiments were carried out in triplicates mean of results is presented here.

The degradation percentage was calculated using following formula:

$$Degradation\ (\%) = \frac{(initial\ concentration - observed\ concentration) \times 100}{initial\ concentration}$$

After complete decolourisation the amount of TiO_2 which can be recovered was studied by centrifuging the mixture and separating the TiO_2. TiO_2 thus separated was collected and then dried in Oven a 104°C for 24 hours and weight was measured when it became constant. Percentage of TiO_2 recovered was calculated using the following formula W_1

$$Percentage\ recovered\ (\%) = \frac{W2 \times 100}{W1}$$

Where,

W1 is the weight of TiO_2 added to the solution

W2 is the weight of TiO_2 recovered after the complete degradation.

3.3.3. Experimental set up for Fenton's oxidation process

Fenton's oxidation process was carried out in conical flask. Solution of 50 mg/l concentration of dye was prepared by dissolving dye in Grade I ultrapure water. 200 ml of this dye solution was taken in conical flask and continuously stirred using magnetic stirrer. pH was measured using pH meter (Hanna instruments) and required pH was adjusted using appropriate amount of Sulphuric acid and/ or Sodium hydroxide. Required amount of Ferrous sulphate ($FeSO_4$) and Hydrogen peroxide (H_2O_2) were added to it. Time of reaction was started after the addition

of H_2O_2. Samples were taken out from reactor at regular intervals of 30 seconds from 1 minute to 5 minutes, and later at an interval of 5 minutes till complete decolourisation of dye was observed using a pipette for analysis. Dye concentration was measured in samples by measuring the absorbance at 490 nm using spectrophotometer (Labtronics Model LT 290). The calibration curve plotted was used to determine the dye concentration in the solution.

The degradation percentage was calculated using following formula:

$$Degradation\ (\%) = \frac{(initial\ concentration - observed\ concentration) \times 100}{initial\ concentration}$$

3.3.3.1. Effect of Hydrogen peroxide concentration

It was observed using constant dosage of $FeSO_4$ (10 mg/l) and constant pH (3.0). Concentration of H_2O_2 was varied from 1 mmol/l to 6 mmol/l.

3.3.3.2. Effect of Ferrous sulphate concentration

It was observed using constant dosage of H_2O_2 (2 mmol/l) and constant pH (3.0). Concentration of $FeSO_4$ was varied from 7.5 mg/l to 150 mg/l.

3.3.3.3. Effect of pH

It was observed using constant dosage of $FeSO_4$ (38 mg/l) and H_2O_2 (5mmol/l). pH was varied from 2.5 to 3.5.

Optimization of experimental parameters was done using Box – Behnken design. Percent removal of dye was considered to be maximised. Concentration of Hydrogen peroxide, concentration of ferrous sulphate, pH and contact time were the parameters to be optimized. Results of preliminary experiments were used to determine the range of these parameters.

3.4. Statistical Analysis

Response Surface Methodology was used for analysis of the effect of independent variables along with the effect of interactions between variables for the Fenton's Oxidation process, as the results obtained for this method were better than that obtained by photo – catalytic degradation.

Response Surface Methodology (RSM) is a statistical tool used for optimization of process variables and can be used for experimental design. It is a multivariate approach. It is be used

to determine relationship between dependent and independent variables. It gives faster results than one factor at a time approach (Arslan – Alaton *et. al.*, 2009). Three level factorial design, Central Composite Design, Box – Behnken Design etc. are some of the types of RSM generally used. In this present study Box – Behnken Design (BBD) is used, as it uses least number of runs compared to Central Composite Design and Three level factorial design (Ay *et. al.*, 2009).

BBD is an independent, rotatable quadratic design with no embedded factorial or fractional factorial points where the variable combinations are at the midpoints of the edges of the variable space and at the centre (Ay *et. al.*, 2009). Mathematical relationship between response (Y) and independent variables (X_i) can be approximated by the following equation:

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1X_1 + b_{22}X_2X_2 + b_{33}X_3X_3 \quad (16)$$

The coefficients (b_i) were obtained by correlating the experimental results with the response with the response functions. The experiments were conducted using statistically designed combinations, these were randomized to reduce the effects of factors that are not included in the study as shown in Table 2.

Table 2 Box – Behnken Design Matrix

Run no	**X1**	**X2**	**X3**
1	0	+1	0
2	0	0	-1
3	-1	+1	0
4	-1	-1	0
5	0	0	0
6	+1	0	+1
7	-1	0	0
8	-1	0	0
9	+1	0	0
10	0	0	-1
11	-1	0	-1

12	0	-1	0
13	-1	0	+1
14	0	0	0
15	0	+1	0
16	0	0	+1
17	0	+1	-1
18	+1	0	0
19	0	-1	0
20	0	0	0
21	0	-1	+1
22	0	+1	+1
23	+1	-1	0
24	0	-1	-1
25	+1	0	-1
26	0	0	+1
27	+1	+1	0

Experimental data were analysed using software minitab version 17 software and fitted to the mathematical equation and coefficients were determined. Analysis of Variance (ANOVA) test was conducted to establish the model adequacy and predictability at 95% confidence level.

4. Results and Discussion

The natural attenuation of AO7 was studied in sunlight and shade to study the effect of exposure to sunlight over the photo – catalytic degradation of dye.

4.1. To study the natural attenuation of Acid Orange 7

The dye concentration was monitored in the morning (before exposure to sunlight) and in the evening (after exposure to sunlight), with sunlight exposure in the range of 7 to 8 hours. As it can be observed from Fig. 6 and 7 that only about 5% degradation was observed for samples under sunlight and under shade after 5 days of exposure. It can also be observed that during the evening to morning period (i.e. no sunlight exposure) some of the degraded dye concentration is recovered, may be due to partial and temporary decomposition of dye under only sunlight. Similar results were obtained by Elías *et. al.* (2011), where only 2% decrease in initial concentration was observed under visible radiation.

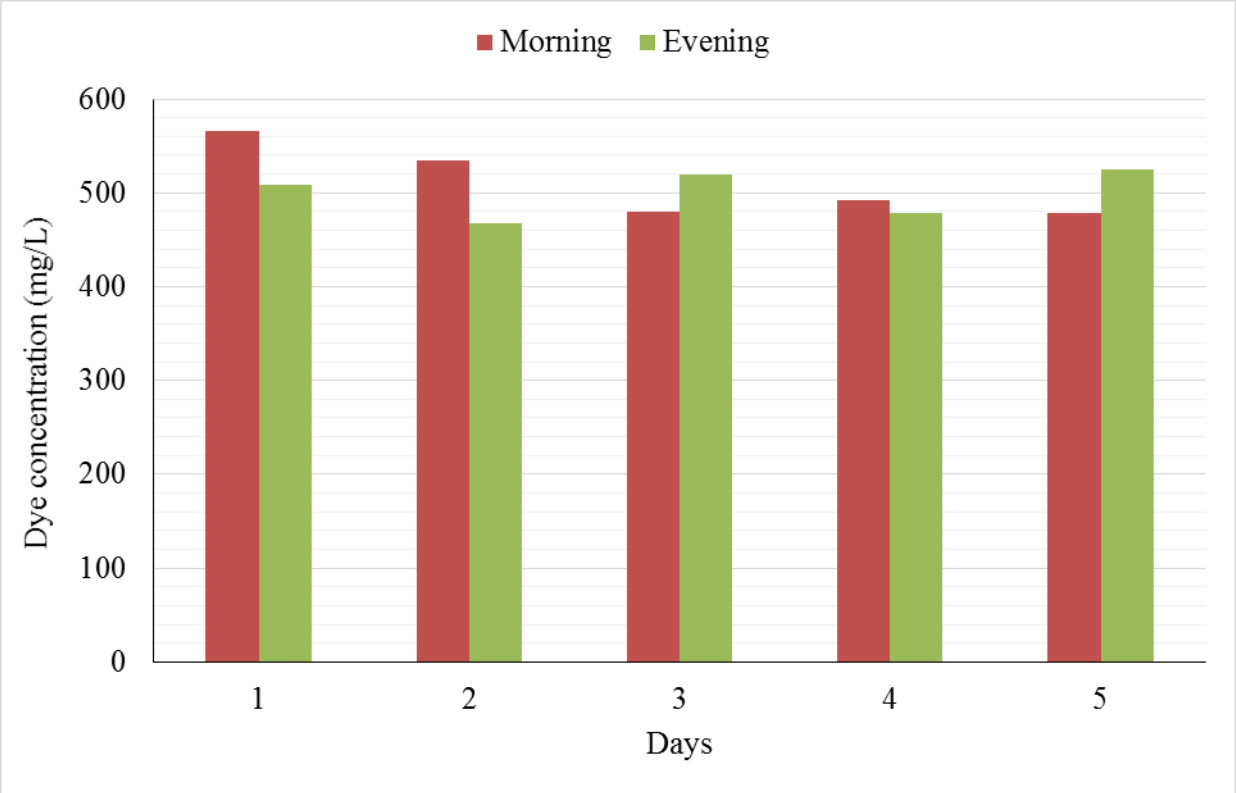

Fig. 6. To study the natural attenuation of AO7 in sunlight

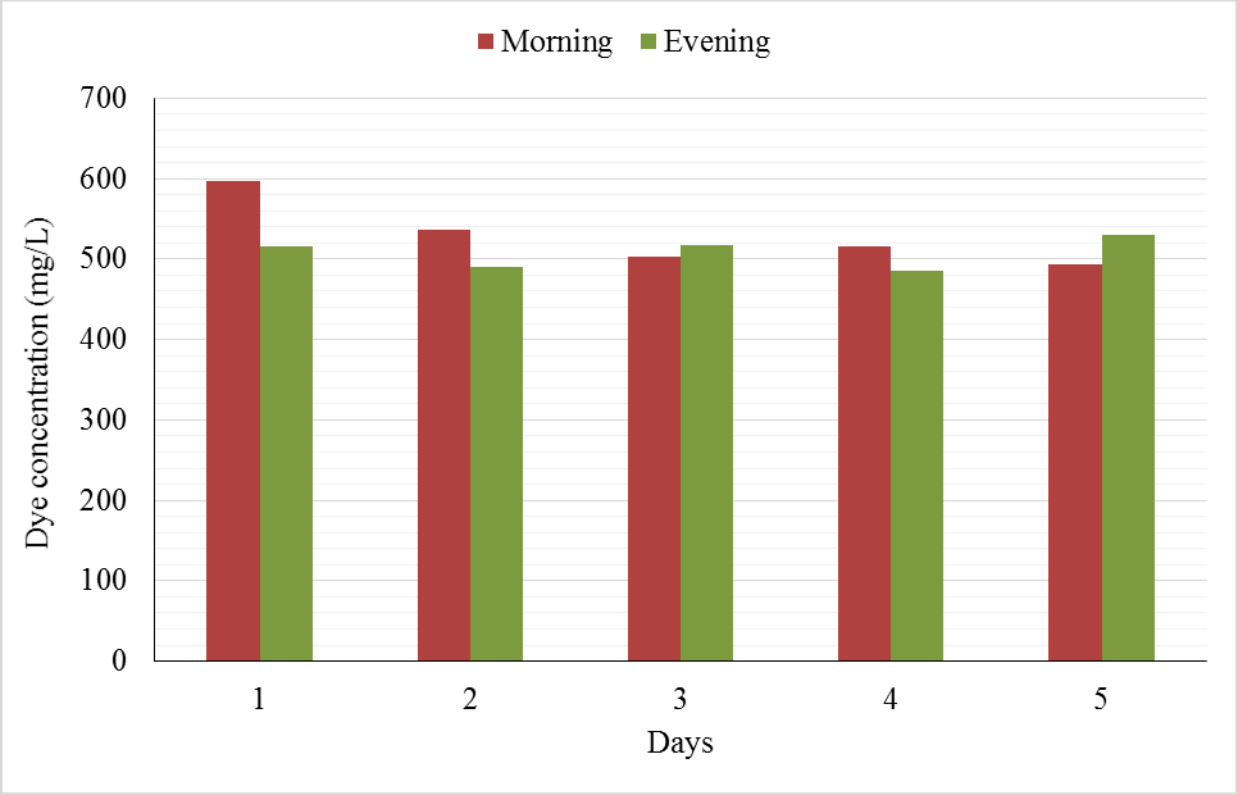

Fig. 7. To study the natural attenuation of AO7 in shade

4.2. Photo – Catalytic degradation of Acid Orange 7

4.2.1. To study the effect of photo – catalyst (TiO_2) on degradation of dye under UV light

It was observed that there was 22.92% degradation after 32 hours when TiO_2 was added @ 2 g/l under UV lamp and sample without any TiO_2 showed zero degradation. Hence, it can be observed that in presence of photo – catalyst (TiO_2), degradation of AO7 improves as compared to without TiO_2.

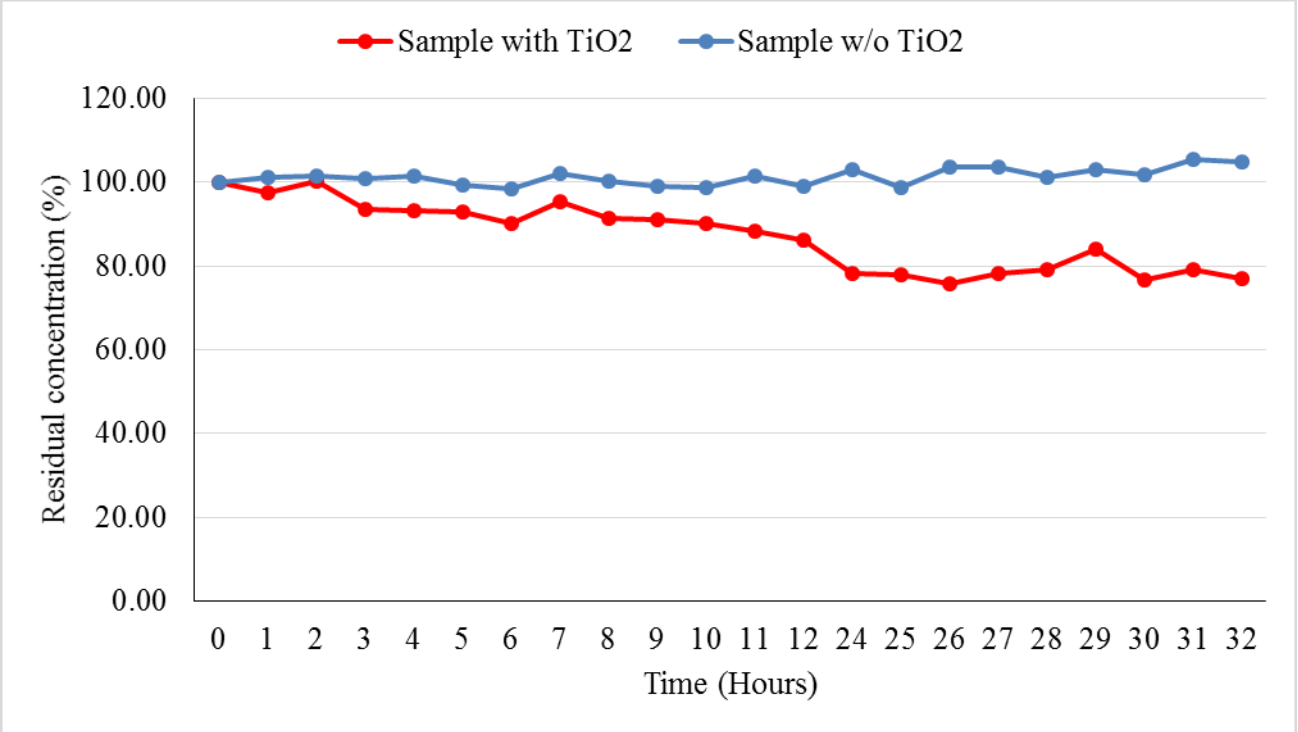

Fig. 8. Effect of photo - catalyst (TiO_2) on degradation of AO7 under UV light

4.2.2. To study the effect of amount of photo – catalyst (TiO2) for degradation of dye

As it can be seen from Fig. 9 after a period of 32 hours maximum degradation of 75.74% was observed when using photo – catalyst concentration @ 1.0 g/l. In a similar study using initial concentration of 25mg/l, 69.09% of degradation in 2.5 hours of irradiation was observed (Bansal *et. al.*, 2010). Difference in time duration may be because of amount of UV irradiation used in the study was due to 4 UV tubes of 30W each and in present study only 1 UV tube 30W is used.

After 32 hours of irradiation the percent degradation is observed to be 22.92%, 75.74% and 61.53% for 2.0, 1.0 and 0.5 g/l of TiO_2 concentration respectively. Percent degradation during first 12 hours are 13.99%, 39.45% and 31.51% for 2.0, 1.0 and 0.5 g/l of TiO_2 concentration respectively. During second 12 hours percent degradation for 2.0, 1.0 and 0.5 g/l of TiO_2 concentration are 8.95%, 37.56% and 22.24% respectively. During the last 12 hours it is observed that percent degradation is 1.58%, 35.83% and 22.24% for 2.0, 1.0 and 0.5 g/l. It can be seen that for TiO_2 concentration of 1.0 g/l percent degradation is maximum even during 12 hours sections and is consistent during the same too.

It is important to study complete degradation of AO7 with time. Hence we use TiO_2 concentration @ 1.0 g/l.

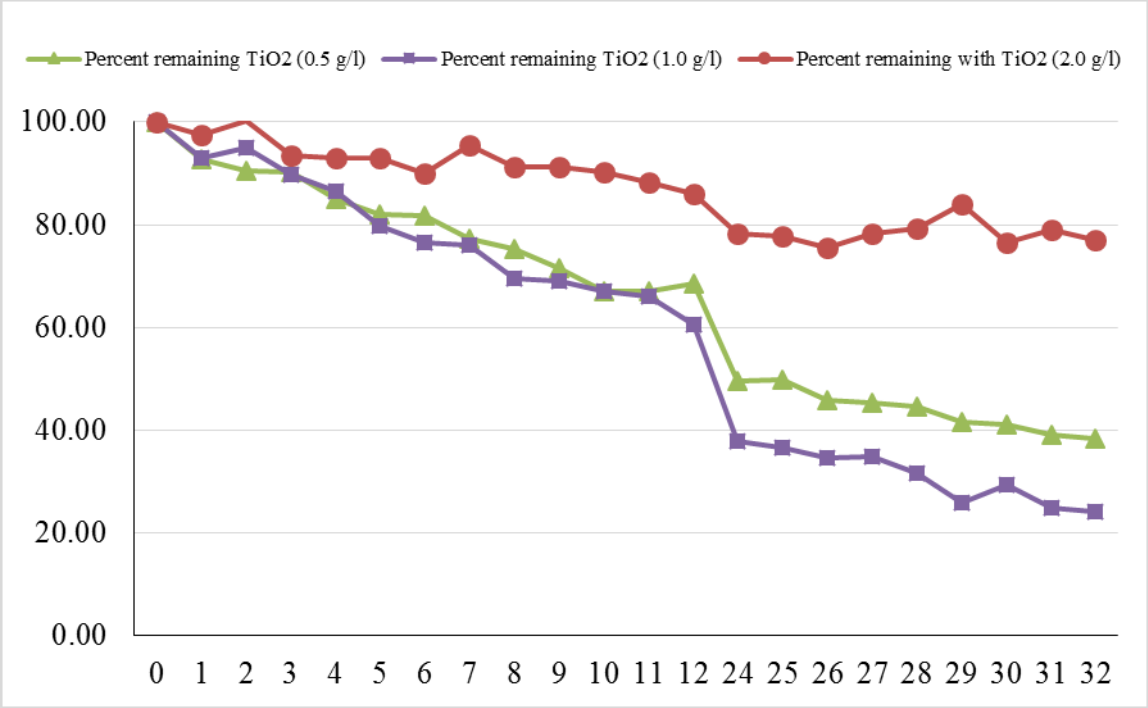

Fig. 9. Effect of different concentration of photo - catalyst on degradation of AO7

4.2.3. To study complete degradation of Acid Orange 7

As it can be seen from Fig. 10 the AO7 is degraded completely after 127 hours. Bansal *et. al.*, (2010) observed complete degradation of AO7 with initial concentration of 25 mg/l in 15 hours.

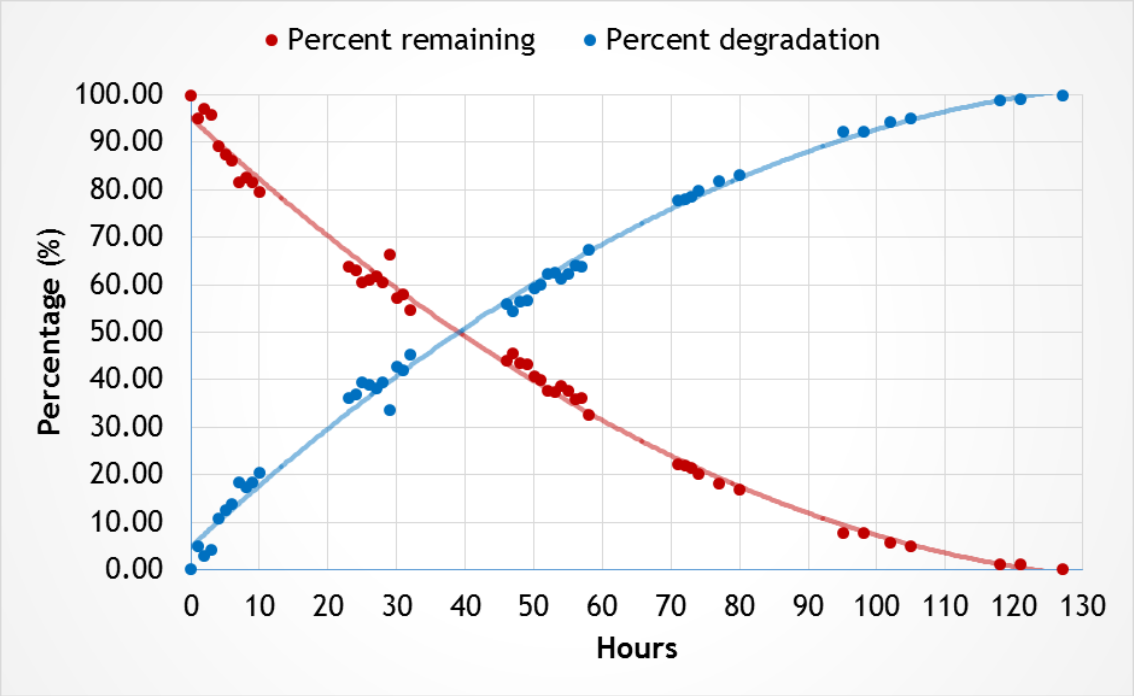

Fig 10. Complete degradation of AO7 by photo – catalytic degradation

4.2.4. Recovery of photo – catalyst (TiO_2)

After the photocatalytic degradation profile of AO7 was studied, the option of regeneration of photo – catalyst was also explored. Since TiO_2 is fine powder, the remaining solution was centrifuged at 5200 rpm for 10 minutes to separate TiO_2 particles. The supernatant was decanted and the pellet was removed; dried at 104°C to remove moisture; and its final weight was determined. The weight difference was used to calculate percent recovery of TiO_2 as given below:

Amount of TiO_2 added, W1 = 0.750 gm

Amount of TiO_2 recovered, W2 = 0.465 gm

$$Percentage\ recovered\ (\%) = \frac{W2\ \times 100}{W1}$$

$$Percentage\ recovered\ (\%) = \frac{0.465\ \times 100}{0.750}$$

Percentage of TiO_2 recovered = 62%.

Based on the recovery of TiO_2, it may be concluded that though the recovery is not very good but it is significant with respect to its fraction for its reuse.

4.3. Fenton's oxidation process for degradation of AO7

4.3.1. Effect of initial concentration of H_2O_2

When initial concentration of H_2O_2 was varied from 1 mmol/l to 5 mmol/l, there was decrease in contact time for complete decolourisation of dye from 230 minutes to 120 minutes. Contact time for complete decolourisation of dye remained constant (120 minutes) after increasing the initial concentration of H_2O_2 more than 5 mmol/l. Hsueh *et. al.*, (2005) also found similar results, they found that for decolourisation of Red MX 5B dye when concentration of Hydrogen peroxide is increased from 20 mg/l up to 200 mg/l time for complete decolourisation decreases and for concentration more than 200 mg/l time remains similar to it. Lucas and Peres (2006), Malik and Saha (2003), Wang (2008), Muruganadhan and Swaminathan (2004) studied decolourisation of different dyes (Reactive Black 5, Direct Blue 54, Direct Red 31, Acid Black 1 and Reactive Orange 4 respectively) and found that for constant contact time, the decolourisation increases with increase in concentration of H_2O_2, reaches at maximum and then remains constant for further increase in concentration of H_2O_2. This can be attributed to the scavenging effect of hydrogen peroxide and recombination of hydroxyl radicals as hydrogen peroxide and hydroxyl radicals were present in excess.

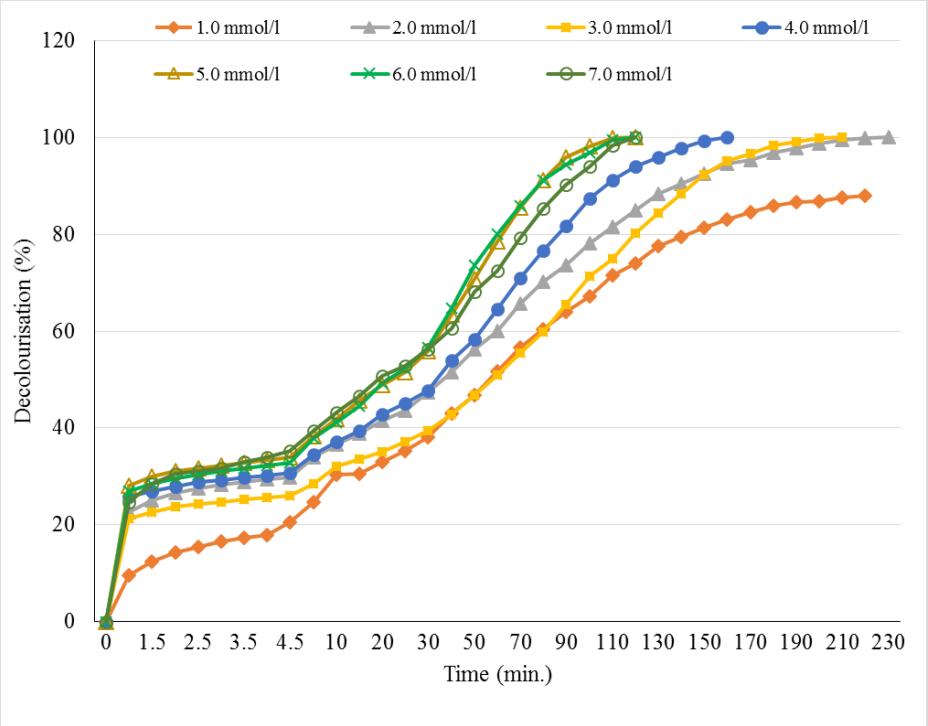

Fig. 11. Effect of H_2O_2 concentration (mmol/l) on decolourisation of AO7 (50 mg/l) at $FeSO_4$ 10 mg/l, and pH 3

4.3.2. Effect of initial concentration of $FeSO_4$

Variation of change in initial concentration of $FeSO_4$ on contact time for complete decolourisation of dye is given in Fig. 12. Initial Concentration of $FeSO_4$ was varied from 7.5 mg/l to 150 mg/l. It was observed that with increasing the initial concentration of ferrous sulphate the contact time for complete decolourisation of dye decreased from 240 minutes to 5 minutes. However for concentrations above 38 mg/l of $FeSO_4$ precipitation and colour due to ferric ions was observed. Similar results were obtained by Ramirez *et. al.*, (2009), they used ferrous ions in the range of 2.5×10^{-6} M to 2.0×10^{-5} M and found that with increasing the concentration of ferrous ions increases the rate of degradation. Chacón *et. al.*, (2006) used 7.16×10^{-5} M, 1.43×10^{-4} M and 1.07×10^{-4} M of ferrous ions and found degradation of 75%, 85% and 90% respectively of Acid Orange 24. Sun *et. al.*, (2009) studied decolourisation of Orange G ferrous dosage was varied from 5.0×10^{-6} M to 3.5×10^{-5} M and percent decolourisation was observed to be between 52% to 92% as ferrous dosage was increased. The increase in ferrous ion concentration increases the rate of hydroxyl radical generation and hence the rate of decolourisation increases.

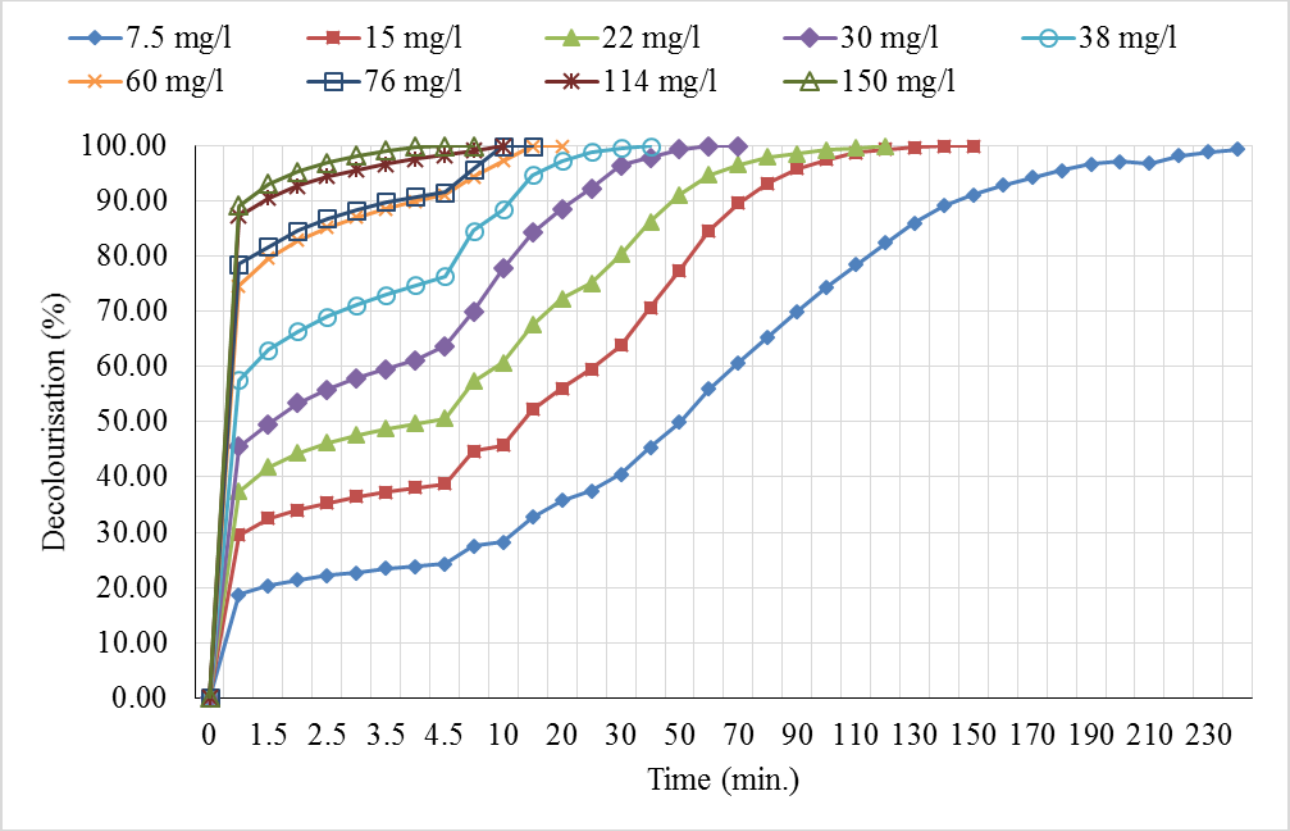

Fig. 12. Effect of $FeSO_4$ concentration (mg/l) on decolourisation of AO7 (50 mg/l) at H_2O_2 2.0 mmol/l, and pH 3

4.3.3. Effect of pH

Variation of pH is very important parameter to be studied as stability of hydrogen peroxide varies with pH. Variation of pH with respect to contact time for complete decolourisation of dye is given in Fig. 13. It was observed that for pH < 3 and pH > 3 the time taken for complete decolourisation of dye was more than that taken for pH = 3 i.e. 10 minutes. Elmorsi *et. al.*, (2010) found similar results for the decolourisation of Mordant Red 73 dye i.e. maximum decolourisation efficiency was observed at pH =3. Peternel *et. al.*, (2007) studied degradation of reactive red 45 and observed that highest TOC removal 74.2% at pH =3. When pH is alkaline hydrogen peroxide is destabilized and forms water and oxygen also iron is precipitated in the form of hydroxide and cannot react with H_2O_2 to form hydroxyl radicals. When pH is strongly acidic (pH<3.0) H_2O_2 is strongly stabilised and doesn't react with ferrous ions. Zhang *et. al.*, (2009) studied degradation of Acid Orange 7 using Fenton oxidation process with ultrasonic irradiation and observed that decolourisation rate increased as pH decreased with maximum value at pH =2. This was observed as they used iron powder in place of iron salt and to dissolve iron powder much more acidic conditions were required.

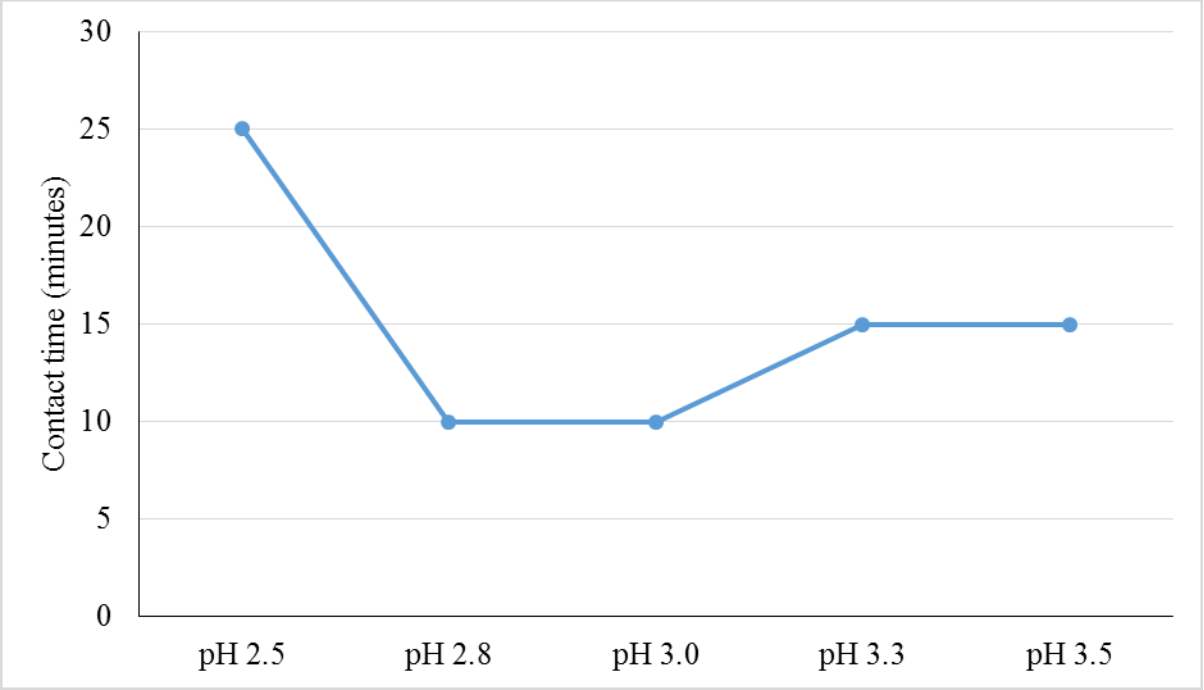

Fig. 13. Effect of pH on contact time for complete decolourisation of AO7 (50 mg/l) at H_2O_2 5.0 mmol/l, and $FeSO_4$ 38 mg/l

4.4. Statistical Analysis

Box – Behnken design (response surface design) was used to optimize the decolourisation of Acid Orange 7, considering the minimum and maximum levels as pH (2.5 – 3.5), H_2O_2 concentration (4 – 6 mmol/l), $FeSO_4$ concentration (30 – 46 mg/l). The ranges considered were chosen from the experiments conducted as shown in this study. The exploratory runs statistically designed by Box – Behnken design are shown in Table 3.

Table 3 Box – Behnken experimental runs and results

Run	Hydrogen peroxide concentration (mmol/l)	Ferrous sulphate concentration (mg/l)	pH	Contact Time (minutes)	Percent decolourisation
1	5	46	3	15	100.0
2	5	38	2.5	5	86.9
3	4	46	3	10	99.7
4	4	30	3	10	95.4
5	5	38	3	10	100.0

Run	Hydrogen peroxide concentration (mmol/l)	Ferrous sulphate concentration (mg/l)	pH	Contact Time (minutes)	Percent decolourisation
6	6	38	3.5	10	100.0
7	4	38	3	15	99.2
8	4	38	3	5	88.0
9	6	38	3	15	100.0
10	5	38	2.5	15	97.6
11	4	38	2.5	10	87.6
12	5	30	3	5	92.2
13	4	38	3.5	10	100.0
14	5	38	3	10	100.0
15	5	46	3	5	96.9
16	5	38	3.5	5	93.5
17	5	46	2.5	10	96.6
18	6	38	3	5	92.4
19	5	30	3	15	100.0
20	5	38	3	10	100.0
21	5	30	3.5	10	97.2
22	5	46	3.5	10	100.0
23	6	30	3	10	98.3
24	5	30	2.5	10	79.8
25	6	38	2.5	10	92.5
26	5	38	3.5	15	100.0
27	6	46	3	10	100.0
28	5	38	3	10	100.0

Factorial fit of the experimental data yielded the following regression equation for the dye decolourisation:

$$Y = -352.5 + 27.8 X_1 + 4.92 X_2 + 156.7 X_3 + 5.85 X_4 - 1.44 X_1X_1 - 0.0167 X_2X_2 - 16.45 X_3X_3 - 0.0895 X_4X_4 - 0.082 X_1X_2 - 2.46 X_1X_3 - 0.182 X_1X_4 - 0.876 X_2X_3 - 0.0297 X_2X_4 - 0.414 X_3X_4 \quad (17)$$

Where Y is percent decolourisation, X_1 is hydrogen peroxide, X_2 is ferrous sulphate, X_3 is pH and X_4 is contact time.

From equation 17 we predicted percent decolourisation. The results obtained are shown in Table 4. It can be seen from these results that a good agreement is obtained between experimental and predicted values for percent decolourisation.

Table 4 Experimental and predicted values of percent decolourisation for each run

Run	**Actual**	**Predicted**
1	100.0	102.0
2	86.9	84.6
3	99.7	99.6
4	95.4	93.2
5	100.0	100.0
6	100.0	98.5
7	99.2	100.0
8	88.0	90.4
9	100.0	100.4
10	97.6	94.5
11	87.6	88.0
12	92.2	89.1
13	100.0	98.7
14	100.0	100.0
15	96.9	96.5
16	93.5	94.9
17	96.6	96.7
18	92.4	94.4

Run	Actual	Predicted
19	100.0	99.3
20	100.0	100.0
21	97.2	99.9
22	100.0	98.0
23	98.3	96.7
24	79.8	84.6
25	92.5	92.6
26	100.0	100.7
27	100.0	100.5
28	100.0	100.0

Table 5 shows the ANOVA results which were obtained for each response. The quality of model fitting was analysed by R^2 value. R^2 value was found to be equal to 0.88 which indicates a good fit by the model as suggested in literature (Torrades *et. al.*, 2014, Arslan – Alaton *et. al.*, 2009). The model F – value was calculated by dividing the model mean square by residual mean square (Arslan – Alaton *et. al.*, 2009). The calculated F – value of model = 6.85 which was greater than tabulated $F_{0.05\,(14,\,13)} = 2.507$, this shows that the model is significant.

Table 5 ANOVA test for response function Y (dye decolourisation)

Source	Sum of squares	Degrees of freedom	Mean square	F – ratio	P – value
Model	663.253	14	47.375	6.85	0.001
X_1 (H_2O_2 concentration)	14.674	1	14.674	2.12	0.169
X_2 ($FeSO_4$ concentration)	77.572	1	77.572	11.22	0.005
X_3 (pH)	205.924	1	205.924	29.78	0.000
X_4 (Contact time)	183.535	1	183.535	26.54	0.000
X_1X_1	12.492	1	12.492	1.81	0.202
X_2X_2	6.843	1	6.8843	0.99	0.338

Source	Sum of squares	Degrees of freedom	Mean square	F – ratio	P – value
X_3X_3	101.497	1	101.497	14.68	0.002
X_4X_4	30.050	1	30.050	4.35	0.057
X_1X_2	1.716	1	1.716	0.25	0.627
X_1X_3	6.027	1	6.027	0.87	0.368
X_1X_4	3.312	1	3.312	0.48	0.501
X_2X_3	49.140	1	49.140	7.11	0.019
X_2X_4	5.641	1	5.641	0.82	0.383
X_3X_4	4.285	1	4.285	0.62	0.445
Residual	89.888	13	6.914		
Lack of fit	89.888	10	8.989		
Pure error	0.000	3	0.000		
Total	753.142	27			

Significant variables and interactions were identified according to the student t – test. It can be seen in Table 6 the obtained results. From these results we can say that X_2 ($FeSO_4$ concentration), X_3 (H_2O_2 concentration) and X_4 (contact time) have a positive effect on the response (P – Values smaller than 0.05 for 95% confidence level) in this study.

Table 6 Estimates of the model regression for percent decolourisation

Term	Effect	Standard error	t – value	P – value
constant		1.31	76.06	0.000
X_1 (H_2O_2 concentration)	2.212	0.759	1.46	0.169
X_2 ($FeSO_4$ concentration)	5.085	0.759	3.35	0.005
X_3 (pH)	8.285	0.759	5.46	0.000
X_4 (Contact time)	7.822	0.759	5.15	0.000

Term	Effect	Standard error	t – value	P – value
X_1X_1	-2.89	1.07	-1.34	0.202
X_2X_2	-2.14	1.07	-0.99	0.338
X_3X_3	-8.23	1.07	-3.83	0.002
X_4X_4	-4.48	1.07	-2.08	0.057
X_1X_2	-1.31	1.31	-0.50	0.627
X_1X_3	-2.46	1.31	-0.93	0.368
X_1X_4	-1.82	1.31	-0.69	0.501
X_2X_3	-7.01	1.31	-2.67	0.019
X_2X_4	-2.37	1.31	-0.90	0.383
X_3X_4	-2.07	1.31	-0.79	0.445

4.4.1. Response surface and contour plots for degradation of AO7

The minitab (version 17) was used to produce 3 – D response surface plots and 2 – D contour plots of the model – predicted responses, while two variables kept constant and the others varying within the experimental ranges. It was utilized to assess the interactive relationships between the process variables and response.

4.4.1.1. Effect of H_2O_2 concentration and $FeSO_4$ concentration on degradation of AO7

Fig. 14 and 15 shows the contour plots and surface plots as a function of initial H_2O_2 concentration and initial $FeSO_4$ concentration: pH and contact time was maintained at 3 and 15 minutes respectively according to the results obtained. It can be seen for a particular concentration of H_2O_2, within the selected range, as $FeSO_4$ concentration increases, percent decolourisation first increases reaches an optimum value then decreases. Decrease may be due to loss of $OH^{\bullet}$ radicals by the following scavenging reaction, as suggested by Ramirez *et. al.*, (2005), Walling 1975:

$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^{-}$

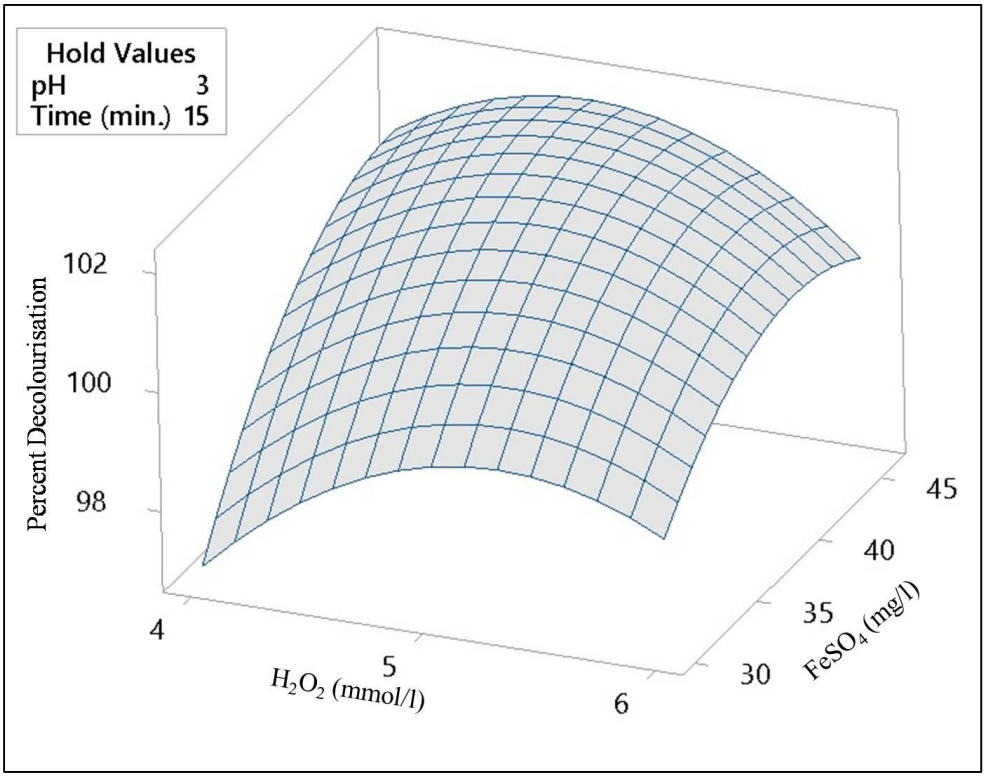

Fig. 14. Surface plot of Decolourisation vs H_2O_2 and $FeSO_4$

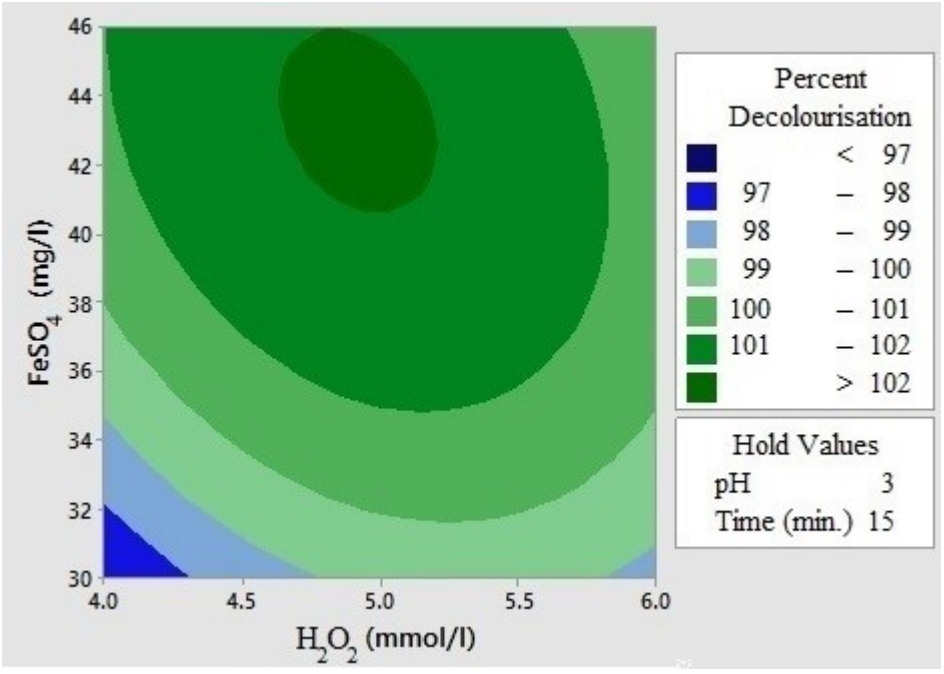

Fig. 15. Contour plot of Percent Decolourisation vs H_2O_2 and $FeSO_4$

4.4.1.2. Effect of H_2O_2 concentration and pH on degradation of AO7

Percent degradation obtained as a function of initial H_2O_2 concentration and pH was depicted in Fig. 16 and 17 in presence of 38 mg/l of $FeSO_4$ solution and contact time of 15 minutes. It can be seen that as pH reaches towards value of 3 the maximum decolourisation was observed for all initial concentration of H_2O_2 then there is slight decrease in value. Result is similar to as obtained by univariate approach.

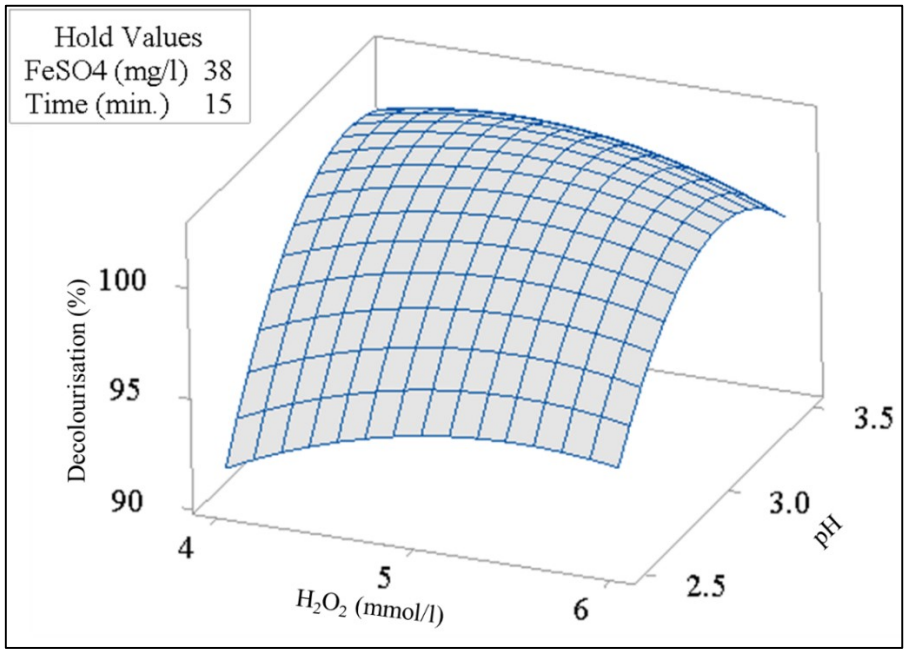

Fig. 16. Surface plot of decolourisation vs H_2O_2 and pH

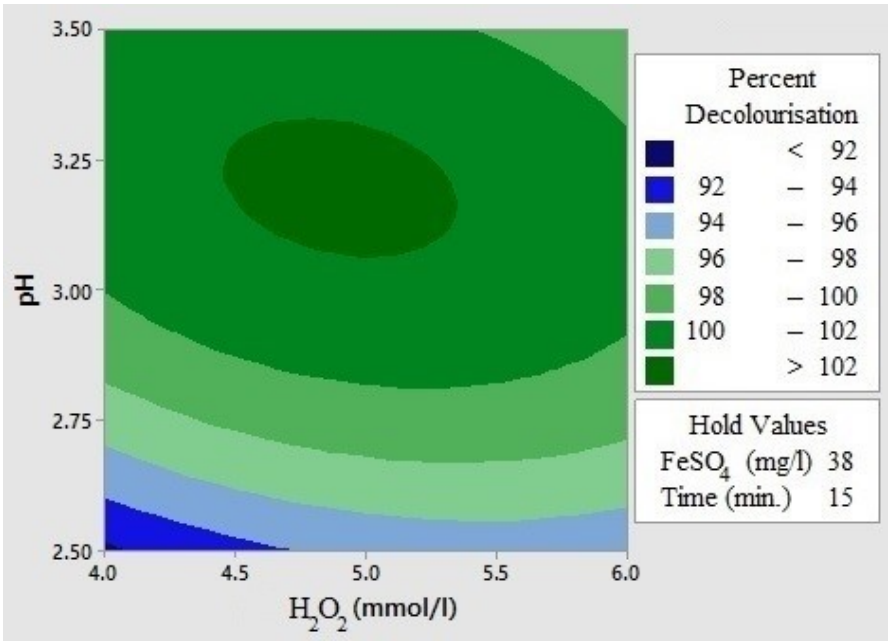

Fig. 17. Contour plot of Percent decolourisation vs H_2O_2 and pH

4.4.1.3. Effect of $FeSO_4$ concentration and pH on degradation of AO7

Fig. 18 and 19 displays 3D and 2D plots for percent degradation as a function of initial $FeSO_4$ concentration and pH (at a fixed H_2O_2 concentration = 5 mmol/l and contact time = 15 minutes). Percent decolourisation at low initial concentration of $FeSO_4$ were highly effected by pH, but at higher initial concentration of $FeSO_4$, pH had little effect. At pH 3 maximum decolourisation can be observed.

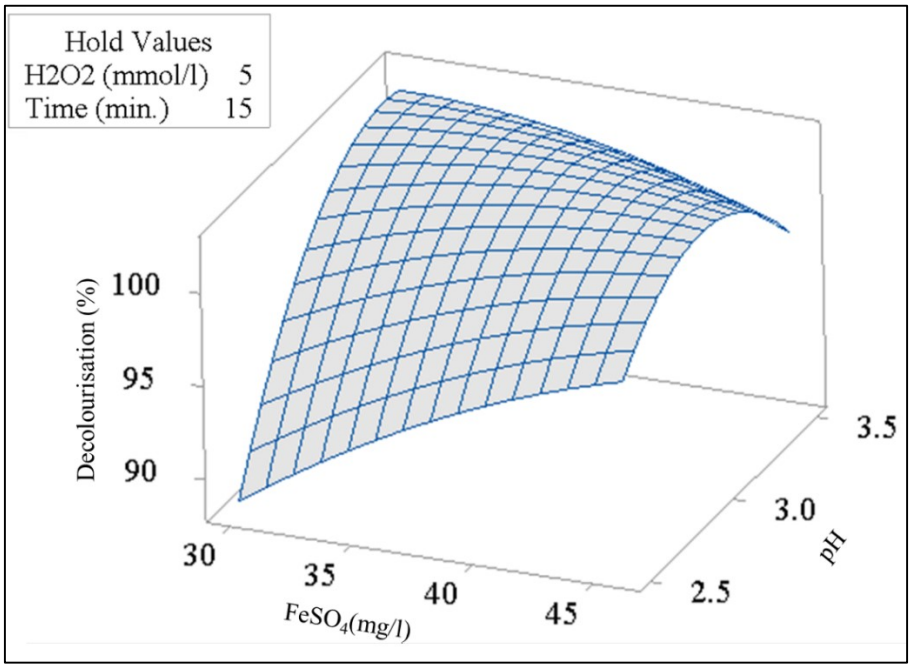

Fig. 18. Surface plot of decolourisation vs $FeSO_4$ and pH

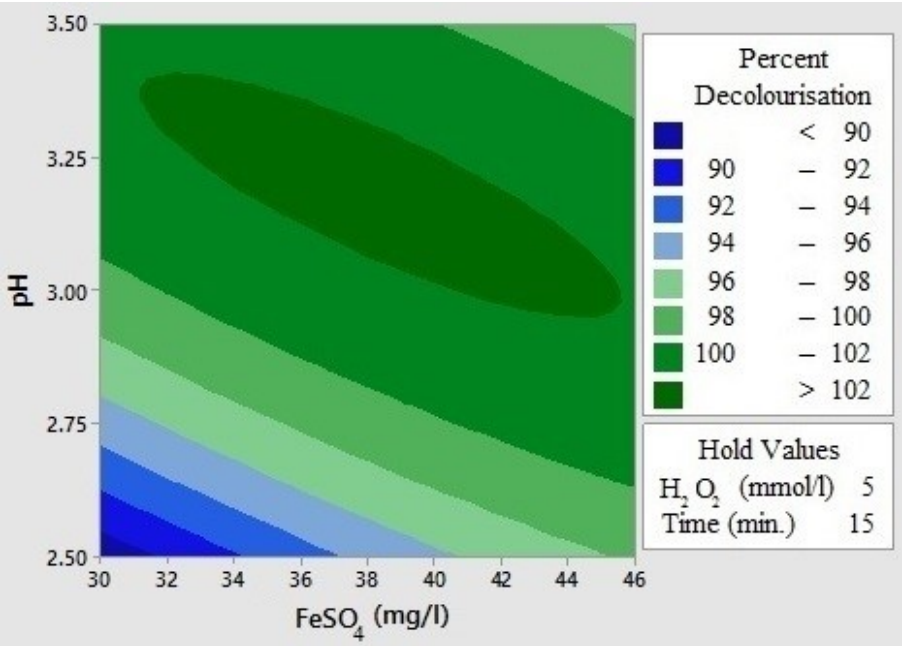

Fig. 19. Contour plot of percent decolourisation vs $FeSO_4$ and pH

4.4.1.4. Effect of $FeSO_4$ concentration and contact time on degradaation of AO7

Fig. 20 and 21 illustrates the effect of initial $FeSO_4$ concentration and contact time upon decolourisation of AO7 for initial H_2O_2 concentration of 5 mmol/l and pH of 3. Maximum decolourisation was obtained after 10 minutes of contact time for all initial $FeSO_4$ concentration.

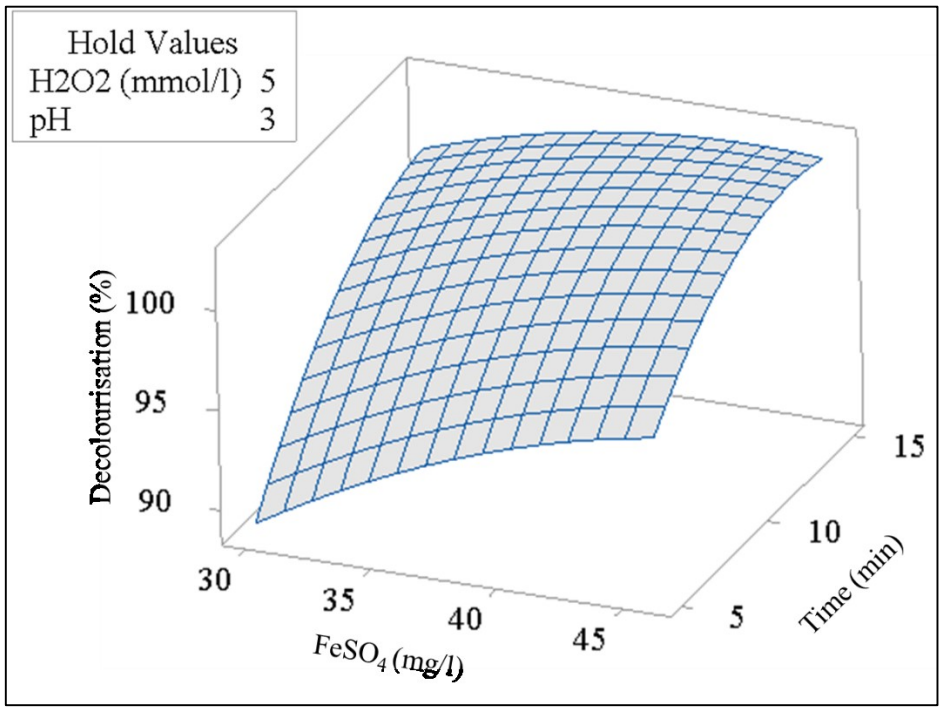

Fig. 20. Surface plot of decolourisation vs $FeSO_4$ and time

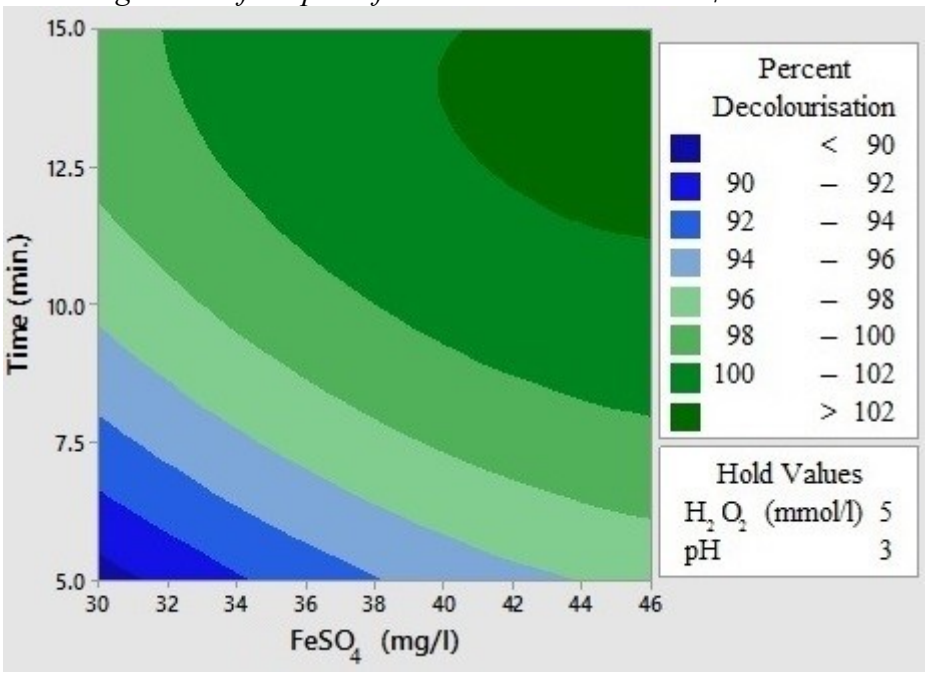

Fig. 21. Contour plot of Percent decolourisation vs $FeSO_4$ and time

4.4.1.5. Optimization of Fenton treatment of AO7

In the present study goal was to maximize the colour removal using optimized values of parameters. Box – Behnken design was used to optimize the parameters. Optimized values obtained were H_2O_2 concentration = 4.97 mmol/l, $FeSO_4$ = 40.83 mg/l, pH = 3.1 and contact time = 13.6 minutes.

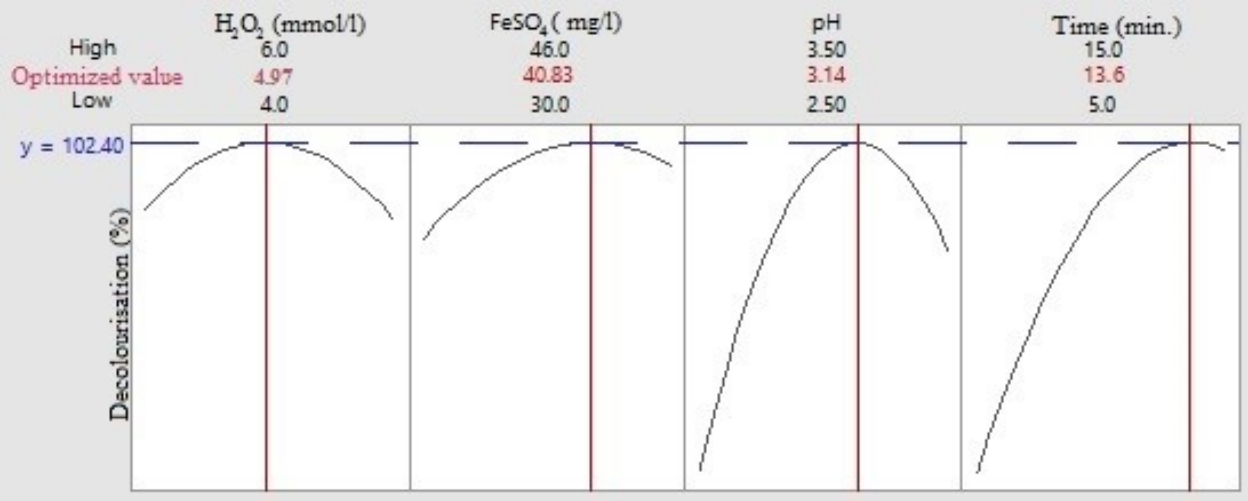

Fig. 22. Optimisation plot of degradation of AO7 by Fenton's oxidation process

4.5. Determination of COD

The degradation of AO7 during the experiment was confirmed on the basis of chemical oxygen demand. The initial COD for 50 mg/L concentration of dye was found to be 340 mg/L. After complete decolourisation during Fenton reaction, the COD was found to be nil. It confirmed that complete degradation of AO7 takes place during the Fenton treatment in a time period of about 15 minutes. The degradation of AO7 was also confirmed on the basis of absorption spectra of dye before and after the treatment. It was observed that there was no absorption by the treated solution in the visible range (420 to 700 nm) and the values were close to baseline as observed for initial de solution (before treatment) (Fig. 23).

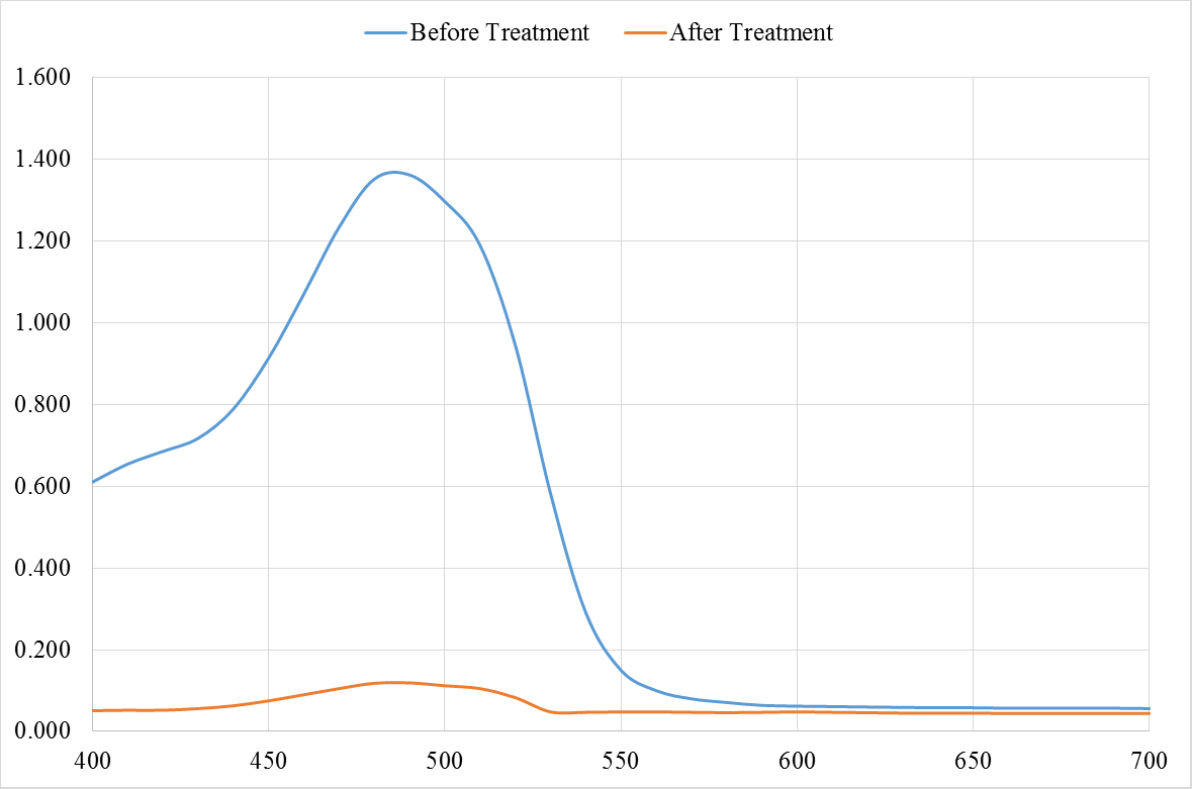

Fig. 23. Absorption Spectra of AO7 dye solution before and after Fenton treatment

5. Conclusion

Based on the experimental results, it is observed that AO7 is persistent in nature and is not degraded under natural conditions. A small fraction of AO7 is photo – chemically broken down to simpler intermediate resulting in some decolourisation but the reaction is reversible and stable AO7 is formed again in dark conditions during the night. The degradation of AO7 can be facilitated using a photo – catalyst like TiO_2 which results in significant improvement in the rate of degradation of AO7. Since TiO_2 is suspended in the solution its recovery after complete degradation of AO7 is imperative, which accounts to ~ 60%. The chemical oxidation (Fenton's reaction) is still effective in regulating the rate of decolourisation of AO7 and its rate was placed above the photo – catalytic degradation using TiO_2. Based on the results obtained it can be concluded that within the experimental conditions used Photo – catalytic degradation using TiO_2 took very long time (127 hours) for complete degradation of AO7, while Fenton's oxidation process showed complete degradation in less than 30 minutes. Hence, it can be concluded that Fenton's oxidation method is an efficient method. The concentrations of H_2O_2 (mmol/l) and $FeSO_4$ (mg/l) are important regulators for decolourisation efficiency at pH ~ 3 H_2O_2 dissociates into OH^- and $^{\bullet}OH$ radicals in presence of Fe^{2+} ions, and $^{\bullet}OH$ radicals results in cleavage of azo bond of AO7, and hydroxylation/epoxidation of aromatic rings resulting in ring opening and subsequent degradation of AO7. The optimised doses of H_2O_2 and Fe^{2+} were found to be less than the reported values in literature resulting in enhanced degradation of AO7 in comparison to other studies. It is important to mention that higher doses of H_2O_2 and Fe^{2+} (higher than the optimised values) are ineffective and result in sequestration of effective oxidising nature of reaction. Concentration of Fe^{2+} increases the efficiency of Fenton's process to a maximum value at optimized conditions. Further increase in Fe^{2+} concentration results in its conversion to Fe^{3+} which is insoluble and precipitates as Ferric hydroxide this results in scavenging of chemical species responsible for decolourisation of AO7. The results so obtained are valid against the predicted values of AO7 degradation using RSM. The experimental and model – predicted response was found to validate each other with a significant R^2 value of 0.88. Final COD is nil and final absorption spectra had no peaks which confirms that complete degradation of dye takes place during Fenton treatment.

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