Introduction and review of literature
Introduction and review of literature

Industrialization is vital for the growth and development of the nation. However, there are associated problems resulting from the generation of industrial waste products. In the last years the pollution problems due to textile industry effluents have increased. Because of lethal and esthetic impacts on receiving waters the treatment of textile effluents is necessary. While much research has been preformed to develop effective treatment technologies for wastewaters containing azo dyes, however no single solution has been satisfactory for remediating the broad diversity of textile wastes (Wallace 2001). The government has provoked due to human and ecological health concerns requires textile effluent discharges to have increasingly lower color and nitrogen levels. Although being aware of the problem, many textile companies have futile to sufficiently eradicate azo dye compounds from their wastewaters. Until dye and textile manufacturers are able to develop efficient technologies, allowing for increased dye-fiber bonding and lower dye house losses (Lewis 1999), the problem of treating these types of wastes will fall to the wastewater treatment facilities.

It is necessary to be aware of the basic composition of azo dyes to predict how these molecules can be destroyed. By knowing the general chemical structure of these compounds, concerns can be given to the toxic potential that they create to the environment. To ensure the safety of effluents, proper technologies need to be used by treatment facilities when degrading azo dyes (Wallace 2001).

Dyes, history

Ever since the beginning of humankind, people have been using colorants for painting and dyeing of their surroundings, their skins and their clothes. All colorants applied were from natural origin till the middle of the 19th century. Inorganic pigments such as soot, manganese oxide, hematite and ochre have been consumed within living memory. Palaeolithic rock paintings, such as the 30,000 year old drawings that were discovered in the Chauvet caves in France provide prehistoric evidence of their application (Chippindale 1998). Organic natural colorants have also an everlasting narration of application, principally as textile dyes. These dyes are all aromatic compounds, originating
usually from plants (e.g. the red dye alizarin from madder and indigo from woad) but also from insects (e.g. the scarlet dye kermes from the shield-louse Kermes vermilio), fungi and lichens (Van der Zee 2002). The English chemist W.H. Perkin in 1856 had tried to synthesize quinine but he obtained a bluish substance with excellent dyeing properties that later became known as aniline purple, tyrian purple or mauveine and started manufacturing the synthetic dyes. 18 years old Perkin has patented his creation and set up a construction line. This concept of research and development was soon to be followed by others and new dyes began to appear on the market, a process that was strongly stimulated by Kékulé’s discovery of the molecular structure of benzene in 1865 (Van der Zee 2002). Welham (2000) stated that in the starting of the 20th century, synthetic dyestuffs had almost completely replaced natural dyes. Dye manufacturing has turn out to be a major part of the chemical industry from the time when thousands of dyes have been manufactured. Currently, when the environment concern is of a major issue, it is appealing to believe that the use of natural colours is an eco-friendly alternative to existing preparation. Several groups have studied on the use of natural dyes in current dyeing industry (Angelini et al. 2003; Bermejo et al. 2003; Hao et al. 2004; Ramalho et al. 2004; Kamel et al. 2005; Singh et al. 2000). There are various benefits of the using natural dyes like the absence of toxicity upon humans, the use of sustainable sources and most importantly they fit into the natural pathways of biodegradation of the released dye-baths.

**Dyes and intermediates**

All aromatic compounds absorb electromagnetic energy but only those that absorb light with wavelengths in the visible range (~350-700 nm) are colored. Dyes are compounds that absorb light with wavelengths in the visible range, i.e., 400-700 nm (Van der Zee 2002). Dyes contain chromophores, delocalized electron systems with conjugated double bonds, and auxochromes, electron-withdrawing or electron-donating substituents that cause or intensify the colour of the chromophore by altering the overall energy of the electron system. Chromophore group is the major structure element responsible for light absorption. The absorption of UV/Vis radiation by an organic molecule is associated with electronic transitions between molecular orbitals. Chromophore normally contains heteroatom as N, O and S, with non-
Introduction and review of literature

bonding electrons. Usual chromophores are -C=C-, -C=N-, -C=O, -N=N-, -NO₂ and quinoid rings. The most important auxochrome groups are: hydroxyl and derivates, -OH, -OR; amino and derivates, -NH₂, -NHR, -NHR₂; sulphonic, -SO₃H; carboxylic, -COOH; and sulphide, -SR (Van der Zee 2002).

Based on the chromophore present, general classes of dyes are shown in Table 1.1.

Based on chromophore or chemical structure, 20-30 different groups of dyes can be recognized. Azo (monoazo, disazo, triazo, polyazo), anthraquinone, phthalocyanine and triarylmethane dyes are quantitatively the most important chromophores Figure 1.1. Other groups are diarylmethane, indigoid, azine, oxazine, thiazine, xanthene, nitro, nitroso, methine, thiazole, indamine, indophenol, lactone, aminoketone and hydroxyketone dyes and dyes of undetermined structure (stilbene and sulphur dyes).

**Figure 1.1: The most important chromophores**

![Azo, Anthraquione, Phthalocyanine, Triarylmethane](image)

Classification of dyes on the basis of colour, structure and application method is principal method adopted by the Colour Index (C.I.). The Colour Index catalogs about 28,000 commercial dye names, used for ~10,500 different dyes, 45,000 of which are currently produced. It is edited since 1924 (and revised every three months) by the Society of Dyers and Colourists and the American Association of Textile Chemists and Colorists. The Colour Index assigned C.I. generic name and defined by its colour and application class.

Dyes may be classified in two ways:

- According to the chemical constitution of the dye molecule, or
- According to the method of application of the dye.

The Colour Index discerns 16 different application classes:
(A) Acid dyes, (B) Azoic dyes, (C) Basic dyes, (D) Direct dyes, (E) Disperse dyes, (F) Mordant dyes, (G) Premetallised dyes, (H) Sulphur dyes, (I) Vat

**Table 1.1: Classification and examples of dyes according to the chromophore present**

<table>
<thead>
<tr>
<th>Class</th>
<th>Chromophore</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitro group</td>
<td><img src="image" alt="Nitro group structure" /></td>
<td>C.I. Acid yellow 24</td>
</tr>
<tr>
<td>Nitroso group</td>
<td><img src="image" alt="Nitroso group structure" /></td>
<td>Fast Green O</td>
</tr>
<tr>
<td>Azo Group</td>
<td><img src="image" alt="Azo group structure" /></td>
<td>Methyl Orange</td>
</tr>
<tr>
<td>Triphenyl methyl dyes</td>
<td><img src="image" alt="Triphenyl methyl dyes structure" /></td>
<td>C.I. Basic Violet 3</td>
</tr>
<tr>
<td>Phthalein dyes</td>
<td><img src="image" alt="Phthalein dyes structure" /></td>
<td>Phenolphthalein</td>
</tr>
<tr>
<td>Indigoid dyes</td>
<td><img src="image" alt="Indigoid dyes structure" /></td>
<td>C.I. Acid Blue 71</td>
</tr>
<tr>
<td>Anthraquinone dyes</td>
<td><img src="image" alt="Anthraquinone dyes structure" /></td>
<td>C.I. Reactive Blue 19</td>
</tr>
</tbody>
</table>
Various attractive forces have the potential of binding dyes to fibres, and often more than one type of chemical bonding can operate with the same dye-fibre combination. The types of bonds established between the dye and the fibre, by increasing relative strength of the bond, can be: Van der waals, hydrogen, ionic or covalent (Guratini and Zanoni 2000; Gomes 2001).

According to the application categories dyes can be classified as seen in Table 1.2.

**Table 1.2: Application categories of dyes (O’Neill et al. 1999 and Gomes 2001)**

<table>
<thead>
<tr>
<th>Type of dye</th>
<th>Characteristics</th>
<th>Chemical structure</th>
<th>Substrates</th>
<th>Current production (yearly)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>When in solution are negatively charged; bind to the cationic NH$_3^+$-groups present in fibres</td>
<td>Sodium salts of sulphonic and carboxylic acids</td>
<td>Nylon, wool, polyamide, silk, modified acryl, paper, inks and leather</td>
<td>~40% of the total ~2300 different acid dyes listed</td>
</tr>
<tr>
<td>Reactive</td>
<td>Form covalent bonds with OH-, NH- or SH-groups</td>
<td>Reactive group</td>
<td>Cotton, wool, silk and nylon</td>
<td>600 of total 1050 different reactive dyes listed</td>
</tr>
<tr>
<td>Metal complex</td>
<td>Strong complexes of one metal ion (usually chromium, copper, cobalt or nickel) and one or two dye molecules (acid or reactive)</td>
<td>Metal complexes</td>
<td>Silk, wool and polyamide</td>
<td>~1/6 of all azo dyes listed</td>
</tr>
<tr>
<td>Direct</td>
<td>Large molecules bound by Van der Waals forces to the fibre</td>
<td>Large molecule with high affinity to fiber (mainly Azo dyes)</td>
<td>Cellulose fibres, cotton, viscose, paper, leather and nylon</td>
<td>~30% of the total 1600 different direct dyes listed</td>
</tr>
<tr>
<td>Basic</td>
<td>Cationic compounds that bind to the acid groups of the fibre</td>
<td>Salts of colour bases</td>
<td>Synthetic fibres, paper and inks</td>
<td>~5% of the total listed dyes</td>
</tr>
<tr>
<td>Mordant</td>
<td>Require the addition of a chemical that combines with the dye and the fibre, like tannic acid, alum, chrome alum, and other salts of aluminium, chromium, copper, iron, potassium, and tin</td>
<td>Require mordant, metal hydroxide and tannic acid etc.</td>
<td>Wool, leather, silk, paper, modified cellulose fibres and anodized aluminium</td>
<td>~23% of the total 600 different mordent dye listed</td>
</tr>
<tr>
<td>Type of dye</td>
<td>Characteristics</td>
<td>Chemical structure</td>
<td>Substrates</td>
<td>Current production (yearly)</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------</td>
<td>--------------------</td>
<td>------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Disperse</td>
<td>Scarcely soluble dyes that penetrate the fibre through fibres swelling</td>
<td>Water insoluble</td>
<td>Polyester, polyamide, acetate, acrylic and plastics</td>
<td>~40% of the total 1400 different reactive dyes listed</td>
</tr>
<tr>
<td>Pigment</td>
<td>Insoluble, non-ionic compounds or insoluble salts that retain their crystalline or particulate structure throughout their application</td>
<td>non-ionic compounds</td>
<td>Paints, inks, plastics and textiles</td>
<td>–</td>
</tr>
<tr>
<td>Vat</td>
<td>Insoluble coloured dyes which on reduction give soluble colourless forms (leuco form) with affinity for the fibre; on exposure to air are reoxidised</td>
<td>Leuco compound (reduced state)</td>
<td>Cellulose fibres, cotton, viscose and wool</td>
<td>–</td>
</tr>
<tr>
<td>Azoic and Ingrain</td>
<td>Insoluble products of a reaction between a coupling component and a diazotised aromatic amine that occurs in the fibre</td>
<td>Insoluble azo dyes (-N=N-)</td>
<td>Cotton, viscose, cellulose acetate and polyester</td>
<td>–</td>
</tr>
<tr>
<td>Sulphur</td>
<td>Complex polymeric aromatics with heterocyclic S-containing rings</td>
<td>Complex polymeric, heterocyclic S-rings</td>
<td>Cellulose fibres, cotton and viscose</td>
<td>15% of the total dyes listed</td>
</tr>
<tr>
<td>Solvent</td>
<td>Non ionic dyes that dissolve the substrate to which they bind</td>
<td>non-ionic mainly diazo compound (lysochrome)</td>
<td>Plastics, gasoline, varnish, lacquer, stains, inks, oils, waxes and fats</td>
<td>–</td>
</tr>
<tr>
<td>Fluorescence brighteners</td>
<td>Mask the yellowish tint of natural fibres</td>
<td>Triazimyl unit and water-solubilizing group</td>
<td>Soaps and detergents, all fibres, oils, paints and plastics</td>
<td>–</td>
</tr>
<tr>
<td>Food</td>
<td>Non-toxic and not used as textile dyes</td>
<td>–</td>
<td>Food</td>
<td>–</td>
</tr>
<tr>
<td>Natural</td>
<td>Obtained mainly from plants</td>
<td>–</td>
<td>Food, cotton, wool, silk, polyester, polyamide and polyacrylonitrile</td>
<td>–</td>
</tr>
</tbody>
</table>
Major noteworthy industrial uses of dyes are in textile dyeing, because of the large amounts used. Dyes are also used in hair colouring, cosmetics, food technology, leather, tanning industry, paper production, agricultural research, photo-electrochemical cells and light-harvesting arrays. Dyes have been working for the control of the usefulness of sewage and wastewater treatment, for the determination of specific surface area of activated sludge and for ground water tracing (Forgacs et al. 2004).

**Azo dyes**

Azo dyes contain at least one nitrogen-nitrogen (N=N) double bond, however many different structures are possible (Zollinger 1991). They are generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocycles or enolizable aliphatic groups (Zollinger 1991). Azo dyes are man-made organic colorants which are symbolized by vast structural variety. They are representing almost 70% of the textile dyestuffs produced (Knackmuss et al. 2002) and most widely used among synthetic azo dyes as dyes for textiles, food and cosmetics. They are easy to manufacture, have low cost, are stable, can be used to colour several materials (textile, leather, plastic, food) and allow a great variety of colours and shades. They are obtained from the coupling of diazonium salts with aromatic amines, phenols, naphthols or aliphatic enols and have in their molecule one or more azo groups. Coupling usually takes place in the para position with respect to the amino or hydroxyl group or in the ortho position if the latter is occupied. The diazonium salts are obtained from the reaction of sodium nitrite with an amine solution with a mineral acid, preferably HCl (Zollinger 1991) – **Figure 1.2**.

The structural class of azo dyes includes dyes from different application classes, namely, acid, basic, metal complex, reactive and mordant.

**Scenario of dye production and discharge**

The textile dyes market is projected to reach $5.9bn by the year 2017, influenced by changing perceptions, technological innovations, consumer spending and population growth, according to a report by Global Industry Analysts (GIA). According to GIA, demand for new dyes is increasing with growing preferences for enhanced performance from new materials, and dye manufacturers and textile producers worldwide are developing innovative
products and processes to comply with strict environmental regulations. 

**Figure 1.2: Synthesis of azo dyes**

Industrialised nations such as the US and Europe witnessed increased shifting of operating bases to emerging markets in Asia. Low manufacturing costs, trained labour and cheaper raw materials in China and India have resulted in the greater concentration of production activity in these two countries, according to the research. Recent statistics on the worldwide production with use of dyes and on the relative distribution between the different dye classes are not readily available. The available data are from the 1993 SRI report (Ollgaard et al. 1998) listed in Table 1.3; show that the relative share of Western Europe is 13% of the world sale. Asia share 42%, US is next with 24% and Europe has around 22% share of the global production in dyestuff production. Due to the greater use of polyester and cotton – based fabrics, there has been shift towards acid dyes, reactive dyes, used in cotton – based fabrics, and disperse dyes, used in polyester. The industries consume the major bulk of the available water. The Indian Chemical Industry, ranks 12th in the world, for the production of chemicals. The Indian textile industry accounts for the largest consumption of dyestuffs, which is 70%. With a production capacity of almost 20% of chemicals and allied items produced in this country, Gujarat is the second largest Industrial state of India. About 2000 big and small scale industries are located in the state, of which 65% are responsible for one or other pollutants added in nature. India has emerged as a global supplier of dyestuff and dyes.
intermediates, particularly for acid, reactive, vat and direct dyes. According to annual report, Department of Chemicals and Petrochemicals, Ministry of Chemical and Fertilizers Government of India 2007-08, India accounts for 6% of the world production.

Table 1.3: Total sales of dyes with the exclusion of solvent and pigment dyes (Øllgaard et al. 1998)

<table>
<thead>
<tr>
<th>DYE CLASS</th>
<th>WESTERN EUROPE (1,000 tonnes)</th>
<th>WORLD (1,000 tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid and mordant</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>Azoic</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td>Basic</td>
<td>8</td>
<td>44</td>
</tr>
<tr>
<td>Direct</td>
<td>9</td>
<td>64</td>
</tr>
<tr>
<td>Disperse</td>
<td>22</td>
<td>157</td>
</tr>
<tr>
<td>Reactive</td>
<td>13</td>
<td>114</td>
</tr>
<tr>
<td>Sulphur</td>
<td>3</td>
<td>101</td>
</tr>
<tr>
<td>Vat</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Sum</td>
<td>85</td>
<td>668</td>
</tr>
<tr>
<td>Relative share (%)</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

About 12% of the synthetic textile dyes used yearly is lost to waste streams during dyestuff manufacturing and textile processing operations. The principal route by which dyes enter the environment is via wastewater (Easton and Cooper 1995). To judge the relative share of the different dye classes in the wastewater of textile-processing industries, dye consumption data should be considered together with the degree of fixation of the different dye classes. These are listed in Table 1.4.

Table 1.4: Estimated degree of fixation for different dye/fibre combinations (Easton and Cooper 1995)

<table>
<thead>
<tr>
<th>Dye class</th>
<th>Fibre</th>
<th>Degree of fixation (%)</th>
<th>Loss to effluent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>Polyamide</td>
<td>80-95</td>
<td>5-20</td>
</tr>
<tr>
<td>Basic</td>
<td>Acrylic</td>
<td>95-100</td>
<td>0-5</td>
</tr>
<tr>
<td>Direct</td>
<td>Cellulose</td>
<td>70-95</td>
<td>5-30</td>
</tr>
<tr>
<td>Disperse</td>
<td>Polyester</td>
<td>90-100</td>
<td>0-10</td>
</tr>
<tr>
<td>Metal-complex</td>
<td>Wool</td>
<td>90-98</td>
<td>2-10</td>
</tr>
<tr>
<td>Reactive</td>
<td>Cellulose</td>
<td>50-90</td>
<td>10-50</td>
</tr>
<tr>
<td>Sulphur</td>
<td>Cellulose</td>
<td>60-90</td>
<td>10-40</td>
</tr>
<tr>
<td>Vat</td>
<td>Cellulose</td>
<td>80-95</td>
<td>5-20</td>
</tr>
</tbody>
</table>
Azo dyes are the largest class with 60-70% of the total organic colorants listed in the Colour Index. Anthraquinone dyes are second largest class (~15%), followed by triarylmethanes (~3%) and phthalocyanines (~2%). The vast majority of the dyes discharged by textile-processing industries are azo dyes and the relative share among reactive, acid and direct dyes is even higher.

**Green environment concern**

The recent years witnessed various dyes being banned across the globe due to environmental concerns. With the increasing demand for eco-friendly textile chemicals from textile manufacturers, companies are recognizing the need to increase R&D spending on development of innovative chemicals and dyes. The environment problems associated with textile activities mainly arise from the extensive use of organic dyes. Dyes are detected in water at concentrations as low as 1 mg/L (Ramalho et al. 2004). Textile-processing effluents are usually with dye content in the range 10-200 mg/L (O'Neil et al. 1999); as a result they are usually highly coloured discharge from processing units in open waters presents an aesthetic problem **Photograph 1.1**.

**Photograph 1.1: Kharicut canal showing the severity of colour containing wastewater discharged effluent of Vatva G.I.D.C., Ahmedabad.**

*Source: Gujarat Samachar Thursday 7 June 2010*

Dyes are intended to be chemically and photolytically stable, so are extremely persistent in natural environments. Therefore, the release of dyes may present an ecotoxic hazard and introduces the likely danger of bioaccumulation that may ultimately affect human by transport through the food chain.
Toxicity considerations of dyestuffs
Dyestuff toxicity has been investigated in numerous researches. Dyes are intended to be chemically and photolytically stable, so are extremely persistent in natural environments. Therefore, the release of dyes may present an ecotoxic hazard and introduces the likely danger of bioaccumulation that may ultimately affect human by transport through the food chain. Ecological and Toxicological Association of Dyes (ETAD) and Organic Pigments Manufacturers promoted systematically research in investigation of bioaccumulation trend of dyestuffs in fish. Seventy five dyes from different application classes were determined for bio-concentration factors (BCF’s) and related to the partition coefficient n-octanol/water (KOW) of each different compound. Some of dyes and intermediates have long been acknowledged toxic in nature. Azo dyes are considered to be xenobiotic compounds that are recalcitrant beside biodegradative process. Acute and short term effects are generally well known that are toxic (Chung and Stevens 1993), carcinogenic, mutagenic (Brown and DeVito 1993; Weisburger 2002), genotoxic, teratogenic or mortal to various microbes, fish (Photograph 1.2) or other aquatic organisms (algae, bacteria, etc.) and mammalian species (Joachim et al. 1995).

Photograph 1.2: Death of fishes due to contamination of pond by green coloured polluted water
Source: Gujarat Samachar, 3rd August, 2004; site: Vinzol village, Ahmedabad
Carcinogenic effect
Primarily focused of studied have been done for so many years for the chronic
effects of azo dyes present in food colorants. The effects of occupational
exposure to dyestuffs of human workers in dye manufacturing and utilizing
industries have received attention. The greatest tragedy of the dyestuff
industry has been the occurrence of occupation tumors of urinary bladder
(papilloma). Dyestuffs in purified form are hardly ever directly mutagenic or
carcinogenic, except for some azo dyes with free amino groups (Brown and
Devito 1993). In mammals, metabolic activation (= reduction) of azo dyes is
mainly due to bacterial activity in the anaerobic parts of the lower
gastrointestinal tract where the released aromatic amines are absorbed by the
intestine and excreted in the urine. Other organs can also reduce azo dyes,
especially the liver and the kidneys. Cleavage of the dye’s azo linkage(s) i.e.
reduction of azo dyes, leads to formation of aromatic amines and several
aromatic amines are known mutagens and carcinogens. The toxic hazard of
aromatic amines is carcinogenesis, especially bladder cancer. The probably
 mechanism for carcinogenicity includes the formation of acyloxy amines
through N-hydroxylation and N-acetylation of the aromatic amines followed by
O-acetylation. Further these acyloxy amines can be converted to nitrenium
and carbonium ions that bind to DNA and RNA, which induces mutations and
tumor formation (Brown and DeVito 1993).

Mutagenic effect
The mutagenic activity of aromatic amines is strongly related to molecular
structure (Ramalho 2005). Brown and DeVito (1993) studied that most of the
dyes on the International Agency for Research on Cancer (IARC) list were
taken out of production now. In 1975 and 1982, the IARC summarized the
literature on suspected azo dyes, mainly amino-substituted azo dyes, fat-
soluble azo dyes and benzidine azo dyes, and a few sulphonated azo dyes
(Peters and Freeman 1982).

Effect of acute toxicity
The action of aerobic and anaerobic bacteria in effluent treatment systems is
carried out to study effects of dyestuffs and dye containing effluents. The
acute toxicity of dyestuffs is generally low. The most acutely toxic dyes for
algae are cationic-basic dyes (Greene and Baughman 1996). The most
acutely toxic dyes for fish are basic dyes, especially those with a triphenylmethane structure. Fish also seem to be relatively sensitive to many acid dyes (Clarke and Anliker 1980). Mortality tests with rats showed that only 1% out of 4461 commercial dyestuffs tested (Clarke and Anliker 1980). Therefore, the chance of human mortality due to acute dyestuff toxicity is probably very low. However, an acute sensitisation reaction to humans by dyestuffs often occurs. Specht and Platzek (1995) found that some disperse dyestuffs have been to cause allergic reactions, i.e. eczema or contact dermatitis.

**Genotoxicity effect**

Genotoxicity is linked with all aromatic amines with benzidine moieties, toluene, aniline and naphthalene moieties. Cartwright had reported that 2-naphthylamine is a carcinogen in 1983; 1-naphthylamine is much less toxic, which suggest that the toxicity of amines depends strongly on the location of the amino-group(s). It is also depends on the nature and location of other substituents like the substitution with nitro, methyl or methoxy groups or halogen atoms may increase the toxicity, whereas substitution with carboxyl or sulphonate groups generally lowers the toxicity (Chung and Cerniglia 1992). According to Jung et al. (1992) studies sulphonated aromatic amines compare to their unsulphonated analogues have low genotoxicity. The possible dangers of sulfonated aromatic amines are particularly important as soluble commercial azo dyestuffs contain one or more sulphonate groups.

**Composition of textile wastewater**

The colour of wastewater released by textile processing industry changes from day to day or several times a day because of the inefficiencies of dyeing process, which ultimately finds its way into the environment. Colour removal has recently become an area of major scientific interest because of its distinct visibility at very low concentrations. Dependant upon the dye application class the amount of dye is lost, varying from only 2% loss when using basic dyes to a 50% loss when certain reactive dyes are used (O’Neill et al. 1999; McMullan et al. 2001; Pearce et al. 2003). The applications of diverse production processes with diverse raw materials create problems in consider wastewater quality and later defining pollution control technologies (Correia et al. 1994). The stages of processing involve desizing, scouring, bleaching mercerizing.
neutralization, dyeing, printing and finishing. Major pollutant types present in
the textile wastewater processing with types of chemical and process of origin
are listed in Table 1.5.

Table 1.5: Major pollutant types in textile wastewater, chemical types
and process of origin (adapted from Delée et al. 1998).

<table>
<thead>
<tr>
<th>Pollutants</th>
<th>Chemical types</th>
<th>Process of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic load</td>
<td>Starches, enzymes, fats, greases, waxes, surfactants and acetic acid</td>
<td>Ds, S, W, Dy</td>
</tr>
<tr>
<td>Colour</td>
<td>Dyes, scoured wool impurities</td>
<td>Dy, S</td>
</tr>
<tr>
<td>Nutrients (N, P)</td>
<td>Ammonium salts, urea, phosphate-based buffers and sequestrants</td>
<td>Dy</td>
</tr>
<tr>
<td>P^H and salts</td>
<td>NaOH, mineral/organic acids, sodium chloride, silicate, sulphate, carbonate</td>
<td>S, Ds, B, M, Dy, N</td>
</tr>
<tr>
<td>Sulphur</td>
<td>Sulphate, sulphite and hydrosulphite salts, sulphuric acid</td>
<td>Dy</td>
</tr>
<tr>
<td>Toxic compounds</td>
<td>Heavy metals, reducing agents (sulphide), oxidizing agents (chlorite, peroxide, dichromate, persulphate), biocides, quaternary ammonium salts</td>
<td>Ds, B, Dy, F</td>
</tr>
<tr>
<td>Refractory organics</td>
<td>Surfactants, dyes, resins, synthetic sizes (PVA), chlorinated organic compounds, carrier organic solvents</td>
<td>S, Ds, B, Dy, W, F</td>
</tr>
</tbody>
</table>

Ds – desizing; S – scouring; W – washing; Dy – dyeing; B – bleaching; M – mercerizing; N – neutralization; F - finishing

Dye removal techniques

A wide range of methods has been developed for the removal of synthetic
dyes from waters and wastewaters to decrease their impact on the
environment. They are divided in three major categories: physical, chemical or
biological (Kirk and Othmer 1993):

1. **Physical**: adsorption, sedimentation, flotation, flocculation, coagulation,
   foam fractionation, polymer flocculation, reverse osmosis/ ultrafiltration,
   radiation and incineration.

2. **Chemical**: neutralization, reduction, oxidation, electrolysis, ion exchange
   and wet – air oxidation.

3. **Biological**: stabilization ponds, aerated lagoons, trickling filters, activated
   sludge, anaerobic digestion and bioaugmentation.

Biological and chemical methods involve the destruction of the dye molecule,
whilst physical methods usually transfer the pollutant to another phase.
Various physical, chemical and biological pre treatment, main treatment and post treatment techniques can be employed to remove colour from dye containing wastewaters (Hao et al. 2004; Robinson et al. 2001). Biological techniques include bacterial and fungal biosorption and biodegradation in aerobic, anaerobic, anoxic or combined anaerobic/aerobic treatment processes.

Physical methods by and large transfer the pollutant from one phase to another phase, while biological and chemical methods involve the destruction of the dye molecule.

Several factors determine the technical and economical feasibility of each single dye removal technique like:

- dye type
- wastewater composition
- dose and costs of required chemicals
- operation costs (energy and material)
- environmental fate
- handling costs of generated waste products.

In general, each technique has its limitations. Sometime, the use of one individual process may often not be sufficient to achieve complete decolourisation.

Dye removal strategies consist mostly of a combination of different techniques. Advantages and limitation of these methods are presented in Table 1.6.

**Physico-chemical Treatment**

Wastewater treatment using physical, chemical and biological methods are available for the dye removal (Hao et al. 2004), each with its technical and economical limitations (Robinson et al. 2001).

Dye treatment using physico-chemical techniques have shortcoming because they are high-priced, have narrow usefulness, are greatly interfered by other wastewater constituents, and/or generate waste products that must be handled separately (Table 1.6) (Anjaneyulu et al. 2005). On the other hand, biological treatment may present a relatively low-priced way to remove dyes from wastewater.
Table 1.6: Advantages and limitations of various decolourisation methods for industrial effluents adapted from Anjaneyulu et al. 2005

<table>
<thead>
<tr>
<th>Treatment methodology</th>
<th>Stage of treatment</th>
<th>Type of industry</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available methods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Physical methods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Adsorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Bagasse</td>
<td>Pre treatment</td>
<td>Sugar/brewery</td>
<td>Waste to treat another waste</td>
<td>Post treatment disposal</td>
</tr>
<tr>
<td>c. Peat</td>
<td>Pre treatment</td>
<td>Any industry as post or pre-treatment</td>
<td>Effective adsorbent due to cellular structure. No activation required</td>
<td>Surface area is lower than activated carbon</td>
</tr>
<tr>
<td>d. Wood chips</td>
<td>Pre treatment</td>
<td>Any industry as post or pre-treatment</td>
<td>Good sorption for specific colourant</td>
<td>Larger contact times and huge quantities are required</td>
</tr>
<tr>
<td>2. Irradiation</td>
<td>Post treatment</td>
<td>Kraftmill/Tannery/distillery/pulp and paper</td>
<td>Effective removal for a wide range of colourants at low volumes</td>
<td>Dissolved oxygen requirement is high. Ineffective for light resistant colourants</td>
</tr>
<tr>
<td>3. Ion-exchange</td>
<td>Main treatment</td>
<td>Any industry</td>
<td>Regeneration with low loss of adsorbents</td>
<td>Specific application</td>
</tr>
<tr>
<td>II. Chemical methods</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Oxidation</td>
<td></td>
<td></td>
<td></td>
<td>Problem with sludge disposal</td>
</tr>
<tr>
<td>a. Fenton’s reagent</td>
<td>Pre/main treatment</td>
<td>Textile/tannery/pulp and paper</td>
<td>Capable of decolourising wide variety of wastes. No alternation in volume</td>
<td>Prohibitively expensive</td>
</tr>
<tr>
<td>b. Ozonation</td>
<td>Main treatment</td>
<td>Textile/tannery/brewery/distillery</td>
<td>Effective for azo dye removal</td>
<td>Not suitable for dispersed dyes. Releases aromatic amines</td>
</tr>
</tbody>
</table>
### Treatment methodology

<table>
<thead>
<tr>
<th>Stage of treatment</th>
<th>Type of industry</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post treatment</td>
<td>Brewery/ distillery</td>
<td>Low temperature requirement</td>
<td>Cost intensive process</td>
</tr>
<tr>
<td>Pre treatment</td>
<td>Kraftmill/ distillery</td>
<td>No additional chemicals required and end products are non-hazardous</td>
<td></td>
</tr>
</tbody>
</table>

| Cogulation and precipitation | Pre/main treatment | Sugar/pulp paper/kraft mill | Complete decolourisation for all class of dyes | Expensive |

### III. Biological methods

<table>
<thead>
<tr>
<th>1. Aerobic process</th>
<th>Main treatment</th>
<th>Textile/ tannery/ kraftmill/ distillery</th>
<th>Colour removal is facilitated along with COD removal</th>
<th>Longer detention times and substrate specific removal. Less resistant to recalcitrant</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Anaerobic process</td>
<td>Main treatment</td>
<td>Distillery/brewery/ pulp and paper/ sugar</td>
<td>Resistant to wide variety of complex colourants. Bio gas produced is used for steam generation</td>
<td>Longer acclimatization phase</td>
</tr>
<tr>
<td>3. Single cell (Fungal, Algal &amp; Bacterial)</td>
<td>Post treatment</td>
<td>Any industry as Post-treatment</td>
<td>Good removal efficiency for low volumes and concentrations. Very effective for specific colourant removal</td>
<td>Culture maintenance is cost intensive. Cannot cope up with large volumes of coloured effluents</td>
</tr>
</tbody>
</table>

### IV. Emerging technologies

<table>
<thead>
<tr>
<th>Main treatment</th>
<th>Textile/ tannery/ distillery</th>
<th>Complete mineralization ensured. Growing number of commercial applications. Effective</th>
<th>Cost intensive process</th>
</tr>
</thead>
</table>

---

Introduction and review of literature
pretreatment methodology in integrated systems and enhances biodegradability

<table>
<thead>
<tr>
<th>Treatment methodology</th>
<th>Stage of treatment</th>
<th>Type of industry</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.Membrane Filtration</td>
<td>Main treatment</td>
<td>Tannery/brewery/distillery</td>
<td>Recovery and reuse of chemicals and water. Wider application for complex wastes</td>
<td>Dissolved solids are not separated in this process. High running cost</td>
</tr>
<tr>
<td>3.Photo-catalysis</td>
<td>Post treatment</td>
<td>Any industry as post or pre-treatment</td>
<td>Process carried out at ambient conditions. Inputs are atoxic and inexpensive. Complete mineralization with shorter detention times.</td>
<td>Effective for small amount of colourants. Expensive process</td>
</tr>
<tr>
<td>4.Sonication</td>
<td>Pre treatment</td>
<td>Any industry</td>
<td>Simplicity in use. Very effective in integrated systems</td>
<td>Relatively new method and awaiting full scale application</td>
</tr>
<tr>
<td>5.Enzymatic treatment</td>
<td>Post treatment</td>
<td>Any industry after biological treatment</td>
<td>Effective for specifically selected compounds. Unaffected by shock loadings and shorter contact times required</td>
<td>Enzyme isolation and purification is tedious. Efficiency curtailed due to the presence of interferences</td>
</tr>
<tr>
<td>6.Redox mediators</td>
<td>Pre/supportive treatment</td>
<td>Any biological treatment</td>
<td>Easily available and enhances the process by increasing electron transfer efficiency</td>
<td>Concentration of mediator may give antagonistic effect. Depends on biological activity of the system</td>
</tr>
<tr>
<td>7.Engineered Wetland Systems</td>
<td>Pre/post treatment</td>
<td>Any industry which releases large volumes of effluents</td>
<td>Cost effective technology and can be operated with huge volumes of wastewater</td>
<td>High initial installation cost. Requires expertise and managing during monsoon becomes difficult</td>
</tr>
</tbody>
</table>

Introduction and review of literature
Biological treatment – a better alternative to physicochemical methods

Remediation of dye containing wastewater can be done by diverse existing techniques consist of various physicochemical techniques, biological and microbiological techniques. An effective method like adsorption on activated carbon is available for the removal of colour; however it is too costly (Fu and Viraraghavan 2001). The key drawbacks of physicochemical techniques has been principally due to the high expenditure, low efficiency, limited versatility, interference by other wastewater constituents, and the handling of the waste generated (Van der Zee and Villaverde 2005; Kaushik and Malik 2009). Conventional treatment technologies have confirmed to be distinctly unproductive for managing wastewater of man-made textile dyes because of the chemical stability of these pollutants (Forgacs et al. 2004). Developing efficient eco-friendly technologies to decrease dye content in wastewater to acceptable levels at affordable cost is of utmost importance (Couto 2009).

Biological methods are generally considered eco-friendly as they can lead to complete mineralization of organic pollutants at low cost (Pandey et al. 2007). It is now known that several microorganisms, including fungi, bacteria, yeasts, and algae, can decolorize and even completely mineralize many azo dyes under certain environmental conditions. The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages; the process is relatively inexpensive, the running costs are low, and the end products of complete mineralization are not toxic (Forgacs et al. 2004). Thus, biodegradation is a promising approach for the remediation of synthetic dyes wastewater because of its cost effectiveness, efficiency, and environmentally friendly nature (Verma and Madamwar 2003; Jirasripongpu et al. 2007; Shedbalkar et al. 2008; Gopinath et al. 2009). As a best alternative, much interest is now focused on biodegradation of dyes (McMullan et al. 2001; An et al. 2002). Bioremediation may be the most effective method of treating industrial dyes containing wastewater (Nozaki et al. 2008). The growing publication of research and review articles dealing with the remediation of environmental pollution caused by synthetic dyes is an indication and proof of the global concern over this issue. During the last two decades, the scientific community in the whole world and especially in India and China has been active in research on the problems caused by this source.
of environmental pollution and its effective remediation. This review is an effort to discuss the science and arts of biodecolourisation and biodegradation of synthetic azo dyes particularly by bacteria.

Biological dye removal techniques are based on microbial biotransformation of dyes. As dyes are designed to be stable and long-lasting colourants, they are usually not easily biodegraded. Destruction of azo dyes can be accomplished by reduction or by oxidation. The reduction of azo dyes generates aromatic amines (Carliell et al. 1995) (Figure 1.3). The microbial removal processes of dyes from effluents of textile and dyestuff manufacturing industry become the dominant technology with distinct the merits of environment benign and cost competitive (Forgacs et al. 2004) over the commonly used physico-chemical methods. The biological removal has proved to be economical and efficient in the treatment of wastewaters containing recalcitrant-chemicals including azo dyes depends on the adaptability and activity of the selected microorganisms. Bioremediation, or the use of microbial techniques to deal with pollution, is a key research area in the environmental sciences. In such approaches microbes acclimatize themselves to the toxic wastes and new resistant strains develop naturally, which then transform various toxic chemicals into less harmful forms. It is an alternative to physicochemical methods because it is eco-friendly, cheaper and publicly acceptable. Many researchers have demonstrated partial or complete biodegradation of dyes by pure and mixed cultures of bacteria, fungi, algae and actinomycetes in recent years that are previously considered non-degradable (Dawkar et al. 2009; Kalme et al. 2007; Saratale et al. 2009 Kalyani et al., 2009; Telke et al., 2009a,2009b; McMullan et al. 2001; Nyanhongo et al. 2002; Chagas and Durrant 2001; Acuner and Dilek 2004; Ramalho et al. 2002; Tekere et al. 2001; An et al. 2002; Chung and Stevens 1993; Bumpus 1995; Delée et al. 1998; Stolz 2001; Pearce et al. 2003; Forgacs et al. 2004).

Because of electron withdrawing nature of azo bond the decolourization and degradation of azo dyes cannot be accomplished by aerobic microorganisms while anaerobic microorganisms have shown that decolourization is possible through cleavage of azo bond, producing corresponding amines (Georgiou et
al. 2003; Méndez-Paz et al. 2005). Azo dye wastewater treatment has been studied in aerobic (Table 1.7) as well as anaerobic treatment processes.

**Figure 1.3: Reduction of an azo dye**

The first step in the biodegradation of azo dyes under anaerobic conditions is reduction that readily precedes which results in the formation of aromatic amines (Carliell et al. 1995). Anaerobic consortia generally do not degrade the aromatic amines but most of the aromatic amines are readily biodegraded under aerobic conditions (Brown and Laboureur 1983). The complete mineralization of azo dyes requires an integrated or sequential anaerobic-aerobic treatment (Field et al. 1995). Biological treatment processes are mainly based on suspended growth process, in which microorganisms are maintained in suspension within the liquid. In attached growth process, microorganisms are attached to some inert medium (known as fixed film process).

**Aerobic Treatment**

It has been reported that azo dyes strongly resist aerobic degradation (Carliell et al. 1995). The studies carried out by Pagga in early 1986 for aerobic degradation of eighty seven dyestuffs did not show any significant biodegradation under aerobic condition. Nevertheless, decolourisation of azo dyes like Reactive red 195 was carried out by *B. cereus* (Modi et al. 2010). But still efforts to isolate microbes capable of degrading dye under aerobic condition have continued.

**Anaerobic Treatment**

The potential of anaerobic microorganism to decolourise dyes is well established (Carliell et al. 1995). In many cases the decolourisation of azo dyes under anaerobic condition is a co-metabolic reaction (Stolz et al. 2001). Anaerobic treatment is a popular for removing biodegradable organic material in industrial and domestic wastewaters due to low sludge production, low cost,
high energy efficiency and process simplicity compared with other treatments. Moreover it offers a green environmental impact as it combines waste stabilization with net fuel production and allows the use of effluent as fertilizer.

**Sequential anaerobic - Aerobic Treatment**

The most promising alternatives for treatment of wastewaters are those offering complete degradation of dyes. Anaerobic bacteria are often able to reduce the azo linkages, but are generally unable to further stabilize the aromatic amines (Figure 1.4). Anaerobic conditions favour dye decolourisation by bacteria (Beydilli et al. 2000). One suggested system appears to be beneficial to follow the combined anaerobic–aerobic process. Aerobic bacteria can oxidize aromatic ring compounds to simpler molecule. An extensive quantity of research has been carried out on anaerobic- aerobic treatment systems used for degrading textile wastewaters (O’Neill et al. 2000a, 2000b).

Decolourisation could be achieved better with a longer anoxic-an aerobic period. Up to 97% decolourisation and 60% COD removal could be achieved with anaerobic treatment and following aerobic treatment can remove an additional 30% COD, which could be caused by the removal of aromatic amines (Field et al. 1995; Delée et al. 1998).

**Figure 1.4: Theoretical representation of the mineralization of an azo dye under anaerobic – aerobic condition**

Source: Sheth 2009
### Table 1.7: Overview of aerobic treatment processes (Sheth 2009)

<table>
<thead>
<tr>
<th>Process</th>
<th>Design/Counterpart</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Referenc- es</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Suspended Growth Processes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated Sludge</td>
<td>Extended aeration system, oxidation ditch, step aeration, contact stabilization, completely mixed aerated system, pure oxygen aeration</td>
<td>Removes ≤ 50-70% Suspended solids, 25-40% BOD</td>
<td>Oxygen limitation, metal toxicity, high cost, sensitive to changes in wastewater, bulking, large quantity of sludge generated</td>
<td>Bitton (1994), Reife et al. (1996)</td>
</tr>
<tr>
<td>Aerated lagoons</td>
<td>Earthen basin, Water depth 2-5 m, surface or diffused aeration</td>
<td>Removes BOD</td>
<td>Expensive in terms of land requirement, Temperature sensitive</td>
<td>Arceivala Soli (1999)</td>
</tr>
<tr>
<td>Waste stabilization ponds</td>
<td>Water depth 1-2 m</td>
<td>Simple</td>
<td>Expensive in land requirement, odour problem, mosquitoes</td>
<td>Arceivala Soli (1999)</td>
</tr>
<tr>
<td><strong>Attached Growth Processes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trickling filters</td>
<td>Cylindrical/rectangular tanks, packed with stone or synthetic material</td>
<td>Effective for highly concentrated organic waste</td>
<td>Filter clogging, Efficiency reduced in cold weather</td>
<td>Bishop and Kinner (1983)</td>
</tr>
<tr>
<td>Rotating biodisc</td>
<td>Series of discs on horizontal shaft</td>
<td>Low residence time and cost</td>
<td>Shaft and bearing failures, odour problem</td>
<td>Bishop and Kinner (1983)</td>
</tr>
<tr>
<td>Root zone reed beds</td>
<td>Phytoplankton, duckweeds, hyacinth and rooted vegetation (reeds)</td>
<td>Purify industrial waste waters</td>
<td>Generates mosquitoes</td>
<td>Arceivala Soli (1999)</td>
</tr>
<tr>
<td>Vermistabilization (composting)</td>
<td>Soils rich in bacteria and earthworms</td>
<td>Degraded organic matter</td>
<td>50-60°C required, toxic chemicals and heavy metals retained</td>
<td>Arceivala Soli (1999)</td>
</tr>
<tr>
<td>Immobilization</td>
<td>Immobilized cells/enzymes, support material</td>
<td>Can be used for treatment of different wastes</td>
<td>Loss of bead integrity due to: cell growth, gas formation, phosphate or Ca⁺ chelating agents</td>
<td>-</td>
</tr>
</tbody>
</table>

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Introduction and review of literature
Microbial decolourisation of dyes

Decolourisation of dyes may occur in two ways: either adsorption on the microbial biomass or biodegradation of the dyes by the cells (Zhou and Zimmermann 1993). Adsorption of dyes may occur on growing/living microbial cells (Fu and Viraraghavan 2001) as well as on dead microbial cells. In biodegradation, the original dye structure is destroyed, and the pollutant is split into fragments by the microbial activity, sometimes achieving complete mineralization, i.e., conversion of the xenobiotic compound into CO₂, H₂O, and some salts of inorganic origin (Ali 2010). From the very nature of the biosorption and biodegradation, biodegradation seems more potential in its operation (Ali 2010). Biosorption of dyes does not eradicate the problem because the pollutant is not destroyed but instead entrapped into the matrix of the adsorbent (the microbial biomass). The disposal of the microbial biomass containing adsorbed dyes itself is a big hurdle in their proposed role in biocleaning of colored waters (Chander and Arora 2007). Thus, biosorption may not be a practical approach for treating large volumes of dye-contaminated industrial effluents because of the problems associated with disposal of the large quantities of biomass after biosorption of dyes from industrial effluents (Kuhad et al. 2004). However, Fu and Viraraghavan (2001) stated that a biosorption mechanism might also play an important role in the decolourisation of dyes by living fungi in addition to biodegradation and the biosorption of dyes may be of interest in biorecovery of these synthetic chemicals from spent dye baths. This is possible through desorption of the adsorbed dyes using suitable solvents or solvent mixtures (Ali 2010).

Biodegradation of dyes

Biodegradation is defined as the biologically mediated breakdown of chemical compounds; it is an energy-dependent process and involves the breakdown of dye into various byproducts through the action of various enzymes (Kaushik and Malik 2009). Biodegradation of synthetic dyes not only results in decolourisation of the dyes but also in fragmentation of the dye molecules into smaller and simpler parts (breakdown products) (Ali 2010). Kaushik and Malik (2009) reported that decolourisation of the dye occurs when the chromophoric center of the dye is cleaved. Various microorganisms, including fungi, bacteria, yeasts and algae, have been used for decolourisation and
degradation of synthetic dyes. They have been shown to have different capabilities for degrading different dyes. Among the different groups of microorganisms used for biodegradation of synthetic dyes, some have specific advantages over others (Ali 2010). The effectiveness of microbial decolourisation depends on the adaptability and the activity of the selected microorganisms (Chen et al. 2003). Development of efficient dye degradation biotechnology requires application of a suitable selected strain and its use under favorable conditions to realize the degradation potential (Novotny et al. 2004b).

**Biodecolourisation and degradation of dyes by bacteria**

Kalyani et al. (2009) stated that in comparison to fungal, bacterial decolourisation is normally faster. It is well known that bacteria degrade azo dyes reductively under anaerobic conditions to colourless aromatic amines. The carcinogenicity of an azo dye may be due to the dye itself or aryl amine derivatives produced during the reductive biotransformation of an azo linkage (Dawkar et al. 2009). These colourless aromatic amines should be degraded further because these may be toxic, mutagenic, and carcinogenic to humans and animals (Chen 2006). This treatment holds promise as a method to completely remove azo dyes from wastewater (Van der Zee and Villaverde 2005). Table 1.8 presents a summary of some studies on biodegradation of dyes by bacteria which was modified from Ali (2010).

Ali (2010) stated that for a general evaluation of dye biodegradability, the dyes chemical structures should be considered rather than their application classes. Bacterial dye biotransformation studies have so far mainly been focused to the most abundant chemical class that of the azo dyes (Ganesh et al. 1994). The electron-withdrawing nature of the azo linkages generally hinders the susceptibility of azo dye molecules to oxidative reactions (Ali 2010). Therefore, azo dyes normally resist aerobic bacterial biodegradation (Ganesh et al. 1994). Under strict aerobic conditions bacteria with specialized azo dye reducing enzymes were found to degrade azo dyes. In contrast, breakdown of azo linkages by reduction under anaerobic conditions is much less specific (Ali 2010). This anaerobic reduction entails decolourisation as the azo dyes are converted to usually colourless aromatic amines and sometime azo dyes are converted to potentially harmful aromatic amines.
Aromatic amines are generally not further degraded under anaerobic conditions.

**Table 1.8: Biodegradation of synthetic dyes by bacteria**

<table>
<thead>
<tr>
<th>Name of Bacteria</th>
<th>Dye (concentration)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>Crystal violet (50 mg L⁻¹)ᵃ, Basic fuchsin, Brilliant green, Malachite green, Acid amaranth, Great red GR, Reactive red KE-3B, Reactive brilliant blue K-GR</td>
<td>Ren et al. (2006)</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>Reactive red 198 (3000 mg L⁻¹)</td>
<td>Chen et al. (2003)</td>
</tr>
<tr>
<td><em>Bacillus sp. VUS</em></td>
<td>Navy blue 2GL (50 mg L⁻¹)</td>
<td>Dawkar et al. (2009)</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Reactive dye</td>
<td>Modi et al. (2010)</td>
</tr>
<tr>
<td><em>Citrobacter sp.</em></td>
<td>Crystal violet (5 µM)ᵃ, Gentian violet, Malachite green, Brilliant green, Basic fuchsin, Methyl red, Congo red</td>
<td>An et al. (2002)</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>Reactive black 5 (1000 mg L⁻¹)</td>
<td>Wang et al. (2009)</td>
</tr>
<tr>
<td><em>Enterobacter sp.</em></td>
<td>Reactive red 195 (30 mg L⁻¹)</td>
<td>Jirasripongpun et al. (2007)</td>
</tr>
<tr>
<td><em>Serratia sp.</em></td>
<td>Reactive red 195 (30 mg L⁻¹)</td>
<td>Jirasripongpun et al. (2007)</td>
</tr>
<tr>
<td><em>Enterococcus gallinarum</em></td>
<td>Direct black 38 (20–250 mg L⁻¹)</td>
<td>Bafana et al. (2008)</td>
</tr>
<tr>
<td><em>Kocuria rosea</em></td>
<td>Malachite green (50 mg L⁻¹)</td>
<td>Parshetti et al. (2006)</td>
</tr>
<tr>
<td><em>Micrococcus glutamicus</em></td>
<td>Scarlet R (150 mg L⁻¹)</td>
<td>Saratale et al. (2009a)</td>
</tr>
<tr>
<td><em>Nocardia corallina</em></td>
<td>Crystal violet (2.3 µM)</td>
<td>Yatome et al. (1993)</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>Scarlet R (150 mg L⁻¹)</td>
<td>Saratale et al. (2009a)</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>Crystal violet (60 µM)</td>
<td>Chen et al. (2007)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa NGKCTS</em></td>
<td>Reactive Red BS C.I. 111</td>
<td>Sheth and Dave (2009)</td>
</tr>
<tr>
<td><em>Bacillus thuringiensis</em></td>
<td>Acid dye</td>
<td>Dave and Dave (2009)</td>
</tr>
<tr>
<td><em>Pseudomonas sp. SUK1</em></td>
<td>Reactive red 2 (1000 mg L⁻¹)</td>
<td>Kalyani et al. (2009)</td>
</tr>
<tr>
<td><em>Shewanella decolorationis</em></td>
<td>Crystal violet (50 mg L⁻¹)</td>
<td>Chen et al. (2008)</td>
</tr>
<tr>
<td><em>Shewanella putrefaciens</em></td>
<td>Reactive black 5 (100 mg L⁻¹)ᵃ, Direct red 81, Acid red 88, Disperse orange 3</td>
<td>Khalid et al. (2008)</td>
</tr>
<tr>
<td><em>Yersinia sp.</em></td>
<td>Reactive red 195 (30 mg L⁻¹)</td>
<td>Jirasripongpun et al. (2007)</td>
</tr>
</tbody>
</table>

ᵃ: Concentration of all dyes used in a given study is the same unless otherwise indicated

Source: Modified from Ali H. 2010
Mechanism of azo dye reduction

The first step in the bacterial azo dye reduction is the reductive cleavage of azo linkages -N=N-, i.e. the transfer of reducing equivalents resulting in the formation of aromatic amines. As aromatic amines are generally colourless, azo dye reduction is also referred to as azo dye decolourisation. This reduction may involve different mechanisms, such as enzymes, low molecular weight redox mediators, chemical reduction by biogenic reductants. The term ‘anaerobic azo dye reduction’ comprises different mechanisms (Figure 1.5) are,

A. Direct enzymatic azo dye reduction,
B. Mediated biological azo dye reduction,
C. Direct chemical azo dye decolourisation by biogenic inorganic compounds.

Figure 1.5: Mechanisms of anaerobic azo dye reduction by bacteria (Adapted from Van der Zee 2002)

When the decolourisation of food azo dyes by lactic acid bacteria isolated from the human gut was reported. It was the first study on azo dye reduction was in print as early as 1937. The formation of toxic aromatic amines in humans is a matter of concern, so research on bacterial azo dye reduction has traditionally chiefly been focused on the activity of (facultative) anaerobic bacteria from mammalian intestines (Chung et al. 1992). Later, when the removal of dyes from wastewater became a topic, bacteria from other origins were also used to investigate anaerobic azo dye reduction, e.g. pure cultures, mixed cultures, anaerobic sediments, digester sludge, anaerobic granular sludge and activated sludge under anaerobic conditions. Several review articles including many studies on bacterial azo dye reduction have been
published (McMullan et al. 2001a; Stolz 2001). The large number of azo dyes that can be reduced by so many different bacteria indicates that azo dye reduction is a non-specific reaction and that the capability of reducing azo dye can be considered as a universal property of anaerobically incubated bacteria. Bacterial biodegradation of non-azo dyes has received little attention so far (Van der Zee 2002).

**Enzymatic and microbial mediated mechanism models**

This mechanism involves enzyme-mediated transfer of reducing equivalents, generated from the oxidation of the substrate to azo dyes. A distinction can be made between direct enzymatic azo dye reduction and indirect azo dye reduction catalysed by enzymatically (re)generated redox mediating compounds. The initial step in bacterial azo dye metabolism under anaerobic conditions involves the reductive cleavage of the azo linkage. This process is catalyzed by a variety of soluble cytoplasmic enzymes with low-substrate specificity, which is known as “azoreductases” (Robinson et al. 2001; Stolz 2001). Rafii suggests an extracellular azoreductase activity in studies done with bacteria isolated from human intestine, mainly *Eubacterium* sp. and *Clostridium* sp. (Rafii et al. 1995). Under anoxic conditions, these enzymes facilitate the transfer of electrons via soluble flavins to the azo dye, which is then reduced. Russ et al. (2000) showed that the cytoplasmic “azoreductases” are presumably flavin reductases and that they have insignificant importance in the *in vivo* reduction of sulfonated azo compounds. Kudlich and co-workers had found a membrane bound “azoreductase” in the cell wall of *Sphingomonas* sp. This strain has both cytoplasmic and membrane-bound azoreductase activities. Since it is highly doubtful that highly charged or polymeric azo dyes can pass through the bacterial cell wall, the chance of non-cytoplasmic azoreductases is then highlighted. The unspecific reduction of azo dyes proposed different model based on studies with *Sphingomonas xenophaga* BN6 bacteria. They have seen an increase in the reduction rate when quinones, like anthraquinone-2-sulfonate or 2-hydroxy-1, 4-naphtoquinone, were added to the culture medium. It was suggested that quinones added in to the medium or some decomposition products released by the cells in to the medium, acted as redox mediators, which were enzymatically reduced by the bacterial cells and that the hydroquinones
formed reduced the azo dye in a purely chemical redox reaction. However, McMullan et al. (2001) had established that the role that such cytoplasmic enzyme in vivo is uncertain.

The isolation of bacteria capable of aerobic decolourisation and mineralization of dyes, specially sulfonated azo dyes, has proven difficult (McMullan et al. 2001) with the notable exception of actinomycetes. For aerobic bacteria to be significant in the reductive process they must be specifically adapted (Pearce et al. 2003). Using this methodology, and testing some analogues of azo dyes as sole source of carbon and energy, several groups manage to isolate and purify “azoreductases” from Pseudomonas strains KF46 and K24 (Zimmermann et al. 1984) and from Xenophilus azovorans KF46F (Blümel et al. 2002). The ability of bacteria to aerobically metabolize other dye classes was described for Kurthia sp., Pseudomonas mendocina MCM B-402 (Sarnaik and Kanekar 1999) and B. cereus (Modi et al. 2010).

**Azo dye decolourisation by biogenic inorganic compounds**

Azo dye decolourisation can occur from purely chemical reactions with inorganic compounds such as sulfide and ferrous ion that are formed as end products of metabolic reactions under anaerobic conditions. Under anaerobic conditions the extracellular reduction of azo compounds is the action of reduced inorganic compounds (e.g. Fe\(^{2+}\), H\(_2\)S), that are formed as end products of certain strictly anaerobic metabolic reactions (Stolz 2001; Van der Zee et al. 2001; Yoo 2002; Van der Zee et al. 2003). Several studies proposed a combined anaerobic-aerobic system for the removal of dyes from wastewaters with a consortium/sludge, as further mineralization of the formed amines is not possible under anaerobic conditions (Supaka et al. 2004; Isik and Sponza 2004). The use of consortia presents significant returns more than the use of pure cultures in the degradation of synthetic dyes. Because, the individual strains may attack the dye molecule at altered site or may utilize the breakdown products formed by another strain for further disintegration. The activated sludge system used as consortium mainly constituted by bacteria, but also with the normal incidence of fungi and protozoa. Nevertheless, the composition of mixed cultures may change during the decomposition process interfering with the control of the system. This thesis...
Chapter 1

will focus on the current problem created by textile effluents and azo dyes. More specifically, azo dyes will be explored and effective techniques for degrading these types of compounds will be described.

Research objectives

The aims of the present investigation were to develop cost-effective, eco-friendly process for decolourisation and degradation of acid dye and dye containing wastewater from commercially available acid azo dyes, synthetic wastewater containing mixture of dyes and actual wastewater using bacteria. Moreover, improvement in decolourisation kinetics to reduce the process time with an application of process scale up from shake flask to 5L capacity indigenously designed bioreactor. Thus, present research was carried out with the following objectives:

1. Screening and isolation of bacteria capable of decolourising and degrading acid azo dyes.
2. Identification of selected isolates by Biochemical tests, Biolog® and 16S rRNA gene analysis.
3. Biodecolourisation kinetic studies of different dyes using pure cultures.
4. Amenability testing of pure cultures and consortiums for different dyes and mixture of different dyes with factorial experiments.
5. Optimization of various physico-chemical parameters for maximum biodecolourisation and biodegradation at shake flask level.
6. Development of culture, which can tolerate high salt and metals concentrations.
7. Ability to decolorize different dyes from different groups.
8. Improved culture for the treatment of synthetic and actual waste of textile industry.
10. Enzyme characterization and molecular mass determination.
11. Elucidation of proposed pathway for degradation of selected dye.
12. Laboratory scale cost effective and simplified bioreactor process development to remove dye from synthetic and actual waste.
14. To study the influence of synthetic and actual waste on selected consortium and optimization of physico-chemical parameters.
15. Analytical methods to confirm degradation.
16. Evaluate the performance of the microorganisms in the reactor, in terms of decolourisation, degradation, COD reduction and detoxification.
17. Phytotoxicity and Microbial toxicity.