1

GENERAL INTRODUCTION
1.1 Dyes: History and Basics

In 1856 William Henry Perkins accidentally discovered Mauve (Fig. 1.1), the world’s first commercially successful synthetic dye and marked the start of synthetic dye industry. In last 160 years, several million different coloured compounds have been synthesized and approximately 15,000 produced on an industrial scale.

![Chemical structure of Mauve](image)

Fig. 1.1 Chemical structure of Mauve

Man has used natural colorant since prehistoric times as reflected by the cave drawings in Europe, Africa, Egypt and China. This clearly shows that colors had and still have profound anthropological, psychological, esthetic, functional and economic impact on society (Zollinger 2003).

A dye can generally be described as a colored substance that has an affinity to the substrate to which it is being applied (Murugesan & Kalaichelvan 2003). The dye is generally applied in an aqueous solution, and may require a mordant to improve the fastness of the dye on the fiber. In contrast with a dye, a pigment generally is insoluble, and has no affinity for the substrate.

1.2 Classification of dyes

The vast array of commercial colorants is classified in the Colour Index (C.I.) which 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 (3rd Edition, issue 2) lists about 28,000 commercial
General Introduction

dye names, representing approximately 10,500 different dyes, 45,000 of which are currently produced. Each different dye is given a C.I. generic name determined by its application characteristics and its colour. Dyes may be classified according to chemical structure or by their usage or application method. The former approach is adopted by practicing dye chemist, who use term such as azo dyes, anthraquinone dyes and phthalocyanine dyes. The latter approach is used predominantly by the dye user. Classification by usage or application is the principal system adopted by the Colour Index. Dyes are now classified according to how they are used in the dyeing process (Hunger 2003).

1.2.1 Acid dyes

Acid dyes are water-soluble anionic dyes that are applied to fibers such as silk, wool, nylon and modified acrylic fibers using neutral to acid dyebaths. Attachment to the fiber is attributed, at least partly, to salt formation between anionic groups in the dyes and cationic groups in the fiber. They bind to the cationic NH\textsuperscript{+} -ions of those fibres. The adjective 'acid' refers to the pH in acid dye dyebaths rather than to the presence of acid groups (sulphonate, carboxyl) in the molecular structure of these dyes. Most synthetic food colors fall in this category.

1.2.2 Basic dyes

Basic dyes are water-soluble cationic dyes that are mainly applied to acrylic fibers, but find some use for wool and silk. Usually acetic acid is added to the dyebath to help the uptake of the dye onto the fiber. Basic dyes are also used in the coloration of paper.

1.2.3 Direct or Substantive dyes

Direct or substantive dyeing is normally carried out in a neutral or slightly alkaline dyebath, at or near boiling point, with the addition of either sodium chloride (NaCl) or sodium sulfate (Na\textsubscript{2}SO\textsubscript{4}). Direct dyes are relatively large molecules with high affinity for especially cellulose fibres. Van der Waals forces make them bind to the fibre. Direct dyes are mostly azo dyes with more than one
azo bond or phthalocyanine, stilbene or oxazine compounds. Direct dyes are used on cotton, paper, leather, wool, silk and nylon. They are also used as pH indicators and as biological stains.

1.2.4 Mordant dyes

Mordant dyes require a mordant, which improves the fastness of the dye against water, light and perspiration. The choice of mordant is very important as different mordants can change the final color significantly. Most natural dyes are mordant dyes and there is therefore a large literature base describing dyeing techniques. The most important mordant dyes are the synthetic mordant dyes, or chrome dyes, used for wool; these comprise some 30% of dyes used for wool, and are especially useful for black and navy shades. The mordant, potassium dichromate, is applied as an after-treatment. It is important to note that many mordants, particularly those in the heavy metal category, can be hazardous to health and extreme care must be taken in using them.

1.2.5 Vat dyes

Vat dyes are essentially insoluble in water and incapable of dyeing fibres directly. However, reduction in alkaline liquor produces the water soluble alkali metal salt of the dye, which, in this leuco form, has an affinity for the textile fibre. Subsequent oxidation reforms the original insoluble dye. The color of denim is due to indigo the original vat dye.

1.2.6 Reactive dyes

Reactive dyes utilize a chromophore attached to a substituent that is capable of directly reacting with the fibre substrate. Reactive dyes are dyes with reactive groups that form covalent bonds with OH-, NH-, or SH-groups in fibres (cotton, wool, silk, nylon). The reactive group is often a heterocyclic aromatic ring substituted with chloride or fluoride, e.g. dichlorotriazine. Another common reactive group is vinyl sulphone. During dyeing with reactive dyes (Fig.1.2), hydrolysis (i.e. inactivation) of the reactive groups is an undesired side reaction that lowers the degree of fixation. It is estimated that 10 to 50% will not react with
the fabric and remain hydrolyzed in the water phase. The problem of coloured effluents is therefore mainly identified with the use of reactive dyes. Most (~80%) reactive dyes are azo or metal complex azo compounds but also anthraquinone and phthalocyanine reactive dyes are applied.

![Reaction of triazyl dye](image)

**Fig. 1.2 Principle of cotton dying with triazyl reactive dye**

### 1.2.7 Disperse dyes

Disperse dyes were originally developed for the dyeing of cellulose acetate, and are water insoluble. The dyes are finely ground in the presence of a dispersing agent and sold as a paste, or spray-dried and sold as a powder. Their main use is to dye polyester but they can also be used to dye nylon, cellulose triacetate, and acrylic fibres. In some cases, a dyeing temperature of 130°C is required, and a pressurized dyebath is used. The very fine particle size gives a large surface area that aids dissolution to allow uptake by the fibre. The dyeing rate can be significantly influenced by the choice of dispersing agent used during the grinding.

### 1.2.8 Sulphur dyes

Sulfur dyes are two part developed dyes used to dye cotton with dark colors. The initial bath imparts a yellow or pale chartreuse color. This is after treated with a sulfur compound in place to produce the dark black we are familiar with in socks for instance. Sulfur Black 1 is the largest selling dye by volume.

### 1.2.9 Azoic dyes

Azoic dyeing is a technique in which an insoluble azo dye is produced directly onto or within the fibre. This is achieved by treating a fibre with both diazoic and coupling components. With suitable adjustment of dyebath conditions
the two components react to produce the required insoluble azo dye. This technique of dyeing is unique, in that the final color is controlled by the choice of the diazoic and coupling components.

1.3 Azo dyes

Azo dyes are the largest group of synthetic dyes and pigments with industrial application due to their relatively simple synthesis and almost unlimited number and types of substituent. Azo dyes contain at least one -N=N- double bond and many different structures are possible. Monoazo dyes have only one -N=N- double bond, while diazo, triazo and polyazo dyes contain two, three or more -N=N- double bonds, respectively. The azo groups are generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocyclic or enolizable aliphatic groups (Zollinger 2003). Azo colorants range in shade from greenish yellow to orange, red, violet and brown. The colors depend largely on the chemical structure, whereas different shades rather depend on physical properties.

1.3.1 Diazotisation

Synthesis of most azo dyes involves diazotization of a primary aromatic amine to give a diazonium salt. The diazonium compound is then coupled with one or more nucleophiles.

Amino- and hydroxyl- groups are commonly used coupling components. The coupling reaction is generally in para position in respect to the amino- or hydroxyl- groups. The fundament of the production of azo dyes was laid in 1858 when P. Gries discovered the reaction mechanism, diazotization, for the production of azo compounds (Zollinger 2003). The general scheme of azo dye synthesis may be divided into two stages (Fig.1.3):
Stage 1 - Diazotisation
This involves a primary aromatic amine, called the diazo component. It is treated in low temperature, acid conditions with sodium nitrite to form an unstable diazonium salt.

Stage 2 - Azo coupling
The diazonium salt is reacted with a coupling component (for example a phenol or an aromatic amine). This forms the stable azo dye.

1.3.2 Chemical structure and colour correlation

Advances in structural theory led to investigations of correlations between chemical constitution and colour. In 1868 German chemists Carl Graebe and Carl Liebermann recognized that dyes contain sequences of conjugated double bonds: \( X=C-C=C-C=C-\ldots \), where \( X \) is carbon, oxygen, or nitrogen. In 1876, German chemist Otto Witt proposed that dye molecules contain two groups; the chromophore and the auxochrome. The chromophore is a group of atoms which control the colour of the dye. At that time, Witt suggested that the auxochrome was a salt-forming group, which helped to improve the colour of the dye. His theory was later modified when it was discovered that the chromophore is usually electron-withdrawing, and auxochromes are normally electron-donating. The two groups are connected by a conjugated system. The chromophore group must be either transition metal complexes or conjugated/delocalized electron systems, whereas auxochromes are acidic (-COOH, -OH, -SO_3H) and basic(-NHR, -NR_2, -NH_2) groups(Fig 1.4).
1.4 World dyes production and Market Scenario

It is estimated that almost $10^9$ kg of dyes produced annually in the world. A market research group Freedonia (2009) in its report estimated that “Global demand for dyes and organic pigments is forecast to grow 3.9 percent annually through 2013. Volume demand will grow 3.5 percent annually. Textiles will remain the largest market, while faster growth will occur in other segments such as printing inks, paint and coatings, and plastics. The Asia/Pacific region will lead gains and increase its market share to one-half of world demand by 2013. China is by far the largest single consumer in the world and the fastest growing national market. India will also post rapid increases, but demand levels will remain well
General Introduction

below those of China. Estimates on the worldwide production volume of different classes of colorants vary greatly (Table 1.1).

Table 1.1 Global Market Share of Different Classes of Dyes (Zollinger 2003).

<table>
<thead>
<tr>
<th>Class of Dye</th>
<th>Market Share (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>23.6</td>
</tr>
<tr>
<td>Acid</td>
<td>18.6</td>
</tr>
<tr>
<td>Disperse</td>
<td>16.2</td>
</tr>
<tr>
<td>Direct</td>
<td>7.7</td>
</tr>
<tr>
<td>Basic</td>
<td>7.1</td>
</tr>
<tr>
<td>Vat, Sulfur, Mordant, azoic dyes and Indigo</td>
<td>16.8</td>
</tr>
</tbody>
</table>

India is among one of the major producers of dyes and dyes intermediate from Asian region and meets the requirement of the world at large. There are about 900 dyes and dyes intermediate producing industries in India. Most of them are in small-scale unorganized industrial sector, and about five thousand dyes and dyes intermediate are in commercial production (Verma 2008).

At many places in India the concept of effluent treatment, by means, of a collective effort, has assumed seriousness by being especially purposeful for cluster of small scale industrial units. Common Effluent Treatment Plant (CETP) not only help the industries in easier control of pollution, but also act as a step towards cleaner environment and service to the society at large. Wastewater of individual industries often contain significant concentration of pollutants and to reduce them by individual treatment up to the desired concentration, become techno-economically difficult. The combined treatment provides a better and economical option because of the equalization and neutralization taking place in the CETP.

1.5 Dyes Discharge

It is estimated that ca. 15% of the total production of colorants is lost during synthesis and processing (Zollinger 2003). During textile processing, inefficiencies in dying result in large amounts of dyestuff being lost to the wastewater. The amount of dye lost is dependent upon the dye application class (Table 1.2).
Particularly Reactive dyes are most inefficient with respect to fixation on fibre (O’Neill et al. 1999; McMullan et al. 2001) and also they are produced highest among all dyes. Hence dye pollution problem is severely associated with reactive acid dyes.

<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>Vat</td>
<td>Cellulose</td>
<td>80-95</td>
<td>5-20</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>
</tbody>
</table>

1.6 Water Pollution

The first contaminant observed in water is the colour (Banat et al. 1996). Many dyes are visible in water at concentrations as low as 1 ppm. The dye manufacturing and dying industry waste water typically contain dye concentration in the range 10-200 ppm (O’Neill et al. 1999). Textile industries consume large volumes of water and chemicals for wet processing of textiles. The chemical reagents used are very diverse in chemical composition ranging from inorganic compounds to polymers and organic products (Zille 2005). Textile dye effluents are complex, containing a wide variety of dyes, natural impurities extracted from the fibers and other products such as dispersants, levelling agents, acids, alkalis, salts and sometimes heavy metals (Laing 1991). In general, the effluent is highly colored with high biological oxygen demand (BOD) and chemical oxygen demand (COD), it has a high conductivity and is alkaline in nature. The presence of very low concentration of dyes in effluent is highly visible and undesirable (Nigam et al. 2000).
1.6.1 Recalcitrant nature of dyes

Due to complex chemical structure, dyes are resistant to fading on exposure to light, water and many chemicals and are highly persistent in natural environment. Many dyes are difficult to decolorize due to their complex structure and synthetic origin. Without adequate treatment these dyes remain stable in the natural environment, for instance half life of hydrolyzed Reactive Blue 19 is about 46 years at pH 7 and 25°C (Hao et al. 2000).

The convectional wastewater treatment, which rely on aerobic biodegradation have low removal efficiency for reactive and other anionic soluble dyes. Due to low biodegradation of dyes, a convectional biological treatment process is not very effective in treating a dyes wastewater. Furthermore, dyes may be inhibitory to the microbial population vital for the functioning ETP and WWTP and may lead to decrease in performance of such treatment plants (Ogawa et al. 1988).

1.6.2 Toxicity aspect of dyes and intermediates

The azo linkage is considered the most labile portion of an azo dye. Degradation of azo dyes can be obtained by reduction or by oxidation. The azo group is a electrophile (electron withdrawing), hence it is more susceptible to the reductive catabolism compare to the oxidative and remain persistent under aerobic conditions (Kanckmus 1996). The reduction releases azo dyes to the colorless component amines. Awareness of pollution potential of textile dyes has been primarily driven by concern over their possible toxicity and carcinogenicity of dyes and their intermediate. This is due to the fact that many dyes are made from known carcinogens, such as benzidine and other aromatic compounds (Clarke & Anliker 1980). Due to the toxicity, mutagenicity and carcinogenicity of azo dyes and their breakdown products, their removal from industrial wastewaters has been an urgent challenge. An analysis of several hundred commercial textile dyes samples revealed that nearly 10 percent were mutagenic in the Ames test. Another study conducted on 45 combined effluents from textile finishing plants showed that 27 percent of the wastewater samples were mutagenic in the Ames test (McCarthy 1997). Most dyes that have been shown to be carcinogenic are no longer used
however, a complete study of all dyestuffs is impossible. Concerns are increasing over the impurities within commercial dye products and the additives used during the dyeing process. Brown and DeVito (1993) predicted possible biological mechanism thought to be responsible for carcinogenic activation of azo dye compounds. Three different mechanisms for azo dye carcinogenicity were identified, all involving metabolic activation to reactive electrophilic intermediates that covalently bind DNA. In the order of decreasing number of published references, these mechanisms are

1. Azo dyes those are toxic only after reduction and cleavage of the azo linkage to give aromatic amines, mostly via intestinal anaerobic bacteria. The aromatic amines are metabolically oxidized to reactive electrophilic species that covalently bind DNA.

2. Azo dyes with structures containing free aromatic amine groups that can be metabolically oxidized without azo reduction.

3. Azo dyes that may be activated via direct oxidation of the azo linkage to highly reactive electrophilic diazonium salts.

Azo toxicity probably cause by more than one mechanisms and it is difficult to develop azo that azo dyes which can not be degraded in to aromatic amines but it is possible to select aromatic amines that are not toxic or less toxic. The release of textile and dye house effluent may cause abnormal coloration of surface waters that captures the attention of both public and the authorities. The untreated effluent bearing dyes when released in to the natural water bodies serious ecological threats to the aquatic flora and fauna. In addition to this sunlight penetration in such a water bodies is hampered leading to the disturbances in the ecological balance. Over 90% of some 4000 dyes tested in an ETAD (Ecological and Toxicological Association of dyestuffs Manufacturing Industry) survey rates of toxicity found amongst basic and diazo direct dyes (Shore 1996) had LD$_{50}$ values greater than 2 x $10^3$ mg/kg. The highest Fish mortality tests showed that 2% out of 3000 commercial dyestuffs tested had LC$_{50}$ values below 1 mg/l. 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 & Anliker 1980). Mortality tests with rats showed that only 1% out of 4461 commercial dyestuffs tested had LD$_{50}$ values below 250 mg/kg body weight 65. Therefore, the
chance of human mortality due to acute dyestuff toxicity is probably very low. However, acute sensitisation reactions by humans to dyestuffs often occurs. Especially some disperse dyestuffs have been found to cause allergic reactions, i.e. eczema or contact dermatitis. Gonzales et al. (1988) stated that workers in the textile industry have a two-fold increased risk of contracting bladder cancer compared to workers in other occupations, for instance aviation, agriculture and construction. The increased risk of contracting colonic and rectal cancers was also noted. However, these cancers related mostly to the synthetic fibre industry.

1.7 Treatment of colored effluent

The world has become more cautious about the environment and also due to ever increasing stringent laws, the textile industry around the world has began using innovate-ve methods for wastewater remediation. Up to now there is no single and economically attractive treatment that can effectively decolorize dyes (Keharia and Madamwar 2003). Therefore dye removal strategies mostly involve combination of different technique. The main methods of dye removal are non-biological i.e. physical and chemical technique or physicochemical (Table 1.3) and alternative research intensive attractive biological method continuously gaining attention.

The majority of physical, chemical and biological color removal techniques work either by concentrating the color into sludge, solid supports, or by the complete destruction of the dye molecule. It is expected that decoloration systems involving destruction technologies will prevail, as the transfer of pollution from one part of the environment to another is prevented (Vandevivere et al. 1998, Hao et al. 2000, Robinson et al. 2001). Physical treatments include adsorption (using activated carbon, peat, wood chips, fly ash and coal mixture, silica gel, etc.), Membrane filtration, Ion exchange, Irradiation and electrokinetic coagulation. Chemical treatment includes oxidative process like use of Fenton reagent (H₂O₂-FeII), Ozone, Sodium hypochlorite (NaOCl), Cucurbituril, photochemical and electrochemical destruction. Table 1.3 refers advantages and disadvantages of these methods.
Biological treatments differ according to the presence or absence of oxygen. In the former case the process is called aerobic (revival of biological sludge in aeration basins) and in the latter anaerobic treatment (decay and rot in stabilizing lagoons). A third mean of biological treatment known i.e. degradation by means of special fungi. Since biological treatment simulates degradation processes that occur in the environment, it is also called biodegradation.

### Table 1.3 Advantages and disadvantages of the current methods of dye removal from industrial effluents (adapted from Robinson et al 2001)

<table>
<thead>
<tr>
<th>Physical/chemical methods</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenton reagents</td>
<td>Effective decolourization of both soluble and insoluble dyes</td>
<td>Sludge generation</td>
</tr>
<tr>
<td>Ozonation</td>
<td>Applied in gaseous state: no alteration of volume</td>
<td>Short-life (20min)</td>
</tr>
<tr>
<td>Photochemical</td>
<td>No sludge production</td>
<td>Formation of by products</td>
</tr>
<tr>
<td>NaOCl</td>
<td>Initiate and accelerate azo bond cleavage</td>
<td>Release of aromatic amines</td>
</tr>
<tr>
<td>Cucurbituril</td>
<td>Good sorption capacity for various dyes</td>
<td>High cost</td>
</tr>
<tr>
<td>Electrochemical destruction</td>
<td>Break down compound are non-hazardous</td>
<td>High cost of electricity</td>
</tr>
<tr>
<td>Activated carbon</td>
<td>Good removal of wide variety of dyes</td>
<td>Very expensive</td>
</tr>
<tr>
<td>Peat</td>
<td>Good adsorbent due to cellular structures</td>
<td>Specific surface areas for adsorption are lower than activated carbon</td>
</tr>
<tr>
<td>Wood chips</td>
<td>Good sorption capacity for acid dyes</td>
<td>Requires long retention times</td>
</tr>
<tr>
<td>Silica gel</td>
<td>Effective for basic dye removal</td>
<td>Side reactions prevent commercial application</td>
</tr>
<tr>
<td>Membrane filtration</td>
<td>Removes all dye type</td>
<td>Concentrated sludge production</td>
</tr>
<tr>
<td>Ion exchange</td>
<td>Regeneration: no adsorbent loss</td>
<td>Not effective for all dyes</td>
</tr>
<tr>
<td>Irradiation</td>
<td>Effective oxidation at lab scale</td>
<td>Require a lot of dissolve O2</td>
</tr>
<tr>
<td>Electrokinetic coagulation</td>
<td>Economically feasible</td>
<td>High sludge production</td>
</tr>
</tbody>
</table>
1.7.1 Physical Method of dye removal

Following section discusses various dye removal techniques used based on physical phenomenon.

i) Adsorption

Carbon adsorption is an effective method for lowering the concentration of dissolved organics in effluent. This process provides an attractive alternative for the treatment of contaminated waters, especially if the sorbent is inexpensive and does not require an additional pre-treatment step before its application. Adsorption is a well known equilibrium separation process and an effective method for water decontamination applications (Dabrowski 2001). Adsorption has been found to be superior to other techniques for water reuse in terms of initial cost, flexibility and simplicity of design, ease of operation and insensitivity to toxic pollutants. Decolourisation is a result of two mechanisms: adsorption and ion exchange (Slokar and Le Marechal 1998), and is influenced by many physio-chemical factors, such as, dye/sorbent interaction, sorbent surface area, particle size, temperature, pH, and contact time (Kumar et al. 1998) Adsorbance of a compound is enhanced by increasing molecular size and number of aromatic rings and by decreasing solubility, polarity and carbon chain branching. Several pilot and commercial systems using activated carbon columns have been developed to decolorize dye effluents (Reife and Freedman 1996). However, in general carbon adsorption of dyes is not very efficient or economical. This is mainly due to poor adsorption of dyes, polar molecules, to carbon, a nonpolar compound. Carbon adsorption is most effective as a finishing step following chemical reduction, which generates by-products with better carbon adsorbability than the parent dyes (Reife and Freedman 1996). Decolourisation is a result of two mechanisms: adsorption and ion exchange (Slokar and Le Marechal 1997). Adsorption also does not result in the formation of harmful substance. Various sorbent reported for their dye removal capabilities like activated carbon (Nasser and El-Geundi 1991; Raghavacharya 1997; Rao et al. 1994), Peat( Poots and McKay 1976), wood chips (Nigam et al.
2000; Poots and McKay 1976), fly ash and coal mixture(Gupta et al. 1990), silica
gel(Robinson et al. 2001) are discussed in literature.

**ii) Membrane filtration**

Ultrafiltration is the most studied membrane filtration process for the
treatment of colored wastewaters (Elliott 1996). This pressure-driven process
separates macromolecules such as dyes from the solvent, by "filtering" the solution
through the membrane. It has some special features unrivalled by other methods;
resistance to temperature, adverse chemical environment, and microbial attack. The
concentrated residue left after separation poses disposal problems, and high capital
cost and the possibility of clogging, and membrane replacements are its
disadvantages. Concentrate consists of intact, reusable dye molecules (Buckley
1992; Crossley 1995) and the water permeate for reuse or discharge. Studies
indicate that the dyes may be concentrated to 0.5 to 1% of the initial volume in the
concentrate to obtain almost colorless water in the permeate (Elliott 1996). Membrane
based processes are used to minimize wastewater volume and recycle
process waters within the mill rather than as an end-of-pipe treatment. Current
economics of membrane technology to treat textile wastewater are high capital
costs hence unfavorable.

**iii) Ion Exchange**

This method is not wide acceptance for the treatment of dye containing
effluents, mainly due to the opinion that ion exchangers can not accommodate a
wide range of dyes (Slokar and Le Marechal, 1997). Wastewater is passed over the
ion exchange resin until the available exchange sites are saturated. Both cation and
anion dyes can be removed from dye containing effluent this way. Advantages of
this method include no loss of adsorbent on regeneration, reclamation of solvent
after use and the removal of soluble dyes. A major disadvantage is cost. Organic
solvents are expensive, and the ion exchange method is not very effective for
disperse dyes (Mishra and Tripathy 1993).
iv) **Irradiation**

A promising alternative to a complete oxidation of biorefractory wastewater is the application of electron beam radiation as pre-treatment for biological oxidation to convert initially biorefractory compounds to more easily biodegradable intermediates. Electron beam radiation technology has been used to enhance the biodegradability of wastewaters containing various biologically refractory organic compounds such as landfill leachate, paper mill wastewater, effluent from a petroleum production, methyl tert butyl ether (MTBE) and 4-chlorophenol (Auslender et al. 2002; Bae et al. 1999; Cooper et al. 2002; Duarte et al. 2004; Yao et al. 2005). Yamazaki et al (1983) proposed a combined system of a $^{60}$Co γ-ray radiation and an activated sludge process for leachate treatment. Application of an integrated system of electron beam radiation and a biological treatment to textile wastewater can be a powerful process. The electron beam radiation can transform refractory organic compounds into easily biodegradable products, thus improving the efficiency and reducing the cost of a further biological treatment step (Kim et al. 2007).

iv) **Electrokinetic coagulation**

One of alternative way that has been used to treat dye wastewater is by using system that used electric energy (Daneshvar et.al 2004). Example of method that used electric energy is electrocoagulation, electrolysis and many more. This system most commonly consists of an electrochemical cell containing a number of steel electrodes separated by gaps. Wastewater flows through these gaps and contacts the steel electrodes. This is a relatively new technique, which was developed in the mid-1990s. It has some significant advantages for use as an effective method for dye removal. There is little or no consumption of chemicals and no sludge build up. The breakdown metabolites are generally not hazardous leaving it safe for treated wastewaters to be released back into waterways. It shows efficient and economical removal of dyes and a high efficiency for Colour removal and degradation of recalcitrant pollutants (Ogutveren and Kaparal 1994; Pelegrini
et al. 1999). Relatively high flow rates cause a direct decrease in dye removal, and the cost of electricity used is comparable to the price of chemicals.

1.7.2 Chemical methods

Water-soluble dyes containing azo or other oxidizable groups can be chemically oxidized by agents in to decolorized products. These chemical techniques are often expensive, and although the dyes are removed, accumulation of concentrated sludge creates a disposal problem. There is also the possibility that a secondary pollution problem will rise because of excessive chemical use. Recently, other emerging techniques, known as advanced oxidation processes, which are based on the generation of very powerful oxidizing agents such as hydroxyl radicals, have been applied with success for the pollutant degradation. Although these methods are efficient for the treatment of waters contaminated with pollutants, they are very costly and commercially unattractive. The high electrical energy demand and the consumption of chemical reagents are common problems.

i) Fenton's reagent

It is a solution of hydrogen peroxide and an iron catalyst that is used to oxidize contaminants or waste waters. It was developed in the 1890s by Henry John Horstman Fenton. Ferrous Iron (II) is oxidized by hydrogen peroxide to ferric iron(III), a hydroxyl radical and a hydroxyl anion. Iron(III) is then reduced back to iron(II), a peroxide radical and a proton by the same hydrogen peroxide (disproportionation).

(1) \( \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\cdot \)

(2) \( \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{OOH}^- + \text{H}^+ \)

Although hydroxyl radicals are powerful species, only Fenton reagent could not reach deep mineralization of organic dyes (Ma et al. 2006; Rehman et al. 2009). Fenton reaction is powerful at initial stage and gradually loses its capability for pollutants degradation with reaction time (Benitez et al. 1999). Such deactivation is
due to the interaction of Fe (III) with degradation intermediates, which is unfavorable to either Fe (III)/Fe(II) recycling (Fernandez et al 2000) or to hydroxyl free radicals generation (Sun and Pignatello 1993). It is well known that Fenton reaction could be accelerated by UV light irradiation, due to photolysis of ferric species, which enhances the regeneration of Fe$^{2+}$ with concomitant production of OH. Thus, it is worthwhile to see how these textile dyes could be degraded in aqueous medium under xenon beam irradiation in the presence of Fenton reagent. Accordingly, our attempt was to use the xenon beam (wavelength $\lambda \leq 320$ nm and intensity $1501 \sim 1532$ lux) with Fenton's reagent to mineralize textile dyes. Fenton oxidation is limited to the fact the textile process wastewaters usually have high pH, while the Fenton process requires low pH. At higher pH, large volumes of waste sludge are generated by the precipitation of ferric iron salts and the process loses its effectiveness (Van der Zee 2002). The usual low efficiency of both colour and COD removals of conventional chemical oxidation techniques have been overcome by the development of advanced oxidation processes (AOP). In this process, oxidizing agents such as O$_3$ and H$_2$O$_2$ are used with catalysts (Fe, Mn and TiO$_2$), either in the presence or absence of an irradiation source (Anjaneyulu et al. 2005). Consequently, an improvement in the generation and use of the free hydroxyl radical (HO') is obtained, which may represent a rate increase of One to several orders of magnitude compared with normal oxidants in the absence of a catalyst (Ince and Tezcanli 1999). Advanced oxidation process (AOP) refers to a set of chemical treatment procedures designed to remove organic and inorganic materials in waste water by oxidation. Contaminants are oxidized by four different reagents: ozone, hydrogen peroxide, oxygen, and air, in precise, pre-programmed dosages, sequences, and combinations. These procedures may also be combined with UV irradiation and specific catalysts. This results in the development of hydroxyl radicals. A well known example of AOP is the use of Fenton's reagent. Advanced oxidation can be defined as oxidation by compounds with an oxidation potential ($E_0$) higher than that of oxygen (1.23 V), i.e. hydrogen peroxide ($E_0 = 1.78$ V), ozone ($E_0 = 2.07$ V) and the hydroxyl radical ($E_0 = 2.28$ V). Hydrogen peroxide alone is not powerful enough (Solozhenko et al. 1995). The four AOPs
that have been most widely studied are ozonation, UV/H₂O₂, Fenton reagent (Fe²⁺/H₂O₂) and UV/TiO₂ (Alpin and Wait 2000).

**ii) Ozonation**

Ozone, one of the strongest oxidants, decolorizes dyes by attacking the most reactive site in the molecule. This generates colorless byproducts, which can be removed physically by flocculation or coagulation or further degraded biologically. Ozone destroys the conjugated chains of the dye molecules to impart color. The decoloration of azo-dyes is fast and thus, the kinetic constants for a second order reaction obtained by different methods are 10⁵-10⁷ L/mol (Ledakowicz et al. 2001). The oxidation potential of ozone (2.07 V) is 1.52 times higher than that of chlorine which allows degrading most organic compounds. The oxidizing ability of ozone is derived from the third, or nascent, oxygen atom. Ozone and hydroxyl radicals (OH⁻) generated in the aqueous solution are able to open the aromatic rings. The use of ozone in textile effluent treatment appears as a very attractive alternative within considerable application potential. Ozone is a powerful oxidising agent (Eₒ=2.08V) and can react with several classes of compounds through direct or indirect reaction. A major limitation of the ozonation process is the relatively high cost of ozone generation process coupled with its very short half-life (Gogate and Pandit 2004a).

**iii) Photochemical**

These processes result in complete mineralization with operation at mild conditions of temperature and pressure. This method degrades dye molecules to CO₂ and H₂O (Yang et al. 1998; Peralto-Zamora et al.1999) by UV treatment in the presence of H₂O₂. Degradation is caused by the production of high concentrations of hydroxyl radicals. UV light may be used to activate chemicals, such as H₂O₂, and the rate of dye removal is influenced by the intensity of the UV radiation, pH, dye structure and the dye bath composition (Slokar and Le Marechal 1997). The photo-activated chemical reactions are characterized by a free radical mechanism initiated by the interaction of photons of a proper energy level with the molecules.
of chemical species present in the solution, with or without the presence of the catalyst. The radicals can be easily produced using UV radiation. UV light has been tested in combination with H$_2$O$_2$, TiO$_2$, Fenton reagents, O$_3$ and other solid catalysts such as for the decolorization of dye solutions (Hao et al. 2000, Gogate and Pandit 2004b).

**iv) Sodium hypochlorite**

Chemical oxidation of coloured wastewaters is also possible with Cl compounds. Electrophilic attack at the amino group by Cl$^+$ initiates and accelerates the subsequent azo bridge cleavage. Namboodri et al. (1994) reported the satisfactory decolorization of acid and direct dyes. Treatment of reactive dyes required longer times, while solutions of metal-complex dyes remained partially coloured. Disperse dyes do not decolourise with NaOCl. Decolorization rate increases with increasing chlorine concentration and decreasing pH of medium. According to Omura (1994) dyes containing amino or substituted amino groups on the naphthalene ring, i.e. dyes derived from aminonaphthol and naphthylamine sulphonic acids) are most susceptible for chlorine decoloration.

**iv) Cucurbituril**

A cucurbituril is a macrocyclic molecule consisting of several glycoluril [=C$_4$H$_2$N$_4$O$_2$=] repeat units, each joined to the next one by two methylene [-CH$_2$-] bridges to form a closed band. The oxygen atoms are located along the edges of the band and are tilted inwards, forming a partly enclosed cavity. The name is derived from the resemblance of this molecule with a pumpkin of the family of Cucurbitaceae. Buschmann (1992) showed extraordinarily good sorption capacity of Cucurbituril for various types of textile dyes. Cucurbituril is known to form host-guest complexes with aromatic compounds (Mock, 1995) and this may be the mechanism for reactive dye adsorption. Another proposed mechanism is based on hydrophobic interactions or the formation of insoluble Cucurbituril-dye-cation aggregates since adsorption occurs reasonably fast. To be industrially feasible,
Cucurbituril would need to be incorporated into fixed bed sorption filters (Karcher et al. 1999). Like many other chemical methods, cost is a major disadvantage.

\textit{v) Chemical reduction}

Another chemical approach is to carry out the reductive cleavage of water soluble dyes containing azo or other reducible groups by agents such as sodium dithionate, sodium hydrosulfite, thiourea dioxide and sodium borohydride to generate aromatic amines (Cook 1996). These compounds are readily metabolized in activated sludge, chemically oxidized or adsorbed on carbon or precipitated by polycationic agents. A major azo dye manufacturer has successfully implemented a sodium borohydride reduction process to remove effluent color and stabilize resulting solids at one of its plants (Cook 1996).

\textbf{1.7.3 Biological treatment}

The Bioremediation is the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes, to minimise the risk to human health and the environment. Biological treatment is often the most economical alternatives when compared with other physical and chemical processes. Often this involves decolorization by means of Biodegradation methods such as microbial degradation and adsorption by (living or dead) microbial biomass and bioremediation systems are commonly applied to the treatment of industrial effluents because many microorganisms such as bacteria, yeast, algae and fungi are able to accumulate and degrade different pollutants (McMullan et al. 2001 and Fu and Viraraghavan 2001). However, their application is often restricted because of technical constraint. According to Bhattacharyya and Sharma (2003) biological treatment requires a large land area and is constrained by sensitivity toward diurnal variation as well as toxicity of some chemicals, and less flexibility in design and operation. Many organic molecules are degraded, many others are recalcitrant due to their complex chemical structure and synthetic organic origin (Kumar et al. 1998). In particular, due to their xenobiotic nature, azo dyes are not totally degraded.
i) **Biodecoloration vs. biodegradation**

Biodecoloration of dyes may occurred due complete mineralization i.e. decomposition of the molecule into H₂O, CO₂, inorganics and biosorption resulting in to phase change of the dye molecules. Biodegradation involves the degradation of the chromophore and may not indicate mineralization of the molecule. This should be distinguished from sorption of the dye to the microbial biomass. There are few known microorganisms that have the ability to reductively cleave azo bonds under aerobic conditions.

ii) **Organisms involved in Bio-decolorization**

Over the Past decades, Biological decolorization has been investigated as a method to transform, degrade or mineralize azo dyes. Moreover, such decolorization and degradation is an environmentally friendly and cost competitive alternative to chemical decomposition possess.

a) **Bacteria**

Efforts to isolate bacterial cultures capable of degrading azo dyes started in the 1970s with reports of *Bacillus subtilis* (Horitsu et al. 1977), followed by *Aeromonas hydrophila* (Idaka & Ogawa 1978) and *Bacillus cereus* (Wuhrmann et al. 1980). Numerous bacteria capable of dye decolorization, either in pure cultures or in consortia, have been reported.

The ability of bacteria to metabolise azo dyes has been investigated by a number of research groups (Cao et al. 1993; McMullan et al. 2001; Bhaskar et al. 2003; Junnarkar et al. 2006; Kalme et al. 2007; Dawkar et al. 2008; Kalyani et al. 2008). Many reports suggest the use of Pure cultures (Table 1.), either whole cells or specific enzymes, for better insight of azo dye reduction mechanism, which are not fully understood yet. Microbial decolourisation requires an unspecific enzymatic capacity ubiquitously found in a wide diversity of microorganisms (Chung and Stevens 1993). This has been mainly demonstrated with microorganisms present in the intestine such as *Clostridium*, *Salmonella*., *Bacillus*.
**Eubacterium** and *Escherichia coli*, which are able to reduce the dyes. Several reports proposed mixed bacterial cultures from a wide variety of habitats to decolourise the diazo linked chromophore of dye molecules. (Knapp and Newby 1995). Nigam and Marchant (1995) and Nigam et al. (1996) demonstrated that a mixture of dyes were decolourised by anaerobic bacteria in 24±30h, using free growing cells or in the form of biofilms on various support materials. Ogawa and Yatome (1990) also demonstrated the use of bacteria for azo dye biodegradation. The semi microbial systems have the drawback of requiring a fermentation process, and are therefore unable to cope with larger volumes of textile effluents. Reports regarding aerobic degradation of azo dyes (Cripps et al. 1990; So et al.1990; Govindaswami et al. 1993; Jian & Bishop1994; Wong & Yuen 1996; Coughlin et al. 1997; Tepper et al.1997) are not common. Kulla (1981) reported the ability of *Pseudomonas* strains to aerobically degrade certain azo dyes. Kodam et al.(2005) reported a 100% decolorization of the sulfonated azo dyes by an unidentified bacterium, KMK48. Optimum decolorization took place strictly under aerobic conditions, which is contrary to other well documented reports. However, the intermediates formed by these degradative steps resulted in disruption of metabolic pathways and the dyes were not actually mineralised. Under anaerobic conditions, such as anoxic sediments, many bacteria gratuitously reduce azo dyes reportedly by the activity of unspecific, soluble, cytoplasmic reductases, known as azo reductases. These enzymes are reported to result in the production of colourless aromatic amines which may be toxic, mutagenic, and possibly carcinogenic to animals. Keck et al. (1997) proposed an anaerobic non enzymatic cleavage of azo bonds by extracellular redox mediators (no coenzymes) naturally produced during the aerobic metabolism of a xenobiotic compound (2-naphthalene-sulfonate) by a strain of *Sphingomonas* sp. Thus, other possible redox mediators or reduction equivalents gratuitously formed during various metabolisms of different bacteria may be involved in the azo dye reduction. Recent literature evidence suggests that additional processes may also be involved in azo dye reduction. It has been reported that many bacteria reduce a variety of sulfonated and non-sulfonated azo dyes under anaerobic conditions without specificity of any significance. In addition many highly charged and high molecular sized sulfonated and polymeric azo dyes are unlikely to pass the cell membrane. Taken together both pieces of evidence
point to the existence of a reducing activity which is not dependent on the intracellular availability of the azo dye (Keck et al. 1997). Due to unsuitable redox potentials complex structure, or steric hindrance of the molecules, the reductive cleavage reaction generally represents one of the rate limiting steps in the overall mineralization of an azo dye (Dos Santos et al. 2004a). It has been suggested that redox mediators could increase the rate of dye decolorization as an electron shuttle from the primary electron donor to the azo dye (Rau and Stolz 2003). Theoretically, feasible redox mediators for biological azo dye reduction must have redox potentials between the half reactions of the azo dye and the primary electron donor (Van der Zee et al. 2003). Unfortunately the standard redox potential ($E_0'$) for most azo dyes is unknown.
Table 1.4 Facultative and strictly anaerobic bacterial cultures, which were able to decolourise azo dyes under anaerobic conditions (adapted from dos Santos et al. 2007)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Dyes</th>
<th>Activity</th>
<th>Decolor. (%)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Clostridium perfringens</td>
<td>Amaranth</td>
<td>0.74</td>
<td>-</td>
<td>Dye</td>
</tr>
<tr>
<td></td>
<td>Methyl Orange</td>
<td>0.62</td>
<td>-</td>
<td>Concentration of 0.033mM</td>
</tr>
<tr>
<td></td>
<td>Orange II</td>
<td>0.70</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tartrazine</td>
<td>0.67</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2. Bacteroides fragilis</td>
<td>Amaranth</td>
<td>-</td>
<td>66.0</td>
<td>Activity after 6 h of incubation.Dye</td>
</tr>
<tr>
<td></td>
<td>Orange II</td>
<td>-</td>
<td>37.0</td>
<td>Concentration of 0.1mM</td>
</tr>
<tr>
<td></td>
<td>Tartrazine</td>
<td>9.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3. Pseudomonas GM3</td>
<td>Acid Violet 7</td>
<td>-</td>
<td>97.4</td>
<td>After 72 h of incubation.Dye</td>
</tr>
<tr>
<td></td>
<td>Reactive Blue 2</td>
<td>-</td>
<td>18.3</td>
<td>Concentration of 100mg/l</td>
</tr>
<tr>
<td></td>
<td>Acid Green 27</td>
<td>-</td>
<td>75.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acid Red 183</td>
<td>-</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indigo carmine</td>
<td>-</td>
<td>69.0</td>
<td></td>
</tr>
<tr>
<td>4. Sphingomonas xenaphaga BN6</td>
<td>Acid Red 27</td>
<td>0.01</td>
<td>-</td>
<td>Dye concentration of 0.1mM</td>
</tr>
<tr>
<td></td>
<td>Acid Orange 20</td>
<td>0.01</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acid orange 7</td>
<td>0.30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acid Red 14</td>
<td>0.20</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acid yellow 23</td>
<td>0.10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acid Black 1</td>
<td>0.30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5. Enterococcus faecalis</td>
<td>Aux 10⁻²/mg protein</td>
<td>99.4</td>
<td>-</td>
<td>After 20 h of incubation.Dye</td>
</tr>
<tr>
<td></td>
<td>Methyl Red</td>
<td>1.81</td>
<td>95.1</td>
<td>Concentration of 0.2mM</td>
</tr>
<tr>
<td></td>
<td>Orange II</td>
<td>1.39</td>
<td>64.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orange G</td>
<td>1.20</td>
<td>99.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amaranth</td>
<td>1.37</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6. Eubacterium biforme</td>
<td>Tartrazine</td>
<td>-</td>
<td>4.0</td>
<td>After 150 minutes of incubation.Dye</td>
</tr>
<tr>
<td></td>
<td>Sunset Yellow</td>
<td>-</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Methyl Orange</td>
<td>-</td>
<td>79.0</td>
<td>Concentration of 2 mM</td>
</tr>
<tr>
<td></td>
<td>Orange II</td>
<td>-</td>
<td>81.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amaranth</td>
<td>-</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allura Red 40</td>
<td>-</td>
<td>11.0</td>
<td></td>
</tr>
</tbody>
</table>

1Semde et al.(1998); 2Bragger et al.(1997); 3Yu et al.(2001); 4Rau et al.(2002); 5Chen et al.(2004); 6Chung et al.(1978).

In a screening of redox potential values for different azo dyes, it was found that $E'_0$ values are normally between -430 and -180mV (Dubin and Wright 1975).
Rau et al. (2002) cites that the NAD(P)H cofactor, which has the lowest $E_0'$ value of -320mV, seems to set the lower limits of redox mediators application.

The reason is that mediators with a more negative $E_0'$ value will not be reduced by the cells and mediators with $E_0'$ higher than -50mV will probably not efficiently reduce the azo bond at high rates. Although this observation was confirmed with a model compound (Rau et al.2002), it has yet to be shown in the case of complex textile wastewater.

Azo dye reduction can take place directly (Fig-1.10) by aerobic or facultatively aerobic bacteria with specific or non specific azo reductase. These bacteria use simple azo compounds as sole source of carbon and energy (Kulla et al. 1983a). There is no clear evidence for the existence of specific azoreductases in anaerobically grown bacteria. However, also under anaerobic conditions, non-specific enzymes may be responsible for the almost ubiquitous capacity of many strains of anaerobic, facultative anaerobic and even aerobic bacteria to reduce azo dyes.

![Diagram of different mechanisms of anaerobic azo dye reduction](adapted from Van Der Zee 2002)

Fig. 1.5 Schematic representation of the different mechanisms of anaerobic azo dye reduction

RM = Redox Mediator, ED = Electron Donor  b = bacteria(enzyme)

Indirect azo dye reduction takes by reduced electron carries. Recent research has revealed that flavin reductases are indeed anaerobic azo reductases (Russ et al. 2000). Also other reduced enzyme cofactors capable of direct azo dye reduction have been reported, e.g. NADH (Nam and Renganathan 2000), NADH and NADPH (Hernandez et al. 1967) and an NADPH-generating system (Semde et al. 1998)

Bacterial mineralization of azo dyes generally takes in two steps:
Step 1: Two mechanisms for the decolorization of azo dyes under anaerobic conditions in bacterial systems have been proposed. The first one consists of direct electron transfer to azo dyes as terminal acceptors via enzymes during bacterial catabolism, connected the ATP generation (energy conservation). The second one involves a free reduction of azo dyes by the end products of bacterial catabolism, not linked to ATP generation (e.g., reduction of the azo bond by reduced inorganic compounds, such as Fe$^{2+}$ or H$_2$S, that are formed as the end product of certain anaerobic bacterial metabolic reactions). Fig. 1.5 shows a possible pathway for the degradation of azo dyes under anaerobic conditions with whole bacterial cells.

Step 2: During anaerobic degradation, a reduction of the azo bond in the molecules is observed. Then, aerobic conditions are required for the complete mineralization of the reactive azo dye molecule. The aromatic compounds produced by the initial reduction are degraded via hydroxylation and opening in the process is necessary in which oxygen is introduced after the initial anaerobic reduction of the azo bond has taken place. The optimum pH for colour removal is around pH 7-7.5. The rate of colour removal tends to decrease rapidly under strongly acid or strongly alkaline conditions. The optimum cell culture growth temperature is between 35 and 45°C.

b) Fungi

The potential of fungi to reduce azo dyes is associated with formation of exoenzymes such as peroxidases and polyphenol oxidases. Peroxidases are hemoproteins that catalyse reactions in the presence of hydrogen peroxide (Duran et al. 2002). In recent years there has been an intensive research on fungal decolorization of dye wastewater. It is turning into a promising alternative to replace or supplement present treatment processes. The most studied fungus is the white rot fungus *Phanerochate chrysosporium*, which is able to decolorize various dyes (Swamy et al. 1992). The use of species of the genera *Pleurotus*, *Bjerkana*, *Tremetes*, *Poyporus* and *Phelinus* and the species *Iprex lacteus*, *Fungalia trogii*, *Ganoderma* sp. and *Thelephora* sp. have been also investigated (Selvam et al. 2003; Wesemberg et al. 2002; Yesilada et al. 2003; Revankar et al. 2006; Fu and Viraraghavan et al. 2002). Knapp et al. (1995) reported that adsorp-
tion of dyes to the microbial cell surface is the primary mechanism of decolorization. The basidiomycete Phanerochaete chrysosporium is one of the ligninolytic fungi that was extensively used to degrade dyes (Cripps et al. 1990; Goszczynski et al. 1994; Spadaro et al. 1992; Wesenberg et al. 2003). This fungus produces several extracellular ligninolytic enzymes that have been associated with the degradation of dyes (Jarosz Wilkolazka et al. 2002; Wesenberg et al. 2003). Other species of ligninolytic fungi, such as Bjerkandera adusta, Pleurotus ostreatus and Irpex lacteus also showed potential for decolorization of various dyes including azo dyes (Novotny et al. 2001; Robinson et al. 2001b; Zhao 2004), however the biochemical pathways involved in azo dye degradation and decolorization by white rot fungi are still unclear (Wesenberg et al. 2003).

White-rot fungi produce various isoforms of extracellular oxidases including laccase, MnPeroxidase and lignin peroxidases (LiP), which are involved in the degradation of lignin in their natural lignocellulosic substrates. This ligninolytic system of white rot fungi (WRF) is directly involved in the degradation of various xenobiotic compounds and dyes. The existing literature and research work done suggests that there is a great potential for developing microbiological decolorization systems with total color removal in some cases within few hours (Balan and Monterio 2000). Development of efficient dye degradation requires a suitable strain and its use under favorable conditions to realize the degradation potential.

c) Yeast

Scientific literature lack details on yeast color removal abilities. Kwasniewska (1980) reported Rhodotorula sp and R. rubrum degraded Crystal Violet completely within 4 days. A strain of ascomycete yeast Candida zeylanoides isolated from contaminated soil was reported to reduce model azo dyes (Martins et al. 1999, Ramalho et al. 2002). The characterisation of an enzymatic activity is described in studies with the yeast Issatchenka occidentalis (Ramalho et al. 2004), and the enzymatic system involved is presented in a work with Saccharomyces cerevisiae (Ramalho et al. 2005). Compared to bacteria and filamentous fungi, yeasts have many advantages. They not only grow rapidly like bacteria, but like
filamentous fungi they also have the ability to resist unfavourable environments. Furthermore, some yeast have been found to be efficient in treating high strength organic wastewaters, such as food industry effluents (Yang et al. 2003). To the present, however, the use of yeast strains in treating dye wastewater has been very limited. Only a few reports on the degradation of azo dyes or anthraquinone dyes by yeasts have appeared (Itoh et al. 1996; Martins et al. 1999; Meehan et al. 2000). Recently Jadhav andGovindwar (2006) reported speedy biotransformation of Malachite green by *Saccharomyces cerevisiae* and another azo dye Methyl Red (Jadhav et al. 2007).

d) Algae

Limited numbers of reports are available on the biological treatment by algae (Venkan-mohan et al. 2003; Aksu and Tezer 2005), in spite of the ubiquitous distribution and their central role in the fixation and turnover of carbon and other nutrient elements (Semple et al. 1999). In particular, no research attention has been focused on utilization of macro algae for dye removal. Macro fresh water algae, a renewable natural biomass proliferates ubiquitously and abundantly in the many parts of the world. Treatment of mono-azo dye, Tectilon Yellow 2G (TY2G), by *Chlorella vulgaris* was investigated and suggested azo bond cleavage (Acuner and Dilek 2004). Mohan et al reported decolorization Direct Brown 2(diazo) by *Spirogyra* species and suggested mechanism of removal due to biosorption and/or bioconversion and/or biocoagulation.

e) Phytoremediation

Phytoremediation, the use of plants to clean up contaminated soil and water, has a wide range of applications and advantages. Constructed wetlands are engineered, man-made systems designed to treat wastewater. Wastewater treatment capability of plants that inhabit natural and constructed wetlands is known there is little information in the literature on the dye removal capability of submersed plants in a continuous flow system. Determination of the dye removal capability of submersed plants may contribute to system design approaches to constructed
wetlands. In constructed wetlands and aquatic plant systems, the photosynthetic tissue of aquatic macrophytes is entirely submerged. These plants are able to assimilate nutrients from polluted waters. The prime potential use of submersed aquatic macrophyte based wastewater treatment systems is "polishing" in secondary treatment effluents. The presence of these plants depletes dissolved carbon in the water and increases the content of dissolved oxygen during the periods of high photo-synthetic activity (Keskinkan and Goksu 2007). Transgenic tobacco plants with Lignin Modifying Enzyme (LME) genes from WRF are currently proposed for the removal of hazardous chemicals from contaminated environments (Iimura et al. 2002) and could be applicable to the case of textile dyes. In one effort an azo dye, acid orange 7 (AO7), was employed to study the role of *Phragmites australis* (*P. australis*) peroxidases (POD) activity in its degradation in a vertical flow constructed wetland (VFCW). Crude plant extract was found to degrade AO7 and its aromatic amines, after 120 h in contact with H₂O₂. The VFCW was found to be suitable to treat an effluent containing an azo dye (Davies et al. 2005). CWs have many advantages such as low construction and operation costs, simple installation and maintenance. CW is being used for both small and large applications. Parac- Osterman et al. (2006) reported *Phragmites australis* for reduction of measured biological parameters (COD, BOD₅, TOC, AOX, el. conductivity, pH, NH₄⁺, NO₃⁻, NO₂⁻, total P and the amount of Cl⁻ ions). Tissue cultured shrub plants of *Blumea malcolmii* was employed to decolorize Malachite green, Red HE8B, Methyl orange, Reactive Red 2 and Direct Red 5B at 20 mg L⁻¹ concentration to varying extent within three days. A significant induction in the activities of lignin peroxidase, tyrosinase, DCIP (2,6-dichlorophenol-indophenol) reductase, azoreductase and riboflavin reductase in the roots was observed during the decolorization of Direct Red 5B, which indicated their crucial role in the metabolism of the dye (Kagalkar et al. 2009).

### 1.8 Combined anerobic and aerobic treatment of azo dyes

For the complete mineralization of azo dyes using microorganism both aerobic and anaerobic treatments are essential which subsequently performed in different reactor system called as sequential anaerobic aerobic treatment (Zitomer
and Speece 1993) and may also implemented in same reactor system called as integrated anaerobic aerobic treatment system (Field et al. 1995).

1.8.1 Sequential Anaero/aerobic treatment

This involved two stage reactor systems. Earlier work done by Haug et al. (1991) demonstrate mineralization of an azo dye Mordant Yellow 3 (MY3) by a bacterial co-culture under sequential anaerobic/aerobic batch conditions where in anoxicogenic condition MY3 was degraded to 6A2NS and 5AS. These metabolites totally degraded and utilized by the culture when aerobic conditions were restored (Fig. 1.6) Rajaguru et al. (2000) reported complete degradation and mineralization of sulfonated azo dyes Orange G, Amido balck 10B, Direct red 4BS and Congo red using glucose as co-substrate. Ong et al. (2004) studies on sequential anaerobic and aerobic-sequencing batch reactor system found that the circulation of mixed liquor between the anaerobic reactor and the carbon-packed column enhanced the chemical AO7 removal efficiencies from 88 to 96%, under simultaneous adsorption and biodegradation process. Particularly in anaerobic exposure presence of co-substrate is must and its major fraction is consumed by culture for reductive gratuitous cleavage of azo bond. Consequently the aromatic amines provided as a main substrate for the organisms in the aerobic bioreactor for utilization and mineralization. The poor biodegradability of sulfonated aromatic amines reported by Tan et al. (2000) under the laboratory conditions suggests that these compounds may not be adequately removed during biological wastewater treatment and its degradation occur only under aerobic condition.

1.8.2 Integrated Anaerobic aerobic treatment

The combined activity of anaerobic and aerobic bacteria can also be obtained in a single treatment step if the bacteria are immobilized in particulate matrices (e.g. biofilm, soil aggregate, etc.). Due to the rapid uptake of oxygen by aerobes and facultative bacteria compared to the slow diffusion of oxygen, oxygen penetration into active biofilms seldom exceeds several hundred micrometers. The anaerobic microniches established inside the biofilms can be applied to the
General Introduction

reduction of electron withdrawing functional groups like azo bonds in order to prepare recalcitrant aromatic compounds for further mineralization in the aerobic outer layer of the biofilm (Field et al. 1995). By maintaining proper condition like low diffusion and oxygen concentration and co-substrate single biofilm can be created where integrated anaerobic and aerobic micro niche are included. Due to the limited diffusion of the oxygen compared to that of the co-substrate a part of the co-substrate will be consumed via oxidative processes in the periphery of the biofilm, while the inner layers of the biofilm will remain anaerobic. In this system, the reduction of the azo dyes and the aerobic mineralization of the aromatic amines will proceed side by side in the same biofilm. In this way occurrence of the unwanted process of autoxidation of the aromatic amines is not likely (Field et al. 1995). Although integrated anaerobic/aerobic systems suffers the operational imbalance between oxygen and co-substrate which is difficult to achieve. In one study by Kudlich et al. (1996) *Sphingomonas* sp. BN6 was immobilized in calcium alginate bead, the cells in the anaerobic center of bead converted MY3 in to 6-amino-naphthalene-2-sulfonate (6A2NS) and 5 amino-salicylate and that 6A2NS was oxidized to 5-amino salicylate by those cells that were immobilized in the outer aerobic zones of the alginate beads. A co-immobilisate of *Sphingomonas* sp. BN6 with a 5-aminosalicylate degrading bacterium completely degraded MY3 (Fig 1.6).

In Nutshell, review decolorization methods mentioned above have advantages and drawbacks. No one method is universally accepted for the treatment of colored water and their selection will depend on the wastewater characteristics like class and concentration of dye, pH, salinity and toxic compounds. Bioremediation of colored effluent using granular sludge and mixed culture of bacteria always is looked upon as most lucrative option but its application is limited due to vague knowledge of microbial interrel-ationship. Therefore, it will be dream treatment if consortium of microbes having potential role in azo dyes mineralization are employed. Hence screening, identifying and characterizing potential isolates from effluent knowing their biodegradation profile, mechanism of biodegradation of dyes and toxicity of biodegraded effluent needs special addition and attention in the scientific literature.
A) anaerobic

B) aerobic

Fig. 1.6 Proposed pathways for biodegradation of the azo dye Mordant Yellow 3 by a mixed bacterial community (adapted from Haug et al. 1991).
1.9 Research Objectives

The objectives of thesis are

i) To isolate, identify, characterize potential microorganisms for obtaining bioremediation of azo dyes and their laboratory simulated effluent.

ii) To study the influence of various physicochemical parameters on dye decolorization.

iii) To analyse biodegradation mechanism of dyes

iv) To know the enzymes involved in biodegradation of dyes.

v) To test toxicity of dye and its biodegradation products.

The thesis is divided into seven chapters.

Chapter 1 presents a literature review on textile wastewaters, mainly covering the aspects of health and environmental concerns and decolorization techniques, with an emphasis on the biotechnological approaches. Chapter 2 describes isolation, screening and characterization of dye decolorizing organisms and dye decolorization profile of selected microorganism. Chapter 3 deals with the effect of various physiological conditions on the dye decolorization efficiency. Influence of various physicochemical factors like temperature, pH, dye concentration, salinity, inoculum size, oxygen relationship and co-substrate are evaluated. It also evaluates the suitability of selected isolate to the immobilization system. Chapter 4 probes the biodegradation mechanism underlined by selected bacteria for decolorization of model azo dye Methyl red revealed using analytical instrumentations. Chapter 5 investigates various enzymes involved in biodegradation of dyes. Chapter 6 seeks the assessment of toxicity of treated and untreated dye on the seed of plants and on beneficial microorganisms. Chapter 7 incorporates the vital findings of the previous chapters which are discussed in a critical way, and significance of present work is argued in line with the current research in dyes biodegradation arena.