2.0 Mechanism of dye decolorization and degradation

The earliest method of colour removal by bacterial cells was by adsorption of the dye onto the biomass, which was similar to other physical adsorption mechanisms (Bras et al., 2001). Colour removal using adsorption was not suitable for long term treatment, because during adsorption the dye gets concentrated onto the biomass, which would become saturated with time, and the dye-adsorbent composition should also be disposed. The association between the dye and bacterial cells has been the first step in the biological reduction of azo dyes, which is a destructive treatment technology (Southern et al., 1995).

The process of bacterial azo dye degradation consists of two stages. The first stage involves the reductive cleavage of the dyes’ azo bond (–N=N–), resulting in the formation of aromatic amines, that is generally colorless but potentially hazardous. The second stage involves degradation of the aromatic amines under aerobic conditions. Azo dye reduction with the help of azoreductase under anaerobic conditions, involves transfer of four-electrons (reducing equivalents), which proceed through two stages at the azo linkage and in each stage two electrons are transferred to the azo dye, which acts as a final electron acceptor resulting in dye decolorization. The resulting intermediate metabolites are further degraded aerobically or anaerobically (Chang et al., 2000; Chang 2004). The presence of oxygen usually inhibits the azo bond reduction activity since aerobic respiration may dominate utilization of NADH; thus hindering the electron transfer from NADH to azo bonds (Chang et al., 2004).

![Mechanisms involved in dye degradation](image)

**Figure 2.1** Mechanisms involved in dye degradation
Anaerobic bio-reduction of an azo dye comprises of three different mechanisms as described by earlier researchers (Figure 2.1).

a) Direct enzymatic reduction
b) Indirect/mediated reduction
c) Chemical reduction (Organic and inorganic compounds)

A direct enzymatic reaction or a mediated/indirect reaction is catalyzed by biologically regenerated enzyme cofactors or other electron carriers. The azo dye chemical reduction can also result from purely chemical reactions with biogenic reductants like sulphide. These azo dye reduction mechanisms have been shown to be greatly accelerated by the addition of many redox-mediating compounds, such as anthraquinone-sulfonate (AQS) and anthraquinone-disulfonate (Cervantes FJ (2002), Guo J (2006), Van der Zee FP (2002). The mechanism of anaerobic azo dye reduction by bacteria is presented in Figure 2.2.

![Figure 2.2 Mechanisms of anaerobic azo dye reduction by bacteria](image)

(RM\text{ox}=oxidised redox mediator; RM\text{red}=reduced redox mediator; ED=electron donor; ED\text{ox}=oxidised electron donor; B=bacteria (enzyme system) (adapted from van der Zee, 2002).

a) Direct enzymatic dye decolorization

The bacterial decolorization of dyes is being facilitated by various reductive enzymes such as azoreductase, NADH-DCIP reductase and MG reductase and oxidative enzymes like lignin peroxidase and laccase (Parshetti \textit{et al.}, 2006; Kalme \textit{et al.}, 2007; Kalyani \textit{et al.}, 2009).

Azoreductase

Azoreductases are flavoproteins (NAD (P) H: flavin oxidoreductase). They are localized to intracellular or extracellular site of the bacterial cell membrane. These azoreductases require NADH or NADPH or FADH as an electron donor for the reduction of an azo bond (Russ \textit{et al.}, 2000). The substrate specificity of azoreductases depends on the functional group present near azo bond. The oxygen
sensitive Orange II azoreductase from *Pseudomonas* sp. KF46 showed highest specificity towards the carboxy group substituted sulfophenyl azo dyes (Zimmermann et al., 1982). The induction of azoreductase during decolorization of azo dyes under static condition was reported earlier (Dawkar et al., 2008; Dhanve et al., 2008). Azoreductase generated toxic amines after reduction of an azo bond.

**NADH-DCIP reductase and MG reductase**

NADH-DCIP reductase belongs to the bacterial mixed function oxidase system and takes part in the detoxification of xenobiotic compounds (Salokhe et al., 1999). The NADH-DCIP reductase reduces the DCIP using NADH as an electron donor. DCIP is blue in its oxidized form and becomes colorless after reduction. The significant induction of nonspecific reductase in the biodegradation of malachite green was termed as MG reductase that reduced the malachite green into leucomalachite green using NADH as an electron donor. (Parshetti et al., 2006).

**Lignin peroxidase (LiP)**

This enzyme belongs to the family of oxidoreductases, specifically those acting on peroxide as an acceptor (peroxidases) and is included in the broad category of ligninases. LiP catalyzes several oxidations in the side chains of lignin and related compounds by one-electron abstraction to form reactive radicals (Tien and Kirk, 1983; Kersten et al., 1985). The cleavage of an aromatic ring was also reported earlier (Umezawa and Higuchi, 1987). The purified LiP from *Brevibacillus laterosporous* MTCC 2298 and *Acinetobacter calcoaceticus* NCIM 2890 efficiently decolorized several sulfonated azo dyes (Gomare et al., 2008; Ghodake et al., 2009b).

**Laccase**

Laccase is one among the numerous members of multicopper oxidase protein family. In the presence of oxygen as an electron donor, it catalyzes the oxidation of substituted phenolic and nonphenolic compounds (Sharma et al., 2007). The first report of prokaryotic laccase was from the rhizospheric bacterium, *Azospirillum lipoferum* (Givaudan et al., 1993). Another laccase has been reported from a melanogenic marine bacterium, *Marinomonas mediterranea* producing two different polyphenol oxidases (PPO), a multi-potent PPO that oxidizes substrates characteristic of both tyrosinase and laccase (Solano et al., 1997). Laccase-like activity has also been reported in *CopA* protein from *Pseudomonas syringae* and *Pedomicrobium* sp. (Ridge et al., 2007). Laccase decolorizes azo dyes through a highly nonspecific free
radical mechanism, thereby avoiding the formation of toxic aromatic amines (Chivikula and Renganathan, 1995). The purified laccase from *Pseudomonas desmolyticum* NCIM 2112 showed 100% decolorization of various dyes, including Direct Blue 6, Green HE4B and Red HE7B (Kalme *et al*., 2009).

**b) Mediated biological dye decolorization**

High molecular weight sulfonated azo dyes are unable to pass through the cell membrane and therefore the reduction of these dyes occur through the mechanism that is not dependent on the transport into the cell membrane (Levine, 1991). The earlier reports showed the role of redox mediators in an azo bond reduction using bacteria under anaerobic conditions (Keck *et al*., 1997; Van der Zee *et al*., 2001; Dos Santos *et al*., 2007). Riboflavin in small amounts significantly enhanced the reduction of Mordant Yellow 10 using anaerobic granular sludge (Field and Brady, 2003). 1-amino-2-napthol, one of the constituent amines of an azo dye, Acid Orange 7, increased its decolorization rate, possibly by mediating the transfer of reducing equivalents (Mendez-Paz *et al*., 2005). The addition of synthetic electron carriers such as anthraquinone-2-6-disulphonate could also greatly enhance the decolorization of many azo dyes (Van der Zee *et al*., 2001). Keck *et al*., (1997) reported anaerobic cleavage of azo dyes by redox mediators formed during the aerobic degradation of a xenobiotic compound. Cell suspensions of *Sphingomonas* sp. strain BN6 grown aerobically in the presence of 2-naphthyl sulfonate (NS) exhibited 10-20 fold increase in the decolorization rate of an azo dye, amaranth under anaerobic conditions. Even the addition of culture filtrates of these cells could enhance anaerobic decolorization by cell suspensions grown in the absence of NS. The redox intermediates generated during aerobic degradation of aromatic compounds could also enhance the dye decolorization (Keck *et al*., 1997). The addition of culture supernatant containing metabolites of a dye-decolorizing *E. coli* NO₃ strain enhanced the azo dye decolorization rate (Chang *et al*., 2004).

**c) Dye decolorization using biogenic inorganic compounds**

Dye decolorization can also occur by chemical reactions with inorganic compounds (sulphide and ferrous ion) that are formed as end products of metabolic reactions under anaerobic conditions. It was reported that H₂S generated by sulphate reducing bacteria resulted in the extracellular decolorization of azo dyes (Yoo *et al*., 2000; Diniz *et al*., 2002). Sulphate influenced dye reduction correlated with biogenic
sulphide formation under methanogenic conditions. In the absence of sulphur compounds, dye decolorization readily occurred in the presence of granular sludge, demonstrating the importance of enzymatic mechanisms. An analysis of decolorization kinetics in the batch reactors and in the laboratory scale anaerobic sludge bed reactors indicated the relative importance of chemical dye reduction mechanisms in high rate anaerobic bioreactors (Van der Zee et al., 2003). Various inducers and stabilizers of oxidoreductive enzymes, such as CaCO$_3$, indole, o-toulidine, veratrole and vanillin enhanced dye decolorization (Dawkar et al., 2008).

2.1 Aerobic decolorization and degradation of azo dyes

Aerobic decolorization of dye containing wastewaters is inefficient for two reasons: Firstly, the dyes are highly stable to biological oxidation and secondly the poor adsorption of the dyes on to the activated sludge (Greaves et al., 1999). Several pure cultures of bacteria have been reported to decolorize azo dyes under aerobic condition. A bacterial strain *Klebsiella* sp. VN-31 was reported to decolorize monoazo dye RY107 and RR198 in 72 and 96 h respectively; the diazo dye RB5 and triazo dye DB71 were decolorized by the same culture in 120 and 168 h, respectively (Franciscon et al., 2009). Similarly, Wang et al., (2009) reported decolorization of Reactive Red 180 (200 mg/L) by *Citrobacter* sp. (CK3) within 36 h of incubation. Use of mixed bacterial cultures for aerobic degradation of dyes has also been reported. An et al., (2002) reported aerobic degradation of triphenylmethane dyes using a mixed culture consisting of *Pseudomonas mendocina* and *Pseudomonas alcaligenes*. Consortia of *Aeromonas caviae*, *Proteus mirabilis* and *Rhodococcus* sp. has been reported to decolorize Acid Orange 7 in 96 h (Joshi et al., 2008). Specialized peroxidase producing bacterial strains like *Streptomyces* and *Sphingomonas* were shown to degrade various xenobiotics including dyestuffs (Molina-Gujiarro et al., 2008). Most of the reports on microbial dye decolorization involve the use of electron rich substrates. Co-substrates such as glucose, yeast extract, hydrolyzed starch, acetate and propionate have served as electron donors and the extent of decolorization also varied depending upon the co-substrate employed in the process (Nigam et al., 1996; Van der Zee et al., 2000).

Several fungal genera have been recognized for aerobic degradation of organic pollutants including azo dyes. Most studies on azo dye degradation by fungi have focused on lignin degrading white rot fungi. Cripps et al., (1990) and Swamy and Ramsay (1999) reported the role of different white rot sp. like *Bjerkandera* sp.,...
Phanerochaetae chrysosporium and Trametes versicolor in decolorization of azo dyes. Novotny et al., (2001) described the decolorization of synthetic dyes by Irpex lacteus. The ability of the strain Trametes modesta to decolorize textile dyes was demonstrated by Nyanhongo et al., (2002). In fungal dye degradation process, the fungal ligninolytic enzyme system, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase have been reported to be involved in the bio-oxidation of dyes (Gold and Alic, 1993). Sporulating fungi such as Aspergillus, Penicillium, Rhizopus, Geotrichum sp. have also been reported for the decolorization of synthetic dyes. Ramya et al., (2007) reported decolorization and degradation of Reactive Blue by Aspergillus sp. These examples prove the role of fungi in decolorization of azo dyes. Nevertheless, the low pH requirement for an optimum activity of enzymes and the long hydraulic retention time for complete decolorization are the limitations in the use of fungal cultures for dye degradation (Banat et al., 1996; Swamy and Ramsay, 1999).

2.2 Anaerobic decolorization and degradation of azo dyes

Under anaerobic conditions, many bacteria reduce the highly electrophilic azo bond in the dye molecule through non-specific enzymatic action (Stolz, 2001; Pearce et al., 2003). Anaerobic reduction of azo dyes by bacteria seems to be an effective and economical process for colour removal as the reactions take place at neutral pH and are extremely non-specific when low molecular weight redox mediators are available. Under static condition, depletion of oxygen is easily accomplished thereby allowing obligate and facultative anaerobic bacteria to reduce azo dyes. Sewage systems often provide additional carbon sources which generally increase the reduction rates (Van der Zee et al., 2002). These carbon sources also facilitate formation and regeneration of reducing equivalents through their oxidation. The reduction of azo compounds has been reported in different anaerobic systems such as anaerobic sludge, sediments and also in anaerobically incubated pure cultures and enrichment cultures. Rafii et al., (1990) reported that certain bacterial species belonging to human intestine like Eubacterium sp., Butyvidbio or Bacteroides sp. were able to decolorize sulfonated azo dyes. The major drawback of anaerobic azo dye reduction is that the aromatic amines formed from reductive cleavage are recalcitrant to further anaerobic degradation (Chung et al., 1992; Van der Zee, 2002). Reduction of azo compounds under anaerobic conditions may be attributed to the presence of reduced inorganic compounds like Fe$^{2+}$ and H$_2$S that are formed as end products of certain strictly
anaerobic bacterial metabolic reactions (Stolz, 2001; Van der Zee et al., 2001; Yoo, 2002; Van der Zee et al., 2003). It has been reported that formation of H$_2$S by sulfate-reducing bacteria resulted in the reduction of such azo dyes as Reactive Orange 96 and Reactive Red 120 (Yoo et al., 2001; Stolz, 2001).

### 2.3 Sequential anaerobic/aerobic treatment of azo dye

Anaerobic decolorization of dyes is inadequate with respect to mineralization of the degradation products. Aromatic compounds like aniline, carboxylated aromatic amines, chlorinated aromatic amines, and substituted benzidines were found to be degraded more efficiently under aerobic conditions and could be further converted into simple end products (CO$_2$ and H$_2$O) (Pinheiro et al., 2004; Eckci et al., 2001), while certain aromatic amines like phenylenediamines, aminonaphthol and o-amino hydroxynaphthalene sulfonic acid tend to auto oxidize under aerobic conditions (Kudlich et al., 1999). Auto oxidation is a process in which oxygen reacts with the aromatic products via free radical reactions and eliminates the aromatic amines but in certain cases the end products which are formed may be toxic, mutagenic and non biodegradable in nature (Field et al., 1993). Figure 2.3 depicts the decolorization and mineralization of azo dyes under anaerobic-aerobic conditions. The sequential anaerobic-aerobic treatment can thus be the most feasible biological strategy for the complete removal of azo dyes from wastewater (Van der Zee et al., 2000; Dafale et al., 2010). Elisangela et al., (2009) reported sequential degradation of textile azo dyes using *Staphylococcus arlettae* strain VN-11. Biodegradation of Reactive Blue 13 was obtained in two-stage anaerobic/aerobic system with *Pseudomonas* sp. by Lin et al., (2010).

Different reactor configurations were used for anaerobic-aerobic steps, including the sequential anaerobic-aerobic reactor system (Zitomer and Speece, 1993) and integrated anaerobic-aerobic reactor system (Field and Brady, 2003). Sequential anaerobic/aerobic conditions can readily be imposed to wastewater by first exposing it to anaerobic conditions and next aerobic conditions using an anaerobic reactor followed by an aerobic reactor. The sequential treatment method for azo dye degradation has been studied by numerous researchers (Van der Zee and Villaverdeb, 2005; Isik and Sponza, 2008). In anaerobic conditions, the reductive cleavage of azo dye resulted in the formation of aromatic amines. After re-aeration of the synthetic dye wastewater, these amines were further degraded by the same isolates. Thus, total degradation of reactive azo dyes was achieved by anaerobic-aerobic treatment (Isik and Sponza, 2006).
2.4 Factors influencing decolorization of dyes

The textile wastewater produced during the processing of fabrics varies in chemical composition such as salts, organics, sulphur compounds, nutrients and different toxic substances. In biological treatment process, several factors affect the decolorization and degradation of dyes which include various physico-chemical parameters such as temperature, pH, dissolved oxygen, dye structure, dye concentration, electron donors and redox mediators which directly influence the efficiency of microbial dye decolorization. Figure 2.4 shows the various factors affecting the decolorization of dyes.

Figure 2.4 Schematic representation of decolorization and mineralization of azo dyes under anaerobic-aerobic conditions (Adapted from Dafale et al., 2010)

Figure 2.4 Factors affecting decolorization of dyes
2.4.1 Effects of oxygen on dye decolorization

The most important factor in the decolorization process is the effect of oxygen on cell growth and dye reduction. During the cell growth phase, oxygen will have significant effect on the physiological characteristics of the cells. During the dye reduction, if the extra cellular environment is aerobic, the high redox-potential electron acceptor oxygen may inhibit the dye reduction process (Chang et al., 2004). This is because the electron liberated from the oxidation of electron donors by the cells are preferentially used to reduce oxygen rather than the azo dye and the reduction product water is not a reductant (Yoo et al., 2001). Additionally, the intermediates of the dye reduction process include the hydrazine form and the azo anion free radical form of the dye which tend to be reoxidized by molecular oxygen (Zimmerman et al., 1982). On the basis of these observations, for efficient colour removal aeration that increases the concentration of oxygen in solution should be avoided (Chang and Lin, 2000). Similar results were observed in studies on pure bacterial strains such as Pseudomonas luteola, Proteus mirabilis, Pseudomonas sp. SUK1 and Micrococcus glutamicus NCIM-2168 (Chang et al., 2001b; Chen et al., 1999; Kalyani et al., 2008; Saratale et al., 2009 b). The intermediates formed during azo dye reduction reaction, like the simple aromatic compounds, are degraded via hydroxylation and ring-opening in the presence of oxygen (Chang et al., 2001b; Pandey et al., 2007).

2.4.2 Effects of temperature on dye decolorization

Microbial decolorization process is directly influenced by temperature because different groups of microorganisms need a defined range of temperature to perform their activity efficiently. Previous literature suggests that, the rate of color removal increases with an increasing temperature up to certain limit; afterwards there is marginal reduction in the decolorization activity. The temperature range of 30 - 45°C is generally required for optimal growth of the microorganisms and this also corresponds to maximal colour removal. Temperature changes lead to a sudden alteration in its activation energy (Yu et al., 2001). Therefore, growth rate, biomass yield and reaction mechanism need optimum temperature for their maximum efficiency. It has also been observed that the decolorization efficiency usually decreases at higher temperature and this decline could be attributed to the loss of cell viability or denaturation of an azo reductase enzyme (Chang et al., 2001a; Saratale et
al., 2009a). However, with certain whole cell preparations the azoreductase enzyme was found to be relatively thermostable and exhibited activity up to 60°C for a short time period (Pearce et al., 2003). Immobilization of the cell culture in a support medium results in a shift in the optimum colour removal temperature towards high values because the micro environment inside offers protection for the cells (Diaz et al., 2002).

2.4.3 Effect of pH on dye decolorization

The pH has a profound effect on the efficiency of dye decolorization. In earlier studies, it was observed that the optimum pH for colour removal is often at a neutral pH or at slightly alkaline pH. The rate of colour removal was higher at only optimum pH but tends to decrease rapidly at strongly acid or strongly alkaline pH. Colour removal was maximum in the pH range of 6.0 - 10.0 as reported by several researchers for different dyes studied (Junnarkar et al., 2006; Guo et al., 2007; Kilic et al., 2007). In case of biological reduction of azo dyes, breakage of the azo bond can result in an increase in the pH due to the formation of aromatic amines, which are more basic than the original azo dyes (Willmott, 1997; Pearce et al., 2003). Effective decolorization of Reactive Red 190 dye by *Citrobacter* sp.CK13 in strong acidic (pH 4.0) and strong alkaline (pH 12.0) conditions has been reported by Wang et al., (2009). *E. coli* and *P. luteola*, exhibited maximum decolorization rate over a wide range of pH between 7.0 and 9.5 (Chang and Lin, 2001). pH tolerance over a wide range is quiet important, as it makes them suitable for application in biological treatment of dye containing textile effluents (Aksu and Donmez, 2003). In an earlier study, it was reported that the dye reduction rate increased nearly 2.5 fold as the pH was raised from 5.0 to 7.0 while the rate became insensitive to pH in the range of 7.0 - 9.5 (Chang et al., 2001).

2.4.4 Effects of dye concentration on dye decolorization

The dye decolorization rate decreases gradually with an increase in dye concentration. This may be attributed to the toxic effects of the dye on the individual bacterium or consortia leading to an inadequate cell to dye ratio. The concentration of dye substrate can influence the efficiency of dye removal through a combination of factors including the toxicity of the dye (and co-contaminants) at higher concentrations, and the ability of the enzyme to recognize the substrate efficiently at very low concentrations that may be present in some wastewaters (Saratale et al., 2006; Jadhav et
The higher the dye concentration, the longer the time required to remove the colour. Dyes with concentrations of 1-10 µM were easily decolorized, but when the dye concentration was increased to 30 µM, colour removal was reduced (Sani and Banerjee, 1999). In one of the previous studies, rapid rate of decolorization by *Pseudomonas* sp. SUK-1 was observed in the initial stages, which decreased gradually with the increase in the concentration of the dye Reactive Red 5 (Kalyani *et al*., 2008). Saratale *et al*., (2009b) reported that the toxic effect of high concentration of dye was reduced when bacterial co-culture was used instead of pure cultures and this might be due to the synergistic effect of the microorganisms which may reduce the toxic metabolites concentration or alter their toxic effect. In contrast, Dubin and Wright, (1975) reported no negative impact of dye concentration on decolorization rate which may be due to non-enzymatic reduction mechanisms that are controlled by processes which are independent of dye concentration (Pearce *et al*. 2003).

**2.4.5 Effects of dye structure on dye decolorization**

Varied structures of azo dyes and the presence of different functional groups significantly affect the decolorization capability in the form of reduction and degradation. Dyes with simple structures and low molecular weights are more susceptible to decolorization at higher rates, whereas the rate of colour removal is lower in the case of dyes substituted with an electron withdrawing group such as –SO₂H, -SO₂NH₂ in the para position of the phenyl ring, relative to the azo bond and high molecular weight dyes (Sani and Banerjee, 1999). The colour removal rate is faster in the case of monoazo dyes as compared to diazo and triazo dyes (Hu, 2001). Azo compounds specifically, with hydroxyl or amino groups are more easily degradable than those with methyl, methoxy, sulpho or nitro groups (Nigam *et al*., 1996). Thus, the sulfonated reactive group of azo dyes is generally considered to be more recalcitrant than carboxylated azo dyes. The rate-limiting step during bacterial decolorization of sulfonated azo dyes is the permeation of dyes through the bacterial cell membrane (Kodam *et al*., 2005; Lorenzo *et al*., 2000). Aerobic degradation of 25 sulfonated azo dyes with their chemical structures was studied by Suzuki *et al*., (2001), who reported that the steric effect of chemical structure strongly affected the colour removal efficiency. Hsueh *et al*., (2009) reported that contribution of hydrogen bonding directly influenced the azo hydrazone tautomerism of hydroxyl azo compounds. The presence of substituent in the para position makes the azo linkage
more susceptible for decolorization compared to the ortho and meta positions of the phenyl ring (Hsueh et al. 2009). Zimmermann et al., (1982) reported that the electron-withdrawing groups present on the phenyl ring accelerate the colour removal. Moreover, in terminal non-enzymatic reduction mechanism, reduction rates are influenced by changes in an electron density in the region of the azo group, causing increase in the reduction rate (Rau et al., 2002). Hitz et al., (1978) concluded that (a) acid dyes exhibit low colour removal due to the number of sulphonate groups in the dye, (b) direct dyes exhibit high levels of colour removal that is independent of the number of sulphonate groups in the dye and (c) reactive dyes exhibit low levels of colour removal (Hsueh et al., 2009; Pearce et al., 2003).

2.4.6 Effects of electron donors on dye decolorization

The azo dyes and other organic materials in textile wastewater are insufficient in quantity to act as effective substrate for the growth of anaerobic and aerobic bacteria and thus for complete decolorization of dyes. Therefore, it is necessary to have an external substrate (electron donor) to enhance decolorization potential. A prerequisite for the azo dye reduction is the presence and availability of the electron donor. Electron donors and electron acceptors are naturally present in the environment and it is important to study the effects of these on bacterial azoreduction (He and Sanford, 2003). The electron donors like sodium acetate, sodium formate, sodium succinate, sodium citrate and sodium pyruvate are reported to enhance the azo dye decolorization of C.I. Reactive Orange 16 by Bacillus sp. ADR (Telke et al., 2009) and various azo dyes by Shewanella decolorationis S12 (Hong et al., 2007). The addition of electron donors such as glucose, acetate, butyrate ions apparently induces the reductive cleavage of azo bonds (Bras et al., 2001). Glucose has a significant role in the colour removal rates, probably because of the increased concentration of H₂ (or formate) that is likely to be formed during glucose fermentation (a maximum of 4 mol H₂ per mol of glucose). Determination of physiological electron donors for each biological colour removal process is important because the donors not only induce the reduction mechanism, but also stimulate the enzymatic system responsible for the reduction process (Pearce et al., 2003; Van der Zee et al., 2001).
2.4.7 Effect of Redox Mediators on dye decolorization

Redox mediators (RM) are molecules that reversibly oxidize and reduce by conferring the capacity to serve as an electron carrier in multiple redox reactions. They are known to accelerate reactions by lowering the activation energy of the total reaction (Stolz et al., 2001). However, in certain cases the presence of RM may even be a prerequisite for the reaction to take place. Flavin based compounds, such as flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN) and quinone based compounds such as anthraquinone 2,6-disulfonate (AQDS), anthraquinone-2-sulfonate (AQS); riboflavin (Vitamin B2), cyanocobalamin (Vitamin B12) and lawsone (2-hydroxy-1,4-naphthoquinone) are well known as redox mediators (Dos Santos et al., 2004). A very small concentration of redox mediator is sufficient to accelerate the electron transfer and is characterized by a redox potential ranging from -200 to -350 mV (Semde et al., 1998). The stimulating performance of the redox mediators is dependent on the redox potential. Therefore, an electron shuttling compound will only be effective as a redox mediator for azo dye reduction if it lowers the activation energy of reaction. The effect of redox mediators on the decolorization rates has been investigated with azo compounds as well as textile wastewaters. The initial studies focussed on the application of RM on reductive biotransformation of contaminants during decolorization of azo dyes in high rate UASB reactors (Cervantes et al., 2002; Van der Zee et al., 2001).

2.4.8 Effect of Redox Potential on dye decolorization

The redox potential is a measure of ease with which a molecule will accept electrons and can be reduced. The redox potential of the electron donors and acceptors influences the colour removal process because the rate-controlling step involves redox equilibrium between the dye and the extracellular reducing agent. Therefore, the more positive the redox potential, the more readily the molecule gets reduced (Bragger et al., 1997). It follows that the rate of colour removal will increase with increasing half-wave potential of the azo substrate. Bragger et al., (1997) showed that there is a linear relationship between the logarithm of colour removal rate and the half-wave potential of the substrate. This correlation suggests that the rate determining step in bacterial dye reduction does not involve a structure-specific phenomenon like selective membrane permeation or enzyme binding (Dubin and Wright, 1975). Under anaerobic conditions, the establishment of low oxidation-reduction potentials (<400 mV) for the
system is required for high colour removal rates. This has an effect on the profile of metabolites that are generated during the reduction process (Lorenco et al., 2001). The colour removal rate is maximum when the redox potential of the system is at its extreme negative and the rate falls as the redox potential of the system rises (Bromley-Challenor et al., 2000).

2.5 Microbial Degradation of Textile Dyes – A Review

2.5.1 Bacterial decolorization and degradation of dyes

Chen et al., (2003) reviewed the decolorization potential of Aeromonas hydrophila, isolated from sludge samples and mud lakes. Even though the strain displayed good growth in aerobic condition, colour removal was the maximum in anoxic or anaerobic culture. The most suitable culture conditions for dye decolorization were pH 5.5 - 10.0 and temperature 20 - 35°C. More than 90% of the dye, RED RBN was decolorized within 8 days with dye concentration of 3,000 mg/L. The strain could also decolorize the media containing a mixture of dyes within 2 days of incubation. Nitrogen sources such as yeast extract or peptone could greatly enhance the decolorization efficiency. Glucose as carbon source inhibited decolorization activity because the consumed glucose might have converted into organic acids and decreased the pH of the culture medium, thus inhibiting the cell growth and decolorization activity.

Moutaouakkil et al., (2003) explored the potential of Enterobacter agglomerans, isolated from dye-contaminated sludge to decolorize the azo dye Methyl Red (MR) under optimum conditions. The bacterial strain completely decolorized 100 mg/L of MR within 6 h of incubation in synthetic medium at temperature of 37°C. The rates of decolorization of MR by E. agglomerans under shaking and static conditions were almost equal, indicating that stirring of the culture medium did not have any influence on the decolorization of the dye. However, the kinetics of the bacterial growth of E. agglomerans was slightly influenced by stirring the culture medium. The strain also decolorized approximately 90% of MR after 6 h of incubation in the presence of 1%, 2% and 4% of glucose at pH 9.0.

In the study, carried out by Supaka et al., (2003), a sequential anaerobic-aerobic treatment process based on mixed culture of bacteria, isolated from textile dye effluent-contaminated soil was used to degrade three reactive azo dyes namely, Remazol Brilliant Orange 3R, Remazol Black B and Remazol Brilliant Violet 5R.
Incorporating the sequential treatment process showed that majority of colours were removed by the anaerobic process and COD was reduced in the subsequent aerobic process. Samples taken from combined anaerobic-aerobic system at the beginning of anaerobic process, after anaerobic process and after subsequent aerobic process were analyzed by HPLC. The results revealed that, under anaerobic conditions, the azo dyes were reduced and the aromatic amines were generated by the bacterial biomass. After re-aeration of the synthetic dye wastewater, these amines were further degraded by the same isolate. Thus, total degradation of reactive azo dyes was achieved by using dual system.

Verma and Madamwar, (2003) studied the decolorization of Ranocid Fast Blue (azo group) and Procion Brilliant Blue-H-GR (anthraquinone group) by using Serratia marcescens. More than 90% decolorization of two dyes was achieved in 5 - 8 days at 26°C with pH 7.0 under static conditions.

Four bacterial species were isolated from wastewater treatment plant that was capable of degrading Methyl Red, an azo dye by Adedayo et al., (2004). Complete decolorization using a mixed-culture was achieved at pH 6.0 and temperature 30°C within 6 h at 5 mg/L of methyl red concentration, and 16 h at 20 - 30 mg/L of dye. The two identified bacteria Vibrio logei and Pseudomonas nitroreducens were capable of utilizing methyl red as the sole carbon source. The Vibrio showed highest dye degradation under the optimum conditions of pH 6 - 7 and 30 - 35°C temperature.

The decolorization ability of anaerobic sludge bacteria obtained from cattle dung slurry was investigated with 17 different dyes in batch assay system using sealed serum vials in a study carried out by Keharia et al., (2004). Experiments conducted using Reactive Violet 5 (RV 5) showed that the bacteria could effectively decolorize dye concentrations up to 1000 mg/L with a decolorization efficiency of above 75% during 48 h incubation at temperature of 37°C. Lower concentrations of RV 5 (up to 500 mg/L) were found to be stimulatory to the methanogenic activity of sludge bacteria. Among the sixteen other dyes tested, nine dyes - Reactive Black 5, Blue 31, Blue 28, Red HE8B, Yellow, Golden Yellow, Mordant Orange, Novatic Olive R S/D and Navilan Yellow GL were decolorized with more than 88% efficiency; Orange II, Navy Blue HER and Novatic Blue BC S/D were decolorized with 50 - 65% efficiency, whereas Procion Orange H2R, Procion Brilliant Blue HGR & Novatic
Blue BC S/D were decolorized with less than 40% efficiency. Ranocid Fast Blue was decolorized up to 92.5%, and this was by sorption, whereas the other dyes were decolorized by biotransformation.

The enrichment of microbial population in the presence of triphenylmethane (TPM) dye Acid Violet-17 (AV-17) was carried out by Sharma et al., (2004) using various soil and sludge samples collected from the vicinity of textile dyeing industries and waste disposal sites. Five bacterial isolates belonging to Bacillus sp., Alcaligenes sp. and Aeromonas sp. were selected out of the twenty five different isolates screened for their ability to decolorize AV-17 dye (10 mg/L) in mineral salts medium agar plates. On the basis of their high decolorization ability, the isolates were used to develop a bacterial consortium. Decolorization of various TPM dyes achieved by the consortium was Acid Violet-17 (86%), Acid Blue-15 (85%), Crystal Violet (82%), Malachite Green (82%) and Brilliant Green (85%).

Study by Kodam et al., (2005) showed 100% decolorization of azo dyes Reactive Red 2, Reactive Red 141, Reactive Orange 4, Reactive Orange 7 and Reactive Violet 5 by an unidentified bacterium KMK 48. High decolorization was obtained within 36 h of incubation at room temperature and pH 7.0. Maximum decolorization was achieved under aerobic conditions.

Khehra et al., (2005) reported isolation of bacterial strains capable of decolorizing azo dyes present in soil/sludge samples collected from waste disposal sites of local textile industries. Four bacterial isolates (Bacillus cereus (BN-7), Pseudomonas putida (BN-4), Pseudomonas fluorescence (BN-5) and Stentorophomonas acidaminiphila (BN-3) capable of completely decolorizing C.I. Acid Red 88 (AR-88) were used to develop a consortium designated as HM-4. The combined metabolic activity of these four isolates led to complete decolorization of AR-88 (20 mg/L) in 24 h, whereas individual cultures took more than 60 h for complete decolorization of the dye. The consortium was also screened for its ability to decolorize different concentrations of other commonly used azo dyes. It was able to decolorize 78% of C.I. Acid Red 88, 99% of C.I. Acid Red 119, 94% of C.I. Acid Red 97, 99% of C.I. Acid Blue 113 and 82% of C.I. Reactive Red 120 dyes at an initial concentration of 60 mg/L in mineral salts medium in 24 h.
Moosvi et al., (2005) investigated soil samples from contaminated sites of Vatva, Gujarat, India and were screened for dye decolorizing organisms. A bacterial consortium RVM11.1 was developed on the basis of rapid dye decolorization potential of the isolates. The consortium exhibited 94% decolorization of Reactive Violet 5 (RV5) (200ppm concentration) within 37 h under a wide pH range from 6.5 to 8.5 and temperature ranging from 25 to 40°C. The bacterial consortium was able to decolorize RV5 under static conditions in the presence of glucose and yeast extract and also showed decolorization with replacement of glucose by starch. Bacterial consortium RVM11.1 showed ability to decolorize 10 different dyes tested.

Pseudomonas aeruginosa NBAR12 was isolated from dye contaminated soil obtained from industrial estate of Ahmedabad, Gujarat, India by Bhatt et al., (2005). The strain was capable of decolorizing twelve different dyes with decolorization efficiency varying between 80 and 95%. Maximum rate of decolorization of Reactive Blue 172 (RB 172) was observed when glucose (2 g/L) and yeast extract (2.5 g/L) were supplemented in the culture medium. The optimum conditions for decolorization were pH 7.0 and temperature 40°C. The decolorizing activity was found to increase with increase in dye concentration from 50 to 400 mg/L. At 500 mg/L dye concentration, the decolorization activity was strongly inhibited. HPLC analysis indicated that dye decolorization occurred due to the breakdown of dye molecules into colorless end products.

Parshetti et al., (2006) reported a study on malachite green (50 mg/L) which was completely decolorized under static anoxic condition within 5 h by bacterium Kocuria rosea MTCC 1532. Decolorization was not observed under shaking condition. K. rosea has also shown decolorization of azo, triphenylmethane and industrial dyes (Cotton Blue, Methyl Orange, Reactive Blue 25, Direct Blue 6, Reactive Yellow 81, and Red HE4B). Semi-synthetic media containing molasses, urea and sucrose have shown 100, 91, 81% decolorization, respectively. Induction of malachite green reductase and DCIP reductase was observed during MG decolorization suggesting their involvement in the decolorization process.

Samples collected from various effluent-contaminated soils in the vicinities of dyestuff manufacturing units of Ahmedabad, India, were studied for screening and isolation of organisms capable of decolorizing textile dyes by Junnarkar et al., (2006). A bacterial consortium was developed based on the rapid decolorization of
Direct Red 81 (DR 81). The bacterial consortium exhibited 90% decolorization ability within 35 h when starch (0.6 g/L) and casein (0.9 g/L) were supplemented in the medium. The optimum conditions for decolorization were pH 7.0 and incubation temperature of 37°C with dye concentration of 200 mg/L. Decolorization of DR 81 was monitored by HPLC, which indicated that dye decolorization was due to its degradation into unidentified intermediates.

The ability of *Rhodopseudomonas palustris* AS1.2352 possessing azoreductase activity to decolorize azo dyes was investigated by Liu *et al.*, (2006). It was reported that anaerobic conditions were necessary for bacterial decolorization. The optimal conditions of pH and temperature were 8.0 and 30 - 35°C, respectively. Decolorization studies of dyes with different molecular structures were performed to compare their degradability. The strain could decolorize azo dye up to 1250 mg/L, and the correlation between the specific decolorization rate and dye concentration was described by Michaelis-Menten kinetics. Cell extracts from the strain revealed oxygen-insensitive azoreductase activity in vitro.

A study on biodegradation potentials of textile effluent-adapted and non-adapted bacteria was carried out by Olukanni *et al.*, (2006). The bacteria were isolated from textile industries wastewater and drains (textile effluent adapted bacteria-T strains) and from a municipal landfill (effluent non-adapted bacteria-N strains). They discovered effluent adapted strains of *Acinetobacter*, *Bacillus* and *Legionella* with potentials for colour removal and strains of *Acinetobacter*, *Bacillus* and *Pseudomonas* with COD removal potential. The T-strains showed 40.95% COD removal with 40.62% decolorization whereas N-strains showed 55.89% COD removal with 43.95% decolorization.

Among the 27 strains of halophilic and halotolerant bacteria isolated from textile industry effluent by Azad *et al.*, (2007), three showed remarkable ability in decolorizing azo dyes. The three strains were able to decolorize azo dyes in a wide range of operational conditions; NaCl concentration (up to 20% w/v), temperature (25 - 40°C), and pH (5.0 - 11.0) after 4 days of incubation in static culture. The strains could decolorize the pure dyes and mixture of dyes. They decolorized up to 5000 ppm of the dye and could tolerate up to 10,000 ppm of the dye. UV-Visible spectrophotometric analyses pre and post decolorization and the colorless bacterial
biomass after decolorization suggested that decolorization was due to biodegradation, and not by inactive surface adsorption. HPLC analysis showed that the principal decolorization was due to reduction of the azo bond, followed by cleavage of the reduced bond.

Kalme et al., (2007) reported study on Red HE7B (RHE7B, 100 mg/L), a sulfonated azo dye, which was decolorized by Pseudomonas desmolyticum NCIM 2112, at static condition in 72 h with 71% reduction in COD. Acclimatization of the bacterial strain had reduced the time required for complete decolorization of 100 mg/L dye from 168 to 72 h. Decolorization of RHE7B was 95% at static condition as against 53% at shaking condition. Growth was observed to be more at shaking (0.46 g/L) as compared to static condition (0.32 g/L). It was shown that extracellular lignin peroxidase had played a crucial role in breakdown of the dye by asymmetric cleavage and reductases in the initial 24 h incubation to break azo bonds of the dye molecule.

Four facultative bacteria of Enterobacter sp., Serratia sp., Yersinia sp. and Erwinia sp. were investigated for their ability to degrade C.I. Reactive Red 195 in solid and liquid medium by Jirasripongpun et al., (2007). Their decolorization ability in liquid medium under anaerobic conditions was superior compared to the aerobic conditions. Enterobacter sp. removed the dye up to 90% within 2 days of incubation. The decolorizing activity was highly dependent on the dye concentration, the presence or absence of glucose and/or peptone and pH. Shaking or static conditions had no discernible effects on the decolorization process. It was demonstrated that the dye removal by Enterobacter sp. was only partial due to dye adsorption to cell mass which represented decolorization rather than further complete degradation of the aromatic amines.

Uddin et al., (2007) studied the microbial decolorization of azo dye Acid Red B under high salinity conditions by Gracilibacillus sp. GTY. Decolorization experiments were carried out using growing and resting cells, as well as by extracting azo reductase. Strain grown in the media containing 15% (w/v) of NaCl showed best decolorization. Strain grown in very low or high concentrations of salt did not show good decolorization, implying that salt concentrations in the surroundings control the production of azo reductase. The optimum conditions for the strain were temperature ±30°C, pH 7.2 and 10 -15% w/v of NaCl.
Dawkar et al., (2008) studied the degradation of disperse dye Brown 3REL by Bacillus sp. VUS. The strain was able to degrade 100% of the dye within 8 h at static anoxic condition. A significant increase in the activities of lignin peroxidase, laccase and NADH-DCIP reductase was observed up to complete decolorization of Brown 3REL. The optimum conditions for degradation were temperature 40°C and pH 6.5 - 12.0. Phyto-toxicity tests and COD revealed non-toxic products of dye degradation. The final products 6,8-dichloro-quinazoline-4-ol and cyclopentanone were characterized by GC-MS. Bacillus sp. VUS also decolorized 80% of the textile dye effluent within 12 h.

Deng et al., (2008) worked on isolation and characterization of Bacillus cereus DC11, capable of decolorizing three main groups of dyes. Operational variables such as pH, temperature, oxygen concentration and carbon source were used to comparatively study the decolorizing effects on anthraquinone, triphenylmethane, and azo dyes, respectively. High decolorization efficiency (95 - 98%) was achieved within 6 h of incubation for 100 mM Acid Blue 25 (anthraquinone dye), 4 h for 55 mM Malachite Green (triphenylmethane dye), and 2 h for 750 mM Basic Blue X-GRRL (azo dye) at 20 - 45°C temperature and neutral pH under anaerobic conditions. Among the decolorized products, there was an intermediate metabolite after the degradation of Acid Blue 25 dye. Malachite Green was degraded into 4, 40-bis (dimethylamino) benzophenone and benzophenone, and the decolorization of Basic Blue X-GRRL was probably due to the reduction of azo bonds.

A study on decolorization of Acid Black 210 by a bioluminescent bacterium, Vibrio harveyi TEMS1, isolated from coastal seawater of Izmir Bay, Turkey was reported by Ozdemir et al., (2008). Maximum rate of decolorization of Acid Black 210 was observed when Luria Bertani medium was used. Decolorization of Acid Black 210 was 38.9% and 93.9% at 24 h under shaking and static conditions, respectively. The optimum dye-decolorizing activity of the culture was obtained at 100 ppm of dye concentration and incubation temperature of 20°C. Vibrio harveyi TEMS1 was also tested for its ability to decolorize four azo dyes namely, Acid Black 24, Acid Blue 7, Acid Green 20 and Acid Yellow 36.

Khadijah et al., (2008) reported isolation, screening and development of local bacterial consortia with azo dye decolorizing potential. Nine isolates from a total of
1540 bacterial colonies were screened for their ability to degrade selected azo dyes. Several combinations of microbial consortia were developed and tested for their effectiveness. Overall the consortia were able to remove 70 - 100% of colour within 72 h compared to 60 - 97% colour removed by individual isolates. A microbial consortium labelled C15 showed good growth in agitation culture but the colour removal was best in static culture with 80 - 100% colour removal in less than 72 h. Based on the 16S rRNA sequencing, two of the bacterial isolates in C15 were found to belong to *Chryseobacterium* genus while the other one belonged to *Flavobacterium* genus.

In an investigation by **Ayed et al., (2009)**, *Sphingomonas paucimobilis* was isolated from the soil sample collected in the vicinity of a textile industry located in KsarHellal, Tunisia. The effect of inoculum size, dye concentration, temperature and initial pH of the solution were studied. The strain was able to decolorize 82.49% of Malachite Green (50 mg/L) within 4 h under shaking condition at pH 9.0 and temperature 25°C. Bacterial inoculum size of 1 OD showed maximum decolorization.

CK3, a bacterial strain with a potential to decolorize the Reactive Red 180, was isolated from the activated sludge collected from a textile mill by **Hui et al., (2009)**. Molecular characterization indicated that the bacterial strain belonged to the genus *Citrobacter*. CK3 showed strong ability to decolorize various reactive textile dyes, azo and anthraquinone dyes. The optimum decolorizing conditions were glucose 4 g/L, pH 7.0 and temperature 32°C under anaerobic conditions. *Citrobacter* sp. CK3 showed 95% decolorization in 36 h in high concentration of dye (200 mg/L) and could tolerate up to 1000 mg/L of dye. Spectrophotometric analysis indicated that the decolorizing activity of *Citrobacter* sp.CK3 was through biodegradation, rather than inactive surface adsorption.

**Gopinath et al., (2009)** reported biodegradation of Congo Red by mutant *Bacillus* sp. by random mutagenesis approach. The mutants of the *Bacillus* sp. were screened based on their decolorization performance and best mutants were selected for further studies. Improved decolorization was observed with dye concentration in the range of 100 -1000 mg/L for wild species whereas mutant strain was found to offer better decolorization up to 3000 mg/L. Mutant strain offered 12 - 30% reduction in time required for the complete decolorization. The optimum pH and temperature were found to be 7.0 and 37°C, respectively. Two efficient strains *Bacillus* sp. ACT 1
and *Bacillus* sp. ACT 2 were isolated from various mutants obtained. *Bacillus* sp. ACT 2 showed improved enzymatic production and *Bacillus* sp. ACT 1 showed improved growth compared to wild strain. The enzyme responsible for the degradation was found to be azoreductase. About 53% increased production of enzyme was achieved with mutant species.

**Vasileva et al., (2009)** carried out a study on the activated sludge samples collected from wastewater treatment plant of a textile factory in Giorgetti Bulgaria AD and they were exploited for isolation of dye decolorizing bacteria. A microbial strain AZO29 was selected based on its efficiency, showing maximum and faster decolorization of Amaranth dye. AZO29 (700 mg/L) showed 100% decolorization after 24 h and degraded up to 1400 mg/l of the azo dye in 72 h in liquid medium. The decolorization rate increased with increase in Amaranth concentration from 0.165 to 0.265 mM. Over 90% conversion was achieved by AZO29 for a range of azo dye concentration up to 0.265 mM.

A statistically valid Plackett-Burman experimental design was incorporated to study the decolorization potential of *Pseudomonas* sp. strain DY1 on heavy metal-containing dye Acid Black 172 by **Du et al., (2010)**. Important parameters such as pH, temperature, concentrations of Fe$^{3+}$ and NaH$_2$PO$_4$ were optimized. A quadratic model obtained from the response surface design was constructed on experimental data. The optimal conditions for decolorization were found to be pH 6.23, temperature 30°C, 8.0 mM of Fe$^{3+}$ and 10 g/L of NaH$_2$PO$_4$. The confirmatory experiments (86.5% decolorization) verified the accuracy of the experimental model. Moreover, the decolorization under the optimal conditions fitted the logarithmic model well ($R^2=0.964$).

**Dubey et al., (2010)** studied the dye degrading ability of *Bacillus* species isolated from soil contaminated with textile mill effluent. The strain utilized Golden Yellow HER, an azo dye as sole source of carbon and nitrogen. Complete decolorization (100%) of the dye (1%) was obtained on day 6 at 30°C temperature and pH 7.0. The azo-nitrogen of the dye substrates provided nitrogen required by the organism in cultures in the absence of nitrogen. Ring opening of the aromatic moiety of the dye produced the carbon source required for the organism. The competition between (NO$_3^-$) and the chromophoric group for the redox equivalents were evident which resulted in preferential reduction of (NO$_3^-$) relative to the chromophoric group.
Jadhav et al., (2010) studied the decolorization potential of *Pseudomonas aeruginosa* strain BCH isolated from sediments contaminated with dyestuff. The decolorization of Direct Orange 39 (Orange TGLL) at 50 mg/L within 45 ± 5 min gave 93.06% decolorization, while it could decolorize 1.5 g/L of dye within 48 h with 60% decolorization at an optimum pH of 7.0. Induction in the activities of lignin peroxidases, DCIP reductase as well as tyrosinase was observed, indicating the significant role of these enzymes in biodegradation of Direct Orange 39.

Modi et al., (2010) studied the potential of isolated bacterial strain from dye house effluent to decolorize sulfonated azo dyes and the influence of environmental factors on dye decolorization process. Among seven bacterial isolates, M1 (*Bacillus cereus*) and M6 were found to be more potent for decolorizing sulfonated azo dyes under aerobic conditions. The decolorization efficiency of M1 (*B. cereus*) was enhanced with maltose as carbon source and peptone as nitrogen source. M6 showed maximum decolorization (87%) at pH 7.0. HPTLC studies proved that more than 97% of the dye (Reactive Red 195) was degraded by bacteria after 72 h of incubation.

Nosheen et al., (2010) studied the effect of added carbon and nitrogen sources on decolorization of reactive dyes (Black B and Orange 16). Reactive Black-B showed maximum decolorization of 74% by bacterial strain B1 and 87% by B2. The results indicated that additional nutrient sources were effective in increasing dye decolorization rate only at lower concentrations of 2 mg/ml. At higher concentrations they decreased colour removal up to 50%. Moreover, extent of colour removal was greatly affected by type of microbial consortia used and chemical structure of the dye.

Patil et al., (2010) studied the effect of bacterial consortium on decolorization of Red HE3B. The consortium PMB11 consisted of three bacterial species namely, *Bacillus odysseyi* SUK3, *Morganella morganii* SUK5 and *Proteus* sp. SUK7. PMB11 decolorized 99% of Red HE3B (50 mg/L) within 12 h in nutrient broth, while in mineral medium it decolorized up to 97% within 24 h. Induction in the activities of various oxidative and reductive enzymes indicated the involvement of these enzymes in decolorization process. GC-MS analysis of the degraded dye showed presence of metabolites such as 2,5-diaminobenzene 6-aminotriazine, aniline 2-sulfate, aniline 3-sulfate, 2-amino 5-chlorotriazine and naphthalene.
Investigation on decolorization of Crystal Violet by aerobic bacteria isolated from soil collected in the vicinity of a dye industry was reported by Thorat et al., (2010). The study revealed that there was 92.03% decolorization of the dye by an unidentified acclimatized bacterial culture - CD11 at 24 h incubation at room temperature and at neutral pH. No significant increase in decolorization was observed when the bacteria were supplemented with co-substrate (1% Glucose). GC-MS analysis was carried out for confirmation of degradation. Five degradation products were extracted and identified.

Ndasi et al., (2011) reported the dye decolorization potentials of local microbial consortia isolated from dye contaminated soils in Ngaoundere-Cameroon against two azo dyes. Effect of operational parameters on dye decolorization rate was optimized using the full factorial design. Microbial growth and decolorization rate were higher in shaking than static conditions. The bacterial consortium showed highest decolorization for Azo Blue (91.86%) and Azo Orange (93.75%) within 48 h in shaking cultures whereas it was 57.78% and 62.06% respectively after 48 h under static condition. pH in the range of 7.2 - 8.0 and dye concentration above 100 mg/L reduced decolorization rate by the bacterial consortia. Increasing inoculum size increased the extent of decolorization.

Prasad et al., (2011) studied the decolorization of Acid Red 128, an azo dye by Bacillus endophyticus VITABRI13, which was isolated from the textile industry effluent collected from Coimbatore, Tamilnadu, India. Effects of physicochemical parameters such as pH, temperature, carbon and nitrogen sources on dye decolorization by the selected bacterium were analyzed. Decolorization of the dye to the extent of 90% was reported at pH 8.0, 35°C with starch and peptone as carbon and nitrogen sources, respectively and in static condition.

Degradation of mixed textile dyes by bacterial strains isolated from dyewaste effluent collected from common effluent treatment plant (CETP) located in Tiruppur and Telungupalayam, Tamil nadu was reported by Rajeswari et al., (2011). Through an acclimitization study, 5 most effective strains were selected out of 112 strains isolated, based on their decolorization of the dye up to 2700 mg/L, yeast extract (0.5%) was the best nitrogen source for 98% decolorization of the mixed dye. Optimum conditions for decolorization were pH 7.0, temperature 30°C with culture under static condition.
In a report by Ogugbue et al., (2011), a bacterial strain capable of decolorizing triarylmethane dyes was isolated from a textile wastewater treatment plant in Greece. The bacterial isolate was identified as *Aeromonas hydrophila* and was shown to decolorize three triarylmethane dyes tested within 24 h with colour removal in the range of 72 - 96%. Decolorization efficiency of the bacterium was a function of operational parameters (aeration, dye concentration, temperature, and pH) and the optimal operational conditions obtained for decolorization of the dyes were: pH 7 - 8, 35°C and culture agitation. Effective colour removal within 24 h was achieved at a maximum dye concentration of 50 mg/L.

Parshetti et al., (2011) studied the effect of *Agrobacterium radiobacter* MTCC 8161 on decolorization of Crystal Violet. Complete decolorization of the dye was seen within 8 h (10 mg/L) at static anoxic conditions. The decreased decolorization capability of *A. radiobacter* was observed, when the dye concentration was increased from 10 to 100 mg/L. Semi-synthetic medium containing 1% yeast extract and 0.1% NH₄Cl showed 100% decolorization of Crystal Violet within 5 h. A complete degradation of Crystal Violet by *A. radiobacter* was observed up to 7 cycles of repeated addition (10 mg/L). When the effect of increasing inoculum size on decolorization of the dye (100 mg/L) was studied, maximum decolorization was observed with 15% inoculum size. A significant increase in the activities of laccase (184%) and aminopyrine N-demethylase (300%) in cells obtained after decolorization indicated the involvement of these enzymes in decolorization process.

Studies on Congo Red decolorization by local isolate VTII inhabiting dye effluent exposed soil, collected from Baddi (H.P) was carried out by Sawhney et al., (2011). A total of 7 strains were obtained with potential for Congo Red decolorization. On primary screening, VTII, an aerobic gram positive *Bacillus* (*Bacillus* sp.) was found to have maximum observable azo dye decolorization ability. The isolate on secondary screening showed 70% decolorization. Maximum decolorization of 85% was reported under optimal conditions of pH 7.0 and temperature 40°C.

Decolorization of Red 3BN by two bacterial species *Bacillus cereus* and *Bacillus megaterium* was studied by Kumar and Bhat, (2012). The strains have been isolated using mineral effluent consisting of known concentration of the dye in ZZ medium. Optimization of culture condition was carried out using OFAT design.
Optimal condition for *B. cereus* was found to be 1% sucrose, 0.25% peptone, pH 7.0, 37°C and 8% inoculum and for *B. megaterium* it was found to be glucose 1%, 0.25% yeast extract, pH 6, 37°C and 10% inoculum. Under optimal conditions, *B. cereus* and *B. megaterium* showed 93.64% and 96.88% decolorization, respectively.

**Tripathi and Srivastava, (2012)** reported decolorization of azo dye by *Bacillus megaterium* ITBHU01. Response surface methodology involving PB design was used to optimize the operational parameters such as pH, temperature, dye concentration, inoculum size and time. All five parameters showed significant effect (P<0.05) on decolorization of Orange G dye. The values of parameters were optimized by applying central composite design and the optimal values for Orange G decolorization were pH 6.9, temperature 37°C, dye concentration 517 mg/L, inoculum size 5.5 % and time 23.7 h. Under these optimized conditions, decolorization of Orange G was 94.48% in static condition.

**Murthy et al., (2012)** studied the bacterial cultures isolated from Environmental Treatment Plant Naroda G.I.D.C., Ahmedabad, Gujarat. The bacterial cultures were subjected for acclimatization to C.I. Reactive Blue 250 (RB 250), in Bushnell and Haas broth. The most efficient bacterial isolate was used for further degradation studies. The strain SpNB6 showed complete decolorization of the selected dye (RB 250 - 100 mg/L) within 8 h in static condition. The optimum pH, temperature, carbon and nitrogen sources for the decolorization were 7.0, 37°C, glucose (0.2 %) and nitrogen (0.5 %), respectively.

**Waghmode et al., (2012)** developed a new batch method by consortium GGBL using two microbial cultures *Galactomyces geotrichum* MTCC 1360 and *Brevibacillus laterosporus* MTCC 2298, for decolorization of Rubine GFL. *G. geotrichum* and *B. laterosporus* showed 50% and 92% decolorization under aerobic/microaerophilic condition, with 24% and 69% of COD reduction as well as 17% and 55% of TOC reduction, respectively. The combined metabolic activity of these strains led to 100% decolorization of the dye (50 mg/L) within 30 h of incubation with significant reduction in COD (79%) and total organic carbon (68%). The optimum pH and temperature for Rubine GFL decolorization were 7.0 and 40°C, respectively. Consortium showed better decolorization of Rubine GFL as compared to the individual microorganism in aerobic/microaerophilic process. Induction in the
activities of enzymes such as laccase, veratryl alcohol oxidase, tyrosinase, azo reductase, and riboflavin reductase indicated their role in the decolorization process.

A bacterial consortium was developed using five different bacterial strains isolated from the effluent obtained from textile mills with the ability to degrade Acid Blue 113, a diazo dye by Nachiyar et al., (2012). The organisms were identified as Citrobacter freundii (2 strains), Moraxella osloensis, Pseudomonas aeruginosa SUB10 and Pseudomonas aeruginosa CLRI BL22. The consortium degraded 90% of the dye in 22 h in 80% diluted textile effluent when supplemented with glucose and ammonium nitrate. Optimization studies using response surface methodology have confirmed that the degradation process was predominantly influenced by agitation and pH whereas glucose was found to have a negative effect. TLC analyses indicated the presence of metanilic acid and peri acid in 24 h sample which disappeared by 48 h. The GC-MS analysis has confirmed the presence of methyl salicylic acid, catechol and β-ketoacidic acid with the R_T values of 7.71, 10.88 and 15.04, respectively confirming the complete degradation of Acid Blue 113.

Study by Patel et al., (2012) deals with the decolorization of diazo dye Acid Maroon V by consortium EDPA that comprised of two isolates, Enterobacter dissolvens AGYP1 and Pseudomonas aeruginosa AGYP2, obtained from a contaminated site. Decolorization of the dye to the extent of 93% (100 mg/L) was observed in mineral salt medium in 20 h under static incubation condition. The consortium showed the decolorization activity over a wide range of pH values (6.0-9.0) with peak activity at pH 7.0. The consortium also decolorized relatively high concentrations of Acid Maroon V (100 - 2000 mg/L). The addition of sucrose along with ammonium dihydrogen phosphate increased the decolorization rate. The consortium also showed 31% reduction in COD within 20 h. The consortium had the ability to decolorize 16 other textile dyes.

Giwa et al., (2012) reported on decolorization of anthraquinone dye C.I. Reactive Blue 19 (RB19) using B. cereus isolated from contaminated food. Effects of various process parameters such as initial dye concentration, glucose, and temperature on dye decolorization were investigated. The bacterial isolate exhibited 95% decolorization within 72 h. The optimum dye decolorizing activity of the culture was observed at pH 7.0 and incubation temperature of 27°C. Maximum dye decolorizing efficiency was observed at 200 mg/L concentration of RB19.
A decolorization study of textile dye Victoria Blue-B (VB-B) by Babu et al., (2013) highlighted two different approaches: one to remove the colour from the dye and the other is to reuse the decolorized water for coloring the same dye. *Shewanella decolorationis* (MBTD16) isolated from Dona Paula Bay was employed for this purpose; 94.83% decolorization was achieved at dye concentration of 50 mg/L. The colored water treated by free and immobilized bacterial cells tested to reuse (VB-B) gave 35 - 50% more colour than the original. Process parameters optimized to achieve maximum decolorization were pH 7.0, temperature 32±2°C, inoculum size 8 % with co-substrates glucose and yeast extract of 5 g/L. Synthesis of lignin peroxidase and tyrosinase were augmented in *S. decolorationis* only after being exposed to the dye. These results indicated that *S. decolorationis* could be efficiently used for both removal of colour from the dye and recycling it back to gain its original colour by adding around half the quantity of the dye.

Joshi et al., (2013) studied the role of *Bacillus megaterium* isolated from the effluent sample collected near VATVA G.I.D.C. (Gujarat) in decolorization of Turquoise Blue dye (Remazol Blue BB). The dye showed maximum decolorization (95%) within 48 h at pH 7.0 and 37°C with dye concentration of 1mg/ml. The other dyes decolorized were Blue M2R, Safranin, Congo Red, Malachite Green, Orange ME2RL and Yellow M8G. Glucose and NH₄Cl of 1% each were found to be the best carbon and nitrogen sources, respectively for maximum degradation process.

Liu et al., (2013) studied the azo dye decolorization using *Shewanella* strains under saline conditions. Growing cells of *Shewanella algæ* and *Shewanella marisflavi* isolated from marine environments demonstrated better azo dye decolorization capacities than other three strains from non-saline sources. Cell suspensions of *S. algæ* and *S. marisflavi* could decolorize single or mixed azo dyes with different structures. Lactate and formate were identified as efficient electron donors for Amaranth decolorization by the two strains. *S. algæ* and *S. marisflavi* could decolorize Amaranth up to 100 g/L NaCl or Na₂SO₄. Extremely low concentration of NaNO₃ exerted strong inhibition on decolorization. Both strains could remove the colour and COD of textile effluent during sequential anaerobic-aerobic incubation. Lower concentrations of NaCl (20 - 30 g/L) stimulated the activities of azoreductase, laccase, and NADH DCIP reductase. Amaranth decolorization by five different *Shewanella* strains was investigated in the presence of
0 - 70 g/L NaCl. The presence of NaCl significantly restrained the decolorization performance of *S. oneidensis* MR-1 and *S. decolorationis*. In the absence of NaCl, MR-1 removed 98.4 % amaranth in 24 h. However, 31 - 109 h was required by MR-1 to reach similar decolorization efficiency in the presence of 10 - 50 g/L NaCl. Only 42.5 and 32.2 % Amaranth were reduced in 109 h in the presence of 60 and 70 g/L NaCl, respectively. Amaranth was almost completely decolorized in 24 h by *S. decolorationis* under non-saline conditions or in the presence of 10 g/L NaCl. In the same period, only 66.6, 27.0, 13.7, and 4.2 % Amaranth were removed in the presence of 20 - 50 g/L NaCl, respectively. Negligible decolorization was observed when the salinity was higher than 60 g/L. Around 29 - 54 h were taken by *S. decolorationis* to realize complete removal of amaranth in the presence of 20 - 40g/L NaCl. Decolorization efficiencies of 12.7 - 72.9 % were reached in 86 h in the presence of 50 - 70 g/L NaCl. Complete removal of Amaranth in 75 h was observed by *S. putrefaciens* in the absence or presence of 10 - 20 g/L NaCl. However, a further increase of NaCl concentration from 30 - 70 g/L resulted in decrease in its decolorization capacity. Over 120 h were needed to realize complete colour removal when NaCl concentration was higher than 40 g/L.

*Comamonas acidovorans* MTCC 3364 was employed for decolorization of Reactive Orange 16 dye in a study carried out by *Rudakiya and Pawar, (2013).* The purpose of this study was to check the decolorization efficiency required for different dyes and to optimize the condition which gave maximum decolorization of the dye. The effect of various operational parameters such as carbon and nitrogen sources, temperature, pH and dye concentration were assessed. *C. acidovorans* showed 99.03% decolorization of Reactive Orange 16 at 37°C, pH 6.85 within 24 h incubation.

*Saratale et al., (2013)* studied decolorization of C.I. Remazol Red by *Lysinibacillus* sp. RGS isolated from the soil of Ichalkaranji textile industrial area. Under optimal culture conditions of 30°C, pH 7.0, under static condition, there was 92% reduction in COD with 6 h of incubation. Operational parameters such as agitation, pH, temperature and initial dye concentration were optimized to maximize and get faster decolorization. The supplementation of low cost co-substrates (extracts of agricultural wastes) enhanced the decolorization performance of *Lysinibacillus* sp. RGS. Induction in oxidoreductive enzymes presumably indicated the involvement of
these enzymes in degradation process. Application of the isolate to decolorize mixture of dyes and real industrial effluent showed 87% and 72% decolorization with 69% and 62% COD reduction within 48 h and 96 h, respectively.

Shertate et al., (2013) reported the decolorization of Acid Yellow 25 by *Marinobacter gudaonensis* AY-1 isolated from natural marine environment. The decolorization of Acid Yellow 25 in nutrient broth and half strength nutrient broth having 8.0% salt concentration was up to 92% and 90.03% respectively in 24 h. The decolorization of the dye by cell-free extract was found to be up to 80.13% in 24 h. The decolorization of the dye was also studied in presence of different co-substrate, 1% each of glucose, yeast extract and starch and found that decolorization was up to 92.77%, 94% and 92.05%, respectively. The COD reduction of the dye by the strain was 70%. The degradation products formed were analyzed by GC-MS analysis and the degraded dye had the products having molecular weights 98, 70, 112, 125, 140, 168, 186, 128, 141, 83, 111, 154, 72 and 155.

A study by Zuraida et al., (2013) focussed on treatment of batik industry wastewater using *Lactobacillus delbruckii*. The effects of agitation, pH and temperature on the *L. delbruckii* growth and decolorization efficiency were assessed. The bacterial strain was incubated under aerobic conditions in 30% (v/v) batik wastewater in MRS broth at different temperatures and pH ranges for 84 h. The microbial growth and decolorization of batik wastewater were monitored. The strain showed good growth in agitation culture but the colour removal was best in static culture with 45 - 60% colour removed in less than 72 h. The optimum pH and temperature for growth of microbe were 7.0 and 37°C and for decolorization of batik wastewater 6.0 and 37°C, respectively.

Muhammad et al., (2014) reported isolation of thirty bacterial strains with azo dye degrading potential from textile effluents. Among the screened isolates, strain IFN4 identified as *Shewanella* sp. was most efficient in decolorizing Reactive Black-5 (200 mg/L) in mineral salts medium. The colour removal was 92.5% in 4 h of incubation. A mixture of dyes was also subjected to decolorization. Bacterium degraded the dye mixture at varied pH (5.0 - 10.0) and temperature (20 - 50°C), but with different efficiency. Maximum decolorization of dye mixture was recorded at pH 8.5 and 35°C under static condition with 10% (v/v) inoculum concentration. Bacterial growth was more under shaking than static condition.
An investigation by Shah et al., (2014) explored the decolorization potential of azo dyes using bacteria isolated from textile dye effluent. Three predominant bacterial species identified were Bacillus subtilis, Pseudomonas aeruginosa and Psuedomonas putida. The decolorization of azo dyes (500 mg/L) was carried out with trace amounts of nitrogen and carbon sources - yeast extract, glucose and sucrose with different bacterial inoculum and incubated for 4 days. Psuedomonas putida (95%) showed maximum decolorization of Blue RR and Pseudomonas aeruginosa (93%) showed best decolorization of Black B. Bacillus subtilis decolorized 91% of Red RR and 65% of Yellow RR. Pseudomonas aeruginosa decolorized 70.58% of Navy Blue.

A study by Suganya et al., (2014) was carried out to degrade the textile Reactive azo dyes by using bacteria isolated from dye contaminated soil near the Erode effluent discharge points. The effect of parameters such as pH, temperature, carbon and nitrogen sources, inoculum size and incubation time were used to determine the optimal conditions for maximum decolorization. The isolated bacterial strains were Bacillus licheniformis and Pseudomonas putida. The optimum pH and temperature for decolorization of RR 195 by P. putida were 7.0 and 37°C respectively. It showed good decolorization efficiency even in alkaline condition. Glucose and yeast extract were found to be the most suitable carbon and nitrogen sources respectively, for both bacteria. Three percent (v/v) inoculum size and 120 h incubation time were most favourable conditions for highest percentage decolorization by both bacterial strains.

An attempt was made by Suad et al., (2014) to examine the potential of different bacterial strains for biodecolorization of azo dye under aerobic condition. The bacterial strains used in the study were Escherichia coli, Pseudomonas aeruginosa, Bacillus sp. and Klebsiella sp. E coli. showed higher decolorization as compared to other bacteria. Maximum decolorization was observed with 0.1 mg/L of Direct Orange (DO), Disperse Brown (DB) and Reactive Green (RG) with Pseudomonas aeruginosa (94%, 88% and 72% respectively) after 7 days of incubation. The minimum removal was observed with 0.5 mg/L of D.O, D.B and R.G. with Klebsiella sp. (80%, 45% and 29% respectively) after 6, 7 days and 1 day of incubation, respectively.

Abraham and Muraleedhara, (2014) studied the native bacterial strains capable of decolorizing Acid Orange 7 (AO7), a sulphonated mono azo dye, isolated
from soil and water samples collected from dye contaminated sites of a coir industry in Alleppy, Kerala, India. Twenty five bacterial isolates were screened for decolorization potential in nutrient broth of which five isolates (*Bacillus amyloliquefaciens* (A2), *Bacillus subtilis* (A3), *Bacillus amyloliquefaciens* (B1), *Sphingomonas paucimobilis* (B2) and *Acinetobacter genomospecies* (B3) were capable of decolorizing AO7. Of the selected isolates, B1 was the best dye decolorizer with percent decolorization of 84.53, followed by A2, B3, A3 and B2 with an efficiency of 73.31%, 67.26%, 62.32% and 54.65%, respectively.

**Khan et al., (2014)** carried out isolation of dye decolorizing microbes from soil samples of dye-contaminated sites. A novel bacterial mixture, RkNb1 was selected based on its efficiency, showing maximum and faster decolorization of textile dyes. The seven bacterial strains identified from the bacterial mixture by 16S rRNA gene sequence analysis were *Ochrobactrum intermedium* (HM480365), *Ochrobactrum intermedium* strain M16-10-4(HM030758), *Enterococcus faecalis* (HM480367), *Arthrobacter crystallopoietes* (HM480368), *Kocuria flavus* (HM480369), *Bacillus beijingensis* (HM480370), and *Citrobacter freundii* (HM480371). This bacterial consortium showed 98.17% decolorization of Reactive Violet 5 (RV5) (400 mg/L) within 8 h. The culture exhibited good decolorization ability at pH 8.0 and temperature 37°C. PB experimental design was used for elucidation of medium components affecting RV5 decolorization. The results indicated that presence of high levels of ammonium sulfate, peptone, malt extract, and MgSO$_4$ as well as temperature and inoculum size in the growth medium affected RV5 decolorization positively. The optimum conditions for RV5 decolorization by RkNb1 (g/L) were; K$_2$HPO$_4$, 3.0; (NH$_4$)$_2$SO$_4$, 1.5; peptone, 1.5; malt extract, 0.5; MgSO$_4$.7H$_2$O, 0.25; NaCl, 0.05; CaCl$_2$.2H$_2$O, 0.06; pH 7.0; temperature 42°C; inoculum size 5 ml; and dye concentration 100 mg/L.

**Singh et al., (2014)** reported a bacterial strain (RMLRT03) with ability to decolorize textile dye Acid Orange (AO) which was isolated from textile effluent contaminated soil of Tanda, Ambedkar Nagar, Uttar Pradesh (India). The decolorization studies were performed in Bushnell and Haas medium amended with Acid Orange dye. The bacterial strain was identified as *Staphylococcus hominis* on the basis of 16S rDNA sequence. The bacterial strain exhibited good decolorization potential with glucose and yeast extract as co-substrates in static condition. The
optimal conditions for the decolorization of AO dye by Staphylococcus hominis RMLRT03 strain were: pH 7.0 and temperature 35°C in 60 h incubation. *S. hominis* could tolerate up to 600 mg/L of AO dye.

### 2.5.2 Fungal decolorization and degradation of dyes

*Cing et al.,* (2003) reported the use of fungal pellets for decolorization of textile dyeing wastewater. Colour removal of 95% from wastewater within one day was shown by live pellets of *Phanerochaete chrysosporium*. The dye removal capacity was a function of time and was proportional to the agitation rate; the optimum temperature was 30°C. The live and dead pellets were further observed in a repeated-batch mode for 5 days. The decolorization performance of live pellets was high and stable for 5 days and they showed two to three higher decolorization capacity than dead pellets.

*Kasinath et al.,* (2003) reported about white rot fungus, *Irpex lacteus* grown in both stationary and agitated submerged liquid cultures as well as in cultures immobilized on polyurethane foam (PUF) and pine wood (PW) to study the effect of growth conditions on degradation of Remzol Brilliant Blue R (RBBR). The decolorization of (RBBR) dye at 150 µg/ml was 100, 95, 97 and 100% within 6 - 10 days. Stationary culture exhibited higher levels of lignin peroxidase (LiP), manganese-peroxidase (MnP) and laccase than submerged cultures. Analyzing the decolorization potential of PUF and PW immobilized cultures decolorizing RBBR, five-fold MnP levels were detected in the former cultures whereas laccase activities were similar. Both the immobilized cultures were able to rapidly decolorize RBBR and also various textile industry effluents. Reusability and regenerative capacity of the immobilized culture was assessed for application in bioremediation process.

*Ozfer et al.,* (2003) studied decolorization of various dyes by pellets of white rot fungi. Fungal pellets removed more than 75% of the colour of the dyes in 24 h. The decolorization of Astrazine Blue dye by *Funalia trogii* was assessed in terms of the effects of factors such as initial pH, concentration of dye, amount of pellet, temperature and agitation. The longevity of the decolorization activity under optimum conditions was observed in repeated-batch mode experiments. An increase in the amount of pellet positively influenced the longevity of the decolorization activity while an increase in dye concentration decreased the dye decolorization capability of pellets.
Selvam et al., (2003) studied the white rot fungus, *Fomes lividus*, isolated from the logs of *Shorea robusta* in the Western Ghats region, Tamilnadu, India. The decolorization of azo dyes such as Orange G (50 µM) Congo Red (50 µM) and Amido Black 10B (25 lM) were tested by using the fungus. The fungus removed 30.8% of Orange G in the synthetic solution, whereas Congo Red and Amido Black 10B were removed to the extent of 74.0 and 98.9%, respectively. The dye industry effluent was treated by the fungus in batch and continuous mode. In batch mode treatment, 84.4% decolorization was observed on day 4 and 37.5% decolorization in continuous mode on day 5.

A study by Ambrósio et al., (2004) reported the decolorization of dyes with different molecular structures by *Cunninghamella elegans* under different media conditions. The decolorization experiment consisted of adding 72 h old mycelium into the culture medium containing one of the dyes (Orange or Reactive Black or rEactive Red) or a mixture of dyes in the presence or absence of sucrose and/or peptone. The decolorization activity was highly dependent on the incubation time, the molecular structure of the dye and presence or absence of co-substrates. The presence of sucrose or both sucrose and peptone significantly increased the decolorization of the dye. However, the presence of only nitrogen source suppressed the decolorization process.

Itoh and Yatome, (2004) evaluated the decolorization of six xanthene dyes (100 µM) by *Coriolus versicolor* a white rot fungus in liquid culture. The decolorization of Fluorescein, 4 Aminofluorescein, and 5-Aminofluorescein were 85.0, 95.0, and 91.9% after 14 days of incubation, respectively. Further, no decolorization was observed with respect to Rhodamine B, Rhodamine 123 hydrate, and Rhodamine 6G. The first three dyes were decolorized with cell free extracts from *C. versicolor*. The decolorization activity was 10.2, 6.7, and 7.2 µM min⁻¹mg⁻¹, respectively.

Levin et al., (2004) investigated the decolorization potential of 26 white rot fungi from Argentina. Production of ligninolytic enzymes by mycelium growing on solid malt extract/glucose medium supplemented with different dyes (Malachite Green, Azure B, Poly R-478, Anthraquinone Blue, Congo Red and Xyldine), extent of dye decolorization and the relationship between enzyme production and dye decolorization were studied. Only ten strains decolorized all the dyes which produced laccase, lignin peroxidase and manganese peroxidase on solid medium. Further, six of the strains could
not decolorize any of the dyes and these six strains tested negative for lignin peroxidase, and produced less than 0.05 U/g agar of manganese peroxidase. The newly identified fungus *Coriolus versicolor f. antarcticus*, was able to decolorize in an hour 28%, 30%, 43%, 88% and 98% of Xylocene (24 mg/L), Poly R-478 (75 mg/L), Remazol Brilliant Blue R (9 mg/L), Malachite Green (6 mg/L) and Indigo Carmine (23 mg/L), respectively. Laccase activity was 0.13 U/ml, but neither lignin peroxidase nor manganese peroxidase could be detected in the extracellular fluids on the day of incubation.

*Park et al.*, (2004) evaluated the decolorization ability of three commercial dyes, Acid Yellow 99, Acid Blue 350, and Acid Red 114, using 10 fungal strains. Among the fungal strains tested, *Trametes versicolor* KCTC 16781 completely decolorized all dyes in both solid and liquid cultures, and was also able to decolorize the mixture of three dyes in liquid experiments. The secretion of ligninolytic enzymes into the extracellular medium during decolorization by *T. versicolor* KCTC 16781 indicated that decolorization correlated with ligninolytic enzyme levels.

The role of fungus basidiomycete *Pleurotus ostreatus* in the decolorization of the Remazol Brilliant Blue R (RBBR) was investigated by *Palmieri et al.*, (2005). When grown in liquid media supplemented with veratryl alcohol, the fungus completely decolorized RBBR in 3 days. *P. ostreatus* produced other enzymes such as laccases, veratryl alcohol oxidase and dye decolorizing peroxidase but only laccases seem to have been responsible for RBBR transformation. POXC and POXA3, two purified laccases were found to degrade RBBR *in vitro*, in the absence of any redox mediators. There was remarkable improvement in the reaction rate and in the final level of dye decolorization by using a mixture of two laccases. The decolorization also depended on incubation temperature and enzyme concentration. The results showed that *P. ostreatus* was able to decolorize more than 90% RBBR within 3 - 6 days at low (5 µM) and high (50 µM) dye concentrations.

An investigation using *in vitro* and *in vivo* decolorization of nine structurally different direct azo dyes by *Phanerochaete chrysosporium* immobilized on ZnOCl₂-activated pumice in stationary cultures was carried out by *Pazarlioglu et al.*, (2005). Lignin peroxidase activity was not detected in the extracellular medium of *P. chrysosporium*. To support dye degradation, ligninolytic culture filtrate from fungus, containing mainly manganese peroxidase, was treated with dye Direct Blue 15
(DB15, 120 mg/L) in a small-scale packed-bed reactor. The colour removal efficiency in repeated batch mode was 95-100%. With increase in initial concentration of the dye, the rate of colour removal decreased to a significant extent. It was observed that MnP played an important role while there was no obvious role for LiP.

Radha et al., (2005) worked on evaluation of Phanerochaete chrysosporium to degrade several synthetic dyes of varying structures. The decolorization potential of P. chrysosporium for seven dyes namely, Methyl Violet, Congo Red, Acid Orange, Acid Red 114, Vat Magenta, Methylene Blue and Acid Green were tested. The effect of operational parameters such as dye concentration, temperature and inoculum size on maximum percentage decolorization was investigated. Experiments were carried out using free cells and fungal cell entrapped calcium alginate beads of different sizes. An optimum glucose and nitrogen concentrations of 5.0 and 0.05 g/L, respectively, in the basal medium were found to be effective for enhancement of maximum decolorization. Percentage decolorization for all the dyes tested was found to be more than 75.0% with the following optimum conditions: temperature 35°C, pH 4.0 - 5.0 and inoculum size of 2 ml (approximately 1.6 × 10^5 cell/ml).

Four white-rot fungi isolated in Pakistan were used for decolorization of four reactive textile dyes by Asgher et al., (2006). Drimarene Orange K-GL, Remazol Brilliant Yellow 3GL, Procion Blue PX-5R and Cibacron Blue P-3RGR were decolorized by Phanerochaete chrysosporium, Coriolus versicolor, Ganoderma lucidum and Pleurotus ostreatus grown in defined nutrient media for 10 days in agitation condition. Drimarene Orange K-GL was completely decolorized (0.2 g/L after 8 days) only by P. chrysosporium, followed by P. ostreatus (0.17 g/L after 10 days). P. ostreatus also showed good decolorization efficiencies (0.19 - 0.2 g/L) on all dyes except Remazol Brilliant Yellow (0.07 g/L after 10 days). G. lucidum did not decolorize any of the dyes to an appreciable extent except Remazol Brilliant Yellow (0.2 g/L after 8 days).

Lopez et al., (2006) studied the decolorization potential of a fungus on the dyes Remazol Brilliant Blue R (RBBR), Poly R-478 and Poly S-119 isolated from composting piles. The decolorizing capability of Poly S-119 was highest followed by Poly R-478, with RBBR being the least and most difficult to decolorize. The mesophilic fungi showed higher decolorization efficiency with more than 95% for Poly R-478 and Poly S-119 and more than 50% for RBBR. The thermophilic fungus decolorized 12, 14 and 40% of Poly R-478, RBBR and Poly S-119, respectively.
Aspergillus ochraceus (NCIM-1146), a fungal species was assessed by Saratale et al., (2006) for its potential to degrade four triphenylmethane dyes. The strain showed significant ability to degrade all dyes tested viz. Malachite Green (98%), Cotton Blue (92%), Methyl Violet (61%) and Crystal Violet (57%) in 24 h incubation at 150 rpm with temperature of 30°C. Decolorization ability of Malachite Green was assessed by various conditions such as composition of media, concentration of dye, amount of mycelia and agitation. The effect of various media composition for the decolorization of Malachite Green (0.5 g/L) by 96 h grown mycelia of A. ochraceus for 24 h was analyzed. All types of media showed decolorization activity in the range of 90 - 98%, except potato dextrose broth (82%). The best results were obtained when normal water and 50mM phosphate buffer were used as medium. In terms of agitation, the highest decolorization activity was obtained at 150 rpm (96%) in 24 h. It was observed that an increase in amount of mycelia positively affected the durability of decolorization activity. Increasing the dye concentration decreased the dye decolorization capability of mycelia.

A sequential batch culture study for decolorization of reactive dye by Coriolus versicolor was reported by Sanghi et al., (2006). The fungus C. versicolor decolorized reactive dye Remazol Brilliant Violet to almost 90%. In a batch reactor, the fungal mycelia removed colour as well as COD up to 95% and 75%, respectively. Decolorizing activity was observed during repeated reuse of the fungus. Through various operational conditions such as media composition, age of fungus and nitrogen source, it was possible to substantially increase the dye decolorizing capability of the fungus. The fungal pellets were used for eight cycles during the long term operation. The medium and dye were replenished at the end of each cycle and the fungus was recycled. Peptone showed higher decolorization activity compared to urea. Presence of a nitrogen source and nutrient content of media played important roles in sustaining the decolorizing activity of the fungus.

Parshetti et al., (2007) examined the decolorization and degradation of textile dye Reactive Blue - 25 (0.1 g/L) by mycelium of Aspergillus ochraceus NCIM-1146. Shaking condition was found to be efficient for complete and faster adsorption (7 h) and decolorization (20 days) of the dye (100 mg/L) as compared to static condition. Substitution of glucose in the medium showed faster adsorption (4 h) and decolorization of dye from bound (7 days) mycelium. FTIR and GC - MS analyses confirmed the biodegradation of Reactive Blue - 25 into two metabolites, phthalimide and di-isobutyl phthalate.
A study by Revankar and Lele, (2007) reported the decolorization of Amaranth dye by Ganoderma sp., a white rot fungus isolated from bark of dead tree. The culture medium was optimized using a combination of OFAT and orthogonal array method. Amaranth (100 ppm) was decolorized up to 96% at 8 h incubation with optimized medium containing 2% starch and 0.125% yeast extract. The isolate was capable of decolorizing other chemically different dyes. Further, the isolate was evaluated for decolorization of industrial effluent (2% v/v) and it showed complete decolorization in 12 days.

Xian et al., (2007) investigated Aspergillus fumigatus XC6 isolated from mildewing rice straw for its ability to decolorize a dye from industry effluent. The strain was capable of decolorizing the effluent over a range of pH (3.0 - 8.0) with the dye as sole carbon and nitrogen source. The optimum pH was 3.0; however, when supplemented with nitrogen sources (0.2% NH₄Cl or (NH₄)₂SO₄) or carbon sources (1.0% sucrose or potato starch), the strain decolorized the effluent completely at the initial pH of the effluent.

Zope et al., (2007) reported decolorization of textile dyes - Reactive Red 195 and Reactive Green 11 by Aspergillus niger group using synthetic wastewater. Supplementation of co-substrates such as 1g % w/v of glucose, ammonium sulphate (0.2 g% and 0.3 g% respectively for Reactive Red 195 and Reactive Green 11) with 1 g% inoculum size favoured degradation. Static condition enhanced dye decolorization than shaking condition. Under optimized conditions (pH 7.0 and temperature 30°C) degradation by A. niger were found to be: Reactive Red 195, 93.0% and Reactive Green 11, 80%.

The potential of two white rot fungi Schizophyllum commune IBL-06 and Ganoderma lucidum IBL-05 to decolorize direct dye Solar Golden Yellow R was investigated by Asgher et al., (2008). Solar Golden Yellow R was decolorized to the extent of 73% by S. commune IBL-06 after 6 days at pH 4.5 and 35°C whereas 70% decolorization was observed on the 7th day by G. lucidum with same culture conditions. Further, addition of 1% glucose gave the best results with complete decolorization of the dye after 2 days of incubation under optimum conditions. The carbon sources (fructose, maltose, sucrose, starch and molasses) showed decolorization of the dye between 65.31% and 100%. The nitrogen sources (ammonium nitrate, ammonium sulphate, yeast extract, urea and peptone) showed an inhibitory effect on dye decolorization.
Bhatti et al., (2008) conducted a study to understand the decolorization potential of *Schizophyllum commune* IBL-6, a white rot fungus for the degradation of reactive textile dye Cibacron Red FN-2BL. In an acclimatization study (10 days), it was observed that *S. commune* IBL-6 was a better decolorizer of Cibacron Red FN-2BL. Several factors such as composition of basal nutrient medium, pH, temperature, initial dye concentration, and additional carbon and nitrogen sources were optimized to develop an economically feasible decolorization process. Optimum dye decolorization was obtained in basal nutrient medium II containing 0.1% dye supplemented with 1% glucose at pH 4.5 and 30°C after 3 days of incubation. The additional carbon sources were found to enhance decolorization process, whereas nitrogen supplements caused inhibition of fungal growth. Manganese peroxidase was found to be the major enzyme involved in degradation of dye with minor contribution by lignin peroxidase and laccase.

Dayaram and Dasgupta, (2008) reported decolorization of four reactive dyes such as Reactive Blue, Reactive Orange, Remazol Black and Congo Red by treatment with enzyme obtained from *Polyporus rubidus*. A laboratory scale bioreactor with laccase immobilized Na-alginate beads was constructed to treat the effluent. More than 80% of dyes were degraded within 5 days under stationary incubation conditions. The enzyme had a maximum activity of 17.1 U/ml after 3 days and was found to be secreted extracellularly by *P. rubidus*. Ninety percent decolorization of Reactive Blue (100 mg/L) was achieved after five days of incubation.

Six Egyptian soil isolates (*Aspergillus niger, Penicillium* sp., and *Bacillus* sp.) were examined for decolorization activities on the reactive and direct dyes by Husseiny, (2008). *Aspergillus niger* and *Penicillium* sp. were found to be the most efficient dye degraders. Maximum decolorization of Direct Red 81 under static condition by *Aspergillus niger* was 96.69% (pH 4.0, 28°C, 4 days incubation period) and by *Penicillium* it was 89.6% (pH 4.5, 35°C and 4 days incubation period).

The effluent generated from International Textile Industry (Nig) Ltd. Odongunyan Industrial Estate, Ikorodu, Lagos, was examined by Adebayo et al., (2010). The effluent had high levels of chemical parameters. The effluent sample was analyzed for BOD, COD, total solid, suspended solid, dissolved solid, odour and colour intensity before subjecting it to biological treatment with mixed culture of *Aspergillus niger* and *Aspergillus wentil*. Followed by post biological treatment (5
days), the effluent was analyzed for chemical parameters. The results showed that the decolorization process had significantly reduced COD to below 250 mg/L, BOD < 30 mg/L and TSS < 30 mg/L which are the upper limit for disposal into surface water. The result indicated remarkable overall COD reduction from 800 mg/L to 200 mg/L (75%), BOD from 750 mg/L to 20 mg/L (97.3%) and TSS < 30 mg/L (99.5%), DS (99.6%) and SS (99.3%).

Ali et al., (2009) investigated the decolorization of Malachite Green, a triphenylmethane dye by two fungal strains, Aspergillus flavus and Alternaria solani. A. flavus and A. solani were able to decolorize 50 µM Malachite Green to the extent of 97.4% and 96.91%, respectively within 6 days of incubation at temperature of 37.5°C. Dye concentration in the range of 10 to 49 µM had no significant effect on percent decolorization in both the species. It was clear that carbon source in the decolorization medium had no significant effect on percent decolorization for both the species.

A statistical application such as response surface methodology was applied by Margarida et al., (2009) to optimize decolorization of the diazo dye Reactive Black 5 (RB5) by crude laccase from the white-rot fungus Trametes pubescens. The presence of the redox mediator 1-hydroxybenzotriazole (HBT) greatly improved the decolorization level of RB5 by crude laccase from T. pubescens. CCD using RSM with three variables namely redox mediator (HBT), dye (RB5) and enzyme (laccase) concentrations were used to optimize significant correlation between the effects of these variables on the decolorization of RB5. The optimum concentrations of HBT, RB5 and laccase were 1.17 mM, 150 mg/L and 500 U/L, respectively, for maximum decolorization of 60% RB5 in 20 min. A quadratic model was obtained for dye decolorization through this design. The experimental values were in good agreement with the predicted ones and the model was highly significant, the correlation coefficient being 0.965.

Decolorization of Orange II, an azo dye by white rot fungus, Phanerochaete chrysosporium was reviewed by Sharma et al., (2009). Almost 85% of the dye was removed in 7 days (optimum decolorization - 5th day at 28 - 30°C at pH 5.0) in liquid culture under shaking aerobic conditions by P. chrysosporium. The higher dye concentration in simulated wastewater showed inhibitory effects on decolorization. Decolorization of Orange II by P.chrysosporium was correlated to lignolytic enzyme activity.
Amatussalam and Latha, (2010) studied the decolorization of Congo Red by four fungal isolates namely, Aspergillus fumigatus, Cladosporium herbarum, Fusarium solani and Humicola fuscoatra, which were isolated from dye effluent contaminated soil. The experiments were carried out by fungal isolates grown in liquid nitrogen medium amended with Congo Red. C. herbarum decolorized 90% of Congo Red in 10 days at 35°C, pH 6.0, with dye concentration of 0.1 mg/100 ml, followed by H. fuscoatra (80%), F. solani (75%) and A. fumigates (70%) with same culture conditions.

A locally isolated white rot fungus Ganoderma lucidum IBL-05 was used for treating original textile industry effluents by Asgher et al., (2010). Dye-containing effluents of different colours were collected from the Arzoo (maroon), Ayesha (yellow), Ittemad (green), Crescent (navy blue) and Magna (yellowish) textile industries of Faisalabad, Pakistan. Decolorization to the extent of 49.5% was recorded by the Arzoo textile industry (ART) effluent on the 10th day of incubation. Hence, the ART effluent was selected for optimization of its process parameters. Process optimization improved the colour removal efficiency of the fungus to 95% within 2 days. Optimized process was catalyzed by manganese peroxidase (1295 U/mL) at pH 3.0 and 35°C using 1% starch supplemented to Kirk’s basal medium. Addition of nitrogen source inhibited enzyme formation and effluent decolorization.

Fazli et al., (2010) reported about optimization of decolorization process of Reactive Blue 19 using Ganoderma sp. Response surface methodology was used to study the effect of independent variables, such as glycerol concentration, temperature and pH on colour removal efficiency in aqueous solution. Maximum decolorization of 95.3% was obtained under optimum conditions of temperature 27°C, glycerol concentration -19.14 mg/L and pH 6.3. The confirmatory experiments showed 94.89% decolorization. This statistical approach employed in the study enabled the authors to improve dye decolorization process up to 1.27 times higher than non-optimized conditions.

Karthikeyan et al., (2010) studied the effect of various process parameters on decolorization of an azo dye, Congo Red, by a fungal isolate, Aspergillus niger HM11. Central composite design and response surface methodology were applied to design experiments to evaluate the interactive effects of the operating variables on the decolorization of Congo Red. Regression coefficients between the variables were generated. The results showed that pH 6.0, 150 rpm agitation, incubation time of 36 h and glucose concentration of 1.0% were optimal for achieving maximum decolorization (99.8%) of Congo Red.
In a study conducted by Sathishkumar et al., (2010) Pleurotus florida NCIM 1243 produced laccase as the dominant lignolytic enzyme, which was used for decolorization of Reactive Blue 198. Banana peel was the most suitable substrate for extracellular laccase production under solid state fermentation. The maximum activity of laccase (5.4 U/g) was detected on the 10\textsuperscript{th} day and partially purified laccase showed excellent decolorization activity. The optimum pH, temperature and enzyme concentration for maximum decolorization was found to be 4.5, 60°C and 1.2 U/ml, respectively. At lower dye concentrations (50 - 100 ppm) maximum decolorization (96\%) was observed which decreased markedly when the dye concentration was increased beyond 150 ppm.

Decolorization of Direct Red 80 by the Phanerochaete chrysosporium MTCC 787 was investigated using statistical design of experiments by Singh et al., (2010). Media components were screened using PB experimental design followed by optimization using RSM, which resulted in enhancement in the efficiency of dye removal by the fungus. Specific dye removal due to enzyme activity and percent dye decolorization were chosen as the responses from the experiments. The P value was less than 0.05 indicating that the media constituents screened were found to be the most effective factors chosen for decolorization. The results revealed complete dye decolorization and maximum specific dye removal by lignin peroxidase enzyme of 0.24 U/mg with optimum combinations of the media constituents such as: glucose, 11.9 g/L; veratryl alcohol, 12.03mM; KH\textsubscript{2}PO\textsubscript{4}, 23.08 g/L; CaCl\textsubscript{2}, 2.4 g/L and MgSO\textsubscript{4}, 10.47 g/L.

Yousefi and Kariminia, (2010) studied the decolorization of monoazo dye Acid Orange (AO7) by the fungal peroxidase from Coprinus cinereus NBRC 30628. Enzymatic decolorization was affected by temperature, pH, enzyme activity, concentration of H\textsubscript{2}O\textsubscript{2} and dye concentration. Statistical methods such as OFAT and RSM to study the combined interactions between the factors were employed. OFAT analysis resulted in optimized conditions such as temperature 25°C, pH 9.0, H\textsubscript{2}O\textsubscript{2} . 3.9 mM and dye concentration - 40 mg/L. In statistical optimization, 2 - factorial design was applied with three factors-temperature, pH and initial dye concentration as significant factors. Response surface analysis was conducted through CCD and a second-order polynomial model ($R^2$=0.9718) was generated. Analysis of variance of this model indicated that the temperature, pH and their interaction were the most
significant factors in decolorization of AO7. The optimum condition through RSM was found to be temperature 25°C, pH 9.0, and dye concentration - 50 mg/L with 100% decolorization.

**Hadibarata et al., (2011)** studied the decolorization potential of white-rot fungus, *Armillaria* sp. strain F022, isolated from the decayed wood of tropical rain forest. Strain F022 was capable of decolorizing azo, triphenylmethane, and anthraquinone dyes, with decolorization obtained after 96 h of culture, with the exception of Brilliant Green. The dyes tested were decolorized by the purified laccase in the absence of any redox mediators. Few of the dyes were completely removed, while others were partially removed even when decolorization time was increased. Among the dyes used, Remazol Brilliant Blue R was the best substrate for the fungal strain. Brilliant Green was not a good substrate for laccase, with only 75% degradation in maximum laccase concentration of 2 U/ml. The laccase from *Armillaria* sp. F022 played an important role in the decolorization process. When dyes were added at 0 h of culture, the maximum dry mycelium weight (DMW) values in the medium containing Brilliant Green were 1/6 of that achieved by the control group. For other dyes, the DMW were similar to control. The optimum conditions for decolorization of three synthetic dyes were pH 4.0 and 40°C.

**Kanmani et al., (2011)** studied the effect of *Aspergillus proliferans* on decolorization of synthetic dyes (70 mg/ml⁻¹) and coloured effluent in liquid culture medium. Synthetic dyes were decolorized between 76 and 89% within 6 days of treatment and 73.5% of colour was removed in industrial effluent within 8 days. The decolorization activity was effectively stimulated by the addition of carbon and nitrogen sources. The high concentration of glucose repressed the decolorization activity. Supplementation of yeast extract had significantly enhanced the effluent decolorization at p<0.05. Laccase showed significant enzyme activity (10,200 U/ml/L) at p<0.005. The crude enzyme decolorized the dyes Aniline Blue and Congo Red (40.9 to 70%) and the effluent (88.6%) in 14 h. The culture free supernatant without the fungal biomass had also effectively decolorized the effluent and synthetic dyes.

**Kumaran and Dharani, (2011)** investigated the different physical and chemical parameters for decolorization of textile dyes by using *Phanerocheate chrysosporium* and *Pleurotus sajor-caju*. Several operational factors such as dye concentration, pH, temperature, and additional carbon and nitrogen sources were used
to evaluate the efficiency of the fungal strains in decolorization of the textile dyes (Navy Hexie, Red GF and Blue B 133). Dye concentration of 50 mg/L showed high percentage of decolorization. Glucose as carbon source and urea as nitrogen source contributed towards enhanced decolorization of the dye by the two strains. High percent decolorization was observed at pH 7.0 and 8.0 and temperature of 35°C.

Khan et al., (2011) studied the decolorization potential of Phanerochaete chrysosporium IBL-03, reactive textile dye, Drimarine Blue K2R. The initial experiment was run for seven days with 0.01% (w/v) of the dye prepared in Kirk's basal nutrient medium. The results showed that P. chrysosporium gave 65% decolorization of the dye in 7 days. By optimization of process parameters, 97% colour removal was observed in three days using 0.005% (w/v) of the dye at pH 4.0 and 30°C with 0.9% (w/v) molasses and 0.2% (w/v) ammonium dihydrogen phosphate added as carbon and nitrogen sources, respectively. Manganese peroxidase showed major enzyme activity (560 U/mL). The dye adsorption studies showed that the dye initially was adsorbed on fungal mats which disappeared later, possibly by the action of MnP secreted by the fungus in secondary metabolism.

Ponraj et al., (2011) studied decolorization of textile dye True Blue by the fungal strains, Aspergillus flavus, Aspergillus niger, Helminthosporium sp., Mucor sp. and Penicillium sp. isolated from textile dye effluent from Elampillai Jalagandapur. The bioprocess variables used to decolorize the dye were different carbon and nitrogen sources, temperature and pH. A. niger showed maximum (96.75%) dye decolorization with maltose as carbon source and A. flavus showed maximum dye decolorization (97.82%) with yeast extract as nitrogen source. A. niger showed maximum (97.26%) dye decolorization at room temperature and at pH 6.0. A. flavus showed highest decolorization of the dye effectively during optimization but predominantly A. niger showed consistent decolorization of the dye.

Four different predominant adapted fungal strains (screened from effluent sample) Aspergillus sp., Penicillium sp., Fusarium sp. and Mucor sp. and 4 predominant non-adapted strains (screened from soil, water and fungal fruiting bodies) Aspergillus sp., Penicillium sp., Fusarium sp. and Rhizopus sp., with potential dye decolorization ability on Reactive Black 5, Amido Black-10B, Red 5B, Reactive Red 120 and Anthraquinone Violet R were reported by Rajendran et al., (2011). These organisms were developed into a consortium which was used to decolorize the
textile effluents containing a mixture of dyes. There was about 67% of colour reduction with 34% of COD reduction by non-adapted fungal consortium while 75% of colour reduction with 50% of COD reduction by the adapted fungal consortium. Analyses of the results through chi-square test, the calculated value (28.712) was higher than the tabulated value (9.49) at 4 degree of freedom.

Shedbalkar and Jadhav, (2011) examined the decolorization ability of *Penicillium ochrochloron* on Malachite Green. Ninety three percent of the dye (50 mg/L) was decolorized in Czapek Dox broth after 14 h with an optimum pH of 7.0 and temperature 30°C. The degradation of Malachite Green was peroxidase-mediated which was shown in the induction of activity of lignin peroxidase enzyme. Fungal culture also decolorized the textile effluent. The dye was detoxified into p-benzyl-N,N-dimethylaniline and N,N-dimethyl-aniline hydrochloride. Degradation metabolites were analyzed by TLC, HPLC, and FTIR and identified by GC-MS analysis. The values of TDS, TSS, COD, and BOD were reduced in the treated samples compared to the control effluent.

Ali and Mohamedy, (2012) reported decolorization activities of some acid and reactive dyes, by six fungal isolates belonging to *Aspergillus niger*, *Penicillium* sp., and *Pleurotus ostreatus*. The isolates were screened for optimum efficiency in terms of temperature and pH. Simulated wastewater and real wastewater samples were used in decolorization experiments. The results obtained indicated that *Pleurotus ostreatus* and *Aspergillus niger* 1 and 2 were more efficient than *Penicillium* sp., with the two kinds of dyes used. The maximum degradation potential of these isolates for acid dyes were 85% at pH 4.0 after 9 days incubation and 90% at pH 5.0 for reactive dyes.

*Ganoderma cupreum* AG-1 isolated from the decayed wood was evaluated for its ability to decolorize azo dye Reactive Violet 1 by Gahlout et al., (2012). With optimum pH of 4.5 and temperature of 30°C, the decolorization obtained was 91.0%. When the decolorization medium was supplemented with appropriate nitrogen source (yeast extract 5 g/L) and carbon source (mannose 2 g/L), the decolorization was 98.0% after 4 days of incubation. Laccase and MnP were found to be the major enzymes involved in biodegradation of the dye.
A white rot basidiomycete *Polyporus brumalis* has been reported to induce two laccase genes under degradation conditions of dibutylphthalate by Kim *et al.*, (2012). When this fungus was grown in a minimal medium, one laccase enzyme was detected by the native polyacrylamide gel electrophoresis. The laccase was purified through ammonium sulfate precipitation and ion exchange chromatography, and the estimated molecular weight was 70 kDa. The optimum pH and temperature of the purified laccase were 4.0 and 20°C, respectively. The K (m) value of the enzyme was 685.0 μM, and the V (max) was 0.147 OD min (-1) unit (-1) for o-tolidine. Purified laccase showed effective decolorization of a dye, Remazol Brilliant Blue R (RBBR), without any laccase mediator. However, this effect was reduced by a laccase inhibitor, kojic acid, which confirmed that the laccase was directly involved in the decolorization of RBBR.

Namdhari *et al.*, (2012) investigated five indigenous fungal strains, isolated from the effluents collected around the discharge site of textile industry situated in Panipat. Decolorization potentials of 3 fungal species *A. allhabadii*, *A. niger* and *A. sulphureus* were evaluated for Reactive Blue MR dye (100 - 300 mg/L) in carbon limited Czapek Dox broth (0.5%). It was found that *A. allhabadii* and *A. sulphureus* showed higher decolorization potential of 95.13±0.11%, and 93.01±0.25%, respectively with 200 mg/l dye, whereas *A. niger* showed higher decolorization of 83.14±0.19% with 100 mg/L after ten days of incubation. Sucrose was the best carbon source for all the fungal strains.

Rengadurai *et al.*, (2012) studied the potential of white rot fungus, *Trametes hirsuta* to decolorize the textile dye Reactive black 5 using Laccase medium. Statistical optimization using RSM incorporating CCD was chosen to evaluate the interactive effects of four variables in different ranges namely, the initial dye concentration, pH, temperature and inoculum size. Experiments were conducted to construct a quadratic model. Validation of the model showed good agreement between the experimental and predicted values. Maximum decolorization of 92.0% was observed at optimum process conditions such as initial dye concentration 220 mg/L, pH 6.0, temperature 30.7°C and inoculum size of 4mm of 3 disc of mycelium.
Rohilla et al., (2012) reported decolorization of reactive dye Orange M2R by four fungal strains, A. flavus, A. niger, A. oryzae, A. terrus and their consortia using Czapek Dox broth. The effects of operational parameters such as carbon source, temperature, pH, shaking vs. static condition on the decolorization potential of Aspergilli and their consortia were investigated with 200 mg/L of the dye. The fungal consortia showed maximum decolorization (89.35±0.63%) with sucrose as carbon source followed by glucose (83.08±1.02%). A. niger decolorized up to 81.37±0.35% in medium containing sucrose. It was found that shaking condition favoured the dye decolorization process. The fungal consortia showed highest decolorization (91.84±0.91%) at an alkaline pH 8.0, whereas A. flavus showed maximum decolorization (83.36±0.63%) at acidic pH 5.0 and A. niger (80.10±0.59%) at pH 6.0.

Fungal consortia (93.61±0.79%) showed maximum decolorization at 35°C; A. niger and A. oryzae showed maximum decolorization at 30°C.

The ability of the fungal strain Aspergillus sulphureus to decolorize a textile dye, Reactive Black HFGR was examined by Salar et al., (2012). The effect of process parameters such as shaking vs static condition, pH, dye concentration and different carbon sources on the degradation of dye was studied in C-limited Czapek Dox broth. Reactive Black HFGR showed highest decolorization (93.04%) under shaking condition at pH 5.0, temperature 25°C and dye concentration of 200 mg/L. The fungal strain showed maximum activity (93.04±1.86%) with sucrose followed by glucose (85.31±0.59%) and fructose (78.71±1.44%).

The role of white rot fungus, Schizophyllum commune IBL-06 in decolorization of direct dye Solar Brilliant Red 80 in Kirk’s basal salts medium was discussed by Asgher et al., (2013). In initial screening, the maximum decolorization of 84.8% of the dye was achieved in 7 days, in shaking condition at pH 4.5 and 30°C. Different factors such as pH, temperature and inoculum density were statistically optimized through Completely Randomized Design (CRD) to enhance dye decolorization efficiency. Under optimum conditions, S. commune IBL-06 completely decolorized the dye using maltose and ammonium sulphate as carbon and nitrogen sources, respectively in 3 days. The three major ligninolytic enzymes lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) were produced by S. commune during the decolorization of the dye. LiP was the major enzyme (944 U/mL) secreted by S. commune IBL-06 along with comparatively lower levels of MnP and laccase.
Batool et al., (2013) studied on Ganoderma lucidum IBL-05 which was used in solid state fermentation (SSF) of wheat straw for lignin peroxidase (LiP) production. The SSF process for LiP production was improved by optimizing some physical parameters at pre-optimized pH 4.5 and temperature 35°C. LiP production by the fungus was substantially improved to 1019 IU/mL after 48h in nutrient medium III with 60% moisture level, 5 mL inoculum size, glucose and urea as carbon and nitrogen sources respectively, in 15:1 C/N ratio, 1 mL of 2 mM Zn\(^{2+}\) as metal ion and 1mL of 4mM 4-MMA as mediator. Surfactants like Tween-80, Tween-20 and SDS suppressed LiP synthesis by G. lucidum IBL-05.

A report on evaluation of 4 fungal species for decolorization of Rubine Toner 12 dye under aerobic condition was presented by Dhanjal et al., (2013). Growth associated decolorization studies were carried out in potato dextrose broth supplemented with Rubine-Toner12. Among the 4 species screened for dye decolorization, Aspergillus niger showed 100% decolorization at pH 6.0, temperature 28°C and dye concentration of 10 mg/L with 120 h of incubation period.

A. niger and Nigrospora sp. were employed for decolorization of Synozol Red HF-6BN in a study carried out by Ilyas et al., (2013). A. niger and Nigrospora sp. were able to decolorize 88% and 96% Synozol Red 6BN, respectively, in 24 days. It was observed that 86% and 90% of Synozol Red containing dye effluent was decolorized by A. niger and Nigrospora sp. after 28 days of incubation at room temperature. A protein with relative molecular mass of 70 kDa was partially purified and examined for enzymatic characteristics. The enzyme exhibited highest activity at temperature ranging from 40 - 50°C and at pH 6.0. The enzyme activity was enhanced in the presence of metal cations. HPLC analysis confirmed that these fungal strains were capable of degradation of Synozol Red dye into metabolites.

In a study by Jonstrup et al., (2013) three fungi, Phanerochaete chrysosporium, Trametes versicolor and Bjerkandera sp BOL13 were compared for decolorization of azo dyes individually or as a mixture. The dye decolorization was evaluated using a lignocellulosic growth substrate. Bjerkandera sp BOL13 showed 89.0% decolorization of Remazol Red RR at an initial pH of 4 - 6 after 13 days of incubation with straw as carbon source. When glucose was substituted as growth-substrate, decolorization efficiencies of 65 - 90% were observed in 12 days in a bioreactor packed with wooden material. The decolorization efficiency was lower when glucose was not fed to the fungus or when a plastic material was used as packing.
Study by Papadopoulou et al., (2013) examined the ability of eleven strains belonging to Pleurotus, Ganoderma and Lentinula mushroom genera in decolorization of eight textile azo and anthraquinone dyes in plate assay. Pleurotus pulmonarius AMRL 177, showing the highest decolorization ability, was further considered for decolorization of Remazol Brilliant Blue R (RBBR) using RSM. The Box-Behnken design matrix for three factors: dye concentration, nitrogen and copper concentration were employed for the optimization of four responses: lag time, mycelium extension rate, decolorization rate and laccase activity. Optimum values of responses obtained were: lag time 3 days, mycelium extension rate 2.8 mm d⁻¹, decolorization rate 2.7 mm d⁻¹ and laccase activity 480 U ml⁻¹.

Sathian et al., (2013) worked on treatment of textile dye wastewater in batch reactor using Ganoderma lucidum. The characteristics of textile dye wastewater were determined. RSM was used to study the effect of process variables like pH, temperature, agitation speed and dye wastewater concentration on dye decolorization and degradation. The optimized conditions were: pH 6.6, temperature 26.5°C, agitation speed 200 rpm and dye wastewater concentration 1:2. Under these optimized conditions, maximum decolorization of 81.4% and COD reduction of 90.3% were observed.

Venugobal et al., (2013) studied the role of mangrove-derived Aplanochytrium sp., on decolorization of malachite green. To enhance the dye removal, a statistical optimization was done by two phases of RSM : PB design and CCD. Aplanochytrium sp. was found to remove the azo dye up to 86.32% within five and half days of incubation under optimized conditions of pH 7.8 and temperature of 27.8°C.

Decolorization of textile dye Reactive Blue 19 (RB19) by Coprinus plicatilis was analyzed by Akdogan et al., (2014). Ninety nine percent decolorization efficiency by RB19 was obtained in media containing intermediate concentrations of ammonium oxalate and glucose (10 g/L) as nitrogen and carbon sources, respectively, at 26°C and pH 5.5. High percent decolorization of the dye was associated with laccase enzyme displaying good tolerance to a wide range of pH values, salt concentrations and temperatures.

An investigation by Haung et al., (2014) reported a nonligninolytic fungal strain Myrothecium sp. IMER1, capable of decolorizing five different synthetic dyes grown on dye-containing agar plates. Five white-rot fungi were compared with Myrothecium sp.
IMER1 for decolorization of Remazol Brilliant Blue R (RBBR) and dye effluents. More than 65% RBBR was removed by *Myrothecium* sp. IMER1. Decolorization of 60 - 95% was observed with white-rot fungi in the acidic pH range of 5.0 - 6.0, whereas colour removal was less than 30% in the basic pH range of 8.0 - 10.0. *Myrothecium* sp. IMER1 showed a more efficient decolorization of the dye in a broad pH range than white-rot fungi tested. With respect to colour removal performance, *Myrothecium* sp. IMER1 was approximately 2 - 5 folds better than white-rot fungi tested in the basic pH range. Decolorization of dye effluents by *Myrothecium* sp. IMER1 at pH 7.0 and 9.0 was 73 and 70%, respectively, while less than 25% of decolorization was observed in the case of white-rot fungi. This study showed that *Myrothecium* sp. IMER1 was a potential candidate for wastewater treatment of dye effluents, especially alkaline dye effluents.

The decolorization potential of *Cordyceps militaris* MTCC 3936, a white rot fungus on reactive dyes was investigated by Kaur *et al.*, (2014). Initial examination in liquid cultures revealed 100% decolorization which was achieved within 3 days of incubation for Reactive Yellow 18 and 6 days for Reactive Red 31, whereas it was 7 days for Reactive Black 8, and 11 days was needed for Reactive Green 19 and Reactive Red 74. RSM was employed to study the effect of three independent variables such as pH, incubation time, and concentration of dye on decolorization process. Statistical analysis revealed that dye decolorization by cell free supernatants of *C. militaris* was more efficient than whole cell. The optimized conditions for decolorization of reactive dyes were, dye concentration - 300 ppm, incubation duration - 48 h and pH 5.5, except for Reactive Red 31. The maximum dye decolorization achieved under optimized conditions for Reactive Yellow 18, Reactive Green 19, Reactive Red 74, and Reactive Black 8 were 73.07, 65.36, 55.37 and 68.59%, respectively.

The potential of *Pereniporia tephropora* MUCL 47500 to decolorize two textile dyes was evaluated by Mounguengui *et al.*, (2014). Optimal conditions for decolorization of Reactive Blue 4 (95.18%) and Methyl Orange (92.03%) were temperature 30°C, pH 5.5 and dye concentration 0.3 g/L. This fungus on liquid media containing Reactive Blue 4 produced different enzymes like laccase and MnP, which had an important role in degradation.

Parmar, (2014) reported decolorization potential of *Aspergillus* species, isolated from textile dye contaminated soil samples on Acridine Red dye.
Decolorization study was conducted by measuring pH and biomass change in the simulated media. Optimization of various parameters like pH, addition of 1.0% co-substrates (glucose and sucrose) as carbon source and dye concentration was carried out in shaking conditions. It was found that the isolate was able to decolorize 95 to 98% dye in Bushnell Haas medium with 1% glucose, sucrose, pH 7.0, 8.0 at room temperature with 100 to 120 ppm concentration of dye in 48 to 72 h on shaking conditions.

Rajendran et al., (2014) examined the ability of seven white rot basidiomycetes fungi, *Ganoderma lucidum* to decolorize three dyes in liquid and solid cultures. Bromophenol Blue (BPB) was tested in liquid culture and Methyl Red (MR) and Methyl Orange (MO) in solid culture for their decolorization ability. All the isolates were able to grow in the dye inoculated culture media and displayed various extents of decolorization. Among the isolates tested, CRS-5 and CRS-3 showed highest decolorization of 98% for BPB as compared to other isolates. The same isolates were also tested for decolorization in the solid medium containing the dyes *viz.*, MR and MO and the actively growing mycelium was able to biosorb the dyes within 5 days.

Yemendzhiev et al., (2014) investigated the decolorization potential of *Trametes versicolor* strain 1 on Reactive Blue 4. The decolorization experiments were carried out with different dye concentrations (50 mg/L and 125 mg/L) and glucose (1, 2 and 3%) in the medium. Lacasse enzyme activity was measured during the process of decolorization. It was shown that there was direct correlation between the observed enzyme activity and the decolorization process. It was established that the best conditions for laccase production and decolorization of 125 mg/L Reactive Blue 4 dye, were in a medium containing 3% glucose. Under these conditions 90% of Reactive Blue 4 was decolorized in 384 h.