Industrial development is one of the main approaches which increase the quality of life in densely populated and less developed regions of the world. However, many people desire to disregard the darker side of industrial development that is the pollution caused by ignoring the principles of cleaner production (Moore and Ausley, 2004). In India, the development of small scale industries to the masses has helped in decentralizing the industrial technology. However, due to the financial constraints posed on the treatment of pollutants, they are discarded into the environment and it contributes to about 40% of the total industrial wastewater (Kaushik and Malik, 2009). One such example of small scale industry is the textile industries which have become a critical environmental concern (Jacob and Azariah, 1998). The textile industry produces a multi-component waste, which is varying daily or even hourly (O’Neill et al., 2000).

2.1. TEXTILE EFFLUENT

Textile industry plays an important role in the world economy and it consumes large quantities (upto 150 l) of water for dyeing one kg of cotton (Hai et al., 2008). Effluents from the textile industries containing highly colored dyes are visualized easily (Kilic et al., 2007). Due to their high solubility, synthetic dyes are represented as common water pollutants and are frequently found in industrial wastewater. Generally dyes are considered as an objectionable type of pollutant since they are toxic (Christie, 2007). Oral ingestion, inhalation and skin sensitization of dyes causes skin irritation which leads to carcinogenicity (Rai et al., 2005). They impart color to water which interferes with the transmission of light and upset the biological metabolism leading to the
destruction of aquatic communities present in ecosystem. Further, the dyes have a tendency to sequester metal and may cause microtoxicity to fish and other organisms (Kuo, 1992).

2.2. COLOR INDEX OF DYE

More than 8,000 chemical products associated with the dyeing process are listed in the Color Index. The color index (C.I) number developed by the society of dyers and colorists is used for the classification of dyes (Color Index, 1971). The understanding of chemical structure of dye helps to assign the five digit C.I number (Reife et al., 1995).

2.3. CLASSIFICATION OF DYES

Dyes are characterized in accordance with their ability to absorb the energy of a particular part of the electromagnetic radiation in which the human eye is sensitive (Rys and Zollinger, 1972). Generally dyestuffs are classified according to origin, chemical/physical properties and characteristics related to the application process (Christie, 2007). Dyes are mainly classified as follows: Anionic (direct, acid and reactive dyes), cationic (basic dye) and nonionic dyes (disperse dye) (Fu and Viraraghavan, 2001). The chromophores in anionic and nonionic dyes are mostly azo groups or anthraquinone types (Gupta and Suhas, 2009). The dyes can also be classified based on the properties (Christie, 2007).

**Acid Dyes:** Acid dyes are widely used to dye acrylics, wool, nylon, silk, nylon/cotton blends, and also to some extent for paper, leather, ink-jet printing, food and cosmetics. Although acid dyes may be used for printing, it is not commercially important since they have poor fastness.
Cationic (Basic) Dyes: Cationic dyes are water-soluble dyes which yield colored cations, mainly used for silk, wool and tannin-modanted cotton. The predominant chemical classes of cationic dyes are diazahemicyanine, triarylmethane, hemicyanine, thiazine, oxazine and acridine.

Disperse Dyes: Disperse dyes are colloidal and have very low water solubilities. Majority of dyes are used for polyester, nylon, cellulose, cellulose acetate and triacetate fibres. These are water-insoluble nonionic dyes used for hydrophobic fibres from aqueous dispersion. Disperse dyes contain azo, anthraquinone, nitro and benzodifuranone groups.

Direct Dyes: Direct dyes are used in the dyeing of cotton and rayon, paper, leather and to some extent to nylon. These dyes are water soluble anionic dyes and commonly dyed from aqueous solutions in the presence of electrolytes and have high affinity for cellulosic fibres. Usually the dyes in this class are polyazo compounds, along with some phthalocyanines and oxazines.

Reactive Dyes: Reactive dyes differ from all other classes of dyes in that they bind to the textile fibres such as cotton by covalent bonds. They are widely used in textile industries regarding favorable characteristics of bright color, water-fast and simple application techniques with less energy consumption making them advantages over direct dyes.

Solvent Dyes: Solvent dyes are used for plastics, gasoline, lubricant, oils and waxes. These dyes are solvent soluble (water insoluble) and are generally non polar or little polar, i.e., lacking polar solubilizing groups such as sulfonic acid,
carboxylic acid or quaternary ammonium. The chemical classes are azo and anthraquinone, but phthalocyanine and triarylmethane are also used.

**Sulphur Dyes:** These dyes are used extensively for cotton and rayon with limited usage for polyamide fibres, silk, leather, paper and wood. Low cost and good wash fastness properties make this class an important and economic one.

**Vat Dyes:** Vat dyes are used for cotton, mainly to cellulosic fibres as soluble leuco salts and also in rayon and wool. These are water-soluble with predominant chemical class containing anthraquinone and indigoids.

**Azo Dyes:** The largest classes (60-70%) of dyes used in industry are azo dyes (Carliell *et al.*, 1995). These dyes are versatile class of dyes which have been used extensively than any other class (Stolz, 2001). Azo dyes are water soluble synthetic organic compounds possessing the characteristic -N=N-, which links the chromophore and auxochrome to form colored molecules of great structural diversity. Usually azo dyes contain one to three azo bonds, linking phenyl and/or naphthyl rings that are usually substituted with some other combination of functional groups including triazine, amino, chloro, hydroxyl, methyl, nitro and sulphonate (Bell *et al.*, 2000).

### 2.4. TOXICITY OF DYE

Dyes usually represent a synthetic origin and complex aromatic molecular structures which is more stable and difficult to biodegrade (Aksu and Tezer, 2005). Azo dyes are typically not removed from waste water by conventional waste-water treatment systems (Isik and Sponza, 2005).
Azo dyes representing sulphonated and unsulphonated compounds not only have a negative aesthetic effect on the wastewater but few of these compounds and their biodegradation products are toxic, carcinogenic and mutagenic (Chung and Stevens, 1993; Carliell et al., 1995). However, some azo dyes can produce toxic end products like 1,4 phenylenediamine, benzidine and substituted benzidines like o-toludine (Rosenkranz and Klopman, 1990). Sulphonated azo dyes show decreased or no mutagenic effect when compared to unsulphonated azo dyes because of their electric charge and low lipophilicity, which prevents them from uptake and metabolic activation (Rosenkranz and Klopman, 1990; Chung and Cerniglia, 1992).

Metal complex dyes usually contain chelated chromium, cobalt, copper and nickel. Few cationic dyes are marketed as zinc salts, where the zinc content will be in the order of 2-3%. Besides these, traces less than 1 ppm of hazardous metals like mercury, cadmium and arsenic are found as impurities from intermediates (Chittaranjan Desai, 1992).

2.5. SCOPE OF TREATMENT METHODS

Color is the first sign of pollution to be recognized in wastewater and it has to be removed before discharging into water bodies or on land. The presence of dyes in water is less than 1 ppm which is highly visible and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies. About 10-15% of the overall production is released into the environment mainly through wastewater (Tan et al., 2000). This has become dangerous because some of the azo dyes or their breakdown products have a strong toxic mutagenic or carcinogenic influence on living organisms (Kalyuzhnyi and Sklyar, 2000).
The growing impact of environmental protection on industrial development promotes eco-friendly technologies which minimize the consumption of freshwater and lower the output of wastewater (Forgacs et al., 2004).

2.6. DYE REMOVAL TECHNIQUES

2.6.1. Chemical methods

In general chemical oxidation involves the use of oxidizing agents such as ozone (O₃), hydrogen peroxide (H₂O₂) and permanganate (MnO₄) to alter the chemical composition of a compound or a group of compounds, e.g. dyes (Metcalf and Eddy, 2003). In advanced oxidation processes (AOP) oxidizing agents such as O₃ and H₂O₂ are used with catalysts (Fe, Mn and TiO₂), either in the presence or absence of an irradiation source (Anjaneyulu et al., 2005).

2.6.2. Physical methods

2.6.2.1. Filtration

Filtration techniques such as ultrafiltration, nanofiltration and reverse osmosis have been used for water reuse and chemical recovery. The main drawbacks of membrane technology are the high investment costs, potential membrane fouling and production of a concentrated dyebath which needs to be treated (Robinson et al., 2001).

2.6.2.2. Activated carbon

Activated carbon is the most common adsorbent and can be very effective with many dyes (Walker and Weatherley, 1997). However, its efficiency is
directly dependent upon the type of carbon material used and the wastewater characteristics, i.e. types of dyes present in the stream (Robinson et al., 2001).

2.7. BIOLOGICAL TREATMENT

Biological dye removal is mainly based on microbial biotransformation of dyes. Microorganisms are employed for the biodegradation of synthetic dyes which is an attractive and simple method by operation, but the biological mechanisms can be complex. Majority of these compounds are chemically stable and resistant to microbiological attack. The isolation of new strains or the adaptation of existing microorganisms to the decomposition of dyes will probably increase the efficacy of bioremediation of dyes. Removal of synthetic dyes by microorganisms from industrial effluents offers considerable advantages. The process is economic, cost effective and the end products resulted from complete mineralization are not toxic. Decolorization of dyes may take place in two ways: either adsorption on the microbial biomass (biosorption) or biodegradation of the dyes by the cells (Zhou and Zimmermann, 1993).

2.7.1. Biosorption

Adsorption of dyes may occur on growing/living microbial cells as well as on dead microbial cells. Biosorption of dyes does not eliminate the problem because the pollutant is not destroyed but instead entrapped into the matrix of the adsorbent (the microbial biomass). The removal of the microbial biomass containing adsorbed dyes itself is a big obstacle in the cleaning of colored waters (Chander and Arora, 2007). The disposal of the large volumes of biomass after biosorption of dyes from industrial effluents is difficult (Kuhad et al., 2004). Adsorption capacity of gram negative bacteria is highly specific to dye which is due to hydrophobic nature of dye and high lipid content in bacteria (Hu, 1992).
Actinomycetes as an absorbent in decolorization of anthraquinone, phthalocyanine and azo dyes containing industrial effluents was reported (Zhou and Zimmermann, 1993).

### 2.7.2. Biodegradation

In biodegradation, the original dye structure is destroyed and the pollutant is split into fragments by the microbial cells, sometimes achieving complete mineralization, i.e., conversion of the xenobiotic into CO₂, H₂O and some salts of inorganic origin. Decolorization of the dye occurs when the chromophoric center of the dye is cleaved (Kaushik and Malik, 2009). A range of microorganisms, including fungi, bacteria, yeasts and algae have been used for decolorization and degradation of synthetic dyes. They have been shown to have different capabilities for degrading different dyes. Development of efficient dye remediation technology requires application of a suitable selected strain and its application under favorable conditions to evaluate the degradation potential (Novotny et al., 2004).

#### 2.7.2.1. Anaerobic degradation

Under anaerobic conditions, low redox potential (≤50 mV) can be achieved which is required for effective decolorization of dyes (Bromley-Challenor et al., 2000). Color removal under anaerobic conditions is also referred as dye reduction which involves the azo bond cleavage i.e. a transfer of four-electrons (reducing equivalents), which proceeds through two stages at the azo linkage. Mordant Orange 1 and Azodisalicylate were decolorized under anaerobic conditions using methanogenic granular sludge (Razo-Flores et al., 1997). Reactive Red 141 was also decolorized under anaerobic conditions in a conventional sewage treatment technology. The chemical identification of the
products of dye degradation showed that decolorization was via reduction mechanism (Carliell et al., 1994).

The reductive decolorization of azo dyes under anaerobic conditions is a combination of both biological and chemical mechanisms. The specialized enzymes azo reductases are present in bacteria that are able to grow using only azo dye as a carbon and energy source. A co-metabolic reaction is most likely the main mechanism of dye reduction, in which the reducing equivalents or reduced cofactors like NADH, NAD(P)H, FMNH₂ and FADH₂ act as secondary electron donor or channel electrons to cleave the azo bond (Dos Santos et al., 2007). The chemical involvement to reductive decolorization of azo dyes under anaerobic conditions may involve biogenic reductants like sulphide, cysteine, ascorbate or Fe²⁺ (Yoo, 2002; Van der Zee et al., 2003).

2.7.2.2. Anaerobic/aerobic degradation

Anaerobic reduction of azo dyes is generally more suitable than aerobic degradation and the intermediate products (carcinogenic aromatic amines) have to be degraded by an aerobic process. Various technologies have been developed for the successive anaerobic/aerobic treatment of dye wastewaters. The removal of dyes from wastewaters in an anaerobic–oxic system involves both decomposition by bacteria and adsorption onto the sludge (Forgacs et al., 2004).

2.7.2.3. Aerobic degradation

Under aerobic conditions, the enzymes mono- and di-oxygenase catalyze the incorporation of oxygen into the aromatic ring of organic compounds prior to ring fission. In most monooxygenases, the electron donor is NADH or NAD(P)H were the direct coupling to O₂ is through a flavin that is reduced by
the NADH or NAD(P)H donor (Madigan et al., 2003). Even though azo dyes are aromatic compounds, their substituent containing mainly nitro and sulfonic groups are quite recalcitrant to aerobic bacterial degradation (Claus et al., 2002). This fact is probably related either to the electron-withdrawing nature of the azo bond and their resistance to oxygenases attack, or because oxygen is a more effective electron acceptor, therefore having more preference for reducing equivalents than the azo dye (Chung et al., 1992; Knackmuss, 1996). However, in the presence of specific oxygen-catalysed enzymes, azo reductases, some aerobic bacteria are able to reduce azo compounds and produce aromatic amines (Stolz, 2001).

Many azo dyes are recalcitrant to aerobic degradation by bacterial cells (Bras et al., 2001). Few microorganisms have the capability to reductively cleave azo bonds under aerobic conditions (Wong and Yuen, 1996; Coughlin et al., 2002). Various bacteria with the ability to decolorize dyes either by pure cultures or in consortia have also been reported (Rajaguru et al., 2000; Coughlin et al., 2002; Pearce et al., 2003; Verma and Madamwar, 2003).

In earlier studies, Acetobacter liquefaciens and Klebsiella pneumoniae were shown to decolorize the toxic azo dye methyl red at aerobic conditions and yielding two colorless compounds namely 2-aminobenzoic acid (ABA) and N, N’-dimethyl-p-phenylenediamine (DMPD) (Wong and Yuen, 1996).

2.7.2.4. Reductive decolourization of azo dyes in the presence of redox mediators

Redox mediators are compounds that accelerate the electron transfer from a primary electron donor to a terminal electron acceptor, which may increase the
reaction rates by one to several orders of magnitude (Cervantes, 2002; Dos Santos, 2005). Redox mediators have shown to be effective not only for reductive decolorization, but also for the reductive transformation of many contaminants such as iron (Lovley et al., 1998), nitroaromatics (Dunnivant et al., 1992), polyhalogenated compounds (O’Loughlin et al., 1999) and radionuclides (Fredrickson et al., 2000). Flavin-based compounds like FAD, FMN and riboflavin, as well as quinone-based compounds like AQS, AQDS and lawsone, have been extensively reported as redox mediators during azo dye reduction (Cervantes et al., 2000; Rau et al., 2002; Field and Brady, 2003; Dos Santos et al., 2005; Encinas-Yocupicio et al., 2006).

2.8. MICROBIAL REDUCTIVE DECOLORIZATION OF AZO DYES

2.8.1. Biodegradation by bacteria

Pure cultures either whole cells or specific enzymes are employed for a better insight of the anaerobic azo dye reduction mechanism (Pearce et al., 2006). Bacterial cultures capable of degrading azo dyes started in 1970’s with reports of Bacillus subtilis (Horitsu et al., 1977), Aeromonas hydrophila (Idaka and Ogawa, 1978) and Bacillus cereus (Wuhrmann et al., 1980). Longer period of adaptation under chemostat conditions were needed to isolate the Pseudomonas strains capable of dye decolorization (Kulla, 1981). The degradation of Orange II dye by microbes were carried out by an azoreductase enzyme and substituting any of the groups near the azo group’s chemical structure hindered the degradation (Zimmermann et al., 1982). Pseudomonas and Aeromonas species which have the capability to decolorize other dyes has been reported (Ogawa and Yatome, 1990).
Kulla et al. (1983) studied the presence of sulfo groups on the aromatic component of few azo dyes which inhibit the biodegradability of the sulfonated azo dyes by bacteria. Klebsiella pneumoniae RS-13 and Acetobacter liquefaciens S-1 capable of decolorizing methyl red have been reported in azo dye containing industrial effluents and the higher activity of K. pneumoniae has been demonstrated (Wong and Yuen, 1996). Magenta, crystal violet, malachite green, pararosaniline and brilliant green decolorization by Kurthia sp. has been reported (Sani and Banerjee, 1999). Bacillus subtilis degraded p-aminoazobenzene under anoxic conditions, producing aniline and p-phenylenediamine as main decomposition products (Zissi and Lyberatos, 1996). Anthraquinone dye degradation by B. subtilis in industrial wastewater was also observed as reduction of dyes to the leuko form and was considered as an initial step in biodegradation (Itoh et al., 1993). The ability of Pseudomonas strains for the decolorization of various dyes has also been studied (Yu et al., 2001). It has been shown that the sulfate reducing bacteria Desulfovibrio desulfuricans can also decolorize reactive orange 96 and reactive red 120 under anaerobic conditions (Yoo et al., 2000).

Azo dye reduction is a ubiquitous capability of many microorganisms under anaerobic conditions (Bromley-Chanellor et al., 2000; Stolz, 2001). The capability to biodegrade azo dyes has also been reported in Shigella (Ghosh et al., 1992) whereas under aerobic conditions azo dyes are generally resistant to attack by bacteria. Azo compounds can also be reduced to amines through co-metabolic process (Haug et al., 1991). The aromatic amines resulted through azo dye reduction was further degraded by multiple-step bioconversion taking place aerobically or anaerobically (Flores et al., 1997). Chang et al. (2001) reported Pseudomonas luteola strain expressing azoreductase activity was utilized to
remove azo dye (reactive red 22) and the effects of various factors such as substrate concentration, medium composition and operation parameters (e.g. pH, temperature, dissolved oxygen etc) was also monitored.

The *Bacillus* OY1-2 rapidly degraded Red B under aerobic and anoxic conditions (Li *et al.*, 2004). Four facultative bacteria namely, *Enterobacter* sp., *Serratia* sp., *Yersinia* sp., and *Erwinia* sp. were selected and studied for their capability to degrade C.I. reactive red 195 (Kalayani *et al.*, 2007). Patil *et al.* (2008) reported that the individual bacterial strains viz., *Bacillus odysseyi* SUK3, *Morganella morganii* SUK5 and *Proteus* sp. SUK7 decolorized reactive blue 59. Francisccon *et al.* (2009) described textile dyes decolorization by *Klebsiella* sp. strain VN-31 under microaerophilic conditions. Zissi *et al.*, (1997) reported that *B.subtilis* co-metabolizes p-aminoazobenzene in the presence of glucose as carbon source, producing aniline and *p*-phenylenediamine, confirming the breakage of azobond. Acid blue 74 was decolorized by *Aeromonas* sp. B-5 under shaking condition (Nobuki *et al.*, 2000).

Moutaouakkil *et al.* (2003) reported that physiochemical parameters such as temperature, stirring, concentration of glucose and pH of the synthetic medium play an important role on methyl red decolorization by *Enterobacter agglomerans* under aerobic conditions. The decolorization of reactive watersoluble azo dyes was achieved under anaerobic conditions using glucose as a carbon source (Carliell *et al.*, 1996). The supplement tapioca starch showed enhanced color removal efficacy from synthetic blue wastewater (Chinkewitvanich *et al.*, 2000). The substrates such as glucose, starch, lactose and sucrose on primary biodegradation of recalcitrant triphenylmethane dyes by *Pseudomonas* sp. was investigated (Oranusi and Ogugbue, 2005a). Hu (1998)
showed that *Pseudomonas luteola* grew well in less glucose concentration (0.125%) and absence of nitrogen source decolorized RP2B to about 95% under shaking incubation process. Parshetti *et al.* (2006) reported complete decolorization of malachite green under static anoxic condition by *Kerstersia rosea* MTCC 1532.

Junnarkar *et al.* (2006) reported 94% decolorization of Reactive Violet 5 (RV 5) by bacterial consortium in the presence of glucose and yeast extract under static condition and it also effectively decolorized dye in the presence of starch. *Shewanella* strain J18 143 isolated from soil contaminated with textile wastewater was able to decolorize azo dyes Remazol Black B and Acid Orange 7. Of various electron donors, acetate, formate, lactate and nicotinamide adenine dinucleotide (NADH) were suggested as the optimal electron donors. Decolorization of the dyes was enhanced by peptone present in the medium (Vijaya *et al.*, 2003).

### 2.8.2. Biodegradation by fungi

White rot fungi are efficient in the biodegradation of xenobiotics, lignin and dyestuffs by their extracellular lignolytic enzyme system (Heinfling *et al.*, 1997). Gill *et al.* (2002) isolated nine white-rot fungal strains for biodecolorization of brilliant green, cresol red, crystal violet, congo red and orange II. Decolorization of dyes by *Trametes hispida* and *Pleurotus ostreatus* in solid state cultures on whole oats has been reported (Rodriguez *et al.*, 1999). Reactive azo dyes have been effectively removed from water by the fungus *Aspergillus foetidus* (Sumathi and Manju, 2000).
Dye removal has been studied on strains of *Aspergillus niger* (Fu and Viraraghavan, 2002) and *Rhizopus arrhizus* (O’Mahony et al., 2002). Biosorption capability of fungal biomass could be increased by some pretreatment such as autoclaving or reacting with chemicals (Fu and Viraraghavan, 2001).

Jin et al. (2007) isolated a strain *Aspergillus fumigatus* XC6 from rice straw which had the capability to decolorize dye effluent with dye as sole carbon and nitrogen sources however when supplemented with appropriate nitrogen or carbon sources, the strain decolorized dye effluent completely. Ramya et al. (2008) reported that *Aspergillus* sp. effectively decolorized reactive blue and other structurally diverse synthetic dyes in the presence of glucose, sucrose and mannitol. Khelifi et al. (2009) reported the decolorizing ability of *Aspergillus alliaceus* 121 C containing carbon and nitrogen sources supplemented with indigo and congo red dyes. The biodegradation of dyes such as Amaranth, New Coccine, Orange G and Tartrazine by *P. chrysosporium* and *Pleurotus sajor-caju* was compared which suggested that Mn-peroxidase, glucosidase and laccase are involved in the decolorization process (Chagas and Durrant, 2001). Addition of activators (Tween 80, veratryl alcohol, manganese (IV) oxide) for the production of lignolytic enzymes by *P. chrysosporium* increased the decolorisation rate of the dye Poly R-478 (Couto et al., 2000). Manamekalai and Swaminathan (2000) showed that the lignin peroxidase of *P. chrysosporium* removes not only dyes but also phenol and chlorophenol from wastewaters.

### 2.8.3. Biodegradation by yeasts

Few studies have been carried out on dye decolorization by yeast (Kuhad et al., 2004; Saratale et al., 2009a). Compared to bacteria and filamentous fungi,
yeasts have many advantages they not only grow rapidly like bacteria, but like filamentous fungi they also have the ability to resist unfavorable environments (Yu and Wen, 2005). Microbial decolorization and degradation of Direct Violet 51 by *Candida albicans* isolated from industrial effluents was reported (Vitor and Corso, 2008).

### 2.8.4. Biodegradation by algae

Algae capable of degrading azo dyes have been reported (Jinqi and Houtian, 1992). Algae such as *Chlorella* and *Oscillatoria* in stabilization ponds play a significant role in aromatic amine removal (Banat *et al.*, 1996). Recent reports on degradation of azo dyes into aromatic amines by breaking the azo linkage by algae such as *Chlorella vulgaris*, *C. pyrenoidosa*, *Spirogyra* and *Oscillatoria tenuis* (Mohan *et al.*, 2002) have been observed.

### 2.8.5. Biodegradation by mixed microbial cultures

The treatment systems composed of mixed microbial populations possess higher degree of biodegradation and mineralization due to synergistic metabolic activities of microbial community and offers considerable advantages over the use of pure cultures in the degradation of synthetic dyes (Khehra *et al.*, 2005). Individual strains (of a microbial consortium) may attack the dye molecule at different positions or may use degraded products produced by another strain for further decomposition (Forgacs *et al.*, 2004). Microbial consortium consisting of both fungi and bacteria may be efficient in biodegradation and mineralization of synthetic dyes and other organic xenobiotics. Mixed culture consortia that are capable of surviving in effluents by utilizing the constituents as sources of carbon, energy and nitrogen would make the process economically feasible (Kuhad *et al.*, 2004). Joshi *et al.* (2008) described bacterial decolorization of
Acid Orange 7 in the presence of organic carbon under microaerophilic condition.

2.9. FACTORS AFFECTING BIODEGRADATION OF DYSES

Ecosystems are dynamic environments with variable abiotic conditions, like pH, temperature, presence of oxygen, metals, salts, etc. Optimization of such abiotic conditions will greatly help in the development of industrial-scale bioreactors for bioremediation (Ali, 2010).

2.9.1. pH

In general, fungi and yeasts show better decolorization and biodegradation activities at acidic or neutral pH whereas bacteria at neutral or basic pH. Nozaki et al. (2008) studied the decolorization of 27 different dyes, including monoazo, diazo, phthalocyanine and triphenylmethane dyes and optimum pH for the decolorization of the dyes was 3.0–5.0. Wang et al. (2009) studied decolorization of Reactive Black 5 by an Enterobacter sp. EC3 at pH 7 for 108 h of incubation.

2.9.2. Temperature

Temperature is an important environmental factor and the biodegradation activities of microorganisms are affected by changes in temperature. Above optimum temperature, the degradation activities of the microorganisms decrease because of slow growth, decreased reproduction rate and deactivation of enzymes responsible for degradation. Wang et al., (2009) studied decolorization of Reactive Black 5 by Enterobacter sp. EC3 at 37°C. Saratale et al. (2009b) found that the 100% decolorization of Scarlet R at 37°C by P. vulgaris and M. glutamicus.
2.9.3. Initial dye concentration

The decolorization of dye decreases with increasing dye concentration. The biodegradation of Congo Red by Bacillus sp., was inhibited at high concentrations (1,500 and 2,000 mg l\(^{-1}\)) (Gopinath et al., 2009). Jirasripongpun et al. (2007) observed that Enterobacter sp. was unable to grow in high Reactive Red 195 dye concentration (50 and 100 mg l\(^{-1}\)).

2.9.4. Agitation

Efficient color removal is observed in shaking cultures because of better oxygen transfer and nutrient distribution when compared to the stationary cultures (Kaushik and Malik, 2009). On the contrary, according to Kalyani et al. (2009), agitated culture of Pseudomonas sp. SUK1 showed almost no decolorization in 24 h, while the static culture decolorized more than 96% of the initial dye concentration (300 mg l\(^{-1}\)) of Reactive Red 2 in 6 h.

2.10. ENZYMATIC DEGRADATION OF AZO DYES

The character of enzymes and enzyme systems in microorganisms that are suitable for the decomposition of dyes has been extensively investigated. Exact knowledge of the enzymatic processes governing the decomposition of dyes is important in the environmental protection both from theoretical and practical points of view (Forgacs et al., 2004).

2.10.1. Peroxidases

Peroxidases are hemoproteins that catalyze reactions in the presence of hydrogen peroxide (Duran et al., 2002). Some peroxidase producing bacterial strains include Streptomyces species and gram negative species such as
Spingomonas chlorophenolicus, Flavobacterium ATCC 39723 decolorize azo dyes (Paszczynski et al., 1992).

2.10.2. Azoreductase

Decolorization of azo dyes by bacteria was initiated by azoreductase, which is capable for the reduction or cleavage of azo bonds in an anaerobic condition. Under aerobic, semi-aerobic (static condition) or anaerobic incubation of bacteria with azo compounds amines are often detected that resulted with a reductive cleavage of the azo bond. For aerobic reduction of azo dyes, specific enzymes (aerobic azoreductases) are necessary to catalyze the reaction in the presence of oxygen. The aerobic azoreductases obtained from carboxy-orange degrading strains K22 and KF46 were purified, characterized and compared (Zimmerman et al., 1982).

Degradation of azo dyes by bacteria is often initiated under anaerobic conditions by an enzymatic biotransformation process that involves cleavage of azo linkages by azoreductase (Kodam et al., 2005) that utilize reduced co-enzyme as an electron donor. Hu (1994) reported that P. luteola can remove reactive azo dye RBB through azoreductase reduction of the azo bond. Pseudomonas sp. and Klebsiella sp. showed azoreductase activity which is involved in the decolorization process (Russ et al., 2000; Jang et al., 2005).

Suzuki et al. (2001) showed that Bacillus sp. OY1-2 tranforms azo dyes into colorless compounds through reductase. Maier et al., (2004) reported that NADH-dependent azoreductase from Bacillus sp. strain exhibited decolorization of azo dyes. Liu et al. (2007) reported azoreductase (AZR) from Rhodobacter sphaeroides AS1.1737 which showed flavodoxin possessing nitroreductase and
flavin mononucleotide reductase activities. Bafana et al. (2008a) described azoreductase from *Bacillus velezensis* has the capacity to degrade azo dye Direct Red 28 (DR28). This enzyme was oxygen-insensitive and required NADH as co-factor. Nachiyar and Rajakumar (2005) isolated an oxygen insensitive intracellular azo reductase from *Pseudomonas aeruginosa* by ion exchange and gel filtration chromatography.

### 2.11. FUTURE PERSPECTIVES

Biodegradation of synthetic dyes using various fungi, bacteria, yeasts and algae is becoming a potential approach for the treatment of dye wastewaters. With the increasing production of synthetic chemicals and their ultimate release into the environment, the natural microbial populations are unable to decompose them in due course of time. To develop a low cost and low-technological bioprocess for the treatment of dye wastewaters, the decolorization of azo dyes with inexpensive co-substrates were carried out and the metabolism of the substrates may generate redox equivalents (electron donors) for the reductive cleavage of the chromophoric group of the dyes. The results provide information for industries to treat their wastewaters before discharging into the environment.

The application of enzyme shows considerable benefits over the direct use of microorganisms. Commercially prepared enzymes can be easily standardized facilitating accurate dosage. The application is simple and can be readily modified according to the character of the dye.

Keeping in mind the increasing production of these chemicals and their persistence in the natural environment, their removal is utmost necessary. By exploiting the biodegradation potentials of different microbes, it is possible to
handle the problem in a better way. A better understanding of biodegradation of synthetic dyes requires knowledge of Chemistry and Microbiology, whereas its application on industrial scale requires knowledge of Biochemical Engineering as well. It is fully hoped that the Science and Technology of biodegradation of organic xenobiotics will emerge as a leading one for control of environmental pollution.

An understanding and knowledge of biodegradation are not only helpful in pollution abatement but also in the production of biofriendly and environment friendly products like biodiesel, bioethanol, biopesticides, biopolymers, etc. The biodegradation abilities of microorganisms can be enhanced by gradually exposing them to higher concentrations of synthetic organic chemicals. Microorganisms exposed to higher levels of pollutants evolve mechanisms and pathways for handling (degrading) them. This happens through expression of genes encoding for enzymes responsible for degradation. Identification, isolation and transfer of genes encoding for degradative enzymes can greatly help in designing microbes with enhanced degradation capabilities.