CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter reviews the literature related to the microbial degradation of dyes and textile wastewater. Microbial degradation methods reported in the literature such as aerobic degradation, anaerobic degradation and fungal degradation are discussed in detail. Literature related to sonochemical degradation of dye and hybrid methods for the destruction of dye are presented. Literature pertaining to the SBR is also analyzed by focusing mainly on textile industry wastewater treatment.

2.2 WATER POLLUTION

Pollution control is presently one of the major areas of scientific activity. The organic compounds that produce colour generally impart a minor fraction of the organic load to wastewater but the colour renders them aesthetically unacceptable. Effluents discharged from textile and dyestuff industries to neighbouring water bodies and wastewater treatment systems are currently causing significant health concerns. Since dyes are designed to resist fading by different physical, chemical and biological agents, they cannot be degraded by conventional treatment processes.

2.3 TEXTILE INDUSTRY WASTEWATER

Textile industries consume substantial volume of water and chemicals for wet processing of textiles. These are used in desizing, scouring, bleaching, dyeing, printing and finishing operations. The chemicals used
range from inorganic compounds and elements to polymers and organic products. There are more than 8000 chemical products associated with the dyeing process listed in the colour index (Society of Dyers and Colourists 1976) while over 100,000 commercially available dyes exist. These dyes include acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal-complex dyes.

2.4 ENVIRONMENTAL IMPACTS

Interest in the pollution potential of textile dyes has been primarily provoked by concern over their possible toxicity and carcinogenicity. This is mainly due to the fact that many dyes are made from known carcinogens, such as benzidine and other aromatic compounds (Clarke and Anliker 1980). It has been shown that azo and nitro compounds are reduced in sediments (Weber and Wolfe 1987) and in the intestinal environment (Chung et al 1978), resulting in the regeneration of the parent toxic amines. Anthraquinone-based dyes are most resistant to degradation due to their fused aromatic structures, which remain coloured for long periods time. Basic dyes have high brilliance and high colour intensity, making them more difficult to decolorize. On the other hand, metal-based complex dyes containing chromium release chromium in to the environment, which is carcinogenic in nature. Some disperse dyes have the tendency to be accumulated biologically in the environment (Anliker et al 1981; Baughman and Perenich 1988). Heavy metal ions from textile effluents have also been reported to be present at high concentrations in both algae and plants exposed to such effluents (Srivastava and Prakash 1991).

2.5 MICROBIAL DECOLORIZATION

Colour removal from wastewater has become major scientific interest, because of its severe environmental impacts. During the past two decades, several physico-chemical decolourization techniques have been
reported and few methods have been accepted by the textile industries. The major impediments for the implementation include high cost, low efficiency and inapplicability to a wide variety of dyes. The ability of microorganisms in decolourising textile dye effluents has received much attention and is a cost-effective method. Recent works reported in the literature have revealed the existence of a wide variety of microorganisms for the decolourization of wide variety of dyes.

2.5.1 Fungal Decolorization

White-rot fungi able to degrade lignin, the structural polymer found in woody plants (Barr and Aust 1994) and these are capable of degrading dioxins, polychlorinated biphenyls (PCBs) and other chloro-organics (Chao and Lee 1994). The first report on aerobic degradation of azo dyes by lignolytic fungi appeared in 1990. Cripps et al (1990) demonstrated that nitrogen-limited cultures of Phanerochaete chrysosporium can be used to decolorize the azo dyes such as Acid Orange 7, Acid Orange 6, and Direct Red 28 (Congo Red). Subsequent work demonstrated that cultures of P. Chrysosporium also decolorize several other azo dyes (Banat et al 1996; Young and Yu 1997). Currently, there is no correlation known between the structure of the azo dyes and P. chrysosporium (Pasti-Grigsby et al 1992; Paszczynski et al 1992). Furthermore, it has been shown that not only P. chrysosporium but also several other fungi (mainly white rot fungi) such as Geotrichum candidum, Trametes versicolor, Bjerkandera adusta, Penicillium sp., Pleurotus ostreatus, Pycnoporus cinnabarinus, and Pyricularia oryzae are also capable of decolorizing complex azo dyes and reactive dyes (Chivukula and Renganathan 1995; Heinfling et al 1997; Kim et al 1995). Comparisons of different fungi suggested that some other fungal species (e.g. Trametes or Bjerkandera species) are superior compared to P. chrysosporium for the decoloration of dyes (Knapp et al 1995; Rodríguez et al 1999;
White-rot fungi produce enzymes such as lignin peroxidases (LiP) and manganese peroxidases (MnP). Other enzymes include glucose-1-oxidase, glucose-2-oxidase, and phenoloxidase enzyme (Archibald and Roy 1992; Thurston 1994; Schliephake and Lonergan 1996; Kirby 1999). These enzymes can also be used for the lignin degradation. Azo dyes, the largest class of commercially produced dyes, are not readily degraded by microorganisms but can be degraded by white-rot fungi (Paszczynski and Crawford 1995). Some fungi, such as, Hirschioporus larincinus, Inonotus hispidus, Phlebia tremellosa and Coriolus versicolor have also been shown to decolorize dye containing effluents (Banat et al 1996; Kirby 1999).

There are some disadvantages with fungal decolorization. The fungal enzymes, which are mainly involved in degradation of phenolic and non phenolic compounds, are lignin peroxidases and manganese peroxidases. In these enzymes, manganese peroxidases must convert Mn$^{2+}$ to Mn$^{3+}$ in order to oxidize phenolic compounds. So, the broth must contain manganese based salts (Glenn et al 1986). Phenoloxidases are oxidoreductases that can catalyze the oxidation of phenolic and other aromatic compounds without the use of cofactors (Duran et al 2002). Laccases are copper-containing enzymes that have a very broad substrate specificity with respect to electron donors (Abadulla et al 2000).

### 2.5.2 Bacterial Decolorization

Aromatic compounds are susceptible to biological degradation under both aerobic and anaerobic conditions (Field et al 1995). Under aerobic conditions, the enzymes mono- and di-oxygenase catalyze the incorporation of oxygen from O$_2$ into the aromatic ring of organic compounds prior to ring fission (Madigan et al 2003). In most mono-oxygenases, the electron donor is
NADH or NADPH, even though the direct coupling to O$_2$ is through a flavin that is reduced by the NADH or NADPH donor (Madigan et al 2003).

Although 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 oxygenase attack (Chung et al 1992; Knackmuss 1996). However, in the presence of specific oxygen catalyzed enzymes called azo reductases, some aerobic bacteria are able to reduce azo compounds and produce aromatic amines (Stolz 2001). These enzymes, after purification, characterization and comparison were shown to be flavin-free. Recently, Blumel and Stolz (2003) cloned and characterized the genetic code of the aerobic azo reductase from Pagmentiphaga kullae K24. This strain was able to grow with the carboxylated azo compound as a sole source of carbon and energy.

In the course of investigating the degradation of azo dyes, it was found that some peroxidase producing bacterial strains also decolorize azo dyes (Cao et al 1993; Paszczynski et al 1992). The oxidation of azo dyes by Streptomyces chromofuscus A11 was due to the productivity extracellular peroxidase, that showed restricted substrate specificity similar to that of the manganese peroxidase from P. Chrysosporium or horseradish peroxidase (Pasti-Grigsby et al 1996).

2.5.3 Reductive Cleavage of Azo Bond

Several bacterial strains have been reported to aerobically decolorize azo dyes by reductive mechanisms. Many of these isolates decolorize the azo compounds only in the presence of other carbon sources and therefore presumably do not use the azo dyes as carbon or energy sources. Bacillus subtilis reductively cleaved p-aminoazobenzene to aniline during aerobic growth in the presence of glucose (Zissi et al 1997). Similarly, strains of Pseudomonas stutzeri, Acetobacter liquefaciens, and Klebsiella pneumonia
were able to degrade Acid Red 2 (Methyl Red) during aerobic growth in nutrient broth or glucose (Wong and Yuen 1996; Yatome et al 1993). The reductive decolorization of sulfonated azo dyes by different bacterial strains under aerobic conditions in the presence of additional carbon sources has also been reported (Coughlin et al 1997; Dykes et al 1994; Jiang and Bishop 1994; Sugiura et al 1999). In aerobic metabolism of azo dyes, the bacterial strains were grown aerobically in complex media or sugars and then incubated without shaking in the presence of different azo dyes (Chang and Lin 2000; Chen et al 1999; Hayase et al 2000).

2.5.4 Azo Dyes as Sole Source of Nutrition

There are several claims in the literature that bacteria with the ability to reduce azo dyes aerobically in a cometabolic fashion can also use these dyes as a sole source of carbon and energy (e.g. Dykes et al 1994; Yatome et al 1993); However, only very few studies demonstrated the utilization of azo compounds as sole source of carbon and energy under aerobic conditions. The ability of bacteria to grow with simple carboxylated azo compounds as sole source of carbon and energy was first shown by Overney (1979), who has isolated a "Flavobacterium", which was found to grow aerobically with the simple model compound 4,4'-dicarboxyazobenzene. From the adaptation processes in continuous cultures, Pseudomonas sp. K22 was obtained after enrichment with carboxy Orange I and Pseudomonas sp. KF46 was obtained from enrichment with carboxy Orange II (Kulla 1981; Kulla et al 1984). These strains showed excellent degradation characteristics by consuming the dye as a sole source of nutrition.

2.5.5 Aerobic Azorecutases

During the aerobic, or semi-aerobic (static culture) or anaerobic incubation of bacteria with azo compounds, amines were often detected. These are originated from a reductive cleavage of the azo bond. The aerobic reductive metabolism of azo dyes requires specific enzymes ("aerobic
azoreductases”) that catalyze these reactions in the presence of molecular oxygen. The aerobic azoreductases from the “carboxy-orange”-degrading strains K22 and KF46 were purified, characterized and compared with each other (Zimmermann et al 1982, 1984). Both azoreductases were monomeric flavin-free enzymes that preferentially used NADPH (significantly higher K_m values NADH) as cofactors and reductively cleaved not only the carboxylated growth substrates of the bacteria but also the sulfonated structural analogues. Both enzymes significantly differed in size (21 kDa vs 30 kDa) and substrate specificity. The azoreductase from strain KF46 (Orange II azoreductase) strictly required the presence of a hydroxy-group in ortho-position in the azo bond. In contrast, the Orange I azoreductase from strain K22 required a hydroxy-group in para-position in the azo bond for catalytic activity.

Surprisingly, neither of the purified enzymes exhibited immunological cross reaction with each other, which suggests that the two enzymes are significantly different (Zimmermann et al 1982, 1984). More recently, the purification and characterization of enzymes from Shigella dysenteriae and Escherichia coli with flavin-dependent aerobic azoreductase activities has been reported (Ghosh et al 1993). The authors used assay conditions that did not allow a clear distinction between a true aerobic and a reaction caused by the intermediate formation of reduced flavins by flavin reductase activities, which then could unspecifically reduce the azo dyes (Russ et al 2000).

Finally, all these analysis provide sufficient knowledge that aerobic degradation of azo and other dyes could be a suitable technique to treat the textile effluent. Bacteria are more effective than fungi due to their adaptability towards adverse environments. Various studies reported in the literature on biological degradation of textile effluents with different organisms are summarized in Table 2.1.
Table 2.1  Microorganisms reported in the literature for the treatment of dye containing wastewater

<table>
<thead>
<tr>
<th>Wastewater / Dye used</th>
<th>Organisms used</th>
<th>Remarks</th>
<th>References</th>
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<tbody>
<tr>
<td>Red 2G azo dye</td>
<td>Streptococcus faeclis</td>
<td>Reduced soluble flavins acting as electron shuttles between NADPH-dependent flavoproteins and azo dye</td>
<td>Gingell and Walker (1971)</td>
</tr>
<tr>
<td>Azo food dyes</td>
<td>Proteus vulgaris</td>
<td>The study concluded the decolorization as zero order reaction and reported that the rate depends on redox potential of dye</td>
<td>Dubin and Wright (1975)</td>
</tr>
<tr>
<td>p-Aminoazobenzene</td>
<td>Bacillus subtilis</td>
<td>Reported the reduction of PAAB under aerobic conditions</td>
<td>Horitsu et al (1977)</td>
</tr>
<tr>
<td>Aminoazobenzene compounds</td>
<td>Aeromonas hydrophilia</td>
<td>54 – 90% color removal was reported with an incubation period of 30 h.</td>
<td>Idaka et al (1978)</td>
</tr>
<tr>
<td>Azo dyes</td>
<td>Bacillus cereus, Sphaerotilus natans, Arthrobacter sp., and Activated sludge</td>
<td>Deals with reduction under anoxic conditions involving non-enzymatic intracellular reduced flavin nucleotides</td>
<td>Wuhrmann et al (1980)</td>
</tr>
<tr>
<td>Acid, direct and basic dyes</td>
<td>Pseudomonas pseudomallei</td>
<td>Azo dyes were decolorized more easily than triphenylmethanes.</td>
<td>Yatome et al (1981)</td>
</tr>
<tr>
<td>p-Aminoazobenzene</td>
<td>Pseudomonas pseudomallei</td>
<td>80% color removal was reported. Metabolism was favored at poor nutrient conditions.</td>
<td>Ogawa et al (1981)</td>
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<tr>
<td>Orange I, Orange II</td>
<td>Pseudomonas sp.</td>
<td>After 7 generations of adaptation to Orange I, about 35% was found to be degraded. After 10 generations, more than 90% of Orange II was degraded.</td>
<td>Kulla et al (1983)</td>
</tr>
<tr>
<td>Orange I, Orange II</td>
<td>Pseudomonas sp.</td>
<td>Comparison of bacterial azoreductases for Orange I and Orange II, differing in their specificity with regard to position of the hydroxyl group.</td>
<td>Zimmerman et al (1984)</td>
</tr>
<tr>
<td>C.I.Acid Orange 12</td>
<td>Pseudomonas cepacia</td>
<td>Multi-stage continuous aerobic cultivation using buffered, low nutrient media. Microbial growth inhibited at high dye concentrations and 90% decolorization was reported in 68 h.</td>
<td>Ogawa et al (1986)</td>
</tr>
<tr>
<td>C.I.Acid Orange 6</td>
<td>Field-collected and laboratory cultures</td>
<td>Aerobic degradation of dye was reported.</td>
<td>Michaels and Lewis (1986)</td>
</tr>
<tr>
<td>C.I.Basic Violet 1</td>
<td>Activated sludge</td>
<td>Aerobic biodegradation was carried out for a short period and around 13% biodegraded and 23% absorbed</td>
<td>Pagga and Brown (1986)</td>
</tr>
<tr>
<td>Various acid, basic, direct, mordant and reactive dyes</td>
<td>Activated sludge</td>
<td>Aerobic biodegradation was carried out for a short period and around 13% biodegraded and 23% absorbed</td>
<td>Pagga and Brown (1986)</td>
</tr>
<tr>
<td>39 reactive dyes with different structures</td>
<td>Immobilized Pseudomonas sp.</td>
<td>Around 22 dyes were decolorized (&gt; 90% decolorization) in 12 h of incubation</td>
<td>Huang et al (1990)</td>
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<td>Nine different azo dyes based on amino-azobenzene and naphthalene-azobenzene</td>
<td>Pseudomonas stutzeri (cell free extract)</td>
<td>80-90% color removal was reported for all the dyes after a 20 h period. Specificity shown for lowest redox potential dyes.</td>
<td>Yatome et al (1990)</td>
</tr>
<tr>
<td>Reactive dyes</td>
<td>Anaerobic sludge</td>
<td>Reactive dyes were decolorized via reduction</td>
<td>Carliell et al (1994)</td>
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<tr>
<td>4 reactive azo dyes</td>
<td>Pseudomonas luteola</td>
<td>37-93% color removal was obtained after 42 h. Color removal was found to be high at static conditions.</td>
<td>Hu (1994)</td>
</tr>
<tr>
<td>C.I. Acid Orange 10</td>
<td>Anaerobic sludge</td>
<td>2-stage anaerobic-aerobic reactor. 65 – 90% decoloration in first stage</td>
<td>Fitzgerald and Bishop (1995)</td>
</tr>
<tr>
<td>Diazo – linked chromophores</td>
<td>Mixed anaerobic culture</td>
<td>85% color removal in 2 days and color removal was due to the reduction of azo bond</td>
<td>Knapp and Newby (1995)</td>
</tr>
<tr>
<td>Various azo and diazo reactive dyes</td>
<td>Thermophilic anaerobic bacterial culture</td>
<td>68 – 84% color removal in 48 h</td>
<td>Banat et al (1996)</td>
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Table 2.1  (Continued)

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<thead>
<tr>
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<tbody>
<tr>
<td>P-Aminoazobenzene</td>
<td>Bacillus subtilis</td>
<td>Concentration decreased from 10.2 to 7.5 mg/l in 25 h in the presence of glucose and nitrate</td>
<td>Zissi and Lyberatos (1996)</td>
</tr>
<tr>
<td>Reactive dyes, diazo dyes, azo dyes, disperse dyes and phthalocyanine dyes</td>
<td>Alcaligenes faecalis, Commonomonas acidovorans</td>
<td>100% decolorization under anaerobic conditions of 5 out of 9 dyes within 48 h</td>
<td>Nigam et al (1996a)</td>
</tr>
<tr>
<td>Cationic, chromium containing azomethine dye</td>
<td>Aerobic mixed bacterial culture</td>
<td>Partial degradation occurred by releasing chromium present in the dye and dye adsorption was also observed partially</td>
<td>Matanic et al (1996)</td>
</tr>
<tr>
<td>Reactive dyes</td>
<td>Alcaligenes faecalis, Commonomonas acidovorans</td>
<td>67 – 89% decoloration of reactive dyes under anaerobic conditions</td>
<td>Nigam et al (1996b)</td>
</tr>
<tr>
<td>Remazol Black B</td>
<td>Alcaligenes faecalis, Commonomonas acidovorans</td>
<td>Microbial consortium immobilized on gravel and over 95% decolorization was reported within 48 h</td>
<td>Oxspring et al (1996)</td>
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<tr>
<td>Amaranth, Orange II, tartrazine</td>
<td>Bacteroides fragilis</td>
<td>80% amaranth, 50% orange II and 20% tartrazine were removed in 8 h, under anaerobic conditions. These different rates of reduction could be correlated with the half-wave potential of the azo dye.</td>
<td>Bragger et al (1997)</td>
</tr>
<tr>
<td>Ramazol Black B, 8 other reactive dyes, anthraquinone dye, industrial wastewater</td>
<td>Shewanella putrefaciens</td>
<td>95% dye removal was observed and 84% color removal was observed from industrial wastewater</td>
<td>Willmott (1997)</td>
</tr>
<tr>
<td>4-Carboxy-4′-sulphoazobenzene</td>
<td>Hydrogenophaga palleronii 5</td>
<td>0.35 mM dye was completely reduced in 40 min under aerobic conditions.</td>
<td>Blumel et al (1998)</td>
</tr>
<tr>
<td>Amaranth, Orange II</td>
<td>Clostridium perfringens</td>
<td>Reduction to below detective level in 50 h under anaerobic conditions.</td>
<td>Semde et al (1998)</td>
</tr>
<tr>
<td>Triphenylmehtane dyes, textile wastewater</td>
<td>Kurthia sp.</td>
<td>98% color removal was obtained for triphenylmethane dyes and 56% color removal was obtained for textile wastewater in 90 h.</td>
<td>Sani and Banerjee (1999)</td>
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Table 2.1 (Continued)

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<thead>
<tr>
<th>Wastewater / Dye used</th>
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<tbody>
<tr>
<td>Simulated textile wastewater containing procion red H-E7B</td>
<td>Sludge from textile wastewater treatment plant (Inclined tubular digester) granules from paper pulp processing plant (UASB reactor)</td>
<td>78% color removal was reported by anaerobic treatment.</td>
<td>O’Neil et al (1999)</td>
</tr>
<tr>
<td>Acid azo dyes, Direct azo dyes and amaranth</td>
<td>Sphingomonas sp. BN6</td>
<td>Cell extracts were used and it showed enhanced activities than whole cells.</td>
<td>Russ et al (2000)</td>
</tr>
<tr>
<td>Reactive Red 3.1</td>
<td>Activated sludge obtained from domestic and industrial effluent treatment plants</td>
<td>Decolorization rates up to 20 – 30 mg/l/h were given by activated sludge under anaerobic conditions.</td>
<td>Challenor et al (2000)</td>
</tr>
<tr>
<td>C.I.Acid Red 42, C.I.Acid Red 73, C.I.Direct Red 80, C.I.Disperse Blue 56</td>
<td>Original seed sludge collected from a municipal wastewater treatment plant</td>
<td>Average removal efficiency for acid dyes was between 80 and 90%. The removal efficiency for C.I.Direct Red 80 was 81%. Removal of C.I.Disperse Blue 56 was unsuccessful</td>
<td>Goncalves et al (2000)</td>
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<tr>
<td>Azo dyes</td>
<td>Mesophilic sludge from a lab-scale UASB reactor</td>
<td>56% removal of azo dye COD was achieved at a volumetric load of 0.3 g azo dye COD / l / day.</td>
<td>Kalyuzhnyi and Sklyar (2000)</td>
</tr>
<tr>
<td>Remazol Black B, textile wastewater</td>
<td>Shewanella putrefaciens</td>
<td>85% Remazol Black B removed in 6.5 h in a suspended growth reactor with continuous flow and 80% color removed from textile wastewater in 24 h</td>
<td>Russ et al (2000)</td>
</tr>
<tr>
<td>Orange G, Amido Black 10B, Direct Red 4BS and Congo Red</td>
<td>Four bacterial strains (Pseudomonas) isolated from dyeing effluent-contaminated soils</td>
<td>The maximum degradation rates reported for Orange G, Amido Black 10B, Direct Red 4BS and Congo red were, 60.9 mg/l/day, 571.3 mg/l/day, 112.5 mg/l/day and 134.9 mg/l/day respectively.</td>
<td>Rajaguru et al (2000)</td>
</tr>
<tr>
<td>C.I Reactive Orange 96</td>
<td>Desulfovibrio desulfuricans</td>
<td>Under anaerobic conditions and in the presence of sulphide, over 95% color removal was obtained in 2 h. The reduction products were aromatic amines and elemental sulphur.</td>
<td>Yoo et al (2000)</td>
</tr>
<tr>
<td>C.I Reactive Red 22</td>
<td>Pseudomonas luteola</td>
<td>A specific decolorization rate of 113.7 mg dye/ g cell/ h and an overall decolorization efficiency of 86.3 mg dye/l/h were obtained.</td>
<td>Chang and Lin (2000)</td>
</tr>
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<tr>
<td>C.I.Reactive Red 22</td>
<td>Pseudomonas luteola</td>
<td>Specific decoloration rates of 18.6 mg/g cell/h for free cells and 3.5 mg/g cell/h for immobilized cells were obtained.</td>
<td>Chang et al (2001)</td>
</tr>
<tr>
<td>C.I.Acid Orange 7</td>
<td>Mixed and methanogenic cultures</td>
<td>The %color removal reported was 94%.</td>
<td>Bras et al (2001)</td>
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<tr>
<td>Industrial wastewater</td>
<td>Bacterial consortia</td>
<td>Continuous operation was carried out and complete removal of dye was achieved.</td>
<td>Plumb et al (2001)</td>
</tr>
<tr>
<td>Hydrolyzed C.I.Reactive Orange 96</td>
<td>Bacterial consortia</td>
<td>The %decolorization reported was 95% after 40 h of incubation.</td>
<td>Yoo et al (2001)</td>
</tr>
<tr>
<td>Hydrolyzed Remazol Brilliant Violet 5R and Remazol Black B</td>
<td>SBR with sludge collected in a full-scale, continuous activated sludge plant</td>
<td>90% color removal was obtained for Remazol Brilliant Violet 5R and 75% color removal was reported in 24 h cycle with sludge retention time of 15 days and an aerated reaction phase of 10 h.</td>
<td>Lorenzo et al (2001)</td>
</tr>
<tr>
<td>Remazol Black B</td>
<td>Paenibacillus azoreducens</td>
<td>98% color removal within 24 h at a dye concentration of 100 mg/l and at a temperature of 37°C</td>
<td>Meehan et al (2001)</td>
</tr>
<tr>
<td>Reactive azo dyes, Direct azo dyes and leather dyes</td>
<td>Pseudomonas luteola</td>
<td>59 – 99% color removal after 2 – 6 days in a static incubator at a dye concentration of 100 mg/l.</td>
<td>Hu (2001)</td>
</tr>
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<tr>
<td>Acid Orange 7</td>
<td>Uncharacterized aerobic biofilm</td>
<td>Acid orange 7 was completely degraded within 1 h in a rotating drum bioreactor containing biofilm and two bacterial strains were able to mineralize, in co-culture, up to 90% of the Acid Orange 7.</td>
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<tr>
<td>Congo red</td>
<td>UASB sludge</td>
<td>Congo red concentrations of 100 – 4000 mg/l were treated and 98% degradation was achieved.</td>
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<tr>
<td>Acid violet-17</td>
<td>Bacterium consortium</td>
<td>Bioreactor, operating at a flow rate of 6 ml/h, resulted in 91% decolorization of 30 mg/l of Acid violet-17 with 94.3 and 95.7% removal of BOD and COD</td>
<td></td>
</tr>
<tr>
<td>Malachite Green</td>
<td>Kocuria rosea</td>
<td>Complete decolorization of 50 mg/l of dye under static anoxic condition within 5 h</td>
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<tr>
<td>Azo dye (Cibacron Red FN-R)</td>
<td>Methanogenic sludge</td>
<td>92 – 97% decolorization was attained during biological degradation, whereas dissolved organic carbon removal was not obtained. Ozonation was practiced as a post treatment to remove dissolved organic carbon successfully from the treated effluent.</td>
<td></td>
</tr>
</tbody>
</table>

References:
- Isik and Sponza (2003)
<table>
<thead>
<tr>
<th>Wastewater / Dye used</th>
<th>Organisms used</th>
<th>Remarks</th>
<th>References</th>
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<tbody>
<tr>
<td>Textile dye Red BLI</td>
<td>Psudomonas sp. SUK1</td>
<td>About 99.28% decolorization was obtained for 50 mg/l of dye within 1 h under static anoxic condition at a pH range from 6.5 to 7.0 and 30°C.</td>
<td>Kalyani et al (2008)</td>
</tr>
<tr>
<td>Methyl orange</td>
<td>Pseudomonas luteola</td>
<td>Decolorization efficiency of 95% was reported at 6 and 9 h for 100 and 350 mg/l, respectively.</td>
<td>Hsueh and Chen (2007)</td>
</tr>
<tr>
<td>Erichrome Black – T</td>
<td>Pseudomonas luteola</td>
<td>86% color removal for 110 mg/l after 10 h, and color removal for 230 mg/l of dye was completely repressed after 2 – 3 h.</td>
<td>Hsueh and Chen (2007)</td>
</tr>
<tr>
<td>Acid orange 7</td>
<td>Enterobacter sp., Psudomonas sp., Morganella sp.</td>
<td>Bacteria were inoculated on activated carbon to form a biofilm and used for degradation experiments. Degradation followed second order kinetics.</td>
<td>Barragan et al (2007)</td>
</tr>
<tr>
<td>Coracryl black,</td>
<td>Daedalea flavida, Polyporus sanguineus, Phanerochaete chrysosporium</td>
<td>Cell free enzyme extracts obtained at different intervals and when incubated with various dyes resulted in 100% of decolorization in 48 h.</td>
<td>Chander and Arora (2007)</td>
</tr>
<tr>
<td>Disperse yellow 3,</td>
<td>Pleurotus ostreatus</td>
<td>The degradation was found to be 50% after 5 days of incubation.</td>
<td>Zhao and Hardin (2007)</td>
</tr>
<tr>
<td>Disperse orange 3</td>
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<tr>
<td>Congo red</td>
<td>Pseudomonas luteola</td>
<td>95% decolorization was observed at 10 and 12 h for 110 and 210 mg/l dye respectively.</td>
<td>Hsueh and Chen (2007)</td>
</tr>
<tr>
<td>Methyl red</td>
<td>Pseudomonas luteola</td>
<td>Methyl red has not been decolorized due to its recalcitrant nature from 100 – 450 mg/l</td>
<td>Hsueh and Chen (2007)</td>
</tr>
<tr>
<td>Azo dye (Direct Black 38)</td>
<td>Textile sludge</td>
<td>A continuous culture experiment showed that the acclimatized sludge decolorized 76% of 100 mg/l dye.</td>
<td>Bafana et al (2007)</td>
</tr>
<tr>
<td>Oxazine, Diazo dyes</td>
<td>Shewanella oneidensis</td>
<td>The results showed that the decolorization is an extracellular reduction process requiring a multi-component electron pathway.</td>
<td>Brige et al (2008)</td>
</tr>
<tr>
<td>Direct Red 5B</td>
<td>Comamonas sp. UVS</td>
<td>About 1100 mg/l of dye in nutrient broth was degraded in 125 h.</td>
<td>Jadhav et al (2008)</td>
</tr>
<tr>
<td>Direct Brown MR</td>
<td>Acinetobacter calcoaceticus NCIM 2890</td>
<td>Activities of lignin peroxidase, laccase and azoreductase were monitored. Degraded products were less toxic than the original dye.</td>
<td>Ghodake et al (2009)</td>
</tr>
<tr>
<td>Reactive Red 2</td>
<td>Pseudomonas sp. SUK1</td>
<td>52% COD reduction was obtained in 24 h. lignin peroxidase and azoreductase activities were detected.</td>
<td>Kalyani et al (2009)</td>
</tr>
<tr>
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<td>Organisms used</td>
<td>Remarks</td>
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<tr>
<td>Reactive Blue 220</td>
<td>Lentinus crinitus</td>
<td>Maximum decolorization efficiency against RB220 achieved in this study was around 95%.</td>
<td>Niebisch et al (2010)</td>
</tr>
<tr>
<td>Triphenylmethane dyes</td>
<td>Staphylococcus epidermidis</td>
<td>750 mg/l of concentrations of various triphenylmethane dyes were degraded with high efficiency with the addition of yeast extract and glucose.</td>
<td>Ayed et al (2010)</td>
</tr>
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</table>
2.6 SONOCHEMICAL DEGRADATION OF DYES

Recent developments in advanced oxidation processes (AOP), which effectively generate free radical species, offer complete colour removal, detoxification and mineralization of the effluents from textile dyeing units under appropriate conditions (Ince et al 1997).

In 1950, Weissler and Cooper found that ultrasonic degradation of carbon tetrachloride leads to chlorine formation. Several authors have studied the ultrasonic degradation of CCl₄ and concluded that the ultrasound allows complete degradation of this compound into chloride and carbon dioxide after a relatively short irradiation time (Ayyildiz et al 2005; Lim et al 2007).

Ince and Tezcanli (2001) studied the degradation of reactive dye by combined sonolysis (520 kHz) and ozonation. This technique was found to be efficient due to the synergistic effect of both the processes. The radical chain reactions taking place during thermolysis of water in collapsing cavities may have contributed chemically to the synergy by providing additional decomposition pathways.

Rehorek et al (2004) used 850 kHz ultrasound at 60, 90 and 120 W for the degradation of industrial azo dyes Acid Orange 5 and 52, Direct Blue 71, Reactive Black 5 and Reactive Orange 16 and 107. The results showed that ultrasound was able to mineralize azo dyes to non-toxic end products. All investigated dyes have been decolorized and degraded within 3 – 15 h at 90 W and within 1 – 4 h at 120 W, respectively. Mass spectrometric investigations showed that hydroxyl radicals destructed the azo dyes by simultaneous azo bond scission, oxidation of nitrogen atoms and hydroxylation of aromatic ring structures.
Gogate et al (2004) developed a novel sonochemical reactor with a capacity of 7.5 l and degraded Rhodamine B. Effect of various operating parameters such as frequency of irradiation, use of multiple frequencies and power dissipation into the system on the extent of degradation were studied. For the degradation of Rhodamine B, power dissipation into the system was found to be the controlling parameter and the extent of degradation is directly proportional to the power dissipation with a coefficient of 74.1.

Vajnhandl and Le Marechal (2007) reported the decolourization and mineralization of reactive dye C.I. Reactive Black 5 by means of ultrasonic irradiation at 20, 279 and 817 kHz. This study reported that the decolorization as well as radical production increased with an increase in frequency, acoustic power and irradiation time. The increase in dye concentrations resulted in decreased decolorization rates.

Bejarano-Perez and Suarez-Herrera (2007) studied the degradation of malachite green by sonolytic, photocatalytic and sonophotocatalytic methods. Carbon tetrachloride was used as an additive in sonochemical degradation experiments. This study showed that CCl$_4$ was found to improve the degradation efficiency of the sonochemical process. The sonochemical and photochemical degradation processes was found to follow first-order kinetics.

The decolorization of Acid Black 210 by ultrasonic irradiation in the presence of exfoliated graphite was investigated by Li et al (2008). Low pH value and large exfoliation volume of exfoliated graphite favored the ultrasonic decolorization of Acid Black 210. The combined method of ultrasonic waves and exfoliated graphite achieved better results than either exfoliated graphite or ultrasound alone. Improved decolorization efficiency was observed in the present system compared to the process using a combination of ultrasound and activated carbon. In the combined method
utilizing ultrasonic waves and exfoliated graphite, approximately 99.5% of Acid Black 210 was removed using 0.8 g/l exfoliated graphite at a pH of 1 within 120 min at 51°C.

Degradation of reactive Brilliant Red K-BP in aqueous solution by means of ultrasonic cavitation was investigated by Wang et al (2008) at different operating conditions. It was found that the degradation of reactive Brilliant Red K-BP in aqueous solution follows pseudo-first-order reaction kinetics and the degradation rate is dependent on the initial concentration of reactive Brilliant Red K-BP, the temperature and acidity of the aqueous medium.

2.7 SEQUENCING BATCH REACTOR FOR TEXTILE WASTEWATER TREATMENT

SBR is more attractive than other bioreactors because of its various advantages: (1) fill and draw systems achieve better degree of treatment than continuous flow systems (2) the volume needed for continuous flow is about twice that needed for corresponding fill-and-draw system and (3) the initial costs are no higher, and probably less, than those of a conventional systems with cost for operation and maintenance about the same as those for conventional systems.

Sirianuntapiboon et al (2005) studied the treatment of vat dye containing wastewater in SBR system. The maximum dye (Vat Yellow 1), COD, BOD\textsubscript{5} and TKN removal efficiencies of the SBR system at an MLSS of 2000 mg/l and an HRT of three days were 98.5 ± 1.0%, 96.9 ± 0.7%, 98.6 ± 0.1% and 93.4 ± 1.3%, respectively. Although, the dye and organic removal efficiencies of the SBR system with real textile wastewater were quite low, they could be increased by adding organic matters, especially glucose. The dye, COD, BOD\textsubscript{5} and TKN removal efficiencies of the SBR system with
glucose (0.89 g/l) supplemented textile industrial wastewater were found to be 75.12 ± 1.2%, 70.61 ± 3.4%, 96.7 ± 0.0%, and 63.2 ± 1.1%, respectively.

The performance of aerobic and anaerobic SBR in treating Orange II containing wastewater was investigated by Ong et al (2005). The results from a specific oxygen uptake rate (SOUR) study showed that the Orange II compound did not exert significant inhibitory effects on the activity of activated sludge microorganisms. The increase in organic loading rate from 2.66 to 5.32 g COD/l day had slightly improved the COD removal efficiency in aerobic SBR but affected the COD removal efficiency in an anaerobic SBR. On the other hand, the increase in organic loading rate improved the Orange II removal efficiency in both the SBR systems. In the case of 100 mg/l Orange II addition, the average fraction of Orange II removed was 15 and 80% in aerobic and anaerobic SBR, respectively.

Kapdan and Oztekin (2006) studied an anaerobic–aerobic SBR, for the treatment of a textile dyestuff (Remazol Rot RR) containing synthetic wastewater at different anaerobic–aerobic residence times and initial COD concentrations (400 to 1800 mg/l). The total reaction time was kept constant at 23 h in all experiments. A dyestuff biodegrading facultative anaerobic bacterial consortium was used as the dominant bacterial culture. The initial COD concentration did not significantly affect the system and 500 mg/l was determined as sufficient to obtain over 90% of the color and more than 85% COD removal efficiency in SBR. Higher concentrations did not improve color removal but decreased the COD removal performance of the system.

Abu-Guhnmi and Jamrah (2006) studied the biological treatment of wastewater discharged from the textile industry using SBR. The characteristics of textile wastewater and the biodegradation of its organic constituents under unsteady state conditions were reported. Then, a bench-scale pilot plant was used to study the performance of SBR by monitoring the
settleability and change in the constituents over time. Results of the study showed that textile wastewater contains different types of pollutants: heat, basicity, suspended solids, organic and inorganic matter, and heavy metals. The factors affecting the biodegradation of organic matter include the reaction time and the ratio of initial substrate to sludge concentrations.

Tantak and Chaudhari (2006) studied two stage sequential Fenton’s oxidation followed by SBR to achieve complete decolorization and mineralization of azo dyes, viz. Reactive Black 5 (RB5), Reactive Blue 13 (RB13), and Acid Orange 7 (AO7). Degradation of dye was assessed based on COD reduction and reduction in aromatic amines. In overall treatment train 81.95, 85.57, and 77.83% of COD reduction was achieved with RB5, RB13, and AO7 dyes, respectively. Fenton’s oxidation process followed by aerobic SBR treatment sequence seems to be viable method for achieving significant degradation of azo dye.

Ong et al (2008) investigated the mineralization of C.I. Acid Orange 7 (AO7) by biological process in oxygen limited condition under GAC-biofilm configured sequencing batch reactor (SBR) operation. The granular activated carbon (GAC) used was immobilized with azo dye degrading microbes through attachment by immersing the GAC into anaerobic bioreactor treating dye-containing wastewater for more than 200 days. The system was fed with 2 l of AO7 containing wastewater and was operated under FILL, REACT, DRAW and IDLE modes in a time ratio of 3:20:0.45:0.15 for a cycle time of 24 h. Nearly complete mineralization of AO7 was achieved with the biological system working at an initial AO7 concentration of 625 mg/l, dissolved oxygen (DO) below 0.25 mg/l and without the presence of external carbon sources.
2.8 SCOPE AND OBJECTIVES OF THE PRESENT INVESTIGATION

The scope of the present study was to analyze the microbial decolourization of a model dye (Congo red) using Bacillus subtilis with a pretreatment (sonolysis) and to improve the biodegradation by mutating the bacteria using random mutagenesis. The main objectives of the present investigation include:

- To isolate the bacterial species and identify the isolates through 16SrRNA analysis, biochemical and colony morphology.
- To study the microbial decolourization of Congo red using the isolate at various operational conditions.
- To study the sonochemical degradation of Congo red at various operational conditions.
- To study the influence of sonolytic pretreatment on microbial decolourization of Congo red.
- To develop improved microbial species by random mutagenesis (UV and chemical (EtBr) mutagenesis) for the treatment of Congo red.
- To compare the performance of biodegradation process using wild and mutant species.
- To study the microbial decolourization in a sequencing batch reactor (SBR).