Chapter-2. Review of Literature
CHAPTER-2.

REVIEW OF LITERATURE

2.1 Anaerobic reduction and biodegradation of azo dyes:

Under anaerobic conditions azo dyes are readily decolorized as a result of the reductive transformation of the azo group which results in the production of colorless aromatic amines. The anaerobic decolorization of azo dyes was first investigated using intestinal anaerobic bacteria (Walker, 1970). Later these compounds were found to become readily decolorized with various other pure cultures (Wuhrmann et al., 1980), mixed cultures (Haug et al., 1991), anaerobic sediments (Weber and Wolfe, 1987), digester sludge (Brown and Laboureur, 1983), anaerobic granular sludge (Razo-Flores et al., 1997) and activated sludge under anaerobic conditions (Bromly-Challenor et al., 2000).

Chung et al. (1978) conducted a study measuring the degradability of seven azo dyes using intestinal and other major anaerobes. The studies were carried out using isolated strains of bacterium in suspended cell mediums containing the different azo dyes. Their findings showed that the reduction of azo compounds could be accomplished by intestinal and other major anaerobes. Furthermore, the presence of aromatic intermediates was also detected in measurable amounts for each dye.

Brown and Laboureur (1983) investigated the anaerobic degradability of 22 commercial dyes. Of the dyes studied, four monazo and six diazo dyes showed moderate to variable reductions.

Brown et al. (1987) studied the degradability of various azo dyes in both anaerobic and aerobic systems.

Later in 1987, Brown and Hamburger conducted a study on 14 azo dyes subjected to anaerobic sludge digestion followed by aerobic treatment.

Although this effectively alters the chromogen and destroys the observed color of the dye, many aromatic groups are not susceptible to anaerobic reduction. However, there is evidence that some azo dye metabolites may be fully stabilized in anaerobic environments (Weber and Wolfe, 1987).
Rafii et al. (1990) reported that a plate assay was developed for the detection of anaerobic bacteria that produce azoreductases. With this plate assay, 10 strains of anaerobic bacteria capable of reducing azo dyes were isolated from human faces. The average rate of reduction of direct blue 15 dye in these strains ranged from 16 to 135 nmol of dye per min per mg of protein. The enzymes were inactivated by oxygen.

The anaerobic decolorization of azo dyes was first investigated using intestinal anaerobic bacteria (Chung et al., 1992).

Chung et al. (1992) demonstrated that azo dye reduction can be carried out by pure cultures of Proteus vulgaris and Streptococcus fecalis, and some other facultative anaerobes, these bacteria constitute only a small portion of the total intestinal microbiota.

In studies conducted by Loyd (1992) and Ganesh (1994), the anaerobic reduction of textile mill effluents and the azo dyes reactive black 5 and navy 106 were investigated, respectively. In both cases, laboratory scale anaerobic reactors were used for dye degradation. The results of Loyd and Ganesh were similar; both observed good decolorization with minimal nutrient removal. While high decolorization of textile effluents is often achieved in anaerobic environments.

A microbial consortium, PDW, decolorized commercially important textile dyes under anaerobic conditions. It reported that decolorization was dependent upon the presence of a carbon and energy source in addition to the textile dyes. PDW removed 76% of color from textile effluent plant after 3 days (Nigam et al., 1996).

Oxspring et al. (1996) reported that an upflow anaerobic filter was developed with a microbial consortium, consisting of predominantly of Alcalignes fecalis and Comamonas acidovorans, immobilized on gravel support, decolorized remazol black B textile dye by 95% within 48 hours at initial dye concentration of 0.5g/l.

Kuldlich et al. (1997) investigated the effect of different artificial redox mediators on the anaerobic reduction of azo dyes by Sphingomonas sp. strain BN6 or activated sludge. Reduction rates were greatly enhanced in the presence of sulfonated anthranquinones. For strain BN6, the presence of both cytoplasmic and membrane-bound azoreductase activity was found.
Anaerobic reduction of azo dyes using microbial sludge can be an effective and economic treatment process for removing color from dye house effluents. Previous studies have demonstrated the ability of anaerobic bacteria to reductively cleave the azo linkages in reactive dyes (Razo-Flores et al., 1997; Chinwetkitvanich, 2000).

Razo-Flores et al. (1997) investigated the fate of Mordant Orange 1 (MO 1) and Azodisalicylate (ADS) under methanogenic conditions using continuous up flow-anaerobic-sludge-blanket (UASB) reactors. Their research focused on the reduction by-products, 5-aminosalicylic acid (5-ASA) and 1, 4-phenylenediamine. Co-substrates, VFA or glucose, were also fed to the reactors in order to supply the reducing equivalents needed for the reduction of the azo bonds. The results of this study demonstrated the ability of an anaerobic consortium to completely mineralize some azo dye compounds. They observed the complete mineralization of ADS with and without a co-substrate, indicating the possibility for aromatic amine destruction in methanogenic environments.

Chinewetkitvanich et al. (2000) performed a study on various reactive dye bath effluents. The research examined the effect of co-substrate and initial color concentrations on fiber-reactive dye reduction efficiencies in UASB reactors. Five different experiments were conducted using a variation of red, blue, and black dye synthetic wastewaters and also real dye house effluents composed of red, blue, and black dyes. Their results showed that by adding a co-substrate, such as tapioca, increased reduction efficiencies could be achieved. However, at high levels of tapioca addition no enhancement was observed. Furthermore, the nonspecific action of anaerobic bacteria allows the biodegradation of a wide range of textile dyes thus making this process more suitable for application on a commercial scale. Anaerobic reduction of azo dyes by bacteria seems to be better suited for the decolorization in sewage treatment systems (Stolz, 2001).

Chang and Lin (2001) reported that a 6.3 DNA fragment containing gene responsible for azo-dye decolorization was cloned and expressed in *Escherichia coli*. The resulting recombinant strain *E. coli* CY1 decolorized azo dye reactive red 22 (200 mg/l). Aeration strongly inhibited the decolorization, but decolorization occurred effectively under static incubation conditions.
Diniz et al. (2002) evaluated the anaerobic decolorization of sulfonated azo dye, congo red by a strain of sulphate reducing bacterium. The results showed that the mechanism of reductive decolorization of this sulfonated azo dye was extracellular and nonenzymatic.

Song et al. (2003) reported that Rhodobacter sphaeroides AS.1737 decolorized more that 90% of several azo dyes (200 mg/l) in 24h under anaerobic condition.

Under anaerobic conditions many bacteria reduce the highly electrophilic azo bond in the dye molecule through nonspecific enzymatic action (Pearce et al., 2003).

Nikolova and Nenov (2004) investigated the transformation of the azo dye schwarz GRS under anoxic conditions in batch reactor. It was found that degradation rates were much higher under anaerobic conditions. The highest biotransformation rate obtained was 542μmol/g under anaerobic conditions in the presence of glucose.

Yemashova et al. (2004) reported the decolorization of three acid dyes i.e. acid orange 6, acid orange 7 and acid orange 52 by methanogenic granular sludge from an anaerobic expanded granular sludge bed reactor. Complete bioreduction was found for all three azo dyes. Sulfanilic acid and 4-aminosorcinol were detected from the decolorizaton of acid orange sulfanilic acid and 1-amino-2-naphttol were detected from the reduction of acid orange 7- and sulfanilic acid and N, N-dimethyl-1, 4-phenylenediamine (DMP) were found to be intermediates of acid orange 52 degradation. Sulfanilic acid and 1-amino-2napthol were persistent in the anaerobic conditions, whereas 4-aminosorcinol was completely mineralized by anaerobic sludge and DMP was transformed into 1, 4-phenylenediamine.

Maier et al. (2004) reported that a Bacillus sp. strain isolated from wastewater drain of a textile finishing company. An NADH-dependent azoreductase of this strain was found to be responsible for the decolorization of azo dyes. They reported that decolorization only took place in the absence of oxygen and was enhanced by FAD.

Sponza and Isik (2004) investigated the decolorization of direct black 38 at concentrations varying between 200 and 3200 mg/l with partially granulated anaerobic mixed culture using glucose as carbon source and electron donor during batch incubation.
Textile wastewater was treated, on site, by means of a two-stage fixed-bed-reactor pilot plant and immobilized anaerobic bacteria on special porous carriers made from reticulated sintered glass. Full decolorization of the textile wastewater can be achieved utilizing this technique (Georgiou et al., 2005).

Ren et al. (2006) reported that Aeromonas hydrophilia strain DN322 decolorized a variety of synthetic dyes including triphenylmethane, azo and anthraquinone dyes. For azo and anthraquinone dyes e.g. acid amaranth, great red GR, reactive red KE-3B and reactive brilliant blue -K-GR (50mg/l) could be decolorized more than 85% within 36 hours under anoxic condition.

Hong et al. (2007) investigated the reduction and biodegradation of mechanism of naphthalene sulfonic azo dye amaranth using a newly isolated Shewanella decolorationis S12. Under anaerobic conditions amaranth was reduced by strain S12 and a stoichiometric amount of two reduction products RP-1 and RP2 were generated. UV/visible spectrophotometric and high performance liquid chromatography (HPLC) indicated that RP1 and RP2 were 1-aminenaphthylene -4-sulfonic acid and 1-aminenaphthylene -2-hydroxy -3, 6- disulfonic acid. The result strongly supports a mechanism of azo dye reduction by the process via the reductive cleavage of the azo bond to form corresponding aromatic amines.

Shewanella decolorationis S12 reduced a variety of azo dyes by use of formate, lactate, pyruvate or H2 as the electron donor. The results provide evidence that the microbial anaerobic azo reduction is linked to the electron transport chain and dissimilatory azoreduction is a form of microbial anaerobic respiration (Hong et al., 2007).

The bacterial isolate Rhizobium radiobacter MTCC 8161 decolorized a sulfonated diazo dye reactive red 141 (50 mg/l) in static anoxic conditions. The degradation efficiency of this strain using urea and yeast extract showed fast decolorization. GC-MS analysis indicated naphthalene diazonium, p-dinitrobenzene and 2-nitrosonaphthol as the final products of reactive red 141. (Telke et al., 2008).

Shewanella putrefaciens completely decolorized 100 mg/l of RB-5 and DO-3 within 6 and 8 hours respectively under static conditions (Crowley et al., 2008).
Attempt have been made to decolorize disperse azo dye Navy Blue-3G by *Brevibacillus laterosporus* within 48 hours under static condition at the concentration of 50 mg/l. Bromobenzene ester substituted aniline derivative, meta-di-nitro benzene and ortho benzene aniline derivative were the degradation products confirmed by GC-MS analysis (Gomare *et al*., 2009).

The *Lactobacillus casei* LA1133, *Lactobacillus paracasei* LA0471 and other lactic acid modified azo dye, tartrazine under anaerobic conditions (Perez-Diaz and McFeeters, 2009).

Olukanni *et al* (2010) reported the decolorization and biodegradation of Reactive Blue 13 (RB13), a sulphonated reactive azo dye, was achieved under static anoxic condition with a bacterial strain identified as *Proteus mirabilis* LAG, which was isolated from a municipal dump site soil near Lagos, Nigeria. This strain decolorized RB13 (100 mg/l) within 5 hours. The formation of aromatic amine prior to mineralization was supported by Fourier transform infrared spectrometry (FTIR), which revealed the disappearance of certain peaks, particularly those of the aromatic C–H bonding at 600–800 cm\(^{-1}\). Gas chromatography–mass spectrophotometry (GC/MS) analysis of the dye metabolite showed the presence of sodium-2 (2-formyl-2-hydroxyvinyl) benzoate, with a tropylium cation as its base peak, this suggested the breakage of naphthalene rings in RB13. The detection of azoreductase and laccase activities suggested the enzymatic reduction of azo bonds prior to mineralization. In addition, phytotoxicity studies indicated the detoxification of RB13 to non-toxic degradation products by this strain of *P. mirabilis* LAG.

### 2.2 Aerobic reduction and biodegradation of azo dyes:

The aerobic reductive metabolism of azo dyes requires specific enzymes (aerobic azoreductases), which catalyze the NAD (P) H-dependent reduction of azo compounds to the corresponding amines. The aerobic azoreductase from the carboxy-Orange II-degrading strain *Pseudomonas* KF46 was purified and characterized. The azoreductase converted not only carboxy-orange II but also several sulfonated structural analogues (Zimmermann *et al*., 1982).

Aerobic microbes cannot reduce azo linkages, their ability to destroy dye chromogen is less than anaerobic bacterium. However, aerobic sludge has been successfully used to stabilize dye metabolites (Brown and Laboureur, 1983).
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Brown (1983) conducted a study on 87 commercial dyestuffs. The tests were performed in a reactor designed to simulate the conditions of and adapted activated sludge wastewater treatment plant. The samples were tested for color and COD removal following 42 days of treatment. Pagga and Brown concluded that dyestuffs are most unlikely to biodegrade in short-term aerobic tests. They further indicated that the primary mechanism for removal for dyes in activated sludge systems may occur by adsorption onto the cell walls. Also, they concluded that COD removal is possible in an aerobic environment.

Evidence for the existence of specialized azo dye reducing enzymes, so-called azoreductases has only been found in some aerobic and facultative aerobic bacteria (Kulla et al., 1983). The existence of enzymes catalyzing azo dye reduction in aerobic bacteria was for the first time proven when two azoreductases from obligate aerobic bacteria were isolated and characterized (Zimmermann et al., 1984).

Shaul et al. (1991) conducted a study on 18 dyes to determine their fate in the activated sludge process. Of these dyes, 15 were acid azo dyes and three were direct azo dyes. The dyes were spiked into pilot-scale treatment system, and effluent and sludge samples were collected. High performance liquid chromatography (HPLC) was used to analyze the samples. Mass balance calculations were performed to determine the amount of the dye in the sludge and in the effluent. Eleven of the dyes passed through the activated sludge system substantially untreated, four were significantly absorbed onto the sludge, and three were apparently biodegraded.

The occurrence of aerobic conversions of sulfonated azo dyes was reported by Heiss et al., (1992). Shaul et al. (1991) reported a complete mineralization of a sulfonated azo dye was found under aerobic conditions.

Loyd (1992) also performed activated sludge treatment tests on two textile wastewaters. The first was a textile dyeing and finishing process water that contained reactive navy 106, and the second was municipal wastewater consisting predominately of textile effluents. Both effluents were fed to laboratory-scale activated sludge reactors. The reactor effluents were analyzed for ADMI color and TOC removal. Loyd concluded that aerobic treatment of the azo dye wastewaters provided significant biodegradation with minimal decolorization, and the biodegradation did not include the azo dyestuffs.
Asides from the specific azoreductases, also non-specific enzymes catalyzing azo dye reduction have been isolated from aerobically grown cultures of *Shigella dysenteriae* (Ghosh *et al.*, 1992), *Escherichia coli* (Ghosh *et al.*, 1993) and *Bacillus* sp. (Rafii and Cerniglia, 1995).

The degradation of azo dyes was also observed in aerobic biofilm reactors (Costerton *et al.*, 1994; Jiang and Bishop, 1994).

In some studies, aerobic color removal of certain azo dyes was achieved, but all these strains required an additional carbon and energy source for their growth (Zissi *et al.*, 1997).

A bacterial strain S5, derived from *Hydrogenophaga palleronii* S1, was able to reduce the azo dye 4-carboxy-4-sulfobenzene and to mineralize the azo dye reduction products 4-aminobenzenesulfonic acid (4-ABS) and 4-aminobenzoic acid (Blumel *et al.*, 1998). Similarly, a bacterial strain MI2, isolated from a biofilm reactor, was able to utilize acid orange 7 and 8 (Coughlin *et al.*, 1997). Furthermore, it was found that *Sphingomonas* sp. strain ICX could use the sulfonated and unsulfonated azo dyes, acid orange 7, acid orange 8, acid orange 10, acid red 4 and acid red 88 as sole carbon and nitrogen source. (Coughlin *et al.*, 1999).

A facultative *Staphylococcus arlettae* bacterium isolated from an activated sludge process in a textile industry successfully decolorized (97%) four different azo dyes under micoraerophilic conditions. Further aeration of the decolorized effluent was performed to promote oxidation of the degradation products. The degradation products were characterized. The presence of aromatic amine in the micoraerophilic stage indicated the presence of azoreductase activity and an oxidative biodegradation process respectively (Elisangela *et al.*, 2009).

The experiments with the recombinant *E. coli* gave further indications that the metabolism of sulfonated azo dyes was apparently often limited by the transport of the highly charged dyes into the microbial cells (Russ *et al.*, 2000).

The successful degradation of acid red 151 was described using an aerobic sequenced biofilm reactor and mineralization experiments showed that 73% of the carbon was transformed into carbon dioxide (Quezada *et al.*, 2000).

Conventional activated sludge treatment of wastes is often an effective and highly economic system for reducing organic pollutants in wastewater. Aerobic treatment of azo dye wastes...
has proven ineffective in most cases, but is often the typical method of treatment used today (Edwards, 2000).

It was reported that a gene coding for an aerobic azoreductase was cloned from *Xenophilus azovorans* KF46F to *Escherichia coli*. The enzyme was heterologously expressed in *Escherichia coli*. The results suggested that whole cells of the recombinant *E. coli* strains were unable to take up sulfonated azo dyes and therefore, did not show in vivo azoreductase activity. However, the reduction of several azo dyes by cell extracts was significantly evident (Blumel *et al.*, 2002).

It suggested that microbial strains with the ability to decolorize sulfonated azo dyes intracellularly will require not only the presence of azoreductases but also a transport system which allows the uptake of the sulfonated dyes into the cells (Van der Zee, 2002).

Most mono-azo dyes tested had higher color removal than the diazo dyes (Chen *et al.*, 2003).

Sandhya *et al.* (2005) reported that facultative micro-organisms (as consortium) under microaerophilic conditions decolorize azo dyes and total biodegradation occurs under aerated conditions.

### 2.3 Combined anaerobic and aerobic biodegradation of azo dyes:

Brown and Hamburger (1987) conducted a study on the ultimate biodegradability of various dyestuffs. Fourteen azo dyes and two other dye types were studied using lab-scale anaerobic and activated sludge reactors. Metabolite production was observed following anaerobic treatment, indicating the presence of eight identifiable aromatic amines.

In one case there exists real proof of mineralization of an azo dye by a bacterial co-culture under sequential anaerobic/aerobic batch conditions (Haug *et al.*, 1991).

Loyd (1992) also performed ANA/AER sequential step-treatment on the two textile effluents. The results generally showed a high decolorization in the anaerobic phase with little TOC, BOD, or COD removal. Loyd also states that there was very little methane or carbon dioxide production in the anaerobic phase. In the aerobic phase, the anaerobic effluent showed a higher degree of TOC removal and less decolorization.
Seshadri and Bishop (1994) investigated the fate azo dyes acid orange 7, acid orange 8, acid orange 10, and acid red 14 in an ANA/ AER sequential step treatment system. They used a bench-scale fluidized-bed anaerobic reactor (FBR) followed by a bench-scale activated sludge reactor as a sequenced second stage treatment step. Their results indicated that the transformation of all the dyes to intermediates was readily achieved by anaerobic reduction, and was assumed to be the result of azo bond cleavage. Complete mineralization was not observed, however, COD and color removals were greatest at HRT’s of 12 and 24 hours, with one hour being the shortest HRT tested and 24 hours the longest. However, the largest cumulative removals occurred in the first two hours of treatment. COD removal was variable depending on the dye, but reductions were seen in both the anaerobic and aerobic phases. Aerobic oxidation of dye intermediates was necessary to decrease COD levels to an acceptable range.

Fitzgerald and Bishop (1995) used three lab-scale reactor system to study the degradation of the azo dyes acid orange 10, acid red 14, and acid red 18. The reactor system included an anaerobic fluidized bed system in the first stage, which was followed by an aerobic Swisher reactor in the second stage. Their results indicated a high degree of degradation of the acid red 18 and acid red 14 in the anaerobic stage, with decolorization greater than 90 percent. Acid Orange 10 was only decolorized by 70 percent. Analysis of the dye intermediates suggested a high degree of removal in the anaerobic stage. Low color loss and COD removal was measured in the aerobic stage.

The proper conditions can be created by keeping the oxygen concentrations and diffusion of oxygen low, so that anaerobic and aerobic microniches are created (Costerton et al., 1994). Supplying oxygen with co-substrate can also create such integrated anaerobic/aerobic conditions. In this system, the reduction of the azo dyes and the aerobic mineralization of the aromatic amines will proceed side by side in the same biofilm. (Field et al., 1995).

The suggested integrated anaerobic/ aerobic conditions can be created by using an aerobic granular sludge with a high tolerance for oxygen as a carrier material for aerobic biofilm (Zitomer and Shrout, 1998). These integrated conditions are in practice and are also possible by use of a support material. Because anaerobic bacteria will develop inside the support material
and in the outer layer of the support material aerobic bacteria will grow, which in this way form a protection barrier for the anaerobic bacteria (Kudlich et al., 1996).

Anaerobic and aerobic microorganisms can cooperate beneficially in one single biofilm (Zitomer and Shrout, 1998).

Anaerobic bacteria are often able to reduce the azo linkages, but are generally unable to further stabilize the dye metabolites; it is advantageous to follow anaerobic treatment processes with an aerobic treatment step. Aerobic microorganisms can oxidize aromatic ring compounds to simpler molecules. A substantial amount of research has been conducted on anaerobic/aerobic sequential step–treatment systems used for degrading textile wastewaters (O’Neill et al., 2000).

O’Neill et al. (2000) conducted a study on reactive azo dye procion red H-E7B. Their research investigated the degradation of procion red H-E7B in an ANA/AER sequential step-treatment system comprised of a lab-scale UASB reactor and an activated sludge tank. To determine the extent of dye degradation, O’Neill et al. used HPLC-UV methods to detect for polar compounds in reactor effluents. This data was then compared with color and total organic nitrogen (TON) removals. The authors observed an increase in polar, UV-absorbing groups in the anaerobic effluent as compared to the reactor feed. The majority of nutrient removal occurred in the anaerobic phase, indicating some degradation of the dye. TON levels were observed to increase after anaerobic treatment followed by a subsequent decrease after aerobic treatment. HPLC also indicated the presence of highly polar compounds in the aerobic effluent, indicating a removal or conversion of the aromatic amine groups to simpler molecules. Based on these findings, O’Neill concluded that procion red H-E7B could be qualitatively shown to degrade to aromatic amine derivatives after anaerobic treatment with subsequent oxidation of these derivatives following aerobic treatment.

The sequential anaerobic and aerobic degradation has been studied for the conversion of azo dyes by numerous researchers (O’Neill et al., 2000). The major part of the organic load (co-substrate) was consumed anaerobically in a sequential anaerobic/aerobic treatment system, and consequently the aromatic amines serve as main substrate for the organisms in the aerobic
bioreactor. Aromatic amines are generally degraded aerobically, but for sulfonated aromatic amines this only is the case for a few of these compounds (Tan and Field, 2000).

A bacterial strain *Pseudomonas luteola* possessing azoreductase activity was utilized to decolorize a reactive azo dye reactive red 22 with fed-batch process consisting of an aerobic cell growth stage and an anaerobic fed-batch decolorization stage. Dissolved oxygen strongly inhibited the azo dye reduction activity; thus aeration should be avoided during decolorization but slight agitation (around 50 rpm) was needed. Overall, decolorization efficiency tended to increase with increasing feeding concentration of dye up to 600 mg dye. Higher initial culture volume resulted in better dye removal (Lin, 2000).

### 2.4 Aerobic biodegradation of aromatic amines:

In an earlier study by Brown and Laboureur (1983b), the aerobic biodegradability of aniline, o-toluidine, p-anisidine, o-dianisidine, and 3, 3’-dechlorobenzindine, was investigated. These compounds are all lipophilic aromatic amines and possible by-products of azo dyes. Because many aromatic structures are non-biodegradable in anaerobic environments and are not hydrophills, they can accumulate in the adipose tissues of organisms. Many aromatics have been identified as possible carcinogens, which make their release into the environment a concern. Previous work by Brown and Labourreur (1983a) indicated that azo dyes may be broken down to their intermediate structures in a reductive environment, but were not amenable to further degradation by anaerobes.

Brown and Laboureur (1983b) concluded that aniline, p-anisidine, p-phenetidine and o-toludine were readily biodegradable by aerobes, while o-dianisidine and 3, 3’-dichlorobenzindine were inherently biodegradable. They suggested that these compounds could be stabilized if released into the environment or directly form a dye house into a conventional wastewater treatment plant.

Various specialized aerobic bacteria were found to degrade several amino-naphthalene sulfonic acids. 6-A-2-NS has been found the most studied amino-napthalene-sulfonic acids and was completely degraded by two co-cultures and one bacterial strain ASL-4 (Nortemann *et al.*, Ph.D. thesis, Narsinghe A.P. (2011), Dr. B. A. M. U. Aurangabad.

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1986; Wittich et al., 1988). Nortemann et al. (1986) isolated *Sphingomonas* sp. BN6 responsible for the initial degradation of 6-A-2-NS out of the co cultures.

Strain S1 degrades 4-ABS via a dioxygenase to 5-sulfocatechol, this compound was then utilized by strain S2. Furthermore, three strains have been described that use the sulfonic acid group of all three aminobenzensulfonic acid isomers as a sulfur source for growth under sulfur-limiting growth conditions with glucose as the carbon source. These bacteria utilized a monoxygenase for the desulfonation (Zurrer et al., 1987).

Thurnheer et al., (1988) used a co-culture of *Comamonas testosteroni* T-2, *C. testosteroni* PSB-4, *Alcaligenes* sp. O-1, strain M-1 and strain S-1 to degrade 2-, 3-, 4-ABS, 4-BOS, 4-TS, benzene-sulfonic acid (BS), and 4-hydroxy-benzene-sulfonic acid (4-HBS) in a continuous stirred tank reactor. A maximum degradation rate of 138 mg/hr/1 was observed for all compounds together. At the end of the experiment after 903 days 4-chloro-benzene-sulfonic acid (4-CBS) was supplied to the reactor and also this compound was degraded.

Two *Pseudomonas* strains, isolated from soil samples by a Japanese group, were able to degrade 2-A-l-NS. Both bacteria degraded the compound via an initial 1, 2-dioxygenase, which lead to the desulfonation and deamination of the molecule (Ohe et al., 1990).

Co-culture consisting of 11 bacterial strains, was enriched from a treatment plant of industrial wastewater was able to degrade 6-A-2-NS. (Rozgaj and Glancer, 1992).

Similarly, a co-culture, composed of the two bacterial strains *Hydrogenophaga palleronii* S1 and *Agrobacterium radiobacter* S2, was found to completely mineralize 4-ABS (Feigel and Knackmuss, 1993). The strain BN6 also was able to to transform 5-, 6-, 7- or 8-, A-2 –NS. These compounds were degraded via an initial dioxygenase, which helped the desulfonation process. After ring cleavage, the corresponding amino-salicylic acids accumulated in the growth medium. Amino-salicylic acid was degraded aerobically by another member of the co-culture (Nortemann et al., 1994). The biodegradation of 5-A-2-NS by strain BN6 resulted in the formation of a dead-end product 5-hydroxy-quinoline-2-carboxylic acid (Nortemann et al., 1993).
Results of the biodegradation experiments, using mixed natural cultures from sludges and sediments, have demonstrated that aromatic sulfonates are sometimes biodegradable. As far as the amino benzene sulfinic acids are concerned, only the simple substituted amino benzene sulfinic acids were found to be aerobically biodegradable (Junker et al., 1994).

Finally, Zissi et al. (1997) investigated the biological oxidation of p-aminoazobenzene (pAAB) by Bacillus subtilis. This was carried out in batch experiments using a suspension medium supplemented with glucose, ammonium chloride, and pAAB under sterile conditions. Cellular growth rates and inhibition, glucose utilization, pAAB degradation, and by-product formation were observed. The results proved that Bacillus subtilis could co-metabolize pAAB in the presence of glucose, breaking the N=N double bond and producing aniline and p-phenylenediamine. Furthermore, evidence was found that suggested pAAB was inhibitory to microbial growth, and that glucose was the growth limiting substrate. The degradation of the dye was the direct result of an oxygen-insensitive azo reductase enzyme found to be present in the soluble fraction of the biomass. This enzyme was also synthesized independently of the presence of pAAB.

Some aromatic amines like phenylene-di-amines, amino-phenols, amino-naphthol and o-amino-hyroxy naphthale-sulfonic acid tend to autoxidize under aerobic conditions (Kudlich et al., 1999).

Van der Zee (2002) reported different bacterial strains including Alcaligenes sp.O-1, M-1, S-1 and S-3 that showed varying abilities of degradation of 2-ABS, 3-ABS, 4-ABS.

2.5 Anaerobic degradation of aromatic amines: 

Of the many different aromatic amines tested, only a few were degraded in anaerobic condition. In one case a complete anaerobic mineralization of the azo dye azodisalicylate was observed under methanogenic conditions. This azo dye was first reduced to the aromatic amine, 5-aminosalicylic acid (5-ASA) and next 5-ASA was anaerobically mineralized (Razo-Flores et al., 1997).

Fermentative bacteria used some specific aromatic sulfonates as a sulfur source under anaerobic condition. Also, one of the sulfonic acid groups of the sulfonated azo dye acid red 1
(disulfonated azo dye) was reported to be removed by *Clostridium strains* (Denger and Cook, 1997). Potentially, the sulfonic acid group could be used as alternative electron acceptors under anaerobic conditions, (Lie *et al*., 1998).

Some aromatic amines, substituted with hydroxyl or carboxyl group were degraded under methanogenic and sulfate reducing conditions (Kalyuzhnyi *et al*., 2000).

2.6 Factors Affecting Dye Biodegradation:

Due to the highly variable nature of biological treatment systems and especially textile effluents, there are a number of factors that may affect the biodegradation rate of azo dyes. Parameters such as temperature, pH, dissolved oxygen or nitrate concentrations, type and source of reduction equivalents, bacteria consortium, and cell permeability can all affect the biodegradation of azo dyes and textile effluents. Dye related parameters such as class and type of azo dye (i.e. reactive-monoazo), reduction metabolites, dye concentration, dye side-groups, and organic dye additives could also affect the biodegradability of azo dye wastewaters (Wallace, 2001).

Wuhrmann *et al.* (1980) investigated the effects of pH, temperature, type and concentration of respiration substrates, and oxygen tension on the rate of biological reduction of a variety of azo dyes. Temperatures can result in the exclusion of a particular group of microorganisms. Using activated sludge, Wuhrmann *et al.* (1980) determined that temperature has an increasing liner relationship with the reduction rate of orange II and Lanasylviolet up to 28°C.

The effectiveness of all the six bacterial isolates in decolorizing 24 different dyes depended on the structure and complexity of the dyes, particularly on the nature and position of constituent in the aromatic rings and the resulting interactions with the azo bond (Zimmerman *et al*., 1982).

The wastewater pH can affect the proper functioning of both anaerobic and aerobic organisms (Grady *et al*., 1999). Loyd (1992) observed an indirect increase in the rate of decolorization of Navy-106, with decreased pH values in anaerobic batch tests.

Glucose found to inhibit the decolorizing activity (Knapp and Newby, 1995).

Nitrate and especially oxygen may play an important role in determining the rate of dye reduction. The presence of oxygen generally inhibits the degradation of azo dye chromogens.
Wuhrmann et al. (1980) demonstrated that obligate aerobes might actually decolorize azo compounds under temporary anoxic conditions. However, nitrate concentrations in the mixed liquor of activated sludge plants could significantly inhibit dye removal. Zissi and Lyberatos (1996) observed Bacillus subtilis to degrade p-aminoazobenzene under anoxic conditions.

Zissi and Lyberatos (1996) demonstrated that Bacillus subtilis was partly able to degrade the disperse azo dye p-aminobenzene under anoxic conditions growing in a batch-reactor. The results proved that Bacillus subtilis co-metabolizes p-aminobenzene under denitrifying conditions in the presence of glucose as a carbon source, producing aniline and p-phenyldiamine.

Hu (1998) reported decolorization of dyes by facultative bacteria. COD, BOD reduction showed that decolorization occurs during the logarithmic growth phase (within 24 hours) and COD/BOD reduction during the maximum stationary growth phase.

Often, bacterial cultures are unable to proliferate when an azo dye is the sole carbon and nitrogen source. Therefore, additional, readily biodegradable sources may be necessary (Wallace, 2001).

However, in other reports glucose addition found to enhance the decolorization process (Kapdan et al., 2000). A bacterial strain A. hydrophila was reported to decolorize RED RBN more than 90% within 8 days at a dye concentration of 300 mg/l in a medium containing a mixture of dyes within 2 days of incubation. Decolorization found a growth related process and was not enhanced significantly by the increase in yeast extract from 8 to 10 g/l after 1 day of incubation. In contrast to a nitrogen source, glucose (higher than 0.15 g/l) inhibited decolorization activity because the consumed glucose was converted to organic acids that might decrease the pH of the culture medium, thus inhibiting the cell growth and decolorization activity. However, addition of phosphate buffer provided a good pH control as well as high decolorization activity and cell growth of A. hydrophila (Chen et al., 2003).

The degradation of navitan fast blue-SSR, a very important commercial diazo dye in the tannery and textile industries was investigated. Pseudomonas aeruginosa decolorized this dye at concentrations up to 122 mg/l. The organism required ammonium salts and glucose to co-metabolize the dye. Organic nitrogen sources did not support appreciable decolorization, whereas,
combined with inorganic nitrogen (NH$_4$NO$_3$), there was observed an increasing effect both on the growth and decolorization mechanism. HPLC analysis confirmed the formation of metanilic acid from the dye, which on further incubation was completely metabolized under shaken culture condition (Nachiyar and Rajkumar, 2003).

Sandhya et al. (2005) reported that facultative microorganisms under microaerophilic conditions decolorize azo dyes and total biodegradation occurs under aerated conditions. Shaking conditions removed COD by 59.20%, but the decolorization was only 20% at 56 mg/l of dye mixture after 72 hours. When the same medium was incubated under static conditions at 30°C, almost 100% decolorization was observed within 16-20 hours, indicated that aeration might have arrested azoreductase enzyme secretion thereby inhibiting decolorization.

The Bacillus sp. strain PS decolorized methyl orange as a model azo dye after 2 days of incubation under aerobic. GC-MS analysis showed that its degradation product were N, N-dimethyl 1, 4-phenylene diamine. (Pourbabaee et al. 2005).

Xu et al. (2007) reported the complete biodegradation of azo dye, fast acid red a under microaerophilic conditions by Shewanella decolorationis S12. A further biodegradation of decolorizing products can only achieved under microaerophilic conditions. Under microaerophilic conditions, S. decolorationis S12 could use a range of carbon sources for azo dye decolorization, including lactate, formate, glucose and sucrose, with lactate being the optimal carbon source. The decolorizing products, aniline, 1, 4-diaminobezene and 1-amino-2-napthol, were confirmed by HPLC and GC-MS analysis.

Bacillus subtilis HM aerobically decolorized 90% of fast red. Addition of glucose improved dye decolorization. Omitting both carbon and nitrogen sources from the medium caused 57.6% decrease in color removal (Mona and Hoda, 2008).

Gomare et al. (2009) reported that Brevibacillus laterosporus showed decolourization of thirteen different azo dyes including methyl red. Decolourization of methyl red was faster (93% within 12 h) under static condition at the concentration 0.2 g l$^{-1}$. Induction in the activities of lignin peroxidase, laccase, aminopyrine N-demethylase, NADH-DCIP reductase and malachite green reductase was observed in the cells obtained after decolourization. Fourier transform
infra-red spectral analysis of products indicated conversion of methyl red into secondary aryl amines and nitrosamines, which further transformed into the aromatic nitro compounds. Gas chromatography–mass spectroscopy analysis suggested conversion of methyl red into high molecular weight complex derivatives. The heterocyclic substituted aryl amine, \( p-(N, N\text{ di formyl})-\)substituted para-di amino benzene derivative and \( p\)-di-amino benzene derivative are the mainly elected biotransformation products. Microbial and phytotoxicity studies suggested nontoxic nature of the biotransformation product.

An isolated bacterium from a textile disposal site, \( Pseudomonas \) sp. SUK1, has the ability to decolorize the reactive textile dyes and methyl orange. This bacterium showed the potential to decolorize the textile dye Reactive Blue 59 at a high concentration (5 g/L\(^{-1}\)), which is frequently used in the textile industry. Induction in the activities of lignin peroxidase, azoreductase, and dichlorophenol indophenol reductase was observed during the decolorization of methyl orange and reactive blue 59. Methyl Orange (as model azo dye) was used to understand the mechanism of biodegradation by \( Pseudomonas \) sp. SUK1. The final product was identified as 1, 4-benzenediamine, N, N-dimethyl by gas chromatography-mass spectroscopy. Microbial and phytotoxicity studies revealed the nontoxic nature of the products of Reactive Blue 59 (Kalyani et al., 2009).

Ola et al. (2010) reported that \( Bacillus cereus \) which was isolated from dye industrial waste, decolorized two reactive azo dyes cibacron black PSG and cibacron red P4B under aerobic conditions. \( B.\) cereus decolorized cibacron red P4B by 81 % using the combination of ammonium nitrate and sucrose, while it decolorized cibacron black PSG by (75%) using yeast extract and lactose.

Saratale et al. (2011) reported that \( Proteus vulgaris \) NCIM-2027 cells immobilized on \( Luffa cylindrica \) (Loofa) completely decolorized C.I. Reactive Blue 172 at 37 °C and pH 8.0 under 5-h static incubation with high total organic carbon (TOC) and chemical oxygen demand (COD) reduction. The repeated-batch decolorization experiments also indicate good reusability of the immobilized biocatalyst. Some oxidoreductive enzymes were shown to be involved in the decolorization and degradation process. Loofa immobilized cells were also able to decolorize a
mixture of reactive dyes in batch mode (in terms of ADMI value) with significant reduction in TOC and COD. Loofa immobilized cells were also used for continuous decolorization of individual and mixture of reactive dyes in a fixed bed bioreactor.

Anjaneya et al. (2011) reported that two different bacterial strains capable of decolorizing a highly water soluble azo dye metanil yellow were isolated. The bacterial strains were identified as Bacillus sp. AK1 and Lysinibacillus sp. AK2 decolorized metanil yellow at 200 mg/l concentration completely within 27 and 12 hours respectively. Various parameters like pH, temperature, NaCl and initial dye concentrations were optimized to develop an economically feasible decolorization process. The maximum concentration of metanil yellow 1000 mg/l was decolorized by strains AK2 and AK1 within 78 and 84 hrs respectively. These strains could decolorize metanil yellow over a broad pH range 5.5-9.0; the optimum pH was 7.2. The decolorization of metanil yellow was most efficient at 40°C and confirmed by UV-visible spectroscopy, TLC, HPLC and GC/MS analysis. Further, both the strains showed the involvement of azoreductase in the decolorization process.