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Review Article

Microbial Decolorization of Azo Dyes – A Mini Review

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The continuous development of industries has resulted in the increase of wastewater leading to consequential decline in levels and quality of natural water in the ecosystem. The textile industries have increased in many of the countries and have shown a significant increase in the use of synthetic complex organic dyes as colouring material. Release of waste waters from the textile industries is a problem in many parts of the world especially the discharge of coloured effluents into the environment is undesirable as many of the dyes and their breakdown products are toxic or mutagenic. Azo dyes are commonly used in textile industries and due to their complex structure they are highly persistent in natural environments. Most of the physicochemical dye removal methods generally used for effluent treatment have many limitations. The treatment of textile dyeing wastewaters has become a challenging task. Microbial decolorization and metabolism of azo dyes has long been known and bioremediation technology has attracted interest. This review paper discusses the abilities of diverse categories of microorganisms (bacteria, yeasts and fungi) in decolorizing and degrading structurally different azo dyes under varying physiochemical conditions. This review also discusses different conditions for azo dye decolorization (aerobic, anaerobic and anoxic) and the various mechanisms adopted by the microbes for metabolism of azo dyes.

INTRODUCTION

The The Environment we live in is composed of atmosphere, earth, water and space. This natural environment in the absence of pollution remains clean and amicable. The composition and complex nature of this stable environment was altered as a result of various human activities such as industrialization, construction and transportation. These major activities though desirable for human development and welfare, have led to the production and release of different kinds of contaminants into the environment causing instability and disorder. A combination of such kinds of unfavourable changes in the environment results in what is known as **Environmental Pollution.** Our biosphere is under constant threat from continuing environmental pollution. Over the last three decades there has been an increasing global concern over the impact of pollution on the environment. Continuing population growth, urbanization and rapid industrialization has led to a dramatic increase in the demand for water resources and increased the intensity in the discharge of contaminated or wastewater around the world [1]. The textile industry is one of the largest and oldest contributing sectors to many Asian economies and the most important in terms of revenue generation. The Indian textile industry accounts for 14% of industrial production, employs 35 million people and accounts for approximately 12% of country's total export business. With the increased demand for textile products, the textile industry and its wastewaters have been increasing proportionally, making it one of the main sources of severe pollution problems worldwide.[2] Textile industries consume large volumes of freshwater for its various wet processes of textile manufacturing and generate equal amounts of wastewater. In addition, the chemical reagents used in textile industries are very diverse in chemical composition ranging from inorganic compounds to polymers and organic products.[3,4] Textile wastewaters generated from the different wet processes are characterized by high pH, temperature, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), detergents, metals, surfactants, suspended and dissolved solids, dispersants, levelling agents, toxic organics (phenols), chlorinated compounds (AOX), sulphide and formaldehyde, may be

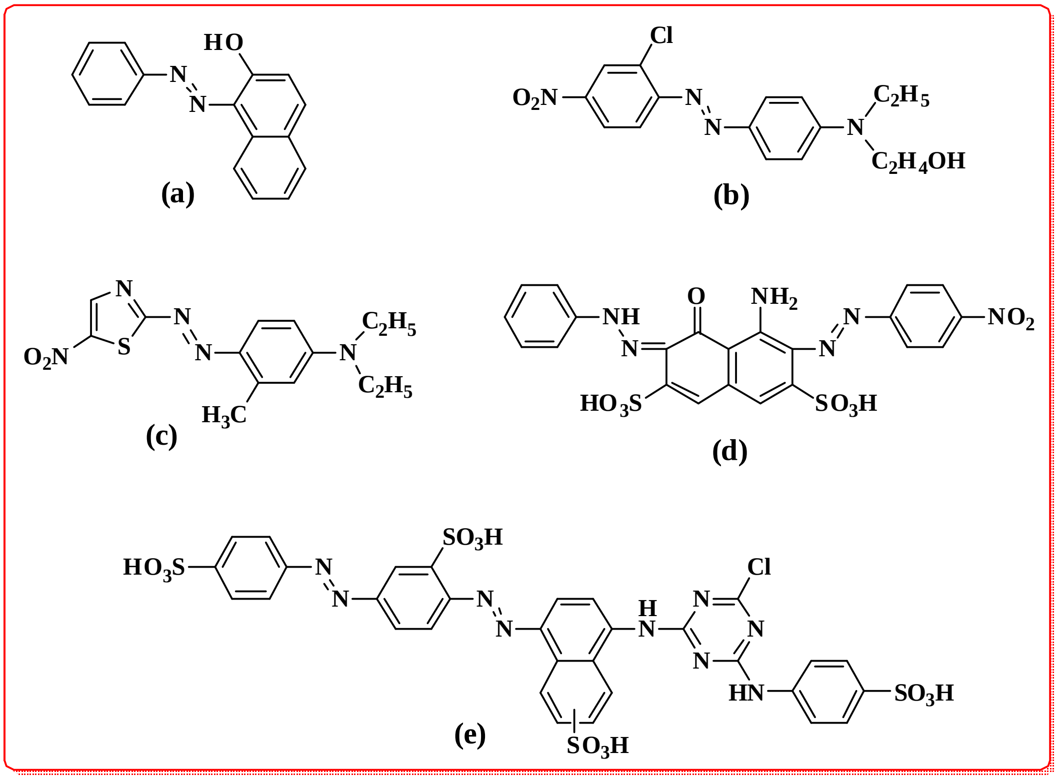

Figure 1. Structures of Azo dyes. (a) Disperse Red 13 (b) Disperse Blue (c) Reactive Brown 1 (d) Direct Green 26 (e) Acid Black 1.

added to improve dye adsorption onto the fibres. The most common textile-processing set up consists of desizing, bleaching, mercerising and dyeing processes.[5,6] Dyeing is the process of adding colour to the fibres, which normally requires large volumes of water not only in the dyebath, but also during the rinsing step. The textile industries use a number of coloured complexes known as dyes, which impart colour to the fabric. A dye is a colored substance that has an affinity to the substrate to which it is being applied.

The dyes possess colour because they 1) absorb light in the visible spectrum (400–700 nm), 2) have at least one chromophore (colour-bearing group), 3) have a conjugated system, i.e. a structure with alternating double and single bonds, and 4) exhibit resonance of electrons, which is a stabilizing force in organic compounds.[7] In addition to chromophores, most dyes also contain an electron withdrawing or donating substituents known as auxochromes (colour helpers). These auxochromes intensify the color of the compounds and provide bonding affinity groups.[8] The most important chromophores are azo (–N=N–), carbonyl (–C=O), methine (–CH=), nitro (–NO_2) and quinoid groups. The common auxochromes include amine (–NH_2), carboxyl (–COOH), sulfonate (–SO_3H) and hydroxyl (–OH). These chromophores and auxochromes together form the basis for the classification of dyes. The dyes were classified into several groups such as azoic dyes, basic dyes, direct dyes, disperse dyes, acid dyes, reactive dyes, solvent dyes, sulphur dyes and vat dyes.[9] These synthetic dyes are extensively used in textile dyeing, paper printing, colour photography, pharmaceutical, food, cosmetic and leather industries.[10-12] Among these, the textile industries have shown a significant increase in the use of synthetic complex organic dyes as the colouring material. Over 10^5 commercially available dyes exist and more than 7×10^5 tonnes of dyestuff are produced annually.[13,14] It is estimated that 280,000 tons of textile dyes are discharged in textile industrial effluent every year worldwide.[15]

AZO DYES

Azo dyes comprise by far the largest family of organic dyes commonly used in textile, food, paper making and cosmetic industries.[16] Azo dyes are characterized by the presence of one or more azo groups (R_1-N=N-R_2). As per 1994 estimates more than half of the annually produced amounts of dyes are azo dyes making them the largest and versatile class of dyes being employed in textile industries.[17,18] Presumably more than 2,000 different azo dyes are currently used to dye various materials such as textiles, leather, plastics, cosmetics and food. Generally azo dyes are composed of one or more azo linkages, substituted with a combination of functional groups including triazine amino, chloro, hydroxyl, methyl, nitro and sulphonated.[19] The largest amount of azo dyes is used for the dyeing of textiles, of which nearly 10–15 % dyestuff remains unbound to the fibre and is therefore released into the environment leading to severe contamination of surface and ground waters

Table 1. Advantages and disadvantages of current methods of dye removal from industrial effluents.

Physical/Chemical methods	Advantages	Disadvantages
Fenton's reagent	Effective decolorization of both soluble and insoluble dye	Sludge generation
Ozonation	Applied in gaseous state; no alteration in volume	Short half life
Oxidation	Rapid process	High energy costs and formation of unwanted by-products
NaOCl	Initiates and accelerates azo bond cleavage	Release of aromatic amines
Adsorption	Good removal of a wide range of dyes, simple can operate continuously	Adsorbent requires regeneration or disposal
Photochemical	No Sludge production	Formation of bi-products
Cucurbituril	Good sorption capacity for various dyes	High operating cost
Electrochemical destruction	Breakdown compounds are non-hazardous	High cost of electricity
Activated carbon	Good removal of variety of dyes	Very expensive to operate
Peat	Good adsorbent due to cellular structure	Specific surface areas for adsorption are lower than activated carbon
Wood chips	Good sorption capacity for acid dyes	Requires long retention times
Silica gel	Effective for basic dye removal	Side reactions prevent commercial applications
Membrane filtration	Removes all dye types	Concentrated sludge production
Ion exchange	Regeneration; no adsorbent loss	Not effective for all dyes
Irradiation	Effective oxidation at lab scale	Requires a lot of dissolved O_2
Electro kinetic coagulation	Economically feasible	High sludge production

in the vicinity of dyeing industries.[20-22] Azo dyes are known to prevalent environmental contaminants, and pose a significant risk to human and environmental health. Some examples of azo dyes and their chemical structures are shown in Figure 1.

Most of the azo dyes are non-toxic except for some azo dyes with free amino groups. These compounds lead to high electrolyte and conductivity concentrations in the dye wastewater, leading to acute and chronic toxicity problems.[23] Occupational sensitisation to azo dyes has been reported in the textile industry.[24] Many of the azo dyes and their degradation intermediates are mutagenic and carcinogenic[25,26] and contribute to the mutagenic activity of ground and surface waters that are polluted by textile effluents.[27-29] Furthermore, dye containing effluents increase biochemical oxygen demand of the contaminated water.[30] Therefore, the removal of colour from textile dyeing effluents is vital before their discharge into surrounding environment.[31] Mounting pressure on the textile industry to treat dye house effluents has led to a host of new and old technologies competing to provide cost-effective solutions.

TREATMENT TECHNOLOGIES

There is no universal method for the removal of colour from the dye wastewater.[32] Due to variation in dye waste water characteristics a number of physical, chemical and biological treatment methods have been employed for its treatment. A number of treatment technologies are available for the remediation of dye wastewaters. These include physicochemical methods like membrane filtration, coagulation/flocculation, precipitation, flotation, adsorption, ion exchange, ion pair extraction, ultrasonic mineralization, electrolysis, advanced oxidation (chlorination, bleaching, ozonation, Fenton oxidation and photocatalytic oxidation) and chemical reduction.[33-35] The coagulation-flocculation method is one of the widely used processes in textile wastewater treatment plants in countries such as Germany and France. It could be used either as a pre-treatment, post-treatment, or even as main treatment system.[36] The chemical oxidation process typically involves the use of an oxidising agent such as ozone (O_3), hydrogen peroxide (H_2O_2) and permanganate (MnO_4) to change the composition of a compound.[37]. A process called selective oxidation, ozone can be designed in such a way that only N=N bond scission occurs. Besides, different advantages, physicochemical techniques have also some serious restrictions such as high cost, low efficiency, limited versatility, interference by other wastewater constituents, and the handling of the waste generated.[38-41] Thus, the removal of colour from textile effluents has been a major concern and there is a great need to develop an economic and effective way of dealing with the textile dyeing waste in the face of ever increasing production activities.[42] This

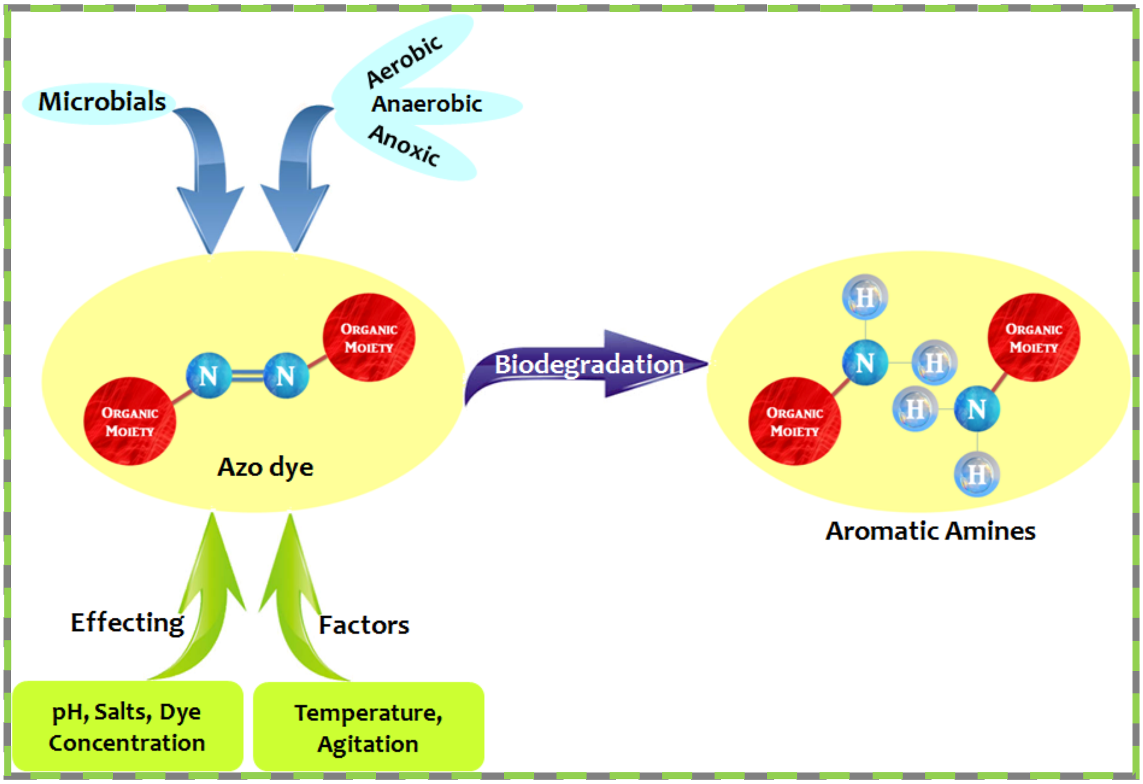

Figure 2. Graphical representation of basic approach for Microbial degradation of Azo dyes.

has resulted in considerable interest in the use of biological systems for the treatment of these wastewaters. These different physico-chemical methods have their own advantages and disadvantages which are summarised in the Table 1.[38]

BIOLOGICALTREATMENT/BIOREMEDIATION

The use of biological methods to bring about remediation of contaminated sites is known as Bioremediation. Bioremediation is a treatment process that uses naturally occurring microorganisms (bacteria, yeast or fungi) to break down, or degrade, hazardous substances into less toxic or nontoxic substances. Biological methods are known to be environmentally friendly as they bring about complete mineralization of organic pollutants at low cost and produce low sludge.[43,44] Biological dye removal techniques are based on microbial biotransformation of dyes. Biodegradation of synthetic dyes not only results in decolorization of the dyes but also in fragmentation of the dye molecules into smaller and simpler parts (breakdown products). Azo dyes are known to be xenobiotic in nature and hence are recalcitrant to biodegradation in conventional activated sludge treatment units.[45,46] Microorganisms play a remarkable role in decomposition and ultimate mineralization of various azo dyes.[47] Bioremediation techniques utilise microorganisms to treat contaminants by degrading organic compounds to less toxic materials, such as CO_2, methane, water and inorganic salts. This is an energy dependent process involving the breakdown of dye into different by-products through the action of various enzymes.[41] These techniques can be carried out *in situ* or *ex situ* under aerobic or anaerobic conditions. Bioremediation of dyeing effluents is considered as a good alternative treatment method due to the extreme variation in composition of textile effluents. Biological methods also offer potential advantages over conventional processes in terms of minimum impact on environment and cost effectiveness.[48] A number of microorganisms including fungi, bacteria, yeasts, and algae have been employed for the decolorization and degradation of synthetic dyes. Their degrading potential varied with different kinds of dyes. The effectiveness of microbial degradation mainly depends upon the adaptability and activity of particular microrganisms.[49] The degradation potential of a selected strain is enhanced by its application under favourable conditions .[50]

BACTERIAL DECOLORIZATION OF AZO DYES

The effectiveness of microbial decolorization depends on the adaptability and the activity of the selected microorganisms.[49] Several microorganisms including bacteria, fungi, yeasts and algae, can decolorize and even completely mineralize many azo dyes. Bacterial decolorization of azo dyes has gained importance in recent times as they are easy to culture and grow more quickly.[51] The role of diverse groups of the bacteria in the decolorization of azo dyes was been extensively studied. Bacterial decolorization of azo dyes is more efficient and faster.[52] Decolorization of azo dyes occurs under anaerobic, anoxic and aerobic conditions or in different combinations by various trophic groups of bacteria. The initial step in the bacterial degradation of azo dyes is the reductive cleavage of the azo bond (-N=N-). Anaerobic conditions are found to be favourable and more satisfactory for the reduction of azo dyes than aerobic degradation,[16] but the resulting intermediate compounds (aromatic amines) are biorecalcitrant under anaerobic conditions and can be toxic and carcinogenic.[18,53] The aromatic amines generated have to be degraded by an aerobic process [54,55] prior to their final discharge to the environment. Hence, the aerobic or at times the anoxic treatment is the only safe method for the biodegradation of azo dyes. Aerobic processes have been recently employed for the treatment of textile wastewater as standalone processes and it is confirmed that they are efficient and cost effective.[56] The most rapid and complete degradation of the majority of pollutants is brought about under aerobic or anoxic conditions. Bacteria are seldom able to decolorize azo compounds in the presence of oxygen; only organisms with specialized azo dye reducing enzymes were capable of degrading azo dyes under fully aerobic conditions, as a result very few reports exists on the aerobic decolorization of azo dyes. [57-60] Many of these isolates require organic carbon sources as they cannot utilize dye as the growth substrate.[18] Figure 2 shows the graphical representation of microbial degradation of azo dyes.

Many studies had been carried out to assess the role of diverse group of bacteria in the decolorization of dyes. Bacterial decolorization is normally faster in comparison to fungal decolorization.[52] The initial step of the bacterial degradation of azo dyes is the reductive cleavage of the –N=N– bond. Bacterial decolorization of azo dyes occurs under anaerobic (methanogenic), anoxic and aerobic conditions by different groups of bacteria. Isolation of bacterial cultures capable of azo dye degradation started in the 1970's with the report of *Bacillus subtilis* [61] followed by *Aeromonas hydrophila* [62] and by a *Bacillus cereus*.[53] Several other decolorizing *Pseudomonas* and *Aeromonas* species were later reported by a Japanese group.[63,64] A host of bacterial cultures such as *Rhodococcus* sp. *Bacillus cereus*, *Plesiomonas* sp. and *Achromobacter* sp. capable of dye decolorization were reported by.[65] Recently, biodegradation of malachite green by *Micrococcus* sp. strain BD15, its biodegradation pathway and enzyme analysis was assessed.[66] A bacterial strain isolated from soil samples around textile factory and identified as *Bacillus* sp. YZU1 exhibited remarkable ability to decolorize Reactive Black 5.[67] An alkaliphilic and halotolerant facultative anaerobe *Planococcus* sp. MC01 efficiently reduced Orange I under alkaline and anaerobic conditions.[49] Biological decolorization of malachite green by *Deinococcus radiodurans* R1 was achieved with higher efficiency and in short time.[68]

BIODEGRADATION OF DYES BY FUNGI

A wide range of aromatics and synthetic dyes are being readily degraded by white rot fungi.[12] White rot fungi produce efficient enzymes (lignin peroxidase, manganese peroxidase and laccase) which are capable of decomposing dyes under aerobic conditions. The first report on dye degradation by white rot fungi was in the year 1983[69] and has been the main subject of research since then. The extracellular non-specific and non-stereoselective enzyme system comprising lignin peroxidase (LiP), manganese peroxidases (MnP) and laccases [11,70] are responsible for the decolorizing activity of synthetic dyes by ligninolytic fungi. The extracellular enzymes of fungi are known to be able to degrade complex synthetic dyes.[71] White-rot fungi are the most intensively studies dye decolorizing microorganisms.

BIODEGRADATION OF DYES BY YEASTS

Scanty reports are available on decolorization of dyes by yeasts.[11,72,73] Yeasts have advantages in growing rapidly like bacteria, but like filamentous fungi, and also have the ability to resist unfavourable conditions. Maximum colour removal of 98% of Remazol Black-B was achieved with the yeast *Kluyveromyces marxianus* IMB3.[74] A couple of simple azo dyes were degraded in batch cultures by a strain of yeast *Candida zeylanoides* [75].

BACTERIAL DECOLORIZATION UNDER ANAEROBIC CONDITIONS

Most of the bacteria degrade the azo dyes reductively under anaerobic conditions to colourless aromatic amines. Dye decolorization under anaerobic conditions is dependent on the added organic carbon source and the dye structure. Simple substrates like starch, acetate, ethanol, glucose have been used for dye decolorization under methanogenic conditions. [76-79] Members of the Gamma Proteobacteria combined with sulphate reducing bacteria were found to be prominent members of mixed bacterial populations in an anaerobic baffled reactor treating industrial dye wastes. Reduction of dyes under anaerobic conditions seems to be unspecific as the rate of decolorization depends on the added organic carbon source as well as the dye structure. [80,18]

BACTERIAL DECOLORIZATION UNDER ANOXIC CONDITIONS

A number of mixed aerobic and facultative anaerobic microbial consortia were been reported to decolorize azo dyes under anoxic conditions.[81-85] Most of these cultures though show growth under aerobic conditions, decolorization was achieved only under anaerobic conditions. Pure bacterial isolates such as *Pseudomonas luteola*, *Aeromonas hydrophila*, *Bacillus subtilis*, *Pseudomonas* sp. and *Proteus mirabilis* were found to decolorized azo dyes under anoxic conditions.[86-88] Decolorization of azo dyes by pure and mixed cultures usually requires complex organic sources such as peptone, yeast extract or a combination of complex organic sources [49,81] Glucose was found to be the preferred substrate in the anaerobic dye decolorization but its use in anoxic conditions seems to vary based on the culture. Mordant Yellow 3 decolorization by *Sphingomonas xenophaga* was enhanced greatly in the presence of glucose and decrease in decolorization was observed in its presence for *Pseudomonas leuteola*, *Aeromonas* sp. and few other mixed cultures.[89,84,59]

BACTERIAL DECOLORIZATION UNDER AEROBIC CONDITIONS

Several bacterial isolates were isolated during the past years which could aerobically decolorize many azo dyes. Many of these isolates could not utilize dye as growth substrate and require organic carbon sources. *Pseudomonas aeruginosa* decolorized Navitan Fast Blue S5R in the presence of glucose under aerobic conditions and also decolorized various other azo dyes.[90] Only a couple of bacteria were found to utilize dye as their sole source of energy. These bacteria reductively cleave –N=N– and utilize the formed aromatic amines as their source of energy. Some of the isolates possessing this trait are *Xenophilus azovorans* KF 46 (Previously *Pseudomonas* sp. KF46) and *Pigmentiphaga kullae* K24 (previously *Pseudomonas* sp. K24), which can grow aerobically on carboxy-orange I and carboxy-orange II.[91, 92] Likewise, an obligate aerobe *Sphingomonas* sp. strain 1CX was isolated which utilised AO7 as sole carbon, energy and nitrogen source and decolorized it.[93] The isolates *Bacillus* sp. OY1-2, *Xanthomonas* sp. NR 25-2 and *Pseudomonas* sp. PR41-1 was also found to utilize AO7 or Acid Red 88 as sole carbon source.[94] In another report, two isolates identified as *Vibrio logei* and *P. nitroreducens* were found to utilize methyl red as sole source of energy.[57]

ANAEROBIC / AEROBIC BIODEGRADATION OF DYES

In order to overcome the problem of recalcitrance of azo dye breakdown products under anoxic conditions, a two stage treatment process is being suggested.[95,20] The first step includes the reductive cleavage of azo bond under anaerobic conditions resulting in colourless aromatic amines, which are then easily metabolized under aerobic conditions. Complete mineralization of azo dye Mordant Yellow 3 was obtained by *Sphingomonas* sp. BN6 co-immobilised with an uncharacterised 5-aminosalicylate degrading isolate with alginate beads. The anaerobic/aerobic treatment process can be carried out sequentially or simultaneously. The sequential processes combine the anaerobic and aerobic steps in the same reaction vessel. The simultaneous systems utilize anaerobic zones within the aerobic bulk phases, as in biofilms, granular sludge, and biomass immobilized in other matrices.[96,97]

BIODEGRADATION OF DYES BY MIXED MICROBIAL CULTURES

With the ever increasing complexity in the structures of xenobiotics it is not possible to find complete catabolic pathways in a single microorganism. A higher degree of biodegradation and complete mineralization can be achieved only when cometabolic activities within a microbial community complement each other. [98,44] The synergistic activity of microbial communities is known to be responsible for the higher degree of mineralization of dyes and also offers considerable advantages over the use of pure cultures.[99] Most of the studies have reported the effectiveness of microbial consortium over pure isolates.[11] The individual isolates of the consortium may target the dye molecule at different positions or utilise the products of decomposition generated by another strain to further the decomposition.[54] [89] described a bacterial consortium capable of mineralizing the sulphonated azo dye Mordant Yellow. Therefore, the process of biodegradation of dyes may be enhanced using mixed microbial cultures due to their synergistic property.

FACTORS AFFECTING BIODEGRADATION OF DYES

The natural ecosystem is a dynamic environment with varying abiotic conditions, like pH, Temperature, presence of oxygen, metals, salts etc. The highly variable nature of the biological treatment systems and the dyeing effluents, a number of factors are known to affect the biodegradation rate of azo dyes. Many researchers have discussed problems associated with dye biodegradation which are least anticipated. Microorganisms playing a major role in the global C, N and S cycles are affected by changes in these parameters and as a result their potential to decompose is also being affected. Thus the effects of these parameters are vital for evaluating the potential of various microorganisms for degrading particular xenobiotics. Therefore, optimization of such conditions would greatly help in the development of large scare bioreactors for the efficient treatment of textile dyeing effluents and for the bioremediation of contaminated sites.

EFFECT OF pH

Fungi and yeast exhibit better decolorization and biodegradation activity at acidic or neutral pH while bacteria at neutral or basic pH. The functioning of anaerobic and aerobic microorganisms is affected by the pH of the wastewater. Decolorization of Reactive Black 5 by *Enterobacter* sp. EC3 was found to be higher at pH 7.0 after 108 h of incubation. Similar efficiency of decolorization was noticed from pH 8.0 –12.0 in 120 h, but decolorization rate was much lower at acidic conditions (pH 4.0 and 6.0). This phenomenon could be due to the fact that the optimum pH for growth of *Enterobacter* sp. EC3 was neutral.[100] Studies on the decolorization of Scarlet R by a microbial consortium GR consisting of *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168 showed the percentage decolorization of 62, 82, 100 and 100 at pH 5, 6, 7 and 8 after 24, 24, 14 and 36 h respectively.[73]

EFFECT OF TEMPERATURE

Temperature is an important factor affecting ecosystem. The biodegradation activities of microorganisms are affected by the changes in temperature. An optimum temperature is required for the growth and multiplication of the decomposers (mostly soil bacteria and fungi). Beyond the optimum temperature, the activity of degradation decreases due to slow growth and reproduction and deactivation of enzymes responsible for degradation. Decolorization of Navy Blue HER by *T. beigelii* (yeast) was studies at various temperatures (30–50°C) and decolorization was found to be faster at 37°C within 24 h incubation.[73] The decolorization rate of Reactive Black 5 by *Enterococcus* sp. EC3 was found to increase with rise in temperature from 22°C to 37°C, and was found to be affected drastically at 42°C. Optimum temperature for decolorization was at 37°C.[100]

EFFECT OF INITIAL DYE CONCENTRATION

The effect of initial dye concentration upon the decolorization of dyes is well researched. The increasing dye concentration brings about decrease in the decolorization rate of dyes. Decolorization rate of Congo Red by *Bacillus* sp. decreased with increase in dye concentration and at high concentrations (1,500 and 2,000 mg/L) inhibition was observed.[101] *Enterobacter* sp. was unable to grow at high concentrations of Reactive Red 195 (50 and 100 mg/L) as it was dead. The dye was found to be toxic to cells at high dye concentrations.[102] Similar toxicity of malachite green to Kocuria rosea MTCC 1532 was observed at higher dye concentrations.[103] This toxicity of dyes to the microbial cells results in the decreased production of microbial biomass. The maximum cell growth yield of *Aeromonas hydrophila* was 1.2-1.6 g/L at dye concentration of 1,000 – 3,000 mg/L of Reactive Red 198, and was found to decrease (0.7–1.0 g/L) at 4,000 – 8,000 mg/L dye concentration.[49] The decolorization rate of crystal violet by *Nocardia corallina* decreased to less than 30% at an increased dye concentration of 4.5 µM.[104]

EFFECT OF SALTS

The textile effluents are characterized by a number of acids, alkalis, salts, or metal ions as impurities.[41] Wastewaters from dyeing units and textile processing industries contain substantial amounts of salts in addition to azo dyes.[47] Dyestuff industry wastewaters contain salt concentrations up to 15–20%.[5] Thus, employing microbial species capable of tolerating salt stress is mandatory for treating such wastewaters. The ability of *Shewanella putrefaciens* strain AS96 in decolorizing four structurally different azo dyes at different concentrations of NaCl was assayed.[47] The azo dyes Reactive Black 5, Direct Red 81, Acid Red 88 and Disperse Orange 3 were decolorized with 100% efficiency when NaCl concentration was 0-40 g/L. Time for decolorization increased with rise in NaCl concentration (60 g/L) and percent decolorization also decreased significantly.

EFFECT OF AGITATION

Contradictory reports exist on the effect of shaking / agitation on microbial decolorization of synthetic dyes. Some authors reported decolorization under shaking conditions and some under static conditions. Higher colour removal is observed under shaking cultures due to better oxygen transfer and distribution of nutrients as compared to stationary cultures. In contrast, agitated cultures of *Pseudomonas* sp. SUK1 showed almost no decolorization in 24 h, whereas static cultures exhibited more than 96% decolorization.[52] Enzymatic activity of static cultures was found to be high.[41] Rate of ligninolytic enzymes and the rate of decolorization increased during the decolorization of Reactive Orange 16 and Remazol Brilliant Blue R by *Irpex lacteus*. Maximal enzyme activity of MnP, laccase, and LiP was noticed after 7 days under static conditions (Novotny *et al.*, 2004). The growth and decolorization of *T. beigelii* was found to be high under static conditions compared to shaking condition.[101]

MECHANISM OF AZO DYE REDUCTION

The primary step in the bacterial degradation of azo dyes in either anaerobic or aerobic conditions, is the reduction of the –N=N– bond. This reduction may involve different mechanisms, such as enzymes, low molecular weight redox mediators, chemical reduction by biogenic reductants like sulfide or a combination of these (Figure 3). The formed intermediate metabolites are further degraded either anaerobically or aerobically. The presence of oxygen usually inhibits the azo bond reduction as aerobic respiration dominates the utilization of NADH; thus obstructing the transfer of electrons from NADH to azo bonds.[105]

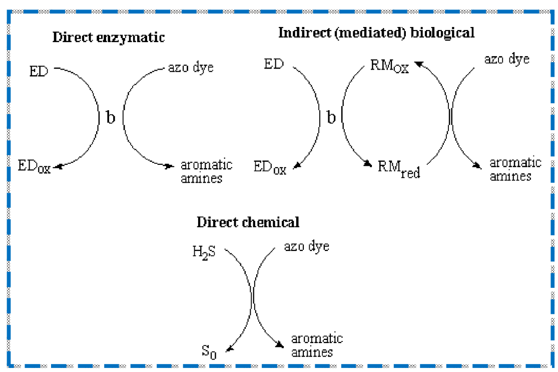

Figure 3. Mechanism of Azo dye reduction.[44]

DIRECT ENZYMATIC REDUCTION OF AZO DYES

This mechanism involves the transfer of reducing equivalents enzymatically generated from the oxidation of the substrate/coenzyme to azo dyes. These enzymes could be specific catalysing only the azo dye reduction, or nonspecific. The nonspecific enzymes catalyze a wide range of substrates. Due to their non-specificity these enzymes readily reduce azo dyes.

ENZYMATIC AZO DYE REDUCTION UDNER ANAEROBIC CONDITIONS

Presence of azoreductases in anaerobic bacteria which decolorized sulphonated azo dyes during growth on solid or complex media was reported by[10] These isolates were reported from *Clostridium* and *Eubacterium*. The azoreductases were found to be oxygen sensitive and released extracellularly. Further investigations with *C. perfringens* showed the azo dye reduction was catalyzed by flavins adenine dinucleotide dehydrogenase reducing nitro aromatic compounds.[106] Another dye decolorization mechanism involves cytosolic flavin dependent reductases which transfer electrons via soluble flavins to azo dyes. A recent study by[107] with a recombinant *Sphingomonas* strain BN-6 showed the reduction of sulphonated azo dye by cytosolic flavin-dependent azoreductase was observed *in vitro* with little importance *in vivo*.

ENZYMATIC AZO DYE REDUCTION UDNER AEROBIC CONDITIONS

The presence of azoreductases from aerobic bacteria were first proven in the isolates *Pseudomonas* K22 (reclassified as *Pigmentiphaga kullae* K24) and *Pseudomonas* KF46 (reclassified as *Xenophilus azovorans* KF46F).[91,108] These intracellular azoreductases exhibited high specificity to dye structures and cleaved their carboxylated as well as sulfonated structural analogues. Similarly, azoreductases from *Bacillus* sp. strain OY1-2 readily decolorized AR88 and AO7 and a group of proprietary reactive dyes.[109] Two other azoreductases from *X.azovorans* KF46 and *P.kullae* were well characterized.[110] Nonspecific enzymes responsible for azo dye reduction were been isolated from aerobically grown cultures of *Shigella dysenteriae*,[111] *E. coli*,[112] *Bacillus* sp.[113] *Staphylococcus aureus*[114] and *P. aeruginosa*.[90]

Conclusions

Much attention has been focused on the remediation of pollutants discharged into the environment form the various processes of the textile industries. The soils and waters are severely contaminated by the release of synthetic dyes into the environment. The wide ranges in chemical structures of dyes often add up to the complication. Azo dyes being the most important group of synthetic colorants are generally considered xenobiotic and are recalcitrant against biodegradation processes. The treatment of dyeing wastewaters and decolorization presents an arduous task. Among the various methods available for treatment of dyeing effluents, the feasible and cost effective biological methods seems to deliver promising solution. The available literature suggests that a combination of anaerobic-aerobic biological methods may be suitable for treatment of dye-containing wastewaters. However, still there is a need to focus on the basic and applied aspects of bioremediation. In order to effectively use the bioremediation tool for degradation and detoxification of dyes a thorough understanding of microbial genetics, biochemistry and physiology is essential. More concerted efforts are required to establish the microbial decolorization process.

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Notes and References

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