



Review Article

Degradation of Toxic Dyes: A Review

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A B S T R A C T

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Dye is an integral part which is used to impart colour to materials. The waste generated during the process and operation of the dyes, contains the inorganic and organic contaminant leading to the hazard to ecosystem and biodiversity causing impact on the environment. The physico-chemical treatment does not remove the color and dye compound concentration. The decolorization of the dye takes place either by adsorption on the microbial biomass or and enzymatic degradation. Bioremediation takes place by anaerobic and/or aerobic process. In the present review the decolorization and degradation of dyes by fungi, algae, yeast and bacteria have been cited. The factors affecting decolorization and biodegradation of dye compounds such as pH, temperature, dye concentration, effects of carbon dioxide and nitrogen, agitation, effect of dye structure, electron donor and enzymes involved in microbial decolorization of dyes have been also highlighted in the review.

Introduction

Environmental pollution due to urbanization and rapid growth of industries has a detrimental effect on human health and ecology. Textile dyes constitute a major source of pollution. Textile industries consume a major share of dyes in India (Guota *et al.*, 2005). Further, the textile industry of India also contributes nearly 14% of the total industrial production of the country.

Certain groups of dyes with their specific chemical character and the methods of dyeing are dependent on each other.

Different methods such as adsorption, precipitation, oxidation, reduction etc. are used for the elimination of these compounds. During disposal, different toxic products can be generated and evacuated through effluents into the environment (Atteke Christiane *et al.*, 2013). It is in this context that many studies have been interested in the optimization of the physicochemical parameters of the medium for new strains with high degradation abilities so, considering all above the factors there is a need to degrade the dyes.

Many dyes and pigments are hazardous and toxic for human as well as aquatic life at the concentration at which they are being discharged to receiving water. The high concentration of dyes is known to cause ulceration of skin, and mucous membrane, dermatitis, perforation of nasal septum, severe irritation of respiratory tract and on ingestion may cause vomiting, pain, haemorrhage and sharp diarrhea (Kumar Praveen G.N. and Sumangala K. Bhat, 2012). Over the last decades, the increasing demand for dyes by the textile industry has shown a high pollutant potential. It is estimated that around 10 -15% of the dyes are lost in the waste water during the dyeing processes. There are various types of dyes which are discussed as follows:

Azo dyes

Azo dye is the largest group of dyes, with $-N=N-$ as a chromophore in an aromatic system. There are monazo, disazo, trisazo, tetrakisazo and polyazo dyes depending upon the number of azo-groups present. Diazotisation of a primary amine, in presence of $HCl + NaNO_2$ at freezing temperature, produces a diazonium salt which in turn coupled with aromatic compounds, producing an azo-dye. Azo pigments are colorless particles (typically earths or clays), which have been colored using an azo compound.

Anthraquinone dyes

Anthraquinone is the basic unit of this class of dyes. It is faint yellow in color which is sufficient to use it as a dye but it cannot be classified as a dye. Dyes containing anthraquinone unit belong to mordant, disperse and vat dyes. Its quinonoid system acts as a chromophore. Anthraquinone dyes have excellent fastness properties.

Disperse dyes

Disperse dyes generally use to dye cellulose acetate, nylon and other hydrophobic fibres. They are also known as acetate dyes. Sulphuric acid (SAR) is used as the dispersing agent. Dispersal and cellitoin are the important dispersing agents. Tatzine (otherwise known as E number E102 or C.I. 19140) is a synthetic lemon yellow azo dye used as a food coloring (P. Chaube *et al.*, 2010).

The concentration of dye contained in the effluent varies between 10-200mg/ml depending on the dyeing process. Dyes are classes of environmental pollutants whose break down products are highly toxic and mutagenic to living organisms. The concentration of dye contained in the industrial effluent varies between 10-200 mg/ml depending upon the dyeing process. There are different class of organic compounds characterized by the presence of unsaturated groups (chromophores) such as $-C=C-$, $-N=N-$ and $-C\equiv N-$, which are responsible for the dye colours, and of functional groups responsible for their fixation to fibres, for example, $-NH_2$, $-OH$, $-COOH$ and $-SO_3H$ (Molinari, *et al.*, 2004). It is quite clear that the change in colour might be due to the biochemical (metabolic) reactions of fungal species.

Dye colors are visible in water concentration as low as 1 mg/l, whereas textile processing wastewater normally contains more than 10-200 mg/l of dye concentration resulting in aesthetic problem, affecting photosynthesis in aquatic plants and have toxic and carcinogenic effect in mammals. About 10-15 % of dyes go unused in textile effluents fungi or their oxidative enzymes can decolorize textile wastewater either by adsorption of dyes on fungal mycelium or

by oxidative. A very small amount of dye in water (10-50 mg/L) is highly visible and reduces light penetration in water systems, thus causing a negative effect on photosynthesis (Cooper, 1993; Vandevivere *et al.*, 1998).

Several factors determine the technical and economic feasibility of each single dye removal technique. These include; dye type and its concentration, wastewater composition, operation costs (energy and material), environmental fate and handling costs of generated waste products. Among the numerous water treatment technologies, research interest in the fungal bioremediation due to their biomass compared to the bacteria, has increased significantly for decolorization and degradation of synthetic dyes (Azmi *et al.*, 1998). Presence of the dyes in aqueous ecosystems diminishes the photosynthesis by impeding the light penetration into deeper layers thereby deteriorating the water quality and lowering the gas solubility. Furthermore the dyes and/or their degradation products may be toxic to flora and fauna (Talarposhti *et al.*, 2001).

Microbial decolouration mechanisms

Microbial communities are of primary importance in degradation of dye contaminated soils and water as microorganisms alter to dye chemistry and mobility through reduction, accumulation, mobilization and immobilization (Kumar *et al.*, 2012). In recent years, biodegradation has become a viable alternative and proven to be a promising technology. Microorganisms have been successfully employed as sources for bioremediation (Khan and Husain 2007). Bioremediation is gaining its significance in utilizing the biological activity of microorganisms to degrade toxic

chemicals in the environment (King *et al.*, 1998) Microbial decolouration can occur via two principal mechanisms: biosorption and enzymatic degradation, or a combination of both have been used to remove dyes by biosorption.

Biosorption

Biosorption is becoming a promising alternative to replace or supplement the present dye removal processes from dye wastewaters (Fu and Viraraghavan, 2003). It involves binding of pollutants to the surface of cell membranes and/or cell walls through physical adsorption, electrostatic interaction, ion exchange, chelation and chemical precipitation (Aksu, 2005). The biosorption capacity of a microorganism is attributed to the heteropolysaccharide and lipid components of the cell wall causing strong attractive forces between the azo dye and the cell wall (Myrna Solís *et al.*, 2012). Sumathi and Phatak (1999) found that the maximal decolouration of several azo dyes using *Aspergillus foetidus* is achieved in the presence of carbon sources and during exponential growth.

Anaerobic and aerobic conditions for decolorization of dyes

Two mechanisms for the decolorization of dyes under anaerobic conditions in bacterial systems have been proposed (Pearce *et al.*, 2003). The first one consists of direct electron transfer to dyes as terminal acceptors via enzymes during bacterial catabolism, connected the ATP generation (energy conservation). The second one involves a free reduction of dyes by the end products of bacterial catabolism, not linked to ATP generation (eg., reduction of the bond by reduced inorganic compounds, such as Fe₂₊ or

H₂S, that are formed as the end product of certain anaerobic bacterial metabolic reactions).

During anaerobic degradation, a reduction of the bond in the molecules is observed. Then, aerobic conditions are required for the complete mineralization of the reactive dye molecule. The aromatic compounds produced by the initial reduction are degraded via hydroxylation and opening in the process is necessary in which oxygen is introduced after the initial anaerobic reduction of the bond has taken place. The optimum pH for colour removal is around pH 7-7.5. The rate of colour removal tends to decrease rapidly under strongly acid or strongly alkaline conditions. The optimum cell culture growth temperature is between 35 and 45°C. Reduction under anaerobic conditions appears to be nonspecific, as most of a varied group of compounds are decolorized, although the rate of decolorization is dependent on the added organic carbon source, as well as the dye structure (Stolz, 2001). Anaerobic conditions and the aromatic amines thus formed have been found to degrade further aerobically.

Different microorganisms are used for decolorization and degradation of dyes which are as follows

Fungus

Fungi has been studied to degrade pollutants due to their extracellular, nonspecific and nonstereoselective enzyme system, including lignin peroxidase (LiP), laccase and manganese peroxidase (MnP) (Hofrichter, 2002). Enayatzamir *et al.*, (2010) reported the ability of the white-rot fungus *Phanerochaete chrysosporium* immobilized into Ca-alginate beads to decolorize different recalcitrant azo dyes

such as Direct Violet 51, Reactive Black 5, Ponceau Xylidine and Bismark Brown R in successive batch cultures. Breakdown of most of organo-pollutants by fungi is closely linked with ligninolytic metabolism. Decolorization of dye is related to the process of extracellular oxidases, particularly manganese peroxidases (Singh *et al.*, 2012). Comparative analysis of the time course of decolorization by the three fungi under their respective optimal conditions has revealed high order of activity by *P.chrysogenum*, and *A.niger* recording almost 100% decolorization. *Cladosporium sp.* also recorded considerably good level of activity shown by Kumar praveen G.N., 2012. Physicochemical parameters of the culture medium (pH, concentration, temperature etc.) as well as the fungal type, affect the decolorization of dyes.

Fungi, due to their excretion of extracellular enzymes, are known to be able to degrade though possibly not completely the structures that are difficult for bacteria to handle (Forss and Welander, 2009). Microbial degradation of Congo red by *Gliocladium virens* (Singh, 2008), various hazardous dyes likes, Congo red, Acid red, Basic blue and Bromophenol blue, Direct green by the fungus *Trichoderma harzianum* (Singh and Singh, 2010) and biodegradation of plant wastes materials (Singh, 2008) by using different fungal strains has been investigated. The results were similar to biodegradation of Congo red and Bromophenol blue by the fungus *Trichoderma harzianam* in semi-solid medium (Singh and Singh, 2010) and biodegradation of Methylene blue, Gentian violet, Crystal violet, Cotton blue, Sudan black, Malachite green and Methyl red by few species of *Aspergillus* (Muthezhilan *et*

al., 2008) in liquid medium. Cripps *et al.*, (1990) also reported the biodegradation of three azo dyes (Congo red, Orange II and Tropaeolin O) by the fungus *Phaenerocheate chrysosporium* (Singh and Ved Pal Singh, 2010). Rahna K. Rathnan *et al.*, 2013 found that the isolated fungus *Aspergillus niger* and *Aspergillus oryzae* and mixed consortium is as an important source for bioremediation of toxic dye. *Aspergillus niger* showed greater decolorisation production during sixteen days incubation (N. Manikandan, 2012).

Algae

It has been reported that more than thirty azo compounds can be biodegraded and decolorized by *Chlorella pyrenoidosa*, *Chlorella vulgaris* and *Oscillatoria tenuis*, with azo dyes decomposed into simpler aromatic amines (Yan and Pan 2004). Algae can play an important role in the removal of azo dyes and aromatic amines in stabilization ponds (Banat *et al.*, 1996).

Yeast

More recently, some studies have shown that yeast species acted as a promising dye adsorbent capable to uptake higher dye concentration, such as *Galactomyces geotrichum*, *Saccharomyces cerevisiae* and *Trichosporon beigeli*, etc. (Jadhav *et al.*, 2008). The first two reports use the ascomycete yeast *Candida zeylanoides* isolated from contaminated soil to reduce model azo dyes (Martins *et al.* 1999, Ramalho *et al.* 2002). The characterisation of an enzymatic activity is described in further studies with the yeast *Issatchenkia occidentalis* (Ramalho *et al.* 2004), and the enzymatic system involved is presented in a work with *Saccharomyces cerevisiae* (Ramalho *et al.* 2005).

Bacteria

Halophiles have been reported to be involved in the dye decolorization (SalahUddin *et al.*, 2007). The moderately halotolerant *Bacillus sp.* were isolated for decolorization of azo dye Red 2G to an extent of 64.89%. This rate of decolorization may be due to the high metabolic

diversity being seen in the halophiles due to their extremophilic nature (Oren *et al.*, 1992; Ventosa *et al.*, 1998). *Brevibacterium sp.* strain VN-15 also dramatically reduced the toxicity of the dye solutions after the static phase of incubation. To achieve this, the bacteria must establish a link between their intracellular electron transport system and high molecular weight, azo dye molecules. For such link to be established, the electron transport components must be localized in the outer membrane of the bacterial cells (in the case of gram-negative bacteria), where they can make direct contact with either the azo dye substrate or a redox mediator at the cell surface (Myers and Myers, 1992).

Nachiyar and Rajkumar (2003) investigated degradation of Navitan Fast Blue dye using *Pseudomonas aeruginosa*. The organisms required ammonium salts and glucose to co-metabolize the dye. Organic nitrogen sources did not support appreciable decolorization, whereas, inorganic nitrogen showed an increasing effect on both growth and decolorization. An oxygen intensive azoreductase was also involved in the decolorization mechanism. Senan and Abraham (2004) reported that an aerobic bacterial consortium consisting of two isolated strains and a strain of *Pseudomonas putida* was also developed for the aerobic degradation of a mixture of textile azo dyes and individual azo dyes at alkaline

pH (9-10) and salinity (0.9 – 3.8 g/l) at ambient temperature (28-2°C). The degradation efficiency of the strains in different media and at different dye concentrations was studied. The enzyme present in the crude supernatant was found to be reusable for the dye degradation. Extent of decolorization recorded by *Bacillus cereus* under ideal conditions was 95% and that by *Bacillus megaterium* was 98% (Maulin P Shah, *et al.*, 2013). Current investigation has confirmed the decolorization of Azo dye red by the bacteria *B. cereus* and *Bacillus megaterium* under “*in vitro*” conditions. Extent of decolorization recorded by *Bacillus cereus* under ideal conditions was 95% and that by *Bacillus megaterium* was 98% (Maulin, 2013).

Enzymatic degradation

Enzymes from certain fungi (laccase, lignin peroxidases and manganese peroxidases) have shown a high ability of degradation of synthetic dyes.

Enzymes for involved in the microbial decolorization and degradation of azo dyes

Laccases

Laccases have been extensively studied for their degradation of azo dyes (Chivukula *et al.*, 1995; Kirby *et al.*, 2000; Peralta *et al.*, 2003; Blanquez *et al.*, 2004; Novotny *et al.*, 2004). Blanquez *et al.*, used *T. versicolour* in the form of pellets to treat a black liquors discharge for detoxifying and reducing the colour, aromatic compounds, and chemical oxygen demand (COD). They found that colour and aromatic compounds were reduced up to 70–80% and COD Enzyme Research 5 was reduced up to 60%. They concluded that *T. versicolour* is able to produce laccase. *T. versicolour* completely decolorizes the

Amaranth, Tropaeolin O, Reactive Blue 15, Congo Red, and Reactive Black 5 with no dye sorption while it partially decolorizes Brilliant Red 3G-P, Brilliant Yellow 3B-A and Remazol Brilliant Blue R with some dye sorption. They found that after decolourization, toxicity of few dyes remained the same while some became nontoxic. Laccase-based hair dyes are less irritant and easier to handle than conventional hair dyes because laccases replace H₂O₂ in the dye formulation. Laccase are also used in dechlorination process. Xylidine is a laccase inducer which increases dechlorination activity due to which dissolved oxygen concentration is reduced. Romero *et al.* found that bacteria *S. maltophilia* decolorizes some synthetic dyes (methylene blue, methyl green, toluidine blue, Congo red, methyl orange, and pink) as well as the industrial effluent (Shraddha, *et al.*, 2011).

Peroxidases: Peroxidases are assisted in the degradation of lignin moieties by auxiliary enzymes and mediators: low-molecular weight compounds that improve lignin biotransformation by readily diffusing into the lignocellulosic matrix and by providing high redox potentials that enhance the variety of substrates that laccases and peroxidases are able to degrade (Wesenberg *et al.*, 2003). Ollikka *et al.*, (1993) reported that Congo red was a substrate for the ligninolytic enzyme lignin peroxidase. The excellent performance of *T. lignorum* and *F. oxysporum* in the biodegradation of textile dyes of different chemical structures reinforces the potential of these fungi for environmental decontamination similar to white rot fungi (Shahid *et al.*, 2013). The capacity of fungi to reduce azo dyes is related to the formation of exo enzymes such as peroxidases and phenol oxidases. Peroxidases are hemoproteins that catalyze

reactions in the presence of hydrogen peroxide (Duran *et al.*, 2002). Lignin and manganese peroxidases (MnP) have a similar reaction mechanism that starts with the enzyme oxidation by H₂O₂ to an oxidized state during their catalytic.

Tyrosines: Tyrosinases are copper-containing dioxygen activating enzymes found in many species of bacteria and are usually associated with melanin production. These proteins have a strong preference for phenolic and diphenolic substrates and are somewhat limited in their reaction scope, always producing an activated quinone as product.

Despite this fact they have potential in several biotechnological applications, including the production of novel mixed melanins, protein cross-linking, phenolic biosensors, and production of L-DOPA, phenol and dye removal and biocatalysis. Although most studies have used *Streptomyces* sp. enzymes, there are several other examples of these proteins that are also of potential interest bacterial tyrosinases: old enzymes with new relevance to biotechnology.

There are various factors affecting decolorization and degradation of synthetic dyes are given in the Table 1 as follows:-

Formula used for dye degradation

Difference between initial and final values using the following formula:

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance value} - \text{final absorbance value}}{\text{Initial absorbance value}} \times 100$$

Initial absorbance value

(A.Tripathi, S.K. Srivastava, 2011).

Phytotoxicity and microbial toxicity of dyes

It is very important to know whether biodegradation of a dye leads to detoxification of the dye or not. This can be done by performing phytotoxicity and microbial toxicity tests of the original dye and its biodegradation products. In phytotoxicity studies, the seeds of model plants can be treated with a particular concentration of the original dye and also with its biodegradation products. Kalyani *et al.* (2009) conducted phytotoxicity study of Reactive Red 2 and its degradation products using *Sorghum vulgare* and *Phaseolus mungo* as model plants. They treated the plant seeds with water, Reactive Red 2 (5,000 ppm), and its extracted metabolite (5,000 ppm) separately and compared percent germination and the lengths of plumule and radicle. From the results, it was found that the metabolites produced after the biodegradation of Reactive Red 2 were less toxic as compared to the original dye.

Dyes may significantly affect photosynthetic activity in aquatic life and Microbial toxicity on *S. paucimobilis* showed growth inhibitory zone (0.8 cm) surrounding the well containing dye, while degradation product did not show inhibitory zone which also confirmed the nontoxic nature of the extracted metabolite. These findings suggest non-toxic nature of the product formed. Previous reports showed Malachite Green G degradation into Leuco-Malachite Green that is equally toxic to MG (Burchmore and Wilkinson 1993) and reduce light penetration. Ayed *et al.*, 2008 Moawad *et al.* (2003) reported the phytotoxicity of different soluble textile dyes estimated by measuring the relative changes in seed germination of four plants: clover, wheat, tomato and lettuce.

Table.1 Factors affecting decolorization and degradation of synthetic dyes which has been shown below

Factors	Descriptions
pH	<p>The pH has a major effect on the efficiency of dye decolorization, the optimal pH for color removal in bacteria is often between 6.0 and 10.0. The tolerance to high pH is important in particular for industrial processes using reactive azo dyes, which are usually performed under alkaline conditions.</p> <p>The pH has a major effect on the efficiency of dye decolorization, the optimal pH for color removal in bacteria is often between 6.0 and 10.0 (Chen <i>et al.</i> 2003; Guo <i>et al.</i> 2007; Kilic <i>et al.</i> 2007).</p>
Temperature	<p>Temperature is also again a very important factor for all processes associated with microbial vitality, including the remediation of water and soil. It was also observed that the decolorization rate of azo dyes increases upto the optimal temperature, and afterwards there is a marginal reduction in the decolorization activity.</p>
Dye concentration	<p>Earlier reports show that increasing the dye concentration gradually decreases the decolorization rate, probably due to the toxic effect of dyes with regard to the individual bacteria and/or inadequate biomass concentration, as well as blockage of active sites of azo reductase by dye molecules with different structures.</p>
Carbon and nitrogen Sources	<p>Dyes are deficient in carbon and nitrogen sources, and the biodegradation of dyes without any supplement of these sources is very difficult. Microbial cultures generally require complex organic sources, such as yeast extract, peptone, or a combination of complex organic sources and carbohydrates for dye decolorization and degradation.</p>
Oxygen and agitation	<p>Environmental conditions can affect the azo dyes degradation and decolorization process directly, depending on the reductive or oxidative status of the environment, and indirectly, influencing the microbial metabolism. It is assumed that under anaerobic conditions reductive enzyme activities are higher; however a small amount of oxygen is also required for the oxidative enzymes which are involved in the degradation of azo dyes.</p>
Dye structure	<p>Dyes with simpler structures and low molecular weights exhibit higher rates of color removal, whereas the removal rate is lower in the case of dyes with substitution of electron withdrawing groups such as $-SO_3H$, $-SO_2NH_2$ in the para position of phenyl ring, relative to the azo bond and high molecular weight dyes.</p>
Electron donor	<p>It has been observed that the addition of electron donors, such as glucose or acetate ions, apparently induces the reductive cleavage of azo bonds. The type and availability of electron donors are important in achieving good colour removal in bioreactors operated under anaerobic conditions.</p>
Redox mediator	<p>Redox mediators (RM) can enhance many reductive processes under anaerobic conditions, including azo dye reduction.</p>

Source: Razia Khan *et al.*, 2013

Table.2 Advantages and disadvantages of the dye removal methods

Physical/chemical methods	Advantages	Disadvantages
Fentons reagent	Effective decolourisation of both soluble and insoluble dyes	Sludge generation
Ozonation	Applied in gaseous state: no alteration of volume	Short half-life (20 min)
Photochemical	No sludge production	Formation of by-products
NaOCl	Initiates and accelerate azo bond cleavage	Release of aromatic amine
Cucurbituril	Good sorption capacity for various dyes	High cost
Electrochemical destruction	Breakdown compounds are non-hazardous	High cost of electricity
Activated carbon	Good removal of wide variety of dyes	Very expensive
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 application
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
Electrokinetic coagulation	Economically feasible	High sludge production

Source : Joshni. T. Chacko et al., 2011

Chen *et al.*, (2008) performed antimicrobial test of crystal violet and its degradation product using *E. coli* strain JM 109 as model microbe. They used crystal violet solution (100 mg/L) before biodegradation, crystal violet solution after incubation (with *Shewanella decolorationis* NTOU1) for 11 h (>98% decolorized) and crystal violet solution

after incubation for 59 h, for growth of *Escherichia coli* strain JM 109. They counted cell number (cells /mL) of the *E. coli* strain JM 109 in the test tubes after incubation with crystal violet or its degradation products for 1, 12 and 24 h. The data showed that crystal violet solution after incubation with the *Shewanella. decolorationis* NTOU1 for 11

h or 59 h was not toxic to the *E. coli* strain JM 109. They concluded that *Shewanella. decolorationis* NTOU1 could detoxify crystal violet during decolourization process. Mathur *et al.*, (2005) performed antimicrobial test of crystal violet against microflora contributing soil fertility as they take part in the biotransformation of organic materials and nutrients.

Different physical and chemical methods have been employed for the treatment of synthetic dyes wastewaters. These methods mostly suffer from serious limitations, like high cost, low efficiency, limited versatility, and production of secondary pollution (sludge), etc. In contrast, bioremediation is a cost-effective, efficient, biofriendly, and environmentally benign method for removal of dyes from industrial wastewaters. Further, to ensure the safety of the decolorized wastewater, studies should be conducted on the toxicity of the treated effluent/dye solution. The aim of the present review is to study about dye, their degradation process and their factors affecting the process for degradation of dyes for future prospects.

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