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Biological methods of dye removal from textile effluents - A review

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Abstract

Textile dyes are molecules designed to impart permanent colours to textile fabrics. They pose an environmental problem due to their toxicity and decrease the aesthetic value of water bodies into which they are discharged. Current physico-chemical technologies for dye removal cannot remove all classes of dyes, and various technologies are usually combined to achieve satisfactory decolourisation efficiencies. Direct biological treatment using fungi or bacteria can also be employed, but nutritional and physiological requirements of microorganisms put constraints on the applicability of such bioremediation processes. The search for efficient and green oxidation technologies has increased the interest in the use of enzymes to replace the conventional non-biological methods. Among the different existing oxidant enzymes, laccase (benzenediol:oxygen oxidoreductases EC 1.10.3.2) has been the subject of intensive research in the past few decades due to its low substrate specificity. Enzymatic treatment using laccase can be simpler and much more efficient than the traditional physical or chemical treatments. This paper reviews conventional biological processes as well as laccase-based processes that might replace the traditionally energy intensive and water-consuming chemical treatment operations in the textile industry.

Keywords: Dyes, Decolourisation, Green Oxidation, Laccase, Textile industry

Introduction

Wastewater from textile industries has been a significant source of environmental pollution and has been often discharged into municipal sewage treatment plant or directly into waterways. Textile wastewater includes dyes, detergents, insecticides, pesticides, grease, oils, sulphates, solvents, heavy metals, other inorganic salts and fibres in amounts depending on the processing regime. Textile dye wastewater has a strong colour, high pH, high temperature, high COD (Chemical Oxygen Demand) and low biodegradability. The complexity of dye structure makes wastewater treatment difficult by conventional physico-chemical process, because of their high cost and low effectiveness in meeting the required levels of decolourisation stipulated by pollution control boards (Latif 2010).

Dyes are recalcitrant organic molecules, resistant to aerobic digestion and are stable upon exposure to light. The problem of coloured effluents has worsened with the use of reactive dyestuffs. Most of the commonly employed dyes in textile industries belong to a class of compounds called as azo dyes, bearing the functional group R-N=N-R' (where R and R' can be either aryl or alkyl). Azo dyes play an important role as colouring agents in the textile, food, and pharmaceutical industry. But due to their toxicity, mutagenicity and carcinogenicity their removal from industrial wastewaters has been a challenge. These are a group of chemicals that are largely resistant to aerobic biodegradation and persist in wastewater treatment processes. The electron-withdrawing nature of the azo bond makes these compounds less susceptible to oxidative biological processes. The removal of dyes has been a challenge to both the textile industry and wastewater treatment facilities.

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Methods for textile dye removal

The technologies for dye removal have been divided into three principal categories: physical, chemical and biological methods.

Physical methods

Different physical methods such as membrane filtration processes (reverse osmosis, ultra filtration, and micro filtration) and adsorption techniques have been widely used. Membrane filtration offers potential applications where treatment processes have to be integrated in plant water circuits rather than a subsequent treatment (Machenbach 1998). Reverse osmosis (RO) membranes have a retention rate of 90% for most types of ionic compounds and produce a high quality of permeate (Ciardelli et al. 2001). RO permits the removal of all mineral salts, hydrolyzed reactive dyes and chemical auxiliaries but the problem has been higher energy consumption. Ultrafiltration has been used as a pre-treatment for

reverse osmosis or in combination with a biological reactor (Marcucci et al. 2001). It has been used for the removal of spin finish compounds, which are hydrophobic in nature, from wastewater resulting from rinsing of textile fibres. Micro filtration has been suitable for treating dye baths containing pigment dyes as well as subsequent rinsing baths (Van der Bruggen et al. 2005). Micro filtration finds applications as a pre-treatment for nanofiltration or RO. Adsorption techniques also serve as an attractive alternative for the treatment of contaminated waters, especially if the adsorbent is inexpensive and does not require an additional pre-treatment step before its application. However, it has been reported that an adsorbent such as activated carbon cannot decolorize solutions in reasonable time and entails high costs.

Chemical methods

Chemical methods include coagulation or flocculation combined with flotation and filtration, precipitation-flocculation with $Fe^{2+}/Ca(OH)_2$, electro-flotation, electro-kinetic coagulation, conventional oxidation methods by oxidizing agents, irradiation or electrochemical processes. Some of the chemical treatment methods involving Fenton's oxidation, photo-catalytic oxidation or ozonation are effective in decolorizing dye solutions, and in some cases, complete oxidation of the dye compounds to CO_2 (Sadr Ghayeni et al. 1998). Ozonation has been reported as the most effective method for reactive dye decolourization, with an efficiency of around 98-99%. But the installation of an ozonation unit involves additional costs. Electrochemical Oxidation and Fenton's Oxidation have been reported to have a very high rate of colour removal with the disadvantage of generating iron oxide sludges (Jennie Perey 2006).Coagulation-flocculation treatments have been generally used to eliminate soluble dyestuffs (Rozzi et al. 1999). Dye decolourisation from synthetic dye solutions using the non-ionic, water soluble, high molecular weight seed gums from the plant *Ipomoea dasysperma* and guar gum as coagulants has shown better results as compared to conventional coagulants. These chemical techniques are often expensive, and although the dyes are removed, accumulation of concentrated sludge creates a disposal problem.

Biological methods

Biological treatment has been the most economical alternative when compared to other physical and chemical processes. Biodegradation methods (Table 1) such as fungal decolourisation (e.g., *Phanerochaete chrysosporium*, *Trametes* sp. and *Aspergillus* sp.), microbial degradation, adsorption by (living or dead) microbial biomass, or bioremediation systems have been commonly applied to the treatment of industrial effluents. Anaerobic biological treatment methods use bacteria (e.g., *Bacteroides* sp., *Eubacterium* sp. and *Clostridium* sp.) to decolourise azo dye solutions through cleavage of the azo bond, yielding aromatic amines as products. Aerobic bacteria have been described to oxidatively decolourise many dyes from several classes, among which azo dyes always turned out to be the most recalcitrant compounds. Dye-degrading fungi find applications in bioreactors for the decolourisation and degradation of azo dyes (Maier et al. 2004). Due to the xenobiotic nature, azo dyes are not totally degraded (Laing 1991; Panswald et al. 2001). Two extra cellular enzymes are primarily involved in the bioremediation of textile effluents azo-reductase and laccase.

Azo-reductase for bio-treatment of textile effluents

Azo-reductase catalyzes a NAD(P)H-dependent reaction in bacteria to metabolize azo dyes to colourless aromatic amines. The faecal enzyme activity of azo-reductase has been commonly considered a marker for pro-carcinogenic activity. The non-specificity of the azo-

Table 1: Microbial method (bacterial & fungal) of dye degradation

Microbial type(s)	Degrading dye(s)	Reference(s)
(I) Bacteria		
Citrobacter sp. CK3	Reactive Red 180	Hui Wang et al 2009
Listeria Sp	Red B5 and Black HFGR	Kuberan.et al 2011
Bacillus subtilis	Acidblue113	Gurulakshmi et al 2008
Klebsiella sp.	Orange 3R	Ponraj1 et al 2011
Salmonella sp.		
Pseudomonas sp.		
Enterococcus faecalis strain YZ66	C.I. reactive yellow 145	Sahasrabudhe et al. 2011
(II) Fungi		
Penicillium chrysogenum, Aspergillus niger Cladosporium sp.	Azo dye- Red 3BN	Kumar Praveen 2012
P. ostreatus (IE8)	Acid black 194,	Elizabeth Rodrı´guez
P. ostreatus (IE8)	Orisol blue BH	et al. 1999
T. hispida (8260)		
Bjerkandera sp.	Amaranth, Remazol	Swamy and
BOS55 P. chrysosporium	Black B, Reactive Blue15,	Ramsay 1999
P. ostreatus	RemazolOrange ,	
T. hirsuta	Tropaeolin O	
T. versicolor		

reductase reaction has been demonstrated by many reports on the decolourisation of azo dyes by sewage sludge under anaerobic conditions. It has been observed that almost all azo compounds tested are biologically reduced under anaerobic conditions, although there are some indications that metal-ion-containing dyes sometimes have reduced decolourisation rates (Maier et al. 2004).

Degradation pathways of 4-carboxy-4-sulphoazobenzene, with reductive cleavage of the azo double bond as the initial degradation step have been established. The aerobic azo-reductases from the carboxyorange-degrading *Pseudomonas* strains K22 and KF46 are monomeric flavine-free enzymes that use NADPH and NADH as cofactors and reductively cleave several sulphonated azo dyes. Although several microorganisms seem to have potential for azo dye degradation, very few strains (*Acetobacter liquefaciens S-1, Aeromonas hydrophilam*, *Bacillus subtilis*, *B. cereus* and *Pseudomonas luteola Acinetobacter sp*.) (Mou et al. 1991; Oxspring et al.1996) can withstand the conditions of dying effluents in terms of pH and temperature (Panswald et al. 2001; Marchant et al. 1994; Zimmermann et al. 1984). Decolourisation takes place due to the production of an extra-cellular enzyme ***azo-reductase*** that reduces the azo bond present in the dyes and subsequently leads to the formation of amines (Mignani et al. 1999). Many researchers also reported that *A. radioresisten*s has the ability to degrade various aromatic hydrocarbons. However, under anaerobic conditions, azo-reductases usually cleave azo dyes into the corresponding amines, many of which are mutagenic and/or carcinogenic.

Laccase for bio-treatment of textile effluents

Another extracellular enzyme that has been found to effectively cleave azo bonds is *laccase* (p-benzenediol: oxygen oxidoreductase, EC 1.10.3.2). Laccase has been regarded to be environmentally friendly and is considered to be an attractive option for the development of new methodologies to treat textile effluents. Laccase, a cuproprotein belongs to a small group of enzymes denominated as 'blue oxidases'. It has been the subject of intensive research in the last decades as it possess low substrate specificity; non-requirement of a cofactor and is an extracellular enzymes making the purification procedures very easy. The rather broad substrate specificity of most laccases may be additionally expanded by the addition of redox mediators, such as ABTS (2,2'-azino-bis(3-

ethylbenzthiazoline) sulphonic acid), 1-hydroxybenzotriazole, or compounds secreted by lignolytic fungi during wood degradation (Ledakowicz et al. 2001) .Laccase oxidatively renders the azo dye more susceptible to nucleophilic attack, and nitrogen being eliminated in molecular form (Chivulka et al. 1995).

Laccases from many different fungi such as *Trametes versicolor*, *T. hirsuta*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus*, *Pyricularia oryzae* or *Phlebia tremell* have been used for the decolourisation of a wide structural variety of dyes. Laccases were detected in insects, bacteria and in white-rot fungi. The lignin-degrading basidomycetes *Trametes versicolor*, *Polyporous pinicitus* and the ascomycete *Myceliophthora thermophila* decolourised synthetic dyes to different extents (Marchant et al. 1994). Laccase acts oxidatively and less specifically on aromatic rings, thus having potential to degrade a wider range of compounds .Fungi *Myrothecium verrucaria* was found to have a strong binding affinity to some azo dyes (Blanquez et al. 2006). *Aspergillus sojae B-1* decolourised the azo dyes Amaranth, Congo Red and Sudan III in a nitrogen-poor media after 3-5 days of incubation (Rodriguez et al. 1999). Fungi *Neurospora crassa* decolourised diazo dyes with 89-91% colour removal within 24 hours of incubation. White-rot basidiomycetous fungi have been efficient in decolourising textile effluents (D'Souza et al. 2006). A white-rot fungus, *Pycnoporus cinnabarinus,* was found to tolerate and decolourise high concentration of dyes in a packed bed reactor within 48-72 hours of incubation. An extracellular oxidase activity in *Pycnoporus cinnabarinus* was also observed which confirmed its suitability for the degradation of dye effluent (Brahimi-Horn et al. 1992). About 96.4% decolourisation by *P. Florida* and 91.1% decolourisation by *T. hirsuta* have been reported on the 10th day of incubation (Sathiya moorthi et al. 2007; Abadalla et al. 2000). Fungal treatment of effluents had a tendency to become very protracted, immobilized enzyme treatment may hold a better potential for dye decolourisation and recycling of effluents without the need for the addition of growth substrates. Direct enzyme treatment was more efficient than the traditional physical-chemical treatments and microbial culture method. Hence, current research emphasises on devising novel processes involving direct enzymatic treatment. The degradation of indigo dyes with purified laccases from *Trametes hirsute* and *Sclerotium rolfsii* was shown to be enhanced to 30% in the presence of the redox mediator acetosyringone (Kandelbauer et al. 2004). A screening using several laccase mediators such as (2,2'-azinobis(3- ethylbenzthiazoline-6-sulphonic acid (ABTS), 1-hydroxybenzotriazole (HBT), *N*-hydroxyacetanilide (NHA), 2,2',6,6'-tetramethylpiperidin-1-yloxy (TEMPO) and violuric acid (VA)) was performed on the degradation of three reactive textile dyes: Reactive Black 5 (RB5), Reactive Blue 114 (RB114) and Reactive Yellow 15 (RY15). The optimum ABTS concentration for RB5 and RY15 decolourisation was determined as 0.1mM, which yielded colour reductions of 73% and 76%, respectively. The optimum temperature and pH for decolourisation were 40ºC and pH 5.0-5.5, respectively, with a maximum decolourisation above 80% for RB114. For RB114 the optimum ABTS concentration was determined as 0.001mM (1µM) with an observed decolourisation of 87%. These results thus revealed the efficacy of a laccase–mediator system (LMS) to treat textile dye effluents (Table 2 and Figure-1&2).

Table 2: Efficacy of a laccase–mediator system (LMS) to treat textile dye effluents

		Dye degradation (%)				
Reactive dye	No mediator	HBT	ABTS	TEMPO	NHA	VA
RB114	ND[a]	0.3	70	ND	1.5	5.0
RB5	ND	0.2	42	1.5	ND	2.5
RY15	ND	2.7	21	2.0	0.8	0.2

ND, Not detected

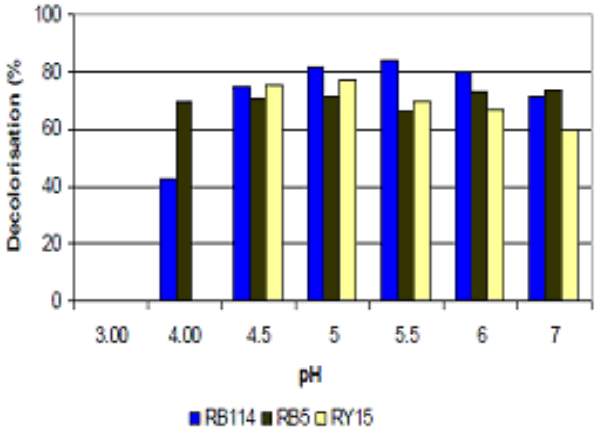

Figure 1: Effect of pH on the decolourisation of reactive dyes: Blue114 (RB114), Black 5(RB5) and yellow 15 (RY15) by laccase mediator (adapted from - Tavares et.al.,2008)

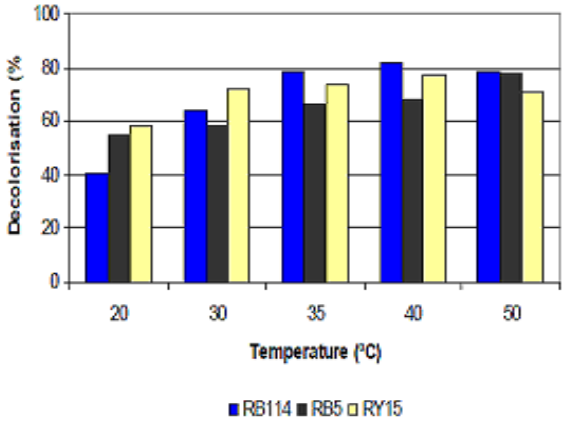

Figure 2: Effect of temperature on the decolourisation of reactive dyes: Blue114 (RB114), Black 5(RB5) and yellow 15 (RY15) by laccase mediator (adapted from - Tavares et.al. 2008)

Conclusion

Various technologies are available for dye removal and application of laccases, show decolourisations at different rates and to different extents and to a wide range of dyes. [25] Little information is available concerning the substrate specificity of laccase. One key parameter for the description of the structure-biodegradability relationship has been identified as the redox-potential of the substrates (Xu et al. 1999). Although chemometrical methods have been employed in order to classify dyes according to their biodegradability, no general models have been deduced so far. However, gaining insights in the degradation pathways of dyes and the structural requirements for biodegradability is important, especially to optimize potential bioremediation systems for industrial textile process water treatment. Although enzyme remediation of dyestuff successfully removed its colour, a potentially harmful organic load remained in the process waters. Thus, an interesting future perspective in the application of laccases for the treatment of dyestuff containing process waters was the polymerization of phenolic dye fragments rather than their oxidative breakdown. If such polymerized fragments were of sufficiently increased molecular weight they could readily be removed by a subsequent filtration step (Couto et al. 2006). Another area of research involves immobilization of laccase on various carrier materials, such as activated carbon, agarose, Eupergit C, Sepharose, and porosity glass, which might increase stability of the enzyme at

high pH, and tolerance to elevated temperatures, and make the enzyme less vulnerable to inhibitors.

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