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Enzymatic Degradation of Azo Dyes – A Review

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ABSTRACT

Synthetic organic colourants (e.g., azo dyes) are used commonly in different industries ranging from food, textile production, printing and pharmaceuticals. The majority of these dyes are recalcitrant, so that they can impart colour on various raw materials. Certain dyes, dye precursors and some aromatic amine metabolites produced through biotransformation of dye compounds have been shown to be carcinogenic. The release of dyes into the environment constitutes a small proportion of water pollution, and the coloured wastewaters represent a serious environmental problem and a public health concern. Colour removal, especially from textile wastewaters, has been a big challenge over the last few decades; until now there is no single and economically attractive treatment that can effectively decolourize textile mill effluent. This review narrates the different enzymatic mechanisms in the reduction of azo dyes.

Keywords: Remediation; azo dyes; enzymes; enzymatic degradation pathways

1. Introduction

The textile mills daily discharge millions of liters of untreated effluents in the form of wastewater into public drains that eventually empty into rivers. Most of them are recalcitrant in nature, especially azo dyes. The stability and their xenobiotic nature of reactive azo dyes makes them recalcitrant hence they are not totally degraded by conventional wastewater treatment processes that involve light, chemicals or activated sludge (Chung *et al*., 1992). The dyes are therefore released into the environment, in the form of coloured wastewater. This can lead to acute effects on exposed organisms due to the toxicity of the dyes, phytoplanktons form abnormal colouration and reduction in photosynthesis because of the absorbance of light that enters the water (Duran and Esposito 2000; Mester and Tien 2000). This also alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and gives the rivers intense colourations and public is greatly concerned about water quality. The presence of unnatural colours is aesthetically unpleasant and tends to be associated with contamination. Without adequate treatment these dyes will remain in the environment for an extended period of time (Olukanni *et al*., 2006).Several methods were adapted for the reduction of azo dyes to achieve decolourization. These include physiochemical methods (Droste, 2004) such as filtration, specific coagulation, use of activated carbon, chemical flocculation etc. Some of these methods (*viz* reverse osmosis, nanofiltration, multiple effect evaporator (MEE)) are found to be effective but quite expensive (Maier *et al*., 2004; Do *et al.*, 2002).Table.1 summarizes the advantages and disadvantages of the current methods of dye removal from industrial effluent (Andre *et al.*,

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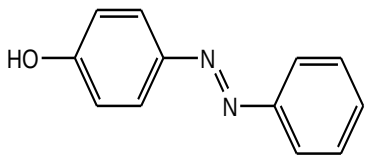
2007). Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluents. A number of microorganisms have been found to be able to decolourize textile dyes including bacteria, fungi, and yeasts (Olukanni *et al*., 2006; Wesenberg *et al*., *2003;* Kirby *et al*., 2000; Martins *et al*., 1999; Banat *et al*., 1996; Paszczynski *et al*.,1991; Gingell *et al*.,1971). They have developed enzyme systems for the decolourization and mineralization of azo dyes under certain environmental conditions (Anjali *et al*., 2006). Although dye molecules display a high structural variety, they are degraded by only few enzymes. These biocatalysts have one common mechanistic feature. They are all redox-active molecules and thus, exhibit relatively wide substrate specificities (Duran and Esposito 2000; Mester and Tien 2000). Preferentially, suitable organisms excrete the active enzymes into the medium. On the other hand dye molecules are transport into the cells; another important requirement for these organisms is its resistance against toxic effects of dyes and other substances present in the effluent. It has been reported after many studies decolourizing rates of dyes by microorganisms' decreases with increasing dye concentrations above certain levels. This may be a limiting factor for bio-elimination. Therefore, in cases where the target molecule or additives inhibit growth, isolated enzyme systems may be preferred.

Table 1: Advantages and disadvantages of the current methods of dye removal from industrial effluent (Courtesy: Andre *et al.,* 2005)

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 accelerates 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

The chemical structure of dyes in general is comprised of a conjugated system of double bonds and aromatic rings. The major classes of dyes have antroquinoid, indigoid, and azo aromatic structures. All of these structures allow strong ∏- ∏* transitions in the UV-visible area, with high extinction coefficients that allow us to consider these structures as dye chromophores. Of all of these structures, the azo aromatic one is the most widespread dye class in the industry. They may have one or more azo (N=N) groups. Azo dyes with one azo group are called mono-azo dyes, with two azo groups, di-azo dyes, followed by tri-azo azo dyes with more than three azo linkages are designated polyazo dyes. The most commercially important are mono-azo dyes and di-azo dyes, tri-azo dyes, whereas polyazo are much less important. The main drawback of this class of dyes is that they are not easily degraded by aerobic bacteria, and with the action of anaerobic or microaerobic reductive bacteria. There is a great environmental concern about the fate of these dyes, and their harmful metabolites especially on reactive dyeing of cellulosic fibers, where large amounts of unbound dye are discharged in the effluent (Pierce**,** 1994). They can form toxic and/or mutagenic compounds such as aromatic amines like naphthylamines, chloro aniline etc… (Chung *et al*.,1992; Wong *et al*.,1996)



A typical azo compound, 4-hydroxyphenylazobenzene

2. Enzymatic Degradation of Azo Dyes

In the case of enzymatic remediation of azo dyes, azo reductases and laccases seem to be the most promising enzymes. Laccases have been shown to decolourize a wide range of industrial dyes (Rodriguez *et al*., 1999; Reyes *et al*., 1999). Low molecular weight compounds like 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) may also be necessary to mediate the actual electron transfer steps of laccases (Wong and Yu, 1999). In the presence of redox mediators decolourization events of degradable dyes could be significantly enhanced (Reyes *et al*., 1999; Abadulla *et al*., 2000; Soares *et al*., 2001).

Similarly, in the case of peroxidases, addition of veratryl alcohol was shown to positively influence the decolourization of azo and anthraquinone dyes catalyzed by lignin peroxidase. However, this effect may either be attributed to the protection of the enzyme of being inactivated by hydrogen peroxide or to the completion of the oxidation-reduction cycle of the lignin peroxidase rather than to just redox-mediation (Young and Yu, 1997).

2.1 Degradation of azo dyes by azo reductases

Azo reductases catalyze the reaction only in presence of reducing equivalents like FADH and NADH. Most of the azo dyes have sulphonate substituent groups and a high molecular weight and they are unlikely to pass through cell membranes. Therefore, the reducing activity referred to the dye is not dependant on the intracellular uptake of the dye (Robinson *et al*., 2001). Russ *et al*. suggested that bacterial membranes are almost impermeable to flavin-containing cofactors and, therefore, restrict the transfer of reduction equivalents by flavins from the cytoplasm to the sulphonated azo dyes. Thus, a mechanism other than reduction by reduced flavins formed by cytoplasmic flavin-dependent azoreductases must be responsible for sulphonated azo dye reduction in bacterial cells with intact cell membranes (Russ *et al*., 2000).

One such mechanism involves the electron transport-linked reduction of azo dyes in the extra-cellular environment. To achieve this, the bacteria must establish a link between their intracellular electron transport systems and the high molecular weight, azo dye molecules. For such a 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). In addition, Gingell and Walker have shown that low molecular weight redox mediator compounds can act as electron shuttles between the azo dye and an NADH (nicotinamide adenine dinucleotide)-dependent azo reductase that is situated in the outer membrane.

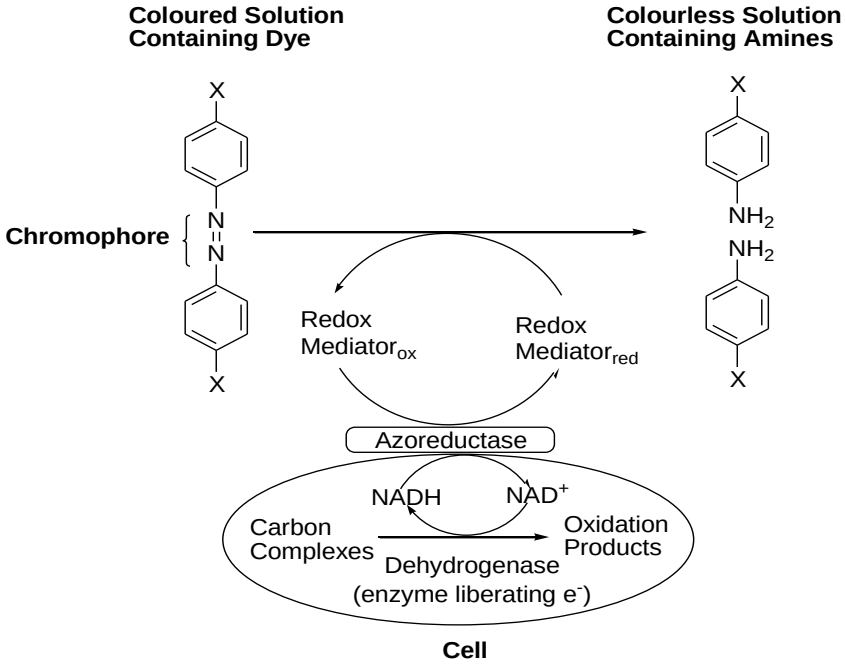


Figure 1: Proposed mechanism for reduction of azo dyes by azo reductase (Courtesy: Keck *et al.*, 1997*)*

These mediator compounds are either formed during the metabolism of certain substrates by the bacteria or they may be added externally (Russ *et al*., 2000). However, if the extra-cellular environment is aerobic, this reduction mechanism will be inhibited by oxygen, due to the preferential oxidation of the reduced redox mediator by oxygen rather than by the azo dye. Kudlich *et al*., support the suggestion that the membrane-bound azo reductase activity, mediated by redox compounds, is different from the soluble cytoplasmic azo reductase that is responsible for the reduction of non-sulphonated dyes that permeate through the cell membrane. Therefore, the membrane-bound and the cytoplasmic azo reductases are two different enzyme systems (Kudlich *et al*.,1997).Fig. 1 shows a proposed mechanism for the redox-mediator-dependent reduction of azo dyes using whole bacterial cells, under anaerobic conditions. Although the final reduction of the azo dyes in the cell supernatants is a dominantly chemical redox reaction, the redox mediators depend on cytoplasmic reducing enzymes to supply electrons (Yoo *et al*., 2001). It is also possible that this chemical redox reaction works in conjunction with a direct enzymatic reaction involving an azo reductase, which may be a dehydrogenase enzyme that is synthesized throughout the cytoplasm and secreted without accumulation inside the cell (Bragger *et al*., 1997)

2.2 Azo dye reduction by 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).

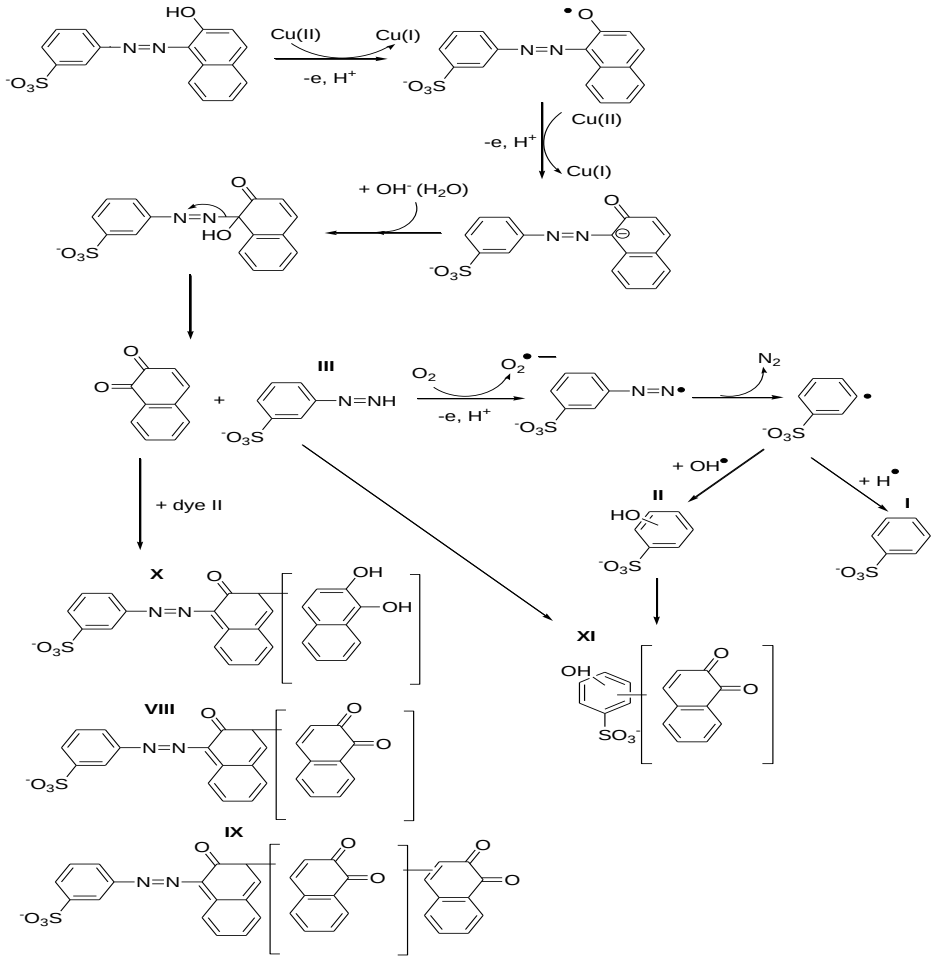


Figure 2: Proposed mechanism of laccase degradation of 3-(2-hydroxy-1-naphthylazo) benzenesulfonic Acid (Courtesy: Andrea *et al*., 2005)

These enzymes are multicopper phenol oxidases that decolourize azo dyes through a highly nonspecific free radical mechanism forming phenolic compounds, thereby avoiding the formation of toxic aromatic amines (Chivukula *et al*.,1995; Wong and Yu, 1999).Fig.2 represents the suggested model for the activity of laccase on one of the azo dyes, 3-(2-hydroxy-1-naphthylazo) benzenesulfonic Acid.

According to this suggested model, laccase oxidizes the phenolic group of the phenolic azo dye, with the participation of one electron generating a phenoxy radical which is sequentially followed by oxidation to a carbonium ion. A nucleophilic attack by water on the phenolic ring carbon bearing the azo linkage to produce 3-diazenyl-benzenesulfonic acid (III) and 1, 2-naphthoquinone then takes place (Susana *et al*., 2005). The radicals which are formed by the one-electron oxidation of dye by laccase will react with 1, 2-naphthoquinone rather than be oxidized. The formed radicals can undergo oxidation to yield compound VIII, reduction to yield compound X, or further polymerization and again oxidation to form compound IX. According to the reported mechanism, cross-coupling between the reactive species results in the formation of C-C and C-O bonds between phenolic molecules and of C-N and N-N bonds between aromatic amines. By phenolic cross-coupling, an electron is removed from the hydroxyl group, generating an alkoxy radical. Alkoxy free radical forms dimers in the *ortho* and *para* positions with the hydroxyl groups. Phenolic radicals can be further oxidized to yield oligomeric products. Under certain conditions, the C-C-formed dimers can take part in coupling reactions to form extended quinines (Andrea *et al*., 2005).

2.3 Azo dye reduction by peroxidases

Fungal systems appear to be most appropriate in the treatment of azo dyes (Ezeronye and Okerentugba, 1999). 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 H_2O_2 to an oxidized state during their catalytic cycle. The first ligninolytic peroxidases were isolated from *P. chrysosporium* and called lignin peroxidase (LiP) (Glenn *et al*., 1983; Tien *et al*., 1983) and manganese peroxidase (MnP) (Kuwahara *et al*., 1984; Wariishi *et al., l*1988). LiP catalyzes the oxidation of non phenolic aromatic compounds like veratryl alcohol. MnP preferentially oxidizes Mn^{2+} to Mn^{3+}, and the Mn^{3+} is responsible for the oxidation of many phenolic compounds (Glenn *et al*., 1986). Mn^{2+} is necessary for completion of the catalytic cycle of MnP. The MnP isoenzymes from *Pleurotus* species and *Bjerkandera adusta* differ from the isoenzymes isolated from *P. chrysosporium* because they are able to oxidize 2, 6-dimethoxyphenol (DMP) and veratryl alcohol in a Mn^{2+}-independent reaction (Glenn *et al*., 1983; Tien *et al*., 1983**;** Ezeronye and Okerentugba, 1999). Several reports have shown that LiP or MnP from *P. chrysosporium* is directly involved in the degradation of various xenobiotic compounds and dyes (Paszczynski *et al*., 1991). In many studies preferential degradation of different sulfonated azo dyes either by MnP and Mn^{2+} or by LiP was demonstrated (Paszczynski *et al*., 1991; Pasti *et al*., 1992). Metabolic pathways for azo dye degradation by fungal peroxidases have been postulated in Fig.3 (Goszczynski *et al*., 1994; Spadaro *et al*., 1994).

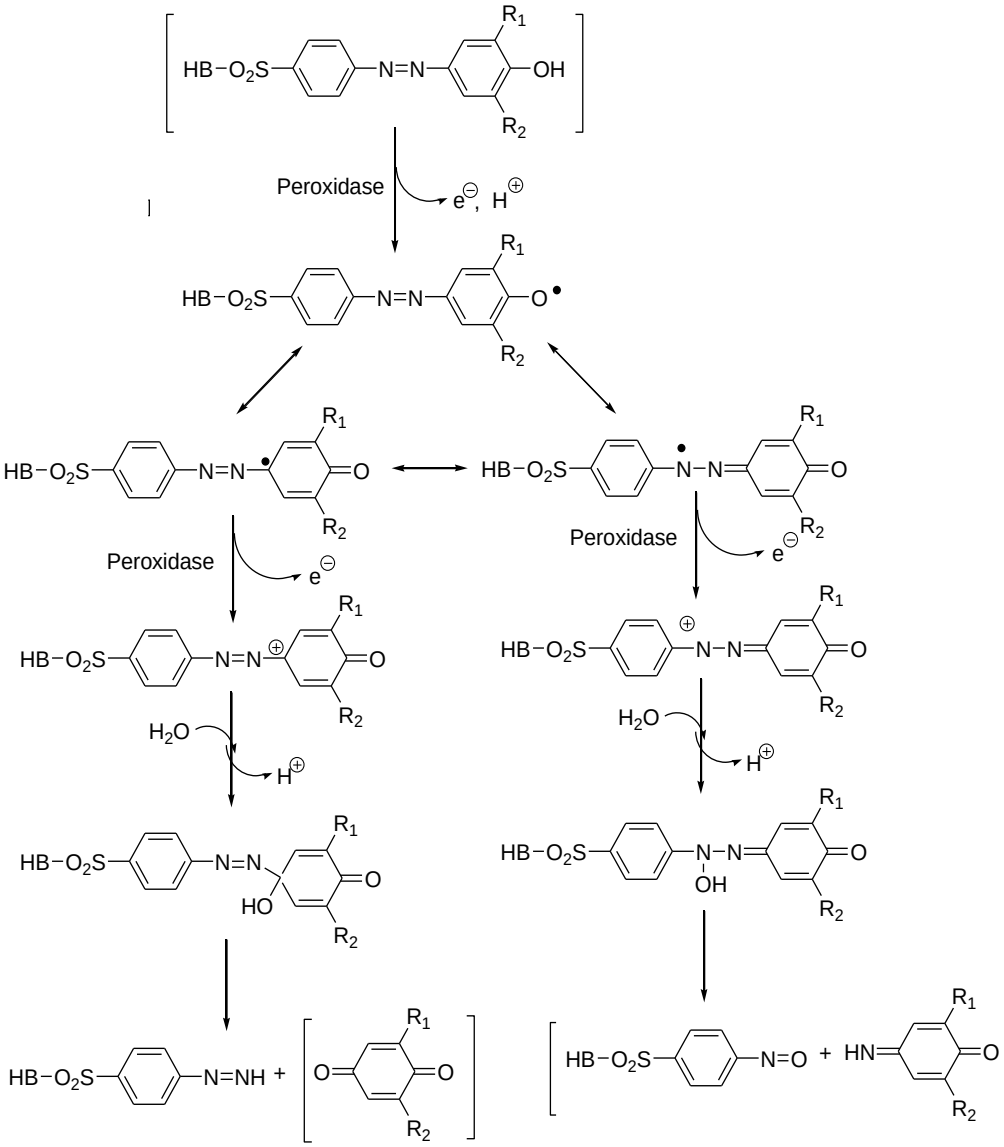


Figure 3: Proposed mechanism for alternative asymmetrical and symmetrical cleavages of sulfonated azo dyes by P. chrysosporium and S. chromofuscus peroxidases (ligninases). The compounds represented by structures in brackets were found in the reaction mixture. Azo dye 1, R, = R2 = CH3 and B = 0; azo dye 2, R, = H, R2 = OCH3, and B = NH.

3. Conclusion

Enzymatic processes are very promising for the decolourization of synthetic azo dyes. Here we have reviewed the initial steps in the pathways for the enzymatic degradation of recalcitrant synthetic azo dyes. To understand the decolourization and degradation mechanism of azo compounds under aerobic conditions, detailed information is needed about the initial enzymatic transformation of azo linkages. The authors in our laboratory are involved in the isolation of potent solo bacterium and bacterial consortium for efficient decolourization of azo dyes from textile mill effluents. Together with this isolation, we too extend our studies to understand of how chemical structure influences the susceptibility of these man-made compounds to degradation, these studies could help to develop a new generation of readily degradable dyes; such research could also exemplify how a new generation of less recalcitrant chemicals might be produced in the future.

4. References

1. Abadulla. E., Tzanov. T., Costa. S., Robra. K.H., Cavaco-Paulo. A.,and Gübitz. G.M., 2000, Decolourization and detoxification of textile dyes with a laccase from *Trametes hirsute,* Applied Environmental Microbiology, 66, pp 3357–3362.

2. Adosinda. M., Martins. M., Lima. N., Silvestre. A.J.D.,and Queiroz. M.J.,2003, Comparative studies of fungal degradation of single or mixed bioaccessible reactive azo dyes, Chemosphere 52, pp 967–973.

3. Andrea Zille., Barbara Go´rnacka., Astrid Rehorek., Artur Cavaco-Paulo., 2005, Degradation of Azo Dyes by *Trametes villosa* Laccase over Long Periods of Oxidative Conditions, Applied Environmental Microbioly, pp 6711–6718.

4. Anjali Pandey., Poonam Singh. , Leela Iyengar, Review on bacterial decolourization and degradation of azo dyes. Department of Chemistry, I.I.T Kanpur .

5. Banat, I. M., Nigam, P., Singh. D., Marchant, R ., 1996,. Microbial decolourization of textile dye containing effluents a review, Bioresource Technology 58, pp 217–227.

6. Blanquez, P., Casas, N., Font, X., Gabarrell, X., Sarra, M., Caminal G,Vicent, T., 2004, Mechanism of textile metal dye biotransformation by *Trametes versicolour*, Journal of Water Research, 38, pp 2166–2172.

7. Bragger, J.L., Lloyd, A.W., Soozandehfar S.H., Bloomfield, S. F., Marriott, C., Martin, G. P., 1997, Investigations into the azo reducing activity of a common colonic microorganism. International journal of pharmaceutics, 157, pp 61–71.

8. Chivukula, M., Renganathan, V., 1995, Phenolic azo dye oxidation by laccase from *Pyricularia oryzae,* Applied Environmental Microbiology, 61, pp 4347–4377.

9. Chung, K. T., Cerniglia, C.E., 1992, Mutagenicity of azo dyes: structure activity relationship. Mutation. Research, 277, pp 201–220.

10. Chung, K.T., Steven,s S.E., 1993, Decolourization of azo dyes by environmental microorganisms and helminthes, Environmenta. Toxicology and Chemistry, 12, pp 2121–2132.

11. Do, T., Shen, J., Cawood, G., Jeckins, R., 2002, Biotreatment of textile effluent using *Pseudomonas* spp. Immobilized on polymer supports. In: Advances in biotreatment for textile processing. Hardin IR; Akin DE & Wilson JS (Eds). University of Georgia Press.

12. Droste, R.L., 2004, Theroy and Practice of water and wastewater treatment. John Wiley & Sons, Inc; Singapore.

13. Duran, N., Esposito, E., 2000, Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review, Applied Catalysis B: Environmental, 28, pp 83–99.

14. Duran, N., Rosa, M.A., D'Annibale A., Gianfreda, L., 2002, Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review. Enzyme. Microbial Technology, 31, pp 907–931.

15. Ezeronye, O. U., Okerentugba, P.O., 1999, Performance and efficiency of a yeast biofilter for the treatment of a Nigerian fertilizer plant effluent, World. Journal of .Microbiology and Biotechnology, 15, pp ,515-516.

16. Gingell, R., Walker, R., 1971, Mechanisms of azo reduction by *Streptococcus faecalis*. II. The role of soluble flavins, Xenobiotica, 1(3), pp 231–239.

17. Glenn, J. K., Akileswaran, L., Gold, M. H., 1986 . Mn(II) oxidation is the principal function of the extracellular Mn-peroxidase from *Phanerochaete chrysosporium*, Archieves of Biochemistry and Biophysics. 251, pp 688–696.

18. Glenn, J. K., Morgan, M. A., Mayfield, M.B., Kuwahara, M., Gold, M.H., 1983, An extracellular H_2O_2-requiring enzyme preparation involved in lignin biodegradation by the white-rot basidiomycete *Phanerochaete chrysosporium*, Biochemical and Biophysical Research. Communications. 114, pp 1077–1083.

19. Goszczynski, S., Paszczynski, A., Pasti-Grigsby, M.B., Crawford, R.L., Crawford, D. L., 1994, New pathway for degradation of sulfonated azo dyes by microbial peroxidases of *Phanerochaete chrysosporium* and *Streptomyces chromofuscus*, Journal of Bacteriology, 176, pp 1339–1347.

20. Keck, A., Klein, J., Kudlich, M., Stolz, A., Knackmuss, H. J., Mattes, R., 1997, Reduction of azo dyes by redox mediators originating in the naphthalenesulfonic acid degradation pathway of *Sphingomonas sp*. strain BN6, Applied Environmental Microbiology; 63(9) pp 3684–90.

21. Kirby, N., Marchan, R., McMullan, G., 2000, Decolourization of synthetic textile dyes by *Phlebia tremellosa*, FEMS Microbiology Letters 188, pp 93–96.

22. Kudlich, M., Keck, A., Klein, J,. Stolz, A., 1997, Localization of the enzyme system involved in anaerobic reduction of azo dyes by *Sphingomonas sp*. strain BN6 and effect of artificial redox mediators on the rate of azo dye reduction, Applied Environmental Microbiology, 63(9), pp 3691–3694.

23. Kuwahara, M., Glenn, Morgan, K.J.M. A., Gold, M.H., 1984, Separation and characterization of two extracellular H_2O_2-dependent oxidizes from ligninolytic cultures of *Phanerochaete chrysosporium* , FEBS Letters, 169, pp 247–250.

24. Maier, J., Kandelbauer, A., Erlacher, A., Cavaco Paulo, A., Gubits, G.M., 2004, A new alkali – thermostable azoreductase from *bacillus sp.* Strain SF, Applied Environmental Microbiology, 70, pp 837 – 844.

25. Martins, M.A.M., Cardoso, M.H, Queiroz, M.J., Ramalho, M.T., 1999, Biodegradation of azo dyes by the yeast *Candida zeylanoides* in batch aerated cultures, Chemosphere ,38, pp 2455–2460.

26. Maximo C., Costa-Ferreira, , 2004, Decolourization of reactive textile dyes by *Irpex lacteus* and lignin modifying enzymes, Process Biochemisry, 39, pp 1475–1479.

27. Mester, T., Tien, M ., 2000, Oxidative mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants, International Biodeterioration and Biodegradation, 46, pp 51–59.

28. Myers, C. R., Myers, J.M., 1992, Localization of cytochromes to the outer membrane of anaerobically grown shewanella putrefaciens MR-1, Journal of Bacteriology, 174(11),pp 3429–38.

29. Novotny, C., Svobodova, K., Kasinath, A., Erbanova, P., 2004, Biodegradation of synthetic dyes by *Irpex lacteus* under various growth conditions International Biodeterioration and Biodegradation, 54,pp 215–223.

30. Olukanni OD., Osuntoki , A. A., Gbenle, G.O., 2006, Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria, Africal Journal of Biotechnology, .5,pp 1980-1984.

31. Pasti-Grigsby MB, Paszczynski A, Goszczynski S, Crawford DL, Crawford R L (1992). Influence of aromatic substitution patterns on azo dye degradability by *Streptomyces* spp. and *Phanerochaete chrysosporium*. Applied Environmental Microbiology, 58, pp 3605–3613.

32. Paszczynski, A., Crawford, R. .L., 1991, Degradation of azo compounds by ligninase from *P. chrysosporium*, Biochememical and Biophysical Research Communications, 178, pp 1056–1063.

33. Paszczynski, A., Pasti, M. B., Goszczynski, S.D., Crawford, L., Crawford, R. L., 1991, New approach to improve degradation of recalcitrant azo dyes by *Streptomyces* spp. and *Phanerochaete chrysosporium*, Enzyme and Microbial Technology, 13, pp 378–384.

34. Peralta-Zamora, P., Pereira, C. M., Tiburtius, E.R.L., Moraes, S.G., Rosa, M. A., Minussi, R. C., Duran, N., 2003, Decolourization of reactive dyes by immobilized laccase, Applied catalysis B: Environmental, 42, pp 131–144.

35. Reyes, P., Pickard, M.A., Vazquez-Duhal, R., 1999, Hydroxybenzotriazole increases the range of textile dyes decolourized by immobilized laccase, Biotechnology Letters, 21, pp 875–880.

36. Robinson, T., McMullan, G., Marchant, R., Nigam, P., 2001, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, Bioresource Technology, 77,pp 247–55.

37. Rodrigue, E., Pickard, M.A., Vazquez-Duhal,t R., 1999, Industrial dye decolourization by laccases from ligninolytic fungi, Current Microbiology, 38,pp 27–32

38. Russ, R., Rau ,J., Stolz, A., 2000,.The function of cytoplasmic flavin reductases in the reduction of azodyes by bacteria, Appied Environmental Microbiology, 66(4),pp 1429–34.

39. Soares, G.M., Pessoa d'A., Costa-Ferreira, M., 2001, Use of laccase together with redox mediators to decolourize Remazol Brilliant Blue R, Journal of Biotechnology, 89,pp 123–129.

40. Spadaro JT, Renganathan V., 1994. Peroxidase-catalyzed oxidation of azo dyes: mechanism of Disperse Yellow 3 degradation Archieves of Biochemistry and Biophysics. 312:301–307.

41. Susana Camarero., David Ibarra., Marı´a Jesu´s Martı´nez., Angel, T.M., 2005, Lignin-Derived Compounds as Efficient Laccase Mediators for Decolourization of Different Types of Recalcitrant Dyes,. Applied Environmental Microbiology, pp 1775–1784.

42. Tien,M., Kirk, T.K., 1983, Lignin-degrading enzyme from the hymenomycete *Phanerochaete chrysosporium* Burds. Science, 221, pp 661–663.

43. Wariishi, H., Akileswaran, L., Gold, M.H., 1988, Manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*: spectral characterization of the oxidized states and the catalytic cycle, Biochemistry, 27, pp 5365–5370.

44. Wesenberg, D., Kyriakides, I., Agathos, S.N., 2003, White-rot fungi and their enzymes for the treatment of industrial dye effluents, Biotechnology. Advances, 22, pp 161–187.

45. Wong, P.K., Yuen, P.Y., 1996, Decolourization and biodegradation of methyl red by *Klebsiella pneumoniae* RS-13, Water Research. 30, pp 1736–1744.

46. Wong, Y., Yu, J., 1999,. Laccase-catalyzed decolourization of synthetic dyes, Water Research, 33,pp 3512–3520.

47. Yoo, E.S., Libra, J., Adrian, L.,2001, Mechanism of decolourization of azo dyes in anaerobic mixed culture, Journal of Environmental Engineering and Science , 127(9), pp 844–849.

48. Young, L., Yu, J., 1997, Ligninase-catalyzed decolourization of synthetic dyes. Water Research, 31,pp 1187–1193.