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ENHANCEMENT OF DYE DEGRADATION ACTIVITY BY MICROORGANISMS – A REVIEW

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ABSTRACT

A variety of synthetic dyestuffs released by the textile industry pose a threat to environmental safety. Existing effluent treatment procedures are unable to remove these dyestuffs completely from effluents because of their colour fastness, stability and resistance to degradation. Microbial processes for the treatment of textile wastewater have the advantage of being cost-effective, environmental friendly and producing less sludge. The most promising microorganisms for wastewater treatment are those isolated from sites contaminated with dyes or from the sludge of treatment plants because they have adapted to survive in adverse conditions. The mechanism of microbial decolourisation occurs from adsorption, enzymatic degradation or a combination of both. The enzymes like reductases and oxidases are involved in the microbial degradation process. The goal of microbial treatment is to decolourise and detoxify the dye-contaminated effluents. This paper provides a detailed review on the enhancement of dye degradation activity by microorganisms.

KEYWORDS: **Decolourization, Dye degradation, Dyestuffs, Microbial degradation and Wastewater treatment.**

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1. INTRODUCTION

The textile industry produces large quantities of highly coloured effluents, which are generally toxic and resistant to degradation by biological treatment methods. Textile wastewater, being mostly non-biodegradable under both natural and sewage treatment plant conditions, is a potential nuisance to the environment. Therefore, it is necessary to find an effective method of wastewater treatment capable of removing colour and toxic organic compounds from textile industry effluents. An extensive use of dyes in textile and other industries has created problems in terms of acute ecological effects. On the other hand, certain dyestuffs exhibit toxic effects on microbial population and carcinogenic potential to human beings, because certain classes of dyes are known to enzymatically degrade or biotransformed in the human digestive system, producing carcinogenic by products. The environmental problems created by the textile industries have received increased attention for several decades because this industry is one of the largest generators of contaminated effluents, which mainly arise from dyeing and finishing processes and is associated with the water pollution caused by the discharge of untreated or poorly treated effluents. Wastewaters resulting from these processes have adverse impacts in terms of Total Organic Carbon (TOC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), suspended solids, salinity, colour, a wide range of pH (5–12) and the recalcitrance of organic compounds, such as azo dyes[55].Dye containing wastewater is treated conventionally by physico-chemical processes. It includes physico-chemical flocculation combined with floatation, electrochemical destruction, precipitation, ion exchange, adsorption and membrane processes. However, these techniques seem to be either inefficient in removing colour, expensive or less suited to a wide range of dye wastewaters[4] .Recently, a number of studies have shown the feasibility of biological treatment systems for dye removal, even though they are toxic to the microorganisms. These microorganisms which include, bacteria, fungi and algae are reported to remove dyes by biosorption and biodegradation mechanisms. Biological methods are environmentally friendly, produce less sludge than physical and chemical systems, and are relatively inexpensive, as the running cost is low. Microbial decolourisation can occur via biosorption, enzymatic degradation or a combination of both. This review focuses on microbial decolourisation of textile dyes and the methods used for enhancement of dye degradation activity by microorganisms.

2. Dyes

Dyes are coloured, aromatic organic compounds which show an affinity towards the substrate to which it is being applied. They are applied to numerous substrates like textiles, leather, plastic, paper, etc in liquid form. Dyes are classified according to their application and chemical structure. They are composed of a group of atoms responsible for the dye colour, called chromophores, as well as an electron withdrawing or donating substituents that cause or intensify the colour of the chromophores, called auxochromes[11]. The most important chromophores are azo (–N=N–), carbonyl (–C=O), methine (–CH=), nitro ($–NO_2$) and quinoid groups. The most important auxochromes are amine ($–NH_3$), carboxyl (–COOH), sulfonate ($–SO_3H$) and hydroxyl (–OH). It is worth to mention that the sulfonate groups confer very high aqueous solubility to the dyes. The auxochromes belong to the classes of reactive, acid, direct, basic, mordant, disperse, pigment, vat, anionic and ingrain, sulphur, solvent and disperse dye[94] . It is estimated that almost 10^9 kg of dyes are produced annually in the world, of which azo dyes represent about 70% by weight[100] .This group of dyes is characterised by reactive groups that form covalent bonds with OH–, NH–, or SH– groups in fibres (cotton, wool, silk, nylon). Azo dyes are mostly used for yellow, orange and red colours[11].To obtain the target colour, normally a mixture of red, yellow and blue dyes is applied in the dyebaths. These three dyes do not necessarily have the same chemical structure. They might contain many different chromophores, in which azo, anthraquinone and phthalocyanine dyes are the most important groups[33]. Anthraquinone dyes constitute the second most important class of textile dyes, after azo dyes [5] . Anthraquinone dyes have a wide range of colours in almost the whole visible spectrum, but they are most commonly used for violet, blue and green colours[11, 21]. Normally colour is noticeable at a dye concentration higher than 1 mg/L and an average concentration of 300 mg/L has been reported in effluents from textile manufacturing processes[27, 58]. Over $7×10^5$ ton and approximately 10,000 different dyes and pigments are produced world-wide annually, about 10% of which may be found in wastewaters[14]. Colour interferes with the penetration of sunlight into the waters, retards photosynthesis, inhibits the growth of aquatic biota and interferes with gas solubility in water bodies[4]. In addition, many dyes are believed to be toxic, carcinogenic or found to be prepared from known carcinogens such as benzidine or other aromatic compounds that might be formed as a result of microbial metabolism[56, 42]. Hence, removal of these dyes from the effluents is necessary.

2.1 Environmental problems caused by dyes

Due to large scale production and extensive application, synthetic dyes can cause considerable environmental pollution and serious health-risk factors[20]. The untreated dye containing effluents that are directly used in agriculture have a serious impact on the environment and human health [69] .Disposal of the untreated dye containing effluents, without any treatment, in water bodies can cause serious environmental and health hazards[78]. Industrial effluents containing synthetic dyes reduces light penetration in water bodies and affects the photosynthetic activities of aquatic flora, thereby badly affecting the food source of aquatic organisms. The thin layer of discharged dyes formed over the surface of a water body also decreases the amount of dissolved oxygen in water, thereby affecting the aquatic fauna. Furthermore, dye-containing effluents increase the biochemical oxygen demand of contaminated water[3]. Many dyes are visible in water at concentrations as low as 1 mg L^{-1} [62]. Thus, apart from affecting the health of plants and animals,

synthetic dyes are also undesirable to water bodies from an aesthetic point of view. Of all known dyestuffs in the world, azo dyes make up about a half, making them the largest group of synthetic colorants and the most common synthetic dyes released into the environment[99]. The fast coloured dyes are a major source of concern to environmentalists, since such pollutants, besides causing aesthetic damage to sites, are also toxic and carcinogenic[53]. In recent years, interest in environmental control of dyes has increased, due to their possible toxicity and carcinogenicity; this is because many dyes are comprised of known carcinogens, such as benzidine and other aromatic compounds[32]. Many of the dyes used by textile industries are known carcinogens and teratogens. Dyes are introduced into the environment through industrial effluents of these industries. There are sample evidences of their harmful effects. Triple primary cancers involving kidney, urinary bladder and liver in a dye worker have been reported[50]. The textile sector contains many hazards and risks to workers, ranging from exposure to noise and dangerous substances, to manual handling and working with dangerous machinery. Each processing stage from the production of materials to the manufacturing, finishing, colouring and packaging poses risks to workers and some of these are particularly dangerous to women's health. Many different groups of chemical substances are used in the textiles sector, including dyes, solvents, optical brighteners, crease-resistance agents, flame retardants, heavy metals, pesticides, and antimicrobial agents. They are used in dyeing, printing, finishing, bleaching, washing, dry cleaning, weaving slashing/sizing, and spinning. Respiratory and skin sensitizers can be found in the textiles industry, for example textile fibres, reactive dyes, synthetic fibres, and formaldehyde. The exposure of workers to dusts from materials such as silk, cotton, wool, flax, hemp, sisal, and jute can occur during weaving, spinning, cutting, ginning, and packaging. Exposure to loud noise can result in permanent hearing damage such as noise-induced hearing loss and tinnitus. These effluents not only contain high concentration of dyes, but also contain chemicals used in various processing stages. Some trace metals such as Cr, As, Cu and Zn are present in these effluents and are capable of causing several health problems including haemorrhage, ulceration of skin, nausea, severe irritation of skin and dermatitis. There is a great need to develop an economic and effective way of dealing with the textile industry effluents in the face of the ever-increasing production activities[63].

2.2 Need for dye degradation

A major class of synthetic dyes includes the azo, anthroquinone and triphenylmethane dyes. Dyes are difficult to degrade biologically, so the degradation of dyes has received considerable attention. About 10-15% of all dyes directly enter the wastewater during the dyeing process[64]. Thus, the wastewater must be treated before releasing into the natural environment. For the biological treatment of wastewater containing dyes, the microbial decolourization and degradation of dyes has been of considerable interest. Increase in colour fastness, stability and resistance of dyes to degradation have made colour removal from textile wastewaters even more difficult[18, 93, 75]. Removal of colour from dye containing wastewaters is a current issue of discussion and regulation in many countries because of the awareness that water is a valuable asset and that should be protected. Nowadays dyed wastewaters are mainly treated by physical and chemical procedures which have many shortcomings[4]. Not only aesthetic problems occur due to dyes, but also bio toxicity and the possible mutagenic and carcinogenic effects of azo dyes have been reported. The colour of dye affects the photosynthetic activity in water bodies. Further, the released dyes on degradation form toxic amines in sediments. Although some of the dyes are adsorbed on to aerobic sludge in wastewater treatment plants, the applied aerobic microbial process cannot readily remove it from wastewater. Additionally the dye reduces the treatment efficiency in these plants, which may lead to a collapse of the biological treatment facility. Therefore physico-chemical or physical methods have been investigated to overcome the aforementioned problems. The physico-chemical methods have the limitations of high operational costs and generation of large quantities of sludge for disposal. Biological treatment of dyes seems to be a cost effective alternative to the physico-chemical methods.

2.3 Degradation and decolourisation of dyes by various methods

There are many different biological and non biological methods used for the removal of dyes from the textile industry effluents. They are discussed below.

2.3.1 Non biological methods used for dye decolourisation

2.3.1.1Physical-Chemical Methods

In physical–chemical methods coagulants like ferric salts or aluminium polychloride are used to form flocs with the dyes, which are then separated by filtration or sedimentation. Polyelectrolyte can also be dosed during the flocculation phase to improve the floc settleability[86]. The coagulation–flocculation method is one of the most widely used processes in textile wastewater treatment plants in many countries such as Germany and France. It can be used either as a pre-treatment, post-treatment, or even as a main treatment system[22, 48]. The coagulation–flocculation methods were successfully applied for colour removal of sulphur and disperse dyes, whereas acid, direct, reactive and vat dyes showed very low coagulation–flocculation capacity[48]. On top of the problem of low colour removal efficiency with some dyes, physical–chemical methods demand large chemicals inputs, and produce high volumes of polluted sludge, which then must be treated[75, 2] .

2.3.1.2 Chemical methods

Chemical oxidation 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 chemical composition of a compound or a group of compounds, e.g. dyes[52]. Among these oxidants, ozone is the most widely used because of its high reactivity with many dyes, usually providing good colour removal efficiencies[1]. In a process called selective oxidation, ozone can be designed in such a way that only –N=N–

bond scission occurs, and biodegradable compounds remain non-oxidised[7]. However, disperse dyes and those insoluble in water represent a drawback for the process, as well as the high cost of ozone[34, 2]. The usual low efficiency of both colour and COD removals of conventional chemical oxidation techniques have been overcome by the development of the so-called advanced oxidation processes (AOP). In this process, oxidizing agents such as O_3 and H_2O_2 are used with catalysts (Fe, Mn and TiO_2), either in the presence or absence of an irradiation source[2]. At present, many different combinations of these AOP have been investigated for colour removal, all of which are capable of producing the free hydroxyl radical (HO). The first example is a reaction called the Fenton reaction, in which hydrogen peroxide is added in an acid solution (pH 2–3) containing Fe^{2+} ions

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO + HO^-$

In comparison with ozonation, this method is relatively cheap and also presents high COD removal and decolourisation efficiencies[87]. The main process drawbacks are the high sludge generation due to the flocculation of reagents and dye molecules[75], as well as the need for decreasing the bulk pH to acidic conditions. The UV-based methods in the presence of a catalyst, e.g. a semiconductive material such as TiO_2, have also been shown to distinctly enhance colour removal[79, 30]. Thus, different combinations such as ozone/TiO_2, ozone/TiO_2/H_2O_2 and TiO_2/ H_2O_2 have been investigated, but they are enormously influenced by the type of dye, dye concentration and pH[23]. Recently, the utilization of solar technologies instead of UV-based methods has been attracting attention[92].

2.3.1.3 Physical methods

Filtration methods such as ultrafiltration, nanofiltration and reverse osmosis have been used for water reuse and chemical recovery. In the textile industry these filtration methods can be used for both filtering and recycling not only pigment-rich streams, but also mercerising and bleaching wastewaters. The specific temperature and chemical composition of the wastewater determine the type and porosity of the filter to be applied[68]. The main drawbacks of membrane technology are the high investment costs, the potential membrane fouling, and the production of a concentrated dye bath which needs to be treated[75]. The recovery of concentrates from membranes, e.g. recovery of the sodium hydroxide used in the mercerising step or sizing agents such as polyvinyl alcohol (PVA), can attenuate the treatment costs[68].

2.3.1.4 Adsorption methods

Adsorption methods for colour removal are based on the high affinity of many dyes for adsorbent materials. Decolourisation by adsorption is influenced by some physical–chemical factors like dye-adsorbent interactions, adsorbent surface area, particle size, temperature, pH and contact time[51, 2]. The main criteria for the selection of an adsorbent should be based on characteristics such as high affinity and capacity for target compounds and the possibility of adsorbent regeneration[41]. Activated carbon (AC) is the most common adsorbent and can be very effective with many dyes[91]. However, its efficiency is directly dependent upon the type of carbon material used and the wastewater characteristics, i.e. types of dyes present in the stream[75]. Additionally, AC is relatively expensive and has to be regenerated offsite with losses of about 10% in the thermal regeneration process. In order to decrease the adsorbent losses during regeneration, new adsorbent materials have been tested for their ability for on-site regeneration. Many alternative materials such as zeolites, polymeric resins, ion exchangers and granulated ferric hydroxide were studied[41]. It was found that zeolites and microporous resins were unsuitable due to their low sorption capacity. Although the ion exchangers provided good sorption capacity, regeneration was sometimes difficult. A number of low-cost adsorbent materials like peat, bentonite clay and fly ash, have been investigated on colour removal[72, 2]. However, the efficiency of these materials varied with the dye class. For instance, fly ash presented high sorption affinity for acid dyes, whereas peat and bentonite presented high affinity for basic dyes.

2.3.2 Biological methods used for dye decolourisation

The application of microorganisms for the biodegradation of synthetic dyes is an attractive and simple method by operation. However, the biological mechanisms can be complex. Large number of species has been tested for decolouration and mineralization of various dyes. Unfortunately, the majority of these compounds were chemically stable and resistant to microbiological attack. The isolation of new strains or the adaptation of existing ones to the decomposition of dyes will probably increase the efficacy of bioremediation of dyes in the near future. The use of microorganisms for the removal of synthetic dyes from industrial effluents offers considerable advantages. The process is relatively inexpensive, the running costs are low and the end products of complete mineralization are not toxic. The various aspects of the microbiological decomposition of synthetic dyes have been previously reviewed[80]. Microbial decolouration can occur via two principal mechanisms: biosorption and enzymatic degradation, or a combination of both.

2.3.2.1 Biosorption

Biomass from algae, yeast, filamentous fungi and bacteria has been used to remove dyes by biosorption. The biosorption capacity of a microorganism is attributed to the heteropolysaccharide and lipid components of the cell wall, which contain different functional groups, including amino, carboxyl, hydroxyl, phosphate and other charged groups, causing strong attractive forces between the azo dye and the cell wall. It was found that, the[81] maximal decolouration of several azo dyes using Aspergillus *foetidus* is achieved in the presence of carbon sources during exponential growth. Some pretreatment processes can modify the adsorption capacity of the biomass, such as autoclaving, as high temperature causes cell rupture with a consecutive increase in surface area, and treatment with acid, formaldehyde, NaOH, $NaHCO_3$ or $CaCl_2$ which change the surface of the microorganism and increase or decrease the capacity of the binding sites. Dead cells have advantages as biosorbents over living cells because, for example, they do not require nutrients, can be stored and used over extended

periods, and can be regenerated using organic solvents or surfactants. The effectiveness of biosorption depends on the following conditions: pH, temperature, ionic strength, time of contact, adsorbent and dye concentration, dye structure and type of microorganism.

2.3.2.2 Enzymatic degradation

Biological systems are able to bring about the degradation of the target chemicals primarily due to their enzymes. Hence enzymes, both intracellular and extracellular, are being explored as biochemical means of wastewater treatment. In general, enzymes are highly specific and extremely efficient catalysts. They can selectively degrade a target pollutant without affecting the other components in the effluent. Therefore, enzymatic treatment is suitable for effluents that contain relatively large amounts of the recalcitrant target pollutants in comparison to others. More importantly, they can operate under mild reaction conditions, especially temperature and pH. In this respect, enzymes outperform the regular catalysts (transition elements like Cu, Ni etc.). From the environmental perspective, enzymes are more acceptable due to their biodegradability. Considering that colorants such as azo dyes can be degraded physico-chemically by oxidation (i.e. AOPs), a majority of the enzymes that are being investigated for their dye degradation potential belong to the enzyme class Oxidoreductases. These enzymes are involved in electron transfer reactions. In the case of reactions wherein the target pollutant is oxidized, the enzyme receives one or more electrons from the substrate and donates these electrons to an electron acceptor. Hence, at the end of the reaction the enzyme is regenerated and is available for the next catalytic cycle. Some of the oxidative enzymes such as the peroxidases require hydrogen peroxide (H_2O_2) or alkyl peroxide (R_2O_2) to act as the electron acceptor. Others such as laccases utilize molecular oxygen for this purpose[40].

2.3.2.3 Fungal decolourisation and degradation of textile dyes

So far the most widely studied dye-decolourising microorganisms are the white-rot fungi. This group of organisms is central to the global carbon cycle as a result of their ability to mineralise the woody plant material lignin, which has a complex polymeric structure. In addition to their natural substrate, white-rot fungi have been found to be capable of mineralising a diverse range of persistent organic pollutants, which distinguishes them from biodegradative bacteria that tend to be rather substrate-specific[74]. The ability of these fungi to degrade such a range of organic compounds results from the relatively non-specific nature of their ligninolytic enzymes, such as lignin peroxidase (LiP), manganese peroxidase (MnP) (EC 1.11.1.13) and laccase. These enzymes and their catalytic properties are reviewed elsewhere[35], but briefly, LiP catalyses the oxidation of non-phenolic aromatic compounds such as veratryl alcohol, whilst MnP oxidises Mn^{2+} to Mn^{3+} which is able to oxidise many phenolic compounds[26]. Laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) is a copper-containing enzyme that catalyses the oxidation of phenolic substrates by coupling it to the reduction of oxygen to water[19]. In 1990, the first report appeared in the literature of sulfonated azo dyes being decolourised, again by *P. Chrysosporium*,[13] and a degradation pathway for this isolate was elucidated[28]. Whilst *P. chrysosporium* remains the most widely studied of white-rot fungi, *Trametes* (*Coriolus*) *versicolor*, *Bjerkandera adusta*, *Pleurotus* and *Phlebia* species, as well as a variety of other isolates are increasingly being studied[12, 36, 45, 66, 82].

2.3.2.4 Bacterial decolourisation and degradation of textile dyes

The bacterial decolourisation and degradation of dyes has been of considerable interest since it can achieve a higher degree of biodegradation and mineralization. It is inexpensive and environmentally-friendly, and produces less sludge[43, 71, 76, 90]. Moreover, bacterial decolourisation is normally faster compared to fungal systems with regard to the decolourisation and mineralization of azo dyes. It has been observed that mixed cultures are particularly useful in this area, as some microbial consortia can collectively carry out biodegradation tasks that no individual pure strain can undertake successfully[67]. However, mixed cultures only provide an average macroscopic view of what is happening in the system, and the results are not easily reproduced, making thorough, effective interpretation of the results quite difficult. Efforts to isolate pure bacterial cultures capable of degrading azo dyes started in the 1970s with reports of *Bacillus subtilis*, *Aeromonas hydrophila* and in *Bacillus cereus* [95]. Recently a substantial amount of research on the subject of colour removal has been carried out using single bacterial cultures like *Proteus mirabilis*, *Pseudomonas luteola* and *Pseudomonas sp.*, and isolated *Pseudomonas sp. SUK1* has shown very promising results for the azo dye degradation under anoxic conditions[8,10,38,98]. In addition, there are also several studies describing the decolourization of reactive azo dyes mediated by pure bacterial cultures. *Pseudomonas aeruginosa* decolourized a commercial tannery and textile dye, Navitan Fast Blue S5R, in the presence of glucose under aerobic conditions. The use of a pure culture system ensures reproducible data, and thus interpretation of experimental observations becomes easier. It is also becoming easier to determine the detailed mechanisms of biodegradation using the tools of biochemistry and molecular biology, and this information could be useful to regulate the enzyme system in order to produce modified strains with enhanced enzyme activities.

2.3.2.5 Laccase mediated dye decolourisation

Laccases (benzenediol:oxygen oxidoreductases, EC1.10.3.2), a family of blue multicopper oxidases, are capable of oxidizing a wide range of aromatic compounds, with concomitant reduction of molecular oxygen to water[83, 85]. They contain four copper atoms: a type I copper (blue copper), a type II copper, and a pair of type III copper centers. Their extensive substrate range makes laccases excellent candidates for various industrial and biotechnological applications, such as biological bleaching in the pulp and paper industry, textile dye decolourization, construction of biosensors for detecting phenolic pollutants, detoxification of recalcitrant environmental pollutants and

bioremediation. Laccases are widely distributed among fungi, higher plants, and bacteria. To date, most laccases studied are of fungal origin, and only fungal laccases are used in industrial processes. However, fungal laccases are usually unstable at high temperatures and alkaline conditions. This characteristic limits their practical applications. Although bacterial laccases have a lower redox potential than fungal laccases and are less frequently investigated, they are more stable to high temperatures and a wider pH range, less dependent on metal ions, and less susceptible to inhibitory agents. Thus, bacterial laccases have significant potential in various industrial applications.

2.3.2.5.1 Structure of active site of laccase

Laccases contain four copper atoms termed T1 (where the reducing substrate binds) and trinuclear copper cluster (T2/T3) (where oxygen binds and gets reduced to water). The four copper ions are classified into three types referred to as Type 1(T1), Type 2(T2) and Type 3(T3). Three types of copper can be distinguished using UV/Visible and Electronic Paramagnetic Resonance (EPR) Spectroscopy. Type 1 Cu at its oxidized resting state is responsible for the blue colour of the protein at an absorbance of approximately 610 nm and is EPR detectable. Type 2 Cu does not confer any colour but is EPR detectable and Type 3 Cu consists of a pair of copper atoms in a binuclear conformation that gives a weak absorbance in the near UV region but no detectable EPR signal[83]. The Type 2 and Type 3 copper sites are close together and form a trinuclear centre that are involved in the catalytic mechanism of the enzyme (Fig.1).

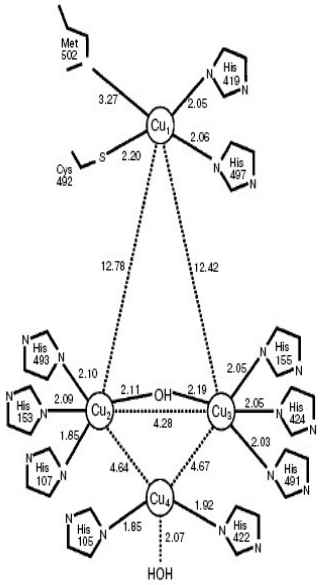

Figure 1
Scheme of T1 and T2/T3 copper sites of CotA laccase from Bacillus subtilis with indicated distances between the most important atoms (Morozoa et al., 2007)

The T2/T3 trinuclear site is the site where the reduction of molecular oxygen takes place by accepting the electrons from T1 site[70].

2.3.2.5.2 Mode of action of laccase enzyme

There are three major steps in the laccase catalysis. The Type 1 Cu is reduced by a reducing substrate, which is then oxidized. The electron is then transferred internally from Type 1 Cu to a trinuclear cluster made up of Type 2 and Type 3 Cu atoms (Fig. 2). The oxygen molecule binds to the trinuclear cluster for asymmetric activation and it is postulated that the oxygen binding pocket appears to restrict the access of oxidizing agents other than oxygen. Hydrogen peroxide is not detected outside of laccase during steady state laccase catalysis indicating that a four electron oxidation of oxygen to water is occurring[25].

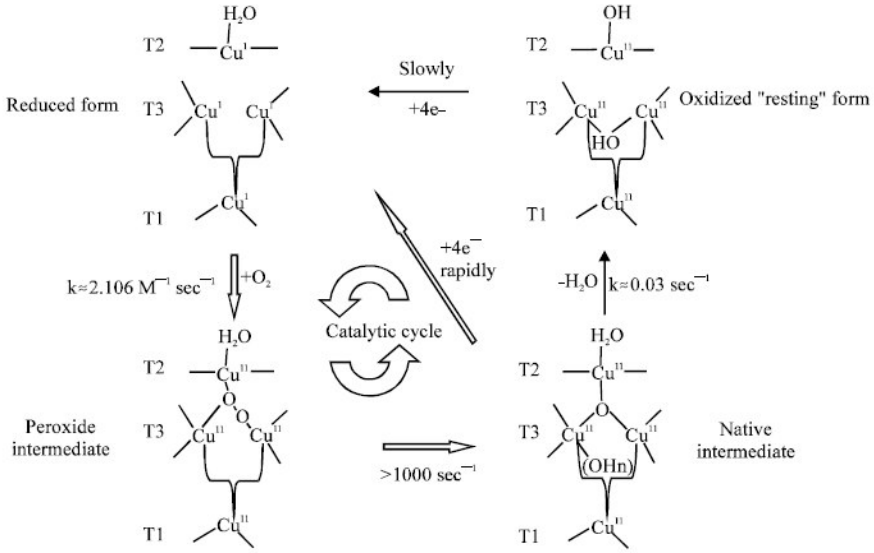

Figure 2
Catalytic cycle of a four copper laccase (Osma, 2009)

As one electron substrate oxidation is coupled to the four electron reduction of oxygen, the reaction mechanism cannot be entirely straightforward.

3. Methods for enhancing the dye degradtion activity of laccase derived from microorganisms

Although numerous laccases, especially microbial laccases have been well characterized, their applications in industrial processes have so far been limited. Since most industrial processes may require harsh conditions, such as high temperatures, extremely acidic/alkaline pH and high ionic strengths, laccases usually lose their activities under these conditions. Therefore, it is necessary to develop robust laccases that show high resistance to these adverse conditions.

3.1 Random mutagenesis

A practical strategy for altering enzyme properties is to introduce random base substitutions into the gene sequence and then select or screen for variants that express the desired phenotype(s). Features that have been enhanced by random mutagenesis include catalytic activity [29], activity in organic solvents[9], thermostability[7, 46, 65], alkaline stability [6] and substrate specificity[57, 29]. The bird's nest fungus *Cyathus bulleri* has been extensively studied[88] for laccase production under shaken and static cultivation conditions and decolourization of polymeric and triphenylmethane dyes. However, low levels of extracellular laccase production by *C. Bulleri* [89] are not sufficient for commercial applications. To enhance laccase production various conventional and modern experimental approaches such as mutagenesis and recombinant DNA technology are being targeted. The effect of Ethidium bromide (EtBr), a chemical mutagen and a model DNA intercalator, on laccase production from *Cyathus bulleri* was studied[15]. The white-rot fungus, *C. bulleri* was initially grown on MEA supplemented with various concentrations of EtBr for 7 days and the ability to produce laccase was tested in MEB, under static cultivation conditions. Treatment with EtBr at 1.5 µg/ml resulted in a variable capable of substantially higher laccase production 18.8 U/ml as compared to the untreated control 4.4 U/ml. The enhanced production of laccase, an oxidoreductase enzyme, by EtBr-treated cultures of *C. bulleri* could possibly be due to the respiratory stress induced in the cells[15]. It has been reported[44] that both the mutated and wild-type genomes are present in a heteroplasmic form in a eukaryotic cell. This phenomenon could have played a significant role in maintaining the 2.5-fold enhanced laccase production by EtBr-treated culture of *C. bulleri* when grown in the absence of EtBr for 3 months The efficiency of laccase production by the wild fungal strains *Pycnoporus cinnabarinus* was investigated[73] by the treatment with physical mutagen (Ultraviolet (UV) and X-rays) and chemical mutagens (Ethidium bromide, Colchicine and Hydrogen peroxide). The improved strain of *Pycnoporus cinnabarinus* showed 15% increase in yield.

3.2 Site Directed Mutagenesis

Two different site-directed mutagenesis studies have been carried out using the endospore-coat laccase *CotA* from *Bacillus subtilis*[16]. The main goal was to evaluate how the redox potential and catalytic efficiency of this enzyme are influenced by modifying residues involved in the coordination and stabilization of the T1 Cu site. Initially, the weakly coordinating Met of the T1 Cu was substituted with non-coordinating Leu and Phe residues[16]. The geometry of the T1 copper centre in these two variants (M502L and M502F) was similar to that of the wild-type, yet both the redox potential and the catalytic activity were significantly altered. The $E^0_{,T1}$ of the two mutants increased by around 100 mV with respect to that of the native laccase but the *k*cat was negatively affected. The M502L mutant displayed a 2 to 4-fold decrease in the *k*cat for phenolic and non-phenolic substrates while in the M502F variant the effect was even more pronounced: the *k*cat values were 10 to 1,840-fold lower to those of the wild-type enzyme. However, a direct correlation between the enhancement in the redox potentials and the decrease in activity was not observed. Subsequently, two hydrophobic residues in the vicinity of the T1 Cu (Leu386 and Ile494) were mutated to Ala, generating

two mutants with strongly altered spectral properties of the copper centres compared to the wild-type[17]. Additionally, these mutants showed a decrease in the E^0_{T1} (approx. 60 and 100 mV for L386A and I494A mutants, respectively). According to the crystal structures of these two site-directed mutants, the replacement of hydrophobic residues by Ala in the neighbourhood of the T1 Cu increased the solvent accessibility affecting the E0' T1. The late 1990's saw the first attempts to engineer fungal laccases by rational approaches when several residues surrounding the catalytic copper centres of laccases from *Myceliopthora thermophila* and *Rhizoctonia solani* were subjected to site directed mutagenesis to determine the parameters responsible for the catalytic activity and redox potential[61, 96, 97] . First, four different mutants of the medium-redox potential laccases from *R. solani* and *M. thermophila* were designed, carrying either a single or a triple mutation in a highly conserved pentapeptide which corresponds to the sequence 512HLHMGM517 of the *Zucchini* ascorbate oxidase (zAO)[96]. More specifically, the L470F and L466V/E467S/A468G variants from *R. solani* laccase and the L513F and V509L/S510E/G511A mutants of *M. Thermophila* were evaluated. The single mutations neither significantly altered the spectro-electrochemical properties (redox potential, cooper geometry), nor the biochemical ones (kinetic parameters, pH activity profiles, fluoride inhibition). On contrast, the triple mutant showed a different behaviour: shifted pH activity profiles, lower *k*cat values for both syringaldazine and ABTS, and higher tolerance against inhibition by fluoride ions at low pH values.

4. CONCLUSION

Synthetic dyes released into the environment cause considerable water and soil pollution because they may be toxic, carcinogenic, mutagenic, and clastogenic to living organisms. Over the last two decades, awareness and concern about the environmental and health hazards of synthetic dyes is increasing in the global community. Consequently, environmental and government legislations are becoming more and more tight regarding the removal of these pollutants from industrial wastewaters. 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, bio friendly and environmentally benign method for removal of dyes from industrial wastewaters. Bioremediation is the application of microorganisms (fungi, bacteria, actinomycetes, yeasts, and algae) for the removal of xenobiotics (synthetic organic compounds, which are not found in nature and are thus foreign and new to the biota) from polluted environments. Microorganisms are our microscopic allies, which can help us clean the contaminated soils and waters. They are tiny biological reactors, which can convert harmful synthetic chemicals (organic pollutants) into simple, less toxic, or completely benign products. Microorganisms can even mineralize organic pollutants, that is, to completely degrade them and convert into water, carbon dioxide, and salts. The biodegradation of synthetic dyes is affected by many factors like pH, temperature, dye concentration, nitrogen content in culture medium, presence of salts, agitation, aeration, etc. Therefore, these factors are to be taken into account while evaluating the biodegradation abilities of different microorganisms. Microorganisms capable of using the dye molecules as a sole source of carbon, nitrogen, and energy are of special interest and significance because they consume the dye for their growth and activities while at the same time eliminate the pollutant in a real sense. Such microorganisms are a valuable gift from nature. Their biodegradative potentials can be exploited to deal with the problem of synthetic dyes pollution and explore new horizons for further research. Over the past two decades, much attention has been focused on the biodegradation of synthetic dyes using different groups of microbes. The science of bioremediation is emerging as a unique tool to deal with the removal of synthetic dyes and other xenobiotics from the environment. Molecular biology techniques may also be used to improve the strains so that rapid mineralization of aromatic amines can be achieved. Their use, however, requires caution. It may also be necessary to combine AOP with biological processes to achieve the required degree of treatment of dye-containing wastewaters so that regulatory standards can be met.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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