DECOLOURISATION OF TEXTILE EFFLUENT BY LACCASE- A REVIEW

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Abstract: *Textiles and allied industries play pivotal role in economy of any country but they are associated with number of harsh chemicals and auxiliaries. Enzymes have great capacity to replace harsh chemicals and are well known for their eco-friendly applications in textile industries. The extensively used enzymes in textiles are amylase in desizing; pectinase and cellulase in bio scouring; catalase in removal of hydrogen peroxide from spent bleaching bath; cellulase in biopolishing, carbonisation of wool and fading of denim; laccase in decolourisation of effluent and denim bleaching; protease in softening of wool; sericinase in degumming of silk; etc.*
Even a small amount of dyes particles (1mg/lt) in effluent may cause irregular colouration of surface water as well as pollute the water with toxic compounds. The presence of dyes particles changes the absorption and reflection of sunlight into the water and hinders photosynthesis reactions of aquatic plants and algae. The oxidoreductase enzyme "laccase" has great potential in decolourisation of textile effluent. The present paper offers an over view of the laccase applications in textile industries up to date.

Keywords*: Enzymes, Laccase, mediator, effluent, decolourisation of dyes molecules*

1. Introduction

The textile wet processing operations comprises preparatory wet processing, dyeing, printing and finishing. In each step different types of chemicals are applied on textile material by help of water. The final effluent of wet processing industries consisting hundreds of chemicals such as acids, alkalis, reducing agents, oxidising agents, dyeing and printing auxiliearies, finishing agents, etc. The dyes classes which are being used in textile industries are acid, basic, direct, disperse, mordant, reactive, sulphur, azoic, and vat dyes. These dyes are designed to resist exposure to sweat, light, water, oxidizing agents and microbial attack to achieve better colour fastness properties. Therefore, decolourisation of dye residue in effluent becomes difficult task [1].

The final effluent which consist many unfixed dyes and toxic chemicals has light absorbance in the wavelength range of 350 to 500 nm exhibit high intensity of colour [2]. The rivers depend on their colour and on the clarity of water. Even minor releases of coloured effluents (1mg/L) may cause irregular colouration of surface waters and pollute the water with toxic compounds. Photosynthesis reactions of aquatic plants and algae are tremendously affected due to the change in absorption and reflection of sunlight of the water.
The decolourisation of effluent is carried out in generally by expensive physical and chemical methods, which are also, produce toxic by products. The majority of physical, chemical and biological colour removal techniques work either by concentrating the colour into sludge, solid supports, or by the complete destruction of the dye molecule. It is expected that decolouration systems involving destruction technologies will prevail, as the transfer of pollution from one part of the environment to another is prevented. Such methods are often very costly and accumulation of concentrated sludge creates a disposal problem. Biological and/or mixed treatment systems that can effectively remove dyes from large volumes of wastewater at a low cost are a preferable alternative. Biological techniques include biosorption and biodegradation in aerobic, anaerobic, anoxic or combined anaerobic/aerobic treatment processes with bacteria, fungi, plants, yeasts, algae and enzymes.

A number of biotechnological approaches have been suggested with potential interest in combating this pollution source in an eco-efficient manner [3]. It is known that lignolitic enzymes (e.g. Mn peroxidise, Lignin peroxidise and laccase) could be used to decolorize textile [4]. Laccases which are highly regarded to be environmentally friendly are considered to be an attractive technology for development of new methodologies of dye degradation from textile industries. The Laccase can be used alone or with mediator to oxidise the aromatic compounds. Laccases play an important role in food industry, paper and pulp industry, textile industry, synthetic chemistry, cosmetics, soil bioremediation and biodegradation of environmental phenolic pollutant. The following contents of the review paper will explore about the properties of enzyme, "Laccase", its catalytic mechanism and various applications one by one.

2. Enzymes

The enzymes are high molecular weight proteins capable of catalysing the chemical reactions of biological processes and hence are known as “Bio-catalyst”. An enzyme is a protein complex which is composed of about 200 to 250 amino acids. They are different from chemical catalysts in that they are thermolabile (temperature sensitive), have relatively low energy of activation, active over a narrow range of pH , biodegradable and very specific to substrate as work on lock and key principal [5]. The extensively used enzymes in textiles are amylase in desizing; pectinase, lipase and cellulase in bio scouring; catalase in removal of hydrogen peroxide from spent bleaching bath; cellulase in bio polishing and fading of denim; laccase in decolourisation of effluent and denim bleaching; protease in softening of wool; sericinase in degumming of silk; etc.

3. Laccase

In the recent years, the use of enzymes in the diverse fields of industrial application has been increased. Many of such potential enzymes are widely distributed in the nature; laccases are one among them.

In contrast to the generally high specificity of enzymes, laccases are rather unspecific. Laccase is one of the few enzymes that have been studied since the end of the last century. Laccase was first discovered by Yoshida in plant [6]. Laccase is a cuproprotein belonging to a small group of enzymes denominated blue oxidases. Laccase (E.C. 1.10.3.2) is an oxidoreductase able to catalyse the oxidation of various aromatic compounds (particularly phenols) with the concomitant reduction of oxygen to water. In general, laccases exhibit four copper atoms, which play an important role in the enzyme catalytic mechanisms.

Laccases not only catalyze the removal of a hydrogen atom from the hydroxyl group of methoxy-substituted monophenols, *ortho*- and *para*-diphenols, but also can oxidize other substrates such as aromatic amines and non-phenolic compounds to form free radicals. Beside proteineous These enzymes contain 15–30% carbohydrate and have a molecule mass of 60–90 kDa with acidic isoelectric point around pH 4.0. This feature may contribute to the high stability of the enzyme.

3.1 Sources of Laccase

Laccases are widely distributed in higher plants, fungi and in a few bacteria. In higher plants, laccases are involved in lignification of xylem tissues, however, various functions have been reported of laccases such as pigment biosynthesis and maturation.

3.2 Mediator for Laccase

The use of laccase has been expanded by the introduction of laccase-mediator systems. Due to the random polymer nature of lignin and to the laccase lower redox potential, with respect to other ligninolytic enzymes, laccase can oxidize only phenolic fragments of lignin. Small natural low-molecular weight compounds with high redox potential (>900 mV) called mediators may be used to oxidize the non-phenolic residues from the oxygen delignification [7].

Simply, a mediator is a small molecule that acts as a sort of ‘electron shuttle’: once it is oxidized by the enzyme, generate a strong oxidizing intermediate, the co-mediator (Med), it diffuses away from the enzymatic pocket and in turn oxidizes any substrate that, due to its size, could not directly enter the enzymatic pocket (Figure 1) (Banci *et al.* 1999) [8].

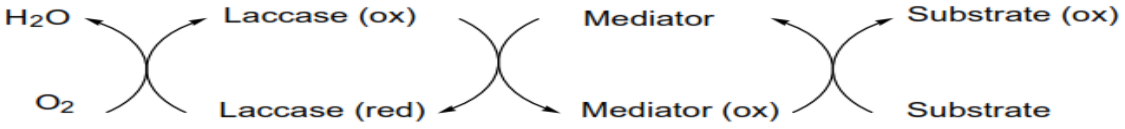

Figure 1: Catalytic cycle of a laccase- Mediator oxidation system

More than 100 possible mediator compounds have been reported but the most commonly used are the azine 2,2´-azino-bis-(3-ethylbenzothiazoline-6sulfonic acid) (ABTS) and the triazole 1-hydroxybenzotriazole (HBT) (Figure 2). Specific mediators usually denominated by mediated systems, are being searched by

investigators to extend the number of dyes decolorized. Considerable resources were exploited in the quest for a cheap, non-toxic mediator that could be used on an industrial scale [9].

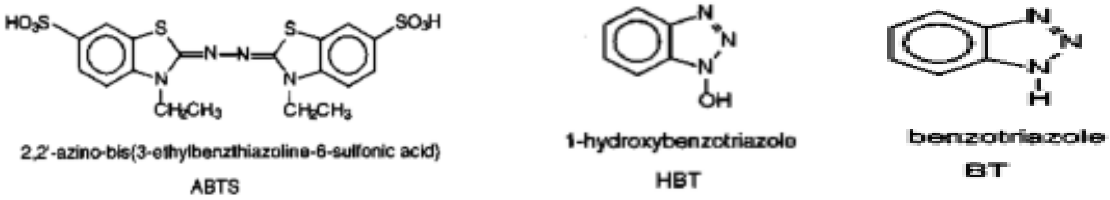

Figure 2: Chemical structure of few mediators

3.3 The reactions mechanism of laccase

Laccase is a polyphenol oxidase (p-diphenol oxidase, EC 1.10.3.2). The reduction of oxygen to water is accompanied by the oxidation, typically, of a phenolic substrate. laccases are remarkably non-specific as to their reducing substrate and the range of substrates oxidized varies from one laccase to another.
The enzyme contains four coppers (Cu) with one near the active copper (T1) and a buried cluster of three coppers with one type (T2) and two type (T3) coppers. The T1 copper extracts electrons from the reducing substrate and transfers it to the tri-nuclear T2/T3 copper cluster where molecular oxygen is reduced to water at T2 copper and T3 coppers act as electron reservoirs.

3.4 Applications of Laccase

Although oxidation reactions are essential in several industries, most of the conventional oxidation technologies have the following drawbacks: non-specific or undesirable side reactions and use of environmentally hazardous chemicals. Therefore, laccase oxidation techniques have potential within a great variety of industrial fields including the pulp and paper, textile and food industries.
Concerning their use in the biotechnology area, Laccases play an important role in food industry, paper and pulp industry, textile industry, synthetic chemistry, cosmetics, soil bioremediation and biodegradation of environmental phenolic pollutant.

3.4.1 Removal of Lignin and pulp bleaching [10]

The separation and degradation of lignin in wood pulp are carried out by ClO_2 and O in the industrial preparation of paper. The oxygen delignification process has been industrially introduced in the last years to replace conventional and polluting chlorine-based methods. These chemical methods also depolymerise cellulose polymers. But the pre-treatments of wood pulp with laccase provide milder and cleaner delignification process without loss in cellulose polymer structure. Lignocellulose is a common substrate for laccase and the laccase ability to break down nonphenolic ligno-cellulose is provided by certain phenolic compounds acting as mediators.

3.4.2 Bio bleaching [11]

The purpose of cotton bleaching is to decolourise natural pigments so a pure white appearance to the fibres can be obtained. Mainly flavonoids are responsible for the colour of cotton. The most common industrial bleaching agent is hydrogen peroxide and chlorine based chemicals, which are usually applied at alkaline pH and temperatures close to boiling. These bleaching agents also react with the fibre and lead to decrease in the degree of polymerisation of cellulose and thus weaken the textile material. Therefore, replacement of hydrogen peroxide or chlorine based bleaching agents by an enzymatic bleaching system would not only lead to better product quality due to less fibre damage but also to substantial savings on washing water needed for the removal of hydrogen peroxide.

Tzanov et. al. reported for the first time the enhancement of the bleaching effect achieved on cotton fabrics using laccases in low concentrations.

3.4.3 Application in food industry [12]

Different chemical compounds such as ethanol, organic acids (aroma), salts and phenolic compounds (colour and taste), etc are present in wines and food beverages. Selective polyphenol removal from wine is

essential to avoid an undesirable alteration in the wine. The Polyphenolic compounds can be eliminated selectively by application of laccase. The flavour quality of vegetable oils or other food items can be also improved with laccase by eliminating dissolved oxygen in them. Laccase can also deoxygenate food items derived partly or entirely from extracts of plant materials.

3.4.4 In Synthesis of organic chemicals and dyes [13-16]

Laccase can catalyse cross-linking reactions which can be used in coating of polymeric films and surface modification of the fabrics. The ability of laccases to generate colour “in situ” from originally non-coloured low-molecular substances makes their use an alternative to the conventional dyeing processes. The enzymatic modification and dyeing processes can be applied in several natural substrates like cotton, sisal, wool, flax and wood.

3.4.5 Biosensors and medical applications [17-19]

A biosensor is an integrated biological-component probe with an electronic transducer, thereby converting a biochemical signal into a quantifiable electrical response that detects, transmits and records information regarding a physiological or biochemical change. A number of biosensors containing laccase have been developed for immunoassays, glucose determination, aromatic amines and phenolic compound determinations. Laccase can be also used in the synthesis of complex medical compounds as anesthetics, anti-inflammatory, sedatives, etc.

3.4.6 Bio bleaching and Fading of Denim in Textile finishing [20]

Laccase is used in commercial textile applications to improve the whiteness in conventional bleaching of cotton and recently fading of denim. Cellulases were used to partially replace the load of pumice stones and laccases could bleach indigo dyed denim fabrics to lighter shades.
In anti-shrinkage treatment of wool, the laccase can also replace toxic chlorination treatment in eco-friendly way. Few researchers also reported efficient discharge printing with laccase enzymes.

3.4.7 Dye Decolourisation or dye degradation

The textile effluents are extremely variable in composition as they contain dyes, chemical auxiliaries, finishes and reaction by products. The presence of so many ingredients can inhibit the enzyme activity and enzymatic decolourisation process. Therefore, decolourisation of textile effluents requires suitable type of enzyme as well as of reactor environment [21,22]. The capability of laccases to act on chromophore compounds such as azo, triarylmethane, anthraquinonic and indigoid dyes leads to the suggestion that they can be applied in industrial decolorization processes [23-25].

3.4.8 Removal of toxic chemical from effluent [26, 27]

Most of phenolic compounds, especially phenolic organohalogens, are poorly degradable, and could impose toxic, carcinogenic, mutagenic, and teratogenic effects upon animals and humans even at low concentrations.

Recent studies propose several degradation mechanisms for phenolic and non-phenolic azo dyes. In few model azo dyes are degraded without direct cleavage of the azo bond through a highly non-specific free radical mechanism forming phenolic type compounds, thereby avoiding the formation of toxic aromatic amines, which might be useful to control environmental pollution. However, some substrate specificity can be found in laccase reactions, which limits the number of azo dyes that can be degraded. To solve this problem laccase / mediator systems are normally used to broaden the range of azo dyes and to increase the decolorization rates. Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. However, the capacity to evaluate the laccase degradation potentials remains incomplete since there is not a complete knowledge on dye decolorization pathways, dye mineralization mechanisms and formation of potentially toxic accumulating intermediates.

Laccases have also shown to be useful for the removal of toxic compounds through oxidative enzymatic coupling of the contaminants, leading to insoluble complex structures. Immobilized laccase was found to be useful to remove phenolic and chlorinated phenolic pollutants. Laccases from white rot fungi have been also used to oxidize alkenes, carbazole, N-ethylcarbazole, fluorene, and dibenzothiophene in the presence of HBT and ABTS as mediators.

4. Conclusion

Pollution free processes are gaining ground all over the world. In this scenario, enzymes emerging as the best alternative to the polluting textle processing methods. Enzymes are not only beneficial from ecological point of view but they are also saving money by reducing water and energy consumption. The laccase has a great potential application in paper, food, medical, textile and allied industry. It can be foreseen that the number of laccase-based industrial oxidation processes will increase significantly and at the same time, there will be an increasing interest in their synthetic exploitation. Laccases are promising enzymes to replace the conventional chemical processes of the textile and allied industry. The physical and chemical process of dye decolourisation also produces toxic by products. Thereore, the laccase appeared to be the most versatile biocatalyst for the decolorization of the dyes.

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