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Research Article

Azo dye degradation by fungi – Review

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Abstract

Fungal based treatment system for the cleaning of dye industry effluent and for bioremediation of dye contaminated soil using fungal based primary and secondary metabolites. The extraction and purification and development of commercial products from efficient stains of these isolates are under progress.

Keywords: Fungi, Azo dye , Biodegrading of azo dyes.

Introduction

Ever since the beginning of mankind, people have been using colorants for painting and dyeing their surroundings, their skins and their clothes. The first evidence of the use of colorant materials by man goes as far as 15000-9000 BC, in the walls of the Altamira cave in Spain. The drawings were performed with inorganic pigments like soot, manganese oxide, hematite and ochre. Historically there is a dye, derived from animal sources (molluscs), that is very important, although presently has no relevance and it's not commercially available. It is Tyrian Purple (see Text Figure 1) and the pigment itself is not in the mollusc; however, when the precursor is extracted it can be converted to the dye by air or light. The presence of this dye goes as far as 1400 BC in the Late Bronze Age as found recently in Lebanon. It has always been rare and costly being used by Roman emperors and high ranking ecclesiastics (Clark *et al.* 1993).

Another ancient dye that is still in use, although not from natural origin nowadays, is indigo. It was extracted from *Indigofera tinctoria* by fermentation and had a characteristic blue colour. It was used as a pigment by the Romans because it had to be chemically reduced to become water soluble. It was

firstly synthetically produced by Adolf von Baeyer in 1880, and actually is used to dye denim (Clark *et al.* 1993).

Up to the end of the nineteenth century natural dyes, obtained mainly from plants (roots, stems, leaves, flowers, fruits, seeds and lichens – Ingamells 1993), were the main colorants available for textile dyeing procedures. The main disadvantages of the use of natural dyes are the need for several steps in the dyeing process, the diversity of sources and related application procedures, the rapid change in trends and the demand for good fastness properties on different substrates that would require a complete database describing possible applications (Bechtold *et al.* 2003).

The pioneering synthesis of mauveine by W. H. Perkins started the era of synthetic dyes, with chemical and physical properties better suited to contemporary demands, better level of quality and more reproducible techniques of application. It also allowed the development and extension of the use of particular products. For example, the development of synthetic fibres such as polyester and cellulose triacetate would

have been severely hindered without the design and synthesis of dyes with appropriate properties (Ingamells 1993).

Since then thousands of dyes have been synthesised, and dye manufacture has become a significant part of the chemical industry. Nowadadys, when care of the environment is a major issue, it is tempting to assume that the use of natural colours is an environmental friendly alternative to present-day practice. There are several groups studying the use of natural dyes in modern dyeing industry (Tsatsaroni and Liakopoulos-Kyriakides 1995, Angelini *et al.* 1997, Ishigami and Suzuki 1997, Angelini *et al.* 2003, Bermejo *et al.* 2003, Kim *et al.* 2004b, Paul *et al.* 2004, Kamel *et al.* 2005, Singh *et al.* 2005). Some of the advantages of the use of this type of compounds are the absence of toxicity upon humans, the use of sustainable sources and the fit into the natural pathways of biodegradation of the released dyebaths.

Structures and uses of dyes

Dyes are compounds that absorb light with wavelengths in the visible range, i.e., 400 to 700 nm (de las Marias 1976, van der Zee 2002)**.** The major structure element responsible for light absorption in dye molecules is the chromophore group, i.e. a delocalised electron system with conjugated double bonds. The absorption of UV/Vis raiation by an organic molecule is associated with electronic transitions between molecular orbitals. The energy of the absorbed radiation is given by:

$$E = E_1 - E_0 = h\nu = hc/\lambda$$

where E_0 is the energy corresponding to the fundamental state of the molecule (J), E_1 is the excited state energy (J), h is the Planck's constant ($6,626*10^{-34}$ Js), ν is the electromagnetic adiation frequency (Hz), c is the light velocity ($3*10^8$ m.s^{-1}) and λ is the wavelength (nm). The more extended the electronic delocalisation, the lower is the transition energy and the higher is the wavelength. To allow delocalization of the electrons double bonds must alternate with single bonds. In the case of synthetic dyes, delocalization is also promoted by benzene or naphthalene rings (Rocha Gomes 2001). Chromophores frequently contain heteroatoms as N, O and S, with non-bonding electrons. By incorporating these electrons into the delocalised system in the aryl rings, the energy of the electron cloud is modified, the

wavelength of the absorbed radiation will shift towards the visible range, and the compound will be coloured. In many cases dyes contain additional groups called auxochromes, which are electron withdrawing or electron donating substituents that cause or intensify the colour of the chromophore by altering the overall energy of the electron system. The most important auxochrome groups are: hydroxyl and derivates, -OH, -OR; amino and derivates, -NH$_2$, -NHR, -NHR$_2$; sulphonic, -SO$_3$H; carboxylic, -COOH; and sulphide, -SR (de las Marias 1976, van der Zee 2002). Some auxochromes also increase the dye affinity for the fibre (natural or synthetic). Natural fibres are based on cellulose (polymeric linear chains of glucose) – cotton and linen - or proteins – wool and silk. Synthetic fibres are for instance viscose, cellulose acetate, polyamide, polyester and acrylic (Guaratini and Zanoni 2000). Common classes of dyes, based on the chromophore present, are shown in Table 1.

According to the Colour Index dyes can be classified on the basis of colour and application method. Various attractive forces have the potential of binding dyes to fibres, and often more than one type of chemical bonding can operate with the same dye-fibre combination. The dominant force depends on the chemical character of the fibre and the chemical groups in the dye molecule. The types of bonds established between the dye and the fibre, by increasing relative strength of the bond, can be: Van der Waals, hydrogen, ionic or covalent (Ingamels 1993, Guaratini and Zanoni 2000, Rocha Gomes 2001). According to the application categories dyes can be classified as seen in Text Table 2.

Dyes are used in textile industry, leather tanning industry, paper production, food technology, agricultural research, light-harvesting arrays, photoelectrochemical cells, hair colouring and cosmetics. Moreover these compounds have been employed for the control of the efficacy of sewage and wastewater treatment, for the determination of specific surface area of activated sludge and for ground water tracing (Forgacs *et al.* 2004). Due to the large amounts used, the most significant industrial use is in textile dyeing.

Azo dyes are the most widely use among synthetic dyes, representing almost 70% of the textile dyestuffs produced (Knackmuss 1996). They are easy to synthesize, have low cost, are stable, can be used to colour several materials (textile, leather, plastic, food) and allow a great variety of colours and shades. They

have in their molecule one or more azo groups. They are obtained from the coupling of diazonium salts with aromatic amines, phenols, naphthols or aliphatic enols. Coupling usually takes place in the *para* position in respect to the amino or hydroxyl group or in the *orto* position if the latter is occupied. The diazonium salts are obtained from the reaction of sodium nitrite with an amine solution with a mineral acid, preferably HCl (de las Marias 1976, Zollinger *et al.* 1991) – Text Figure 3. The structural class of azo dyes includes dyes from different application classes, namely, acid, basic, metal complex, reactive and mordant (de las Marias 1976).

Biological treatments

The fate of environment pollutants is largely determined by abiotic processes, such as photooxidation, and by the metabolic activities of microorganisms. Since catabolic enzymes are more or less specific, they can act on more than their natural substrate. This explains why the majority of xenobiotics are subject to fortuitous metabolism (cometabolism) (Knackmuss 1996) and several groups explore these microbial capacities for the bioremediation of dyes.

The limitations of biological processes are mainly caused by limited biodegradability of primarily xenobiotic compounds like dyes, by toxic or inhibitory effects of pollutants for the microbial population and by the slow rate of biodegradation of particular pollutants (Jeworski and Heinzle 2000).

Enzymes

In the studies of biological degradation of dyes an effort as been made in order to identify, isolate and test the enzymes responsible for the decolourisation. In the case of extracellular fungal enzymes, like manganese and lignin peroxidases and laccases (Text Figure 6), or cytosolic azoreductases from bacteria, this has been achieved by several groups (Dass and Reddy 1990, Gosh *et al.* 1992, Spadaro and Renganathan 1994, Heinfling *et al.* 1998a, Rafii and Coleman 1999, Schliephake *et al.* 2000, Campos *et al.* 2001, Suzuki *et al.* 2001, Nyanhongo *et al.* 2002, Blümel and Stolz 2003, Ryan *et al.* 2003, Maier *et al.* 2004).

The application of enzyme preparations shows considerable benefits over the use of microorganisms.

Commercial preparations can be easily standardized, facilitating accurate dosage. The application is simple and can be rapidly modified according to the character of the dye or dyes to be removed (Forgacs *et al.* 2004). Nevertheless the use of whole cells rather than isolated enzymes is advantageous, because costs of purification are extremely high and the cell offers protection from the harsh process environment to the enzymes. Also, degrading is often carried out by a number of enzymes working sequentially and not by one single enzyme (Pearce *et al.* 2003).

Fungi

The most widely studied dye-decolourising microorganisms are the white-rot fungi like *Phanerochaete chrysosporium* (Text Figure 7) (Glenn and Gold 1983, Bumpus and Brock 1988, Pasti-Grigsby *et al.* 1992, Chao and Lee 1994, Conneely *et al.* 1999, Chagas and Durrant 2001, Kunz *et al.* 2001, Martins *et al.* 2002, Mielgo *et al.* 2002), *Trametes versicolor* (Field *et al.* 1992, Borchert and Libra 2001, Tekere *et al.* 2001), *Coriolus versicolor* (Kapdan *et al.* 2000, Kapdan and Kargi 2002) and *Bjerkandera adusta* (Field *et al.* 1992, Heinfling-Weidtmann *et al.* 2001).

Several other non-white-rot fungi can also successfully decolorize dyes like *Aspergillus niger* (Abd El-Rahim and Moawad 2003), *Geotrichum candidum* (Kim *et al.* 1995, Kim and Shoda 1999), *Pleurotus ostreatus* (Martins *et al.* 2003, Palmieri *et al.* 2005) and *Cunninghamella elegans* (Cha *et al.* 2001, Ambrósio and Campos-Takaki 2004) among others (Fu and Viraraghavan 2001). White-rot fungi constitute a diverse ecophysiological group comprising mostly basidiomycetous fungi capable of aerobic lignin depolymerization and mineralization, playing a central role in the global C-cycle (McMullan *et al.* 2001, Wesenberg *et al.* 2003). This ability is correlated to the capacity of these organisms to synthesise lignin-degrading extracellular enzymes such as lignin peroxidases (LiP) and manganese peroxidases (MnP), or laccases (Lac) (Robinson *et al.* 2001, Stolz 2001, Forgacs et al. 2004) which, thanks to their lack of substrate specificity, are also capable of degrading a wide range of xenobiotics (Reddy 1995, McMullan *et al.* 2001, van der Zee 2002, Wesenberg *et al.* 2003, Novotný *et al.* 2004).

Among these are dioxins, polychlorinated biphenyls (PCBs), chlorophenols, polycyclic aromatic hydrocarbons (PAHs) and nitroaromatics, including dyes (Reddy 1995, Robinson *et al.* 2001, Wesenberg *et al.* 2003, Forgacs *et al.* 2004). LiP catalyses the oxidation of non-phenolic aromatic compounds such as veratryl alcohol. MnP oxidizes preferably Mn^{2+} to Mn^{3+} which is able to oxidise many phenolic compounds. Laccase is a copper-containing enzyme that catalyses the oxidation of phenolic substrates by coupling it to the reduction of oxygen to water (McMullan *et al.* 2001, Wesenberg *et al.* 2003). Whilst it is clear that these enzymes play a significant role in dye metabolism, care must be taken not to exclude the possibility of the existence of other degradative mechanism (McMullan *et al.* 2001). Recently a third group of peroxidases, versatile peroxidase (VP), has been recognized in species of *Pleurotus* and *Bjerkandera*, (Heinfling *et al.* 1998 a, Heinfling *et al.* 1998 b).

A number of other enzymes are produced in parallel including H_2O_2-producing enzymes required by other peroxidases (glyoxal oxidase and superoxide dismutase), and enzymes linked to lignocellulose degradation pathways (glucose oxidase and aryl alcohol oxidase) (Wesenberg *et al.* 2003, Novotný *et al.* 2004). Although these fungi have been shown to decolourise dyes in liquid fermentations, enzyme production has also shown to be unreliable mainly due the unfamiliar water environment (Robinson *et al.* 2001). Their performance is also closely related to the operation conditions (concentration of dye, pH and temperature), which is a serious drawback for this type of wastewaters (Fu and Viraraghavan 2001). Nevertheless they have the potential to oxidise substrates that have low solubility which is an advantage for the treatment of non-soluble dyes (like vat for instance). Another advantage of these systems is that the constitutive nature of the enzymes obviates the need for the adaptation (Reddy 1995). In process design and optimization of fungal treatment there are some features that should be considered. As the decolourization of dyes by *P. chrysosporium* and other white-rot fungi occurs in secondary metabolic conditions, the important enzyme system is released by the fungal cells under either carbon or nitrogen limitation (Glenn and Gold 1983, Spadaro *et al.* 1992, Wesenberg *et al.* 2003). Production of LiP and MnP is generally optimal at high oxygen tension but is

repressed by agitation in submerged liquid culture, while laccase production is often enhanced by agitation. Usually more than one isoform of the enzyme system is expressed by different taxa and culture conditions (Wesenberg *et al.* 2003). Due to the complexity of these systems, both dye structures and enzymatic transformations involved, there is a gap in the knowledge of the degradation and mineralization of dyes by these microorganisms.

Yeasts

In literature the ability to degrade azo dyes by yeasts was only described in a few reports. The first two reports use the ascomycete yeast *Candida zeylanoides* isolated from contaminated soil to reduce model azo dyes (Martins *et al.* 1999, Ramalho *et al.* 2002). The characterisation of an enzymatic activity is described in further studies with the yeast *Issatchenkia occidentalis* (Ramalho *et al.* 2004), and the enzymatic system involved is presented in a work with *Saccharomyces cerevisiae* (Ramalho *et al.* 2005).

Synthetic textile dyes are one of the most serious pollutants that contaminate steadily higher amounts of wastewater as industrial effluents (Eichlerova *et al*. 2006). The dyes are highly recalcitrant owing to their chemical structure. They are often toxic, or transform into a toxic product when released into the environment (Kandelbauer *et al.* 2004). One of the major problems with textile effluents is that they have a toxic effect on the germination rates and biomass of many plant species that play important ecological functions. Conventional wastewater treatments are not efficient to remove recalcitrant dyestuffs from effluents. The physical and chemical methods for removing dyes are not suitable, owing to their high cost and low efficiency. Thus, biotechnological approaches have been attracting interest for the textile industry (Eichlerova *et al*. 2006). Yeasts have attractive features for dye decolorization, as they grow faster than most filamentous fungi and have the ability to resist unfavorable environments, when compared with bacteria and filamentous fungi. To date, the use of yeast strains in dye wastewater treatment has been limited (Pajot *et al*. 2007).

The decolorization of Direct Black 22 by *Aspergillus ficuum* has been studied. It was found that *Aspergillus ficuum* could effectively decolorize Direct Black 22

especially when grown as pelleted mycelia. Results showed that the media containing Direct Black 22 at 50 mg/L could be decolorized by 98.05% of the initial color in 24 h. The optimum pH and temperature of decolorization are 4.0 and 33 °C respectively. Aeration was quite beneficial to decolorization. Medium composition and the concentration of Direct Black 22 could affect the rate of decolorization. The dye degraded products assayed by UV-visible spectrophotometer and macroscopic observation showed that the decolorization of Direct Black 22 by mycelial pellets includes two important processes: bioadsorption and biodegradation.The degradation experiment agree with the Michaelis-Menten kinetics equation (Xin-jiao Dong *et al*. 2001).

Basidiomycetous group belonging to the order *Aphylopholares* and the family *Polyporaceae*, the edible mushroom *Pleurotus* is widely distributed and is cultivated in Japan, China and Europe (Senyah *et al*., 1989). *Pleurotus* is used in biotechnological processes of bioconversion and bioremediation, such as the fungal degradation of chlorinated monoaromatics and BTEX compounds (Buswell, 2001), as well as in the biodegradation of xenobiotic compounds (Morgan *et al.,* 1991), in the purification of air, water and soil, in the cleanup of contaminated soils and in the treatment of industrial effluents (Miles; Chang, 1997,Reid *et al.*, 2002). Studies have also shown the use of fungi in processes such as the biodegradation of Tussah (*Antheraea pernyi*) silk fibroin films by a proteolytic enzyme and the oxidation of domestic (*Bombyx mori*) silk fibroin by mushroom tyrosinase (Monti *et al*., 2005). These processes involve the action of important biodegrading enzymes, e.g., ligninase, hemicellulase, cellulase, xylanase and glucosidase, which are produced by *Pleurotus sajorcaju* (Singh *et al.*, 2003).

Another application is the biodegradation of industrial effluents such as dyes. A large number of dyes are released for consumption, including red 40. Although the risks posed by red 40 are unknown and its use is forbidden in some countries, this dye is widely utilized. The possible risk to humans that ingest food containing dyes can be extended to the environment, for factories discharge their residues into nearby rivers and streams (Bontempo, 1985).

Many dyes and other substances present in textile effluents are not readily degraded during their permanency in traditional aerobic treatment systems (Pagga and Brown, 1986 ; Wesenberg, *et al*. 2003). Although many physicochemical techniques of decolorization have been developed over the last 20 years, few have been implemented by the textile industries due to their high cost, low efficiency and inapplicability to a wide variety of dyes. A definitive solution of the color problem of textile effluents would provide a marked competitive advantage for this industrial sector. Since no single process is able to decolorize all textile effluents, a solution for each situation should be considered, possibly involving a combination of different methods (Banat *et al*. 1996).

The success of a biological process for color removal from a given effluent depends in part on the utilization of microorganisms that effectively decolorize synthetic dyes of different chemical structures. Many bacteria, actinomycetes, east and mitosporic fungi are able to decolorize dyes, with color removal by these microorganisms being mainly attributed to adsorption of the dyes (Zhou and Zimmermann, 1993). Basidiomycetous fungi are able not only to decolorize but also to degrade and mineralize a broad spectrum of different dye structures (azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes), in addition to numerous other toxic organic and recalcitrant compounds. The enzymatic system involved in the degradation of pollutants by these fungi is nonspecific and even acts on mixtures of pollutants (Machado *et al*. 2005; Shah and Nerud, 2002; Wesenberg *et al*. 2003).

Due to rapid industrialization and urbanization, a lot of chemicals including dyes are manufactured and used in day-to-day life. Dyes are synthetic and aromatic molecular structural compounds. According to their dissociation in an aqueous solution, dyes can be classified as acid, direct reactive dyes (anionic), basic dyes (cationic) and disperse dyes (nonionic). They are used on several substrates in food, cosmetics, paper, plastic and textile industries. Solutions retain them by physical adsorption by making compounds with metals and salts using covalent bonds. Many chemical dyes have been used increasingly in textile and dyeing industries because of their ease and cost effectiveness in synthesis, firmness and variety in color compared to that of natural dyes. About 100,000 commercial dyes are manufactured including several varieties of dyes

amounts. Laccases belong to the group of phenoloxidases. These copper containing enzymes are

such as acidic, basic, dyes. Over 10,000 dyes with an annual production of over 7 X 105 metric tons are commercially available (Campos *et al*., 2001). Approximately 50% of the dyes are released in the industrial effluents (Zollinger, 1987). Colored industrial effluents from the dyeing industries represent major environmental problems. Unbound reactive dyes undergo hydrolysis due to temperature and pH values during the dyeing processes. The strong color of discharged dyes even at very small concentrations has a huge impact on the aquatic environment caused by its turbidity and high pollution strength; in addition toxic degradation products can be formed.

Dye wastewater discharged from textile and dyestuff industries have to be treated due to their impact on water bodies and growing public concern over their toxicity and carcinogenicity. Many different and complicated molecular structures of dyes make dye wastewater difficult to be treated by conventional biological and physico-chemical process. Therefore, innovative treatment technologies need to be investigated. Decolorization of dye wastewater by fungal metabolic activities is the subject of many studies.

Fungi from the Basidiomycetes group, known as white rot fungi are a hetereogenous group of microorganisms but have in common the capacity to degrade lignin as well as other wood components (Kirk and Farrell, 1987). The white rot fungi are by far the most efficient liginiolytic microorganisms. They are able to degrade a wide variety of recalcitrant pollutants including various types of dyes. Most information on the biodegradation of synthetic dyes by ligninolytic fungi has been obtained with *Phanerochaete chyrsosporium* (Paszczynski and Crawford, 1995). White rot fungus showed some capacities to remove dyes from industrial effluents. The fungus has been studied for their ability to degrade recalcitrant organopollutants such as polyaromatic hydrocarbons, chlorophenols and polychlorinated biphenyl. The decolorization of phenol red, methylene blue, coomassive blue, dextran blue etc., has been used to indicate ligninolytic activity.

Laccase based decolorization treatments are potentially advantageous to bioremediation technologies since the enzyme is produced in larger

oxidative enzymes detected in many plants and secreted by numerous fungi.

Azo dyes are released in large quantities into the environment from textile industries. These dyes are recalcitrant to microbial degradation, causing problems in the usual biological treatment of the industrial effluents (Swamy and Ramsay, 1999). Despite this, microbial degradation of azo dyes has been reported using different microorganisms (McMullan *et al.* 2001), bacteria (Rajaguru *et al*. 2000), yeasts (Martins *et al*. 1999) and filamentous fungi, such as the white rot fungi (Martins *et al.* 2001; Pointing, 2001). Due to the fungi oxidative mechanisms it is possible to avoid the formation of hazardous anilines, formed by reductive cleavage of the azo dyes, by other microorganisms such as bacteria (Chung and Stevens, 1993).

White rot fungi produce several enzymes that have been related to their ability to degrade natural polymers, such as lignin and cellulose, but can also degrade different synthetic chemicals, usually recalcitrant to biodegradation (Field *et al.* 1993; Knapp *et al*. 1995). One of the well-characterized white rot fungi for industrial use is the basidiomycete *Phanerochaete chrysosporium*. The interest in this fungus is mainly due to the expression of some non-specific extracellular enzymes, as the ligninolytic peroxidases, that have been implicated in the degradation of dyes (Spadaro *et al.* 1992; Ollikka *et al.* 1993). The promising results obtained with this ligninolytic fungus, lead to the study of the potentialities of other species of ligninolytic basidiomycetes.According to this, biodegradation studies using *Trametes* and *Pleurotus* spp. have reported that production of laccase was highly related to lignin and dyes degradation (Platt *et al.* 1984; Thurston, 1994; Abadulla *et al.* 2000). Although Basidiomycetes assume a noticeable importance in possible industrial application, other fungi, such as Deuteromycetes, have also been studied. *Aureobasidium pullulans* is an example of a deuteromycete with the ability to degrade industrial aromatic compounds such as the lignin breakdown products (Schoeman and Dickinson, 1997).

Dyes are commonly used in the textile, food and cosmetic industries and they are released into the environment in industrial effluents such as textile and dyestuff industries (1). Dyes used in this study are: 1) azo dye (Orange II), 2) triphenyl methane dye (Bromphenol Blue), 3) heterocyclic dye (Methylene Blue) and 4) starting material for polymeric dyes (Remazol Brillant Blue R). Various heterocyclic, azo and triphenyl methane dyes used widely by, e.g., the textile and dyestuff industry, are often resistant to biological wastewater treatment, and thus they are released into aqueous environment (Ollikka *et al.* 1993). The microbial degradation and decolorization of dyes have received considerable attention from the viewpoint of treating industrial wastewater containing dyes. Azo dyes are the largest class of dyes (Cripps *et al.* 1990). They are not readily degraded by microorganisms. Microorganisms that are able to degrade azo dyes anaerobically, have been isolated. However aromatic amines produced by all these anaerobic microorganisms may be toxic and carcinogenic (Meyer, 1981). Wastewater treatment facilities are often unable to completely remove commercial dyestuffs, thus contributing to the pollution of aqueous habitats (Bumpus and Brock, 1988). Some triphenylmethane dyes have been shown to be carcinogenic . The lignin degrading white rot fungi mineralize a wide variety of structurally diverse environmental pollutants (Paszczynski *et al.* 1992; Yadav and Reddy , 1993; Yesilada and Fıskın, 1995; Yesilada *et al.* 1995). Due to high oxidative potential of many of the enzymes associated with white rot fungi, e.g., ligninase, laccase, Mn-peroxidase, they have shown to exert a positive effect upon many potential environmental pollutants (Paszczynski and Crawford 1992; Paszczynski *et al,* 1991). There is considerable number of studies on decolorization and degradation of dyes by white rot fungi especially *P. chrysosporium* (Pasti-Grigsby et al. 1991; Spadaro et al. 1992). H2O2 dependent dye decolorization with concentrated culture filtrates has also been reported (Greene and Gould, 1986; Vyas and Molitoris, 1995). So far, decolorization of dyes by culture filtrate has been investigated only in *P. chrysosporium*. There is not much information about the effects of the culture filtrate of white rot fungi on decolorization of dyes. Also there is almost no study about the effect of culture filtrates taken of different time intervals on the decolorization of the dyes.

Excess dyestuff in process water is highly undesirable because of environmental concerns. Due to their chemical structure, dyes are highly recalcitrant and often toxic or can lead to toxic transformation products when released into the environment. Therefore, their complete mineralisation is desired. Various physical and chemical methods for the elimination of dyestuff have been developed during the past decades. However, they all inevitably cause high consumption of chemicals and energy (Slokar and Le Marechal, 1998; Robinson *et al* . 2001). Biotechnological approaches are gaining increasing interest in the textile industry (Cavaco-Paulo and Gübitz, 2004). Bioremediation techniques for textile wastewater are currently evolving as promising sustainable alternatives (Binkley and Kandelbauer, 2003;) and a huge number of microbial systems, both, aerobic and anaerobic have been described in this context (McMullan *et al* . 2001; Stolz 2001). Fungi, with their ligninolytic enzyme systems are also being applied in the biological decolorization of textile dyestuff (Martins *et al* . 2003; Kapdan and Kargi, 2002; Fu and Viraraghavan 2001). Besides adsorption onto fungal mycelia and merely removing dyestuff physically from the effluent (Sumathi and Manju, 2000), oxidative biodegradation takes place upon action of enzymes such as peroxidases and laccases (Wesenberg *et al* . 2003).

Xian-Chun Jin *et al. ,* (2006) reported tha the strain *Aspergillus fumigatus* XC6 isolated from mildewing rice straw was evaluated for its ability to decolorize a dye industry effluent. The strain was capable of decolorizing dyes effluent over a pH range 3.0–8.0 with the dyes as sole carbon and nitrogen sources. The optimum pH was 3.0; however, supplemented with either appropriate nitrogen sources (0.2% NH4Cl or (NH4)2SO4) or carbon sources (1.0% sucrose or potato starch), the strain decolorized the effluent completely at the original pH of the dyes effluent. Therefore, A. *fumigatus* XC6 is an efficient strain for the decolorization of reactive textile dyes effluents, and it might be a practical alternative in dyeing wastewater treatment.

White rot fungi, are however efficient in the biodegradation of recalcitrant compounds like xenobiotics, lignin and dyestuffs by their extracellular ligninolytic enzyme system (Brodkorb and Legge 1992; Heinfling *et al.* 1997). White rot fungi offer

significant advantages over bacteria. Their extracellular enzyme systems including lignin peroxidase (LiP), Mn-dependent peroxidase (MnP), laccase and Mn-independent versatile peroxidases (VP) being nonspecific can attack a wide variety of complex aromatic dyestuffs (Barclay *et al.* 1990; Nagai *et al.* 2002; Boer *et al.* 2004; Kamitsuji *et al.* 2005). Because the enzymes are extracellular, the substrate diffusion limitation into the cell, generally observed in bacteria, is not encountered. White rot fungi do not require preconditioning to particular pollutants because enzyme secretion depends on the nutrient limitation, nitrogen or carbon, rather than the presence of pollutant. The extracellular enzyme system also enables white rot fungi to tolerate high concentrations of pollutants (Knapp *et al.* 1997).

A majority of the previous studies have focused on the lignin-degrading enzymes of *P. chrysosporium* and *T. versicolor*. Recently, however, there was a growing interest in studying the lignin-modifying enzymes of a wider array of white rot fungi, not only from the standpoint of comparative biology but also with the expectation of finding better lignin degrading systems for use in various biotechnological applications.

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