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AZO DYES DECOLORIZATION USING WHITE ROT FUNGI

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ABSTRACT

Wastewater from different industries is one of the major environmental concerns in the present scenario. The textile industry uses many kinds of synthetic dyes as azo, anthraquinone, polycyclic compounds and triphenylmethane and among them azo dyes are most commonly preferable. Azo dyes cause a serious environmental issue because these dyes are obstinate to biodegradation. Textile industries discharge large amounts of dyes about 10-200 mg/L and 10 -20% of the dye along with organic and inorganic accessory chemicals because the uptake of these dyes by fabrics is very poor. Industrial effluents containing about 5-10% of dyestuffs, which is usually discharged into water bodies. This highly colored textile wastewater severely affects photosynthesis in the plant. It also has an impact on aquatic life due to low light penetration and oxygen consumption. So, this textile wastewater must be treated before their discharge. Physical or chemical methods are costly, energy consuming, low efficient to the environment and generate secondary sludge. Thus biological degradation most preferable for textile dyes degradation will be eco-friendly, do not generate secondary sludge and cost-effective method. Fungi especially white rot fungi (WRF), produces Peroxidases (Lignin peroxidase, LiP, and Manganese peroxidase, MnP) and Phenol oxidase (Laccase) can be used for bioremediation of Azo dyes. In this article, decolorization and biodegradation of Azo dyes, abilities of WRF are reviewed.

KEYWORDS: *Azo, Anthraquinone, Biodegradation, Lignin Peroxidise, Manganese Peroxidase*

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INTRODUCTION

Due to rapid industrialization and urbanization release of industrial effluent into water bodies causing major environmental pollution. A large amount of wastewater released from textile industries as effluent is one of the major problems of water pollution (Kaur *et al*, 2010). Textile industries release a huge amount of colored effluent in a surrounding water body without any proper treatment causing major environmental pollution. A dye is used to modifying the color characteristics of the different substrate such as fabrics, paper, and leather. Natural dyes are generally used in before 19th century that is obtained from animals and vegetables. However natural dyes are replaced by the synthetic dye at the beginning of the twentieth century (Figure 1). Among all dyes used for colorization in textile industries, Azo dyes are most demandable. Azo compounds are xenobiotic, contains aromatic compound and very recalcitrant to biodegradation (Vandevivere et al 1998) without of more than 900,000 metric tons of dyes 60-70% of total dyes. Synthetic azo dyes may be converted to toxic, recalcitrant and carcinogenic products under anaerobic conditions by the presence of −N=N− bond (Saratale et al 2011).

Impact of Azo Dyes on the Environment

The global consumption of dyes and pigments approximates 7x105tons/year and only in the textile industry it consumes about two-thirds of all the world production. It has been estimated that in textile industries about 80% of azo dyes are used dyeing process (Sudha et al 2014) rest of the Azo dye app 10-15% do not take binding with fiber so discharged into water bodies (Baban et al 2003). Synthetic azo dyes that released as colored effluent contain toxic substances and hazardous chemicals that cause serious water pollution problems by increasing in chemical oxygen demand (COD), biological oxygen demand (BOD) and alteration in pH of water. The colored waste of textile industries causes an acute effect on aquatic ecosystem results loss of environmental balance due to low penetration of light and oxygen consumption. It also misbalances organic-inorganic chemical content of the environment and is affective for the biotic content present in water. When dye gets mixed with water, light penetration efficiency decreased inside the water and complete water ecosystem gets affected. Toxic compounds of azo dye mix with water bodies and enter into fishes or other aquatic animals which are further taken up by human causing hypertension, sporadic disorder, cramps, etc. with prolonged effect. Due to easy inhalation or its ready solubilization in water, azo dyes cause fast absorption by the skin leading to the risk of an allergic reaction, cancer, eye irritation, etc (Pandey et al 2007).

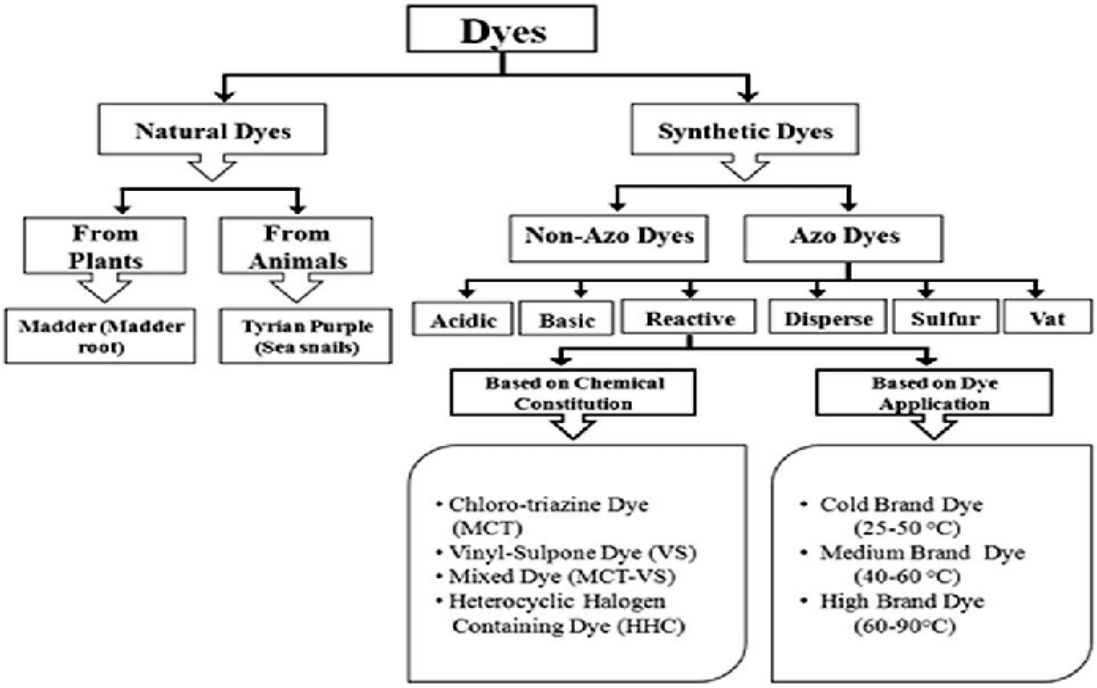

Figure 1: Dye Classification on the Basis of Dye Chemical Constitution

Azo dyes are synthetic organic compounds characterized by the presence of one or more azo (-N=N-) bonds in association with one or more aromatic systems. Biological methods are getting more attention for decolorizations of these dyes. Since it is effective, specific, less energy-intensive, eco-friendly, cost-effective, environment benign and produces less secondary sludge (Singh et al 2012). It results in partial or complete bioconversion of pollutants to stable nontoxic compounds. White rot fungi are efficient biodegraders for a recalcitrant compound such as dyestuffs, xenobiotics, and lignin due to the extracellular secretion of enzymes (Ahlawat et al 2011). Three major classes of enzymes designated lignin peroxidases (LIPs), manganese-dependent peroxidases (MNPs) and laccases play an important role in the fungal degradation of lignin. These extracellular enzymes are non-specific and degrade a wide range of complex aromatic

dyestuffs (Boer et al 2004).

Chemical Properties of Azo Dyes

Dyes are soluble in aqueous solution and produce the color due to the presence of chromophore groups present in its chemical structure. These colored dyes are used in textile, paper, leather or food industries. Azo dyes are highly soluble in water. It is characterized by the

presence of one or more azo groups (-N=N-), usually in number of one or four, linked to phenyl and naphthyl radicals, which are usually replaced with some combinations of functional groups including: amino ($-NH_2$), chlorine (-Cl), hydroxyl (-OH), methyl ($-CH_3$), nitro ($-NO_2$), sulphonic acid and sodium salts (- SO_3Na) [18]. Monoazo dyes contain one nitrogen bond (-N=N-); likewise, diazo dyes contain two -N=N- bonds, triazo dyes contain three -N=N- bonds, and polyazo dyes contain more than three N=N bonds. The presence of the linkage N=N, reduces thepossibility of unpaired electron pairs in nitrogenatoms, are readily reduced to hydrazines and primary amines, functioning as good oxidizing agents [USEPA. 2008.]

Types of Azo Dyes

In textile industries, synthetic dyes, basic dyes, azo dyes, sulphur dye, oxidation dye, anthraquinone dye, acridine dye are used for colorants (Sarkar et al 2017). Depends on characteristics of the processes in which they are applied Azo dyes can be acid dyes, direct dyes, reactive dyes, disperse dyes or others. Acid dyesconsists of anionic groups; these are water soluble, low molecular weight and having sulfonate groups. The main application of these dyes is to dyeing of proteins, that is animal hair fibers (wool, silk) and synthetic fiber (nylon). The term acid used because the dyeing process takes place in weakly acidic solution (pH 2-6). The cationic groups in the fiber: animal protein fibre and nylon fibers responsible for attachment with anionic groups in the dyes. After dyeing a certain amount of dyestuff is released into water bodies(Table 1).

Table 1 Types of Dye (Kirk-Othmer 1979)

Dye Class	Characteristics	Fiber	Pollutants
Acidic	Water soluble anionic Compounds	Wool, nylon, cotton blends	color: Organic acids, unfixed dyes
Basic	Water soluble, applied in weakly acidic dye baths, very bright dyes	Acrylic, cationic, polyesters, nylon, cellulosic and protein fibers	Nil
Direct	Water soluble, anionic compounds, applied without mordants	Cotton, rayon cellulosic fibers	Collur salts, unfixed dye, cationic fixing agents, surfactants, deformer, retarding agents.
Dispersive	Insoluble in water	Polyester, acetate, moddacrylic, nylon, triacetate and olefin fibers	Colour organic acids, carriers, leveling agents, phosphates, lubricants, dispersants, diluents
Reactive	Water soluble, anionic compounds, largest class	Cotton, cellulosic, wood fibers	Colours, salts, alkali, unfixed dye, surfactants
Sulphur	Organic compounds containing Sulphur	Cotton, cellulosic fibers	Colours, alkali, oxidizing agents, reducing agents, infixed dye
Vats	Oldest dyes, chemically complex, water insoluble	Cotton, cellulosic, wood fibers	Colours, alkali, oxidizing agents, reducing agents

Direct dyes are used to color cotton, paper, leather, silk, and nylon.Direct dyes also consistsof sulfonate groups responsible for solubility. The dyeing process is normally carried out in neutral or slightly alkaline dye bath. Washing is easy and fast. Reactive dyes are so called because they consists of reactive groups that react with the –OH groups of cellulose or with $–NH_2$ and –SH groups of protein fiber s by covalent bonds. Disperse dyes do not contain acidic or basic groups for attachment so these are water insoluble. They are finally ground mixed to a dispersed agent. The dyeing rate is influenced by particle size and chosen dispersing agent.

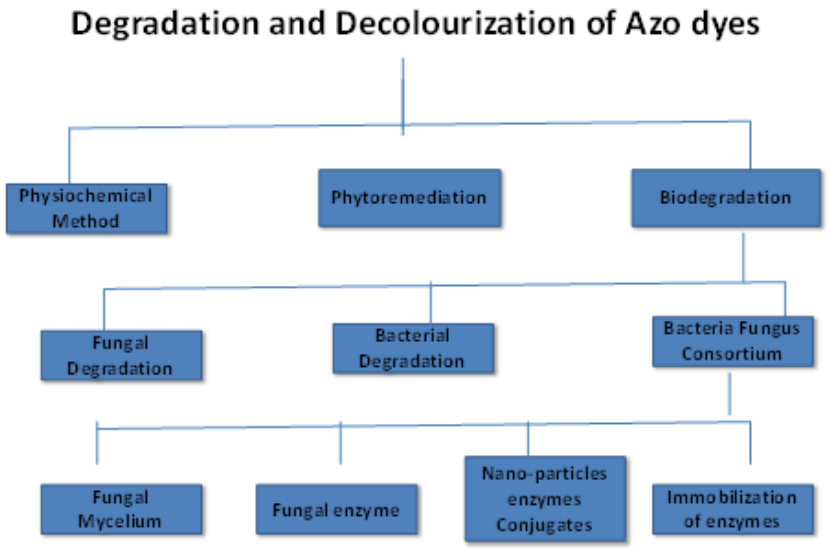

Figure 2: Different Possible Methods of Degradation and Decolorization of Azo Dyes (Sarkar Et Al. 2017)

Different Factors Affecting Mineralization of Azo Dyes

Oxygen availability plays a very vital role for complete mineralization of Azo dyes. There are two common steps to biodegrade Azo dyes: a first step which is anaerobic cleavage diazo bonds in Azo dyes which released aromatic amines, the second step usually occurs aerobically in which degradation of aromatic amines takes place. The first step usually occurs in anaerobic condition but it can be carried out by several aerobic bacteria by producing azoreductase which cleaves azo groups in the presence of molecular oxygen. Under aerobic conditions, fungal degradation of Azo dyes has also been described, chiefly by lignin-degrading fungi mainly white rot fungi (Stolz, 2001) (Figure 2,3).

The hydrophobicity and hydrophilicity are two main factors that influence the bioavailability. Polar substances such as sulfonated azo dyes cannot pass through the plasma membrane. Indeed only the biological system in which reductive enzyme (that cleave azo bonds) are extracellular are most effective for mineralization of Azo dyes. The hydrophobic Azo dye can pass through the plasma membrane and can be degraded in the cytoplasm. But degradative microorganism shows high activity in an aqueous phase where the availability of hydrophobic dyes is low. Several fungi (i.e., *Phanerochaete chrysosporium, Geotricum candidum, Trametes versicolor, Bjercandera adusta, Penicillium sp., Pleurotus ostreatus, Pycnoporus cynnabarinus, Pyricularia oryzae*) are able to degrade azo dyes by extracellular enzymes production: lignin-degrading fungi are able to decolorize several kinds of even complex molecules of sulfonated and nonsulfonated azo dyes, mainly by synthesizing Isoenzymes such as lignin peroxidases (LiP), manganese peroxidases (MnP) and laccases (Chen H, 2006, Stolz A, 2001). In *Pleurotus sajor-caju* cultures grown over solid medium, it was observed that soluble azo dyes were degraded, even if incompletely, while insoluble azo dyes were not degraded during

mycelial growth (Munari et al 2008).

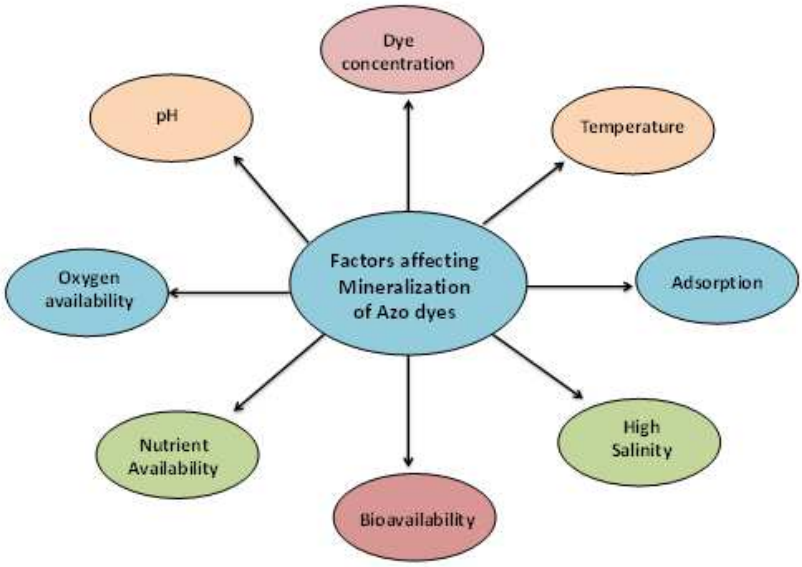

Figure 3: Different Factors Affecting the Complete Mineralization of Azo Dyes

Adsorption of dye is necessary during biological reduction by biomass. Dye adsorbed on biomass occurs until it reaches its saturation; the amount of dye being absorbed is proportional to the amount of biomass. Initial adsorption of the dyes on fungal biomass followed by degradation was observed in cultures of *Irpex lacteus, Phanerochaete chrysosporium, Trametes versicolor*, and *Trichophyton rubrum* (Maximo et al 2004, Wang et al 1998 and Tatarko et al 1998)

Azo dye and their degraded components are toxic to the biological system. Thus to achieve complete mineralization of azo dyes it ensures that the concentration should be in optimum range before the process started.

Azo Dyes Decolorization Capability of White Rot Fungi

Usually, by limiting the carbon and nitrogen source the expression of ligninolytic enzymes by white rot fungi can increase. These enzymes are extracellular and usually form during secondary metabolism (Stolz, 2001). It was also observed that the rate of degradation changes with different dyes changed. The ligninolytic enzymes producing *Irpex lacteus* produced an increased amount of Manganese Peroxidase (MnP) with the addition of addition high concentration of manganese and dye but not the Azo dyes. At low concentration of manganese no increase in production of MnP. This shows that the expression of ligninolytic enzymes is inducible (Susla et al 2008).

To mineralize reactive dyes high pH tolerantly is important because decolorization of these dyes takes place in alkaline condition. Expression of lignin peroxidase (LiP) from ligninolytic fungi seems to be higher at optimum pH range (4.5-5) (Stolz 2001). Before the application of fungi on site for decolorization of Azo dyes, the effect of pH and temperature should be examined on the rate of biodegradation in vitro. For the microbial population the rate of decolorization generally related to optimum cell growth temperature and pH, with an increase of decolorization proportional to the increase of temperature within the optimum temperature range (Wong et al 1996, Ozdemir et al 2008, Adedayo et al. 2004, Angelova et al 2008, Chang et al 2000 and Mali et al 2000).

Wastewater released from dyestuff industries consist of high salt concentration up to 15-20%. Most of the microorganisms are not able to biodegrade Azo dyes in these conditions because they cannot tolerate high salt concentration. To achieve decolorization of Azo dyes in high saline concentration, selection of halophile or halotolerant microorganism is an option. These microorganisms are capable to degrade Azo dyes even in high saline concentration (Asad et al 2007).

Table 2: Selected White Rot Fungus and their Extracellular Enzymes Able to Decolorize Azo Dyes

Whit Rot Fungus	Enzyme	Dye	References
Phanerochaete Chrysosporium	LiP	Diazo dyes	Paszczynski A et al. 1991
	-	Direct Red 80 Mordant Blue 9	Singh et al. 2013
	-	Direct Violet Reactive Black 5 Ponceau Xylidin Bismark BRrown	
	LiP	Reactive Brilliant Red	Adosinda et al. 2001
	-	Orange 2	
	-	Amido Black	Senthikumar et al. 2014
	LiP and MnP	Amaranth, Orange G	Yu et al. 2006
	LiP and MnP	Congo Red	
Trametes versicolor	-	Reactive Red 2	Nilson I at al. 2006
	Laccase	Direct Black 38 Direct Blue 15 Direct Orange 26 Direct Green 6 Direct Yellow 12	Pazarlioglu et al. 2010
	-	Remazol Black B	Aksu et al. 2007
	Laccase	Orange 2 and Acid orange 6	
	-	Remazol brilliant Yellow 3-GL (RBY3-GL)	Asgher et al. 2016
	MnP	Reactive Green 19	Sari et al. 2012
Cerrena spp. WICC F39	-	Methylene Blue, Reactive Black 5	Hanapi SZ et al. 2018
Cerrena unicolor BBP6	MnP	Congo Red, Methyl Orange, Ramazol Brilliant Blue, Bromophenol Blue, Crystal Violet	Zhang et al. 2018
Cerrena unicolor	Laccase	Congo Red, Ramazol Brilliant Blue R, Lanset grey, Poly R-478	Moilanen et al. 2010
Trametes ljubarkyi	Laccase	Reactive violet 5 (RV 5)	Goh et al. 2017
Trametes gibbosa sp. WRF 3	-	Coralene Golden yellow, Coralene Navy Blue, Coralene Dark Red	Kunjadia et al. 2016
	-	Reactive Black 5	Adnan et al. 2014
Trametes trogii	Laccase	Reactive Black 5, Reactive violet 5	Sayahi E et al. 2016
	Laccase	Remazol Brilliant Blue R Reactive Blue 4 Acid Blue 129 Acid Red 1 Reactive Black 5	Zhang et al. 2011

Table 2: Contd.,			
Genoderma sp.	LiP	Amaranth	Revankar et al 2007
	-	Reactive Blue 19	Fazli et al. 2010
	-	Remazol Black 5	Sudiana et al. 2018
	-	Reactive Orange 16	
Pleurotus sajar-caju	MnP	Congo Red	Yehia RS 2014
	Laccase	Amaranth, new coccine, and Orange G	Chagas et al. 2001
	Laccase	Reactive Black 5	Murugesan et al. 2007
Genoderma lucidum	Laccase	Congo Red	
	Laccase	Reactive Black 5	Forss et al. 2009
Phanerochaete Sordid	-	Reactive Black 5	Permpornsakul et al. 2016
	MnP	Reactive Red 120	Harazono et al. 2003
Armillaria sp. F022	-	Acid Red 27	Adnan et al. 2014
	Laccase	Reactive Black 5 Remazol Brilliant Blue R	Hadibarata et al. 2011
Ceriporia lacerata New isolated WRF	-	Congo Red	Wang et al. 2017
Irpex lacteus		Methyl Red and Congo Red Reactive Orange 16, Congo Red, Reactive Black 5, Naphthol Blue Black, Chicago Sky Blue	Novotony et al. 2001
Pleurotus ostreatus	Laccase	Drimarene Blue	Erkurt et al. 2007
	-	Acid Orange 7 Acid Orange 8 Mordant Violet 5	Lu Y et al. 2008
	LiP	Disperse Orange 3 Methyl Red and Congo Red	Zhao et al. 2006
	-	Synazol Red HF6BN	Ilyas et al. 2012
	LiP	Disperse Orange 3 Disperse Yellow 3	Zhao et al. 2007
	Laccase MnP	Direct blue 14	Singh et al. 2010
Coriolus versicolor	Laccase	Drimarene Blue	Erkurt et al. 2007
	-	Orange 7	Hai et al. 2013
Ischnoderma Resinosum	Laccase	Orange G	Eichlerova et al. 2005
Dichomitus squalens	Laccase MnP	Orange G	Eichlerova et al. 2005
Pleurotus eryngii F032	LiP MnP Laccase	Reactive Black 5	Hadibarata et al. 2013
Pleurotus calyptratus	Laccase	Orange G	Eichlerova et al. 2005
Datronia sp. KAPI0039	Laccase	Rezamol Brilliant Blue R Reactive Black 5	Vaithanomsat et al. 2010
Lentinula edodes	MnP	Congo Red, Trypan Blue, Amido Black	Boer et al. 2004
Bjerkandera adusta Dec 1	MnP LiP	Amaranth	Gomi et al. 2011
Funalia trogii	Laccase	Astrazone Blue	Yesiladalil et al. 2007
	Laccase	Drimarene Blue	Erkurt et al. 2007
Alternaria alternata CEMRI F6	-	Congo Red	Chokraborty et al. 2013

Textile dyes are recalcitrant and can persist in the environment up to a long period of time because of high thermal and photostability. The major environmental concern of these dyes is their absorption and reflection of sunlight entering the water. Due to this reason the photosynthetic activity of aquatic plant and algae lost which influences the food chain. (Zaharia et al 2009).

Table 3: Example of Some Commonly used Azo Dyes in the Textile Industry and their Hazardous Effects

Types of Azo Dye	Hazardous Effect	References
Reactive brilliant red	Inhibit function of human serum albumin,	Li WY et al. 2010
Acid Violet 7	Chromosomal aberration, acetylcholinesterase activity inhibition, membrane lipid peroxidation	Mansour HB et al. 2010
Malachite Green	Carcinogenesis, mutagenesis	Srivastva S et al. 2004
Reactive Black 5 (sulfonated azo dye)	Restrict nitrogen use efficiency of plant, decrease the urease activity, chance mutagenecity, and carcinogenicity increase	Dave et al. 2015,Topac et al. 2009, Gottlieb et al. 2003
Disperse Red 1 and Disperse Red 13	Mutagenic to human, may affect the activity and composition of microbial communities	Mahmood et al 2016, Chequer et al. 2015 and Ferraz et al. 2011
Congo red	Carcinogenic and mutagenic Effect	Gopinath et al. 2009

Many dyes and their break down products are carcinogenic, mutagenic and/or toxic to life. The presence of very small amounts of dyes in the water be highly visible, seriously affects the quality and transparency of water bodies such as lakes, rivers, and others, leading to damage to the aquatic environment. Azo dyes have toxic effects, especially carcinogenic and mutagenic. They enter the body by ingestion and are metabolized by intestinal microorganisms causing DNA damage.

CONCLUSIONS

Azo dye degradation depends on their different ligninolytic enzymes, primarily laccases in response to white rot fungus.WRF degrade a wide variety of organic compounds, including polymers such as lignin, chlorinated phenols, dioxins, chloroanilines and dyes that depends on their unspecific action and great oxidative ability. White rot fungi can withstand toxic levels of most organic pollutants (Aust et al 2004). Thus, their application in several biotechnology fields and in the bioremediation of pollutants will undoubtedly be important in the future.

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