DEGRADATION OF SYNTHETIC DYES BY LACCASES – A MINI-REVIEW

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Abstract: Laccases provide a promising future as a tool to be used in the field of biodegradation of synthetic dyes with different chemical structures. These enzymes are able to oxidize a wide range of phenolic substrates without the presence of additional co-factors. Laccases have been confirmed for their potential of synthetic dye degradation from wastewater and degradation products of these enzymatic reactions become less toxic than selected dyes. This study discusses the potential of laccase enzymes as agents for laccase-catalyzed degradation in terms of biodegradation efficiency of synthetic dyes, specifically: azo dyes, triphenylmethane, indigo and anthraquinone dyes. Review also summarizes the laccase-catalyzed degradation mechanisms of the selected synthetic dyes, as well as the degradation products and the toxicity of the dyes and their degradation products.

Key words: laccase, synthetic dye, biodegradation, toxicity, degradation products.

1. Introduction

Synthetic dyes have a nearly exhaustible range of application in various types of industry including food, pharmaceutical, textile, printing, paper or chemical. It has been estimated that approximately 5-10 % of dyes used in the industrial sector could remain persistent in wastewater (DAFALE *et al*., 2008). Untreated dyeing effluents are a serious environmental problem in the twenty first century. The release of dyes into the environment is harmful due to toxicity, carcinogenic and/or mutagenic effects on living organisms. The presence of synthetic dyes in wastewater can cause an increase of BOD (biochemical oxygen demand) and COD (chemical oxygen demand) levels. Moreover, the chromophoric groups strongly absorb sunlight and therefore, photosynthetic activity of organisms is inhibited (DA SILVA *et al*., 2010; KAGALKAR *et al*., 2010). The negative impact of synthetic dyeshas been observed in the oestrous cycle and reproductive system in rats (NATH *et al*., 2015), in biochemical markers of vital organs, such as the liver and kidney (AMIN *et al*., 2010) and to foetal growth (WAN *et al.*, 2011; GOPINATHAN *et al.*, 2015). A correlation between the presence of synthetic dyes and high clastogenic activity in bone marrow cells has been observed in rats (RAJAGURU *et al*., 1999). Additional studies have shown that a correlation exists between synthetics dyes and mitotic abnormalities (AZMI *et al*., 1998), as well as cirrhosis from chronic consumption of selected dyes (AXON *et al*., 2012).

Because of the aforementioned reasons, wastewater treatment containing synthetic dyes is a global problem requiring an immediate yet cost-effective solution. Degradation of synthetic dyes using biological methods is a promising, environmental-friendly process and these methods are presented as a cheaper alternative to the

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expensive physicochemical methods producing large amount of sludge, which must be degraded by other processes. Using biological methods, the degradation of synthetic dyes can occur in a cost efficient, eco-friendly format with certain advantages. Not only are the products of enzyme-catalyzed reaction less toxic than synthetic dyes (CAMPOS *et al*., 2001; TELKE *et al*., 2011; ADNAN *et al*., 2015), but the degradation products can be utilized by various natural organisms.

These biological methods are founded on enzymes, which are produced by organisms. In the case of enzymatic degradation, azoreductases, peroxidases and phenol oxidases have a potential for dye biodegradation, but in certain aspects each of these enzyme falters in their biodegradation efficiency. Azoreductases (EC 1.7.1.6) require additional co-factors such as $NADH_2$, $NADPH_2$ and $FADH_2$ for the activation of the catalyst. Additionally, azoreductases can degrade only azo dyes via reductive cleavage of azo bonds (PANDEY *et al*., 2007). One of the main disadvantages of this enzyme-catalyzed degradation processes the formation of toxic products (PLATZEK *et al*., 1999). It is known that azoreductases are intracellular enzymes and their use in the pure form without production organisms is problematic due to issues of stability and necessary regeneration of co-factors. Furthermore, the direct application of microorganism produced azoreductases can result in additional problems from the inability of dye diffusion through cell membranes, due to the molecular weight of azo dyes (ROBINSON *et al*., 2001). Peroxidases (EC 1.11.1.X), such as horseradish peroxidase, chloroperoxidases, lignin peroxidases and manganese peroxidases, are other enzymes that could be useful for synthetic dye biodegradation but have a factor that limit their benefits in synthetic dye degradation. All of these enzymes belong to hemoproteins which catalyse the chemical reactions in the presence of hydrogen peroxide (DURAN *et al*., 2002). These reactions require specialized attention from because the presence of hydrogen peroxide at too high of a concentration can cause the inactivation of peroxidases (AITKEN *et al*., 1994). The last enzyme group described in the literature as useful for synthetic dye decolorization includes phenoloxidases. These enzymes belong to oxidases which catalyse the oxidation of phenolic compounds in the presence of oxygen without additional co-factors. One of these enzymes, laccase (EC 1.10.3.2), is a member of the multicopper oxidase family (TELKE *et al*., 2011). Laccases catalyse the removal of a hydrogen atom from the hydroxyl group via electron oxidation (BOLLAG, 1992), which generates non-toxic products during synthetic dye biodegradation (CAMPOS *et al*., 2001; TELKE *et al*., 2010; ADNAN *et al*., 2015). Although the biodegradation potential of laccases is mostly evaluated by the change of a absorption spectrum of dye (SUZUKI *et al*., 2001; BIBI *et al*., 2011; CHMELOVÁ and ONDREJOVIČ, 2015), the destruction of chromophores can led to dye degradation (CHEN and TING, 2015).

In this mini-review, the synthetic dye degradation of laccase-catalyze reaction has been observed with regards to their degradation products and their potential toxicity.

2. Laccases

Laccases (EC 1.10.3.2, *p*-diphenol: dioxygen oxidoreductases) are classified as phenol oxidases which are able to catalyse one-electron oxidation of the substrate associated with the simultaneous reduction of oxygen in water. These enzymes are

produced by a wide spectrum of organisms such as bacteria, filamentous fungi or plants (RODRÍGUEZ-COUTO and HERRERA, 2006; BIBI *et al*., 2011; ZHOU and XIANG, 2013). Laccases are monomeric, dimeric or tetrameric glycoproteins with four copper atoms per monomer located in catalytic sites. Type 1 copper (Cu T1, ligated by at least one Cys and two His) is paramagnetic and responsible for the characteristic blue colour and the oxidation of the substrate. Type 2 copper (Cu T2, ligated by two His) and two copper atoms of type 3 (Cu T3, each ligated by three His) conforming trinuclear cluster play a key role in the reduction of molecular oxygen to two molecules of water (DAVIES and DUCROS, 2006; MOT and SILAGHI-DUMITRESCU, 2012).

Laccase can oxidize different organic compounds such as mono- and di-phenols or their derivatives with hydroxy-, carboxy-, methoxy-, amino- or sulpho- functional groups by radical mechanism. Phenols are typical substrates for laccases due to their low redox potential which allows for electron abstraction by the Cu T1. Therefore, the ability for laccase enzymes to oxidize molecules is determined by the redox potential of Cu T1 (E^0 Cu T1). E^0 values have been determined using potentiometric titrations for various laccases (Table 1). According to E^0 Cu T1, the laccases can be classified into three categories: low-, medium- and high-redox potential laccases. This redox potential is a result of combined factors such as copper-ligand interactions, the effect of desolvation around the T1 site, the intermolecular electrostatic interactions, and the restrictions in protein folding (LI *et al*., 2004). Bacterial and plant laccases have low-redox potential (below +460 mV vs. NHE – normal hydrogen electrode) while fungal laccases belong to medium- and high-redox potential laccases (MATE and ALCALDE, 2015). Laccases with medium-redox potential (+460 to +710 mV vs. NHE) are produced mainly by ascomycetes and basidiomycetes. Laccases with high-redox potential (+730 to +790 mV vs. NHE) are commonly found in white-rot fungi (basidiomycetes). For industrial application, laccases with a high-redox potential are the most relevant and most useful. The efficiency of synthetic dye oxidation by laccases depends on differences between the redox potential of dye and potential of enzyme (YANG *et al.*, 2015).

For phenolic substrate, the reaction starts with the deprotonation of the phenolic hydroxyl group. This results in the formulation of unstable phenoxy radicals which lead to quinone formation. This process combines the oxidative transformation without the coupling of forming products, where oxidation cleavage leads to a reduction of product molecular mass and oxidative decarboxylation of different products (POLAK and JAROSZ-WILKOLAZKA, 2012).

For diversification of substrate specificity, laccases need the presence of low-molecular weight compounds (redox mediators) because they usually are not able to oxidize non-phenolic compounds or molecules with high redox potential (GIARDINA *et al*., 2010). These compounds act as intermediate substrates for laccases. The initial step is the oxidation of a redox mediator to forming a radical (1) (FABBRINI *et al.*, 2002). The substrate is attacked the radical thereby giving rise to product and producing mediator back.

$$\text{Med-OH} \xrightarrow{\text{laccase}} \text{Med-OH}^{\bullet +} \longrightarrow \text{Med-O}^{\bullet} \qquad (1)$$

Table 1. The values of redox potential E^0 (Cu T1) for laccases isolated from various organisms.

Organism	MW (kDa)	pH	E^0 (Cu T1) (mV vs. NHE)	Reference
Rhus vernicifera	-	5.5-8.5	0.410	JOHNSON *et al*., 2003
Trametes ochracea	64±2	3.7- 4.9	0.79±0.1	SHLEEV *et al*., 2004; SHLEEV *et al*., 2005
Trametes hirsuta	70±2	3.5-4.5	0.78±0.1	SHLEEV *et al*., 2004; SHLEEV *et al*., 2005
Coprinus cinereus	58	5.5	0.55	SCHNEIDER *et al*., 1999
Cerrena maxima	67±4	4.0-6.0	0.75±0.05	SHLEEV *et al*., 2004; SHLEEV *et al*., 2005
Coriolopsis fulvocinerea	65±2	3.9-5.2	0.78±0.1	SHLEEV *et al*., 2004; SHLEEV *et al*., 2005
Ganoderma sp.	62	3.0-5.0	0.63	SHARMA *et al*., 2013
Marasmius guercophilus C30	65	5.7	0.73	KLONOWSKA *et al*., 2002
Melanocarpus alomyces	-	8.0	0.46±0.01	KRUUS *et al*., 2003
Pleurotus ostreatus	-	3.0	0.588	DAI *et al*., 2016
Pycnoporus sanguineus	67	4.5	0.747	ZIMBARDI *et al*., 2016

Redox mediators can be natural or synthetic.3-hydroxyanthranilic acid, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol, syringaldehyde, sinapinic acid, acetovanillon, ferulic acid, vanillin, *p*-coumaric acid (MOREIRA *et al*., 2014) belong to the group of natural mediators. Generally these compounds are phenolic substrates produced by white-rot fungi. Synthetic redox mediators are 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), 1-hydroxybenzotriazole, N-hydroxyphthalimide, violuric acid or N-hydroxyacetanilide. The application of artificial mediators has several disadvantages such as high cost and toxicity (CANAS and CAMARERO, 2010).

Laccases are able to degrade various organic pollutants with different chemical structures via direct oxidation (BOLLAG, 1992). This ability depends on the enzyme as well as dye structures. The presence and the location of different functional groups in dye structure can affect degradation of selected dyes. For most laccases, the presence of *orto*- and *meta*- substituents in dye structure is preferable for laccase oxidation as these in *para*-position (BALDRIAN, 2006; TAUBER *et al*., 2008). Electron-donating substituents (-OH, $-CH_3$, $-NH_2$, $-N(CH_3)_2$) contribute to increased biodegradability while electron-withdrawing substituents (-COOH, $-SO_3H$, $-NO_2$, -Cl, -Br) make a ring less susceptible to biological oxidation (SUZUKI *et al*., 2001). For example CHIVUKULA and RENGANATHAN (1995) found that laccase from *Pyricularia oryzae* oxidizes only azo dyes with methyl- or methoxy- substituents in azo structure while non-substituted 4-(4′-sulfophenylazo)-phenol and its 2-chloro- and 2-nitro-analogs were not oxidized. The negative effect of halogen groups (-Cl, -Br) in the structure of triphenylmethane dyes such as Bromophenol Blue and

Bromochlorophenol Blue to their decolorization by laccase was confirmed also in our recent work (CHMELOVÁ and ONDREJOVIČ, 2015).

3. Synthetic dyes

Synthetic dyes are complex aromatic compounds which are produced via chemical synthesis which provide a wide range of colours. The most industrially used synthetic dyes can be classified as azo dyes, anthraquinone, triphenylmethane and indigo dyes (Fig. 1) according to the main structure which often includes chromophores (-C=C-, -C=O, -C=N-, -NO_2, -N=N- and quinonoid rings) responsible for absorption of visible light.

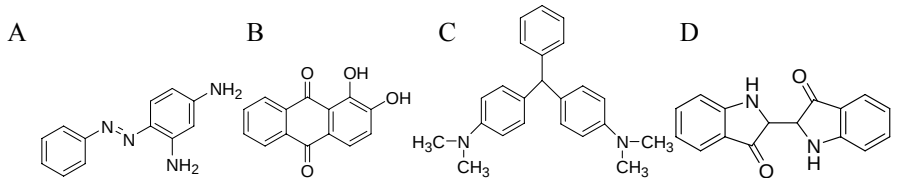

Fig. 1. The basic structure of selected synthetic dyes. A – azo dye (Chrysoidine), B – anthraquinone (Alizarine), C – triphenylmethane (Malachite Green) a D – indigo dye (Indigo).

Diversion of compounds belonging to individual synthetic dye groups is caused mainly by the auxochrome groups e.g. $(CH_3)_2N$-, -NH_2, -OH, -OCH_3, CH_3CO-, CH_3-, -NO_2, -SO_3H, -COR, -CO_2H, halogens (FORGACS *et al*., 2004).

3.1 Mechanisms of azo dye degradation

Azo dyes belong to the group of aromatic compounds which contain one or more azo bonds (-N=N-). Azo dyes are produced by the copulation of diazonium salts with amines or phenols or naphtol, respectively. Azo bonds are substituted with benzene or naphthalene which can contain different functional groups such as -Cl, -CH_3, -NO_2, -NH_2, -OH and -CO. In the enzymatic degradation pathway, azo dyes can be cleaved symmetrically or asymmetrically (TELKE *et al*., 2009) through a highly non-specific free radical mechanism, forming phenolic products. The biggest problem with enzymatic cleavage of azo dyes is the formation of toxic products, mainly amines. Therefore, it is important to identify and evaluate toxicity and/or mutagenicity of degradation products (CHHABRA *et al*., 2009).

Azo dye degradation by laccases starts by asymmetric cleavage of the azo bond followed by oxidative cleavage, desulfonation, deamination, demethylation and dihydroxylation, depending on dye structure (TELKE *et al*., 2009; TELKE, *et al*., 2011; ADNAN *et al*., 2015; YANG *et al*., 2015; ZHENG *et al*., 2016). Some authors describe degradation of azo dyes without the cleavage of the azo bond (CHEN, 2006; PEREIRA *et al*., 2009). This mechanism includes the formation of phenolic type compounds resulting from highly non-specific free radical mechanism (CHEN, 2006). The inability of laccase to cleave azo bond in the structure of dyes may be related to

the redox potential of laccase. PEREIRA *et al.* (2009) suggested the proposed mechanism for biotransformation of the mono azo dye Sudan Orange G by laccase from *Bacillus subtilis*. The enzymatic oxidation of Sudan Orange G results in the production of oligomers, and possibly polymers through radical coupling reactions without the cleavage of azo bond. Laccase from *B. subtilis* has a low-redox potential (455 mV vs. NHE) (DURAO *et al*., 2006), so it belongs to the group of low-potential laccases. On the other hand, laccases from fungal organisms are more applicable for degradation of azo dyes because they belong to the group of high-redox potential laccases (MATE and ALCALDE, 2015). An example can be found in the work of TELKE *et al*. (2010) which describe the degradation of azo dye (Methyl Orange) by laccases from *Aspergillus ochraceus* NCIM-1146 (Fig. 2).

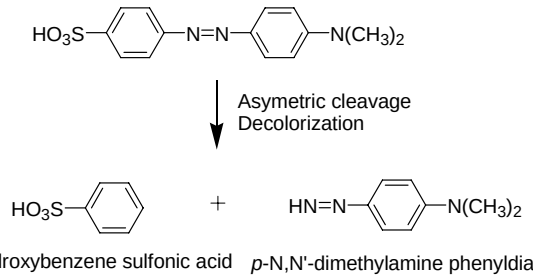

Fig. 2. Degradation mechanism of mono azo Methyl Orange by laccase from *Aspergillus ochraceus* (TELKE *et al*., 2010).

The first step of mono azo dye decolorization by laccases is carbocation, the formation of an electron-deficient reaction centre and therefore highly reactive intermediates. These can be subject to the nucleophilic attack by $-SO_3$, -OH or halogen nucleophiles resulting in asymmetric cleavage of azo bond (TELKE *et al*., 2010). Degradation products formed by laccase from Methyl Orange in this way are *p*-N,N'-dimethylamine phenyldiazine and *p*-hydroxybenzene sulfonic acid (Fig. 2). Although many authors marked these compounds as toxic (WANG *et al*., 2008), DU *et al*. (2015) concluded that degradation products (amine and amide derivatives) formed by biological treatment of Methyl Orange in water solution by *Aeromonas* sp. strain DH-6 have lower phytotoxicity levels tested on Chinese cabbage.

Degradation of bis azo dyes is a more complicated process. In the same way, azo bonds are cleaved asymmetrically by laccases (SI *et al.*, 2013; ADNAN *et al.*, 2015; ZHENG *et al.*, 2016) but this reaction requires electrons for reduction (NAM and RENGANATHAN, 2010). Laccases can transfer electron to azo dye because laccases contain four histidine-rich copper binding domains in the catalytic centre (ZHENG *et al*., 2016). Biodegradation of the bis azo dye Congo Red by high-redox potential laccases from *Trametes pubescens* (E^0 Cu T1 = 738 – 745 ± 5 mV vs. NHE; SHLEEV *et al*., 2007) led to the formation of naphthalene amine (Fig. 3) but phytotoxic test have shown that the purified laccase can detoxify azo dye Congo Red (SI *et al*., 2013). This means that Congo Red degradation does not finish in this phase but probably continues forming other non-toxic degradation products.

Similarly, biodegradation of the bis azo dye Reactive Black 5 by laccase from *Trichoderma artroviride* F03 was initiated by the cleavage of the bis azo bond and followed by deamination, hydroxylation and sulphonation. The genus *Trichoderma* is characterized by production of laccases with medium-redox potential (SADHASIVAM *et al*., 2008). The biodegradation mechanism continued with the aromatic ring fission of naphthalene-1,2,8-triol, where its oxygenated ring at C1 and C2 position was cleaved to 2-(2-carboxy-ethyl)-6-hydroxy-benzoic acid via 8-hydroxy-[1,2]-naphthoquinone. 2-(2-carboxyethyl)-6-hydroxy-benzoic acid can be degraded via two possible pathways (i) it undergoes decarboxylation and methylation to form 2,4-ditertbutylphenol (detected at 7th day) and (ii) it is transformed to benzoic acid by decarboxylation mechanism (ADNAN *et al.*, 2015). Moreover, ADNAN *et al.* (2015) found out that laccase from *T. artroviride* D03 did not generate toxic aromatic amines. Similarly, ZHENG *et al.* (2016) found that Acid Black 172 biodegradation by laccase does not produce toxic amines.

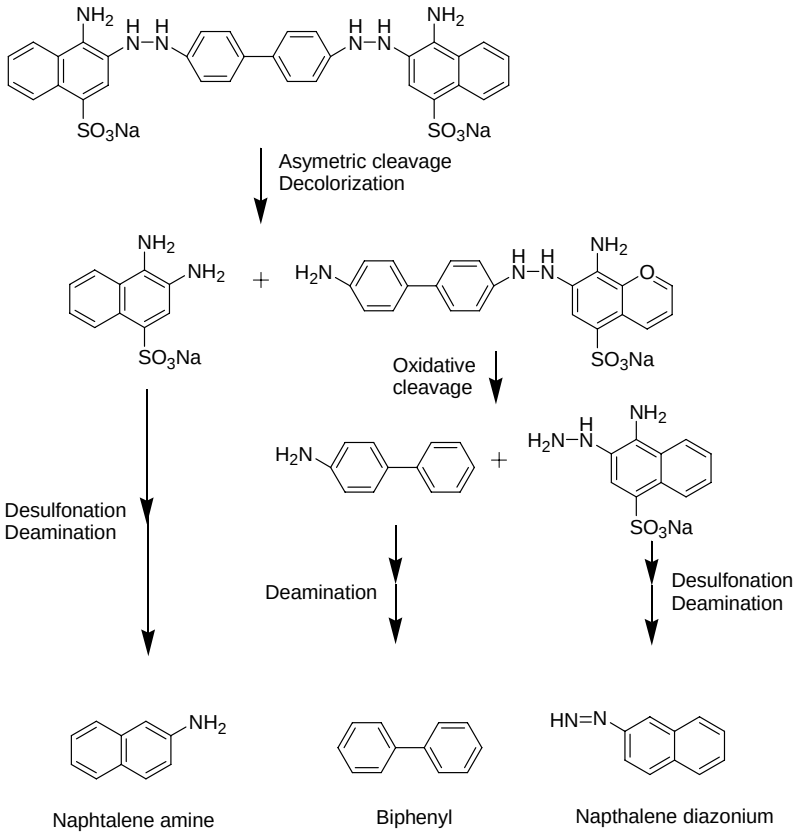

Fig. 3. Degradation mechanism of Congo Red by purified laccases from *Trametes pubescens* (SI *et al*., 2013).

3.2 Mechanisms of indigo dye degradation

Indigo dyes are organic compounds with a characteristic blue colour obtained from plant material such as *Indigo feratinctoria* L., *Indigofera suffructicosa Mill., Isatis tinctoria* L. and *Polygonum tinctorium.* At present these dyes are produced by chemical synthesis via Baeyer-Drewson reaction. Indigo dyes are non-soluble in water, alcohols and ether because these dyes contain intra- and inter-molecular hydrogen bonds. For the highest solubility of Indigo dyes in water it is necessary to solubilize the dye in water with a base such as sodium hydroxide. Indigo and its derivatives such as Indigo Carmine play a key role in various sectors such as the food, textile or cosmetic industries and medicine because of their colour but they are highly toxic and contact with skin or eyes may cause irritation.

The oxidation of Indigo dyes by laccases is observed as the sequential taking of four electrons from the molecule of Indigo. The first step in Indigo and Indigo derivatives degradation is electrochemical oxidation to dehydroindigo (BEGGIATO *et al*., 1993) followed by an attack of nucleophile (e.g. water) which lead to the incorporation of O-atoms into the degradation products (CAMPOS *et al*., 2001). The proposed pathway of Indigo degradation by laccase from *Trametes hirsuta* is shown in Fig. 4-A (CAMPOS *et al*., 2001). Laccases are able to degrade Indigo via the formation of isatine (indol-2,3-dion). Next isatine is degraded to anthranilic acid (2-aminobenzoic acid) spontaneously via decarboxylation of isatic acid, which is intermediate formed hydrolytically after isatine degradation.

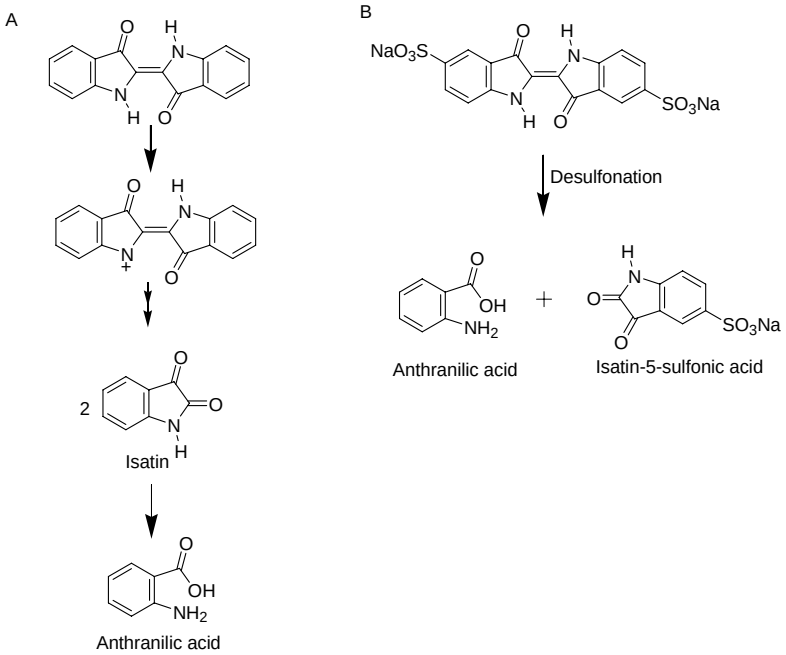

Fig. 4. Degradation mechanism of Indigo (A) and Indigo Carmine (B) by laccase from *Trametes hirsuta* (CAMPOS *et al.*, 2001; SINGH *et al.*, 2007).

YOUNES and SAYADI (2013) found that enzymatic degradation of Indigo Carmine by laccase from the fungus *Scytalidium thermophillum* reached 98 % decolorization after 24 hours. Toxicity level of degradation products was lower toward bacteria *Escherichia coli* and *Bacillus megaterium* than Indigo Carmine itself. The bacterial strain γ-proteobacterium JB and the white-rot fungus *T. hirsuta* were able to effectively degrade Indigo Carmine to anthranilic acid and isatin-5-sulfonic acid (SINGH *et al*., 2007), but higher amount of anthranilic acid was measured in the reaction system catalyzed by fungal laccase (98.3 %) than bacterial laccase (56.4 %) (Fig. 4-B). Additionally, laccase from *T. hirsuta* had the higher rate of reaction. These results were confirmed by CAMPOS *et al.* (2001) which found that degradation of Indigo is faster in the presence of laccase from the fungal strain *T. hirsuta* than laccase from the bacterial strain *Sclerotium rolfsii*, but the same degradation products (anthranilic acid and isatin) were created after biodegradation by both laccases. This difference in the reaction rate was caused by the different redox potential of selected laccases because fungal laccases have higher E^0 Cu T1 potential than bacterial laccases (MATE and ALCALDE, 2015).

3.3 Mechanisms of triphenylmethane dye degradation

Triphenylmethane (TPM) dyes belong to the group of older dyes with intense colour to be used in the textile, paper, food, cosmetic and leather industries and medicine. The basic structure of these dyes is triphenylmethane. TPM dyes are known as resistant to enzymatic decolorization and the process requires more time for dye degradation (FOROOTANFAR *et al*., 2012). Although SRIVASTAVA *et al.* (2004) did not confirm mutagenicity of TPM dyes such as Basic Green 4 and Acid Violet 17 (SRIVASTAVA *et al*., 2004), Malachite Green (MG) is a typical TPM dye used in farmed fish for controlling of protozoan and fungal infection. Degradation of MG by laccase as well as toxicity of MG and its degradation products has been extensively studied by many authors (KUMAR *et al*., 2012; ZHUO *et al*., 2015; YANG *et al*., 2015). Compared with phytotoxicity level of MG expressed by inhibition of seed germination, phytotoxicity levels of its degradation products were decreased. Similarly, decrease toxicity of TPM dyes after laccase treatment was observed in other studies (KALYANI *et al*., 2008; SATHISHKUMAR *et al*., 2013; YANG *et al*., 2015).

Laccases can oxidize the methyl carbon attached in TPM dye structure, giving stable products which are affected by *p*-substituted phenyl. BIBI *et al.* (2011) suggested that *N*-demethylation was the key factor of TPM degradation. CASAS *et al.* (2009) found that laccases are able to degrade TPM dyes, but non-substituted TPM dyes were not completely degraded by laccases. Laccase from *Cyathus bulleri* is able to degrade Basic Green 4 via demethylation. YANG *et al.* (2015) proposed two parallel degradation pathways of MG by laccase of *Cerrena* sp. (Fig. 5).

The first pathway starts with demethylation of MG followed by degradation or polymerization of MG for chromophore destruction. In the second pathway, MG is firstly hydroxylated to its carbinol form (FISHER *et al.*, 2011) which is quickly broken down. The presence of both pathways contributes to fast and efficient degradation of MG by laccases. The pathway is probably depended on laccase type and reaction conditions.

KUMAR *et al.* (2012) confirmed the formation of four different non-identified degradation products of MG after treatment by laccase from *Pleurotus ostreatus*. On the other hand, after degradation of Brilliant Green 1 closing related to MG by laccase from *Trametes versicolor* (E^0 Cu T1 = 785 mV vs. NHE; REINHAMMAR *et al*., 1972), the formation of only two compounds (benzoic acid and diethylamine) were observed (CASAS *et al*., 2009).

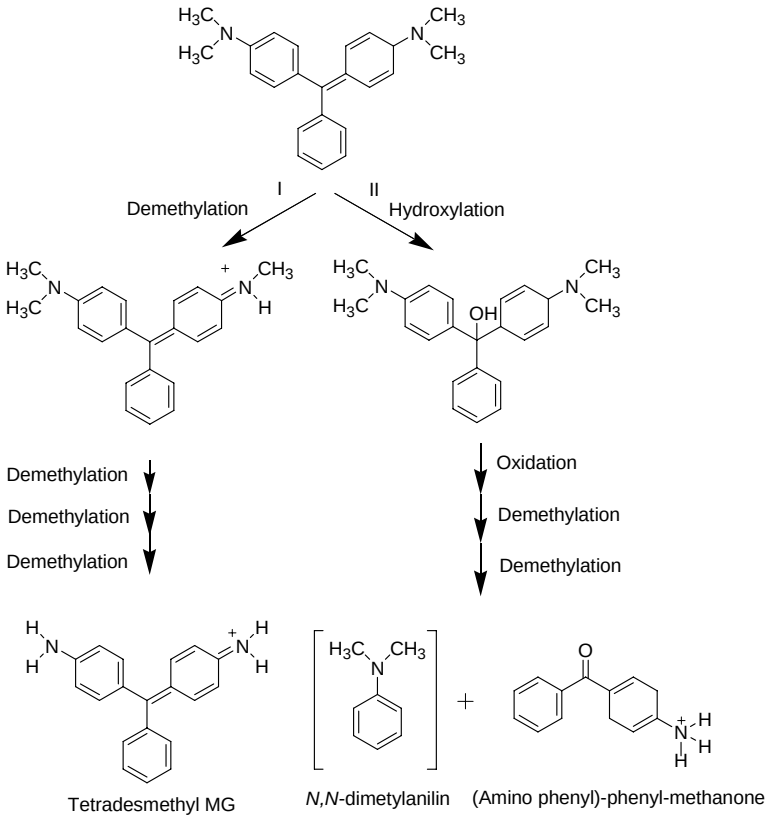

Fig. 5. Pathways (I, II) for Malachite Green (MG) degradation by laccase of *Cerrena* sp. (YANG *et al*., 2015).

3.4 Mechanisms of anthraquinone dye degradation

Anthraquinone dyes are the second most important class of textile dyes (BAUGHMAN and WEBER, 1994). They provide a wide range of colours including violet, blue and green and show excellent long-term colour stability (MENG *et al*., 2003). The oldest dye from this group is Alizarin which was extracted from the root of durable plant *Rubia tinctorum* for the first time. The π-conjugated electron system is delocalized in the core and in the substituents. The important anthraquinone dyes

commonly used in textile production process are Acid Blue 129 and Remazol Brilliant Blue R (RBBR). These dyes were used as reactive dyes for polymeric dye production and represent the class of often toxic and recalcitrant pollutants.

There are several experimental works (YANG *et* al., 2009; ZENG *et al*., 2012; AFREEN *et al*., 2016) concerning the study of an anthraquinone dye decolorization by laccases. Laccases have been shown to decolorize anthraquinone dyes more efficiently than other classes of dyes (ZENG *et al.*, 2011). Additionally ZENG *et al.* (2012) described anthraquinone dyes acting as redox mediators for azo dye decolorization catalyzed by laccases. During the degradation of anthraquinone dyes by laccase, the chromophore of dye can be broken down forming smaller molecules with potential lower toxicity levels. OSMA *et al*. (2010) observed biodegradation of RBBR by laccase from *T. pubescens* and suggested the reaction pathway including the identified intermediates and degradation products (Fig. 6-A). During RBBR degradation by laccase was observed reduction, hydroxylation, deamination, and oxidation reactions. In the later work of HADIBARATA et al. (2011) confirmed the effective decomposition of RBBR dye by laccase from *Polyporus* sp. S133 (Fig. 6-B) (HADIBARATA *et al*, 2011). The analogous deamination reaction is not observed in RBBR biodegradation by laccase from *Polyporus* sp. S133 resulting in products which contain an amino group in their structure.

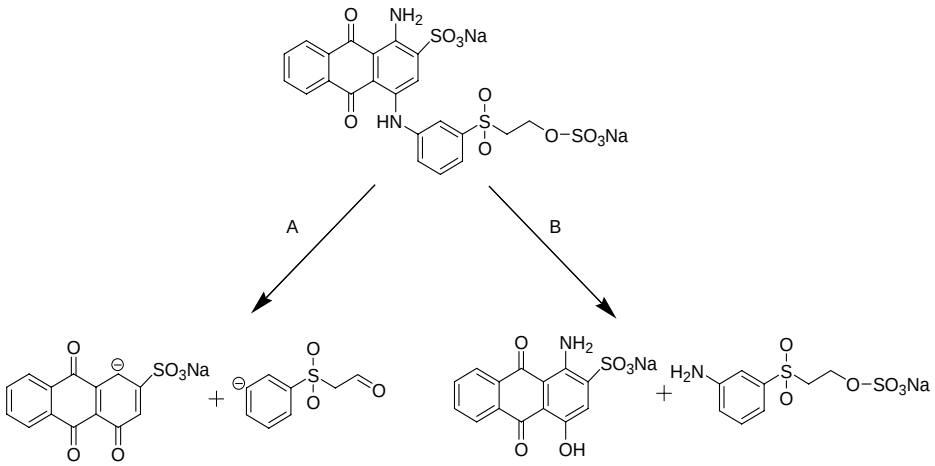

Fig. 6. Pathway for Remazol Brilliant Blue R degradation by the laccase of *Trametes pubescens* (A) (OSMA *et al*., 2010) and laccase of *Polyporus* sp. S133 (HADIBARATA *et al*, 2011).

Although HADIBARATA *et al.* (2011) found the presence of low-molecular weight products after laccase-catalyzed reaction but toxicity levels of these products were not lower. On the other hand, OSMA *et al*. (2010) evaluated phytotoxicity of RBBR and its degradation products after laccase treatment using ryegrass as a representative of grasslands species. Degradation products of RBBR were still toxic for ryegrass (germination index – GI of 69 %) but less than original dye (GI of 26 %).

4. Conclusion

It has been demonstrated that laccases are able to degrade synthetic dyes with different chemical structures. The efficiency of biodegradation is primarily affected by type of laccase-producing organism which determines the redox potential of laccases, different substrate specificities and, last but not least, rates of dye biodegradation. While bacterial and selected plant species produce laccases with a low-redox potential, filamentous fungi produce laccases with medium- or high-redox potential. The white-rot fungi group is particularly interesting in this respect, as producer of high-redox potential laccases. The prediction of degradation products formation after laccase treatment of synthetic dyes is a valuable tool to the evaluation of their potential toxicity. The experimental results presented in the literature suggest that degradation products that form in laccase-catalyzed reaction with synthetic dye have lower toxicity than dye itself.

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