Fungal Laccase as a Green Catalyst

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Abstract—Laccases belongs to multinuclear copper containing oxidase and can act as a variety of aromatic and non-aromatic compounds. Due to their broad substrate specificity, they are considered as a promising candidate in various industrial and biotechnological sectors. They are regarded as a green catalyst in biotechnology. The present review focuses on structure, reaction mechanism, categories, applications, economic feasibility, limitations, and future prospects of fungal laccases. Thus, this review would help in understanding laccases along with the areas, which has not been focused and requires attention. Since past, immense work has been carried out on laccases; yet, new discoveries and application are ever increasing which includes biofuel, biosensor, fiber board synthesis, bioremediation, clinical, textile industry, food, cosmetics, and many more.

Key words—Bioremediation, Catalyst, Mediator, Oxidoreductase, Multicopper, Antibiotics.

I. INTRODUCTION

Laccases are p-diphenol:dioxygen oxidoreductase [EC 1.10.3.2] belonging to the family of multicopper proteins. It was first isolated from the sap of Japanese lacquer tree Rhus vernicifera [1] and are widely distributed in nature being found in plants.

Fungi [2-4] insects and bacteria first bacterial laccase was discovered in Azospirillum lipoferum. Since bacterial laccases have low redox potential [5] fungal laccases [6] are more preferred owing to their high redox potential [7-9]. Laccases have the ability to oxidize a wide range of aromatic and non-aromatic compounds which includes substituted phenols, some inorganic ions, and variety of non phenolic compounds [10] Due to its low substrate specificity it can act on a broad range of substrates and has attracted considerable attention in different environmental, industrial and biotechnological sectors [11-12]. Laccases have been regarded as a Green Tool, because they require molecular oxygen (O₂) as the only co-substrate for bio-catalysis and not hydrogen peroxide H₂O₂. Laccases have the ability to reduce dioxygen to water by oneelectron oxidation of substrate [13] which is mainly substituted phenolic compounds.

Laccases have high catalytic efficiency and are used for technical applications in various industrial and biotechnological domains [14-15] which includes improving properties of fibers, biosynthesis, energy exploitation, environmental protection, biodetection, degradation of synthetic dyes, printing and dyeing industry, biopulping in paper industry, conversion of aromatic compounds [16] and removal of phenols which causes cancer and teratogenicity when present in waste water [17]. In addition, it is also used in fast moving consumer goods as toothpaste, mouthwash, detergent, soap, and diapers in cosmetics as deodorants; in beverage and food industry for wine and juice stabilization [18-19] in dough or baked products to increase strength of gluten structures in pharmaceutical industries as anesthetics, anti inflammatory drugs, antibiotics, and sedatives and in nanobiotechnology as nanoparticle based biosensors. However, laccases are not able to exhibit full efficiency under harsh conditions. Therefore, novel strains which can tolerate harsh conditions and give maximum enzyme production with minimum energy consumption are in huge demand.

The present review is an attempt to provide cumulative information on various aspects of fungal laccases, which includes information pertaining to the structure, reaction mechanism, categories, and industrial and biotechnological
application of laccase.

II. STRUCTURE AND REACTION MECHANISM OF LACCASE

Laccases are known to exist in a variety of forms; they can be monomeric, homotetrameric, heterodimeric, and multimeric. Their molecular weight ranges from 50 to 130 kDa depending upon the organism [20]. The plant laccases approximately contains 45% carbohydrate content, whereas for fungal laccases, it is 10-30% of molecular weight [21]. It is assumed that the carbohydrate portion of laccase ensures the conformational stability of the protein part and protects the enzyme from proteolysis and inactivation by radicals. The primary structure of laccasses consists of Greek key β barrel topology which constitutes of approximately 500 amino acid residues organized in three consecutive domains. These amino acids are distributed in three domains: first domain with 150 initial amino acids, second domain with 150 and 300 amino acid, and third domain with 300-500 amino acids. The stabilization of laccase structure is due to the presence of disulphide bonds between domains I and II and between domains I and III [22]. However, in Melanocarpus albomyces, three disulfide bridges are present of which one is inside domain I, another between domain I and domain III and the last one between domain II and III [23]. Laccases are known to exist in four different Cu catalytic forms per protein unit. These four Cu ions are divided into three types of structures:

TYPE-1: PARAMAGNETIC BLUE COPPER

Copper type-1 confers blue color to multicopper proteins, which is due to the intense electronic absorption caused by the covalent copper cysteine bond. Due to its high redox potential of +790 mV substrate oxidation takes place at the type-1 copper site and has an absorbance at 610 nm type-1 copper has a trigonal coordination, with two histine and a cysteine as conserved equatorial ligands and one position usually variable, and in case of fungal laccases, the axial ligand is leucine or phenylalanine. It has even been argued that the axial position ligand influences the oxidation potential of the enzyme, which possibly provides the mechanism for regulating its activity [24].

TYPE-2: PARAMAGNETIC NORMAL/NON-BLUE COPPER

Copper type-2 or normal Cu site is characterized by the lack of strong absorption features in the visible region and reveals usual electron paramagnetic resonance (EPR) spectra. Type-2 copper is coordinated by two histidines residues and is strategically positioned close to type-3 copper.

TYPE-3: DIAMAGNETIC SPIN COUPLED COPPER-COPPER PAIR

It is a binuclear center regulated by six histidines and spectroscopically characterized by an electron adsorption at 330 nm oxidized form and absence of an EPR signal due to the strong antiferromagnetical coupling between the two type-3 copper atoms which is related to the presence of a hydroxyl bridge. The type-2 copper and type-3 copper form a trimuclear cluster where molecular oxygen is reduced and release of water takes place. An example of one electron oxidation of phenolic hydroxyl groups, while reducing oxygen and forming phenoxy radicals along with water are represented below [25]:

\[
4\text{Phe—OH} + \text{O}_2 \xrightarrow{\text{Laccas}} 4\text{Phe—O}^\cdot + 2\text{H}_2\text{O}
\]

The Type-3 copper centers also have common feature of another protein superfamily which includes the tyrosinases and haemocyanins [26] figure-1.

REACTION MECHANISM OF LACCASE: DIRECT AND INDIRECT OXIDATION

The basic reactions catalyzed by laccase can be of two types: direct oxidation and in direct oxidation. The direct oxidation involves the oxidation of substrate to the corresponding radical as a result of direct interaction that occurs with copper cluster. However, in certain reactions, direct oxidation is not feasible as laccase can only oxidize those compounds whose ionization potential does not exceed redox potential of T1 copper ion. Nevertheless, the limitation can be overcome by the use of mediator which is a two step process: first enzyme catalyzes the oxidation of the mediator and then the oxidized mediator oxidizes the substrate. However, for the reaction to occur without any obstruction, certain features should be exhibited by the mediator: (a) the reaction should occur without any hindrance it must be good substrate for laccase both in its oxidized and reduced forms, should be stable; it must not inhibit enzymatic reaction, and conversion must be cyclic in nature [27] shown in figure.2
Besides mediators, the use of inducer to enhance laccase production has been widely practiced in fungi especially in the white rots where metals, aromatic compounds, and phenolic compounds [28] have been used as inducers. Conversely, there are certain substances which can inhibit the production of laccase and are known as the inhibitors such as sodium azide and dithiothreitol DTT.

YELLOW LACCASE

Yellow laccase is artificially reduced blue laccase as it does not have absorption at 600 nm and EPR spectrum [29]. Alteration of yellow to blue laccase can occur by the reduction of type-I copper site by aromatic product of lignin degradation or binding of specific amino acid of enzyme polypeptide to a molecule of modified product.
produced by lignin degradation; it can also be due to heterogeneity induced by glycosylation. The modified molecule bound to the apoenzyme performs the function of electron transfer mediator analogous to the role of 2,2'-azinobis (3-ethylbenzthiazoline - 6-sulfonic acid) diaminonium salt (ABTS) or other compounds in the reaction of blue laccase, hence having high redox potential which allows them to oxidize non phenolic compounds without any mediators and having greater industrial potential [30]. The change in protein conformation may explain the sensitivity of yellow laccase to CO and other inhibitors, e.g., P. tigrinus. At present, information about the purification and characterization of yellow laccase is extremely limited and their catalytic properties are still seldom investigated. Much work has not been done on the yellow laccase, but a few strains which are reported for the production of yellow laccase are as follows P. tigrinus [29], S. aeruginosa, G. fornicatum [30], P. ostreatus [31], Panustigrinus, Phlebia radiate, Phlebia tremellosa, Pleurotus ostreatus D1 (YLPO), Sclerotinia sclerotiorum, and Panustigrinus [30].

WHITE LACCASE

The white laccase exhibits neutral pH and has anomalous metal content which is responsible for its unique characteristic [32]. It exhibits absorption peak at 400 nm but absence of peak at 605 nm T1 copper site and 330 nm (T3 binuclear copper). White laccase does not exhibit EPR spectra as well as T1 and T2 signals [33]. The reason for the colorlessness iron, but it was conferred that the lack of absorbance at 605 nm can be due to the incomplete oxidation state of copper which has fully occupied electron configuration of d10 and no d-d transition [33] and may be responsible for extra high activity of protein. White laccase has been considered under laccase family, because the primary structure of the white laccase is identical to those of known laccase and it uses oxygen (O2) as an oxidative substrate. There is the absence of the formation of hydrogen peroxide (H2O2) as the product of catalyzed reaction and substrate specificity exhibited is also the same as that of known laccase [32], e.g., Lepista nuda molecular mass of 56 kDa [34].

Few strains reported for white laccase production include Pleurotus ostreatus [32] and Myrothecium verrucaria NF-05 [33]. These strains besides single copper atom consist of various.

LACCASE MEDIATOR

The efficiency of substrate oxidation by a laccase depends on the difference between the redox potentials [35] of the substrate and the T1 Cu. Due to the lower redox potentials of laccases (<1V) [36] laccases are originally thought to be able to oxidize only the phenolic ligninmoiety, with the majority of lignin being nonphenolic and with higher redox potentials. Low molecular- weight redox mediators are used to expand the laccase substrate range or increase the reaction rate, especially for substrates with higher redox potentials or too large to fit in the enzyme’s active site. Commonly used laccase mediators include synthetic mediators such as 2,2’-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and 1-hydroxybenzotriazole (HBT) as well as natural phenolic mediators such as syringaldehyde and acetosyringone. Despite the proven efficiency of artificial mediators, natural mediators (believed to be true mediators of fungal laccases in nature) are considered to be alternatives to the artificial ones because they are more economically feasible and environmentally friendly. Laccase oxidation of the substrate may proceed differently with a mediator. However, it is not always the case. Malachite green degradation products in the presence and absence of ABTS have been shown to be identical or different, depending on the enzyme.

Different types of mediators have different catalytic mechanisms ABTS mediated substrate oxidation proceeds via an electron transfer route. ABTS is first oxidized to its radical cation (ABTS+) and then to the dication (ABTS2+) with redox potentials of 472 and 885 mV, respectively. Unlike ABTS, an N-OH type mediator (such as HBT and violuric acid) forms the N-oxy radical upon laccase oxidation and subsequent deprotonation the radical in turn abstracts the benzylic hydrogen atom from the substrate. Similarly, phenolic mediators also follow a radical hydrogen abstraction mechanism, but with the intermediate being a phenoxy radical. The effect of a mediator on laccase oxidation varies with the laccase and substrate and depends on the radicals formed, recyclability of the mediator and stability of the laccase in the presence of the mediator [37]. Regardless of the reaction mechanism, mediators incur additional costs, and can cause toxicity [38] and laccase inactivation [39]. Although, laccases without the requirement for facilitating mediators, the laccase/mediator system is regarded as a feasible industrial solution, ideal mediators that are cheap, green, effective, stable, recyclable, not toxic, or enzyme-inactivating should be ascertained [40].

LACCASE IMMOBILIZATION

Laccases are immobilized for recycling, operational stability, and resistance to application conditions. Immobilization techniques include entrapment, adsorption, covalent binding, self immobilization as well as combinations of the aforementioned techniques. Activity recovery varies based on the enzyme, the
immobilization method of choice, and preparation parameters. Compared with their free counterparts, immobilized laccases are more tolerant to high temperatures and storage and can be reused multiple times [41-42]; they are also more resistant to inhibitors such as NaCl. Immobilization sometimes improves the catalytic activity of laccases [43-45] despite the common concern of reduced enzyme flexibility, steric hindrance and diffusion limitations [46]. Readers can refer to reviews on preparation and applications of immobilized laccases [41-42].

III. LACCASE APPLICATIONS IN BIODEGRADATION OF PPCPs

The value of fungi as well as fungal enzymes in pollution control and environment management has been recognized. Examples of environmentally important enzymes comprise hydrolases, laccases, lyases, peroxidases, tyrosinases, and P450 cytochrome mono oxidases [47-50]. The ability of laccases to effectively degrade and detoxify a variety of persistent organic pollutants (POPs) has received considerable attention in the field of bioremediation [51-53] and laccases can also be used in enzymatic biosensors for environmental pollution monitoring [48]. A summary of environmental contaminants as laccase substrates is provided in (Table-1). The contaminants investigated include dye stuffs [54-55], polycyclic aromatic hydrocarbons (PAHs) [56], endocrine disrupters [51, 57] and pesticides [58] shown in figure-3.

Pharmaceuticals and personal care products (PPCPs) are becoming ubiquitous in the environment and are recognized as emerging trace organic contaminants [59-61]. Laccases can be employed for their removal [51]. Laccases have been used in PPCPs as an ingredient; many products generated by laccases have antimicrobial, anticancer, antioxidant, detoxifying, or other activities [62]. Specifically, laccases can be used to synthesize novel antibiotics and laccase based antimicrobial formulations are considered as a green alternative to chemical decontamination. Nonetheless, the focus of this review lies in the degradation and detoxification of PPCPs contaminants with laccases.

DEGRADATION OF ANTIBIOTICS

Antibiotics constitute one of the most used classes of drugs in the world; they are used in human and veterinary medicine as well as livestock farming. Antibiotics that are not metabolized enter the environment. Conventional water treatment processes cannot effectively remove antibiotics [61], while more efficient advanced treatment methods have disadvantages such as high costs and secondary pollution. Antibiotics pose health risks by selecting for antibiotic resistance bacteria (ARB). Antibiotics, ARB, and antibiotic resistant genes have been detected in soil, sediments, and water bodies including waste water drinking water and marine water. There has been a fast growth in the literature describing laccase
utilization in antibiotic removal, especially within the past 2 years, but this topic has not been properly reviewed.

Target antibiotics under investigation include penicillins, tetracyclines, sulfonamides, quinolones, trimethoprim, sulfamethoxazole and tetracycline are two most studied (Table-2). The removal time ranges from minutes to hours, depending on the laccase, antibiotic and treatment parameters. Mediators such as HBT, ABTS, and SA are often used to enable or accelerate antibiotic conversion by laccases. Infact, significant antibiotic removal within 1 hrs usually requires involvement of an appropriate mediator [63]. Manganese peroxidase was more efficient in tetracycline conversion than laccase, but the addition of HBT can promote laccase catalysis to a rate higher than that of manganese peroxidase although still slower than that of lignin peroxidase (95%) degradation efficiency in 5 min. Interestingly, mediators, i.e., ABTS, SA, and AS, are consumed without observed catalytic activity during degradation of sulfamethoxazole [64]. Sulfonamides and tetracyclines are more easily attacked by laccase compared with quinolones [65].

| Compound | Laccase | Enzyme From | Mediator | Reference |
|----------|---------|-------------|----------|-----------|
| Phenol   |         |             |          |           |
| Chlorophenols, cresols, nitrophenols | Trametessanguineus laccase expressed in Trichoderma atroviride | In culture | - | [54] |
| Technical nonylphenol | Phomasp. UHH5-1-03 | Free | SA | [76] |
| Oxybenzone, pentachlorophenol | P.ostreatus | Free | ABTS, HBT, HPI, TEMPO, SA, VA, VAN | [30] |
| 4-tert-butylphenol,4-tert-octylphenol | Myceliophthorathermophilalaccase expressed in Aspergillus oryzae (Novozyme) | Enzymatic membranereactor | SA | [72] |
| 2,4-dichlorophenol | Pycnoporus sanguineus CS43 | Free | - | [37] |
| DYESTUFFS | | | | |
| Bromo phenol Blue, Congo Red, Coomassie Blue, Tripan Blue | T. sanguineus laccase expressed in T. atroviride | Free | - | [54] |
| Acid Black 172, Congo Red, Crystal Violet, Direct Fast Blue FBL, Indigo Blue, Naphthol Green B, Methylene Blue, Neutral Red, Reactive Brilliant BlueX-BR, Remazol Brilliant Blue Reactive (RBBR) | T. pubescens | Chitosanbeads | - | [10] |
| Acid Orange 67, Basic Red18, Basic Yellow 28, Direct Black 166, Direct Yellow 107, Disperse Yellow 79 | Paraconiothyrium variabile | Free | HBT | [64] |
| Substance | Organism | Condition | Reaction | Ref. |
|-----------|----------|-----------|----------|------|
| Brilliant Blue-G, Brilliant Blue-R, Bromo phenol Blue, Coomassie Blue R250, Crystal Violet, Malachite Green, Methylene Blue, Xylene Cyanol, RBBR | *P.sanguineus* | Free | VA | [30] |
| RBBR | *Cerrena* sp.HYB07 | Cross-linked enzyme aggregates | - | [43] |
| Coomassie Blue-R 250 | *Cerrena* sp.HYB07 | Cross-linked enzyme aggregates | ABTS, AS, HBT, SA, SYA | [73] |

**ENDOCRINE DISRUPTERS**

| Substance | Organism | Condition | Reaction | Ref. |
|-----------|----------|-----------|----------|------|
| Bisphenol-A (BPA) | *Coriolopsis gallica, Bjerkanbactera adusta, T. versicolor* | Free | HBT | [38] |
| BPA | *T.sanguineus* laccase expressed in *T. atroviride* | In culture | - | [54] |
| BPA | *T.versicolor* laccase expressed in *S.Cerevisiae* | Surface display | ABTS | [61] |
| BPA | *M.thermophila* laccase expressed in *A. oryzae* (Novozyme) | Ongranular activated carbon, continuous flow packed-bed reactor | - | [72] |
| BPA, 17α-ethyl estradiol | *T.versicolor* | Polyamide- 6/chitosan fibers | - | [72] |
| BPA, 17α-ethyl estradiol | *Phomasp. UHH5-1-03* | Free | SA | [77] |
| BPA, 17α-ethyl estradiol, 17α-estradiol, 17α-estradiol 17- acetate, estriol, estrone | *M.thermophila* laccase expressed in *A. oryzae* (Novozyme) | Enzymatic membrane reactor | SA | [72] |

**POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)**

| Substance | Organism | Condition | Reaction | Ref. |
|-----------|----------|-----------|----------|------|
| All-15 USEP ApriorityPAHs | *B.subtilis* Cot Aexpressed in *E.coli* | Free | ABTS | [56] |
| Naphthalene, phenanthrene | *T.versicolor* (Sigma-Aldrich) | Non ionic surfactant-modified clay | - | [56] |
| Benzo [a] pyrene, phenanthrene | *T.sanguineus* laccase expressed in *T. atroviride* | Free | - | [54] |

**PESTICIDES**

| Substance | Organism | Condition | Reaction | Ref. |
|-----------|----------|-----------|----------|------|
| Atrazine | *P.ostreatus* | Free | ABTS, HBT, HPI, TEMPO, SA, VA, VAN | [30] |
| Atrazine, isoproturon | *O.sativa* laccases expressed in *P.pastoris* | Inculture | - | [58] |
| Compound                          | Laccase                                      | Enzyme from                              | Reaction parameters                                                                 | Efficiency     | Toxicity after treatment              | References |
|----------------------------------|----------------------------------------------|-------------------------------------------|-------------------------------------------------------------------------------------|----------------|----------------------------------------|------------|
| **PENICILLINS**                  |                                              |                                           |                                                                                     |                |                                        |            |
| Amoxicillin, ampicillin,         | *T. versicolor*                               | Enzymatic membrane reactor               | 10µg/L antibiotics, 1m MSA, starting pH 6, 25°C, 0.07m/s flow, tangential configuration | 54–100% after 24h | Increased (B.subtilis and V. fischeri) | [39]       |
| cloxacillin, penicillin-G,       | (Sigma-Aldrich)                              |                                           |                                                                                     |                |                                        |            |
| penicillinV, oxacillin           |                                              |                                           |                                                                                     |                |                                        |            |
| **SULFONAMIDES**                 |                                              |                                           |                                                                                     |                |                                        |            |
| Sulfapyridine, sulfathiazole     | *T. versicolor*                              | Free                                      | 16-20 mg/L antibiotic, 50-55 U/L laccase, 0.8 mM VA, pH 4.5, 25°C, 135 rpm            | 100% after 8h  | NR                                     | [74]       |
| Sulfadimethoxine, sulfamonometh oxine | *Perenni poriastrain* TFRI 707               | Free                                      | 50 mg/L antibiotic, 6 U/mL, 1 mM ABTS or VA, pH4.1.30°C, 8% glycerol                 | T (1/2)        | NR                                     | [79]       |
| Sulfadimethoxine, sulfamonometh oxine | *Perenni poriastrain* TFRI 707               | Free                                      | 50 mg/L antibiotic, 6 U/mL laccase, 8% glycerol; 1 mM ABTS, pH 4, 50-60°C; 1 mM VA, pH 4; 2m MSA, pH 6, 50°C | 100% after 30 min with ABTS; 100% after 15 min with VA; >95% after 60 min with SA | Reduced (V.fischeri) with VA and HBA; increased with ABTS and SA | [40]       |
| Sulfamethoxazole                 | *T. versicolor*                              | Free                                      | 1,100 µg/L antibiotic, 1mM HBT, 25°C, 70 rpm                                         | 41% after 22 h | NR                                     | [48]       |
| Sulfamethoxazole                 | *M. thermophila* laccase expressed in A. oryzae (Novozyme) | Enzymatic membrane reactor               | 830 µg/L d antibiotic, 70-100 µM/ min laccase, 5µ MSA, 3g/L granular activated carbon | 65%            | Increased (Tox Screen3 assay with *Photobacterium leiognathi*), which can be reduced by granular activated carbon addition | [69]       |
| Sulfadiazine, sulfamethazine,    | *Echinodontium taxodii*                      | Oriented immobilization on                | 50 mg/L antibiotic, 0.2 U/mL laccase, 1m MAS, SA or SYA, pH 5                       | >95% after 30 min | Reduced (E.coli and S. aureus)        | [67]       |
| sulfamethoxazone                 |                                              |                                           |                                                                                     |                |                                        |            |
| Antibiotic | Animal source | Immobilization | Conditions | Reduction (%) | Notes |
|------------|---------------|----------------|------------|---------------|-------|
| Sulfamethoxazole | *T. versicolor* | Free | 73-93 µM antibiotic, mediator/laccase ratio: 1.1 (ABTS), 1.7 (SA) or 2.4 (AS), pH 6, 25°C, static | 100% | Reduced (algae *Pseudokirchneriella subcapitata*) |
| Sulfamethoxazole, sulfathiazole | *T. versicolor* | On porous silica beads | 50 mg/L antibiotic, 1U/mL laccase, 1mM HBT, pH 5.40°C, 50rpm | 76-85% after 1h | Reduced (E. coli, *P. aeruginosa*, *H. influenzae*, *S. enterica*, *S. aureus*, *S. pneumoniae*) |
| Sulfadimethoxine | *T. versicolor* | Free | Pergram soil: 2µg antibiotic, 10 U laccase, 8µmol ABTS or HBT, 1mg peat; room temperature | >90% after 72 h | NR |
| Sulfamethoxazole | *T. versicolor* Lac3 expressed in *S. cerevisiae* | Surfacesisplay | 30 µM antibiotic, 0.25 U/mL laccase, pH 5, 37°C, 250 rpm | 44% after 30h | NR |

**TETRACYCLINES**

| Antibiotic | Animal source | Immobilization | Conditions | Reduction (%) | Notes |
|------------|---------------|----------------|------------|---------------|-------|
| Tetracycline | *T. versicolor* (Sigma-Aldrich) | Enzymatic membrane reactor | 20 mg/L antibiotic, 0.002g/L laccase, pH 6, 25°C, batch | 0.34 mg/h for 10d | NR |
| Tetracycline | *T. versicolor* (Sigma-Aldrich) | Enzymatic membrane reactor | 20 mg/L antibiotic, 10 g/L laccase, 1.4µm poresize, 25 cm length, tangential (10L/h), 25°C, 8 L/h/m² permeation | >200 mg/h/m² for 24h | NR |
| Tetracycline | *T. versicolor* (Sigma-Aldrich) | Free | 100 µg/mL antibiotic, 17.5 µg/mL laccase, pH 7, 20°C | 78% after 18h | Reduced (*B. subtilis*) |
| Chlorotetracycline, doxycycline, oxytetracycline | *T. versicolor* (Sigma-Aldrich) | Enzymatic membrane reactor | 10µg/L antibiotics, 10µ MSA, starting pH 6, 25°C, 0.07 m/s flow, tangential | 85–98% after 24h | Increased (*B. subtilis* and *V. fischeri*) |
| Antibiotic          | Organism                        | Configuration                              | Concentration | pH | Temperature | Oxidation Efficiency | Change in Bacteria (Ref) |
|---------------------|---------------------------------|--------------------------------------------|---------------|----|-------------|----------------------|-------------------------|
| Tetracycline        | Cerrena sp.HYB07                | Magnetic cross-linked enzyme aggregates    | 100 µg/mL     | 6  | 25°C        | 80% after 12h         | Reduced (E.coli and B. licheniformis) [66] |
|                     |                                 |                                            | 40U/mL antibiotic, pH 6, 25°C               |               |             |                      |                         |
| Flumequine          | T. versicolor (Sigma-Aldrich)   | Free                                       | 90 mg/L      | 6  | 39°C        | 98% after 2h          | NR                      | [65]                    |
|                     |                                 |                                            | antibiotic, 6U/mL laccase, pH4, 1.35mM ABTS |               |             |                      |                         |
|                     |                                 |                                            | pH 6, 60°C, 200 rpm ultrasound (75 W, 22 kHz, 50% duty cycle) |               |             |                      |                         |
| Ciprofloxacin       | A. oryzae                       | Free                                       | 10 mg/L      | 6  | -           | 51% after 5h          | NR                      | [76]                    |
|                     |                                 |                                            | antibiotic, 0.02% (w/v) laccase, pH 6, 60°C, 200 rpm ultrasound (75 W, 22 kHz, 50% duty cycle) |               |             |                      |                         |
|                     | T. versicolor (Sigma-Aldrich)   | Enzymatic membrane reactor                 | 10 µg/L      | 6  | 25°C        | 0-84% after 24h       | Increased (B. subtilis and V. Fischeri) [39] |
|                     |                                 |                                            | antibiotics, 10 or 1,000 µM SA, starting pH 6, 25°C, 0.07 m/s flow, tangential configuration |               |             |                      |                         |
|                     | T. versicolor (Fluka)           | Magnetic cross-linked enzyme aggregates    | 100 µg/L     | 7  | 20°C, 125 rpm | 47% after 6h; 60% after 12 h | NR                      | [69]                    |
|                     |                                 |                                            | antibiotic, 1 U/mL laccase, 0.1 mM ABTS, pH 7, 20°C |               |             |                      |                         |
|                     | Metronidazole                   | Enzymatic membrane reactor                 | 10 µg/L      | 6  | 25°C        | 25.9% after 24h       | Increased (B. subtilis and V. Fischeri) [39] |
|                     |                                 |                                            | antibiotic, 10 µM SA, starting pH 6, 25°C, 0.07 m/s flow, tangential configuration |               |             |                      |                         |

This is presumably due to the strong electron donating aromatic amine group in sulfonamides and the phenol group in tetracyclines, which are not found in quinolones. However, identified tetracycline transformation intermediates suggest that the phenol group is not the primary target for laccase oxidation, and that oxygen...
addition, demethylation, water elimination reactions occur during laccase treatment [66]. For sulfonamides, increasing electro negativity of the substituents is accompanied by decreased oxidation. Two sulfonamides, namely sulpyridine and sulfathiazole, are desulfonated by laccase. Covalent cross coupling of sulfonamides is observed with laccase and mediator SA or AS [67], but not ABTS [68]. Trimethoprim has 2 amine groups and 3 methoxy groups and is usually administered in combination with sulfamethoxazole. Little [69] to over 60% [70] degradation of this antibiotic without a mediator have been reported. Furthermore, SA at 1.000 µM, but not 10 µM, increases trimethoprim removal from 27 to 67%; nearly complete elimination of sulfamethoxazole is achieved under the same conditions [71]. Some antibiotics (e.g., penicillins) are unstable in aqueous solutions, and attention should be paid to sample preservation and quantification [71]. Laccase from T. versicolor, especially the product sold by Sigma-Aldrich, is most frequently used in biodegradation studies of antibiotics as well as other trace organic contaminants. Other laccases include laccases from basidiomycetes Cerrena sp. HYB07, Echinodon tiuntaxodii, Perenniporia strain TFR1 707 and P. sanguineus, from ascomycetes Phomasp. And Myceliophthora thermophila (recombinantly expressed in Aspergillus oryzae) and from action bacteria Streptomyces ipomoeae (expressed in E. coli). Laccases immobilized by different methods have been used for antibiotic degradation; including enzymatic membrane reactors [72-74] granular activated carbon [75] silica beads oriented immobilization [67] magnetic cross linked enzyme aggregates and cell surface display [65]. In particular, enzymatic membrane reactors (gelatin ceramic membranes grafted with commercial T. versicolor laccase) in tetracycline degradation have been evaluated in depth with respect to membrane preparation, efficiency, kinetics, and economics [71]. Mathematical cost estimation indicates that the enzymatic process is still economically uncompetitive. Improvements should be made in terms of enzyme kinetics, reactor effective temperature and regeneration costs. For example, a pore diameter of 1.4 µm, in contrast to 0.2 µm, increases enzyme loading of the membrane reactor, avoids extensive membrane area, and facilitates tetracycline degradation.

Occasionally, laccases do not participate in antibiotic removal by white rot fungi; for instance, laccase was not responsible for oxy tetracycline degradation by P. ostreatus or T. versicolor or sulfamethoxazole degradation by aquatic ascomycete Phomasp. UHH 5-1-03. In these cases, other enzymes, such as cytochrome P450, may be resorted to for biodegradation. It should still be pointed out that even extracellular laccase is not able to directly oxidize sulfamethoxazole, when a mediator is added significant removal is achieved. Laccases are also applied in combination with other processes in antibiotic treatment, such as ultrasound [76] and soil adsorption. The involvement of other processes facilitates degradation of antibiotics, e.g. quinolone antibiotics, which are recalcitrant to laccase oxidation. Laccase can also improve efficiency and stability of antibiotic removal by other organisms. When sulfamethoxazole is the transformed by non laccase producing bacteria Alcaligenes faecalis, the efficiency drops when some metabolites such as N4 acetyl sulfamethoxazole are transformed back to the parent compound. The removal efficiency does not decrease when the coculture of A. faecalis with laccase producing P. sanguineus is used or when cell free laccase was added to A. faecalis culture [77]. Toxicity of antibiotics after laccase treatment is commonly accessed via growth inhibition assay or bioluminescence inhibition test. Antibiotic degradation by laccase mostly leads to reduced toxicity. A good example comes from the comparison of the sulfamethoxazole transformation products and their toxicity by A. faecalis with or without exogenous laccase, N-hydroxy sulfamethoxazole (HO-SMX), a toxic and recalcitrant intermediate of sulfamethoxazole, is formed upon A. faecalis treatment. Additional laccase, on the other hand, eliminates HO-SMX along with the toxicity. However, sometimes laccase/mediator catalyzed antibiotic transformation results in even higher toxicity and this seems to frequently associate with the mediator SA [77]. It is postulated that the enhanced toxicity can be derived from oxidation of aromatic structures, especially phenols, to quinonoids.

The majority of studies on antibiotic degradation were carried out in aqueous environments, but there have been a few studies on remediation of soil, river sediment and sludge [78]. Laccase containing extract from spent mushroom compost of Pleurotus eryngii and extract containing micro capsules enhanced degradation of three tetracyclines in river sediment as well as degradation of three sulfonamides in sewage sludge. Sulfonamide antibiotics can form stable covalent bonds with humic constituents, and laccase can catalyze unreactive hydroquinone moieties in hemic acid to reactive, electrophilic quinone moieties which in turn react with the antibiotic. This will affect the fate, bioactivity, and extractability of sulfonamides in soils.

**IV. CONCLUSION**

This review clearly professed that laccase is one of the standout enzymes being used in peculiar areas of biotechnology such as medicine, bioremediation,
mediator which includes improving properties of fibers, biosynthesis, energy exploitation, environmental protection, biodetecion, degradation of synthetic dyes, printing and dyeing industry, biopulping in paper industry, conversion of aromatic compounds and removal of phenols which causes cancer and teratogenicity when present in waste water. In addition, it is also used in fast moving consumer goods (FMCG) as tooth-paste, mouthwash, detergent, soap, and diapers in cosmetics as deodorants; in beverage and food industry for wine and juice stabilization.

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