Immobilized Laccase: A Promising Bioremediation Tool for the Removal of Organic Contaminants in Wastewater

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Contents

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Abstract Laccase, an incredible enzyme, has a wide prospective in bioremediation processes, mainly due to its relative broad oxidation capacity, the lack of requirement of cofactors, and the use of readily available oxygen as the final electron acceptor. However, the large-scale application of laccases in bioremediation necessitates immobilization/insolubilization of the biocatalysts to enhance their operational stability. With the burgeoning use of laccases in wastewater treatment, several state-of-the-art methods have been developed over the past few years to immobilize laccase, derived from various microbial sources, in order to enhance the selectivity, activity, stability, and reusability. Recent advances in these immobilization methods offer promising solutions to the limitations of soluble enzymes, such as poor reusability due to poor recoverability, low stability, and high costs, to name a few. This article is intended to review the various recent methods employed for immobilization or insolubilization of laccase and its use in treating various types of organic contaminants in wastewaters including those from olive mill, pulp and paper, biorefinery, municipal, hospital, and textile industries. Furthermore, to improve the potential of the laccase-based biocatalytic system against wastewater/pollution treatment, co-immobilization of enzymes such as tyrosinase, peroxidase, and glucose oxidase, with laccase, would serve as a promising bioremediation tool for treating the organic contaminants in industrial and municipal wastewater. The concept and approach of this review also renders knowledge on a yet unexplored focus on the pioneering advances on the development of immobilized laccase-based reusable biocatalysts, which could be employed for treatment of industrial and hospital wastewater.

1 Laccase: A Bioremediation Tool

The global rise in environmental contaminants has led to a reduction in the quality of life, which has become a major concern in recent years. The root of these contaminants can be mainly traced to the waste treatment plants, and the source and type of treatment is determined by the nature of these contaminants (Pal et al. 2010). Typically, wastewater treatment focuses on remediation of the effluent in order to meet the regulations of the effluent rather than using the treated water in the industrial process. Despite the development of several wastewater remediation processes, effective and rapid large-scale water treatment still remains a challenging problem (Dignac et al. 2000). On the other hand, with the advances in enzyme technology over the past decade, an increased interest in the use of enzymes in wastewater treatment has been noted. Among these enzymes, laccase (EC 1.10.3.2) has been widely exploited as a biocatalytic tool for various industrial applications including wastewater treatment due to its ability to utilize a broader substrate range and molecular oxygen for catalysis (Gasser et al. 2014). Moreover, the ability of laccases to transform phenol and non-phenolic compounds by oxidation process makes them an excellent choice for the remediation of a wide range of contaminants

(Couto and Herrera 2006). Laccases are multicopper oxidases, with a potential to oxidize a broad range of phenolic compounds including amino phenols, *ortho*- and *para*-diphenols, polyphenols, and aromatic and aliphatic compounds, which are coupled with electron reduction of O_2 to H_2O (Strong and Claus 2011). Laccases can degrade potential contaminants, making them less toxic and imparting high bioavailability, which can be removed by conventional physical or mechanical methods. During the laccase-mediated bioremediation process, the redox potential (E°) difference between the enzyme and the substrate is one of the crucial factors that determine the oxidation rate of the substrate, thus limiting the use of laccase. On the other hand, with the use of redox mediators, which act as a diffusible shuttle from laccase enzyme to substrate, the scope of laccase on the remediation of high redox potential pollutants has broadened to a greater extent (Morozova et al. 2007; Frasconi et al. 2010). The best examples for laccase-mediated removal of contaminants include the removal of PAHs such as anthracene or benzopyrene (Zeng et al. 2016; Wu et al. 2008), recalcitrant dyes (Kumar et al. 2014), and organophosphorus compounds, such as nerve agents VX or Russian VX (Amitai et al. 1998). However, large-scale treatment processes require a large amount of the enzyme which is not considered economical (Osma et al. 2011). Moreover, several factors such as pH and chemical compounds present in wastewater hinder the enzyme activity by inhibiting the activity site of the enzyme or inducing modification in the amino acid residues or chelation of the copper atoms of the enzyme, leading to the reduction or inhibition of the enzyme activities (Johannes and Majcherczyk 2000; Bollag and Leonowicz 1984). In addition, the high ionic strength imparted due to the presence of halide groups (e.g., F^-, Cl^-, Br^-), heavy metals (e.g., Hg^{2+}, sn^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}), surfactants and some organic solvents (acetone, acetonitrile, dimethyl sulfoxide), or proteases may inhibit the activity of laccase (Madhavi and Lele 2009; Yaropolov et al. 1994; Couto and Herrera 2006; Cabana et al. 2007). The difficulty in the recovery and reusability of the enzymes has also posed as a major limitation in the large-scale application of laccase. Thus, development of an immobilized laccase system not only overcomes the drawbacks but also enhances the stability of the enzyme to a greater extent.

2 Laccase Immobilization

Irrespective of the general definition of immobilization, the prime functionality of the immobilization process addresses two main functionalities: first, to aid in effectively stabilizing the functionality of the enzyme within a desired time and space (catalytic function) and, second, to ease the separation of the enzyme, in turn enabling reusability of the biocatalytic system (non-catalytic function). The enzyme immobilization process depends on the interaction of two functional compounds, the enzyme and the carrier (carrier-based immobilization). The amino acid and the functional groups on the side chain of laccases play a pivotal role in enhancing the stability. Moreover, the presence of the side chain influences most of the surface

properties of the enzyme, including the net charge, resultant of the individual amino acid dissociation constants (pKa) that dictate the interaction of the enzyme with the carrier particle (Secundo 2013; Halling et al. 2005). In fungal laccases, the three main amino acid residues that are reported to be present are histidine (His), cysteine (Cys), and methionine (Met). As Met is a non-polar amino acid with a sulfur moiety, it is not involved in the cross-linking reaction in contrast to His and Cys (Ba et al. 2013; Habeeb and Hiramoto 1968). On the other hand, cysteine along with histidine, lysine, and tyrosine residues is found to interact with the functional group present on the carrier molecule (Ba et al. 2013). In addition, the stability and flexibility of the immobilized particle can be varied by modifying the chemical bonds between the enzyme and carrier particle, for example, the introduction of a covalent bond by cross-linking agents. In most cases, the efficiency of the immobilized enzyme mostly depends on the properties of the carrier particle (Sheldon and van Pelt 2013). Considering this, it is generally accepted that the carrier should have a large accessible surface area (i.e., >100 m^2 g^{-1}) combined with a pore size of approximately three times larger than the average diameter of the enzyme (i.e., >30 nm). The latter results in better enzyme loading, retention, and reduced substrate and cofactor diffusion constraints (Cao 2005).

Ideally, the carrier material should be inert and prepared to correlate with the surface property of the enzymes, with a large porous surface area to accommodate a large number of enzyme molecules. Moreover, the carrier particles should be chemically and mechanically stable. For wastewater application, the carrier particles should also be economically feasible, environmentally friendly, and more widely available, which makes their selection a pivotal step in the immobilization process. However, the presence of carrier particles may hinder the mass transfer rate leading to a decrease in catalytic activity, which has led to the development of a carrier-free immobilization technique by cross-linking the biocatalyst with cross-linking agents such as glutaraldehyde (Sheldon and van Pelt 2013).

3 Immobilization Methods

Considering the surface properties of enzymes and their application, several immobilization methods have been introduced (Fig. 1). These methods have been broadly divided into five main types for ease of understanding:

1. Covalent immobilization
2. Cross-linked enzyme immobilization
3. Adsorption
4. Entrapment
5. Encapsulation

This chapter presents the various laccase immobilization technologies that exist for bioremediation of wastewater (Table 1), for which most of the studies were conducted either at a lab-scale or at a pilot-scale level.

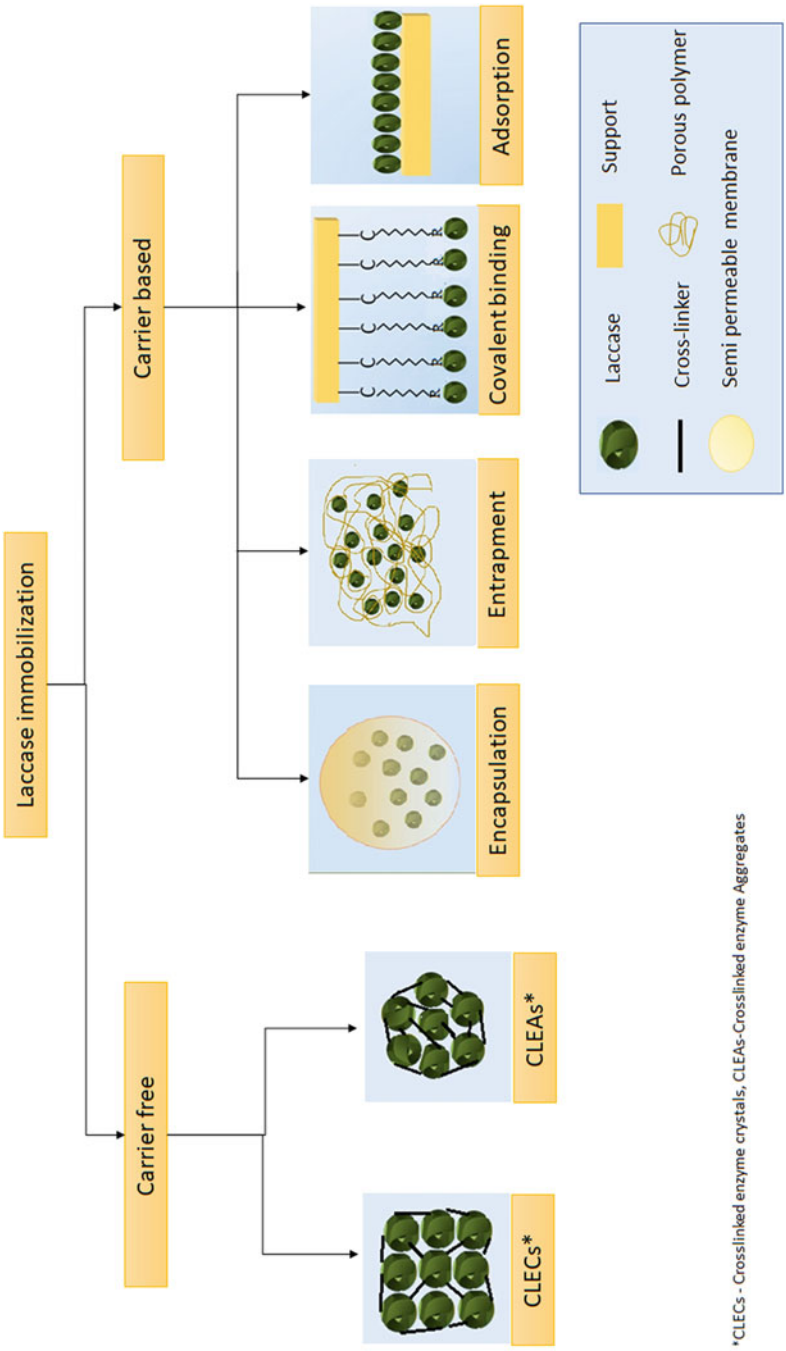

Fig. 1 Schematic representation of types of immobilization

Table 1 Laccase-based immobilized systems used for wastewater remediation

Microorganism	Immobilization process	Support	Yield (%)	Biocatalytic load (U/mg)	Reusability (cycle)	Application	References
White-rot fungi family	Adsorption	Woodchips	NP[a]	NP	NP	Industrial crude effluent and ozonated effluent	Vanhulle et al. (2005)
T. villosa	Adsorption with carrier modifier	Aminopropyltriethoxysilane alumina spherical particle	~50	NP	4	Reactive black 5 dyeing effluent	Zille et al. (2003)
T. versicolor	Physical adsorption	Halloysite nanotubes and chitosan	NP	123.1 mg/g	10	Phenols in wastewater	Yao et al. (2015)
Coriolus versicolor	Adsorption and entrapment	Laccase adsorbed on activated carbon entrapped in calcium alginate bead	NP	NP	8	Dichlorophenols in wastewater	Zhang et al. (2006)
Lactarius volemus	Adsorption	Microsilica	NP	NP	NP	Textile wastewater containing reactive black 5	Kalkan et al. (2014)
T. versicolor	Adsorption	Surface modified magnetic silica particles	NP	58.3	NP	COD[b] reduction in papermaking wastewater	Liu and Wang (2014)
		Magnetic silica particles	NP	34.7	NP		
Paraconiothyrium variabile	Entrapment	Gelatin–alginate	NP	NP	5	Decolorization of industrial synthetic dyes	Mogharabi et al. (2012)
T. versicolor	Entrapment	Copper alginate and iron oxide	85.5	NP	NP	Synthetic pollutants in wastewater	Le et al. (2016)
Trametes sp.	Entrapment	Manganese ferrite ($MnFe_2O_4$)	NP	0.0165	NP	Dye wastewater	Shojaat et al. (2016)
Pleurotus ostreatus IBL-02	Encapsulation	Hydrophobic gel entrapment	NP	NP	NP	Decolorization of different dyes and local textile wastewaters	Asgher et al. (2012)

T. versicolor	Encapsulation	Multi-walled carbon nanotube	88.9	NP	NP	Phenolic organics from water	Dai et al. (2016)
Ganoderma lucidum	Sol gel	Trimethoxysilane and propyltrimethoxysilane	~90	NP	NP	Wastewater effluent	Irshad et al. (2012)
Lentinula edodes	Covalent	Eupergit	45	NP	8	Phenols in olive mill wastewater	D'Annibale et al. (2000)
L. edodes	*Covalent*	Chitosan	NP	0.520	NP	Polyphenols, *ortho*-diphenols, and dye in olive mill wastewater	D'Annibale et al. (1999)
T. hirsuta	Covalent	Aminopropyltriethoxysilane alumina	68	0.14 mg/g	NP	Textile dyes and dying effluent	Abadulla et al. (2000)
Pycnoporus coccineus	Covalent	Acrylic epoxy-activated resins, Eupergit C 250 L	NP	0.110	NP	Olive oil mill wastewater phenolic compounds	Berrio et al. (2007)
Coriolopsis polyzona	Covalent	Amino-modified silica nanoparticles	61.4	2.67	NP	Real-time wastewater	Zimmermann et al. (2011)
Phoma sp. UHH 5-1-03	Cross-linking	PVDF membranes by electron beam irradiation	NP	NP	NP	PhAC in wastewater	Jahangiri et al. (2018)
T. versicolor	CLEAs[c]	Magnetic mesoporous silica microbeads (MMSMB)	39	1.53	NP	PhAC in wastewater	Arca-Ramos et al. (2016b)
T. versicolor (laccase) and *Myceliophthora thermophila* (laccase)	Co-immobilization	Fumed silica nanoparticles	89.9	1.10	10	Micropollutants from wastewater	Arca-Ramos et al. (2016a)
T. versicolor (Laccase) and *M. thermophila* (Laccase)	Combi-CLEAs	Functionalized chitosan	23.3 (Lac) 115.6 (Try)	NP	NP	COD of paper mill wastewater	Ba et al. (2012)

(continued)

Table 1 (continued)

Microorganism	Immobilization process	Support	Yield (%)	Biocatalytic load (U/mg)	Reusability (cycle)	Application	References
T. versicolor laccase, *Bjerkandera adusta* versatile peroxidase (VP), *Aspergillus niger* glucose oxidase (GOD)	Combi-CLEAs	Chitosan	NP	NP	NP	Pharmaceuticals from urban wastewater	Touahar et al. (2014)
T. versicolor laccase (TvL) *Mushroom tyrosinase (Tyr)*	Combi-CLEAs	Chitosan	10.6 (lac) 61.8 (Tyr)	NP	NP	Acetaminophen in hospital and municipal wastewater	Ba et al. (2014a)

[a]*NP* not provided
[b]*COD* chemical oxygen demand
[c]*CLEAs* cross-linked enzyme aggregates

3.1 Covalent Binding

Covalent immobilization provides strong bonding and helps in overcoming the drawbacks of leaching, specifically when working in aqueous media consisting of denaturing factors (wastewater). This robustness of the enzyme could be due to the restriction in the conformation changes by resisting the enzyme folding and thermal vibrations by multiple bonding between the carrier and enzyme molecule (Brady and Jordaan 2009). The covalent association of laccase with the support occurs due to the presence of the amino acids in the side chain (eg. methionine, cysteine, lysine, histidine), whose reactivity depends on the functional groups, such as hydroxyl, imide, phenolic, indolyl, etc. (Sheldon and van Pelt 2013; Singh et al. 2015). The specific activity of the immobilized laccase depends on the surface area of the support material, which acts as a decisive factor. Similar to adsorption, several synthetic and naturally occurring carriers can be used for the covalent immobilization of laccase. The most common synthetic carriers, which include magnetic nanoparticles (Wang et al. 2012), mesoporous silica (Salis et al. 2009), silica (Zawisza et al. 2006), Eupergit® (Lloret et al. 2012), etc. along with the naturally occurring carrier molecules such as chitosan (Kalkan et al. 2012), chitin, activated coal (Davis and Burns 1992), and coconut fiber (de Souza Bezerra et al. 2015), were commonly used for immobilization and subsequent bioremediation purposes. The occurrence of steric hindrance, a major limitation, due to direct coupling of support with enzymes can be overcome by the introduction of spacer arms such as glutaraldehyde, which separate the enzyme from the support molecule. On the contrary, the carrier molecules containing carboxylic acid, such as polymers of acrylic acid, require activators, such as carbodiimide, which under slightly acidic conditions react with the carboxylic acid group to form highly reactive *O*-acylisourea derivatives. Among many commercially available water soluble carbodiimides, cyclohexyl-3-(2-morpholino-ethyl)-carbodiimide (CMC), 1-ethyl-3-(3-dimethylamino propyl)-carbodiimide (EDC), and 1-cyclohexyl-3-(2-morpholino-ethyl)-carbodiimide (CMC) are commonly reported. Among the other functional groups, amine-bearing supports were widely used as carrier particles for covalent immobilization. The amino functionalization can be introduced by either aminosilane attachment or polyethyleneimine coating.

Coupling of laccases with the modified support can be achieved by various methods, which include the use of bifunctional groups such as dialdehydes, di-imidate esters, and diisocyanates. Among the various bifunctional groups, glutaraldehyde was the most commonly used cross-linking agent during covalent immobilization. Glutaraldehyde forms a complex Schiff base with an amine functional group on the carrier to form α,β-unsaturated carbonyl groups on which enzyme may attach. Arıca et al. (2009) used a bifunctional glutaraldehyde for the immobilization of laccase on non-porous poly glycidyl methacrylate and ethylene glycol dimethacrylate beads with a maximum biocatalytic load of 4.9 mg/g along with bioremediation application of the textile industrial dye Reactive Red 120 (Arıca et al. 2009). Over the past decade, an increase in the number of covalently immobilized

laccase particle-based reports for bioremediation application reflects the popularity of the covalent immobilization technique. One such report includes the use of covalently immobilized *Lentinula edodes* laccase on Eupergit (D'Annibale et al. 1999), which proves its effective removal of phenolics from olive mill wastewater in lab scale (D'Annibale et al. 2000). Despite its merits, diffusion limitation of the substrate, intense aggregation, and settling along with the possibility of structural alternation due to covalent bond formation on the active site of laccase limits the use of covalent-based laccase immobilization.

3.2 Cross-Linking of Laccase

Enzyme immobilization by cross-linking the reactive NH_2 group of the enzyme using bi- or multifunctional cross-linking agents resulted in the strategical development of carrier-free immobilization. Carrier-free immobilized biocatalysts can be produced directly by cross-linked enzyme crystals (CLECs) or cross-linked enzyme aggregates (CLEAs). However, as CLECs require highly purified crystalline enzyme, CLEAs established itself as a potential alternative in developing a stable biocatalyst that gears up activity to the maximum level possible. CLEAs is a rapid, gentle, and cost-effective method for the production of carrier-free biocatalysts (Sheldon 2011). In recent years, CLEAs have become a novel and potential biocatalytic system on both the lab and industrial scale and are generally considered to be the next-generation biocatalyst for their applicability in the elimination of emerging contaminants (Cabana et al. 2009; Ba et al. 2012, 2014a; Kumar et al. 2012). The main advantage associated with CLEAs includes simultaneous purification and immobilization of an enzyme particle in a simpler method in a short duration of time, with the aid of a salting-out agent, ammonium sulfate, in the presence of organic solvents, such as polyethylene glycol (Cabana et al. 2009), tert-butanol (Kumar et al. 2012), and isopropanol (Matijošytė et al. 2010) under optimum physiological conditions of the enzyme, along with cross-linking agents, such as glutaraldehyde (Cabana et al. 2009). The resultant aggregates are separated by simple centrifugation followed by washing with a buffer solution. Formation of linking bonds among lysine amino acid residues by the action of dialdehyde group (glutaraldehyde) was reported to be a cause for the formation of smart biocatalysts, CLEA particles, with tunable physical properties (Cabana et al. 2009). Since the immobilization technique does not require a carrier particle as support, high biocatalytic activity can be achieved at a lower production cost (Kumar et al. 2012). Another additional benefit of the CLEAs technology is that it stabilizes the quaternary structure of multimeric enzymes and enhances the operational stability of the biocatalytic system (Kumar et al. 2012). Generally, the resultant CLEAs exhibit stability in both aqueous and solvent phases, making them suitable immobilization processes (Sheldon 2011; Ba et al. 2012).

However, the gelatinous nature of CLEA particles leads to low reproducibility and low mechanical stability. As a result, the CLEAs are often bound to a carrier to

improve the operational stability of CLEAs or to ease the separation process. Thus, the process can be upgraded by introducing carrier particles such as mesoporous silica particle or magnetic nanoparticle (Kumar et al. 2014). CLEAs have been extensively used in various applications, including bioremediation. With the use of modern nano-engineered technologies, a creative fusion of biocatalysis, chemical engineering fundamentals, and nanotechnology has opened up attractive horizons towards immobilization techniques. This amalgamation resulted in the development of magnetic nanoparticle-based CLEAs of laccase to bolster long-term mechanical stability and could be recycled by the application of a magnetic field (Kumar et al. 2014). The operational stability of the CLEAs was enhanced by coating with a chitosan and 3-aminopropyltriethoxysilane polymer network (Hassani et al. 2013). Due to the limitation of restricted mass transfer of substrate in CLEAs, porous cross-linked aggregates (*p*-CLEAs) with enhanced biocatalytic activity were developed and proved to be effective with recyclability up to 15 cycles (Kumar et al. 2012).

Consequently, CLEAs are considered as the most attractive carrier-free method of immobilization for the effective treatment of wastewater. As an application in real matrix, magnetic CLEAs (mCLEAs) from *T. versicolor*, which is known for its potential in the effective biotransformation of pharmaceutically active compounds—acetaminophen, mefenamic acid, fenofibrate, and indomethacin—claimed to exhibit an enhanced stability against chemical denaturants in real wastewater matrix (Arca-Ramos et al. 2016b). The potential application of CLEAs laccase in wastewater was widely discussed by Ba et al. (2013). Despite scientific advances in immobilization by cross-linking, most of the application of CLEA particles was restricted to simulated wastewater treatment rather than real matrix, which includes mCLEAs of laccase for decolorization of dye (Kumar et al. 2014) and antibiotics (Yang et al. 2017) and porous CLEAs for reactive dyes (Kumar et al. 2012). However, low yield of the enzyme and absence of required mechanical strength due to the lack of carrier particle act as a major drawback for a CLEA-based laccase system.

3.3 Adsorption

Adsorption of enzymes involves physical interaction of the enzyme with the supports through dipole–dipole, hydrogen bonding, electrostatic, or hydrophobic and hydrophilic interactions (Jesionowski et al. 2014). For adsorption, the desired adsorbate (support) is made to contact with the laccase for a fixed time period at suitable conditions, and the unabsorbed enzymes are removed by washing. In spite of the weak interaction of the enzyme and adsorbent, high catalytic activity, less or no requirement of chemicals, and provision for reusability of support make this technique popular among others. Depending on nature and occurrence, the carrier molecules can be categorized into organic carriers, which include naturally occurring compounds such as chitosan, chitin, cellulose, alginates, etc. and inorganic carriers such as silica, titania, hydroxyapatite, etc. (Jesionowski et al. 2014). Laccase generally exhibits higher affinity towards organic carriers in comparison with the

inorganic carrier molecule (Jesionowski et al. 2014) and is found to be more selective towards chitosan due to the enhanced ionic interactions. The work with chitosan as a carrier molecule along with itaconic acid and Cu(II) as a carrier modifier displayed an enhanced catalytic loading, which in turn improved the efficiency bioremediation of contaminants (Bayramoglu et al. 2012). Similarly, laccase immobilized on various organic carriers—chitosan (Yang et al. 2006), wood fiber (Saarinen et al. 2008), inorganic carrier—alumina (Abadulla et al. 2000), quartz (Saarinen et al. 2008), metals (zirconium) (Li et al. 2018), and porous support—mesoporous silica (Shao et al. 2009), mesoporous molecular sieve MCM-41 (Fernández-Fernández et al. 2013) was reported for its subsequent application towards bioremediation of various contaminants. On the other hand, use of magnetic nanoparticle as a carrier opened a new window in the metal affinity adsorption-based immobilization method. Metal ions, such as poly crystal gold, metal (gold and silver)-coated electrodes, indium tin oxide films, magnetic nanoparticle, and metal chelates have been tested as ion exchange supports for laccase immobilization based on adsorption.

Among the metal chelates, application of laccase-based copper-chelated magnetic nanoparticles for the treatment of coking wastewater was considered as the best example for metal affinity adsorption (Wang et al. 2012). The other metal-based adsorptions include the use of either magnetic copper nanoparticles (Wang et al. 2008) or magnetic chitosan microspheres (Fang et al. 2009). The affinity of the enzyme and carrier is considered as the most important criterion for adsorption and its use in wastewater treatment. In most cases, the weaker interaction of carrier particle can be enhanced using intermediate agents (carrier modifiers) or by modification of the carrier surface. The histidine functional group containing support molecules was reported to exhibit a favorable adsorption condition for laccase immobilization (Fernández-Fernández et al. 2013). The bifunctional (glutaraldehyde and ethylene glycol-*N*-hydroxysuccinimide) and monofunctional (citraconic anhydride) groups were reported as widely used surface modification agents to introduce functional group on the surface of carrier for the enhanced adsorption of laccase (Fernández-Fernández et al. 2013). Liu and co-workers modified the magnetic nanoparticle surface using a bifunctional agent and showed a two-fold improvement in the biocatalytical loading with application in reduction of COD levels in real-time papermaking (pulp and paper) effluent (Liu and Wang 2014). On the other hand, with global awareness on eco-friendly processes, researchers are working on renewable carrier supports such as coconut fibers with good water holding along with cation exchange properties, modified Kaolin with chemical acetylation, etc. As an important aspect, it should be noted that enhanced operational stability was observed in most of the support's molecules, with the exception of some supports such as aluminum hydroxide (Ahn et al. 2007). Despite its relatively simple and inexpensive method, with industrial potential, the presence of a weak interaction leading to desorption of laccase and reduction in the biocatalytic loading under the harsh conditions of wastewater treatment acts as a major limitation. The exposure of laccase to the microbial attack that is present in the wastewater environment acts as another major drawback. On the other hand, the competitive adsorption of the

pollutants to the adsorbent reduces the catalytic loading of laccase in real matrix (Alshabib and Onaizi 2019). Moreover, the adsorbed enzymes are shielded by the hydrophobic interface which hinders the interactions with substrates (Spahn and Minteer 2008).

3.4 Encapsulation

Encapsulation is considered as the simplest and the most straightforward method involving entrapment of the biomolecules in a polymer matrix without any association of bonding between the network and the biomolecules (Mohidem and Mat 2012). Similar to entrapment, this method has the advantage of reusability and permeability of the substrates. The widely applied sol–gel technology and bioencapsulation are considered as two different encapsulation methods with an application in bioremediation (Irshad et al. 2012). The sol–gel is chemically inert and exhibits a remarkable half-life period along operational stability making this method popular (Mohidem and Mat 2012). Among many immobilization methods, laccase encapsulation was quite popular in bioremediation along with its other applications, which include as selective coating for optical and electrochemical biosensors and as a stationary phase of an affinity chromatography. With advances in polymer science, several studies have been reported on laccase immobilization with a wide range of natural and synthetic polymers. For enhanced shelf life period, doping of additives was introduced to protect laccase from denaturation by minimizing the nucleophilic attraction of water and also maintained the hydration levels by modifying the microenvironment of laccase (Fernández-Fernández et al. 2013). Mohidem and co-workers demonstrated the effect of additive agents polyvinyl alcohol, polyethylene glycol, and (3-aminopropyl)triethoxysilane in the enhancement of enzyme stability (Mohidem and Mat 2012). Addition of these agents as additives during the pre-gelation process is aimed at preserving the laccase catalytic activity during the gelation process. The literature regarding laccase encapsulation for the elimination of real-time pollutants in wastewater is limited. Encapsulated laccase in hydrogels by the sol–gel method exhibited an enhanced degradation of the dye in industrial effluent in comparison with its free counterpart from *P. ostreatus* (Asgher et al. 2012). Furthermore, with the invention of the electrospinning method, several eco-friendly and recoverable fibrous membranes were developed, which in turn were used for the entrapment of laccase using in situ emulsion electrospinning immobilization technology (Niu et al. 2013). Even though encapsulation of laccase exhibits enhanced operational stability along with resistance against inactivating agents, the leakage of the enzyme and diffusion limitation hindered their industrial usage (Ba et al. 2013).

3.5 Entrapment

Enzyme entrapment is considered a simple immobilization method achieved using a polymer network such as a natural or organic polymer. The classic example of enzyme entrapment is the polymerization of sodium alginate with calcium chloride, which leads to the formation of interfacial polymerization with the precipitation of calcium alginates (Fraser and Bickerstaff 1997). The gel obtained is considered to be chemically inert and mechanically stable, which protects the enzyme from the external environment (Fraser and Bickerstaff 1997; Bickerstaff 1997). In addition, the open lattice structure of the immobilized beads allows a greater mass transfer due to high porosity ranging from 200 to 150 nm in diameter (Fraser and Bickerstaff 1997). In addition, laccase entrapment can be performed through thermo-reversal polymerization by using either natural or synthetic polymers such as alginate, gelatine, polyvinyl acetate, beta-carrageenan, and acrylic acid (Fraser and Bickerstaff 1997). As a wastewater treatment application, this immobilization method is limited due to the presence of chelating agents such as EDTA, citrate, Mg^{2+}, and phosphate in the wastewater, which may destabilize the calcium alginate beads (Fraser and Bickerstaff 1997). Some techniques, such as the use of stabilizing agents or stabilizing by cross-linking with other polymers such as chitosan (Lu et al. 2007), were found to increase the stability of laccase. For instance, alginate was reported as a stabilized agent with gelatine as a natural polymer and demonstrated an enhanced stability of up to 85% of residual activity after five successive cycles of decolorization of industrial synthetic dye (Mogharabi et al. 2012). In parallel with the knowledge on the laccase and their dependence on the copper molecule, Teerapatsakul et al. investigated the use of copper sulfate as an alternative to calcium chloride for the effective formation of copper alginates, with enhanced laccase activity. In comparison with calcium alginate, copper alginate proved to be a better support with enhanced residual activity and reusability (Teerapatsakul et al. 2008). In spite of several advancements in the entrapment of laccases, their usage is limited due to low catalytic loading and excessive leakage of laccases during the process (Spahn and Minteer 2008; Cao 2005). Moreover, due to the high thermal conductivity of the polymers used in the entrapment process, a rapid reduction in the thermal stability of the laccase during the bioremediation process was assumed to be another common drawback of the entrapment process (Cao 2005).

3.6 Co-immobilization

The knowledge about enzyme chemistry along with studies on their reaction mechanisms led to the strategic development of a novel immobilization technique where more than one enzyme is used during immobilization. This process is called co-immobilization. Among the co-immobilization strategies, multipurpose CLEAs involving more than one enzyme, multi-CLEAs or combi-CLEAs, are gaining

prominence for their sole process, the multi-enzyme cascade process. By definition, combi-CLEAs are defined as CLEAs of different proteins/enzymes. In addition, requirement of less space with high economic and eco-friendly benefits of the combi-CLEAs made this technique preferable. Due to substrate limitations, changing operating conditions, and the synergistic action that results from the combination of different enzymes, it is preferable to use, for example, multiple oxidoreductase enzymes like laccase (Lac), tyrosinase (Tyr), peroxidase (VP), and glucose oxidase (GO) to eliminate a wide range of trace level organic contaminants like endocrine-disrupting compounds and pharmaceuticals (Anderson et al. 2018).

These versatile biocatalysts can be comprised of oxidative and supporting enzymes that interact in a cascade of reactions, which makes them a perfect bioremediation tool for dye decolorization, lignin bioprocessing, elimination of pharmaceuticals, and endocrine-disrupting compounds (Taboada-Puig et al. 2011; Abadulla et al. 2000; Ba et al. 2012, 2013, 2014a; Touahar et al. 2014). Even though several enzymes have been immobilized by this technique, immobilization of laccase along with other oxidoreductase enzymes for bioremediation application is limited. Ba et al. used a bi-enzymatic system, with laccase and tyrosinase immobilized as combi-CLEAs that showed improved efficiency in the transformation of acetaminophen from municipal and hospital wastewaters, respectively, at pH 7.0 (Ba et al. 2014a). Immobilization of the versatile tri-enzymatic system consisting of laccase, versatile peroxidase, and glucose oxidase by CLEAs technique (Touahar et al. 2014) exhibited an enhanced stability of the enzymes. The resultant biocatalyst showed an increase in the oxidation spectrum of 14 pharmaceutical compounds such as acetaminophen, diclofenac, mefenamic acid, atenolol, epoxy carbamazepine, fenofibrate, diazepam, trimethoprim, ketoprofen, indomethacin, carbamazepine, caffeine, and naproxen in synthetic wastewater, which was more efficient than the free enzymes (Touahar et al. 2014). By exploring the advantage of laccase with varying redox potential, a multi-laccase system from *Thielavia* genus, *Coriolopsis polyzona*, *Cerrena unicolor*, *Pleurotus ostreatus*, and *Trametes versicolor* was immobilized onto fumed silica nanoparticles for the oxidation of trace organic contaminants, namely, carbamazepine, diclofenac, sulfamethoxazole, ibuprofen, gemfibrozil, benzophenone-2, benzophenone-4, and bisphenol A (Ammann et al. 2014). On the other hand, the pollutants, gemfibrozil and benzophenone-2, which resist oxidation by the sole action of *T. versicolor* or *C. polyzona* laccase, were reported to be successfully remediated by co-immobilized *C. polyzona* and *T. versicolor* laccase nano-biocatalyst. This reflects the importance of the co-immobilization technique in bioremediation. Due to the variation in the pH of the wastewater, a multipurpose immobilized biocatalyst was developed with subunits of laccase from *C. polyzona*, with an acidic pH optimum, and from *C. cinerea*, with a neutral pH optimum, which showed biocatalytic activity through a broad pH range, implying their suitability for the treatment of micropollutant-contaminated real wastewaters of varying pH (Agathos 2012). In the same context, Ba et al. insolubilized two commercial laccases, from a fungus and a bacterium, using combi-CLEAs, and claimed to achieve a 70% reduction in total COD of a pulp and paper mill wastewater (Ba et al. 2012).

4 Immobilized Laccase-Based Reactors for Wastewater Treatment

The selection of the reactors for the immobilized laccase and establishment of its operation strategy for the efficient treatment of wastewater is considered a major challenge. Depending on the mode of immobilization and treatment strategies of wastewater, various bioreactors, along with multifunctional hybrid reactors, have been designed, which makes the categorization of reactors a difficult task. However, based on the design, the immobilized enzyme-based bioreactors are grouped into:

1. Stirred tank reactors (STR)
2. Membrane-based reactors (MBR)
3. Packed bed reactors (PBR)
4. Fluidized bed reactors (FBR)

These enzyme reactors generally operate either in batch, fed batch, or continuous mode. However, the batch reactor is considered a more versatile tool in determining the degradation kinetics of wastewater, which makes it a prime choice of reactor design at a laboratory scale or pilot scale.

Despite the extensive work and potential applications of immobilized laccase in degradation of contaminants, limited studies were reported on the remediation of real-time wastewater using immobilized laccase in a bioreactor (Table 2). Generally, packed bed reactor, fluidized bed reactor, and membrane reactors are considered to be ideal reactors for immobilized enzyme systems (Wanga and Zhonga 2011; Stanbury et al. 2013; Messing 2012). However, with technological advancements, some bioreactors are reported to have a suspension system, which includes stirred tank reactors, airlift, and bubble column bioreactors. For instance, Osma et al. used a continuous stirred tank reactor (CSTR) for the degradation of textile dye in simulated wastewater using covalently immobilized laccase (Osma et al. 2010).

The selection of a bioreactor generally depends on some key fundamental principles, such as transport phenomena, which include mass transfer along with the efficiency to maintain the optimum conditions required by the immobilized laccase (Stanbury et al. 2013). However, in most cases, it is difficult to design an ideal reactor that meets all the requirements, and hence some of these requirements can be packed accordingly. For example, it is crucial to balance the agitation speed and the mass transfer in STR in order to avoid the reduction in biocatalytic load due to shear force (Wanga and Zhonga 2011). Accordingly, several bioreactors and hybrid reactors have been designed for wastewater treatment which is briefly discussed in the next sections.

Table 2 Various strategies of laccase immobilized based reactors for the remediation of contaminants in wastewater

Microorganism	Immobilization strategy	Reactor	Design	Flow rate (ml/min)	Hydraulic retention time HRT (h)	Application	References
Genetically modified *Aspergillus oryzae*	Physical adsorption on granular activated carbon	Packed bed reactor	Width: 1 cm Height: 22 cm Bed volume: 17 mL	2.4	NP[a]	Micropollutants in simulated water and wastewater	Nguyen et al. (2016)
T. versicolor	Metal affinity adsorption on copper-chelated mesoporous silica nanoparticle	Magnetically stabilized fluidized bed bioreactor	Glass column Height: 20 cm Width: 1.5 cm Power supply: 0–10 A	5.5–10.8	NP	Phenols from coking wastewater	Wang et al. (2012)
T. versicolor	Calcium alginate bead-based entrapment	Packed bed reactor	Glass column Height: 50 cm Width: 2 cm Temperature: 30 ± 2 °C	1	NP	Simulated paper industrial effluent	Niladevi and Prema (2008)
T. versicolor	Encapsulation on poly(D,L-lactide-co-glycolide) (PDLGA) by emulsification of laccase	Laccase-loading spider-type reactor (LSTR)	Spider-type reactor	NP	NP	PAHs in wastewater	Niu et al. (2013)
Coriolopsis gallica	Covalently immobilized on mesoporous silica particle	Continuous fed batch reactor	Working volume: 50 mL	0.6	1.25	Organic contaminant in wastewater	Nair et al. (2013)
Myceliophthora	Covalently immobilized on silanized alumina pellets	Fixed bed reactor	Working volume: 31.4 ml Width: 2 cm Length: 55 cm	10	NP	Maillard products from distillery wastewater	Singh et al. (2015)

(continued)

Table 2 (continued)

Microorganism	Immobilization strategy	Reactor	Design	Flow rate (ml/min)	Hydraulic retention time HRT (h)	Application	References
P. ostreatus	Covalently immobilized on TiO_2 particle	Hybrid membrane reactor	rpm: 150 Temperature: 25 °C	~40	24	Bisphenol-A and carbamazepine in sewage	Ji et al. (2017)
T. pubescens	Covalently immobilized on surface-modified alumina spheres	Fluidized bed bioreactor	Glass column Height: 20 cm Width: 4.5 cm Working volume: 200 mL Air flow: 0.5 vvm	NP	NP	Simulated textile effluent	Osma et al. (2010)
		CSTR	Glass column Height: 20 cm Width: 7 cm Working volume: 200 mL Air flow: 0.5 vvm	NP	33		
T. versicolor	Covalently immobilized on hydrophilic PVDF microfiltration membrane	Microfilter membrane reactor	Feed solution: 200 mL Air flow: 1.26 mMol/L Temperature: 25 °C	6	NP	Phenylurea pesticide in wastewater	Jolivalt et al. (2000)

C. versicolor	Covalently immobilized on functionalized activated carbon	Packed bed reactor	–	30	12	Pulp mill and bleach plant effluent	Davis and Burns (1992)
T. versicolor	Covalently immobilized on TiO_2 modified particles with APTES	Membrane hybrid reactor	–	NP	96	Carbamazepine in effluent	Ji et al. (2016)
T. versicolor	Particle CLEAs on chitosan	Hybrid bioreactors	Reactor volume: 1 L rpm: 300	0.9	NP	Aromatic pharmaceutical from wastewater	Ba et al. (2014b)

[a]*NP* not provided

4.1 Stirred Tank Reactor

An STR is considered as the most commonly used bioreactor design in bioprocess industries for its ease in fabrication and simple operation with minimal process controls (Fig. 2a). An STR consists of a simple tank containing a sparger for aeration along with an agitator or impeller with a wide range of functionalities like heat and mass transfer (Messing 2012). Depending on the transport phenomena, power consumption, and fluid dynamics, numerous impellers were designed, which were grouped into axial and radial flow impellers. For a shear-sensitive immobilized laccase system, the conventional impeller may adversely affect the system due to either high hydrodynamic shear force or air bubble produced by the gas sparger (Messing 2012). On this aspect, a number of alternative forms of impeller, like Intermig, Prochem Maxflo T, and Scaba 6SRGT, were developed with improved mixing efficiency at low tip speed (Wanga and Zhonga 2011), which are assumed to be suitable for immobilized laccase. Besides the impeller, the design of STR for wastewater treatment follows the standard geometrical specifications that are required in conventional STR, which were widely reported by some authors (Stanbury et al. 2013; Kargi and Shuler 1992).

Depending on the mode of operation, a STR can be operated either in batch or in continuous mode. In batch mode, the recovery of the immobilized laccase is often accompanied by centrifugation or filtration. However, the formation of clumps, especially in CLEAs, leads to a reduction in the efficiency of immobilized particles, which acts as a major limitation in the treatment of wastewater (Arca-Ramos et al. 2016b). To address this setback, Arca-Ramos et al. developed laccase cross-linked magnetic silica microbeads for successful treatment of pharmaceutically active compound (PhAC) in wastewater (Arca-Ramos et al. 2016b), which was based on the design of magnetic-assisted laccase immobilization reported (Kumar et al. 2014). In the continuous stirred tank reactor (CSTR), hydraulic retention time (HRT) is considered a key design variable, which is defined as the amount of time spent by the wastewater in the reactor (Osma et al. 2010). Similar to a batch reactor, CSTR can also be operated for effective wastewater treatment. However, reduction in the biocatalytic load due to shear force and requirement of further downstream processing for the separation of the immobilized laccase in CSTR increases the overall process cost of wastewater treatment using immobilized laccase, thus limiting its usage on a large scale.

4.2 Membrane Bioreactor

A membrane bioreactor is a simple device that combines the concept of biocatalytic conversion with membrane separation (Fig. 2b) (Stanbury et al. 2013). Restriction of the enzymes within the reactor, which further reduces the washing loss of the enzymes, is considered a major advantage of a membrane bioreactor (Wanga and

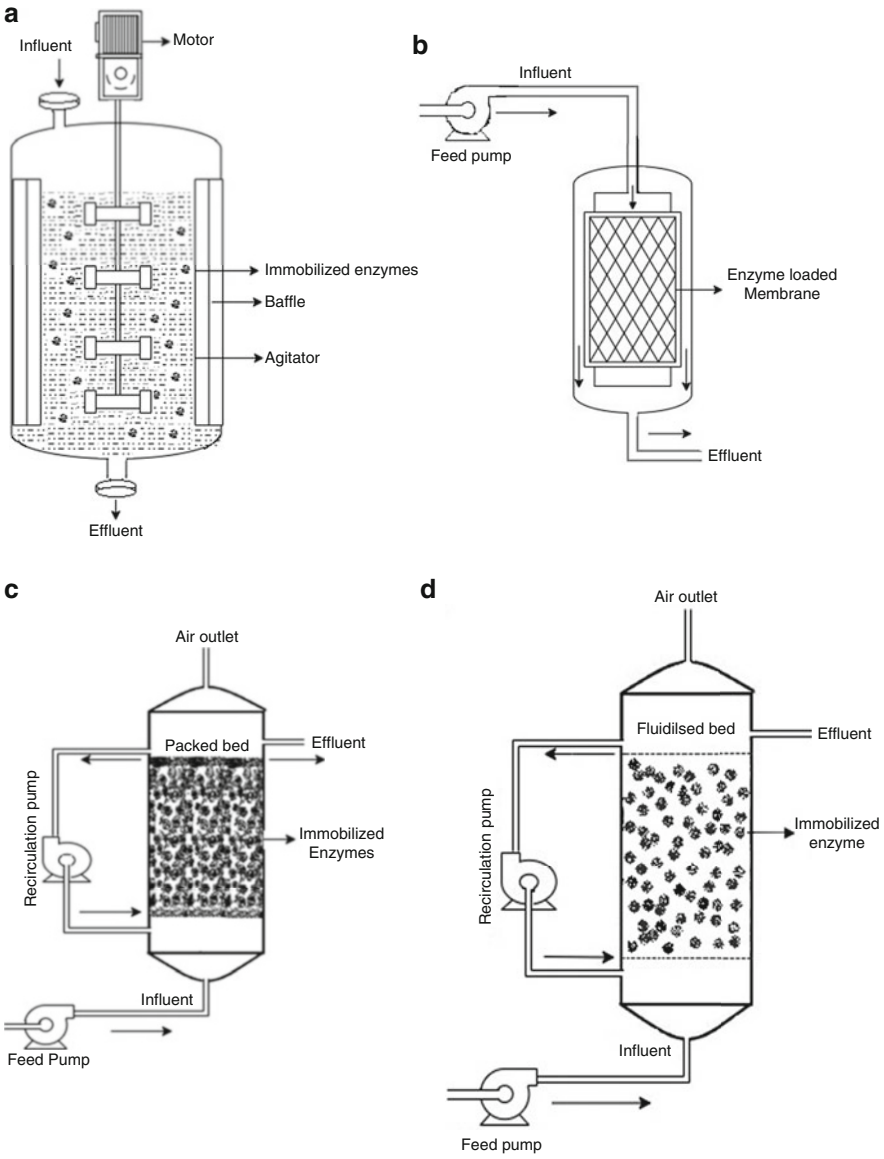

Fig. 2 Schematic representation of (**a**) stirred tank reactors (STRs), (**b**) membrane-based reactors (MBR), (**c**) packed bed reactors (PBR), and (**d**) fluidized bed reactors (FBR)

Zhonga 2011). With the advancement in immobilization techniques, biocatalytic-dense membranes have successfully replaced conventional membranes and are considered a promising technology in the field of wastewater treatment using a membrane bioreactor. Additionally, obligatory interaction of the pollutants with the immobilized laccase during filtration makes this reactor an obvious choice in

the treatment of pollutants (De Cazes et al. 2014). The membrane is selected depending on the application and the immobilization strategy, and some of the common membranes include polysulfone, cellulose, polytetrafluoroethylene, ceramic, polyvinylidene difluoride (PVDF), and polypropylene. On the other hand, pore size of the membrane acts as a decisive property in arbitrating the mass transfer rate of the substrate into the membrane (Kargi and Shuler 1992; Stanbury et al. 2013). Depending on the mode of membrane packing, these membrane reactors can be grouped into any of the following modules: plate and sheet, spiral wound, and tubular or hollow-fiber modules.

The biocatalyst, laccase, can be either be entrapped inside the porous structure or adsorbed onto the surface of the membrane. The immobilization techniques used in a membrane bioreactor are mainly classified as non-covalent immobilization, covalent immobilization on the surface of the membrane, and entrapment of laccase in the membrane (Arca-Ramos et al. 2018). Entrapment of laccase in the membrane was reported to be a benign approach when compared to the other immobilization techniques, as it shields the enzyme from harsh conditions that avail in the wastewater treatment process (Wanga and Zhonga 2011). The work on laccase-mediated remediation of PAHs in real-time wastewater matrix, where laccase was encapsulated on poly(D,L-lactide-co-glycolide) in spider type bioreactors, showed the effectiveness of the entrapped laccase system. This immobilized laccase exhibited enhanced stability when compared to the free enzymes and retained more than 80% of its initial activity for about 60 days of incubation (Niu et al. 2013).

On the contrary, during entrapment, the limited volume porosity leads to a reduction in the biocatalytic load, which was considered a common limitation. As an alternative, immobilization on the surface of the membrane, specifically by covalent immobilization, was considered as a strategic solution. For instance, Jolivalt et al. and Ji et al. covalently immobilized laccase and showed an enhanced remediation of micropollutant in sewage water in a membrane reactor (Ji et al. 2016, 2017; Jolivalt et al. 2000). In spite of several advantages, such as low maintenance and low sludge generation with a higher degradation rate of organic pollutants, MBR has limited use due to the fouling of membrane and limited contact time between laccase and contaminants. This phenomenon may decrease the permeate flux or surge in the transmembrane pressure, which increases the overall operational cost and decreases the membrane's life span. Additionally, most of the previous studies reported were mostly on simulated wastewater bioremediation using laccase-assisted membrane reactors, among which only limited reports have been published on real wastewater matrices (Table 2).

4.3 Packed Bed Reactor

A packed bed reactor (PBR) also known as a fixed bed reactor is the most common type of bioreactor used for an immobilized enzyme system owing to its simplicity and high reaction rates (Fig. 2c). The conventional PBR consists of a vertical column

packed with appropriate carrier particles loaded with biocatalysts, forming a submerged bed. The feed can be introduced either from the top or from the bottom, and in most laboratory scale reactors, the feed is given from the bottom due to the ease in maintaining the liquid levels above the bed (Sondhi et al. 2018). However, on an industrial scale, to avoid the use of a pump and to reduce power consumption, the feed is often supplied from the top under the influence of gravitational force. Reversal of the feed flow has the advantage of maintaining the compactness of the bed and also aids in clearing the loaded biocatalyst (Illanes 2008). Ideally, the flow of the liquid is parallel to the reactor axis, thereby avoiding the back mixing (Arca-Ramos et al. 2018). Due to the ease in operation and simple design, these reactors are widely used as model reactors for the treatment of wastewater, irrespective of the laccase immobilization strategy (Niladevi and Prema 2008; Nguyen et al. 2016; Davis and Burns 1992). Furthermore, with the development of co-immobilization technology, Krastanov developed a simple biocatalytic system by co-immobilizing *Pyricularia oryzae* laccase and mushroom tyrosinase on Mikroperl support for the removal of various phenolic compounds, such as 4-methoxyphenol, 2,6 dimethoxyphenol, 2,4 dichlorophenol, 4-choloro-3-methylphenol, 4-chlorophenol, naphthol, chlorogenic acid, 3-methoxyphenol, 2-chlorophenol, guaiacol, *m*-cresol, *p*-cresol, *o*-cresol, 3-chlorophenol, phenol, catechol, catechin, and DOPA in a packed/fixed bed tubular bioreactor (Krastanov 2000).

However, relatively poor mass and heat transfer due to low liquid velocity are considered the major drawback of PBR (Wanga and Zhonga 2011). Another drawback of PBR includes accumulation of stagnant gas packets, which leads to poor transfer phenomena (Wanga and Zhonga 2011). As a solution to this setback, Shiotanni and Yamane proposed a shallow horizontal packed bed reactor with free head space, and the designed reactor was successful in removing the produced carbon dioxide accumulated in the head space above the packed bed due its buoyancy. This design can be used as a better alternative in wastewater treatment applications to avoid the formation of stagnant gas pockets during the remediation process.

4.4 Fluidized Bed Reactor

A fluidized bed reactor (FBR) is considered an effective reactor used in the wastewater treatment process due to its low cost, high mass transfer rates, and uniform mixing (Fig. 2d) (Bello et al. 2017). The FBR is specifically used for high viscous effluent or low-soluble pollutant that creates clogging problems in PBR (Arca-Ramos et al. 2018; Lema Rodicio et al. 2014). The basic principle of FBR involves the passage of the fluid through the static bed of immobilized biocatalysts with a sufficient superficial velocity, such that the immobilized particles behave like a fluid. When the fluid velocity is less than the drag and gravitational force of the bed, the reactor behaves as a packed bed reactor. However, at particle velocity, the applied fluid velocity balances the weight of the particle and, thus, completely suspends the

particles in the fluid. The fluid velocity required to move the bed is termed as minimum fluidization velocity (Bello et al. 2017). Depending on the minimum fluidization velocity, the FBR is categorized as smooth fluidization, slugging fluidization, bubbling fluidization, turbulent fluidization, or pneumatic fluidization (Yang 2003).

Based on the application and required transport phenomena, the FBR can operate either in a two-phase flow system (solid–gas or solid–liquid) or in a three-phase flow system (solid–liquid–gas) (Grace and Bi 1997). However, in wastewater treatment, a solid–liquid system is commonly used, where the solid phase can be immobilized laccase and the liquid phase wastewater effluent. This configuration was successfully applied by Osma et al., for the remediation of simulated textile wastewater using laccase covalently immobilized on silica particle (Osma et al. 2010). Additionally, the reports on remediation of pollutants from coking wastewater claimed more than 95% remediation of phenols using laccase immobilized on magnetic mesoporous silica particle (Wang et al. 2012). Similarly, Cabana et al. used CLEAs of laccase from *Coriolopsis polyzona* for the effective elimination of BPA, nonylphenol, and triclosan in a fluidized bed reactor (Cabana et al. 2007). However, lack of proper oxygen transfer to the immobilized laccase in a two-phase flow system can be a major limitation (Chao et al. 2011). In this situation, the three-phase flow system, which includes an inlet for aeration or oxygenation, acts as a suitable alternative. In addition, the fluidization can be achieved by providing either current or countercurrent of either effluent or gas, which makes it a suitable reactor for the wastewater treatment using immobilized laccase (Chao et al. 2011; Wanga and Zhonga 2011; Grace and Bi 1997). Despite these advantages, limited research has been reported with the three-phase flow FBR due to high shear force and elutriation of particles at a high flow rate.

4.5 Other Miscellaneous Reactors

A perfusion bioreactor was designed to overcome the disadvantages of mechanical shear in reactors and reduction in the reusability of the particles (Fig. 3). Based on metallic filtrations like membranes, a perfusion basket reactor, filled with immobilized laccase particles, was developed for the effective degradation of endocrine-disrupting chemicals (EDCs) (Cabana et al. 2009). This unbaffled and 3-blade marine propeller showed more than 80% of degradation efficiency of EDCs in a hydraulic retention time (HRT) of 325 min (Cabana et al. 2009). Applying a similar concept, a perfusion basket reactor equipped with mCLEAs was developed for continuous degradation of synthetic dyes (Kumar et al. 2014). This reactor was reported to perform in continuous mode and could be used for the effective treatment of wastewater on a large scale.

Based on conventional bioreactor functionalities, several hybrid reactors were introduced for the effective remediation of wastewater, which combined two or more functionalities of different reactors. One such hybrid reactor was designed by Ba

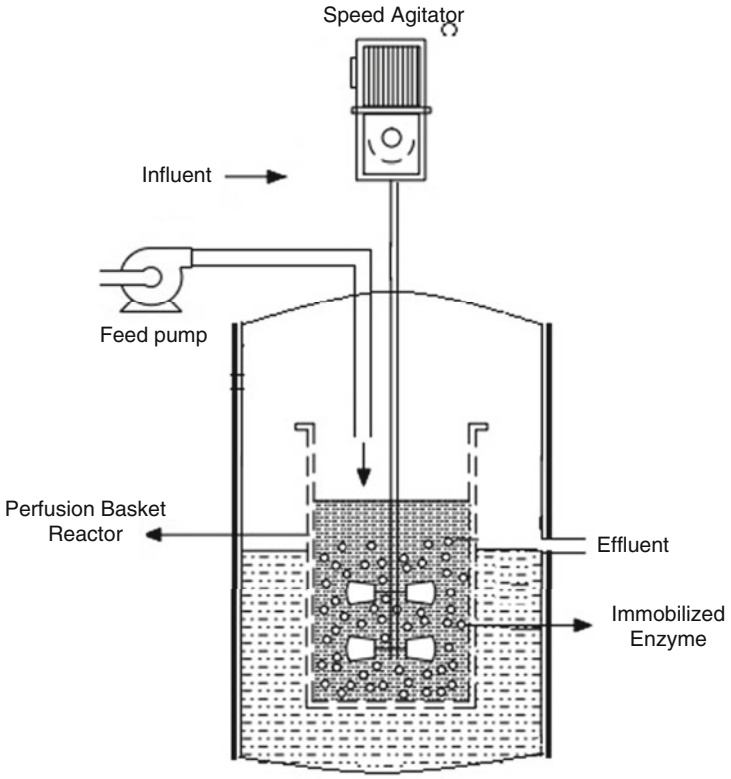

Fig. 3 Schematic representation of perfusion basket reactor with immobilized enzyme

et al. with a combination of simple STR (loaded with CLEAs of laccase) and a membrane filter unit (Ba et al. 2014b). This hybrid reactor reported more than 93% elimination of carbamazepine in 72 h along with complete removal of acetaminophen and mefenamic acid from municipal wastewater (Ba et al. 2014b). Although the proof of concept of these reactors was successful in laboratory scale, lack of extensive research on these reactors (with immobilized laccase) for the treatment of wastewater acts as a major drawback in commercialization of these reactors.

5 Conclusion

Laccase has emerged as a potential biocatalytic tool in the degradation of a broader spectrum of contaminants due to its high redox potential. However, the lack of sufficient availability of the enzyme impedes its commercial application for the treatment of wastewater, which can be overcome by the insolubilization process. An understanding of different types of immobilization strategies, along with their

interactions with the amino acid moiety of laccase, is considered highly indispensable for enhancing the effectiveness of the immobilization strategies for their use in large-scale applications. This biocatalytic system generally shows a greater potential for the biotransformation of contaminants into innocuous products when used in a suitable bioreactor. Despite the advancements in wastewater treatment, numerous challenges still exist that have to be addressed in order to make laccase-mediated systems a commercially competitive technology. However, with a clear understanding of the challenges, it will be possible in the future to design a desired laccase system without compromising its stability and activity, for the continuous remediation of wastewater in an environmentally friendly and economically feasible way.

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