Review

Mitigation of bisphenol A using an array of laccase-based robust bio-catalytic cues – A review

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ARTICLE INFO

Article history:

Received 25 May 2019

Received in revised form 23 June 2019

Accepted 24 June 2019

Available online xxx

Editor: Paola Verlicchi

Keywords:

Bisphenol A

Laccase

Enzyme immobilization

Oxidative degradation

Acute toxicity

Estrogenicity

ABSTRACT

Bisphenol A (BPA) is a known endocrine disruptor that poses concerning environmental and human-health related issues and ecological risks. It has been largely used as an intermediate in the manufacture of epoxy resins and polycarbonate plastics. Traces of BPA can reach into the environment through inadequate or inefficient removal during wastewater treatment, uncontrolled landfill leachates, and leaching out from the discarded BPA-based materials. Several physicochemical treatment methods including adsorption, Fenton, ozonation, electrochemical and photochemical degradation, and membrane filtration, have been applied for BPA elimination. However, these methods are not adequate for large-scale treatment due to some inherent limitations. Benefiting from high catalytic efficiency and specificity, enzyme-based bio-catalytic degradation strategies are considered quite meaningful alternative for efficient and effective BPA removal from different routes. Among various oxidoreductases, i.e., laccases exhibited a superior potential for the remediation of BPA-containing wastewater. Enzymatic oxidation of BPA can be boosted by using various natural or synthetic redox mediators. Immobilized enzymes can expand their applicability to continuous bioprocessing and facilitates process intensification. Therefore, optimized formulations of insolubilized biocatalysts are of strategic interest in the environmental biotechnology. In this review, recent research studies dealing with BPA removal by the laccase-catalyzed system are presented. At first, the presence of BPA in the ecosystem, sources, exposure, and its impact on the living organisms and human beings is summarized. Then, we highlighted the use of crude as well as immobilized laccases for the degradation of BPA. In addition to toxicity and estrogenicity removal studies, the unresolved challenges, concluding remarks, and possible future direction is proposed in this important research area. It is palpable from the literature reviewed that free as well as immobilized forms of laccases have displayed noteworthy potential for BPA removal from wastewater.

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1. Introduction

Endocrine disrupting chemicals (EDCs) have recently drawn scientific and public attention due to evident environmental issues. These compounds disrupt hormonal activities and adversely affect human health and aquatic organisms by disrupting growth, development, and reproduction. Some EDCs are also known to exhibit toxic, mutagenic, and carcinogenic effects (Huang and Weber, 2005; Barrios-Estrada et al., 2018a). Bisphenol A (BPA), also known as 2,2'-bis-(4-hydroxyphenyl) propane or 4,4'-isopropylidenediphenol, is the most commonly detected emerging pollutants in the environment and aquatic ecosystem (Ying et al., 2009; Barrios-Estrada et al., 2018b; Bilal et al., 2019a). BPA has been extensively studied because

of its widespread application as monomers in the manufacture of epoxy resins and polycarbonate plastic (Fig. 1). These epoxy resins are utilized in preparing polycarbonate baby feeding bottles and epoxy food-can coatings (Dekant and Völkel, 2008). However, it has been categorized as an EDCs by the US Environmental Protection Agency (EPA) because it interferes with production, secretion, transport, mechanism of action or removal of natural hormones associated with the homeostasis, behavior or reproduction in living organisms. It exhibits acute toxicity to the aquatic creatures in the range of 1000–10,000 µg/L and proven to be estrogenic at concentrations below 1 µg/m³ (Kang and Katayama, 2008; Careghini et al., 2015). Therefore, the release of BPA to aquatic setting has become a tangible challenge for the researchers and the scientific community over the past decade.

The techniques for removal of EDCs from wastewater can be divided into three categories including physical treatment, biological removal and advanced oxidation processes (AOPs) including Fenton reaction (Mohapatra et al., 2011), photocatalytic oxidation

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Fig. 1. Persistence, applications, transmission routes and preventive measures to BPA exposure.

(Kanigaridou et al., 2017), ozonation (Wang et al., 2014), and ultrasonic oxidation (Yu et al., 2014). Though these wastewater treatments can remove several EDCs, significant shortcomings such as elevated costs and generation of undesirable compounds hindered their applications. Other commonly used treatment approaches i.e., activated carbon adsorption are restricted due to the low hydrophobicity of BPA (Choi et al., 2006). These facts necessitate a versatile treatment approach to completely eliminate EDCs that may pose risks even at extremely low levels (Liu et al., 2009; Bilal and Iqbal, 2019a; Rasheed et al., 2019).

BPA can be biodegraded and transformed by the use of microbial enzymes, including manganese peroxidase, lignin peroxidase, horseradish peroxidase, polyphenol oxidases, and tyrosinases. Application of the laccases and peroxidases are of exceptional interest owing to broad-spectrum substrates range, and capability for environmental applications (Bilal et al., 2017; Bilal et al., 2018a; Chauhan and Jha, 2018; Bilal et al., 2019b). Laccases are multi-copper polyphenol oxidases with an omnipresent distribution in nature, ranging from various fungi to higher plants (Baldrian, 2006). Owing to proficiency, specificity, and eco-sustainability, these biocatalysts have found wider applications in the food industry, bioremediation, paper, and pulp industry, biofuel industry, textile industry, and other industries (Alcalde, 2015; Bilal et al., 2017; Mate and Alcalde, 2017; Vera et

al., 2019). The ability of laccases to oxidize phenolic and non-phenolic compounds can be enhanced in the presence of a suitable natural or synthetic redox mediator that acts as an 'electron shuttle' between the enzyme and pollutant. The broad application of native laccases tends to be hindered by elevated processing costs, problematic separation, easy deactivation, and susceptibility of the enzymes under industrial settings (Bilal et al., 2018b; Bilal et al., 2018c; Bilal and Iqbal, 2019b; Bilal et al., 2019c). Lack of adequate functional stability of enzyme due to activity loss is another limiting factor for larger scale exploitation of laccase enzymes. Immobilization of enzyme provides an excellent means to solve many of these associated drawbacks (Bilal and Iqbal, 2019c; Bilal et al., 2019d). Increased catalytic stability and repeatability of the insolubilized biocatalysts reduced their application costs. Numerous studies have reported the enhanced ability of the immobilized laccases in biodegradation and biotransformation of EDCs. Therefore, substantial efforts have been made in the past years to developing optimized formulations of immobilized biocatalysts for bioremediation purposes. However, to date, there has been no review focused on the degradation and elimination of BPA from water by laccase catalyzed system. This review summarizes the current progress regarding the application of soluble as well as immobilized laccase enzymes from different microbial source for the biotransformation and removal of BPA from the aqueous media. After a

description of the potential source, occurrence, and associated detrimental effects on the ecosystem/human health, the use of free and immobilized laccase-based biocatalytic systems for the degradation and detoxification of BPA is highlighted.

2. Sources, exposure and health-related consequences of BPA

BPA is a high-value chemical with worldwide production of over 2 Metric ton (Mt) in 2003 (Burridge, 2003). Reports have shown that 95% of BPA was globally consumed in the manufacture of polycarbonates and epoxy resins used in adhesives, epoxy surface coatings, electrical laminates, and composites. The polycarbonate plastics are typically employed in food and drink packing, whereas epoxy resins are generally employed to coat metal products such as bottle tops, food cans, and water supply pipes (Rivas et al., 2009). Among the total industrial production, approximately 100 tons might have been discharged into the environment (Rubin, 2011). BPA has been identified in wastewater effluent and sludge in wastewater treatment plants (WWTPs). WWTPs are conceived as one of the leading secondary routes of BPA pollution because of its inadequate degradation or elimination by physicochemical and biological treatment methods. It can enter industrial and municipal wastewater through direct discharge to sewers and stormwater run-off (Mohapatra et al., 2010). Besides, it can be introduced into the ecosystem from paper mill effluents, landfill leachate, and polycarbonate plastics degradation (Rigol et al., 2002; Mohapatra et al., 2010). Agronomic and industrial wells can also result in the discharge of BPA to groundwater. Fig. 2 represents the possible routes of BPA contamination to ground and surface water.

Among the potential sources of human acquaintance to BPA, foods are considered as the main exposure source. Whereas, transdermal absorption and inhalation of polluted airborne dust are likely to be the secondary routes. Heating, acid and basic environments can intensify the migration rate of BPA from food packing to foods generat-

ing pronounced threat for public health (Geens et al., 2011). Identification and detection of BPA in the amniotic fluid, urine, serum, and placental tissues indicates its continuous exposure to humans. The occurrence of BPA in wastewater effluents at low but environmentally pertinent concentrations reveals that the classic treatment technologies are not satisfactorily effective in the removal of these toxic compounds (Ikezuki et al., 2002).

In 1938, BPA was first recognized to be estrogenic, in MCF-7 human breast cancer cell culture (Dodds and Lawson, 1938; Sohoni and Sumpter, 1998). Based on the laboratory investigations, BPA can act as an EDCs by binding to estrogenic receptors in living entities (Rochester, 2013). BPA, even at a very low level (1–10 µg/L) is intensely noxious to freshwater and marine organisms. It can cause feminization in different animal species (i.e., fish) and has shown adverse health effects in experimental rats (Vom Saal and Hughes, 2005). Though there is a dearth of studies reporting the influence of BPA on humans, nevertheless, its adverse effects on animals reveal potentially adverse impacts in humans as demonstrated by studies correlating BPA with miscarriages and ovarian diseases (Sugiura-Ogasawara et al., 2005). Apart from the reproduction, developmental, behavioral, and neurochemical effects, exposure of BPA is also thought to cause cardiovascular disease, human prostate and breast cancer, impaired brain development, hormonal imbalance, type-2 diabetes, proliferation of tumor cells and anomalies in hepatic enzymes (Wetherill et al., 2002; Anderson et al., 2012). Maternal acquaintance to BPA results in post-delivery alterations in DNA methylation changes the expression of some specific genes in children and interrupts epigenetic programming of gene expression during child growth (Kundakovic and Champagne, 2011). It was also reported to be oncogenic with the potential mechanisms including endocrine disruption, promoting tumorigenic development, genotoxicity, and growth reprogramming that upsurges vulnerability to other cancer-causing events (Keri et al., 2007). Fig. 3 illustrates the adverse effects of BPA exposure on human health.

Fig. 2. Representation of possible routes of BPA contamination to ground and surface water.

Fig. 3. Adverse effects of BPA exposure on human health.

3. Crude laccases for degradation of BPA

Laccases are the oxidoreductive enzymes that are extensively used in diverse industrial applications owing to their unique biocatalytic properties of easy availability, high stability, and broad-spectrum substrate specificity. These enzymes can oxidize a broad range of contaminants, such as aromatic, anilines, dye pollutants, and phenols, etc. However, the elevated purification cost of enzymes has become the major hindrance for widespread applications of purified laccases. Laccases-assisted processes will become much less expensive by bypassing the purification step. Therefore, the development of methods using crude laccases for the degradation of BPA is of prime interest. Apart from this, the purified laccases display low BPA oxidizing capability because of the absence of redox mediators. Such mediators naturally exist in the crude extracts, while chemical mediators must be incorporated during the use of purified laccases. Mediators are small-molecular-weight natural and synthetic compounds that substantially improve the catalytic performance of laccases (Cañas and Camarero, 2010). Several studies have utilized the laccases-based homogeneous enzyme-catalyzed reactions for the biotransformation and biodegradation of BPA from wastewaters with and without adding redox mediators (Table 1). For example, Chairin et al. (2013) achieved 12% degradation of 100 mg/L BPA after 12 h treatment of a purified laccase (0.64 U/mL) from *T. polyzona*. Whereas, BPA was eliminated

in 3 h when the synthetic mediator 1-Hydroxybenzotriazole (HBT) was added from the reaction combination. Furthermore, maximal BPA removal was reached by a purified *T. versicolor* laccase only by adding 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) mediator to the reaction mixture (Atacag Erkurt, 2015). Highly efficient biodegradation of BPA by non-dialyzed crude laccase extracts compared with the dialyzed extracts evidently demonstrate that the natural mediators are essential in combination with the enzymes for complete BPA removal. However, the use of natural mediators is much more cost-effective and eco-sustainable than that of artificial compounds. Similarly, de Freitas et al. (2017) developed a safe, cost-effective, and eco-friendlier method in the bioremediation of soils, water, and industrial wastes polluted with endocrine disruptor BPA using crude laccases from *Pleurotus* strains. After 1 h of reaction treatment by laccase (8.0 U L^{-1}) from *P. ostreatus* and *P. pulmonarius*, 100% and 85% BPA was removed at a final concentration of 100 mg/L and 200 mg/L, respectively. Thirteen aliphatic and aromatic degradation metabolites of BPA, including ethyl-3-ethoxy propanoate, methylpent-3-ic acid, 4-ethyl-2-methoxyphenol, and *p*-isopropenylphenol, were identified by high performance liquid chromatography and gas chromatography–mass spectrometry (GC–MS) analyses. Different authors have identified various biodegradation metabolites during the removal of BPA, and thus proposed different pathways for BPA degradation. de Freitas et al. (2017) identified thirteen degradation metabolites and proposed a possible mechanism of

Table 1

Biodegradation and biotransformation of BPA by crude/free laccases under different reaction conditions.

Enzymes sources	Treatment time (min)	BPA removal (%)	Intermediate products	References
<i>Trametes versicolor</i> JSRK13	LPST strategy using HBT acting as a redox mediator. The reaction containing 1 mL BPA (250 µM) and 1 mL laccase (100 U/mL) was performed for 24 h at 28 °C.	Above 90%	NR	Singh et al. (2019)
<i>Trametes versicolor</i>	1 h reaction at pH 5.2, Temp. 44.6 °C, initial BPA concentration (5 mg/L).	97.68%	Ethylbenzene, <i>p</i> -xylene, and cyclohexanone 1-methyl-4-isopropenyl-2-cyclohexanol	Hongyan et al. (2019)
<i>Phanerochaete chrysosporium</i>	The reaction mixture (3 mL) consisted of 1 mL BPA (1, 4 mM) at pH 9.0 and 30 °C for up to 3 h.	93%	NR	Nejad et al. (2019)
<i>Pleurotus ostreatus</i>	Enzyme (3 to 10 U) was incubated with BPA (50 to 200 mg/L) at different pH 5.0 and 28 °C.	100% at an initial of 50 mg/L BPA 90% at a concentrations of and 100 mg/L	<i>p</i> -Isopropenylphenol, ethylpent-3-oic acid, ethyl-3-ethoxy propanoate, and 4-ethyl-2-methoxyphenol.	de Freitas et al. (2017)
<i>Pleurotus pulmonarius</i>	Enzyme (3 to 10 U) was incubated with BPA (50 to 200 mg/L) at different pH 5.0 and 28 °C.	80%	Thirteen aliphatic and aromatic including <i>p</i> -isopropenylphenol, methylpent-3-oic acid, ethyl-3-ethoxy propanoate, and 4-ethyl-2-methoxyphenol.	de Freitas et al. (2017)
<i>Coriolopsis gallica</i>	The reaction mixture contained 0.01% BPA and 1.0 U/mL in 100 mM sodium acetate buffer at pH 5.0 and 45 °C and conducted for 24 h.	100%	Tartaric acid and β-hydroxybutyric acid generated from oxidative reactions using HBT as a redox mediator and without HBT, respectively.	Daâssi et al. (2016)
<i>Trametes versicolor</i>	The initial concentration of BPA was 50 mg/L. The reaction was carried out at 40 °C and 150 rpm using the purified laccase.	30%	Nonane, 4-ethyl-2-methoxy-phenol, and 5-(2-methyl propyl)	Atacag Erkurt (2015)
<i>Funalia trogii</i>	Reaction mixture contained BPA (50 mg/L) and crude laccase and incubated at 40 °C and 150 rpm in the dark.	100%	Nonane, nonadecane 5-(2-methyl propyl), and 4-ethyl-2-methoxy-phenol	Atacag Erkurt (2015)

HBT—1-hydroxybenzotriazole; LPST—laccase production and simultaneous transformation; NR—not reported.

laccases for BPA biotransformation and its degradation products by *P. ostreatus* (Fig. 4). It is well documented that the redox potential of laccases from different white-rot fungi (WRF) varies between 0.4 and 0.8 V vs. NHE (Fei et al., 2015), and thus influence the relative amount of each intermediate. The laccases-mediated oxidative biodegradation of BPA producing *p*-isopropenylphenol as an intermediate has also been reported in various former studies (Fukuda et al., 2001). Fukuda et al. (2004) revealed BPA oxidative condensation pathway generating oligomers with C—O or C—C bonds between phenolic groups followed by its cleavage resulting in the formation of *p*-isopropenylphenol. Other reports also substantiated condensation pathway-based degradation of BPA by laccases by identifying some high molecular weight degradation products and polymeric structures (Torres-Duarte et al., 2012; Cabana et al., 2007). The acute toxicity of *P. ostreatus* laccase-assisted BPA was also significantly reduced from 85% to <5.0%. In contrary, *P. pulmonarius* laccase did not decrease the toxicity, and at least one BPA metabolite showed toxicity as the parent compound itself (de Freitas et al., 2017). Daâssi et al. (2016) analyzed the potential of *Coriolopsis gallica* laccase as a biocatalyst for the rapid oxidation and removal of BPA. GC–MS profile revealed carboxylic acid derivatives including tartaric acid as the major BPA degradation metabolites by laccase-mediated reaction using HBT as a redox mediator, whereas oxidative reactions lacking HBT resulted in a β-hydroxybutyric acid generation. BPA degradation profile showed that BPA was entirely bio-transformed within 3 h of reaction. Fig. 5 depicts the proposed pathways for BPA degradation by laccase from *C. gallica* in the presence and absence of HBT (Daâssi et al., 2016).

4. Immobilized laccase for degradation of BPA

As stated above, the rising importance in applications of laccases is based on its great biotechnological versatility and catalytic potential to oxidizing a wide spectrum of substrates. Recently, laccases

have been employed to degrade and transform an array of xenobiotic compounds including synthetic dyes, pesticides, EDCs, polycyclic aromatic hydrocarbons, and pharmaceuticals (Taheran et al., 2017). Several factors, such as the high production cost, loss of functional stability, and non-recyclability make the soluble laccases-based treatment economically offensive in the biodegradation of above-mentioned xenobiotic. Immobilization technology may overcome, at least in part, the drawback for larger scale enzyme implementation. Therefore, substantial research efforts have been directed in the last few years to developing insolubilized laccases potentially useful in bioremediation purposes. Remarkably, laccase immobilization results in numerous improvements including increased operational and storage stabilities, better control of the enzymatic reaction, and the possibility of multiple uses. A variety of insolubilized laccases have been conceived as suitable biocatalysts for biodegradation of BPA (Couto and Herrera, 2006; Ba et al., 2013; Upadhyay et al., 2016). Various solid supports including silica, hydrogels, sodium alginate, kaolinite, chitosan, graphene oxide and recently magnetic iron oxide nanoparticles, and magnetic nanoflowers have been exploited for laccases immobilization and used for the exclusion of BPA in batch and continuous reactions (Table 2). Immobilization of laccase enzyme within these insoluble support matrices can be accomplished through physical and chemical means. Attachment of enzymes to insoluble supports via hydrophobic or electrostatic interactions and encapsulation or entrapment within the support network is the most common physical methods. Whereas chemical immobilization is attained through generating covalent bonds between the solid support and enzyme molecule.

4.1. Biopolymers-based hydrogels for laccase immobilization and removal of BPA

Naturally derived or biopolymers-based smart hydrogels are well known for providing biocompatible environments for the immobiliza-

Table 2

Biodegradation and biotransformation of BPA by immobilized laccases under different reaction conditions.

Enzyme source	Support/bioreactor	Immobilization method	Operating conditions	BPA removal (%)	References
<i>Trametes versicolor</i>	Bifacial EMR	'Reverse filtration' of laccase followed by PDA coating	Biocatalytic membranes were immersed in 20 mL of BPA solution (2, 5 and 10 mg·L ⁻¹) for 6 h under shaking (100 rpm).	Much higher and stable BPA removal when EMR operated in flow-through mode compared with the contact mode for 9 reuse cycles	Cao et al. (2018)
<i>Cyberlindnera fabianii</i>	Calcium alginate beads	Entrapment	100 µM BPA and 250-µL laccase were added to 10 mL distilled water and 1 mL Folin-Ciocalteu reagent. Degradation was monitored for 24 h at 3 h intervals.	71%	Olajuyigbe et al. (2019)
<i>Cyberlindnera fabianii</i>	Copper alginate beads	Entrapment	Laccase (250 µL) and BPA (100 µM) were mixed and enzymatic degradation was measured for 24 h.	65.5%	Olajuyigbe et al. (2019)
<i>Pleurotus ostreatus</i>	MANAE-agarose	Adsorption	Enzyme (5 U/mL) was reacted with BPA (100 mg·L ⁻¹) in 50 mM acetate buffer (pH 5.0) for 1 h at 28 °C and 120 rpm	>90% of degradation after 15 cycles of reuse	Brugnari et al. (2018)
<i>Trametes versicolor</i>	FBR using stabilized laccase in mesoporous silica	Cross-linking	Laccase (68 U·L ⁻¹) was reacted with BPA (25 mg·L ⁻¹) at pH 5 for 2 h in FBR	53.8%–81.0%	Piao et al. (2019b)
<i>Trametes versicolor</i>	Mesoporous silica	Cross-linking	Laccase (480 U·L ⁻¹) was reacted with BPA (25 mg·L ⁻¹) at pH 5.0 for 2 h	90.1%	Piao et al. (2019a)
<i>Trametes versicolor</i>	Cu(II)-ion-chelated magnetic microspheres	Adsorption	Reaction was conducted for 12 h in phosphate buffer (pH 5.0, 0.2 mol/L) containing 50 mL BPA solution and 0.1 g of immobilized laccase.	85%	Lin et al. (2016)
<i>Trametes versicolor</i>	Mn(II)-ion-chelated magnetic microspheres	Adsorption	Reaction was conducted for 12 h in phosphate buffer (pH 5.0, 0.2 mol/L) containing 50 mL BPA solution and 0.1 g of immobilized laccase.	88%	Lin et al. (2016)
<i>Trametes versicolor</i>	MWCNTs-LCEfMs	Encapsulation	Support loaded with 2 mg enzyme and 30 mL of BPA solution (10 mg/L) were incubated at 25 °C for 5 h under shaking (150 rpm).	85%	Dai et al. (2016)
<i>Trametes pubescens</i>	Ca-alginate beads	Cross-linking + entrapment	Optima operating conditions were pH 5, 30 °C, 20 mg/L BPA and 1500 U·L ⁻¹ laccase for 2 h	99%	Lassouane et al. (2019)
<i>Trametes sp.</i>	Hollow fiber membranes	Covalent cross-linkage	Solutions of 10 ppm BPA in the presence of with 0.5 mM redox mediators were permeated.	100% degradation using ABTS at a low space velocity	Mokhtar et al. (2019)
<i>Trametes versicolor</i>	TiO ₂ sol-gel coated PVDF membrane	Chemical coupling	40 mL of BPA (150 mM) in 0.1 M acetate buffer (pH 5.5) solution was reacted for 24 h	>80%	Hou et al. (2014)
<i>Aspergillus oryzae</i>	Enzymatic membrane reactor	–	The reactor was operated at pH 6.8 and 28 °C for 24 h by maintaining 3.0 mg/L dissolved oxygen.	Higher than 85% and 95% without and adding syringaldehyde, respectively	Nguyen et al. (2014)
<i>Pycnoporus sanguineus</i>	Multi-channel ceramic membrane based bioreactor	Covalent binding	Active membrane incorporated bioreactor was operated at a fixed flow of 10 L/h of BPA (20 mg/L) at 25 °C.	100%	Barrios-Estrada et al. (2018b)
<i>Trametes versicolor</i>	Multi-channel ceramic membrane based bioreactor	Covalent binding	Active membrane incorporated bioreactor was operated at a fixed flow of 10 L/h of BPA (20 mg/L) at 25 °C.	100%	Barrios-Estrada et al. (2018b)
<i>Trametes versicolor</i>	Magnetic nanoflowers	Covalent binding	MNFs were added into 120 mL BPA (10 to 100 mg·L ⁻¹) and shaken at 120 rpm for 45 min.	100% within 5 min	Fu et al. (2019)
<i>Trametes versicolor</i>	TiO ₂ nanoparticles	Physical adsorption	BPA concentration: 0.15 mg mL ⁻¹ and 50 mg of the nanocomposite were placed at 21 °C.	93.3%	Tapia-Orozco et al. (2018)
<i>Trametes versicolor</i>	TiO ₂ nanoparticles	Covalent linkage	BPA concentration: 0.15 mg mL ⁻¹ and 50 mg of the nanocomposite were placed at 21 °C.	91.3%	Tapia-Orozco et al. (2018)
<i>Coriolopsis polyzona MUCL38443</i>	Mesoporous silica particles	Sequential adsorption-cross linking	The reactor was constantly fed with 50 mM BPA solution at a flow rate of 27 mL/h.	90% in CSTMR	Demarche et al. (2012)
<i>Rhus vernificera</i>	(HPEI/PES) electrospun nanofibrous membranes	Covalent binding	BPA (100 µg/L) was passed through the membranes at a constant pressure of 600 kPa.	89.6%	Koloti et al. (2018)
<i>Pleurotus ostreatus</i>	Functionalized TiO ₂ nanoparticle surface hybrid membrane reactor	Covalent binding	50 mL BPA solution was poured into the membrane cell and Mixed by a magnetic stirrer. The experiments continued for 24 h at a constant temperature of 25 °C.	90% within the first 6 h	Ji et al. (2017)
<i>Trametes versicolor</i>	PEG hydrogel microparticles	Encapsulation	30 mg of microparticles were incubated with 0.5 mL BPA solution (25 mg/L) at room temperature and agitation speed of 150 rpm.	Over 90%	Piao et al. (2019a)

BPA–PDA–polydopamine; EMR–enzymatic membrane reactor; MANAE–agarose—monoaminoethyl-N-amino ethyl; FBR–fluidized bed reactor; MWCNTs–LCEfMs–Multi-walled carbon nanotubes modified electrospun fibrous membranes; PVDF– hydrophilic polyvinylidene fluoride; ABTS–2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); MNFs–magnetic nanoflowers; CSTMR–continuous stirred-tank membrane reactor; HPEI/PES–hyperbranched polyethylene mine/polyether sulfone; PEG–polyethylene glycol.

tion of biomolecules in a desired polymeric network. Therefore, such hydrogels have shown great potential as support matrices for enzyme entrapment to upgrade their catalytic performance, stability, and reusability. Gel entrapment has been envisioned as one of the paramount immobilization techniques because of gel polymers non-toxicity, mild process, and no damage to the intrinsic confirmation of the biocatalyst. For example, Koklukaya et al. (2016) entrapped laccase in poly (acrylamidecrotonic acid)/ χ -carrageenan, poly (acrylamide-crotonic acid)/sodium alginate, or poly(acrylamide-citraconic acid)/ χ -carrageenan polymers-based hydrogels and used for degradation of acid orange 52. The resulting hydrogel-immobilized enzyme led to 73% color removal in the presence of the mediator. Asgher et al. (2017) encapsulated laccase into gelatin, agar-agar, and polyacrylamide-based hydrogels, and evaluated for their catalytic activity, thermal stability, and kinetic characteristics. The as-prepared hydrogels loaded biocatalysts were more active and durable than their free counterparts. In addition to excellent supports for enzymes entrapment, hydrogels can also be good adsorbent materials for pollutants in aqueous solution. Considering the easy efficient bio-catalytic reaction for pollutants elimination, recently, Piao et al. (2019a) synthesized laccase-loaded highly durable, recyclable, and multi-functional biocatalytic micro-particles for BPA removal. For this, the laccase enzyme was encapsulated into polyethylene glycol (PEG) hydrogel by polymerization technique (Fig. 6). The obtained glutaraldehyde (GLU) cross-linked spherical particles presented high enzyme loading, activity recovery, and profound stability at wider pH values (pH 3.0–7.0) compared with the free state of the enzyme. Results showed about 85.6% removal efficiency during the first 12 h by incubating the BPA solution (0.5 mL, 25 mg/L, pH 5.0) with 30 mg laccase

loaded particles at room temperature. After this period, the biotransformation was gradually increased to 93.6% and 100% within 24 h and 36 h, respectively. Moreover, the enzyme-coated particles were recycled for several BPA biotransformation rounds and retained higher than 90% of initial BPA removal efficiency after seven-repeated cycles. Notably, external shaking was not required during the process as the BPA removal occurred inside the microenvironment of the synthesized laccase-particles.

Olajuyigbe et al. (2019) extracted and purified laccase from *Cyberlindnera fabianii* by ion exchange and gel exclusion chromatography, which displayed an approximate molecular weight of 52 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Purified laccase was then entrapped on Cu and Ca-alginate microspheres, characterized and applied for the removal of BPA. Both forms of the gel-entrapped laccases exhibited higher storage stability than free enzyme for over 21 d at 4 °C and was operationally stable for three repeated cycles with retention of >70% residual activity. Particularly, Cu and Ca-alginate entrapped laccases led to 65.5% and 71% degradation of BPA at the end of 14 d. Though entrapment in alginate beads is low-cost, environmentally friendly, and an easy technique for enzyme immobilization, but is limited by the porosity of the gel matrix gel leading to enzyme leaching. To overcome this issue, Lassouane et al. (2019) developed a new and promising strategy that involved GLU cross-linking of crude laccase from *T. pubescens* before their entrapment into Ca-alginate beads. This immobilization method led to a 30% increase in immobilization yield along with 7-fold enzyme leakage reduction compared with the laccase immobilization without cross-linking. Under the optimal conditions of pH 5.0, and 30 °C, the newly developed biocatalyst (1500 U L^{-1} lac-

Fig. 6. Schematics for the preparation of laccase entrapped polyethylene glycol (PEG) hydrogel micro-particles. Reprinted from Piao et al. (2019a), an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>). Copyright (2019) the authors. Licensee MDPI, Basel, Switzerland.

case) could remove >99% BPA (20 mg/L) from aqueous solutions in 2 h reaction time. Moreover, it retained higher than 70% activity even after 10 consecutive BPA removal batches. Insignificant adsorption of BPA onto the alginate beads confirmed that BPA biotransformation was entirely attributed to the laccase action. Recently, Brugnari et al. (2018) immobilized *P. ostreatus* laccase on an activated agarose carrier support i.e. monoaminoethyl-N-aminoethyl (MANAE)-agarose gel to improve its catalytic performance for the removal of BPA. The temperature stability of agarose-immobilized laccase was considerably increased ($p \leq 0.05$) as compared to its free counterpart with half-lives 2.3- and 7.0-folds greater at 40 °C and 55 °C, respectively. Immobilized biocatalyst retained 80% and 70% of its original catalytic performance after 40 and 170 days of storing at 4 °C. In addition, >90% retention of its BPA degradation capability after 15 repeated cycles indicate MANAE-agarose as a cost-effective substitute for scale-up BPA degradation in aqueous solutions.

4.2. Nanosupports for laccases immobilization and removal of BPA

Among the different support carriers attempted for successful laccase immobilization, magnetic iron oxide nanoparticles appeared as highly proficient carriers for laccase immobilization (Bayramoğlu et al., 2010). These support materials are essentially based on the magnetic properties of the solid-phase that facilitate rapid separation in a magnetic field. Furthermore, these magnetic supports can be easily

separated and recycled by externally using a magnetic field, and hence, capital and processing costs can be substantially reduced (Pogorilyi et al., 2014). Nevertheless, the magnetic nanoparticles are required to functionalize with some biocompatible materials to improve the catalytic properties of the immobilized biomolecules. A laccase from *T. versicolor* was adsorbed onto Mn(II)- and Cu(II)-chelated magnetic microspheres and the resulting nanobiocatalyst was applied for the removal of BPA from aqueous solution (Fig. 7) (Lin et al., 2016). Compared with the free enzyme, the storage and thermal stabilities of metal-ion-chelated magnetic microspheres absorbed laccase were considerably enhanced. Immobilized enzyme preserved higher than 38% of its preliminary catalytic activity at 65 °C, while the free laccase completely lost its activity under identical conditions. Under the optimal operating conditions, as-prepared nanobiocatalyst presented a high BPA removal efficiency, and resultantly, >80% of BPA was eliminated under the combined actions of adsorption and biodegradation (Lin et al., 2016). More recently, Piao et al. (2019b) established a highly-efficiency treatment system for BPA in a fluidized bed reactor (FBR) by crosslinking laccase inside mesoporous silica using GLU. The as-prepared developed biocatalytic system displayed better stability than the free laccase over a wide range of pH and temperatures conditions. It also showed robust BPA biotransformation efficiency in a batch reaction relative to the free-state of the enzyme. The cross-linked enzyme exhibited pronounced reusability and could be recycled for 3-times without losing

Fig. 7. Schematic illustration of reversible immobilization of laccase onto magnetic microspheres for BPA degradation. Reprinted from Lin et al. (2016), with permission from Elsevier. Copyright (2015) Elsevier B.V.

any removal efficacy. Great stability, and retention of >50% if its original BPA removal performance after ten times repeated use render mesoporous silica as robust support to developing biocatalysts for continuous degradation of various emerging pollutants compared with the batch reaction.

Electrospun fibrous membranes (EFMs) comprising of non-woven fibers with diameters ranging from 10 to 100 nm are regarded as promising support materials for enzyme immobilization because of their huge specific area, biocompatibility, porosity, permeability, and interconnectivity, etc. (Wang et al., 2009; Tran and Balkus, 2012). Dai et al. (2010) fabricated the core-shell structural electrospun fibers for direct laccase encapsulation by employing an emulsion electrospinning technique and eventually formed the laccase-attached EFMs. Benefiting from the biocompatible polymer shell microenvironment, and moderate encapsulation method, the developed EFMs presented improved stability and preserved above 67% of its free laccase activity. Nevertheless, it still lost higher than 30% of activity after immobilization presumably due to reduced diffusion of substrates/products, and the electron-transferring rate, and contact between enzyme molecule and the substrate (Xu et al., 2013). In order to address the above-stated shortcomings, multi-walled carbon nanotubes (MWCNTs) were utilized as favorably modified materials to enhance the performance of enzymatic electrodes and biofuel cells owing to their excellent mechanical strength and electrical conductivity (Reuillard et al., 2015). Several MWCNTs-modified EFMs (MWCNTs-EFMs) have been developed for enzyme immobilization, and numerous reports have substantiated an effective increase in electron transfer rate between enzymes and membrane electrodes or substrates by using MWCNTs (Gutiérrez-Sánchez et al., 2012; Jose et al., 2012). Dai et al. (2016) successfully fabricated MWCNTs-LCEFMs via emulsion electrospinning by encapsulating an active laccase inside these nanofibers. The as-prepared MWCNTs-LCEFMs exhibited not only high activity recovery but operational and storage stabilities were also improved due to the greater electron transfer during laccase-catalyzed reaction system. The resistance of MWCNTs-LCEFMs towards varying environmental temperature and pH conditions was also increased. Finally, the removal efficiency of the newly synthesized MWCNTs-LCEFMs for BPA reached higher than 90% under wider temperature and pH ranges because of the elevated adsorption and tensile strength.

The TiO_2 nanoparticles (NPs) is another promising immobilization support because of its low price, high surface area, fine-tuned surface structure, coordination ability, good hydrophilicity, biocompatibility and steadiness (Chen and Dong, 2003; Ji et al., 2017). Moreover, the occurrence of hydroxyl groups on the TiO_2 NPs surface allows further functionalization for covalent attachment of enzyme, and the resultant biocatalytic nanoparticles presented greater operative stability for pollutants biodegradation (Durán et al., 2002). Covalent laccase immobilization onto the TiO_2 NPs maintained about 80% of its original activity (Hou et al., 2014). Despite excellent catalytic performance, reutilization of TiO_2 NPs immobilized bio-catalytic system suffered a major hindrance for wastewater remediation. Immobilization of laccase on polymeric and inorganic membranes can address this technical difficulty. Inorganic NPs coated nano-composite membrane exhibits the benefits from organic as well as inorganic components including low price, high flexibility and stability, huge surface area, and well-ordered surface morphology (Zhao et al., 2011). Many attempts have been dedicated to use nanostructured composites to immobilize enzyme for biosensors development. Recently, the TiO_2 NPs coated polyvinylidene fluoride (PVDF) micro-filtration membranes were used as novel nanocomposite support material for laccase immobilization by covalent bonding using APTES and GLU as functionalization agents. The TiO_2 -functionalized nano-membranes presented a significant improvement in BPA elimination efficacy and operational stability under moderate flux conditions. Immobilized biocatalyst preserved >90% of its original BPA removal efficiency after four degradation cycles (Hou et al., 2014). Mokhtar et al. (2019) designed polymer brushes functionalized novel hollow fiber membrane to host laccase for biodegradation of BPA. The radiation-induced graft polymerization was used to synthesize polymer brushes of glycidyl methacrylate using aminoethanol as the functional group to unfold polymer brushes for efficient multilayer laccase immobilization (Fig. 8) (Mokhtar et al., 2019). The as-prepared system substantially improved the stability of the immobilized laccase than free enzyme in methanol-containing solutions by means of aminoethanol hydroxyl groups. The membrane system was capable in the oxidation of BPA resulting in higher levels of substrate degradation. Among the three major redox mediators studied for laccase activity improvement including ABTS, HBT, and 2,2,6,6-tetramethyl-1-piperidinyloxy, only ABTS augmented the oxidation efficiency with 100% BPA removal.

Fig. 8. Scheme for the design of functional membranes using radiation-induced graft polymerization. Reprinted from Mokhtar et al. (2019), with permission from Elsevier. Copyright (2019) Elsevier B.V.

4.3. Magnetic nanoflowers immobilized laccases for removal of BPA

Recently, a simple and novel immobilization approach was unveiled to fabricate self-assembled enzyme-inorganic hybrid nanostructured materials with flower-like shapes (Ge et al., 2012). These hybrid nanoflowers were extremely highly active and durable compared to the free or traditionally immobilized biocatalysts because of their larger surface area and efficient enzyme confinement (Rong et al., 2017). Altinkaynak et al. (2016) reported that the laccase-loaded inorganic hybrid nanoflowers displayed remarkably 650% increase in syringaldazine oxidizing activity than that to the free form of the laccases. Despite the fact of extremely elevated enzyme activity and stability of these nanoflowers, inadequate recyclability and reusability unfavorably hamper their widespread practical applications. The synthesis of recyclable enzyme-inorganic nanoflowers designed by integrating magnetic nanoparticles (MNPs) with nanoflowers might overcome the above-mentioned inadequacies owing to their low cost, simple operation, easy separation, and distinctive magnetism property (Gao et al., 2018). In this context, Fu et al. (2019) recently constructed highly efficient, easily separable and recyclable laccase-incorporated MNFs by coupling amino-functionalized MNPs onto the laccase-inorganic hybrid nanoflowers and applied for the degradation of BPA in aqueous solution. The prepared MNFs were porous, spherical, and flower-like structure with an average diameter of 15 μm as characterized by SEM (Fig. 9) (Fu et al., 2019). Under the optimal operating conditions, the resulted hybrid nanocomposites achieved 100% degradation of BPA within only 5 min of reaction and maintained >90% of its original initial activity after 2 months of storage at 4 $^{\circ}\text{C}$. These nanocomposites possessed great reusability dropping only 5.0% activity after reusing 5 times for BPA degradation.

Few reports have also documented the immobilization of enzymes on dendritic nanostructures, such as polyamidoamine-modified

MNPs, and aldehyde-terminated polyamidoamine dendrimers for the mineralization of organic micro-pollutants (Hamidi et al., 2012; Daumann et al., 2014; Zhu et al., 2016). Hyper-branched polymer-biocatalyst bioconjugates are mounting as compelling support materials in the arena of materials science and biocatalysis (Ge et al., 2007). Koloti et al. (2018) carried out the covalent immobilization of *Rhus vernificera* laccase on highly branched polyethyleneimine/polyether sulfone (HPEI/PES) electrospun nanofibrous membranes for the BPA removal from water. The dendritic membranes anchored laccase displayed 89.6% BPA elimination compared with the 52.4% by pristine PES. It also showed remarkable reusability retaining up to 79% BPA degradation efficiency even after four consecutive filtration cycles. Leaching test revealed insignificant enzyme detachment over four-repeated filtration cycles due to the stabilized carbodiimide bonds between the nanofibrous membranes and the enzyme molecule. Three principal dendritic membranes removal mechanisms might be attributed to the occurrence of hydrophobic nanocavities in the HPEI polymer, hydrogen bonding between the polymer and the BPA, and electrostatic interaction between HPEI polymer, and oxygen atoms in BPA. The hydrophobic HPEI polymer nanocavities created in the laccase-modified membranes can effectively adsorb as well as encapsulate BPA. Fig. 10 illustrates the proposed mechanism for the exclusion of BPA by laccase modified dendritic membranes (Koloti et al., 2018). The BPA dispersed from the bulk water and adsorbed within interior HPEI nanocavities of the membranes during the removal process.

4.4. Enzymatic membrane reactor for continuous removal of BPA

Among the methods widely attempted for micro-pollutants elimination including adsorption, membrane filtration, photo/chemical/electrochemical oxidations, biodegradation, and membrane bioreactor, enzymatic membrane reactors (EMRs) constitute the most attrac-

Fig. 9. SEM images of nanoflowers (a and b) and magnetic nanoflowers (c and d) at different magnifications. Reprinted from Fu et al. (2019), with permission from Elsevier. Copyright (2018) Elsevier B.V.

Fig. 10. The proposed mechanism for removal of BPA in a dead-end filtration system using laccase modified dendritic membranes. Reprinted from Koloti et al. (2018), with permission from Taylor & Francis. Copyright (2018) Taylor & Francis.

tive approach owing to their greater efficacy, environmental friendliness, easy enzyme recovery, and their repeatability in the continuous operation for large-scale application (Lloret et al., 2013; Hou et al., 2014). Laccase (E.C. 1.10.3.2), a ligninolytic enzyme is under significant research for EMRs because of its ability to oxidize an array of toxic pollutants to safe and innocuous products. Hence, significant progress has recently been put forward in micro-pollutants degradation by laccase-mediated EMR (Lloret et al., 2013; Hou et al., 2014; de Cazes et al., 2015). Entrapment of enzymes in membrane greatly improves the enzyme loading and activity retention, an enormous permeability loss, and enzyme leaching was inevitably observed during the bio-application (Giorno et al., 2001). Cao et al. (2018) synthesized three kinds of biocatalytic membranes by 'reverse filtration' of laccase solution to address these drawbacks and improving the overall EMR performance. After this, different mussel-inspired dopamine coating strategies including single dopamine (DA) deposition, DA/polyethyleneimine (PEI) co-deposition, and DA/Cu²⁺ co-deposition were employed to construct bifacial EMR for the highly efficient biotransformation of BPA (Fig. 11) (Cao et al., 2018). The properties of enzyme, i.e. enzyme loading, permeability, activity, and stability, immobilized on biocatalytic membranes fabricated by co-depositing DA/PEI and DA/Cu²⁺ were increased than that to single polydopamine (PDA)-coated membrane. EMR coated with the PDA/Cu²⁺ membrane showed the highest BPA removal efficiency because of copper-assisted greater enhanced electron transfer rate between laccase and BPA substrate. The lowest efficiency, on the other hand, for the PDA/PEI-coated EMR might be ascribed to the higher diffusional limitations of the thick PDA/PEI coating. The pressure-induced convective mass transfer that improved the substrate availability to enzyme along with products removal could overcome the drawbacks of declined BPA removal efficiency following the accumulation of product on the membrane. In another study, a novel EMR was developed for continuous elimination of two widely detected micro-pollutants in wastewater explicitly diclofenac and BPA by a commercial *A. oryzae* sourced laccase (Nguyen et al., 2014). Use of an ultrafiltration membrane prevented enzyme washout and enabled >60% and 80% removal of diclofenac and BPA in the continuous reactor system. The diclofenac and BPA removal rate were further increased

to >80% and >95%, respectively, by the introduction of a natural redox-mediator syringaldehyde (5 mM) to the EMR. However, within 72 h of continuous operation, the enzyme activity was steadily decreased to undetectable levels accompanied by a substantial reduction in micropollutants removal. Barrios-Estrada et al. (2018b) developed immobilized laccase-based membrane bioreactor as a versatile bio-catalytic system for the biotransformation of BPA. Two forms of laccase i.e., in-house extracted from an indigenous *Pycnoporus sanguineus* (CS43) and commercial *T. versicolor* laccase were covalently attached to a multi-channel ceramic membrane in the presence of GLU as a cross-linker as represented in Fig. 12 (Barrios-Estrada et al., 2018b). Results showed that both forms of immobilized laccases in the developed laccase-assisted membrane reactor (Fig. 13) resulted in 100% biotransformation of BPA A in <24 h treatment time. Immobilized biocatalysts presented optimal activity at pH 5.4 and 5.0 with a degradation rate of 79.0 and 204.8 $\mu\text{mol}/\text{min}/\text{U}$ for *T. versicolor* and *P. sanguineus*, respectively (Barrios-Estrada et al., 2018b) (Table 3).

5. Removal of toxicity by laccase-catalyzed treatment

Several BPA degradation intermediate metabolites including, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, *p*-hydroxyacetophenone, phenol, hydroquinone, 2-phenylpropene, and *p*-isopropylphenol are have been recognized to be even highly toxic than the original BPA compound itself resulting in secondary pollution (Lu et al., 2013; Han et al., 2015). The formation and accumulation of intermediates that are more noxious, particularly those comprising aromatic rings, and the synergistic effect among these compounds can contribute to the increased toxicity. Therefore, it is of great significance to address the elimination of toxicity and estrogenic activity of BPA and its associated compounds. Degradation and transformation of these intermediates into less or non-toxic compounds by enzymatic treatment results in a decrease of toxicity. de Freitas et al. (2017) reported that laccase from *P. ostreatus* resulted in a significant toxicity reduction as determined by Microtox kit bioassay. Notably, the acute toxicity of *P. ostreatus* laccase treated BPA samples was reduced from 85% to <5.0%, indicating the possibility of the enzymatic method for eliminating BPA from contaminated environments. In an-

Fig. 11. Schematic diagram of biocatalytic membrane preparation via mussel-inspired coatings. Enzyme immobilization was carried out by ‘reverse filtration’ of laccase and subsequent PDA coating on the support layer via (a) single DA self-polymerization, (b) DA/PEI co-deposition (c) DA/Cu²⁺ co-deposition under alkaline condition. Reprinted from Cao et al. (2018), with permission from Elsevier. Copyright (2017) Elsevier B.V.

other study, the relative acute toxicity of BPA was reduced from 98.9% to 7.5% by 6-h treatment of *Funalia trogii* crude extract using Microtox assay. GC–MS characterization revealed that BPA was not detected in the medium after 2 h, and the relative toxicity was diminished to 17.7%. These findings evidenced the association of toxicity with the presence of BPA that was eliminated in parallel to BPA mineralization from the medium. Moreover, the toxicity of the BPA degraded products, i.e. nonane, 4-ethyl-2-methoxy-phenol, and 5-(2-methyl propyl) by the crude extract was observed much lower than the toxicity of BPA itself (Atacag Erkurt, 2015). Similar toxicity bioassays with various bioluminescent bacteria have been to compare the toxicity status of BPA and its degradation metabolites. A ToxScreen3 assay demonstrated that BPA and DCF solutions were particularly non-toxic to the indicator bacterial strain (*Photobacterium leiognathi*). Lack of a notable increase in toxicity in the EMR permeates revealed that laccase-treatment did not generate any noxious by-products (Nguyen et al., 2014). However, the toxicity of permeate was significantly increased by incorporating the mediators, as previously reported by Kim and Nicell (2006). It is worth noting that toxicity to the *P. leiognathi* does not inevitably indicate that the effluent is toxic to other indicator strains. For example, Cruz-Morató et al. (2013) reported that enzyme-treated media toxic to the *Vibrio fischeri* was not essentially lethal to other aquatic organisms tested. Therefore, additional studies are necessary to shortlist redox mediators, which augment enzymatic biotransformation efficiency without raising the toxicity of treated media. The phytotoxicity of BPA solution before and after Ca-alginate beads immobilized laccase treatment was recently analyzed using *Raphanus sativus* (radish) seeds (Lassouane et al., 2019). Phytotoxicity results revealed the toxic nature of BPA to *R. sativa*, and no phytotoxicity to *R. sativus* was recorded after the laccase-assisted treatment.

6. Elimination of estrogenic activity by laccase-catalyzed treatment

BPA is chemical with potential capability to emulate the female estrogen hormones, which interrupt the body's chemical messengers by directly interacting with the steroid receptors (Uchida et al., 2001; Jobling et al., 2003). It is stated that endocrine disruptors may be associated with diminished sperm counts, anomalies in the reproductive system of female, increasing occurrence of breast and testicular cancer, and other medical abnormalities (Lang et al., 2008). Application of laccases appears as a potentially promising alternative approach in the removal of micro-pollutants, including the suspected and known endocrine disruptors, i.e. BPA. Fukuda et al. (2004) utilized a luciferase reporter assay using COS-7 cells to examine the removal of estrogenic activity of the BPA reaction products by a recombinant *T. villosa* laccase. After centrifugation, the supernatant, as well as precipitate of the laccase-treated reaction mixture, presented no estrogenic activity even when present at extremely higher amounts. The laccase-assisted BPA treatment was found highly effective in the removal of the reproductive toxicity of BPA by using a sufficient level of the enzyme. Arboleda et al. (2013) evaluated the acute toxicity of the enzyme-treated BPA samples using the micro-crustacean *Daphnia pulex*-based toxicity bioassay. Initial BPA solution showed an LC50 value of 10%, whereas, the toxicity was substantially reduced after treatment with the laccases-containing enzyme preparations from *Ganoderma stipitatum*. After 48 h incubation of BPA samples with the enzyme solution, no mortality of *D. pulex* was observed at any of the tested dilutions. Likewise, estrogenicity was also eliminated following the enzymatic treatment, and up to 70% and 50%, estrogenic activity was decreased by 3.5 h laccase treatment from *G. stipitatum* and *L. swartzii*, respectively. A 6-h laccase treatment from both strains eliminated up to 90% estrogenicity of the BPA solution. In an earlier report, the estrogenic activity of BPA solution was completely

Fig. 12. Schematic process of laccase immobilization. Reprinted from Barrios-Estrada et al. (2018b), with permission from Elsevier. Copyright (2017) Elsevier B.V.

vanished by persisting the laccase treatment to 12 h (Tsutsumi et al., 2001). The disappearance of estrogenicity of BPA solution is attributed to the structural modification in the parent compound (Cabana et al., 2007). The laccase-catalyzed BPA oxidation generates phenoxy radicals, which are involved in constituting BPA oligomers (Huang and Weber, 2005; Cabana et al., 2007), and these products were lacking the structural attributes essential to bind to the human estrogen receptor (hER) (Fang et al., 2001; Tsutsumi et al., 2001).

7. Unresolved problems, conclusions, and future perspectives

Application of sustainable and environmentally responsive materials, i.e., enzymes is a promising technology for the remediation of wastewaters containing phenolic pollutants compounds. Potential efficiency at a nano-molar level, biodegradability, non-toxicity, harmless nature, and lack of secondary pollution concern are among the

desirable characteristics of these biocatalysts. However, enzymatic removal of phenolic wastewater is highly expensive and not competitive with the currently utilized methods. Commercial unavailability of most of the relevant enzymes renders the large-scale enzymatic treatment processes a tangible economic and technical hurdle. This fact urgently necessitates the bulk biosynthesis of enzymes to make these biocatalysts economic and commercially available. Though recombinant DNA technology offers great potential for large scale enzymes production at a lower cost. However, it is yet to deploy at industrial level to meet the current demand for many enzymes. In addition, effective separation and purification of enzymes are still bottleneck requiring innovations in this respect. Consequently, careful and well-planned research work for elevated enzyme production accompanied by purification is one of the immediate necessities for establishing enzymatic treatment a practical approach in the near future. Loss of enzyme activity is another serious limitation of enzyme appli-

Fig. 13. Schematic diagram of laccase-immobilized ceramic-based membrane reactor. *Reprinted from Barrios-Estrada et al. (2018b), with permission from Elsevier. Copyright (2017) Elsevier B.V.*

cation for bioremediation of wastewater. Under un-favored and non-physiological conditions, incubation of the enzyme in an aqueous medium, for less than a day, results in a huge loss of its activity, and thereby enzyme in-effectivity (Nicolucci et al., 2011; Dehghanifard et al., 2013). Since real wastewaters harbor a variety of organic/inorganic impurities that might influence the enzyme activity, therefore, it is necessary to construct biocatalytic systems that potentially tolerate the effect of inhibitory compounds. This will indeed circumvent the environmental impact and cost of the pre-treatment processes. Resistant to varying pH values are also essential enabling the enzymatic remediation of acidic as well as alkaline phenolic wastewaters without additional cost for pH amendment.

Extensive literature survey revealed that immobilized form of laccases could be reliable and promising candidates for effective treatment of BPA-containing wastewaters in contrast to free enzymes. After immobilization, these enzymes presented substantially improved catalytic performance, greater thermal and storage stabilities, as well as elevated recovery and recyclability of the enzyme. More recently, the nanostructured materials incorporated immobilized enzymes exhibited a huge impact on enzymatic wastewater treatment technology. Suh nanostructured materials have emerged as a very promising enzyme immobilization matrices in real-time practical applications because of their exceptional physiochemical and structural properties. Therefore, the laccases immobilization on nanostructured materials is expected to be a futuristic approach in wastewater treatment in the future. Despite a large body of evidence that the heterogeneous biocatalytic systems are more proficient compared with the homogeneous enzyme-based reactions in the wastewater remediation, some investigators demonstrated the contradictory trend (Gassara et al., 2013; Wang et al., 2015). Part of these problems might originate from the

fact that almost all studies are not conducted under identical conditions (e.g., the concentration of enzyme, source, and type of enzyme, substrate type, and concentration, operating conditions, reaction duration, etc.). Such an enormous variability is suggested to minimize to acquire meaningful effectiveness of a specified enzyme under the given substrate. In addition, the enzyme-pollutant interactions and the impacts of external factors such as pH, temperature, the presence of inhibitory components, and co-pollutants on such interactions should be characterized on a molecular level.

Another imperative consideration is that the active site of the enzyme should not be buried after immobilization. On the contrary, it must be uncovered to the reaction media enabling the interaction between the pollutants and immobilized enzymes. Therefore, enzyme immobilization has to be carefully carried out to achieve the reaction between the reactive groups of the solid support with functional groups on the catalytic site of the enzyme molecule. If such functional groups are present at the target position on the enzyme molecule, it could be introduced by chemical coupling method (Gahlout et al., 2014; Onaizi, 2017). Nevertheless, it is worth noting that such an enzyme modification will not have any influence on the catalytic activity and stability of the enzyme.

Uncited reference

Liu et al., 2015

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Table 3
Removal of toxicity and estrogenic activity of BPA by laccase-based treatment.

Valuable producer and enzyme name	Toxicity/estrogenicity analysis method	Notable results	Source
Ca-alginate beads immobilized crude laccase from <i>Trametes pubescens</i>	Phytotoxicity using <i>Raphanus sativus</i> (radish) seeds	BPA metabolites obtained after laccase treatment showed no phytotoxicity to the tested radish seeds.	Lassouane et al. (2019)
Crude laccases from <i>Pleurotus ostreatus</i>	Commercial bioassay test (Microtox test kit) based on the inhibition of bioluminescence emitted by the <i>Vibrio fischeri</i>	The acute toxicity of BPA reduced from 85% to <5.0% after laccase treatment.	de Freitas et al. (2017)
Crude enzyme extract of <i>Funalia trogii</i>	SDI Microtox1 test kit (Strategic Diagnostics) based on the inhibition of bioluminescence emitted by the <i>Vibrio fischeri</i>	More than 90% toxicity of BPA decreased after 6 h incubation period.	Atacag Erkurt (2015)
Laccases-containing enzyme preparations from <i>Ganoderma stipitatum</i>	Eco-toxicity bioassay using the microcrustacean <i>Daphnia pulex</i>	No observation of <i>D. pulex</i> death after the 48 h incubation period with the enzyme treated solution at any of the dilutions tested.	Arboleda et al. (2013)
Enzymatic membrane reactor loaded with laccase from <i>Aspergillus oryzae</i>	A ToxScreen3 assay based on bioluminescence inhibition in <i>Photobacterium leiognathi</i>	Treated BPA presented not particularly toxic to the indicator bacteria.	Nguyen et al. (2014)
Laccase from a recombinant <i>Trametes villosa</i>	Luciferase reporter assay using COS-7 cells	Both the soluble and insoluble fractions of the BPA reaction products had no estrogenic activity after treatment even at rather elevated levels	Fukuda et al. (2004)
Enzyme preparations containing mainly laccases, from <i>Ganoderma stipitatum</i> and <i>Lentinus swartzii</i>	Yeast Estrogenic Screen (YES) assay	Up to 70% and 50% reduction in estrogenic activity by reacting BPA solution with laccase from <i>L. swartzii</i> and <i>G. stipitatum</i> , respectively, for 3.5 h.	Arboleda et al. (2013)

Acknowledgment

All authors are grateful to their representative universities and institutes for providing literature facilities.

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