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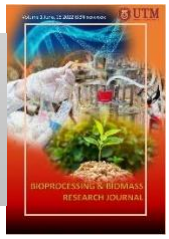

Review Article

A Brief Review of Immobilized Oxidoreductase Enzymes for the Removal of Endocrine-Disrupting Chemicals from Wastewater

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ABSTRACT

Modern technological of human activities in industries or housing areas have created an unhealthy environment, particularly through unmanageable wastewater. For the time being, this kind of pollution is getting serious as it caused the emerging pollutant actively to spread to humans and living organisms. These non-biodegradable pollutants, to be specifically known as endocrine-disrupting chemicals (EDCs) are synthetic or natural chemicals that have high toxicity and persistency which can interfere with the endocrine system in humans and animals. The removal of EDCs has received high attraction among researchers using physical-chemical treatments, however, conventional techniques do not effectively remove EDCs from wastewater. This review aims to discuss research related to biological approaches that have been carried out to efficiently remove EDCs from wastewater using oxidoreductase enzymes, especially via an immobilization strategy. In general, free enzymes have limitations to be applied in industrial scales such as low stability and fragility, and unable to separate from the bulk solution. On the other hand, immobilized enzymes offer better operational stability and reusability in harsh environments. This review also discussed the bioremediation of EDCs using several immobilized oxidoreductase enzymes like lignin peroxidase (LiP), manganese peroxidases (MnP), horseradish peroxidases (HRP), laccases and tyrosinases. The application of immobilized enzymes and factors affecting the bioremediation using oxidoreductase enzymes were also explored to highlight their potential for the removal of EDCs from wastewater.

INTRODUCTION

Freshwater constitutes about 25% of the water sources on the Earth's surface. However, its demand has kept increasing over the years due to urbanization and industrialization requirements and activities (Azizi et al., 2022; Crini & Lichtfouse, 2019; Sarker et al., 2021). Industrial activities undergo technological advancements that generate massive volumes of wastewater containing various dangerous chemicals and toxic pollutants. This contaminated wastewater is released into the environment and threatens natural ecosystems and living organisms.

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(Kumar et al., 2022). The main concern is the production of endocrine-disrupting chemicals (EDCs), which are primary pollutants in the wastewater produced by the industrial sectors and can enter water resources, thereby affecting the quality of freshwater. Therefore, improvements in wastewater treatment and water management need to be given high priority (Rajasulochana & Preethy, 2016).

Emerging pollutants, such as EDCs, have raised an alarm for worldwide environmental issues due to their severity and their ability to cause extensive diseases by disrupting the endocrine systems of humans and animals (Ifelebuegu & Ezenwa, 2011). Exogenous and emerging EDCs include natural chemicals such as estrogens, androgens, and phytoestrogens as natural pollutants. Meanwhile, synthetic chemicals such as synthetic polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), mostly industrial chemicals and their by-products, pharmaceuticals and personal care products (PPCPs), and pesticides are also considered EDCs (Wee & Aris, 2017; Kumar et al., 2022; Surana et al., 2022).

EDCs have high persistence in nature, which makes it difficult to effectively remove or degrade them from wastewater using conventional treatment methods (Liu et al., 2021). EDCs can interfere with normal cellular function by mimicking the action of hormones and driving cells to produce undesired responses (Al-Jandal et al., 2018), They also disturb metabolic processes in the body, impacting the synthesis and regulation of natural hormone levels (Liu et al., 2009; Wee & Aris, 2019). As EDCs can disrupt the endocrine system, they are linked to numerous harmful effects on human health, including changes in sperm quality and fertility, respiratory and metabolic issues, cardiovascular problems, neurological and learning disabilities, and in severe cases, can cause cancerous diseases (Ismail et al., 2020; Pironti et al., 2021). Thus, over the years, many efforts have been made using physical, chemical, electrochemical and biological approaches to remove EDCs from wastewater (Auriol et al., 2006; Surana et al., 2022).

Alternatively, biological techniques using enzymatic treatment have received great attention to reduce the presence of EDCs or remove them from wastewater. Enzymatic processes catalyze reactions with great selectivity under moderate conditions. Oxidoreductase enzymes are highlighted as the preferred strategy for removing EDCs from wastewater because of their capability to degrade various potentially toxic compounds. Examples of endocrine disruptors from many sources are illustrated in **Figure 1** (Alneyadi et al., 2018; Valero et al., 2014). Several types of oxidoreductase enzymes, including laccase, manganese peroxidase, lignin peroxidase, horseradish peroxidase, and tyrosinase, have been focused on for the removal of EDCs.

However, an enzyme immobilization approach has been applied to remove EDCs from wastewater because immobilized enzymes have higher stability under harsh conditions (extreme pH and temperature), are easy to separate and can be reused for several cycles (Bilal et al., 2019). The common method in enzyme immobilization is the physical or chemical technique that can be used to immobilize enzymes to a carrier (Alvarado-Ramírez et al., 2021). This process is where the soluble enzyme is attached to a solid carrier creating a heterogeneous immobilized enzyme. In addition, enzyme immobilization by adsorption technique is a potential physicochemical method for treating textile wastewater as it involved the binding of textile wastewater effluent molecules onto support surfaces. This method is commonly classified as either chemisorption or physisorption (Ali et al., 2012).

In some cases, a continuous system of wastewater treatment plants is quite challenging as the washing step at the early stage caused the biocatalyst leached out from the system (Galliker et al., 2010). Thus, an effort has been conducted to develop cross-linked enzyme aggregates for laccase from while rot fungal strain *Coriolopsis polyzona*

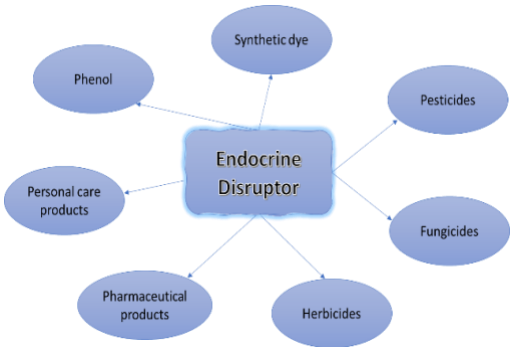

Figure 1 Potential endocrine disruptor from many sources.

which can efficiently degrade nonylphenol, bisphenol A and triclosan (Cabana et al., 2007). On the other hand, immobilized horseradish peroxidase on hydrated titanium oxide has great improvement in stability, thus it can adapt to wastewater composition variations (Ai et al., 2016). Therefore, enzyme immobilization has been proven to enhance the performance of the enzyme biocatalyst for EDCs removal from wastewater. This review discusses the immobilization of various oxidoreductases enzyme, and operational parameters that might affect the efficiency in the removal of EDCs are presented.

IMMOBILIZATION TECHNOLOGY

Immobilization of oxidoreductase enzymes is proposed to produce a potential biocatalyst for the removal of pollutants such as EDCs. It is known to improve storage stability, enhance enzyme stability against wide variations of pH and temperature, increase thermal stability and resistance, suppress enzyme inactivation and introduction of enzyme reusability, as compared to free enzymes (Zdarta et al., 2021). **Figure 2** shows the various immobilization methods which are mainly divided into physical and chemical techniques (Ashkan et al., 2021; Lee et al., 2020). Adsorption is known as the easiest immobilization method because it does not involve any coupling agents or modification steps for immobilization (Chakraborty et al., 2016). This method results from the interaction between the support and the enzyme relying on weak non-specific forces such as hydrogen bonds, Wan der Vaals and ionic interactions (Liu et al., 2018). The immobilization approach does not cause any chemical changes in enzymes and preserves the initial catalytic activity. Nonetheless, one of the main drawbacks of the adsorption technique is enzyme leakage from the support due to weak non-specific interactions (Ashkan et al., 2021).

Furthermore, another immobilization technique is entrapment. In the entrapment process, the enzyme is physically entrapped into the pores of the support and allows the diffusion of the substrate and products through

the support's pores. Entrapment techniques have two different approaches such as fibre entrapping and microencapsulation (Liu et al., 2018). The main advantage of this technique is the enzyme structure remains unchanged. Unfortunately, there are possible in which enzyme leakage, diffusion limitations and support abrasion might occur during the reaction process (Zucca & Sanjust, 2014). Furthermore, covalent binding is another technique in immobilization. This method is developed by the interaction of the support and functional groups on the enzyme surface. Common functional groups in this technique that react with the support are carboxylic, amine, thiol and phenolic groups. These functional groups ensure to form strong bonds between enzyme molecules and support. The main benefit of this method is the strong bonding between enzyme and support prevent the enzyme leakage (Chakraborty et al., 2016; Homaei et al., 2013). Nonetheless, in this approach, the structure of the enzyme is chemically altered and might block the enzyme's active site (Datta et al., 2013).

Immobilization technique using cross-linking is one of the techniques that is widely used by applying two different methods including, first, cross-linking between enzyme and support using a bifunctional reagent. Second, the formation of aggregates enzyme molecules by adding salts, organic solvents or non-ionic polymers (Ahmad & Sardar, 2015; Gorecka, 2011) before cross-linking using bifunctional reagent like glutaraldehyde to form cross-linked enzyme aggregates (CLEAs) (Zucca & Sanjust, 2014). The major advantage of this method is the formation of strong covalent bonding which minimalizes the desorption of enzymes during the reaction process (Chakraborty et al., 2016). However, this method might affect the catalytic properties of enzymes as the enzyme aggregates may disturb the conformation of the enzyme structure. Finally, we should emphasize that there is no universal immobilization technique that applies to any specific enzyme. The selection and optimization processes are crucial to obtain the best and appropriate support material and immobilization technique as it depends on the type of enzyme and biocatalytic properties (Zdarta et al., 2018; Mohamad et al., 2015).

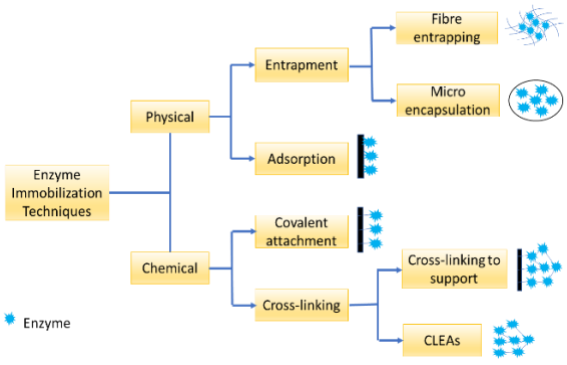

Figure 2 Enzyme immobilization techniques categorized by physical and chemical approaches.

EDCs REMOVAL WITH OXIDOREDUCTASE ENZYME

Oxidoreductase enzymes are categorized as EC 1 in the classification scheme for the enzyme commission numbers, and they catalyze oxidoreduction reactions which are electron transfer reactions from a donor molecule to a reductant molecule. This section reviews the use of immobilized laccase, manganese peroxidase, lignin peroxidase, horseradish peroxidase, and tyrosinase for the removal of EDCs. **Figure 3** illustrated the enzymatic degradation by oxidoreductase enzyme via the oxidation process for the removal of EDCs.

Laccase

Laccase is a multi-copper-containing oxidoreductase enzyme that oxidizes substrate by a single electron in the presence of oxygen molecules that contains four copper atoms bound to 3 redox sites (Ba & Kumar, 2017). This enzyme is very useful and appealing for industrial applications in the paper, food, textile industry and bioremediation as it can catalyze a wide range of aromatic compounds (Kunamneni et al., 2008; Strong & Claus, 2011).

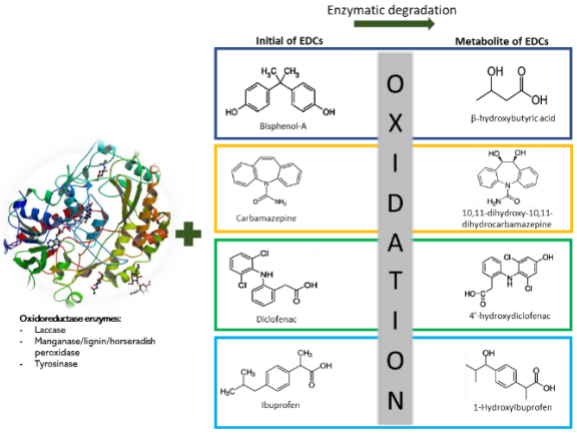

Figure 3 Enzymatic degradation of EDCs using oxidoreductase enzymes via an oxidation process.

The Japanese lacquer tree, *Rhus vernicifera*, was the source of the earliest reports of the laccase in 1883 (Viswanath et al., 2014). Laccases are more common in fungi than in higher plants. Most of the research and application directions were generated for fungal laccase, mainly from white rot fungus may be due to their high redox potential compared with bacterial and plant laccases (Agrawal et al., 2018). Fungal laccase is produced from *Trametes versicolor* (Zainith et al., 2020) and white-rot fungi such as *Phlebia radiate, Pleurotus ostreatus* (Olusola et al., 2012) as well as basidiomycetes like *Phanerochaete chrysosporium* (Arregui et al., 2019; Shraddha et al., 2011), *Theiophora terrestris*, and *Lenzites betulina* (Palaskar et al., 2022). In general, laccase, with its superior catalytic properties and broad substrate range, emerges as a viable strategy for future water purification (Bronikowski et al., 2017; Catherine et al., 2016). Nonetheless, the low stability, short service life, lack of reusability, and high-cost limit the industrial utilization of laccase (Daronch et al., 2020). Scientists have rekindled their interest in using immobilized laccase as a desirable and advantageous green biocatalytic tool (Zhou et al., 2021).

According to Ji et al. (2016), laccase has been immobilized onto modified carbon nanotubes polyvinylidene fluoride (CNTs-PVDF) membrane by physical adsorption and chemical bonding. Physical adsorption of laccase enzyme onto the modified CNTs-PVDF membrane yielded the highest enzyme activity (0.45 U/cm^2). The immobilized laccase successfully removed bisphenol-A (BPA), carbamazepine (CBZ), diclofenac (DCF), clofibric acid (CA) and ibuprofen (IBF). The results showed 90% BPA, 75% DCF, 68% IBF, 49% of CBZ and 46% of CA were removed after

48 hours of incubation (Ji et al., 2016). Therefore, the immobilized laccase onto CNTs-based membranes has good potential in wastewater treatment. Moreover, in another study performed by Lin et al. (2016), laccase was reversibly immobilized via adsorption onto Copper Cu (II) ion chelated magnetic Fe_3O_4/chitosan microsphere. The immobilized laccase exhibited superior thermal and storage stability than that of the free enzyme. The immobilized laccase maintained 38% of its activity while the free enzyme lost its activity at a higher temperature of 65 °C. The immobilized laccase activity also maintained 62% of its activity after 14 days of storage, whereas free laccase activity lost around 83% of its initial activity. The immobilized laccase successfully achieved 85% removal efficiency of bisphenol A after 12 h of incubation (Lin et al., 2016). Therefore, immobilized laccase has a potential future in the remediation of industrial wastewater.

In a recent study by Wu et al. (2019), laccase was immobilized onto an amino-functionalized magnetic metal-organic framework (MOF) by the adsorption and covalent binding method. As an outcome, the immobilized enzyme showed a high degree of tolerance at a broad range of pH and temperature. The immobilized laccase maintained around 80% of its initial activity even at pH 2.0, whereas the free enzyme retained only 7% of its initial activity. At 85 °C, immobilized laccase retained 80% of the initial activity whereas its free enzyme was almost inactivated. For storage stability, the immobilized laccase showed great sustainability for the remaining 89% of its activity after 28 days. The immobilized laccase was used to remove 2,4-dichlorophenol with 87% removal efficiency after 12 h of reaction (Wu et al., 2019).

Lignin Peroxidase

Lignin peroxidases (LiP) was originally produced from *Phanerochaete chrysosporium* (Eriksson & Bermek, 2009), and then it was produced from *Trametes versicolor* (Falade et al., 2017) and *Coriolopsis gallica* (Elisashvili et al., 2017). The LiP is commonly known as diaryl propane oxygenase which catalyzes oxidative cleavage or depolymerizes lignin in the presence of hydrogen peroxide (H_2O_2) (Bilal et al., 2019; Falade et al., 2017). LiP has a high redox potential which can break α- to β-carbon bonds by opening aromatic rings of dye structure. The liP also catalytically degrades a variety of phenolic and non-phenolic compounds. However, using free enzymes have poor stability, is fragile in a harsh environment, is expensive and has no reusability. An efficient and reliable enzyme technology via enzyme immobilization is required as these drawbacks restricted the use of free enzymes, especially in many industrial applications (Hu et al., 2013; Shaheen et al., 2017).

Previously, LiP has been entrapped onto polydopamine (PDA) nanoparticles and remained more than 70% at acidic conditions (pH 2.5 – 4.0), while free LiP only retained 60% of the initial activity under the same conditions (Guo et al., 2019). The immobilized LiP improved thermal stability at temperatures ranging from 40 °C to 60 °C, as compared to the free enzyme. While the free enzyme was nearly inactivated, the entrapment of LiP onto PDA enhanced the storage stability of the immobilized enzyme by maintaining 68% of its initial activity, after 60 days of storage at 4 °C (Guo et al., 2019). They successfully degraded 100% of phenol and 5-chlorophenol after 48 h of reaction using the immobilized LiP. Therefore, immobilized LiP could efficiently remove pollutants and have application potential in wastewater treatment.

Manganese Peroxidase

Manganese peroxidase (MnP) is an oxidoreductase enzyme containing glycosylated heme which is recognized for lignin degradation. MnP was first discovered in *Phanerochaete chrysosporium*. Currently, several studies showed that MnP has been presented in other bacteria such as white-rot fungi (Bilal et al., 2019; Chowdhary et al., 2018). MnP has been considered one of the earliest peroxidases used for the removal of environmental pollutants via the oxidation process. This is because it can catalyze the oxidation from Mn^{2+} to Mn^{3+} of various types of phenolic compounds, polycyclic aromatic hydrocarbons and hazardous dye pollutants (Chang et al., 2021; Kumar & Chandra, 2020). A previous study conducted by Hirano et al. (2000) mentioned that BPA degradation using MnP has occurred via the oxidation of Mn (II) in which the removal of one electron from the substrate to form phenoxy radical by Mn (III) in the presence of hydrogen peroxide as the oxidant, which initiated the removal of one electron from the substrate to form phenoxy radical by Mn (III). Then, the BPA radical would undergo random cleavage at the aromatic rings and C-C linkages to form low molecular weight metabolites.

MnP has been applied in many applications including bioleaching, biopulping, delignification, and bioremediation (Bilal et al., 2016b; Twala et al., 2020). Nevertheless, the use of free enzymes has been restricted for industrial application processes (Acevedo et al., 2010). Alternatively, enzyme immobilization is a preferred option to overcome the issues raised when the free enzyme is used, to enhance the catalytic performance of enzyme biocatalysts (Bilal et al., 2015). MnP has been successfully immobilized onto magnetic Fe_3O_4/Chitosan nanocomposite (Siddeeg et al., 2020). The immobilized MnP was developed to eliminate artificial dyes such as methylene blue (MB) and reactive orange 16 (RO16) in textile wastewater for 50 minutes at 27 °C and pH 7. According to the findings, free MnP and immobilized MnP managed to remove 52% and 96% of MB, and also degraded 65% and 98% of RO16, under the same conditions. Additionally, immobilized MnP preserved more than 80% of its initial activity after 5 successive cycles. In another study conducted by Bilal et al. (2016), MnP was immobilized by entrapping the enzyme in agar-agar. The pH and temperature stability of the immobilized MnP greatly improved. This could be due to the enzyme conformational in agar-agar being protected, thus improving MnP stability. The MnP entrapped in agar-agar effectively decolourized 85% of reactive blue 21 dye in the presence of mediators. It showed that the immobilized MnP are useful for the bioremediation of textile dye effluents (Bilal et al., 2016a).

Horseradish Peroxidase

Horseradish Peroxidase (HRP) is a glycosylated enzyme containing two different metal centres, one heme ion Fe-protoporphyrin (IX) prosthetic group and two calcium ions (Monier et al., 2010). HRP can catalyze the oxidation of phenolic acids, aromatic and non-aromatic compounds using hydrogen peroxide as an oxidant (Mohamed et al., 2013; Spadiut & Herwig, 2013). The main applications of HRP are wastewater treatment containing phenols, environmental remediation, degradation of harmful substances from drinking water, and treatment of dyes and industrial effluents (Adeel et al., 2018; Lai & Lin, 2005).

However, free HRP displayed poor stability and catalytic efficiency in wastewater treatment, thus immobilization method is a promising option to overcome these problems (Bilal et al., 2019).

Chang et al. (2014) have developed cross-linked HRP onto NH_3-modified Fe_3O_4 /SiO_2 nanoparticles surface using glutaraldehyde. The immobilized HRP was further used to remove 2,4-dichlorophenol with the presence of H_2O_2 at pH 6.4 and a temperature of 30 °C. The developed cross-linked HRP achieved 80% removal efficiency which indicated that immobilized HRP could be a potential biocatalyst for removing pollutants. The immobilized HRP also maintained 85% of its initial degradation activity after 4 cycles. On the other hand, another study reported by Bilal et al. (2016) stated that HRP was covalently immobilized in calcium alginate using glutaraldehyde which displayed 68% recovery activity at a higher temperature (80 °C), while free HRP was nearly inactivated. Moreover, the immobilized HRP sustained 40% of its initial activity after 7 successive cycles. The immobilized HRP was used for dye decolourization using a packed bed reactor. The decolourization process using immobilized HRP yielded 72% reactive dye, 87% reactive blue 4 and 80% RO16 of removal efficiency.

Tyrosinase

Tyrosinase is an enzyme that is responsible for the initial steps in melanin biosynthesis, which contains copper for hydroxylating L-tyrosine to the 3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to dopaquinone. There are numerous sources of tyrosinase such as bacteria, fungi, plants and insects (Ba et al., 2017; Roy et al., 2014). Tyrosinase is used in many industrial applications such as food additives, biosensor applications and biosynthesis of DOPA, a common drug for Parkinson's disease (Min et al., 2019; Nawaz et al., 2017). Additionally, tyrosinase has broad specificity to react with various phenolic compounds, thus it has potential application in the bioremediation of phenolic compounds (Bilal et al., 2019). Many researchers have reported that immobilized tyrosinase could be a promising approach for removing phenolic compounds from wastewater (Seetharam & Saville, 2003).

Dincer et al. (2012) have immobilized tyrosinase onto chitosan-clay composite beads using glutaraldehyde as the cross-linker. The immobilized tyrosinase improved its thermal stability compared to the free enzyme. The immobilized tyrosinase maintained 52% of its initial activity while the free enzyme lost 86% of its initial activity. This might be due to the immobilization method by cross-linking using glutaraldehyde could protect the enzyme conformational changes, which makes it more resistant to heat and denaturing agents. Moreover, the immobilized tyrosinase efficiently removed about 100% phenol in the first cycle, and it was further reused for 150 minutes, and the immobilized tyrosinase maintained about 43% of its initial activity after 7 cycles (Dinçer & Aydemir, 2012). In another report by Xu & Yang (2013), the tyrosinase was immobilized in the form of cross-linked enzyme aggregates (CLEAs), and it was used for the degradation of p-chlorophenol, p-cresol and phenol. Based on the result, tyrosinase CLEAs have completely removed p-chlorophenol, p-cresol, and phenol. It was stated that this method is very effective in removing phenol from wastewater either in batch or continuous processes. Moreover, tyrosinase CLEAs have efficiently reduced the toxicity of the phenolic solution, showing it as a promising catalyst for wastewater treatment (Xu & Yang, 2013). Table 1 summarized the use of oxidoreductase enzymes in the degradation of EDCs using different immobilization techniques.

OPERATING PARAMETERS FOR EDCs REMOVAL

The influence of operating conditions such as pH, temperature and time on the efficacy of immobilized enzymes for EDCs elimination was also critically reviewed since the changes in these variables will impact the immobilized enzyme and its catalytic activity.

Time

Pollutant removal effectiveness is greatly influenced by contact time. Pollutant removal efficiency increases over time until levelling off. This might be due to the accumulation of degradation products and the adsorption saturation of the support, which inhibits the catalytic process (Bayramoglu et al., 2013; Liu et al., 2012). In a report by Kalsoom et al. (2022), immobilized manganese peroxidase on iron oxide nanoparticles was used to remove textile dye pollutants like Direct red 31 and Acid black 234. Based on the results, the degradation of Direct red 31 was above 80 % and gradually increased to above 90%, whereas Acid black 234 degradations increased from around 20% to above 70% in 24 hours. The degradation of the pollutants gradually increased with time until reaching the maximum decolourization which was around after 24 hours (Kalsoom et al., 2022).

In another report by Bilal et al. (2016), encapsulated manganese peroxidase in Ca-alginate beads was further used to decolourize textile dye effluents. The decolourization efficiency was 48.7% after 1 hour of contact time. The decolourization efficiency increased with time till 5 h, where maximum decolourization of around 87.5% was achieved, demonstrating the importance of contact time in the process of pollutant degradation. (Bilal et al, 2016). Bayramoglu et al. (2013) also immobilized tyrosinase on modified diatom silica that was applied to remove phenolic compounds such as phenol and phenylacetate. The phenolic compound degradations increased with time to 87 % phenol and 91 % phenyl acetate after 12 h of contact time. The increment of removal efficiency over time is due to a high number of active sites available for reaction at the initial process through random collision to bind with the substrate until it reaches equilibrium due to active site saturation (Mohamad Said et al., 2021).

pH

One of the crucial parameters that can affect the removal of pollutants is pH. Enzyme conformation can be easily disturbed by changes of extreme pH either too acidic or too alkaline conditions. This condition can inhibit enzyme catalytic activity, thus removal efficiency is decreased (Zdarta et al., 2018). Immobilized MnP prepared by Kalsoom et al. (2022) found that maximum degradation of Direct Red 31 and Acid Black 234 occurred at pH 3 with 70% removal efficiency. However, the removal efficiency kept decreasing when the pH was at pH 4-10. This could be due to modifications of enzyme structure caused by hydroxylation and protonation occurred by the pH changes. This phenomenon could block the enzyme active sites, thus leading to enzyme denaturation and loss of functionality (Qureshi et al., 2020). On the other hand, a previous study reported that immobilization of peroxidase on Concanavalin

A wood shaving improved dye decolourization, as compared to free enzyme. The immobilized peroxidase and free enzyme remove 85% and 55% of direct dye. A reduction of free enzyme activity at pH 5 to pH 7 caused by the changes in pH might disrupt intramolecular and intermolecular bonds, thus resulting in catalytic activity decrement (Matto & Husain, 2009). Additionally, immobilized MnP was incubated in the range of 2 to 10 to determine the effect on dye degradation. The immobilized MnP effectively degraded 73% of textile effluent at pH 5 and gradually decreased from pH 6 to pH 10 (Bilal et al., 2016b).

Table 1 Degradation of EDCs by immobilized oxidoreductase enzymes

Enzyme	Immobilization method	Carrier	Targeted EDCs	Efficiency in EDCs removal (%)	Reference
Lignin peroxidase	Cross-linking	Chitosan beads	Sandal Fix Foron Blue (E2BLN)	89.71	(Perveen et al., 2020)
Lignin peroxidase	Entrapment	Ca-alginate beads	Sandal fix red (C4BLN)	93	(Shaheen et al., 2017)
Manganese peroxidase	Entrapment	Ca-alginate beads	Snadal fix red (C4BLN)	78.14	(Bilal & Asgher, 2015)
			Sandal fix Black CKF	87.63	
Manganese peroxidase	Entrapment	Xerogel matrix composed of trimethoxysilane and propyl tetraethoxysilane	Textile dye effluents	98.8	(Asgher et al., 2013)
Horseradish peroxidase	Covalent binding	Poly(methylmeth acrylate-co-ethyl acrylate) (PMMA CEA) microfibrous membrane	BPA	90	(Xu et al., 2013)
Horseradish peroxidase	Covalent binding	Modified reduced graphene oxide (RGO)	Phenol	100	(Vineh et al., 2018)
Laccase	Physical adsorption	CNTs-PVDF membrane	BPA	90	(Ji et al., 2016)
Laccase	Ion adsorption	Cu(II)-chelated magnetic microspheres	BPA	85	(Lin et al., 2016)
Laccase	Entrapment	Sol-gel matrix	Malachite blue	90	(Bagewadi et al., 2017)
Laccase	Cross-linking and entrapment	Alginate beads granular activated carbon	BPA	99	(Lassouane et al., 2019)
Laccase	Covalent binding	FE_3O_4@Cs composite	2,4-Dichlorophenol	91.4	(Zhang et al., 2020)
Tyrosinase	Encapsulation	Silica aerogel	Phenol	90	(Sani et al., 2011)
Tyrosinase	Cross-linking	Polyacrylonitrile beads	Phenol	96	(Wu et al., 2017)

Previous studies have reported that the immobilization of enzymes for pollutant removal can offer stabilization and protection from enzyme denaturation at extreme pH due to the strong intermolecular force between enzyme and support. For instance, immobilized HRP on amine-functionalized glycidyl methacrylate-g-poly (ethylene terephthalate) fibers resulted in changes in the optimum pH of free (pH 8) and immobilized enzyme (pH 6). A small change in pH can lead to an alteration of enzyme conformation and the substrate cannot bind to the active site thus enzyme is unable to perform a catalytic reaction. By immobilization, the enzyme usually results in stabilization at a wide range of pH (Arslan, 2011). In another study, a slight change in the pH optimum of free (pH 8) and immobilized HRP (pH 9). The immobilized HRP also shows higher stability at a wide range of pH (pH 6-10) (Kim et al., 2012). This result suggested that an imbalance of H+ displacement in the microenvironment of immobilized enzymes in the bulk solution leads to higher alkaline conditions for optimal reaction (Monier et al., 2010).

Temperature

The performance of pollutant degradation using immobilized enzymes may be greatly influenced by temperature. Too low or high temperatures can cause a change in the structure of enzyme conformation, thus leading to the inhibition of the enzymatic reaction.

Consequently, the enzyme has low thermal denaturation and removal efficiency (Zdarta et al., 2018). Previously, immobilized HRP showed maximum phenol removal efficiency of 80% at 25 °C to 35 °C. However, the removal efficiency decreased to 47% after incubation at 45 °C, under the same conditions. It was stated that the reduction of removal efficiency could be due to the denaturation of the enzyme's active site at higher temperatures (Bayramoğlu & Arıca, 2008). Based on the studies by Rahmani et al. (2015), immobilized laccase on silica beads was used to remove sulfathiazole and sulfamethoxazole. The removal of the pollutants by immobilized laccase at 50 °C achieved 80% efficiency. However, the removal efficiency gradually decreased when the temperature was at 70 °C. At higher temperatures, it caused enzyme inactivation due to thermal denaturation.

Furthermore, in another study, immobilized HRP on chitosan beads demonstrated that the immobilized HRP functioned effectively even at a high temperature (55 °C). The chemical modification of HRP helps the neutralization of lysine positive charge which resulted in an increment of thermal stability of the immobilized HRP (Liu et al., 2002). The improvement of thermal resistance of immobilized enzymes could be due to the prevention of enzyme unfolding because of the strong bond between enzyme and support (Sanjay & Sugunan, 2006). In another study, entrapped laccase in barium alginate was employed for acetaminophen removal (Ratanapongleka & Punbut, 2018). Notably, the highest degradation rate of acetaminophen was above 90% at temperatures ranging from 25 °C to 35 °C. Nonetheless, the degradation rate of immobilized laccase showed a drastic reduction to 20% at 65 °C. This is because enzyme denaturation could occur beyond a specific temperature (Ratanapongleka & Punbut, 2018). The degradation of EDCs using other immobilized enzymes is listed in **Table 2**.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

The removal of endocrine-disrupting chemicals such as synthetic dye, phenolic compounds, pharmaceutical products and pesticides are critical issues that need to be solved. These chemicals pose a threat to human health and the survival of living organisms. The promising approach using immobilized enzymes including immobilized laccase, tyrosinase, horseradish, manganese, and lignin peroxidase has shown remarkable potential in the removal of recalcitrant compounds from wastewater. The immobilized enzymes possess many advantages compared to free enzymes such as improved catalytic properties and operational stabilities. The catalytic activity of immobilized oxidoreductase enzymes relies on parameters such as pH, temperature and time. Despite the potential, the incorporated immobilized enzymes confront some challenges that need to be tackled while there is still far more opportunity for advancement in the treatment of comprehensive wastewater.

There are several suggestions for the studies of EDCS removal in future research and development, as shown below:

I. Development of novel technologies for the immobilization of oxidoreductase enzyme to improve enzyme catalytic properties, reusability and stability at various process conditions. Innovative and appropriate support for enzyme immobilization promotes the stability of the biocatalyst which is appropriate for the biocatalytic process requirements.
II. Optimization of current technologies to create highly efficient and long-term stable enzyme biocatalysts.
III. Research and development of the advanced solution for pollutant elimination to obtain a cost-effective and efficient process for the degradation of EDCs.
IV. Optimization of the biodegradation process for efficient treatment of pollutants as specific enzymes might have a specific mechanism for the removal of EDCs.
V. Implementation of the immobilized oxidoreductase enzymes in pilot-scale of biodegradation operations using optimum process parameters.

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Table 2 Degradation of EDCs using different immobilized oxidoreductase enzymes and their operating conditions

Enzymes	Targeted EDCs	Removal efficiency (%)	Operating conditions	Reference
Laccase	Phenol	78	25 C, pH 6.0, 100 rpm, 12 h	(Liu et al., 2012)
Tyrosinase	Phenol	96	40 C, pH 7.0, 100 rpm, 12 h	(Wu et al., 2017)
Laccase	BPA	96	30 C, pH 5.0, 100 rpm, 24 h	(Zdarta et al., 2018)
Laccase	2,4-dinitrophenol	90	25 C, pH 5.0, 100 rpm, 12 h	(Dehghanifard et al., 2013)
LiP	Methylene blue	90	25 C, pH 4.0, 100 rpm, 30 min	(Ferreira-Leitao et al., 2007)

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