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7 Unlocking nature's remediation arsenal: the role of polyphenol oxidases in efficient and eco-friendly industrial wastewater treatment

Abstract: Phenol and its derivatives have gained considerable attention recently due to their high toxicity, teratogenicity, and mutagenicity. Petroleum refinery wastewater is a significant source of phenolic compounds. However, conventional techniques used to treat these wastewaters have several drawbacks, such as incomplete or inefficient removal of phenols. In contrast, biocatalytic processes have garnered significant attention as they offer sustainable and effective removal of toxins, including phenols, from wastewater. Among various biocatalysts, polyphenol oxidases have emerged as major biocatalytic enzymes. These enzymes contain copper and catalyze the oxidation of specific phenolic substrates to quinones in the presence of molecular oxygen. Polyphenol oxi-

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dases have a wide range of applications. In the food industry, they are utilized for cocoa and tea production, enhancing coffee flavor and assessing food quality. In medicine, they find applications in treating phenylketonuria, Parkinson's disease, and leukemia. In environmental technology, they play a crucial role in removing phenolic pollutants from industrial wastewater. In the pharmaceutical industry, polyphenol oxidase-immobilized electrodes differentiate between morphine and codeine. This chapter provides comprehensive details about polyphenol oxidases' structure, biochemical properties, and applications, specifically focusing on their role in wastewater treatment.

7.1 Introduction

Several years of industrial activity have resulted in the production and introduction of a variety of toxic chemicals into the ecosystem. These chemicals contaminate natural water sources through wastewater from municipalities and industries, posing a threat to the local and global environment and ecology. Phenol and its associated phenolics are recognized as primary contaminants in industrial wastewater generated by various industries, including metallurgical, coal conversion, petroleum refining, resin, plastic, paper, wood, agricultural, chemical, pharmaceuticals, and textile dyeing industries [Fig. 7.1] [1, 2]. The extensive and uncontrolled use of pesticides, herbicides, and insecticides in agriculture also contributes significantly to the presence of these hazardous substances in groundwater and surface water. Furthermore, the microbial degradation of pesticides or other xenobiotics releases phenols and aromatic amines into the environment [3, 4]. Faust and Aly [5] reported that the concentrations of pollutants generally range from 100 to 1,000 mg/L. Upon entering the human body, these compounds can induce significant toxicity and pose health hazards, including cardiac arrhythmias, kidney disease, skin cancer, and other malignancies [6, 7]. Priority pollutants such as 2,4-dichlorophenol, 2,4,5-trichlorophenol, and pentachlorophenol (PCP) are included in the US EPA's water quality standards database [Fig. 7.2] [8]. Therefore, it is crucial to identify efficient methods to solve this pollution problem and safeguard the quality of life for future generations. Eliminating these chemicals from groundwater and drinking water requires decontaminating them from industrial wastewater before their discharge into natural water bodies.

Various chemical and physical technologies have been developed for decontaminating wastewater, including flocculation, coagulation, and adsorption. Common physical methods include rapid adsorption on activated sludge [9], carbon black [10], powdered activated carbon, pyrolyzed rice, and other low-cost adsorbents. Advanced technologies such as photolysis, reverse osmosis, ion exchange, advanced oxidation, and nanofiltration have also been successfully developed in laboratory settings. However, the cost of treating urban wastewater tends to be prohibitively high. Recently, biological methods for wastewater treatment have gained attention due to their ability to completely oxidize various impurities, including toxins, while requiring simple equipment and low cost. Shi et al. identified the pulsed high-voltage discharge system as a promising physical technology for reducing organic pollutants [11]. The photo-Fenton reaction [12] and the solvent-impregnated resin system [13] have been explored for removing phenols

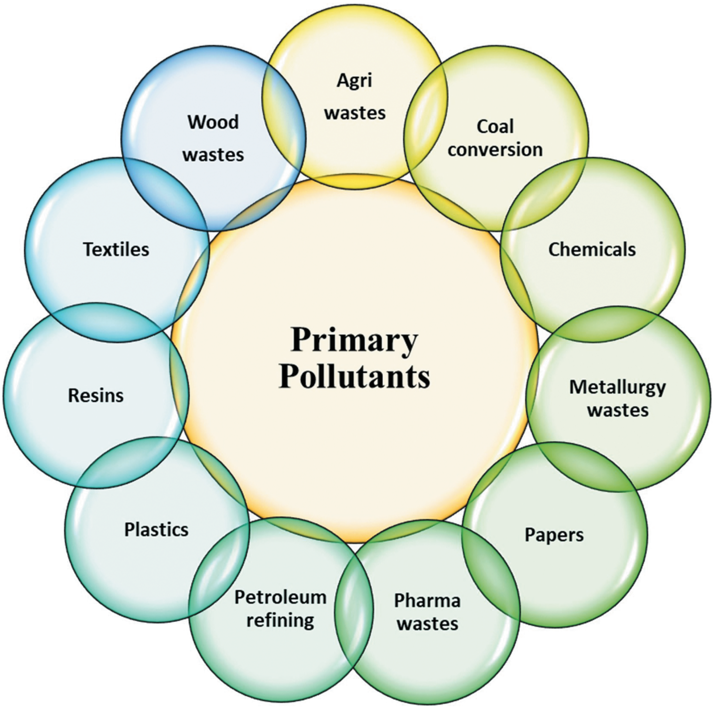
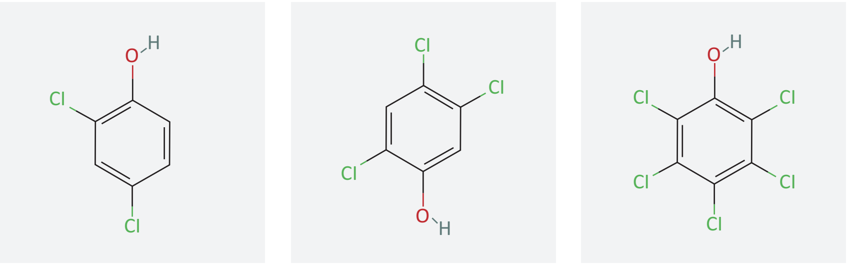


Fig. 7.1: Phenol and its associated phenolics are recognized as primary contaminants in industrial wastewater generated by various industries.



2,4-dichlorophenol 2,4,5-trichlorophenol Pentachlorophenol

Fig. 7.2: Structure of polyphenols considered as major water pollutants as per the US EPA.

and thiophenols from water through the complex synthesis of phosphine oxides and phosphates via hydrogen bonding. Heyl and Jorissen [14] argue that solid polymer electrolytes used as ion exchange membranes can remove pollutants from wastewater electrochemically. Phytoremediation and microbiological clearance are primary biological techniques employed to eliminate phenol. Phytoremediation involves using plants and plant-associated bacteria, including symbiotic and commensal organisms, to degrade phenol in contaminated wastewater or industrial leachate [15]. Algae have also been reported to contribute to phenol degradation, with examples such as *Ankistrodesmus braunii* and *Scenedesmus quadricauda* identified by Pinto et al. [16].

Microbial elimination is a prominent biological approach utilized in the bioremediation of phenolic pollutants commonly found in industrial wastewater and effluents. Microbial phenol degradation, a highly effective bioremediation method, relies on bacteria that utilize phenol as their primary carbon source. Numerous studies have demonstrated the ability of bacteria and fungi to degrade phenols and phenolics. For instance, *Bacillus subtilis* and *Alcaligenes* species [17], *Ralstonia eutropha* [18], and *Sphingomonas* species have been shown to break down chlorophenols. Takeuchi et al. [19] discovered a bacterial strain named *Sphingobium chlorophenolicum*, which exhibits complete digestion of PCP. Additionally, Ueshiba et al. identified *Sphingobium amiense* sp., a rare bacterium capable of digesting nonylphenol [20]. Schwien and Schmidt's research in 1982 revealed that aerobic bacteria can convert mono- and dichlorophenols into chlorocatechol through hydroxylation [21].

Fungi have also proven beneficial in the bioremediation of aqueous phenols found in industrial wastewater and effluents. Examples include *Rhizopus oryzae*, *Pleurotus sajor-caju*, *Phanerochaete chrysosporium*, and *Pleurotus ostreatus* [22–24]. However, standard phenol treatment procedures suffer significant drawbacks, including nonbiodegradability, incomplete removal, high cost, low efficiency, generation of hazardous by-products more toxic than the parent phenol, high energy consumption, and limited applicability within specific concentration ranges [25]. Therefore, there is a clear need for innovative solutions that offer high performance, effectiveness, and wide application in phenol removal from industrial wastewater.

Enzymatic treatment, specifically utilizing peroxidase (POD), represents an optimal approach for the biological removal of phenol from industrial wastewater, as suggested by Klibanov et al. [26]. In addition to POD, several other enzymes, including polyphenol oxidase (PPO), phenolase, cresolase, and cytochrome P450, have been identified and documented (as presented in Tab. 7.2). The effectiveness of these enzymes in the biodegradation of phenolic pollutants found in industrial wastewater and effluents has been well-demonstrated in scholarly literature.

Utilizing cost-effective and reliable PPOs in various practical domains has been an area of significant research interest. However, further investigation is still required to explore the application of PPOs, particularly laccase and tyrosinase, to remove phenolic pollutants from industrial wastewater. The primary objective of this chapter is to provide a comprehensive overview of research outcomes in this specific domain, encompassing unexplored areas. This academic publication presents a detailed summary of the biodegradation mechanism of phenols using PPO enzymes derived from various sources (Tab. 7.1). Furthermore, the chapter emphasizes the advantages of this approach compared to other methodologies.

Tab. 7.1: Current status of bioremediation using various organisms to combat polyphenol toxicity of water.

S. no.	Organism nature	Name of the organisms	References
1.	Algae	*Ankistrodesmus braunii*	[16]
		Scenedesmus quadricauda	
3.	Bacteria	*Bacillus subtilis*	[17–21]
		Alcaligenes sp.	
		Ralstonia eutropha	
		Sphingomonas species	
		Sphingobium amiense	
4.	Fungi	*Rhizopus oryzae*	[22–24]
		Pleurotus sajor-caju	
		Phanerochaete chrysosporium,	
		Pleurotus ostreatus	
		Sphingobium chlorophenolicum	
9.	Plants	*Lycopersicon esculentum* Mill. cv. Pera	[56]

7.2 Biochemical properties of polyphenol oxidase (PPO)

Natural environments contain several enzymes that play a role in the oxidation of phenolic compounds. These enzymes can be classified into two distinct groups: POD and PPO. POD is an enzymatic catalyst that utilizes hydrogen peroxide to facilitate substrate oxidation. Metalloproteinases, known as PODs, are primarily produced by various microorganisms and plants and contain a "heme" prosthetic group. These enzymes utilize hydrogen peroxide to expedite biochemical reactions [27]. The enzyme PPO, with the EC number 1.14.18.1, is found in various microorganisms, plants, and mammals, and it is known to have copper as a cofactor. PPOs are responsible for enzymatic browning and postharvest deterioration in fruits and vegetables. They also play a crucial role in plant and fungal defense against pathogens [28, 29]. Jukanti et al. [30] reported in 2004 that six genes at different chromosomal positions encode PPO.

Previous studies have shown that specific enzymes facilitate the oxidation process of monophenols and diphenols, producing their corresponding quinones [31, 32]. Monophenolase (EC 1.14.18.1) and diphenolase (EC 1.10.3.1) are two distinct enzymes involved in quinone production [27, 33]. PPO enzymes can be classified into two major groups: laccase and tyrosinase. These enzymes oxidize phenolic and nonphenolic aromatic compounds into less toxic and insoluble quinones. The compounds include phenols, *o*- and *p*-cresols, catechins, gallic acid, and *o*-, *m*, and *p*-phenylenediamines.

The enzyme tyrosinase EC 1.14.18.1 monophenol monooxygenase and EC 1.10.3.1 *O*-diphenol oxidoreductase or catechol oxidase are widely distributed across various organisms, from bacteria to mammals. It plays a role in various physiological processes, including melanin biosynthesis in bacteria and animals and promoting plant immunity [34]. Claus reported that tyrosinase catalyzes two consecutive oxygen-dependent reactions [35]. The cresolase activity leads to *O*-hydroxylation of monophenols, resulting in the formation of *O*-diphenols, while catecholase activity oxidizes *O*-diphenols to *O*-quinones. In 2004, Tomowski and Homolka [36] identified a two-nuclear copper complex in the active site of tyrosinase through chemical and spectroscopic tests. On the other hand, laccase (EC 1.10.3.2, *p*-benzenediol:oxygen oxidoreductase) belongs to a different category. This blue oxidase utilizes oxygen molecules to catalyze the oxidation of a wide range of aromatic and nonaromatic substances through a radical-catalyzed reaction mechanism. Previous research has highlighted its significance in the metabolic breakdown of complex organic substrates such as lignin or humic acid substances [37, 38]. It also plays a role in the oxidation of various aromatic substances, including phenolic dyes, phenols, and chlorophenols. Laccases exhibit potent nonspecific oxidation potential, making them effective biocatalysts for biotechnological applications. While laccases were initially thought to be restricted to eukaryotic organisms like fungi, higher plants, and insects, they are now known to have a significantly broader distribution among prokaryotic organisms [39]. The crystal structure of a bacterial-derived laccase has been successfully determined, revealing its phylogenetic relationship with enzymes such as ascorbate oxidase, ceruloplasmin, and bilirubin oxidase [40].

Tab. 7.2: Oxidoreductive enzymes involved in phenol degradation.

S. no.	Enzyme	Phenol substrate	References
1.	Phenol oxidase	Chlorophenol and phenol	[76, 77]
2.	Polyphenol oxidase	Bisphenol A, phenol, *p*-cresol, *o*-cresol	[78–81]
3.	Phenol hydroxylase	Phenol	[82]

Tab. 7.2 (continued)

S. no.	Enzyme	Phenol substrate	References
4.	Catechol 2,3-dioxygenase	Phenol	[83]
5.	Catechol 1,2 oxygenase	Phenol, methoxyphenol, and lignophenols	[84]
6.	Peroxidase	Phenol, lignophenols	[85]
7.	Laccase	Phenol, bisphenol, 2,6-dimethoxyphenol, anthracene, nonylphenol, TNT, benzo[a]pyrene, and 4-AmDNT	[86, 87]
8.	Tyrosinase	Phenol	[88]
9.	Horse radish peroxidase	Phenol	[89]
10.	Cytochrome P450	Bisphenol A	[90]
11.	Lignin peroxidase (Lip)	Pyrene, benzopyrene, amino dinitrotoluenes	[91]
12.	Chloroperoxidase	Halophenols	[92]

7.3 Applications of PPO

PPOs have widespread applications in the healthcare [Fig. 7.3] and food processing industries. They play a significant role in clinical and preclinical diagnostics, as highlighted by Pastore and Morrissi [41]. In the commercial sector, enzymes remove phenolic compounds from wastewater, ensuring their suitability for human consumption. Within the food industry, enhancing the quality and nutritional value of horticultural products is essential during the postharvest period. Enzymatic browning, resulting from the oxidation of endogenous phenolic compounds, is a well-known undesirable attribute. Assessing PPO activity is crucial for determining a fruit’s shelf life and suitability [42]. Evaluating the efficacy and reliability of techniques used to measure enzyme strength in diverse food sources is paramount. Despite perceived limitations in the food sector, Motoda [43] discovered that polyphenols could enhance the sensory attributes of tea and coffee.

Researchers are investigating the health benefits of polyphenol-rich substances, such as extra-virgin olive oil and cocoa beans, which possess antioxidant and antiplatelet properties. Their ability to manage atherosclerotic conditions may contribute to reducing the incidence of cardiovascular diseases, including myocardial infarction and cardiovascular risk factors, as indicated by Loffredo et al. [44]. They propose that polyphenols indirectly regulate cardiovascular risk factors, such as blood pressure, serum cholesterol, insulin sensitivity, platelet aggregation, and endothelial function [44]. Based on recent studies, Zheng et al. [45] hypothesize that polyphenols could be an effective natural therapy for Alzheimer's disease. In a study, five anthoxanthin polyphenols were examined to assess their interactions in preventing amyloid-beta (Aβ) oligomer-induced neuronal responses and their impact on oligomerization and antioxidant activity [45]. Caruana et al. [46] reported that red wine polyphenols exhibit nutraceutical properties and target underlying mechanisms involved in the etiology and neuropathology of Alzheimer's and Parkinson's diseases.

Fig. 7.3: Application of polyphenols in biomedical industry.

7.4 Wastewater treatment approaches using PPO

PPO can be utilized in wastewater bioremediation through one-stage and two-stage approaches. In one-stage bioremediation, the enzyme facilitates the oxidation of phenol to quinone. In two-stage bioremediation, the oxidation reaction is followed by the separation or adsorption of the enzyme-generated products [47]. The one-step oxidation approach is commonly preferred in enzymatic removal processes, which may involve enzyme-protective or -oxidizing agents [48]. The second separation stage is required to eliminate enzyme-generated metabolites from wastewater, which may or may not involve additional chemical additives [49, 50].

Enzymatic water treatment has employed both free and immobilized PPO, with immobilized PPO demonstrating superior efficiency in terms of stability, reusability, and prolonged viability. The combination of enzymatic and biological treatments has also been reported to remove phenol [51]. Immobilization of PPO can be achieved using various matrices, such as SiO_2–alginate hybrid [52], calcium and copper alginate, polyamide membrane [53], chitosan beads [54], cinnamoylated derivative-coated glass beads [55], and others.

7.5 Recent approaches for enzymatic wastewater treatment

Phytoremediation has emerged as a successful strategy for mitigating the impact of phenol and its associated phenolic compounds through genetically modified plant species. These plants exhibit upregulation of specific genes involved in synthesizing enzymes such as POD or PPO, facilitating the phytoremediation of phenolic compounds. Oller et al. [56] reported on using a transgenic tomato (*Lycopersicon esculentum* Mill. cv. Pera) engineered to enhance the activity of enzymes involved in phenol breakdown, effectively eliminating phenol. The increasing demand for wastewater bioremediation has sparked interest in exploring the potential of nanobiotechnology in this field.

Nanotechnology-based water treatment aims to achieve three primary objectives: prevention, treatment or remediation of contaminants, and sensing or detection. Nanobiotechnology finds applications in disciplines that require precise manipulation of target molecules at the molecular level [57]. Various nanotechnological methods have been employed in water treatment, including nanomembranes and nanoporous zeolites. However, the outcomes still need to meet expectations fully. The use of advanced nanostructured biomaterials and enzyme technology holds promise in significantly enhancing application and performance [58]. Novel techniques have been developed, such as the production of individual enzyme nanoparticles, attachment of enzymes onto nanosized structures, and autonomous immobilization of enzymes [59]. Nanoscale carrier materials facilitate high enzyme loading per unit mass, catalyst recycling, and minimal enzyme activity loss. Kim et al. proposed using single-enzyme nanoparticles, composed of a single enzyme molecule wrapped in a porous organic–inorganic framework with a few nanometers of thickness, to enhance enzyme stability [60].

The utilization of enzymes or nanomaterial–enzyme conjugates has emerged as a promising approach for the bioremediation of industrial wastewater. Cloete et al. [61] have proposed that this approach may offer greater effectiveness and cost-efficiency than traditional techniques. Galliker et al. [62] have demonstrated the effectiveness of laccase-modified silica nanoparticles for the biotransformation of phenolic pollutants in wastewater. It is feasible to fabricate enzymes or nanostructured materials based on PPO using benign nanomaterials such as carbon or boron nanotubes, resulting in PPO-based nanowires and nanofibers.

The integration of nanomaterial–PPO conjugates into bioreactors facilitates comprehensive wastewater bioremediation. Corvini and Shahgaldian [63] highlight the use of immobilized laccase on silica nanoparticles in bioreactors for the removal of hormone-disrupting chemicals, including recurrent pollutants or micropollutants, from wastewater, representing a significant advancement in PPO-nanomaterial-based water treatment. Nanoenzyme technology offers a promising alternative to enzymatic water treatment, providing advantages such as affordability, reusability, and improved performance compared to conventional methods.

7.6 Future prospects

Enzymes have garnered significant scientific attention in recent years as potential biocatalysts for large-scale processes in green chemistry. Industrial sectors are now required to adopt environmentally sustainable technologies that do not involve hazardous elements [64]. Biocatalysts offer several advantages over traditional technologies, including their biodegradable nature, sustainable origin, intrinsic characteristics, and ability to operate under mild temperature and pressure conditions. The biodegradation capacity of biocatalysts provides a safeguarding function for wastewater treatment systems, reducing the risk of secondary contamination [65]. Thorough characterization of biocatalysts enables the identification of optimal enzymes for the breakdown of persistent organic compounds present in industrial settings. However, the cost of enzymatic systems using purified enzymes is typically higher than conventional wastewater treatment methods, which limits their practicality [66]. Commercial availability is limited for most biocatalysts used to treat phenolic wastewater, which presents technical and economic challenges for large-scale enzyme-based wastewater treatment. Using recombinant DNA technology is crucial for achieving cost-effective production of biocatalysts and enzymes [67]. Successful implementation of this approach is expected to pose significant challenges in terms of enzyme purification and isolation. While pure enzymes are essential for the technical process of enzymatic water and wastewater treatment, their commercial significance may be limited. Extensive research is needed to improve enzymatic purification and production methodologies to make enzymatic wastewater treatment economically viable [66]. The stability and activity of biocatalysts pose significant challenges in enzymatic treatment methods for efficiently removing pollutants in wastewater [68]. Studies have shown that enzyme activity declines within 24 h of incubation in aqueous solutions, even under unfavorable conditions. Inhibitory chemicals present in the reaction medium can reduce the activity and effectiveness of enzymes [69].

Research indicates that industrial wastewater containing various inorganic and organic compounds can affect the functionality of biocatalysts [69]. Efficient methods such as precipitation, ion exchange, coagulation–flocculation, filtration, and similar processes can effectively remove dissolved and suspended inorganic pollutants. These techniques are comparable to biological wastewater treatment [70–72].

Enzymatic systems that exhibit resilience to specific inhibitory substances are preferred due to their potential to mitigate environmental impact and reduce physical treatment costs. Maintaining pH stability in biocatalysts is crucial for effectively eliminating organic and phenolic contaminants from wastewater, regardless of whether the conditions are acidic or alkaline. This practice eliminates or reduces the expenses of pH adjustment before the enzymatic treatment process. Directed evolution, protein engineering, and enzyme mutagenesis have led to modified enzyme variants that are more stable and exhibit improved performance in different industrial applications [71]. Chemical and immobilization methods are commonly used to enhance the stability and reusability of enzymes [73]. Due to the unique substrate specificities of enzymes, more than a single biocatalyst utilizing a single enzyme would be required to address all categories of pollutants efficiently. The integration of chemical and enzymatic techniques in wastewater treatment has been observed to enhance the efficacy of the process, leading to the complete detoxification and elimination of recalcitrant contaminants from effluent streams. While conflicting findings exist in some studies [74], it has been determined that homogeneous enzymes demonstrate superior effectiveness compared to heterogeneous reaction systems [75]. The discrepancies observed could be attributed to variations in operational parameters used in the different investigations, including enzyme concentration, treatment duration, and enzyme source. Therefore, careful design of these investigations is necessary to minimize variability and accurately assess the effectiveness of a biocatalyst against a phenolic contaminant. This would enhance the understanding of the interaction between contaminants and biocatalysts among researchers and aid in developing modeling approaches for enzyme selection in the degradation of organic pollutants [72]. The data mentioned above contributes to optimizing enzymatic action.

7.7 Conclusion

PPO in wastewater treatment offers cost-effective advantages due to its ability to utilize oxygen to convert phenols into quinones. These quinones possess high reactivity and can undergo successive conversions, forming other intermediates that can be efficiently eliminated through selective sorption or chemisorption on appropriate surfaces. The critical significance of employing PPO in wastewater bioremediation lies in its selective activation of phenols, leading to adsorbable quinones. Future research should focus on the enzymatic removal of phenols using PPO to advance this field further. This can be accomplished by conducting comprehensive literature reviews on the isolation, purification, characterization, and immobilization of PPO from various sources. These highly efficient, cost-effective, and environmentally friendly techniques make the PPO's promising avenues for further exploration and application in wastewater treatment.

References

[1] Ha, S.R., Vinitnantharat, S., and Ozaki, H. 2000. Bioregeneration by mixed microorganisms of granular activated carbon loaded with a mixture of phenols. Biotechnology Letters, 22, 1093–1096. https://doi.org/10.1023/A:1005650612768.

[2] Grady, C.P.L., Jr. 1990. Biodegradation of toxic organics: Status and potential. Journal of Environmental Engineering, 116(5), 805–828. https://doi.org/10.1061/(ASCE)0733-9372.

[3] Berry, D.F., and Boyd, S.A. 1985. Soil Biology and Biochemistry, 17, 631.

[4] Ruggier, O.P., Sarkar, J.M., and Bollag, J.M. 1989. Soil Science, 147, 361.

[5] Faust, S.D., and Aly, O.M. 1983. Chemistry of Water Treatment. 1st Edition, Butterworth Publishers, Stoneham, MA.

[6] Rice, R.H., and Cohen, D.E. 1996. The basic science of poisons. In: Klaassen, C.D., Amdur, M.O., Doull, J. (eds). Casarett and Doull's Toxicology. 5th Edition, McGraw-Hill, New York.

[7] Adeyemi, O., Oginni, O., Osubor, C.C., Oloyede, O.B., Oladiji, A.T., and Adebayo, E.A. 2009. Effect of water contaminated with phthalate, benzene and cyclohexane on Clarias gariepinus' cellular system. Food and Chemical Toxicology, 47(8), 1941–1944.

[8] Sharma, H.A., Barber, J.T., Ensley, H.E., and Polito, M.A. 1997. A comparison of the toxicity and metabolism of phenol and chlorinated phenols by Lemna gibba, with special reference to 2,4,5-trichlorophenol. Environmental Toxicology and Chemistry, 16(2), 346–350.

[9] Zhao, J., Li, Y., Zhang, C., Zeng, Q., and Zhou, Q. 2008. Sorption and degradation of bisphenol A by aerobic activated sludge. Journal of Hazardous Materials, 155(1–2), 305–311.

[10] Dominguez-Vargas, J.R., Navarro-Rodriguez, J.A., de Heredia, J.B., and Cuerda-Correa, E.M. 2009. Removal of chlorophenols in aqueous solution by carbon black low-cost adsorbents, Equilibrium study and influence of operation conditions. Journal of Hazardous Materials, 169, 302–308.

[11] Shi, J., Bian, W., and Yin, X. 2009. Organic contaminants removal by the technique of pulsed high-voltage discharge in water. Journal of Hazardous Materials, 171, 924–931.

[12] Gernjak, W., Krutzler, T., Glaser, A., Malato, S., Caceres, J., Bauer, R., and Fernandez-Alba, A.R. 2003. Photo-Fenton treatment of water containing natural phenolic pollutants. Chemosphere, 50, 71–78.

[13] Cuypers, R., Sudholter, E.J., and Zuilhof, H. 2010. Hydrogen bonding in phosphine oxide/phosphate-phenol complexes. ChemPhysChem: A European Journal of Chemical Physics and Physical Chemistry, 11(10), 2230–2240. https://doi.org/10.1002/cphc.201000084.

[14] Heyl, A., and Jorissen, J. 2006. Electrochemical detoxification of waste water without additives using solid polymer electrolyte (SPE) technology. Journal of Applied Electrochemistry, 36, 1281–1290.

[15] Saiyood, S., Vangnai, A.S., Thiravetyan, P., and Inthorn, D. 2010. Bisphenol A removal by the Dracaena plant and the role of plant-associating bacteria. Journal of Hazardous Materials, 178, 777–785.

[16] Pinto, G., Pollio, A., Previtera, L., Stanzione, M., and Temussi, F. 2003. Removal of low molecular weight phenols from olive oil mill wastewater using microalgae. Biotechnology Letters, 25, 1657–1659.

[17] El-Sayed, W.S., Ismaeil, M., and El-Beih, F. 2009. Isolation of 4-chlorophenol-degrading bacteria, Bacillus subtilis OS1 and Alcaligenes sp. OS2 from petroleum oil-contaminated soil and characterization of its catabolic pathway. Australian Journal of Basic and Applied Sciences, 3, 776–783.

[18] Padilla, L., Matus, V., Zenteno, P., and Gonzalez, B. 2000. Degradation of 2,4,6-trichlorophenol via chlorohydroxyquinol in Ralstonia eutropha JMP134 and JMP222. Journal of Basic Microbiology, 40, 243–249.

[19] Takeuchi, M., Hamana, K., and Hiraishi, A. 2001. Proposal of the genus Sphingomonas sensu stricto and three new genera, Sphingobium, Novosphingobium and Sphingopyxis, on the basis of phylogenetic and chemotaxonomic analyses. International Journal of Systematic and Evolutionary Microbiology, 51, 1405–1417.

[20] Schwien, U., and Schmidt, E. 1982. Improved degradation of monochlorophenols by a constructed strain. Applied and Environmental Microbiology, 44, 33–39.

[21] Ushiba, Y., Takahara, Y., and Ohta, H. 2003. Sphingobium amiense sp. nov., a novel nonylphenol-degrading bacterium isolated from a river sediment. International Journal of Systematic and Evolutionary Microbiology, 53, 2045–2048.

[22] Justino, C., Marques, A.G., Rodrigues, D., Silva, L., Duarte, A.C., Rocha-Santos, T., and Freitas, A.C. 2011. Evaluation of tertiary treatment by fungi, enzymatic and photo-Fenton oxidation on the removal of phenols from a kraft pulp mill effluent: A comparative study. Biodegradation, 22, 267–274.

[23] Rocha-Santos, T., Ferreira, F., Silva, L., Freitas, A.C., Pereira, R., Diniz, M., Castro, L., Peres, I., and Duarte, A.C. 2010. Effects of tertiary treatment by fungi on organic compounds in a kraft pulp mill effluent. Environmental Science and Pollution Research International, 17, 866–874.

[24] Fountoulakis, M.S., Dokianakis, S.N., Kornaros, M.E., Aggelis, G.G., and Lyberatos, G. 2002. Removal of phenolics in olive mill wastewaters using the white-rot fungus Pleurotus ostreatus. Water Research, 36, 4735–4744.

[25] Chung, T.P., Tseng, H.Y., and Juang, R.S. 2003. Mass transfer effect and intermediate detection for phenol degradation in immobilized Pseudomonas putida systems. Process Biochemistry, 38, 1497–1507.

[26] Klibanov, A.M., Alberti, B.N., Morris, E.D., and Felshin, L.M. 1980. Enzymatic removal of toxic phenols and anilines from waste waters. Journal of Applied Biochemistry, 2, 414–421.

[27] Durán, N., and Esposito, E. 2000. Potential applications of oxidative enzymes and phenol oxidase-like compounds in wastewater and soil treatment: Review. Applied Catalysis B, 28, 83–99.

[28] Mayer, A.M. 2006. Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry, 67, 2318–2331.

[29] Seo, S.Y., Sharma, V.K., and Sharma, N. 2003. Mushroom tyrosinase: Recent prospects. Journal of Agricultural and Food Chemistry, 51, 2837–2853.

[30] Jukanti, A.K., Bruckner, P., and Fischer, A.M. 2004. Evaluation of wheat polyphenol oxidase genes. Cereal Chemistry, 81(4), 481–485.

[31] Minussi, R.C., Pastore, G.M., and Durán, N. 2002. Potential applications of laccase in the food industry. Trends in Food Science & Technology, 13, 205–216.

[32] Torres, E., Bustos-Jaimes, I., and Borgne, S.L. 2003. Potential use of oxidative enzymes for the detoxification of organic pollutants. Applied Catalysis B: Environmental, 46, 1–15.

[33] Durán, N., Rosa, M.A., D'Annibale, A., and Gianfreda, L. 2002. Applications of laccases and tyrosinases (phenol oxidases) immobilized on different supports: A review. Enzyme and Microbial Technology, 31(7), 907–931.

[34] Peralta-Zamora, P., Pereira, C.M., Tiburtius, E.R.L., Moraes, S.G., Rosa, M.A., Minussi, R.C., and Durán, N. 2003. Decolorization of reactive dyes by immobilized laccase. Applied Catalysis B: Environmental, 42, 131–144.

[35] Claus, H. 2004. Laccases: Structure, reactions, distribution. Micron, 35, 93–96.

[36] Tomšsovsky, M., and Homolka, L. 2004. Tyrosinase activity discovered in Trametes spp. World Journal of Microbiology and Biotechnology, 20, 529–530.

[37] Witayakran, S., and Ragauskas, A.J. 2009. Synthetic applications of laccase in green chemistry. Advanced Synthesis and Catalysis, 351, 1187–1209.

[38] Duangmal, K., and Apenten, R.K.O. 1999. A comparative study of polyphenol oxidases from taro (Colocasia esculenta) and potato (Solanum tuberosum var. Romano). Food Chemistry, 64, 351–359.

[39] Claus, H. 2004. Laccases: Structure, reactions, distribution. Micron, 35(1–2), 93–96. ISSN 0968-4328, https://doi.org/10.1016/j.micron.2003.10.029.

[40] Ni Eidhin, D., Degn, P., and O'beirne, D. 2010. Characterization of polyphenol oxidase from rooster potato (Solanum tuberosum cv rooster). Journal of Food Biochemistry, 34, 13–30.

[41] Pastore, M., and Morisi, F. 1976. Lactose reduction of milk by fibre-entrapped beta-galactosidase: Pilot plant experiments. Methods in Enzymology, 44, 822–844.

[42] Hamdan, N., Lee, C.H., Wong, S.L., Fauzi, C.E.N.C.A., Zamri, N.M.A., and Lee, T.H. 2022. Prevention of enzymatic browning by natural extracts and genome-editing: A review on recent progress. Molecules, 27, 1101. https://doi.org/10.3390/molecules27031101.

[43] Motoda, S. 1979. Formation of aldehydes from amino acids by polyphenol oxidase. Journal of Fermentation Technology, 57, 79–85.

[44] Loffredo, L., Perri, L., Nocella, C., and Violi, F. 2017. Antioxidant and antiplatelet activity by polyphenol-rich nutrients: Focus on extra virgin olive oil and cocoa. British Journal of Clinical Pharmacology, 83(1), 96–102.

[45] Zheng, H., Fridkin, M., and Youdim, M. 2015. New approaches to treating Alzheimer's disease. Perspectives in Medicinal Chemistry, 7, 1–8.

[46] Caruana, M., Cauchi, R., and Vassallo, N. 2016. Putative role of red wine polyphenols against brain pathology in Alzheimer's and Parkinson's disease. Frontiers in Nutrition, 3, 31.

[47] Mukherjee, S., Basak, B., Bhunia, B., et al. 2013. Potential use of polyphenol oxidases (PPO) in the bioremediation of phenolic contaminants containing industrial wastewater. Reviews in Environmental Science and Biotechnology, 12, 61–73. https://doi.org/10.1007/s11157-012-9302-y.

[48] Erhan, E., Keskinler, B., Akay, G., and Algur, O.F. 2002. Removal of phenol from water by membrane-immobilized enzymes: Part I. Dead-end filtration. Journal of Membrane Science, 206, 361–373.

[49] Cooper, V.A., and Nicell, J.A. 1996. Removal of phenols from a foundry wastewater using horseradish peroxidase. Water Research, 30, 954–964.

[50] Ikehata, K., and Buchanan, I.D. 2002. Screening of Coprinus species for the production of extracellular peroxidase and evaluation of the enzyme for the treatment of aqueous phenol. Environmental Technology, 23, 1355–1367.

[51] Marín-Zamora, M., Rojas-Melgarejo, F., Garc'Ia-C'Anovas, F., and Garc'Ia-Ruiz, P.A. 2007. Effects of the immobilization supports on the catalytic properties of immobilized mushroom tyrosinase: A comparative study using several substrates. Journal of Biotechnology, 131(4), 388–396.

[52] Abadulla, E., Robra, K.H., Gübitz, G.M., Silva, L.M., and Cavaco-Paulo, A. 2000. Enzymatic decolorization of textile dyeing effluents. Textile Research Journal, 70, 409–414.

[53] Peralta-Zamora, P., Pereira, C.M., Tiburtius, E.R.L., Moraes, S.G., Rosa, M.A., Minussi, R.C., and Durán, N. 2003. Decolorization of reactive dyes by immobilized laccase. Applied Catalysis B: Environmental, 42, 131–144.

[54] Shao, J., Ge, H., and Yang, Y. 2007. Immobilization of polyphenol oxidase on chitosan–SiO2 gel for removal of aqueous phenol. Biotechnology Letters, 29, 901–905.

[55] Khan, A.A., and Husain, Q. 2007. Decolorization and removal of textile and non-textile dyes from polluted wastewater and dyeing effluent by using potato (Solanum tuberosum) soluble and immobilized polyphenol oxidase. Bioresource Technology, 98, 1012–1019.

[56] Oller, A.W., Agostini, E., Talano, M., Capozucca, C., Milrad, S.R., Tigier, H.A., and Medina, M.I. 2005. Overexpression of a basic peroxidase in transgenic tomato (Lycopersicon esculentum Mill. cv. Pera) hairy roots increase phytoremediation of phenol. Plant Science, 169, 1102–1111.

[57] Zekić, E., Vuković, Z., and Halkijević, I. 2018. Application of nanotechnology in wastewater treatment. Građevinar, 70, 315–323. https://doi.org/10.14256/JCE.2165.2017.

[58] Prachi, P., Gautam, P., Madathil, D., and Nalinakumari, B.N. 2013. Nanotechnology in waste water treatment: A review. International Journal of ChemTech Research, 5, 2303–2308.

[59] Gehrke, I., Geiser, A., and Somborn-Schulz, A. 2015. Innovations in nanotechnology for water treatment. Nanotechnology, Science and Applications, 6(8), 1–17. doi: 10.2147/NSA.S43773. PMID: 25609931; PMCID: PMC4294021.

[60] Kim, J., Grate, J.W., and Wang, P. 2006. Nanostructures for enzyme stabilization. Chemical Engineering Science, 61, 1017–1026.

[61] Cloete, T.E., Kwaadsteniet, M.D., Botes, M., and López-Romero, J.M. 2010. Nanotechnology in Water Treatment Applications. Caister Academic Press, Norfolk, UK.

[62] Galliker, P., Hommes, G., Schlosser, D., Corvini, P.F., and Shahgaldian, P. 2010. Laccase-modified silica nanoparticles efficiently catalyze the transformation of phenolic compounds. Journal of Colloid and Interface Science, 349, 98–105.

[63] Corvini, P.F.X., and Shahgaldian, P. 2010. LANCE: Laccase-nanoparticle conjugates for the elimination of micropollutants (endocrine disrupting chemicals) from wastewater in bio-reactors. Reviews in Environmental Science and Biotechnology, 9, 23–27.

[64] Valero, E., González-Sánchez, M.-I., and Pérez-Prior, M.-T. 2015. Removal of organic pollutants from industrial wastewater by treatment with oxidoreductase enzymes. In: Jiménez, E., Cabañas, B., Lefebvre, G. (eds). Environment, Energy and Climate Change I: Environmental Chemistry of Pollutants and Wastes. Springer, New York, NY, USA, 317–339. ISBN 978-3-319-12907-5.

[65] Hutchison, J.M., Mayer, B.K., Vega, M., Chacha, W.E., and Zilles, J.L. 2021. Making waves: Biocatalysis and biosorption: Opportunities and challenges associated with a new protein-based toolbox for water and wastewater treatment. Water Research X, 12, 100112. https://doi.org/10.1016/j.wroa.2021.100112. ISSN 2589-9147.

[66] Feng, S., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Cheng, D., Varjani, S., Lei, Z., and Liu, Y. 2021. Roles and applications of enzymes for resistant pollutants removal in wastewater treatment. Bioresource Technology, 335, 125278. https://doi.org/10.1016/j.biortech.2021.125278. ISSN 0960-8524.

[67] Lambertz, C., Ece, S., Fischer, R., and Commandeur, U. 2016. Progress and obstacles in the production and application of recombinant lignin-degrading peroxidases. Bioengineered, 7, 145–154.

[68] Nicolucci, C., Rossi, S., Menale, C., Godjevargova, T., Ivanov, Y., Bianco, M., et al. 2011. Biodegradation of bisphenols with immobilized laccase or tyrosinase on polyacrylonitrile beads. Biodegradation, 22, 673–683.

[69] Jiang, W., Li, W., Xiao, F., Wang, D., and Wang, Z. 2017. Influence of NOM and SS on the BPA removal via peroxidase catalyzed reactions: Kinetics and pathways. Separation and Purification Technology, 173, 244–249.

[70] Ferasat, Z., Panahi, R., and Mokhtarani, B. 2020. Natural polymer matrix as safe flocculant to remove turbidity from kaolin suspension: Performance and governing mechanism. Journal of Environmental Management, 255, 109939.

[71] Farasat, Z., Panahi, R., and Mokhtarani, B. 2017. Timecourse study of coagulation-flocculation process using aluminum sulfate. Water Conservation and Management, 1, 7–9.

[72] Gołub, A., and Piekutin, J. 2020. Removal of inorganic impurities from wastewater after production of soda ash on selected sorbents. Scientific Reports, 10, 4624.

[73] Abdollahi, K., Yazdani, F., and Panahi, R. 2016. Data in support of covalent attachment of tyrosinase onto cyanuric chloride crosslinked magnetic nanoparticles. Data Brief, 9, 1098–1104.

[74] Lin, J., Liu, Y., Chen, S., Le, X., Zhou, X., Zhao, Z., et al. 2016. Reversible immobilization of laccase onto metal-ion-chelated magnetic microspheres for bisphenol a removal. International Journal of Biological Macromolecules, 84, 189–199.

[75] Wang, S., Fang, H., Wen, Y., Cai, M., Liu, W., He, S., and Xu, X. 2015. Applications of HRP-immobilized catalytic beads to the removal of 2,4-Dichlorophenol from wastewater. RSC Advances, 5, 57286–57292.

[76] Robles, A., Lucas, R., de Cienfuegos, G.A., and Galvez, A. 2000. Phenol oxidase (laccase) activity in strains of the hyphomycete Chalara paradoxa isolated from olive mill wastewater disposal ponds. Enzyme and Microbial Technology, 26, 484–490.

[77] Okeke, B.C., Paterson, A., Smith, J.E., and Watson-Craik, I.A. 1997. Comparative biotransformation of pentachlorophenol in soils by solid substrate cultures of Lentinula edodes. Applied Microbiology and Biotechnology, 48, 563–569.

[78] Li, L., and Steffens, J.C. 2002. Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta, 215, 239–247.

[79] Cano, P.M., de Begona, A., Gloria, L., and Mariana, S. 1997. Improvement of Frozen banana (Musa cavendisher, C. Venana) colour by blanching: Relationship between browning, phenols and poly phenol oxidase and peroxidase activities. Zeitschrift für Lebensmitteluntersuchung und Forschung A, 204, 60–65.

[80] Burton, S.G., Duncan, J.R., Kaye, P.T., and Rose, P.D. 1993. Activity of mushroom polyphenol oxidase in organic medium. Biotechnology and Bioengineering, 42, 938–944.

[81] Shashirekha, S., Uma, L., and Subramanian, G. 1997. Phenol degradation by the marine cyanobacterium Phormidium valderianum BDU 30501. Journal of Industrial Microbiology and Biotechnology, 19, 130–133.

[82] Gurujeyalakshmi, G., and Oriel, P. 1989. Isolation of phenol-degrading Bacillus stearothermophilus and partial characterization of the phenol hydroxylase. Applied and Environmental Microbiology, 55, 500–502.

[83] Ali, S., Fernandez-Lafuente, R., and Cowan, D.A. 1998. Meta-pathway degradation of phenolics by thermophilic bacilli. Enzyme and Microbial Technology, 23, 462–468.

[84] An, H.R., Park, H.H., and Kim, E.S. 2001. Cloning and expression of thermophilic catechol 1,2-dioxygenase gene (catA) from Streptomyces setonii. FEMS Microbiology Letters, 195, 17–22.

[85] Xia, Z., Yoshida, T., and Funaoka, M. 2003. Enzymatic degradation of highly phenolic lignin-based polymers (lignophenols). European Polymer Journal, 39, 909–914.

[86] Dodor, D.E., Hwang, H.-M., and Ekunwe, S.I.N. 2004. Oxidation of anthracene and benzo[a]pyrene by immobilized laccase from Trametes versicolor. Enzyme and Microbial Technology, 35, 210–217.

[87] Saito, T., Kato, K., Yokogawa, Y., Nishida, M., and Yamashita, N. 2004. Detoxification of bisphenol A and nonylphenol by purified extracellular laccase from a fungus isolated from soil. Journal of Bioscience and Bioengineering, 98, 64–66.

[88] Xiangchun, Q., Hanchang, S., Yongming, Z., Jianlong, W., and Yi, Q. 2003. Biodegradation of 2,4-dichlorophenol in an air-lift honeycomb-like ceramic reactor. Process Biochemistry, 38, 1545–1551.

[89] Wu, Y., Taylor, K.E., Biswas, N., and Bewtra, J.K. 1998. A model for the protective effect of additives on the activity of horseradish peroxidase in the removal of phenol. Enzyme and Microbial Technology, 22, 315–322.

[90] Sasaki, M., Maki, J., Oshiman, K., Matsumura, Y., and Tsuchido, T. 2005. Biodegradation of bisphenol A by cells and cell lysate from Sphingomonas sp. strain AO1. Biodegradation, 16, 449–459.

[91] Novotny´, C., Svobodova´, K., Erbanova´, P., Cajthaml, T., Kasinath, A., Lang, E., and Sasek, V. 2004. Ligninolytic fungi in bioremediation: Extracellular enzyme production and degradation rate. Soil Biology and Biochemistry, 36, 1545–1551.

[92] Osborne, R.L., Raner, G.M., Hager, L.P., and Dawson, J.H. 2006. C. fumago chloroperoxidase is also a dehaloperoxidase: Oxidative dehalogenation of halophenols. Journal of the American Chemical Society, 128, 1036–1037.