**MICROBIAL ENZYMES: A SUSTAINABLE APPROACH FOR BIOREMEDIATION OF INDUSTRIAL POLLUTANTS**

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**Abstract**

Industrialization and other human activity represent significant environmental hazards. A wide variety of living things can be harmed by toxic pollutants in their specific surroundings. Emerging contaminants in nature are associated with a number of acute and long-term biotic component harms, which subsequently damage the ecosystem and provide major risks. Conventional methods of pollution removal are ineffective and result in the creation of secondary contaminants. Prenatal illnesses, cancer, cardiovascular and mental disorders, allergy diseases, and other adverse impacts are all significantly exacerbated by pollution. By utilization of microorganisms or their enzymes, harmful contaminants are removed from the environment through the efficient remediation method known as bioremediation. Environmental microorganisms frequently produce a wide range of enzymes that can remove dangerous toxins by using them as a substrate for growth and development. Microbial enzymes have the ability to break down and eliminate dangerous environmental contaminants and convert them into non-toxic forms through their catalytic reaction mechanism. Microbial enzymes can easily be modified by pollutant substrate into other relatively harmless products by recognising them under ideal settings (temperature/pH/contact time/concentration). The most representative enzymes involved in bioremediation include cytochrome P450s, laccases, hydrolases, dehalogenases, dehydrogenases, proteases, and lipases, which have shown promising potential degradation of polymers, aromatic hydrocarbons, halogenated compounds, dyes, detergents, agrochemical compounds, etc. For the breakdown of polymers, dyes, aromatic hydrocarbons, detergents, halogenated chemicals, agrochemical compounds, etc., these enzymes have shown great potential. Several [immobilizations](https://www.sciencedirect.com/topics/engineering/immobilisation), genetic engineering strategies, and nanotechnology applications have been developed to improve enzyme performance and reduce pollution removal process costs. Oxidation, elimination, reduction and other numerous mechanisms, favour this type of bioremediation. Through the use of environmentally friendly new technologies, genetically modified microorganisms that generate a variety of recombinant enzymes can improve the considerable breakdown of pollutants. Few microbial enzymes have been utilised thus far, yet a significant amount of microbial diversity remains undiscovered. This chapter focuses on recent trends and prospects for microbial enzymes in the sustainable degradation of harmful contaminants (such as dyes, [poly-aromatic](https://www.sciencedirect.com/topics/engineering/polyaromatics) hydrocarbons, plastics, [heavy metals](https://www.sciencedirect.com/topics/engineering/heavy-metal), and pesticides) in the environment.

**Introduction**

Environmental pollution is one of the world's most significant problems due to the uncontrolled discharge of untreated effluents and pollutants into the environment. Environmental contamination is mostly caused by population increase, industrialisation, exploration, urbanization, and mining (Jie et al., 2023). A considerable quantity of pollutants from untreated sewage to nuclear waste has been released into the environment, posing a severe threat to humanity's survival (Kesari et al., 2021). Anthropogenic activities including industrialization, farming methods, population growth, and unhealthy competition for dominance are having severely negative effects on Earth. These processes result in the production of pollutants, primarily phenols, polyaromatic hydrocarbons, pesticides, azo dyes, polychlorinated chemicals, heavy metals, and other dangerous compounds. These chemicals endanger the biotic components of ecosystems because they are resistant to biodegradation (Elekwachi, 2014). These contaminants have a profound impact on every area of the planet, having carcinogenic, mutagenic, and toxic consequences on people and other living things (Liu et al., 2019).

Pollutants have been cleaned up using a variety of physical and chemical techniques, including oxidising agents, electrochemical processes, pollutant adsorption, ion exchange, and membrane filtering (Ufart et al., 2015). Traditional procedures were adequate for the high concentration of contaminants, but they were insufficient to reduce the pollution to allowable levels (Malik, 2004). Traditional methods for cleaning up pollutants have a number of drawbacks, including their high cost, difficult procedures, stringent international regulations imposed on decontamination, general public rejection, non-specificity, space limitations and potential for secondary pollution creation (Singh et al., 2008). As a result, interest has grown in bioremediation, eco-friendly and biological procedures.

Bioremediation is the transition or deterioration of pollutants by microorganisms into non/less toxic substances (Sharma et al., 2018). The bioremediation method primarily depends on microorganisms that degrade multi-pollutants enzymatically and transform them into less/non-toxic substances/metabolites that may be useful products (Narayanan et al., 2023). Using biological agents for the reduction of pollutants present in the environment is an affordable process and reduces the risk they impose on people’s health and threats to the environment (Kirchhoff, 2003). It mostly uses intracellular accumulation or enzymatic transformation to degrade and detoxify contaminants (Singh et al., 2008). Microbial enzymes are regarded to be innovative, economical, and promising when used in the bioremediation of persistent pollutants (Fig. 1). Because of their metabolic activity and capacity to thrive in a variety of environmental conditions, bacteria, fungi etc. are present throughout the biosphere and produce enzymes. Among the various microbial enzymes, such as those from *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, *Rhodococcus,* *Mycobacterium* has already been shown to break down pesticides and hydrocarbons. The most common microbial enzymes that participated in bioremediation, are cytochrome P450s, dehydrogenases, laccases (Lac), proteases, hydrolases, lipases, and dehalogenases (Bhandari et al., 2021). The bioremediation technique has a number of constraints. Only a few biological agents have the aptness to produce specialised enzymes with sufficient power to break down contaminants, and the process is exceedingly slow and constant. Therefore, because genetically engineered microbes produce required enzymes in huge quantities under ideal conditions, we choose them for bioremediation.

Industrial microbial enzymes currently have a very competitive global market. Numerous novel microbial enzymes are been developed using rDNA technology, meta-genomics and protein engineering for different bioprocesses. In food, pharmaceutical, textile, paper, biotechnological, leather, and other industries, a variety of molecular techniques are implemented to boost the production and effectiveness of different microbial enzymes (Nigam 2013; Singh et al., 2016; Adrio and Demain, 2014). In this chapter, we have focused on the role and mechanism of microbial enzymes in the sustainable bioremediation of industrial pollutants.

A diagram of different types of materials

Description automatically generated

**Figure 1:** Application of microbial enzymes in bioremediation of toxic industrial pollutants into non-toxic compounds

**Microbial enzymes for bioremediation of industrial pollutants**

Microbial enzyme-mediated bioremediation is a sustainable approach in current scenario. Enzymes are present in almost every naturally existing organism i.e. from prokaryotes to eukaryotes. Many microbial enzymes act as catalysts for producing a variety of products from a variety of substrates under controlled circumstances. Additionally, through bioconversion or biodegradation processes, several microbial enzymes can quickly convert hazardous substances into valuable by-products (Fig. 1). The large range of applications for these compounds necessitates the industrial production of microbial enzymes under varied physical and chemical circumstances. Under extreme pH and temperature circumstances, microbial enzymes ought to function properly. During prolonged commercial fermentations, the use of thermostable enzymes lowers the danger of microbial contamination (Adrio and Demain, 2014). Anaerobic bacterial enzymes have been used to de-chlorinate chloroform, trichloroethylene (TCE), and polychlorinated biphenyls (PCBs) (Sharma 2012).

It is possible for a number of endogenous biological events to take place along a well-defined pathway. Credit goes to the superb metabolic catalysts that are enzymes. Enzymes accelerate a range of biological operations that are crucial to sustaining human life by lowering the reaction activation energy without causing any long-term impact. The manufacture of enzymes from microbes has a number of advantages, including easy handling, rapid growth under controlled circumstances, straightforward gene manipulation, elevated manufacturing yield etc. Microbial enzymes are also being employed more frequently in industrial settings as a result of their catalytic activity, non-toxicity, specificity, eco-friendliness, stability, cost-effectiveness and ease of manufacturing. The sources, mechanisms involved and uses of microbial enzymes isolated from different bacterial and fungal species for bioremediation are shown in Table 1 and Table 2 respectively.

**A. *Laccases (* EC 1.10.3.2)**

Laccases are extracellular enzymes that contain several copper ions and are made up of various glycoprotein subunits. By using a one-electron mechanism, laccases (benzenediol oxygen oxidoreductases) oxidise some amines, ethers, and esters as well as some phenolic and aromatic compounds in bacteria, fungi and plants (Shekher et al., 2011). The water molecules are oxidised as a result of this multi-copper blue oxidase coupling the electron transport. The possibility for laccases to be used in many biotechnological processes has been explored because oxygen is used as the final electron acceptor, a wide range of substrates can be used, and cofactors are not required. These qualities have piqued researcher’s interest in using laccases to break down a variety of harmful substances found in wastewater. Most microbial laccase from various microorganisms is recognised, defined, and investigated, particularly the *Streptomyces laccase* from *actinomycetes* (Guan et al., 2018).

The stability of laccases under a range of pH, organic solvents, temperatures, and salt concentrations is among their most notable biochemical characteristics (Guan et al., 2018). Laccase is typically a highly stable, industrially applicable heat-resistant enzyme, in contrast to recombinant Cot A from *E. coli* which particularly has half-life of approximately 120 minutes as seen in Cot A from *B. subtilis* at 75℃ temperature (Chandra and Chowdhary 2015). Laccase has the ability to eliminate xenobiotics and generate polymeric compounds employed in bioremediation procedures. The most prevalent contaminant now understood, PAHs, which are made up of a linearly structured benzene ring, are spread equally in the natural environment (Li et al., 2010; Zeng et al., 2011). Due to their toxicity, persistence, mutagenicity, and carcinogenicity, pollutants and their by-products pose a serious threat to the ecosystem. Pollutants are created by burning industrial waste and fossil fuels. When used with the most efficient laccase mediator 1-hydroxy benzene triazole (HBT), polycyclic aromatic hydrocarbons (PAHs) are transformed by laccase into their quinone form, which is subsequently further broken down to carbon dioxide (Khlifi et al., 2010). Textile industry-produced phenols and dyes can be eliminated and detoxified using laccase (Sondhi et al., 2008).

Agricultural wastes including sawdust, banana peels, and rice bran contain lignin and phenolic substances that increase laccase production (Muthukumarasamy et al., 2015 ). There are numerous biotechnological uses for laccases in a variety of industries, including paper, food, textiles, cosmetics and others. This is because of their high oxidative capability. Laccases are also employed to break down agricultural items like pesticides and herbicides, protecting the environment from dangerous chemicals. Additionally, Laccases have been employed in the bioremediation of soil contaminated by oil hydrocarbons, the treatment of wastewater containing textile industry dyes, and the bio-pulping and bio-bleaching procedures (Annibale et al., 2006 ; Sharma et al., 2014; Martin-Sampedro et al., 2012).

A complicated mixture of phenolic metals, post-methanation detoxification of distillery effluent, colour removal and disintegration of distillery effluent comprising amino carbonyl complex and chloro-lignin-containing pulp paper mill waste have also been described for the microbial laccase. Recombinant Cot A laccase from *E. coli* has been demonstrated in a study to be capable of discolouring simulated textile effluents (STE). Seven structurally distinct dyes were successfully decoloured by recombinant Cot A laccase in both pure and un-purified forms. When simulated textile effluents were buffered at pH 7, pure and un-purified Cot A laccase decolourized more quickly (Wang and Zhao 2017). At basic pH (pH 9.0), the pure recombinant laccase removes colour more than ninety-three percent of the tested colours in about four hours (Lu et al., 2013). An environmentally acceptable alternative to traditional dye degradation techniques that are frequently expensive and result in harmful consequences is laccase-mediated dye degradation (Vaithyanathan et al., 2022). *B. vallismortis strain* fmb103 is responsible for the production of recombinant laccase; that is used in aquaculture wastewater bioremediation(Sun et al., 2017). Effluent from textile printing industry is used for decolourization of recombinant CuO from *E. Coli* K12 produced in *Pichia pastoris* (Ma et al., 2017). Two laccases were found in the cell-free extract of *Pseudomonas putida* strain F6 (soil bacteria) and Cop A was one of them. It was capable of destroying five of the seven dyes tested: Evans Blue, Brom Cresol Purple, Remazol brilliant Blue , Reactive Black 5 & Amido Black 10B (Mandic et al., 2019). Almost ninety-two percent of the BPA (bisphenol A) is converted into C9 H12 O2 ( 4-ethyl-2-methoxy phenol) by *Bacillus sp.* (Rajeshwari and Bhuvaneswari 2016). Similar results were obtained by laccase, isolated Lac15 from marine microbial metagenome, which demonstrated one-hour decolourization of reactive azo dyes at 50 mM, which includes reactive deep blue M-2GE & brilliant orange K-7R (Guan et al., 2014). Laccase produced from microorganisms such as *Pseudomonas stutzeri* and *Streptomyces cyaneus* CECT 3335 was used to evaluate the biological decolourizing of kraft eucalyptus pulps eucalyptus pulps using mediators such as ABTS ( 2,2-Azino-bis-3-Ethyl benzo thiazoline-6-sulfonic acid) etc.

Laccases have also been reported to be produced from filamentous fungi. White rot fungi are responsible for producing the most laccases (Fernandez-Fernandez et al., 2013). Strong Laccase producers include *Pleurotus florida* (Silva et al., 2012*)*, *P.ostreatus* (Alexandrino et al.,2007), *P. pulmonarius* (Tychanowicz et al., 2004), and *P. tailandia* (Menezes et al.,2009). The fungi *Trametes sp*. (Couto and Herrera, 2006), Coriolopsis sp. (Cabana et al.,2007), *Grifola sp.* (Nitheranont et al., 2011),and many others also create laccases. The *Rhus vernicifera* tree served as the first source of laccase isolation (Yoshida 1883). Presently, bacteria such as *E. coli*, insects example *Bombyx*, *Drosophila, Papilio*, *Schistocerca* etc. and plants that produce laccases include mango, peaches and pines (Arora and Sharma 2010; Kalra et al., 2013). As a result, laccase has enormous promise for the cost-effective treatment of wastewater comprising phenolic and non-phenolic chemicals, PHAs (poly-hydroxy alkanoates), artificial colours, and other developing contaminants.

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| Table 1: Bioremediation of industrial pollutants through various bacterial enzymes | | | | | | | |
| S.No. | Name of enzyme | Name of bacteria | Substrate used | Mechanism  involved | Industry | Application | References |
| 1 | *Laccase*  *(* EC 1.10.3.2) | *Streptomyces maltophilia* | Synthetic dyes such as methylene blue, methyl green, toluidine blue, Congo red, methyl orange, and pink | Ring cleavage in aromatic compounds  and reduce one molecule of oxygen in the  water and produce free radicals | Textile | Degradation and decolourization of synthetic dyes in Textile effluents | Romero et al., 2006 |
| 2 | *Streptomyces cyaneus* | 2,2′-Azino-bis-(3-  ethylbenzothiazoline -6-sulfonic  acid (ABTS) | Plastic | Oxidation of Micro-pollutants like BPA (Bisphenol A), DFC (Diclofenac), and MFA (Mefenamic acid) | Margot et al., 2013 |
| 3 | *Geobacillus thermocatenulatus* | ABTS | Textile | Degradation and Decolorization of  Textile dyes, especially  Congo red and  bromophenol blue | Shekhar et al., 2011 |
| 4 | *A*. *gonensis (*cell-free  extracts laccase) | ABTS | Tannery | Bioremediation of  tannery effluents | Bhandari et al.,2021; Shekhar et al., 2011; Yanmis et al., 2016 |
| 5 | *Cytochrome P450*  (EC 1.14.14.1) | *Rhodococcus*  *rhodochrous* | Hexahydro-1,3,5-trinitro 1,3,5-triazine (RDX) | Performs electron transfer reactions and catalysis by reduction or oxidation of heme iron. | Pharmaceutical | Degradation of RDX | Guengerich 2018, Du Plessis-Rosloniec 2011 |
| 6 | *Lipase*  (EC 3.1.1.3) | *Bacillus subtilis* | Olive oil | Catalyzes the hydrolysis of mono-, di-, and triglycerides into fatty acids and glycerol as well as catalyse the esterification  reactions. | Food | Bioremediation of  Wastewater,  Cleaning detergent of tough oil or grease stains. | Bhandari et al.,2021, Haniya et al., 2017; Marty 2012; Saraswat et al., 2017 |
| 7 | *Bacillus pumilus* | Palm oil | Detergent , food, cosmetic | Degradation of palm oil containing  Industrial wastewater | Saranya et al., 2019 |
| 8 | *Dehydrogenase*  (EC1.1.1.1) | *Pseudomonas putida* | 4-Hydroxybenzaldehyde and 4-  hydroxy-3-methylbenzaldehyde | Oxidizing organic compounds and  generating energy | Tannery | Breakdown of 2,4- xylenol. | Chen et al., 2014; Phale et al., 2019; Dotaniya et al., 2019 |
| 9 | *S. rhizophila* | Vinyl alcohol oligomer and polyvinyl alcohol | Textile, paper , food | Polyvinyl alcohol  degradation | Phale et al., 2019; Dotaniya et al., 2019 |
| 10 | *Protease*  (E.C 3.4.21.12)  *Protease*  (E.C 3.4.21.12) | *Bacillus subtilis* | Feather culture medium | Assist in the breaking of protein peptide bonds. | Poultry, tannery | Deterioration of casein as well as feathers. Degradation of proteins like keratin, casein, etc., leather dehairing, and  wastewater treatment | Suh and Lee 2001; Razzaq et al., 2019 |
| 11 | *Chryseobacterium* sp. strain kr6,  *Bacillus pumilus* | Chicken feathers | Poultry | Deterioration of feathers. | Riffel et al.,2003; Razzaq et al.,2019; Refai et al., 2005 |
| 12 | *Streptomyces thermoviolaceus* | Hair, collagen, Muscle, nail,  feathers | Poultry | Hydrolyze the fibrin, collagen, muscle, nail, and hair | Razzaq et al., 2019; Chitte et al., 1999 |
| 13 | *Thermoanaerobacter keratinophilus* | Complex medium without oxygen having merino wool, human hairs, chicken feathers | Poultry | Breakdown of keratin fibers. | Razzaq et al., 2019; Riessen and Antranikian 2001 |
| 14 | *Dehalogenase*  (EC 3.8.1.5) | *Bacillus* sp. | 2,4,6-Trinitrobromophenol  (TBP) | Cleaves the carbon-halogen bond and  eliminates the halogens | Pesticides | Degradation of TBP | Zu et al., 2012; Allpress and Gowland 1998; Jugder et al., 2015 |

Table 2: Bioremediation of industrial pollutants through various fungal enzymes

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S.No. | Name of enzyme | Name of fungus | Substrate used | Mechanism involved | Industry | | Application | Reference |
| 1 | *Laccase*  *(* EC 1.10.3.2)  *Laccase*  *(* EC 1.10.3.2) | *Coniophora puteana* | Syringaldazine (SGZ) | Ring cleavage in aromatic compounds  and reduce one molecule of oxygen in the  water and produce free radicals | Textile | | Deterioration of artificial dye | Shekhar et al., 2011 |
| 2 | *T. versicolour* | Pellets of *T. versicolour* | Textile | | Detoxifying and reducing the colour, aromatic compounds, and chemical oxygen demand (COD) were reduced up to 70–80% and COD was reduced up to 60%. | Shekhar et al., 2011 |
| 3 | *Aspergillus*  *flavus* | Dyes | Textile | Removing  surfactants and dyes | | Ghosh and  Ghosh , 2018 |
| 4 | *T. versicolour* and *Pleurotus ostreatus* | Glucose and fructose | Pharmaceutical | 71% of p-hydroxy benzoic acid and 56% of protocatechuic acid were degraded | | Keum and Li 2004 |
| 5 | *Cerrena unicolosr* | sugarcane bagasse | Paper and pulp | Reducing lignin content | | D’Souza-Ticlo et al., 2009 |
| 6 | *Flavodon flavus, Pycnoporus sanguineus, Trichosporon beigelii* NCIM-3326 | Bromophenol blue , malachite green | Textile | Decolourized several synthetic dyes such as Azure B and Brilliant Blue R in low nitrogen medium, decolourized bromophenol blue and malachite green (azo dyes) | | Soares et al., 2001, Elisachvili and Kachlishvili 2009 |
| 7 | *P. chrysosporium* | Azo black reactive dye | Tannery | Degradation of Azo Black Reactive 5 dye | | Enayatizamir et al., 2013 |
| 8 | *P. chrysosporium* URM 6181 and *Curvularia lunata* URM 6179 | Indigo dye | Textile | Decolourize effluent containing textile indigo dye by approximately 95% for 10 days of treatment 95% reduction of indigo dye | | Miranda et al., 2013 |
| 9 | *Flavodon flavus, Coriolopsis gallica* | Sugar cane bagasse | Pulp and paper | Decolourize the effluent from a Kraft paper mill bleach plant | | Hernandez et al. 2017 |
| 10 | *Cytochrome*  *P450*  (EC 1.14.14.1) | *Phanerochaete chrysosporium* | Polyaromatic hydrocarbon (PAHs) | Performs electron transfer reactions and catalysis by  reduction or oxidation of heme iron. | Pharmaceutical | Catabolize PAHs including anthracene and the endocrine  disrupting alkylphenols, detoxify a variety of pharmaceutical compounds, including antibiotics,anti-inflammatories,  and β-blockers | | Hirosue et al. 2011; Syed et al. 2011;Olicon-Hernandez et al. 2017 |
| 11 | *Phanerochaete chrysosporium, Saccharomyces cerevisiae* |  | Coal | Use for the bioremediation of Mutagenic/carcinogenic fused-ring high molecular weight PAHs (HMW-PAHs) Crude oil aliphatic hydrocarbon n-alkanes Endocrine-disrupting long-chain alkyl | | Naghdi et al.,2018 |
| 12 | *Lipases*  (EC 3.1.1.3) | *Aspergillus sclerotiorum* | Sunflower seed | Catalyses the hydrolysis of mono-, di-, and triglycerides into fatty acids and glycerol as well as catalyse the esterification  reactions. | Dairy | Bioremediation in dairy effluent to reduce oil and greese. It reduced over 90% of the O&G present in the sample | | Dickel et al., 2023 |
| *Aspergillus terreus* | - |
| 13 | Petroleum | Remediate oily polluted soils | | Mahmoud et al., (2015) |
| 14 | *Dehydrogenase*  (EC1.1.1.1) | *Phanerochaete chrysosporium (*preferred acidic conditions) | Sewage-treatment-plant (STP) sludge | Oxidizing organic compounds and  generating energy | Pulp and paper | Used to treat the acid effluent stream discharged from a pulp mill bleach plant and were able to remove colour from their respective effluent sources | | Wingate et al., 2005 |
| 15 | *Humicola insolens*( preferred alkaline conditions) | Sewage-treatment-plant (STP) sludge | Oxidizing organic compounds and  generating energy | Textile | Applied to the effluent discharged from the caustic sewer of the bleach plant and were able to remove colour from their respective effluent sources | | Wingate et al., 2005 |
| 16 | white rot fungus *Funalia trogii* | *Funalia trogii* pellets |  | Textile | Decolourization of textile dyes | | Tilli et al., 2011 |
| 17 | *Catalase*  (EC 1.11.1.6) | *Neurospora crassa* | H2O2 | Convert hydrogen peroxide to water and oxygen | Tannery | Bioremediation of heavy metals from tannery effluent | | Takio et al., 2021 |
| 18 | *Peroxidases*  (E.C. 1.11.1.7) | *Thanatephorus sp., Auricularia sp., Penicillium geastrovirus, Candida*  *tropicalis* | Phenols, hydroquinone, dyes, amines, aromatic alcohols and xenobiotic | catalyze the oxidation of various organic and inorganic substrates by reacting with hydrogen peroxide and similar molecules | Textile | degradation of synthetic dyes such as azo, remazol blue, cibacron  red, remazol brilliant blue, anthraquinone, and so forth | | Sugano et al., (2006), Liers et al., (2010) |
| 19 | *Proteases*  ss(E.C 3.4.21.12) | *Aspergillus flavus, A. oryzae ,*  *A. niger ,*  *Cladosporium herbarum*  *F. solani , P. chrysogenum* | Hides and skins | catalyze hydrolytic reactions that degrade protein molecules down to peptides and eventually to free amino acids | Tannery | Widely used in the  leather processing industries , recycling of waste , wastewater  treatment | | Negi et al., 2011, leng et al., 2013; castro et al., 2015; sethi and Gupta 2015; Jridi et al., 2014; Verma et al., 2016 |
| 20 | *Tannase*  (E.C. 3.1.1.20) | *A. niger,*  *A. oryzae, A. japonicus, A. gallonyces, A. awamori,* | Tannin | Catalyzes  the hydrolysis of ester bonds | Tannery | Used in the degradation of tanneries effluents containing tannins | | Lagemaat et al., 2001 |

**B. *Cytochrome P450* (EC 1.14.14.1)**

Cytochrome P450, a member of the heme enzyme superfamily is widely distributed in all the three domains of life i.e., Eukaryota, Bacteria, and Archaea (Zu et al., 2012). It carries out a variety of tasks, such as the biotransformation of harmful compounds in our ecosystem and synthesising intricate natural products in living systems (Allpress and Gowland 1998). P450s have the innate ability to break down xenobiotics (Jugder et al., 2015) through bioremediation-related chemical processes. They create carbon substrate and oxidised products by combining molecular oxygen with NADH or NADPH. NADH and NADPH act as a cofactor (Yoshida; 1883). They also need ferredoxin and ferredoxin reductase as a source of electrons for catalytic action. Studies on microbial P450s using protein engineering and non-engineering techniques have been conducted for bioremediation of organic contaminants and hydrocarbons. One of the recognised microbial P450s is the *Bacillus megaterium* CYP102A1 (P450BM3) model, which has been proven through protein engineering research to have the ability to oxidise PAHs. Gaseous alkanes, terpenes, and dangerous compounds are routinely detoxified using alkanes (Guan et al., 2018). Many microorganisms mainly bacteria such as *Rhodococcus, Gordonia, Mycobacterium,*  *Pseudomonas* etc. have been found to include some catabolic genes and plasmids that express P450s for the reduction of POPs (persistent organic pollutants) and their elimination from our environment (Silva et al., 2012). The expression of CYP108J1 during the breakdown of PAHs is repressed by NarL (nitrate-dependent two-component regulatory factor), according to further mutational study (Menezes et al., 2009).

**C. Lipases (EC 3.1.1.3)**

Lipases are Triacylglycerol ester hydrolyses. Lipases are enzymes that promote the breakdown of triglycerides into fatty acids and glycerol. Lipases catalyze the reaction in which bonds are broken due to acid (acidolysis), due to alcohol (alcoholysis), and due to amino acids (amino lysis) in addition to hydrolysis. Lipases are the most adaptable biocatalysts. Plants, animals, and microbes all have lipases in varying concentrations. The most common source of lipases is microorganisms. *Bacillus sp.* is one of the most common bacteria that has the best chance of producing lipase. The most prevalent lipase producers are *Bacillus alcalophilus, B. licheniformis, B. stearothermophilus, Serratia rubidaea, Pseudomonas aeruginosa, Staphylococcus caseolyticus*, and *Acinetobacter radioresistens* (Thakur 2012).

Industrial applications for microbial lipases include the processing of resolution of racemic mixtures, detergents, enhancement of flavour via the creation of short-chain fatty acid, esters and alcohols, and amino acid derivatives, and the development of biosensors that serve as diagnostic tools for the detection of various diseases (Hasan et al., 2006). Microbial lipases are fully commercially applicable in the bioremediation of oil residues, petroleum contaminants and effluents, as well as in a number of different industries, including the pharmaceutical, polymerization, pulp and paper, and cosmetic sectors (Arora et al., 2020; Gurung et al., 2013). Lipases can speed up the rate of bioremediation of oily effluents released from a variety of sources that comprise fats, proteins, and oils (Basheer et al., 2011; Hassan et al., 2018). Oil spills, including those involving n-alkanes, aromatic hydrocarbons, and PAHs, have been controlled using lipase from the bacteria *Acinetobacter sp., Mycobacterium sp.,* and *Rhodococcus sp.* (Casas-Godoy et al., 2012). However, *Pseudomonas* lipase is been utilized for the remediation of soil having waste oil released from industries. *Pseudomonas aeruginosa* lipase has been observed to degrade castor oil (Amara and Salem 2009). Biologically remediation of wastewater that is contaminated with crude oil using *P.* *aeruginosa*; Additionally it has been shown to be beneficial by a week-long decrease in oil concentrations and an 80 percent reduction in waste toxicity (Verma et al., 2012). One of the main environmental issues is the contamination of soil due to the deposition of mineral oil hydrocarbons that are released as petroleum by-products. Bacteria that are isolated from soil polluted with motor oil produce lipase that helps in the remediation of hydrocarbon. The principal soil contaminant, the hydrocarbon, can be eliminated with the use of lipase produced by bacterial isolates from soil polluted with motor oil (Mahmood et al., 2017). In household laundry, lipases are used to reduce environmental contaminants and improve the effectiveness of detergent to get rid of stubborn grease or oil stains. To decrease the percentage of phosphate-by products in laundry detergents crude lipase from the *Bacillus subtilis* strain is used (Saraswat et al., 2017). Biodegradable polymers have been hailed as one of the solutions to the growing problem of environmental pollution. The hydrolysis of polylactide (pla) into oligomers and then subsequently into monomers is catalysed by lipase PL, which was isolated from *Alcaligenes sp.* (Hoshino and Isono 2002). It was discovered that *L. plantarum* displayed the highest polycaprolactone (PCL) degradation efficiencyin comparison with other lipases when it came to the breakdown of artificial polyester polycaprolactone (PCL) by co-cultures of *L. brevis* and *L. plantarum* lipases (Wang et al. 2018). Table 1 and 2 lists a few microbial lipases together with information about each one's characteristics and bioremediation function.

In fungus lipase makers are *Rhizopus,* *Penicillium, Aspergillus sp. etc. Rhizopus arrhizus and R. niveus* are among the *Rhizopus* species that generate lipases effectively (Pandey et al., 1999).Based on their wide range of applications in multiple industrial fields, lipases are anticipated to see the greatest growth rate among enzymes.

**D. *Dehydrogenase* (EC1.1.1.1)**

Dehydrogenases are members of the oxidoreductase family and are mainly found in bacteria, yeast, plants, animals and human beings. The bacteria’s cell-free extracts that break down xenobiotics manufactured industrially showed signs of polyethylene glycol dehydrogenase activity. Numerous *Sphingomonas* species Utilize polyethylene glycol as fuel while PPG dehydrogenase (dye-linked), an enzyme present in *Stenotrophomonas maltophilia's* outer membrane or in periplasmic space is responsible for the destruction of high-molecular-weight PPG, the cytoplasmic enzyme is functioning to metabolise low-molecular-weight PPG (Tachibana et al., 2002). Xenobiotic polyvinyl alcohol which is water soluble in nature is broken down by recombinant polyvinyl alcohol dehydrogenase, which is used to oxidise glycols (Hirota-Mamoto et al., 2006). It has been discovered that aldehyde dehydrogenase is involved in the anabolic and catabolic processes of aromatic compounds. Aldehyde dehydrogenase is extensively and specifically expressed, according to a protein expression analysis of *Amycolatopsis tucumanensis* DSM 45259's biodegradation of phenanthrene (Bourguignon et al., 2019). Table 1 lists a few dehydrogenases together with information about each one's characteristics and function in bioremediation.

**E. *Protease* (E.C 3.4.21.12)**

The hydrolase family of enzymes, which includes protease, catalyses the peptide bonds in proteins. Proteases are mainly secluded from *Bacillus* and *Aspergillus* species. Proteases that are isolated from microorganisms are very pivotal due to their inexpensive price, high production, and useful function. They are extensively employed in sectors like wastewater treatment, the food sector, and the leather sector (Kumar and Sharma 2019). Protease is used in the removal of polymers since it can break down ß-ester bonds, ß-ester bonds produced by poly hydroxyl butyrate (PHB) depolymerase, and c- linkages (Haider et al., 2019). Because of the presence of keratin protein which is insoluble in nature, animal horns, nails, poultry faeces, and the shedding and moulting of appendages are resistant to breakdown. Along with their unpleasant odour, they are responsible for environmental contamination. Keratinase, a protease enzyme, may break down keratin proteins and be used in the bioremediation of chicken waste by breaking down and recycling keratinous wastes into beneficial by-products. *Stenotrophomonas maltophilia* KB13's keratinase, a protease enzyme, has demonstrated notable activity in the biological degradation of chicken feathers (Bhange et al., 2016). *Bacillus sp.* FPF-1's keratinase enzyme has demonstrated its capacity to utilise resistant keratinous waste biomass from the agricultural industry (accession number MG214993) by degrading chicken feathers at an 82 percent rate (Nnolim et al. 2020). The breakdown of keratin was successfully accomplished by the collaboration of two enzymes from the bacteria *Stenotrophomonas sp.,* serine protease and disulphide reductase. Similar to this, considerable hydrolysis of keratinous wastes from chicken has been demonstrated by keratinase enzymes generated by *Pseudomonas species* and *Bacillus species.* (Mazotto et al., 2011). By-products of feather degradation can be employed as ferric ion reducers, fertilisers for plant development, feed additives, free radical scavengers (Laba et al., 2018), and hazardous hexavalent chromium reducing agents (Bhange et al., 2016) since they are rich in amino acids and minerals. In the leather business, keratinase is utilised in place of the conventional chemicals CaO and Na2S in environmentally friendly enzymatic de-hairing operations, which reduce pollution by preventing the release of harmful waste into water bodies (Akhter et al., 2020). Additionally, the bioremediation of marine crustacean wastes employed in the de-proteinization phase of chitin extraction uses protease enzymes. By producing alkaline protease, *Bacillus licheniformis* strain MP1 reduces the protein content of shrimp waste by 75% (Jellouli et al., 2011). On a 7-day fermentation, the microbe *S. marcescens* FS-3de-proteinized up to eighty-four percent of the proteins in crab shells (Jo et al., 2008), and 72 per cent of the protein is removed by *P. aeruginosa* strainK-187 from shrimp and crab shell powder (SCSP), 78 percent from natural shrimp shells (NSS), 45 percent from SCSP that has been acid-treated (Oh et al., 2000). The water-dispersible polyurethane Impranil, which weighs 3 g/L, is broken down by *Pseudomonas fluorescens* in four to five days due to an enzyme with beta action. At an ideal pH of 5.0, the impurified enzyme was reported to be a protease (Howard and Blake 1998). Similar to this, *Pseudomonas chlororaphis* decomposed the Impranil substrate while exhibiting protease activity (beta clearing zone) and esterase activity (alpha clearing zone) at a predominate pH between 7 and 8 and at a temperature of 30°C (Howard et al.1999). By breaking down and transforming marine crustacean debris and keratinous waste products into beneficial molecules, proteases lessen environmental pollution.

**F. Peroxidases (E.C. 1.11.1.7)**

Peroxidases are oxidoreductases that utilize a free radical mechanism for transforming a variety of chemical substances into oxidized or polymerized products. Specifically, ferricyanides and ascorbic acid are converted into harmless components by peroxidase activity by being given electrons. Through the bio-remediation of phenol, cresol, and chlorinated phenolic chemicals in wastewater, peroxidases have the potential to reduce water pollution. Peroxidases are suitable enzymes for the creation of enzyme-linked immunosorbent assay (ELISA) kits, which are employed in the detection of many diseases because they may produce chromogenic products at low concentrations. Peroxidases are helpful in a number of industrial and analytical bioprocesses because of their strong reduction potential. Degradation of artificial colors like azo, remazol blue, Cibacron red, and remazol brilliant blue is one of these processes. Peroxidases are also utilized in the paper industry to degrade wood components by hydrolyzing lignin and cellulose into carbon dioxide and water, respectively (Regalado et al., 2004).

Peroxidases are affirmed from a variety of sources including plants, animals, and microbes. Various plant sources such as horseradish (*Armoracia rusticana*), banana (*Musa paradisiaca*), bare (*Acorus calamus*), papaya (*Carica papaya*), and so forth have been reported for peroxidase production. Among the bacterial strains, *Bacillus* sp. (Dawkar et al., 2008; Bansal and Kanwar 2013), *Escherichia coli* (Di-Gennaro et al., 2014), and *Pseudomonas* sp. (Kalme et al., 2007; Telke et al., 2010) are predominant peroxidase producers. Whereas, in the case of fungal strains, *Pleurotus ostreatus* (Faraco et al., 2007), *Thanatephorus* sp. (Sugano et al., 2006), *Auricularia* sp. (Liers et al., 2010), *Umbelopsis isabellina* (Yang et al ., 2003), and *Penicillium geastrovirus* (Yang et al., 2003) have been stated as effective peroxidase producers. Peroxidases have also been reported from yeasts such as *Debaryomyces polymorphus,* *Candida tropicalis* (Yang et al., 2003) and many more.

**G. Cellulases (E.C. 3.2.1.4)**

The cellulase enzymes aids in the hydrolysis of cellulosic substrates into monomeric products. They are produced by microbial strains as they grow on cellulosic materials and hydrolyse the β-1, 4-glycosidic bonds of cellulose. Three different types of cellulases- endoglucanase (1,4-D-glucan-4-glucanohydrolase, E.C. 3.2.1.4), exocellobiohydrolase (1,4-D-glucan glucohydrolase, E.C. 3.2.1.74), and β-glucosidase (D-glucoside glucohydrolase, E.C. 3.2.1.21) are required. Cellulases have been discovered in a number of bacterial, yeast and fungal strains. Fungi are the main cellulase-producing microbial groups, as they can utilize secondary pathways and yield higher cellulase activity. Fungal genera such as *Aspergillus* and *Trichoderma* are well-known cellulase producers. *A. niger, Aspergillus* *protuberus* (Yadav et al., 2016), *A. fumigatus* (Das et al., 2013), *A. ellipticus* (Agrawal et al., 2016), and so forth are reported as efficient cellulases producing Aspergilli. *Trichoderma viride* ( Nathan et al., 2014) as well as *T. asperellum* (Raghuwanshi et al., 2014) have also been reported as potent cellulase producers. Other fungal strains that exhibit potential cellulase activity include *Myceliophthora thermophile* (Pereira et al.,2015), *Penicillium echinulatum* (Camassola and Dillon 2014), and *Rhizopus oryzae* (Kupski et al., 2014), and From bacteria, *Thermomonospora sp*., *Cellulomonas sp*., *Microbispora sp., Clostridium sp., Cellvibrio sp*., and *Ruminococcus sp.* are reported as potent cellulose-producing genera (Saranraj et al., 2012).

**Mechanism of microbial enzymes in bioremediation**

In nature, there are a number of genes that are known to express different types of enzymes and that regulate how these enzymes function and what kind of structure they should adopt to perform a certain function. The numerous protein folds of the enzyme are regulated by highly distinct sets of genes, which not only adapt the structure of the enzyme for a variety of uses, but also determine the catalytic mechanism, which establishes the function of enzymes at a given site. Figure 2 shows the mechanism of action for some of the most important microbial enzymes utilized in bioremediation**.**

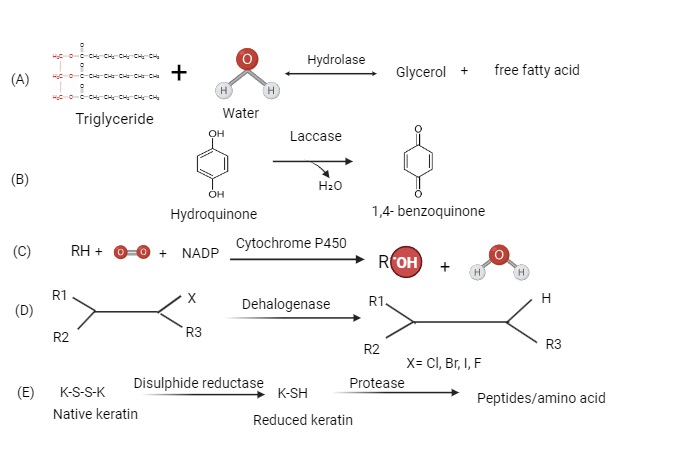


Figure 2: Some microbial enzymes used in bioremediation; general enzymatic reactions catalyzed by (a) Hydrolase, (b) Laccase, (c) Cytochrome P450, (d) Dehalogenase, (e) Protease

With a variety of aromatic compounds, such as polyphenol (Bourbonnais and Paice, 1990), aromatic amines, and methoxy-substituted monophenols (Bourbonnais et al., 1995), one oxygen molecule is reduced to water along with the oxidation of one electron. According to Gianfreda and Bolla (1999), laccases have four copper atoms called Cu T1 (where the reducing substrate binds) and trinuclear copper cluster T2/T3 (where oxygen is reduced to water and electrons are transferred from type I Cu to type II and type III Cu trinuclear clusters). These four copper ions are divided into Type 1 (T1), Type 2 (T2), and Type 3 (T3) categories. Electronic paramagnetic resonance (EPR) spectroscopy and UV/visible spectroscopy can be used to distinguish between these three categories.

A trinuclear centre made up of Type 2 and Type 3 copper is involvrd in the mechanism of the enzyme. O2 reduction is seen at fixed state (Gianfreda & Bolla.,1999). For the complete reduction of molecular O2 to water, four reducing substrate molecule should be oxidized. Free radicles are produced when laccase oxidizes the substrate. By adding mediators, laccase-mediated catalysis can be extended. Mediators are low-molecular-weight organic compounds that are oxidized by laccase. N-hydroxyphthalimide (NHPI), 1-hydroxy benzotriazole (HOBT), 2,2- azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), and 3 hydroxyanthranilic acid are most widely used synthetic mediators (Bourbonnais et al.,1995; Gochev & Krastanov., 2007).

The glycerol backbone of a lipid substrate serves as the typical site of lipase activity in the case of the lipase enzyme. The mechanism of lipase maturation occur by two steps: the first would involve folding of the monomer, which starts right after the N-terminus of the lipase emerges through the Sec61 translocon (i.e., co-translocational); the second would involve assembly of fully folded monomers into homodimers. Classically, lipases are involved as a catalyst in the hydrolysis of triglycerides:

Triglyceride + H2O → fatty acid + diacylglycerol.

Diacylglycerol + H2O → fatty acid + monacylglycerol

Monacylglycerol + H2O → fatty acid + glycerol

Dehydrogenase enzymes transfer electrons from the substrate to an electron carrier; what carrier is used depends on the reaction taking place. NAD+, FAD, and NADP+ are common electron acceptors utilized by this subclass. In this process, electron carriers are reduced and are regarded as oxidizers of the substrate. Dehydrogenase enzymes catalyze the reaction with co-enzymes like flavin group, nicotinamide adenine dinucleotide (NAD), flavin mononucleotide (FMN) or nicotinamide adenine dinucleotide phosphate (NADP) (Phale et al., 2019). The dehydrogenase enzyme which is present in all living organisms transports hydrogen atoms from organic transporters to electron-acceptor substances (Dotaniya et al., 2019).

Hydroxylase enzymes have active sites containing metal that can catalyze the reactions (Fitzpatrick., 2000; Di Gennaro., 2011). The metal in the activator role changes the oxidation state of the substrate. Iron metal transfers its electron through electron transfer chain and is used to change the oxidation state (Di Gennaro., 2011). Iron metal has the ability to form OH radicals as well as can transfer oxygen to a compound (Massart & Vercauteren 1959).

Dioxygen molecules can serve as an electron acceptor for oxidases to use in catalyzing processes (Phale et al., 2019). In other words, according to Fetzner and Steiner (2010), oxidases utilise molecular oxygen as an electron acceptor. Oxidases transfer electrons using a variety of substances, including metals and cofactors. These substances consist of amine oxidases, metals based on alcohol or flavin, or both (Martin et al., 2000).

Cytochrome P450 involve using molecular oxygen and utilizing NADH or NADPH as a cofactor producing carbon substrate and oxidized products (Guengerich., 2018). They also use ferredoxin and ferredoxin reductase as a source of electrons for catalytic function.

## Recombinant microbial enzymes produced by different expression systems

Studies have demonstrated effective methods to create various microbial enzymes by diverse microorganisms (Table 3) using a variety of study approaches that have been improved in recent years. Due to its capacity to oxidize a variety of substrates, recombinant laccase has demonstrated considerable promise in the degradation of numerous types of pollutants. Laccase-mediated degradation has been investigated for the treatment of a number of pollutants, including dyes (Kesebir et al., 2021), phenolic compounds, insecticides, and polycyclic aromatic hydrocarbons. Endo-1,4-glucanase-encoding *Aspergillus fumigatus* gene (Afu6g01800) was cloned in the pET-28a (+) vector and expressed in the Rosetta TM (DE3) strain of *E. coli.* The findings of the study demonstrated that the afegl7 enzyme belongs to the GH7 family. The afegl7 gene encodes a protein of 460 amino acids, a CBM1 domain at residues 424–460, and a molecular weight of 52 kDa.

Table 3: Microbial enzymes produced by recombinant DNA technology for enhanced bioremediation.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S.no. | Recombinant enzyme | Host | Origin | Expression vector | Function | References |
| 1. a. | Laccase cot A | *E.coli* DH 5 | *B. subtilis* | pMD18-T | Degradation of artificially produced dye | Guan et al., 2014 |
| 1. b. | Laccase CueO | *P. pastoris GS115* | *E. coli K12* | pHBM905BDM | Decolorization of effluent from textile industry | Ma et al., 2017 |
| 1. c | Cellobiohydrolase | CBH1 | Aspergillus niger | Pichia pastoris | Degradation of pulp, cellulose etc. | Li et al. 2012 |
| 1. d | Endoglucanase | ReEG I | Trichoderma reesei | Pichia Shepherds | Degradation of pulp, cellulose, oat xylan, birch xylan, corn straw | Tao et al. 2019 |
| 1. e | Laccase (Fmb- rL 103) | *E. coli* BL 21 | *B. vallismortis* fmb-103 | pMD19-Tlac103 | Degradation of triphenyl methane dye | Sun et al., 2017 |
| 1. f | Cytochrome P105D1 | *Acinetobacter calcoaceticus* | *Streptomyces griseus* | pSP19g10L | Deterioration of pollutants herbicides | Lamb et al., 2000 |
| 1. g | CYP 153 | *E. coli* | *Acinetobacter sp*. EB104 | pUC18 | Introduction of hydroxyl group (Hydroxylation) of unsubstituted alkanes | Maier et al., 2001 |
| 1. h | Dehydrogenase | *E.coli* | *Pseudomonas putida* S12 | pET28 | Helps in the styrene catabolic pathway by catalysing the conversion of phenyl-acetaldehyde to pheny-lacetic acid. | Crabo et al., 2017 |
| 1. i | Dehydrogenase | *E. coli* | *Azoarcus evansi KB740* | pMal-c2x | Oxidizes the ring cleavage product | Gescher et al., 2006 |
| 1. j | Dehydrogenase | *E. coli* | *Rhodococcus sp*. P14 | pET-32a | Bioremediation of steroids | Ye et al., 2019 |
| 1. k | Dehalogenase | *E. coli* BL21 (DE3) | *Ochrobactrum species* | pET30a-a6 | Degradation of TBBPA (tetra bromo bisphenol A ) | Liang et al., 2019 |

**Conclusion**

Exposure to increasing pollutants degrades the ecosystem even at low concentrations over extended periods of time because they are clearly linked to multiple chronic damages to biotic components. At the right temperature, pH, contact time, and concentration, a particular pollutant substrate is recognised by a particular microbial enzyme, enabling efficient enzymatic transformation into a variety of harmless products through a variety of enzymatic reactions. High-molecular-weight PAHs and poly-halogenated aromatics interact with the cytochrome P450 enzyme's active site and undergo oxidation to create non-toxic compounds. Higher thermo-stability and less substrate specificity Antibiotics, synthetic colours, PAHs, and phenolic contaminants were all converted by laccase’s halotolerant capabilities through the oxidation process. Dehalogenase enzymes have the ability to cleave carbon-halogen bonds either hydrolytically, reductively, or oxygenolytically. By removing halides, halogenated contaminants are successfully converted into a substrate that reduces the amount of chlorinated environment. The enzyme dehydrogenase selectively converted various hydroxyl groups found in synthetic polymers and medium-chain secondary alcohols into analogous aldehydes. The protease enzyme breaks down biomass of keratinous waste, colours, waste produced by marine crustaceans, biodegradable plastics and protein polymers by using the hydrolysis reaction mechanism. Microbial hydrolases break down additives like plasticizers, cyanides, and compounds containing nitrile to produce less dangerous by-products through condensations and alcoholysis reactions. Microbial lipase effectively decomposed the co-polymers, synthetic polyester, and parabens into a variety of biodegradation products due to its substrate specificity and optimum stability. Thus, it can be concluded that using microbial enzymes to biodegrade hazardous organic and inorganic contaminants during bioremediation is a sustainable, safe, effective, and eco-friendly way to restore the damaged environment.

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