Communities of Microbial Enzymes and Biodegradation of Persistent Environmental Pollutants

Oluwafemi Adebayo Oyewole, Muhammed Muhammed Saidu, Abdullahi Dabban Idris, Japhet Gaius Yakubu, and Aisha Bisola Bello

Abstract Enzymes are biocatalysts that potentiate the rate of substrate conversion into products. They are composed of amino acids with one or more polypeptide moieties. Microbial enzymes are the various enzymes of microorganisms' source, which have wide scope of applications in medicine and industries, including the degradation of persistent environmental wastes. Persistent environmental pollutants have become a global environmental and health concern. Owing to the rapid technological advancement and development in industries, large quantities of persistent environmental pollutants are being let out into the ecosystem posing serious threats to living organisms, thereby deteriorating the environment. Several microbial enzymes are widely used in the decomposition of recalcitrant organic and inorganic wastes. Oxidoreductases and hydrolases constitute the major class of microbial enzymes utilized in biodegradation of environmental pollutants; oxygenases, laccases, and peroxidases are the superfamilies of the oxidoreductase class, whereas lipases, cellulases, and proteases constitute the superfamilies of hydrolytic enzymes widely employed for bioremediation. Bioremediation involves the use of enzymes of microbial origin or the whole cell in the breakdown or transformation of environmental pollutants into less toxic or nontoxic products. Polymeric compounds such as polyethylene, polypropylene, polystyrene, polyvinyl chloride (PVC), polyurethane (PUR), and polyethylene terephthalate (PET) have been degraded using microbial enzymes. The biodegradation process is, however, often impeded due to the incapability of microbial enzymes to hydrolyze the functional groups present.

O. A. Oyewole (✉) · M. M. Saidu · J. G. Yakubu
Department of Microbiology, Federal University of Technology Minna, Minna, Nigeria
e-mail: oa.oyewole@futminna.edu.ng; muhammad.m1603943@st.futminna.edu.ng

A. D. Idris · A. B. Bello
Department of Biological Sciences, The Federal Polytechnic Bida Niger State, Bida, Nigeria

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1 Introduction

The conjoined effect of the upsurge in human population and the growing industrial sector has mounted pressure on the limited natural resources (land, air, and water). The environment has not been adequately and judiciously managed and maintained since the industrial and technological development. Therefore, there have been indiscriminate increases in environmental pollution globally as a result of the increasing population, rapid industrialization, and other anthropogenic factors including agricultural practices that are potentially hazardous. These have led to the accumulation of a variety of persistent environmental pollutants such as polymeric compounds, organic wastes, industrial effluents, heavy metal-containing wastes, and other inorganic wastes. These pollutants often contain traces of heavy metals (such as chromium, lead, cadmium, nickel, iron, zinc, copper), petroleum products, pesticides, and various organic compounds such as hydrocarbons, organophosphorus, and phenolic compounds (Megharaj et al., 2011).

Persistent environmental pollutants are highly lipophilic compounds, which are potentially harmful to humans and affect the stability of the ecosystem when they biomagnify through the food chain. The level of environmental quality greatly influences the safety and well-being of every living organism in that environment. Persistent environmental pollutants have become a global environmental and health concern (Guo et al., 2019). Science and technological advancements coupled with the industrial revolution have, however, led to large quantities of recalcitrant pollutants with varying degrees of toxicity including organic and radioactive waste being effluxed into the environment, thereby endangering several life forms and affecting the quality of the environment. An effective means of getting rid of these persistent pollutants is of immense significance (Aislabie et al., 2005; Guo et al., 2019). In ancient times, wastes were usually eradicated from the environment by burying them in soil. Owing to the inadequacy of new places to dump and bury wastes, this technique of waste treatment became obsolete. With the advent of new techniques, the method of waste burying was gradually replaced with techniques like use of elevated temperatures to incinerate waste and chemical decomposition of waste, examples of which include ultraviolet (UV) oxidation and base-catalyzed dechlorination. These technologies, despite their effectiveness at reducing pollutants, have their own drawbacks. They are not environmentally friendly, are uneconomical and complex, and are not generally acceptable. The use of microbial enzymes to bioremediate pollutants is now seen as a suitable alternative to elevated temperature and use of chemicals for waste eradication.

Degradation of environmental pollutants by making use of microorganisms or microbial enzymes has gained increasing recognition over the years as a promising technique for waste removal. Generally, bioremediation approaches of waste removal are environmental transformations, in the form of supply of nutrients (biostimulation) and oxygen (bioventing or biosparging) and introduction of effective degraders (bioaugmentation). The enzymatic degradation of recalcitrant environmental pollutants offers numerous merits and has high preference over the

physical and chemical waste remediation approach, particularly for less toxic and large quantities of wastes. One of the most attractive advantages of the bioremediation strategy is the in situ treatment, which entails treating the contaminated soil at the contamination site without transporting the contaminated material. Ryan et al. (1991) recounted that several reports have been documented on the successful treatment of petroleum-contaminated sites using microbial enzymes. Microbial enzymes have been extensively utilized in the management and treatment of hazardous wastes such as polychlorinated biphenils, trichloroethylene, and benzene, toluene, ethylene, and xylene (BTEX).

2 Biodegradation of Persistent Environmental Pollutants

Biodegradation is the breakdown, disintegration, or transformation of pollutants into nontoxic or less toxic substances by the metabolic or enzymatic action of microorganisms. The use of various microorganisms in the degradation of a wide variety of environmental wastes has been documented (Vidali, 2001; Leung, 2004). The biodegradation process is solely dependent on the enzymatic action of microorganisms to biotransform pollutants into nontoxic or innocuous substances. The effectiveness of bioremediation is a measure of the suitability of environmental and nutritional conditions for the proliferation and activity of microorganisms, and as such oftentimes requires the conditions to be manipulated to achieve a faster rate of pollutant degradation.

Various microorganisms having numerous hereditary determinants such as extrachromosomal DNA and catabolic genes adapt to adverse environmental conditions by genetic recombination, duplication, mutation, and hypermutation. Chakraborty and Das (2016) reported that the microbial metagenome constitutes the largest genetic reservoir with miscellaneous enzymatic activities implicated in biodegradation. Some major persistent environmental pollutants catabolic genes involve in biodegradation include biphenyl dioxygenase for degradation of biphenyl, 2,3-dioxygenase for breaking down of organochlorine pesticides and angular dioxygenase for degradation of dioxins or furans (Chakraborty & Das, 2016). However, bioremediation is a gradual process, with only few strains of bacteria and fungi proven to be potent pollutant degraders, while others could only be effective under laboratory conditions.

2.1 Principles of Biodegradation

Biodegradation involves the disintegration and detoxification of toxic environmental pollutants by the action of living organisms such as plants, animals, and microorganisms (including bacteria, fungi, and algae), which biodegrade or detoxify and transform the toxic environmental pollutants into products such as CO_2, H_2O, and

metabolites, which are mostly innocuous or less toxic than the original compound. During bioremediation, the degrading species can be from the site of contamination or may be introduced from elsewhere to the site for bioremediation through the process of bioaugmentation. Microorganisms obtain energy for their growth and development by degrading or transforming these pollutants through enzymatic action.

The rate of biodegradation is influenced by a complex of interwoven factors, which are population density of the degrading species, environmental conditions, the complexity of the pollutants, and, particularly, the bioavailability of the pollutants to the organisms; therefore, the manipulation of environmental conditions is immensely important to bring about an increase in microbial growth rate and thus fast and effective biodegradation.

2.2 *Limiting Factors of Bioremediation*

Bacterial growth, which ultimately determines the rate of bioremediation, is often limited by various factors such as:

- pH
- Oxygen
- Temperature
- Poor bioavailability of pollutants
- Moisture
- Soil structure
- Inadequate nutrients
- Other bactericidal or bacteriostatic compounds may be present

Microorganisms can thrive in extreme environments; however, a vast majority of bacterial and fungal species prefer optimal conditions of growth. Such a situation is not easily set up and maintained in the field or site (Dua et al., 2002; Dana & Bauder, 2011). The bioremediation process usually takes place under aerobic conditions, although microbial degradation of recalcitrant pollutants can also occur in anaerobic environments. The involvement of various microbial enzymes is extremely crucial for degradation of persistent lignin and organic pollutants by bacteria and fungi, respectively (Vidali, 2001; Lehninger et al., 2004).

2.3 *Microbial Enzymes in Bioremediation*

Enzymes are biochemical substances that catalyze the rate of substrate transformation into products by lowering the energy of activation of the reaction. Enzymes are usually composed of several amino acids; hence, they are proteinous in nature with one or more polypeptide moieties. The catalytic site of an enzyme in which a

reaction takes place is called the active site. A holoenzyme is composed of an apoenzyme (glycoprotein moiety of an enzyme) and a prosthetic group (nonprotein moiety of an enzyme).

Microbial enzymes are enzymes derived from microorganisms, bacterial, algal, or fungal species, with numerous uses in the field of medicine and in several industries. Microbial enzymes are widely used in the degradation of recalcitrant pollutants.

2.4 *Sources of Microbial Enzymes*

The application of microbial enzymes has gained attention over the years due to their versatility, specificity, selectivity (chemo–regio–enantio), and catalysis of diverse reactions. A number of enzymes are produced by various microorganisms that catalyze the degradation of complex natural polymeric compounds into simple ones. Microorganisms producing extracellular enzymes are ubiquitous in nature, and studies have reported their isolation from various environments. Pollutant-degrading strains are widespread in the environment including air, water, soil, sludge, industrial effluents, and also as normal flora in the human body. Several microbial enzymes known to degrade pesticides and hydrocarbons are produced by strains of *Mycobacterium*, *Alcaligenes*, *Sphingomonas*, and *Pseudomonas* amongst other aerobic bacterial species. Lipases, a group of microbial enzymes capable of degrading polyurethane and other recalcitrant pollutants, are produced by various microbes such as *Comamonas acidovorans* TB-35, *Curvularia senegalensis*, *Aureobasidium pullulans*, *Fusarium solani*, *Cladosporium* sp., *Pseudomonas chlororaphis*, *Pseudomonas stutzeri*, and *Pestalotiopsis microspora* (Roohi Kulsoom et al., 2017). Different bacterial and fungal species such as *Bacillus* sp., *Pseudomonas* sp., *Aspergillus nidulans*, *Aspergillus niger*, *Penicillium simplicissimum* YK, and *Enterobacter* sp. produce esterases, a class of hydrolases involved in the breakdown of polyvinyl chloride (PVC) and polyethylene amongst other plastics. The microbial flora, which secrete extracellular enzymes for the degradation of plastics, include *Streptococcus*, *Pseudomonas*, *Staphylococcus*, *Micrococcus*, and *Moraxella* among the bacterial species, and the fungal species are *Aspergillus niger* and *Aspergillus glaucus*, whereas *Actinomycetes* sp. and *Saccharomonos poragenus* constitute the yeast species. Chlorinated aliphatics including di- and trichloroethylene have also been degraded by certain aerobic methylotrophs. Complete degradation of chloroform, PCBs (polychlorinated biphenyls), and chlorinated solvents has been achieved by various extracellular enzymes produced by certain anaerobes (Roohi Kulsoom et al., 2017).

3 Classes of Microbial Enzymes in Bioremediation

The various classes of microbial enzymes include:

3.1 Microbial Oxidoreductases

Oxidoreductases in bacteria and fungi mediate the detoxification of toxic pollutants by oxidizing substrates (Gianfreda et al., 1999). Microorganisms derive carbon and energy by oxidizing the contaminants into completely harmless compounds; this is achieved using oxidoreductases to hydrolyze the linkages so as to aid electron transfer from the donor (usually the reduced substrate) to the recipient (another chemical compound).

The humification of various phenolic compounds obtained from degradation of lignin is catalyzed by oxidoreductases. In addition, various toxic xenobiotic compounds can be detoxified by oxidoreductases; detoxification of phenol or aniline-containing compounds is carried out by polymerizing the compound with other substrates or by linking the compounds to humic substances (Park et al., 2006). Degradation and decolorization of azo dyes by microbial enzymes have also been reported (Vidali, 2001; Husain, 2006). Chlorinated phenolic compounds are recalcitrant pollutants and the major constituents of wastes from the pulp and paper industry. During pulp bleaching process, incomplete breakdown of lignin results in the formation of chlorinated phenolic compounds, and they are removed from the contaminated environment by many fungal species using various extracellular oxidoreductases produced in the mycelium such as laccases, manganese peroxidases, and lignin peroxidases. Rubilar et al. (2008) observed that fungi degrade soil pollutants more readily than do bacteria due to their filamentous nature.

Oxidoreductases have the ability to catalyze the coupling of carbon to carbon and carbon to other elements like oxygen and nitrogen, and, sometimes, coupled with polymerization of carbon units and removal of methyl or halogen group, they have the ability to form reactive radicals that hydrolyze various chemical bonds. These reactions facilitate the purification and transformation of recalcitrant environmental pollutants by enzymes into nontoxic or less toxic substances that can be easily evicted from the environment in subsequent treatment procedures. Various studies have shown the potential applications of oxidoreductases mainly in bioremediation of soil pollutants and industrial wastewater remediation. However, intracellular enzymes commonly produced by most fungi, such as cytochrome P450 monooxygenase, may catalyze degradation of various organic pollutants (Bezalel et al., 1996); white rot fungi produce several ligninases, which are extracellularly active and so are better agents of bioremediation of highly polar pollutants (Field et al., 1993). Therefore, laccases and peroxidases are the main enzymes that have been used, whereas tyrosinases and catechol oxidases have been found to have limited applications in bioremediation.

Microbial Oxygenases

Oxygenases are microbial enzymes belonging to the oxidoreductases class of enzymes. They oxidize the reduced substrate by incorporating an oxygen molecule

using flavin adenine dinucleotide (FAD)/nicotinamide adenine dinucleotide (NADH)/nicotinamide adenine dinucleotide phosphate (NADPH) as the cofactors. They are classified into two based on the number of oxygen atoms used to oxidize the substrate, namely, monooxygenases and dioxygenases. The most studied groups of microbial enzymes used in bioremediation are bacterial mono- and dioxygenases. They play important roles in the breakdown of organic compounds by their increased reactivity, high catalytic efficiency, and water solubility, to bring about the cleavage of the aromatic ring by introducing oxygen atoms into the organic compound. Oxygenases act on diverse group of substrates including chlorinated aliphatics. Oxygenases catalyze the degradation of numerous halogenated compounds, which constitute the largest group of persistent environmental pollutants owing to their widespread use as pesticides, hydraulic and heat transfer fluids, plasticizers, and intermediates for chemical synthesis. Dehalogenation of alkyl halides such as methyl halide, ethyl halide, and halogenated ethylene is catalyzed by oxygenases with conjoined action of multifunctional enzymes (Fetzner & Lingens, 1994).

Microbial Monooxygenases

Monooxygenases oxygenate substrates by adding an oxygen molecule to them. Monooxygenases, owing to their high regioselectivity and stereoselectivity on a broad range of substrates, function as biological catalysts in the bioremediation process. Monooxygenases catalyze various reactions such as removal of sulfur, halogen, and nitrate and incorporation of ammonia and hydroxyl group as well as transformation of degradation of aliphatic and aromatic compounds. Several researches have been carried out on monooxygenases for their high catalytic efficiency in biodegradation of aromatic compounds; the degradation of phenol by monooxygenase is shown in Fig. 1. Of all the monooxygenases, methane monooxygenase is the best and most used monooxygenase. The enzyme catalyzes the transformation and degradation of hydrocarbons such as tetrachloromethanes, alkanes, cycloalkanes, alkenes, haloalkenes, ethers, and aromatic and heterocyclic hydrocarbons (Fox et al., 1990; Grosse et al., 1999). Monooxygenases catalyze the oxidative dehalogenation reactions under aerobic conditions and reductive dechlorination at low oxygen levels.

Monooxygenases are subdivided into flavin-dependent monooxygenases and P450 monooxygenases on the basis of the cofactor present. Flavin is present in flavin-dependent monooxygenases as a prosthetic group, and, so, NADP or NADPH is required as a coenzyme. P450 monooxygenases are found in eukaryotes and prokaryotes and are known to contain heme. Most of the monooxygenases that

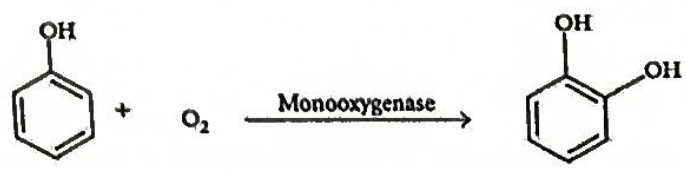

Fig. 1 Degradation of phenol by monooxygenase (Arora et al., 2010)

have been previously identified have a cofactor; however, certain monooxygenases have the unique ability of functioning independently of a cofactor. These monooxygenases only require molecular oxygen for their activities, utilizing the substrate as a reducing agent (Arora et al., 2010). Monooxygenases include a versatile superfamily of enzymes that are involved in the oxidation of a diverse group of substrates ranging from simple hydrocarbons as alkanes to steroids, fatty acids, and other complex molecules.

Microbial Dioxygenases

Dioxygenases oxidize substrates by incorporating oxygen molecule into the substrate. Dioxygenases are a group of Rieske nonheme iron oxygenases. They are involved in the oxidation of a broad range of substrates, mainly aromatic compounds, and, so, they have important application in bioremediation of environmental pollutants. Dioxygenases contain one or two electron transport proteins, which precede their oxygenase components. The presence of the Rieske (2Fe–2S) cluster and mononuclear iron in the crystal structure of naphthalene dioxygenase has been confirmed in each alpha subunit (Dua et al., 2002).

Catechol dioxygenases form part of nature's strategy for degrading aromatic molecules in the environment. These enzymes are present in various soil bacterial species, and they transform aromatic precursors into aliphatic products. The enzymes that cleave intradiol use Fe(III), whereas the enzymes that cleave extradiol make use of Fe(II) and Mn(II), as illustrated in Fig. 2 (Que & Ho, 1996).

Microbial Laccases

Laccases are a group of multicopper oxidase enzymes widely distributed in higher plants, insects, and especially found in various bacterial and fungal species. They catalyze the reduction of oxygen molecules to water and oxidation of reduced phenolic compounds (Gianfreda et al., 1999; Mai et al., 2000). Microbial laccases

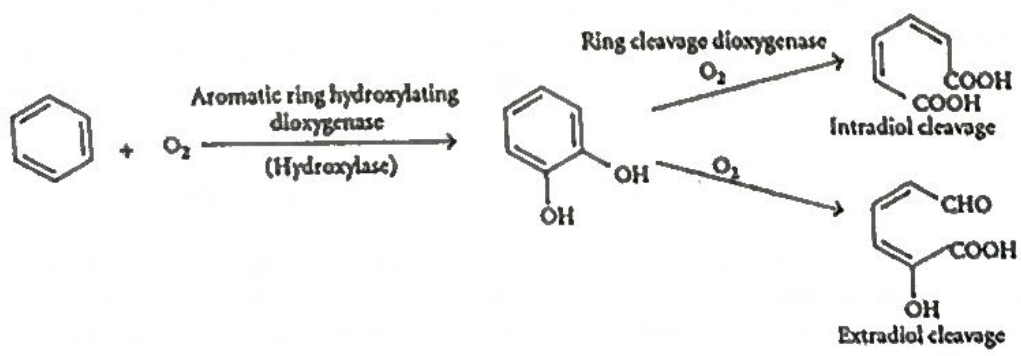

Fig. 2 Degradation of aromatic compounds by dioxygenase (Que & Ho, 1996; Arora et al., 2009)

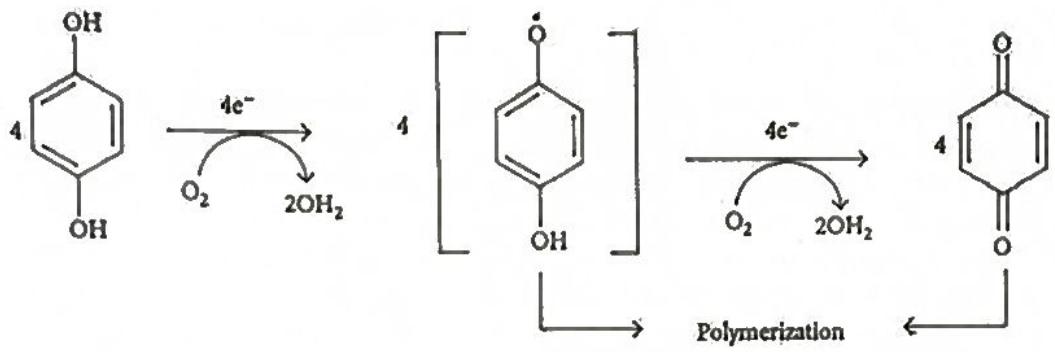

Fig. 3 General reaction mechanism for phenol oxidation by laccase (Dedeyan et al., 2000)

are a glycosylated polyphenol group of oxidoreductases containing four Cu ions in each molecule, which catalyze the oxidation of phenols and other aromatic compounds. Each polymeric laccase molecule has type 1, type 2, and type 3 Cu subunits, of which type 2 and type 3 form a trinuclear Cu cluster. Certain bacterial and fungal species produce numerous intracellular and extracellular laccases, which have the potential to oxidize O- and P-diphenol, aminophenols, polyphenols, polyamines, lignins, and aryl diamines as well as some inorganic ions as depicted in Fig. 3 (Ullah et al., 2000; Couto & Toca Herrera, 2006). Isolation of laccases has been reported from various species of Ascomycetes, Deuteromycetes, and Basidiomycetes fungi having more than 60 fungal strains. Laccases play a vital role in biodegradation of phenolic pollutants and removal of endocrine disruptors (Couto & Toca Herrera, 2006). Laccases are widely used for breaking down lignin into pulp, degradation of insecticides and pesticides, organic synthesis, detoxification of wastes, and transformation of textile dyes. When a laccase oxidizes a compound, a single electron is lost often in the form of a free radical, which may undergo further oxidization or other nonenzymatic reactions such as hydration, disproportionation, and polymerization (Faccelo & Cruz, 2008). Oxidation, decarboxylation, and demethylation of phenolic and methoxyphenolic acids using microbial laccases have been reported. Lignins are also depolymerized by microbial laccases to yield a variety of phenols. Laccases express the unique ability to degrade a broad range of environmental pollutants and offer great potential for bioremediation applications (Gianfreda et al., 1999). The specificity and affinity of laccases to substrates vary with pH. Various reagents are capable of inhibiting laccase enzymes, such as halides (with the exception of iodide), azides, cyanides, and hydroxides (Xu, 1996).

Properties of Microbial Laccases

Laccases are glycoproteins composed of one, two, or numerous monomers. Glycosylation enhances the ability of laccases to retain copper, remain unchanged with temperature variation, and be susceptible to degradation and secretion. Laccases show considerable heterogeneity upon purification. The growth medium composition determines the glycoprotein composition and glycosylation content.

Sources of Microbial Laccases

The major sources of laccases are higher plants and fungi; however, various laccases have been recently isolated from different bacterial species, particularly *Streptomyces* sp. and *Marinomonas mediterranea* (Arias et al., 2003; Jimenez-Juarez et al., 2005). Fungi, however, have been found to have more laccases than higher plants. Various Basidiomycetes like *Phanerochaete chrysosporium*, *Theiophora terrestris*, and *Lenzites betulina* (Viswanath et al., 2008) and other species of white rot fungi (Kiiskinen et al., 2004a, b) such as *Phlebia radiata* (Niku-Paavola et al., 1998), *Pleurotus ostreatus* (Palmieri et al., 2000), and *Trametes versicolour* (Bourbonnais et al., 1995) have shown laccase activity. Laccases have also been isolated from many species of *Trichoderma* including *Trichoderma atroviride*, *Trichoderma harzianum* (Holker et al., 2002), and *Trichoderma longibrachiatum* (Velazquez-Cedéno et al., 2004). Laccase isolated from a species of Ascomycetes (*Monocillium indicum*) was the first laccase enzyme to be studied and characterized, which showed peroxidase activity (Thakker et al., 1992). Laccase produced by *Pycnoporus cinnabarinus* has lignin-degrading potential, whereas laccase isolated from *Pycnoporus sanguineus* has the ability to oxidize phenolic compounds (Pointing & Vrijmoed, 2000). Contrary to the role of laccase in plants, which catalyzes lignification, laccase in fungi plays various roles in lignin breakdown, formation of spores, pigment and fruiting body, and causing of various plant diseases (Yaver et al., 2001).

Mechanism of Microbial Laccase Activity

Laccases catalyze reactions by reducing an oxygen molecule to water and oxidizing an electron with a broad range of aromatic compounds including polyphenols, methoxyphenols, and aromatic amines. Laccases have copper atoms depicted as Cu T1 (to which the substrate binds) and a trinuclear copper cluster T2/T3 (shuttling of electron between the three Cu ions that results in oxygen reduction to water) (Gianfreda et al., 1999). The Cu ions are grouped into three types: Type 1 (T1), Type 2 (T2), and Type 3 (T3). Electron paramagnetic resonance (EPR) spectroscopy and UV light are commonly used to differentiate between the three types. A trinuclear center is formed by Type 2 and Type 3, which catalyzes various reaction mechanisms. Asymmetric activation of the trinuclear center is brought about by the binding of the oxygen molecule to prevent the oxidizing agents from binding. The catalytic activity of laccase involves the reduction of oxygen in a steady state. Laccase serves as a power house that stores electrons for oxidation reactions to bring about reduction of the oxygen molecule. Therefore, for the complete reduction of molecular oxygen to water to occur, four reducing substrate molecules must be oxidized by laccase enzymes. Free radicals are generated when laccase oxidizes the substrate.

Catalysis of the substrate mediated by laccase is applied to non-phenolic compounds with the addition of a mediator. A mediator is an organic compound with low molecular weight oxidized by laccase. The highly active cation radicals oxidize the

non-phenolic compounds that laccase alone cannot oxidize. 1-Hydroxy benzotriazole (HOBT), *N*-hydroxyphthalimide (NHPI), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS), and 3-hydroxyanthranilic acid are the most common synthetic mediators (Gochev & Krastanov, 2007).

Production of Microbial Laccases

Laccases are produced extracellularly by several species of fungi during secondary metabolism, with the exception of Zygosaccharomyces and Chytridiomycetes (Morozova et al., 2007). Some species of soil and freshwater Ascomycetes have been reported to produce laccase (Junghanns et al., 2005). Moreover, laccase has been isolated from various organisms including *Gaeumannomyces graminis*, *Magnaporthe grisea*, *Melanocarpus albomyces*, *Monocillium indicum*, *Neurospora crassa*, *Ophiostoma novo-ulmi*, and *Podospora anserina* (Iyer & Chattoo, 2003; Palonen et al., 2003). A dimethoxyphenol oxidizing enzyme produced by *Botryosphaeria* is a true laccase. In plant biomass decay, syringaldazine is oxidized by the laccase-producing Ascomycetes species (Lyons et al., 2003). Phenols and aminophenols are oxidized by laccase produced by a Basidiomycetes yeast (*Cryptococcus neoformans*), although tyrosine is unaffected. *Saccharomyces cerevisiae* produces oxidase with a membrane-bound multicopper plasma, which is homologous to fungal laccase (Stoj & Kosman, 2003). Studies have shown that the fungal species known for producing appreciable amounts of laccases in varying quantities are Basidiomycetes and saprotrophic fungi (Hatakka, 2001). Laccase is also produced by *Pycnoporus cinnabarinus* as the only ligninase that breaks down lignin (Eggert et al., 1996). Although the laccase-producing capacity of brown rot fungi remains largely unknown and no laccase undergoes purification, research has shown that *Coniophora puteana* oxidizes the syringaldazine (Lee et al., 2004) and assists in oxidizing ABTS in *Laetiporus sulphureus* (Schlosser & Hofer, 2002). Gayazov and Rodakiewicz-Nowak (1996) concluded that laccase production is usually influenced by a number of factors, which include the cultivation type (either submerged or solid-state), limiting factor, and carbon and nitrogen source.

Microbial Peroxidases

Peroxidases belong to a subclass of oxidoreductases that catalyzes the reduction of peroxide compounds such as hydrogen peroxide (H_2O_2) and also oxidize several organic as well as inorganic compounds. Peroxidases are a widely available group of enzymes derived from numerous sources, which bring about degradation of lignin and several phenolic compounds by reducing the hydrogen peroxide in the presence of a mediator. Toxic compounds such as ferricyanide and ascorbate are also degraded by peroxidases to yield nontoxic compounds by donating electrons that bind to the substrate (Hamid & Rehman, 2009). Peroxidases are either heme or nonheme proteins. Peroxidases also regulate hormone and immune responses in

mammals. Peroxidases are widely used to reduce pollutants, including remediation of industrial effluents heavily laden with phenols, cresols, and chlorinated phenols, and also in degrading and decolorizing synthetic dyes. Extracellular peroxidase secreted by white rot fungi catalyzes the degradation of lignin by the unspecific free radical, which brings about oxidation reactions (Lundell et al., 2010). Peroxidases oxidize various compounds including amines, dimethoxybenzene, lignin, phenols, and several other aromatic alcohols without the presence of a mediator (Mn(II)). Phenolic as well as non-phenolic compounds are also oxidized by peroxidases; a dye-decolorizing peroxidase from *Agaricus* was found to oxidize dyes and phenolic compounds (Hofrichter et al., 2010). Studies have shown great potential of *Phanerochaete chrysosporium* to degrade a broad range of pollutants (including dioxins, PCBs, hydrocarbon compounds, industrial effluents, pesticides, and trinitrotoluene commonly used in making munitions), which is attributed to various peroxidases, which are nonspecific in activity (Marco-Urrea & Reddy, 2012). Heme peroxidases are broadly classified into two categories; the first category is found only in animals, plants, fungi, and prokaryotes, whereas the second category has three subclasses based on sequence comparison. Class I includes intracellular enzymes like yeast cytochrome c peroxidase, ascorbate peroxidase from plants, and catalase peroxidase from bacterial duplicated genes. Class II consists of peroxidases secreted by fungi such as lignin peroxidase (LiP) and manganese peroxidase (MnP) by *Phanerochaete chrysosporium* and *Coprinus cinereus* peroxidase or *Arthromyces ramosus* peroxidase (ARP). Class II peroxidases are responsible for lignin degradation in wood. Class III includes peroxidases of plant origin including from horseradish (HRP), barley, or soybean. These peroxidases are biosynthetic enzymes that catalyze various processes in plants such as plant cell wall formation and lignin formation (Hiner et al., 2002; Koua et al., 2009).

There is no evolutionary link between nonheme peroxidases, so they constitute five broad independent families, which include thiol peroxidases, alkylhydroperoxidases, nonheme haloperoxidases, manganese catalases, and NADH peroxidase. The largest of the five families is thiol peroxidase, which is further subdivided into two subfamilies, glutathione peroxidases and peroxiredoxins (Koua et al., 2009).

Sources of Microbial Peroxidases

Peroxidases are ubiquitous and are obtained from numerous sources including a variety of plants, animals, and microorganisms. Microbial peroxidases are isolated from bacteria, cyanobacteria, fungi, actinomycetes, and yeasts. The predominant peroxidase-producing bacterial species include *Bacillus* sp., *Pseudomonas* sp., and *Citrobacter* sp., whereas *Phanerochaete chrysosporium*, *Candida krusei*, and *Coprinopsis cinerea* are the main peroxidase-producing fungi, *Streptomyces* sp. and *Thermobifida fusca* constitute the *Actinomycetes* species, and cyanobacteria include various species of *Anabaena*. Yeasts are used in the biomineralization of pollutants, decolorization of dyes, feed production, as bioindicators, and are also

used extensively as raw materials in the food, chemical, paper, and pulp industries for degradation of lignin, decolorization of textile dyes, and remediation of sewage.

Properties of Microbial Peroxidases

Peroxidases are oxidoreductases involved in the catalysis of various reactions, particularly peroxide reduction and also the oxidation of several organic as well as inorganic compounds. They are heme proteins with a prosthetic group containing iron (III) protoporphyrin IX. Peroxidases include various specific enzymes such as NADH peroxidase, glutathione peroxidase, and iodine peroxidase and other nonspecific enzymes.

Subclasses of Microbial Peroxidases

Microbial peroxidases are subdivided into many types based of their source and activity. The most studied microbial peroxidases include lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), and versatile peroxidase (VP), attributed to their high catalytic ability and ubiquity.

Microbial Lignin Peroxidases

Lignin peroxidases (LiPs) are heme peroxidases that are produced by *Phanerochaete chrysosporium* during secondary metabolism. LiPs act on lignin along with several phenolic substrates in the presence of hydrogen peroxide as a cosubstrate and veratryl alcohol as the mediator. During the process, reduction of hydrogen peroxide to water occurs when it accepts an electron from LiP while the LiP gets oxidized. The LiP thereafter accepts an electron from veratryl alcohol and gets reduced to its original form, whereas veratryl alcohol forms veratryl aldehyde. Finally, the veratryl aldehyde accepts an electron from the substrate and gets reduced to veratryl alcohol. The overall process yields oxidized halogenated phenolic compounds or polycyclic or other aromatic compounds accompanied by a series of nonenzymatic reactions (Yoshida, 1998; Ten Have & Teunissen, 2001).

LiPs play an important role in the breakdown of the lignin in the plant cell wall. Aromatic compounds are also oxidized by lignin peroxidases having redox potentials higher than 1.4 V normal hydrogen electrode (NHE) by single-electron abstraction, although the exact mechanism of the redox reaction is not well understood (Piontek et al., 2001).

Microbial Manganese Peroxidases

Manganese peroxidases (MnPs) are extracellular heme peroxidases secreted by the lignin-degrading Basidiomycetes species of fungi. They oxidize the manganate (II) ion [Mn^{2+}] to manganate (III) ion [Mn^{3+}] in a series of reactions. Manganese peroxidase production is triggered by the manganate (II) ion, which acts as the substrate for manganese peroxidase. The manganate (III) ion formed as a result of oxidation of the manganate (II) ion mediates the oxidization of a variety of phenolic compounds (Ten Have & Teunissen, 2001).

Microbial Versatile Peroxidases

Versatile peroxidases (VPs) are enzymes that can directly oxidize manganate (II) ion, methoxybenzenes, phenolic aromatic substrates similar to lignin peroxidase, manganese peroxidase, and horseradish peroxidase, but, unlike other peroxidases, versatile peroxidases are capable of oxidizing these substrates without manganese as the mediator. Versatile peroxidase has exceptionally broad substrate specificity and is found to catalyze the oxidation of phenolic as well as non-phenolic compounds (Ruiz-Duenas et al., 2007). As such, large-scale production of versatile peroxidases is required in industrial processes for various biotechnological applications and biodegradation of recalcitrant wastes (Tsukihara et al., 2006; Wong, 2009).

Applications of Peroxidases in Degradation of Environmental Pollutants

Biodegradation of Synthetic Dyes

Synthetic dyes constitute a problematic and recalcitrant class of environmental pollutants, which are not easily degraded (Ong et al., 2011). Dyes have important applications in the textile, paper and pulp, and petroleum industries and in color photography, amongst others. These compounds greatly contribute to environmental pollution when released into industrial effluents. The white rot fungus is considered a valuable alternative to biodegradation of environmentally hazardous compounds. Oxidative enzymes such as laccase, LiP, and MnP produced by *Phanerochaete chrysosporium* are found to oxidize the substrates. Peroxidases and oxidases are used as efficient oxidizing agents, which degrade dyes. Synthetic textile dyes have been completely decolorized using several bacterial peroxidases. *Brevibacterium* has been used to completely remove chromate Cr (VI) and azo dye Acid Orange 7 (AO7) under nutrient-limiting conditions. The reducing enzyme of *Brevibacterium casei* uses the AO7 as an electron donor to reduce Cr (VI). A purple color complex intermediate is formed by the oxidized AO7 and the reduced Cr (III) (Ng et al., 2010). Studies have shown the unique ability of *Phanerochaete chrysosporium* RP 78 to decolorize several azo dyes under optimal conditions (Ghasemi et al., 2010). Dawkar et al. (2008) observed that the *Bacillus* sp. VUS from soil contaminated with

textile effluents expressed the potential to degrade various dyes. Several other peroxidases have been isolated from microorganisms in addition to LiPs that biodegrade synthetic dyes. The decolorization of Remazol Brilliant Blue was achieved by the extracellular peroxidase isolated from *Pleurotus ostreatus* along with other synthetic dyes including triarylmethane, heterocyclic azo, and polymeric dyes. The decolorization of bromophenol blue is best at 98%, and methylene blue as well as toluidine blue O both decolorize best at 10%.

Bioremediation of Wastewater

Industrial pollution is a major global concern being one of the principal sources of environmental pollutants, which deteriorate surface and groundwater and affect the general well-being of the environment. Pure water serves as a deterrent to infectious agents, which are the causative agents of numerous waterborne diseases. Microbial peroxidases have been effectively used to bioremediate wastewater containing various aromatic compounds (Hamid & Rehman, 2009; Ong et al., 2011). The major constituents of wastewater of several industries include phenols, aromatic amines, and other aromatic compounds (Kaušpediene et al., 2010).

Peroxidases are a class of oxidoreductase enzymes involved in detoxification of a wide range of phenolic compounds through oxidative coupling reactions (Mui et al., 2010). *Phanerochaete chrysosporium* produces a lignin peroxidase, *Bjerkandera adusta* produces lactoperoxidase, a versatile peroxidase, and *Caldariomyces fumago* produces chloroperoxidase that catalyzes the oxidative dehalogenation of pentachlorophenol to tetrachloro-1,4-benzoquinone in the presence of H_2O_2. *Pleurotus eryngii* and *P. ostreatus* produce another versatile peroxidase that oxidizes Mn^{2+} into Mn^{3+}, in a similar manner as MnP, and like the action of LiP, it oxidizes the high redox potential of aromatic compounds and has broad specificity and also oxidizes non-phenolic compounds (Ruiz-Duenas et al., 2009).

Mechanism of the Horseradish–H_2O_2–PhOH Reaction

The reaction of horseradish peroxidase with phenolic compounds follows a cyclic path. The summary of the reactions is given as:

$$E + H_2O_2 \rightarrow Ei + H_2O, \tag{1}$$

$$Ei + PhOH' \rightarrow Eii + PhO, \tag{2}$$

$$Eii + PhOH'' \rightarrow E + PhO + H_2O. \tag{3}$$

The reaction begins with the original form of the enzyme (E), and, thereafter, the enzyme gets oxidized by H_2O_2 to yield compound A (Ei), an active intermediate compound. Compound A then oxidizes one phenol molecule (PhOH) to form its free radical (PhO), and, then, compound A becomes compound B (Eii). The second phenol molecule is further oxidized by compound B to yield another free radical of

phenol (PhO). The enzyme returns to its original state (E) to complete the cycle. The free radicals (PhO) are polymerized to yield an insoluble precipitate (Mossallam et al., 2009). The equation is given as:

$$\text{PhO} + \text{PhO} \rightarrow \text{Aromatic polymer.} \quad (4)$$

Biodegradation of Polycyclic Aromatic Hydrocarbon (PAH) Pesticides

Pesticides include all classes of synthetic chemical compounds employed in the control of pest organisms such as insects (insecticides), rodents (rodenticides), birds (avicides), weeds (herbicides), and fungi (fungicides). Several health malignancies are associated with exposure to different pesticides, which include, but are not limited to, memory disorders, dermatological conditions, respiratory disorders, neurological deficits, cancer, depression, miscarriages, and birth defects (McCauley et al., 2006). Biodegradation of pesticides using microorganisms or their enzymes is seen as the most promising approach to eliminate the toxic products of pesticides amassed in the environment. Microorganisms bring about the structural change and degradation of these compounds in the environment by physically and chemically interacting with the substrates. Fungal peroxidases have been used to effectively detoxify various pesticides into innoxious compounds. Studies have shown the great potential of *P. chrysosporium* to transform organophosphorus pesticides (Jauregui et al., 2003), and the enzyme chloroperoxidase from *Caldariomyces fumago* has also been found to biotransform organophosphorus pesticides. Polycyclic aromatic hydrocarbons are acted upon by phenol oxidases and peroxidases to reduce them to simpler and less toxic forms that can easily be degraded. Peroxidases such as LiPs and MnPs catalyze the oxidation of polycyclic aromatic hydrocarbons (Harford-Cross et al., 2000).

Biodegradation of Chlorinated Alkanes and Alkenes

Chlorinated alkenes such as trichloroethylene (TCE) and perchloroethylene (PCE) widely utilized as degreasing solvents have contributed significantly to soil and aquifer contamination, thereby posing severe threat to human health. The LiP of *P. chrysosporium* catalyzes the in vitro reductive dehalogenation of TCE to yield its corresponding chlorinated radicals in the presence of hydrogen peroxide, tertiary alcohol, and oxalate (or ethylenediaminetetraacetic acid (EDTA)) (Yadav et al., 2000). An imazethapyr (IMZT)-degrading strain of bacterium IM-4 was isolated from the soil heavily laden with IMZT. In addition to imazethapyr, several imidazolinone herbicides including imazapic, imazapyr, and imazamox are also degraded by the strain (Huang et al., 2009). *Tinea versicolor*, which produces an extracellular hydroxyl radical through quinone redox cycling, also has the ability to reduce PCE and TCE (Marco-Urrea et al., 2009). *P. chrysosporium* cultures grown under aerobic conditions are capable of mineralizing TCE.

Biodegradation of Phenoxyalkanoid Acid and Triazine Herbicides

Herbicides are generally used in agricultural settings around the world, and the most widely used herbicides include 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyaceticacid). Agent Orange used by the US forces as defoliants in the Vietnam War contains 2,4-D and 2,4,5-T. 2,4-D is readily eradicated from the environment since it are easily degraded by bacterial species. On the contrary, 2,4,5-T is difficult to biodegrade by microorganisms, and, so, it persists more in the environment. Serious illnesses were attributed to 2,4,5-T during the Vietnam War for their exposure to Agent Orange. They are extremely toxic to humans and are most often considered as mutagenic agents. *P. chrysosporium* and *Dichomitus squalens* produce ligninolytic peroxidases that are involved in detoxification of 2,4-D- and 2,4,5-T-chlorinated phenolic intermediates. Laccases and peroxidases produced by *P. chrysosporium* were reported to biodegrade atrazine, which is a commonly used triazine herbicide (Bending et al., 2002).

Biodegradation of Chlorinated Dioxins

Polychlorinated dibenzodioxins are highly toxic environmental pollutants that are carcinogenic in nature and are found bioaccumulating in humans and animals as a result of their lipophilic properties. Various species of white rot fungi have been used to degrade chlorinated compounds like polychlorinated dibenzodioxins and polychlorinated dibenzofurans, indicating the activity of LiP and MnP (Kasai et al., 2010). Dioxins have also been effectively degraded using MnP produced by *Phanerochaete sordida*.

Biodegradation of Chlorinated Insecticides

Lindane was extensively used as an insecticide in the past century with a global production of approximately 600,000 tons between the year 1950 and 2000. Lindane has been banned globally, owing to its high resistance to degradation leading to its persistence. Under ligninolytic conditions, lindane is partially mineralized by *P. chrysosporium* in a broth medium and in corncob-amended soils (Quintero et al., 2008). However, degradation of lindane has not been extensively studied in vitro using LiP and MnP from *P. chrysosporium*. Excessive use of dichlorodiphenyltrichloroethane (DDT) (the first of the chlorinated organic insecticides) was reported after World War II. Heavy contamination of agricultural soils with DDT poses severe threats to the safety of food and to human health. DDT has been found to be susceptible to attacks by *P. chrysosporium*, *P. ostreatus*, *T. versicolor*, and *Phellinus weirii*.

3.2 *Microbial Hydrolytic Enzymes*

Soil and water pollution by industrial effluents having ample amount of hydrocarbons and heavy metals is a weighty problem of the modern world. These compounds constitute the major contaminants of aquatic and terrestrial environments, as a result of their extensive use. The technique of eliminating them by the use of microorganisms or microbial enzymes is seen as a safe and cost-effective approach. The action of bacteria on the pollutants is the major process of breakdown of organic pollutants. Vasileva-Tonkova and Galabova (2003) reported that the activity of extracellular enzymes is the basic step in the biodegradation of these organic compounds.

Hydrolases constitute the Class III enzymes and are further subdivided based on the type of bond they hydrolyze. The chemical bonds in toxic pollutants are cleaved by the action of hydrolytic enzymes, which ultimately reduce their toxicity. Oil spills and insecticides (carbamate and organophosphate) are degraded by cleavage of bonds in the compounds. Hydrolases are also involved in condensations and alcoholysis amongst several other related reactions. Hydrolase enzymes have numerous advantages such as ubiquity, lack of cofactor stereoselectivity, and tolerance to water-miscible solvents. Hydrolytic enzymes such as lipases, cellulases, proteases, and amylases amongst other extracellular enzymes are widely used in various industries including the food and beverage industry, as feed additives, and in the pharmaceutical and chemical industry (Sanchez-Porro et al., 2003). Important enzymes such as hemicellulase, cellulase, and glycosidase are widely used in biomass degradation (Schmidt, 2006). The various microbial hydrolytic enzymes in bioremediation include microbial lipases, microbial cellulases, and microbial proteases.

Microbial Lipases

Lipase enzymes degrade lipids. Lipase is found in bacteria, actinomycetes, and in plant and animal cells, but, of all these, microbial lipase is the most versatile and widely used for industrial applications. Sharma et al. (2011) stressed the involvement of lipase in a series of reactions including hydrolysis, esterification, interesterification, aminolysis, and solvolysis with alcohol. Lipases are widespread and are obtained from numerous sources; they are involved in the breakdown of triacylglycerols to yield fatty acids and glycerol. Lipases are responsible for the depletion of hydrocarbon compounds in contaminated soils, and, as such, lipase is considered the most effective indicator parameter for measuring the rate of degradation of hydrocarbons in soil (Riffaldi et al., 2006). Lipase is not only used in assessing the rate of bioremediation but also has several potent industrial applications such as in the food processing industry, chemical and detergent manufacturing, cosmetic formulation, and the paper and pulp industry; however, its industrial uses are limited by the cost of its production (Joseph et al., 2006; Sharma et al., 2011).

Sources of Microbial Lipases

Lipases have widespread occurrence in a variety of plants and animals; however, they are more abundant in microbial flora comprising bacteria, fungi, and yeasts. Lipases of microbial origin have gained much attention, and they have the most biotechnological applications among various classes of microbial enzymes, as lipase is produced by numerous microbial strains. Prominent lipase-producing microorganisms include *Candida* sp., *Pseudomonas* sp., and *Rhizopus* sp. *Candida rugosa* is the most frequently used organism for lipase synthesis. The commonly adopted in situ techniques for lipase synthesis are submerged and solid-state fermentations. Various microorganisms including filamentous fungi, yeasts, and bacteria have been extensively studied for the production of lipases.

Filamentous Fungi

The production of lipase by filamentous fungi is influenced by various factors such as the strains, growth medium composition, and conditions including temperature, pH, oxygen content, and source of carbon and nitrogen (Cihangir & Sarikaya, 2004). Commercially important lipase-producing fungi include strains such as *Rhizopus*, *Aspergillus*, *Penicillium*, *Geotrichum*, *Mucor*, and *Rhizomucor*.

Yeasts

Vakhlu and Kour (2006) concluded that the main terrestrial lipase-producing yeasts include the species of *Candida*, *Rhodotorula*, *Pichia*, and *Yarrowia lipolytica*. Wang et al. (2007) reported the cloning and overexpression of the lipase-coding genes in the species of *Candida*, *Geotrichum*, *Trichosporon*, and *Y. lipolytica*. There is widespread use of lipase produced from different strains of *C. rugosa* and *Candida antarctica*; however, recent research has shown the potential of other yeasts to produce lipases. It has been recently discovered that the contamination of freshly produced olive oil with a rich microflora has resulted in the synthesis of enzymes that regulate the organoleptic and physicochemical attributes of the oil (Ciafardini et al., 2006). Strains including those of *Saccharomyces cerevisiae*, *Candida wickerhamii*, *Candida boidinii*, and *Williopsis californica*, in addition to other strains of yeasts, were identified among the microorganisms isolated from the contaminated oil, with *S. cerevisiae* and *W. californica* reported as effective lipase-producing strains. Lipase production in *S. cerevisiae* was intracellular, whereas extracellular lipase activity was found in *W. californica*.

Bacteria

Several bacterial species have been explored of which the *Bacillus* species expressed the extraordinary ability of lipase production, making them immensely recommended strains for biotechnological applications. Prominent lipase-producing Bacilli include *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus licheniformis*, *Bacillus coagulans*, and *Bacillus alcalophilus*. Other species of bacteria capable of producing lipase enzymes are *Pseudomonas* sp., *Burkholderia* sp., and *Staphylococcus* sp.

Ertugrul et al. (2007) studied 17 different strains of bacteria that can utilize tributyrin in a tributyrin medium and concluded that the best lipase-producing strain was a *Bacillus* strain.

Microbial Cellulases

Cellulose is a complex carbohydrate with numerous industrial applications such as in the manufacture of textiles, paper, explosives, and pharmaceutical products and therefore contributes to the economic development worldwide. Raw materials are largely composed of celluloses, which constitute more than 94% of cotton and more than 50% of wood. Cellulose is extensively used as a major raw material in numerous industries; however, abundant quantities of cellulosic materials are yet to be well explored or used more efficiently. Agricultural wastes, industrial effluents, and biosolids have been reported to contain substantial quantities of cellulose, which serve as a carbon source for anaerobic degradation of biosolids to yield methane. Celluloses in combination with other complex carbohydrate polymers such as hemicellulose and lignin along with traces of extractives constitute the cellulosic biomass.

The conversion of cellulose waste material into food by cellulases to meet the demands of the growing population has become a field of research interest (Bennet et al., 2002). Cellulose is hydrolyzed by a variety of microorganisms using a multienzyme system. Some microorganisms secrete cellulases that are cell-bound, associated with envelope or extracellular cellulases. Some bacterial and fungal species express low levels of extracellular cellulases, hemicellulases, and pectinases (Adriano-Anaya et al., 2005). Several enzymes usually make up the cellulase class of enzymes, with at least three distinct groups of cellulases catalyzing any hydrolysis reaction:

1. Endoglucanase (endo-1,4-D-glucanohydrolase) hydrolyzes cellulose from the region of low crystallinity, resulting in free chain ends.
2. Exoglucanase (1,4-β-D-glucan cellobiohydrolase) acts by extracting the cellobiose units from various free chain ends to further hydrolyze cellulose.
3. β-Glucosidase acts along with other enzymes to produce several glucose units by breaking down cellobiose.

Sun and Cheng (2002) reported that cellulose is degraded by cellulase enzyme to simple sugar, which is further acted upon by bacteria and/or yeasts to yield ethanol. Crystalline cellulose is also degraded by cellulase enzymes to glucose.

Sources of Microbial Cellulases

Fungi are the major source of microbial cellulase; however, cellulase has been isolated from few bacterial and actinomycete species. Cellulase-producing microorganisms generally degrade carbohydrates but lack the ability to break down proteins and lipids to obtain energy for growth and development (Lynd et al., 2002). The bacterial species *Cellulomonas* and *Cytophaga* and most other fungal species amongst the cellulolytic microbes have the ability to degrade other carbohydrates besides cellulose; however, cellulolytic anaerobes are found to utilize only cellulose and products of cellulose hydrolysis. Certain fungal strains produce a variety of extracellular cellulases attributed to the large amounts of extracellular proteins they secrete. *Trichoderma reesei* has been extensively studied and was found to hydrolyze both natural and synthetic celluloses to glucose. Prominent cellulose-degrading microorganisms include *Aspergillus* sp., *Humicola* sp., *Penicillium* sp., *Trichoderma* sp., *Phanerochaete chrysosporium*, *Fusarium solani*, and *Talaromyces emersonii* amongst the fungal species, whereas the bacterial species are *Cellulomonas* sp., *Cellvibrio* sp., *Microbispora bispora*, and *Thermomonospora* sp. amongst the aerobes and *Acetivibrio cellulolyticus*, *Bacteroides cellulosolvens*, *Bacteroides succinogenes*, *Clostridium thermocellum*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* constitute the anaerobic cellulolytic bacteria. Cellulose is metabolized by numerous fungi for energy; however, the cellulase–enzyme complex required for hydrolyzing cellulose is produced only by few strains. Certain species of fungi including *Aspergillus* sp., *Penicillium* sp., *Humicola* sp., and *T. reesei* are capable of yielding substantial quantities of extracellular cellulases. Cellulolytic aerobic bacteria such as *Cellulomonas* and *Cytophaga* have the ability to degrade cellulose in pure cultures (Lynd et al., 2002). However, the microorganisms that are commercially used for the extraction of cellulases are mainly *T. reesei*, *Humicola insolens*, *A. niger*, *Thermomonospora fusca*, and the species of *Bacillus*. Saranraj et al. (2012) recounted that several researches has been conducted on bioremediation of organic wastes using bacteria, actinomycetes, and fungi (including *Trichoderma* sp., *Penicillium* sp., and *Aspergillus* sp.) to synthesize cellulase enzymes.

Mechanism of Cellulase Activity

Microorganisms bring about the degradation of cellulose by making use of several microbial enzymes, which constitute the multienzyme complex. These organisms include bacterial and fungal species consisting of aerobic and anaerobic and mesophilic and thermophilic organisms inhabiting different environments. A number of extracellular enzymes are secreted by aerobic bacteria, with unique binding

sites to accommodate different conformations of cellulose. Cellulosome, an extracellular multienzyme complex composed of several enzymes, is produced by anaerobic bacteria. The activity of the single components of the multienzyme complex is stimulated toward the crystalline substrate following its binding to a noncatalytic structural protein (scaffolding). *Clostridium thermocellum*, which is a thermophilic bacterium, is the most complex and best investigated cellulosome.

Cellulase enzymes are found to degrade filter paper and other natural celluloses as well as synthesized celluloses like carboxymethyl or hydroxyethyl cellulose. Cellulases attack cellulose at 1,4-β linkages, whereas lignin and cereal are at β-D-glucans. The newly generated chain ends of cellulose are acted upon by exoglucanases to mainly produce cellobioses, which are repeating units of disaccharides with glucose units joined with a 1,4-β linkage, whereas β-glucosidase acts on cellulose from the ends by cleaving the terminal β-D-glucose residues. Cellulose usually occurs alongside other components like hemicellulose, lignin, and pectin, which are degraded by cellulase enzymes. Several D-glucose units present in polysaccharides linked by 1,4-α-D-glucosidic linkages are hydrolyzed by amylases, whereas the 1,4-α-D-galactosiduronic linkages in galacturans are randomly broken down by pectinases. Environmental pollutants containing lignocellulosic materials are degraded by a variety of cellulases (Saranraj et al., 2012).

Microbial Proteases

Microbial proteases constitute a group of microbial enzymes that cleave peptide linkages in aqueous solution and catalyze the synthesis of peptide linkages in a nonaqueous medium. Proteases catalyze the hydrolysis of peptide bonds in substances high in protein content, which are often released into the air by shedding and molting of feathers and other appendages, decay of dead animals, and as by-products of various industries including the textile and food industry. Singh (2003) and Beena and Geevarghese (2010) recounted that proteases are widely used in several industries including the food, textile, detergent, and pharmaceutical industry. Proteases are divided, on the basis of their catalytic action on peptide bonds, into endopeptidases and exopeptidases. Endopeptidases are further subdivided, on the basis of the position of the active site, into metallopeptidases and serine, cysteine, and aspartic endopeptidases.

Sources of Microbial Proteases

Proteases are widely available in a variety of plants, animals, and microorganisms; however, protease from a microbial source has more preference over other sources due to its easy accessibility, cost-effectiveness, and large-scale production as a result of fast reproducibility of microorganisms and easy manipulation for generation of recombinant enzymes with unique properties. Kumar and Takagi (1999) affirmed that the two-third share of global production of commercial proteases in the enzyme

market is of microbial origin. Microorganisms degrade proteins and utilize the products for growth and development. Proteinases (endopeptidases) produced by various species of microorganisms initiate the degradation process, and peptidases (exopeptidases) catalyze further hydrolysis at various locations within and between cells. Different varieties of microbial proteases are produced by various species of an organism and also by different strains of the same species. The most common class of microbial proteases, produced by all groups of microorganisms (bacteria, fungi, yeast, and actinomycetes), are the alkaline serine proteases.

Fungal proteases: Proteases of fungal origin are of great interest to researchers due to their specificity to a wide range of substrates, high diversity, and thermostable nature. A vast array of fungal proteases produced by various species of fungi, such as *Aspergillus* sp., *Chrysosporium keratinophilum*, *Conidiobolus coronatus*, *Entomophthora coronata*, *Fusarium eumartii*, *Paecilomyces lilacinus*, *Scedosporium apiospermum*, *Rhizopus oligosporus*, *Cephalosporium* sp. KSM 388, and *Tritirachium album Limber*, has been studied (Veloorvalappil et al., 2013). Separation of the mycelium can be achieved by simple filtration, making it extremely advantageous.

Bacterial proteases: Bacterial proteases have extensive uses in various industries, such as the food, textile, and pharmaceutical industries, and are generally utilized in biodegradation of protein-containing wastes as a result of their large production scale and catalytic efficiency. The maximum catalytic efficiency of bacterial protease is achieved at a high pH level (8–12) with an optimum temperature of 50–70 °C. These unique features of bacterial proteases make them more suitable for biodegradation of pollutants. Prolific sources of microbial enzymes include several bacterial species including *Alteromonas* sp., *Mycobacterium* sp., *Pseudomonas* sp., *Streptomyces* sp., *Thermoactinomyces* sp., *Arthrobacter protophormiae*, *Lactobacillus helveticus*, *Xanthomonas maltophilia*, *Vibrio alginolyticus*, *Brevibacterium linens*, *Staphylothermus marinus*, and *Salinivibrio* sp. strain AF-2004.

4 Microbial Degradation of Plastics

Biodegradation of polymeric substances (polyethylene, polypropylene, polystyrene, polyvinyl chloride) is greatly impeded due to the absence of hydrolyzable functional groups in their backbone (Restrepo-Florez et al., 2014; Krueger et al., 2015). The decomposition and mass reduction of polymeric compounds is initiated by the conjoined action of microorganisms and climatic factors including temperature, humidity, rainfall, pressure, and other physical factors (Eubeler et al., 2010; Restrepo-Florez et al., 2014). Koutny et al. (2006a, b) and Fontanella et al. (2010) observed that the effects of UV irradiation and oxidizing agents result in the formation of carbonyl groups, which are easily accessible for further microbial attacks.

Numerous researches on biodegradation of the various types of polyethylene have been conducted in the past few decades (Restrepo-Florez et al., 2014; Sen &

Raut, 2015). Ligninases, which degrade lignin (a complex noncarbohydrate aromatic compound) in the cell wall of plants (Suhas et al., 2007), have been found to degrade polyethylene (Restrepo-Florez et al., 2014; Krueger et al., 2015); however, there is difficulty in attaining a complete and efficient degradation, since the degradation of lignin requires a lower redox potential compared to the homologous covalent linkages in the backbones of polyethylene (Krueger et al., 2015). Examples of such microbial enzymes are lignin peroxidases, manganese peroxidases, and laccases.

UV-irradiated polyethylene films in cell extracts as well as culture supernatants were degraded by a thermostable laccase produced by *Rhodococcus ruber* C208 in the presence of copper (Santo et al., 2013). Similarly, the molecular weight of a polyethylene membrane according to Fujisawa et al. (2001) has been effectively reduced by laccase produced from *Trametes versicolor* in the presence of 1-hydroxybenzotriazole, which acted as a cofactor in the reaction.

4.1 Microbial Degradation of Polyurethane

Polyurethane (PUR) is a polymeric substance containing urethane (carbamate) linkages between adjacent polyols and di- or polyisocyanate (Seymour & Kauffman, 1992). Polyurethanes are classed as either polyether polyurethanes or polyester polyurethanes depending on polyol (which is the amorphous part of the compound composed of a polyether or polyester) used for polycondensation reactions (Urgun-Demirtas et al., 2007). Loredo-Trevino et al. (2012) and Cregut et al. (2013) asserted that various microbial enzymes capable of hydrolyzing urethane linkages to depolymerize polyurethane include microbial ureases, esterases, and proteases. A variety of enzymes have been isolated from bacteria (Howard et al., 2012) and fungi (Russell et al., 2011) with the potential to degrade polyester polyurethane. Several studies have shown that carbamate and amide linkages are hydrolyzable by proteases and ureases that cleave the urea linkages (Matsumiya et al., 2010). The major enzymatic depolymerization of polyester polyurethane is the cleavage of the ester bonds by esterases and proteases (Howard, 2002). Christenson et al. (2006) reported that the urethane bonds present in polyether polyurethane may be hydrolyzed by hydrolases from bacteria and fungi; however, the class of polyurethane is much more resistant to enzymatic degradation than polyester polyurethane.

4.2 Microbial Degradation of Polyethylene Terephthalate

Polyethylene terephthalate (PET) is a polymeric substance made from polymerization of ester-linked terephthalic acid (an aromatic dicarboxylic acid) and ethylene glycol (Webb et al., 2013). The production of polyethylene terephthalate according to Research and Markets (2015) had surpassed 41.6 million tones worldwide as of

2014, and it is widely used in manufacturing beverage bottles, as packaging materials, and in the textile industry. Polyethylene terephthalate is highly durable and resistant to microbial degradation due to the repeating units of aromatic terephthalate in its backbone (Marten et al., 2003, 2005). The polyethylene terephthalate polymer is semicrystalline in nature (partially crystalline and partially amorphous); this also contributes to its resistance to degradation.

5 Conclusions

Environmental pollutants, which have become a serious global concern, are treated either by various physicochemical procedures or by enzymatic degradation. Application of microbial enzymes in degradation of persistent pollutants is more effective, efficient, economical, and eco-friendly and thus is the more acceptable and preferable approach to elimination of environmental pollutants. Microbial enzymes used to efficiently biodegrade persistent environmental pollutants include oxygenases, laccases, and peroxidases amongst the oxidoreductases, whereas the hydrolytic enzymes are lipases, cellulases, and proteases. Although enzymatic degradation is slow, it ensures complete depletion of conversion of pollutants into less harmful products. However, with the advent of recombinant DNA technology, microorganisms can easily by manipulated to produce enzymes with a broad spectrum of activity that will catalyze the depletion or conversion of persistent environmental pollutants into value-added products at less time.

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