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Understanding the functional attributes of different microbial enzymes in bioremediation

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Abstract

Bioremediation uses biological organisms and their metabolic processes in order to degrade contaminants present in water, soil etc. Microbes have the vast potential are the major resource for bioprocess of using microbial enzymes reduces the toxicity of pollutants caused by the waste materials like pesticides, insecticides, plastics, other hydrocarbon-containing substances and obtain novel useful substances for mankind and the environment. Enzymes produced by bacteria, fungi, plants play a key role in the biodegradation of toxic organic compounds. The purpose of bioremediation processes that will an eco-friendly and cost-effective mechanism. The aim is to develop an advanced technique in bioprocesses that will help to minimize toxin risk and thereby acquire new, usable substances. Some of the bioremediation-related compounds like oxidoreductases hydrolases, dioxygenase, peroxidases, and laccase are most widely considered. The aim of the review is to express the role of microbial enzymes on the bioremediation of toxic, hazardous environmental pollutants.

Keywords: Bioremediation; Environment; Enzymes; Hydrocarbons; Pollutants;

Introduction

Microorganisms are widely distributed in the biosphere due to their metabolic activity is very impressive and they can grow in any nutritional condition. The enzyme plays a very important role in the sustainability of all life forms (Abatenh et al., 2017). Living organisms produce enzymes that act as a catalyst in chemical reactions. The term 'Enzyme' was first coined by Wilhelm Friedrich Kühne (Sheehan and Himmel, 1999). Nowadays human population, urbanization and industrialization are increasing that are associated with constantly elevating pollution levels (Kekkonen, 2017). One important issue is a huge amount of waste water generated from dairy, food industries, oil refinery, poultry house and wool processing factories (Kumar et al., 2019; Ara et al., 2019; Singh et al., 2019). Microorganisms act as a significant pollutant removal in soil, water, sediments (Bajaj and Singh, 2015). Enzymes are of great importance in the development of industrial bioprocesses as they play a crucial role as metabolic catalysts (Singh et al., 2019). The majority of currently used industrial enzymes are hydrolytic in action that is used for the degradation of various natural substances (Kirk et al., 2002). Other enzymes include carbohydrates, primarily amylases, proteases, lipases, proteases, xylenes and celluloses used in various industries such as starch, textile, detergent, baking and food industry (Gurung et al., 2013; Tripathi et al., 2020).

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Microbial enzymes have been extensively used for the biodegradation of pollutants including both organic and inorganic pollutants. This process is known as bioremediation that uses metabolic processes of biological organisms in order to degrade contaminants so that they remain no longer in harmful form (Canak et al., 2018). Instead of simply collecting the pollutant and storing it, bioremediation is a well-organized procedural activity that is applied to break down or transform to less toxic or non-toxic elemental and compound forms (Abatenh et al., 2017). Bioremediation involves utilization of biological agents to clear the contaminated or polluted sites (Sharma, 2019). A study showed that bacteria, fungi and archaea are the prime organisms that are used as a biochemical agent in the bioremediation process (Abatenh et al., 2019). Both bacteria and fungi rely on the participation of different extracellular and intracellular enzymes respectively for the process of bioremediation (Karigar and Rao, 2011). Utilizing microbial enzymes is an eco-friendly, least harmful and cheaper method to remove toxic products from the environment (Sharma et al., 2019). An attempt has been made in this review to highlight the different categories of microbial enzymes and their role in bioremediation.

Types of microbial enzymes

Enzymes are biocatalysts that carry out chemical reactions and depending upon the type of reaction these are classified into six different categories like oxidoreductase, transferase, hydrolases, lyases, ligases and isomerases. Each above of the following enzyme has been discussed in the following section.

Oxidoreductases

Oxidoreductase is a large family of enzymes that catalysed the biological oxidation-reduction processes. Various bacteria, fungi and higher plants carry out detoxification of toxic organic compounds through oxidative coupling with help of these oxidoreductases. As a result, heat or energy is generated and oxidoreductase will bring about degradation of pollutants. The heat is utilized by microorganisms for their metabolic activity (Medina et al., 2017). Microbes extract energy via energy-yielding biochemical reactions mediated by oxidoreductase and break the chemical bond that assists the transfer of electrons from a donor to acceptor. During the oxidation-reduction process the contaminations are finally oxidized into harmless compound. Oxidoreductase have been utilized in the bioremediation of numerous natural and anthropogenic pollutants. Oxidoreductase detoxifies various synthetic organics such as phenolic, azo rings, and aniline substances which are the essential for xenobiotics or soil environment.

A gram-positive bacteria *Bacillus safensis* CFA-06 produces oxidoreductase to degrade the petroleum compounds (Fonseca et al., 2015). After lignin degradation various harmful phenolic compounds are released in the environments which are degraded by oxidoreductases through polymerization and co-polymerization (Husain, 2006). Colour compounds produced from textile

industries are released into the environment and are degraded by various

Table 1. List of microbial enzymes with source and their usage in different bioremediation processes

S. No.	Enzyme	Source organism	Application	Reference
1	Oxidoreductase	*Bacillus safenis*	Used for bioremediation of contaminated soil, xenobiotics, decolorization and degradation	Husain, 2006; Bansal And Kanwar, 2013;
2	Monooxygenease	*Bacillus megaterium* BM3	Degrade hydrocarbons like substitute methanes, alkanes, haloalkanes and aromatic heterocyclic hydrocarbon	Roccatano, 2015
3	Diooxygenease	*Pseudomonas putida* F1	Degrade aromatic compounds into aliphatic products	Mukherjee and Roy, 2013
4	Laccase	*Rhizoctonia praticola, Trametes hispida, Bacillus vallismortis fmb103, Pleurotus ostreatus*	Depolymerization of lignin to an array of phenols and degradation of bisphenol A	Dodar et al., 2004; Rodriguez et al., 1999; Legerska et al., 2016; Strong and Claus, 2011
5	Peroxidase	*Escherichia coli* *Bacillus* sp, *Pseudomonas* sp, *Thanatephorus* sp, *Auricularia* sp, *Pleurotus ostreatus*	Degrade lignin and oxidises manganese, methoxybenzenes, and phenolic aromatic substrate	Bansal and Kanwar, 2013; Abdel-Hamid et al., 2013
6	Lignin peroxidase	*Phanerochaete chrysosporium, Trametes versicolor*	Oxidise various compounds and biodegrade plant cell wall constitute lignin.	Xuet al., 2014; Abdel-Hamid et al., 2013; Behbahani et al., 2016; Piontek et al., 2001
7	Manganese Peroxidase	*Trametes sp, Peniophora incarnata*	Oxidise lignin and other compounds	Zhanga et al., 2016; Lee et al., 2016
8	Versatile Peroxidase	*Pleurotus eryngii*	Oxidized both phenolic and non-phenolic lignin model dimers	Koch et al., 2017
9	Hydrolase	*Microbacterium* sp	Bioremediation of pesticides, insecticides	Karigar & Rao, 2011; Lei et al., 2017
10	Lipase	*Pseudomonas aeruginosa,*	Degrade cooking waste	Verma et al., 2012; Sharma et al., 2011

11	Cellulase	*Clostridium,Cellulomonas, Thermomonospora, Trichoderma, Aspergillus, Humicola*	Convert waste cellulosic material into food, paper and pulp industry	Kuhad et al., 2011; Hmad and Gargouri, 2017
12	Protease	*Aspergillus* sp, *Bacillus licheniformis, Bacillus* sp	Hydrolyze peptide bonds	Pandey et al., 2017; Tripathi et al., 2020

oxidoreductase enzyme (Novotny et al., 2004).

Oxygenases

Oxygenase belongs to the oxidoreductase group of enzymes. They play a key role in the aerobic degradation of aromatic compounds, catalyses the cleavage of the ring in aromatic compounds by adding one or two molecules of oxygen. On the basis of the number of oxygen atoms are used they are grouped into two categories the monooxygenase and dioxygenase. One of the bacterial microbe *Pseudomonas* sp. LBr produces glyphosate oxidase (GOX) which is involved in the bioremediation of pesticides. GOX converts glyphosate into amino methyl phosphonate (AMPA) and releases the keto acid glyoxylate (Scott et al., 2008). Some marine bacteria also produce oxygenase for the degradation of organic pollutants (Sivaperumal et al., 2017).

Monooxygenase

Monooxygenases incorporate one atom of the oxygen molecule into the substrate in the metabolic pathways. Monooxygenase are classified into two types based on the presence of cofactor: flavin dependent monooxygenase and P450 monooxygenase. Flavin dependent monooxygenase used for the degradation of chlorine containing pesticides like endosulfan (Bajaj et al., 2010). P450 monooxygenase isolated from the bacterium *Bacillus megaterium* BM3 has the capacity to degrade a variety of substrates such as fatty acid and aromatic compounds (Roccatano, 2015). Monooxygenase act as biocatalysts in bioremediation process due to their highly regioselectivity and stereoselectivity on wide range of substates. Monooxygenase carry out desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, biotransformation and biodegradation of various aromatic and aliphatic compounds (Arora et al., 2010).

Dioxygenase

Dioxygenases are multicomponent enzymes systems that introduce molecular oxygen into their substrate. Dioxygenases are primarily oxidizing aromatic compounds. They are the key enzymes in pathways for the bacterial degradation of aromatic hydrocarbons. On the basis of their mode of action, they are classified into (1) aromatic ring hydroxylation dioxygenases (ARHDs) (2) aromatic ring cleavage dioxygenases (ARCDs) (Parales and Ju, 2011). Toluene dioxygenase (TOD) produced by *Pseudomonas putida* F1 catalyses the degradation of toluene (Mukherjee and Roy, 2013). The catechol dioxygenases are found in the soil bacteria causes biotransformation of aromatic and

aliphatic products (Muthukamalam et al., 2017). Various aromatic compounds released into the environment from different industries. Dioxygenase breaks down the aromatic ring at 1 and 2 position (Guzik et al., 2013). Naphthalene dioxygenase isolated from *Pseudomonas putida* involve the naphthalene degradation (Gennaro et al., 1997).

Laccases

Laccases are copper containing oxidases produced by certain plants, fungi, insects, and bacteria, catalyses the oxidation of a wide range of reduced phenolic and aromatic substrates followed by reduction of molecular oxygen to water. It is found in multiple isoforms and is found both inside and outside of the cell (Mai et al., 2000). Laccase isolated from fungus *Trametes hispida*is able to be decolorize azo dyes by oxidizing their bonds and transform into less harmful substances present in the environment (Rodriguez et al., 1999; Legerska et al., 2016). Laccase produced by *R. praticola* have the ability to degrade and biotransform phenolic compounds (Strong and Claus, 2011). Laccase isolated from fungus *Trametes versicolor* is a powerful enzyme for the bioremediation of a wide range of pollutants like phenolic and aromatic compounds (Chakroun et al., 2010).

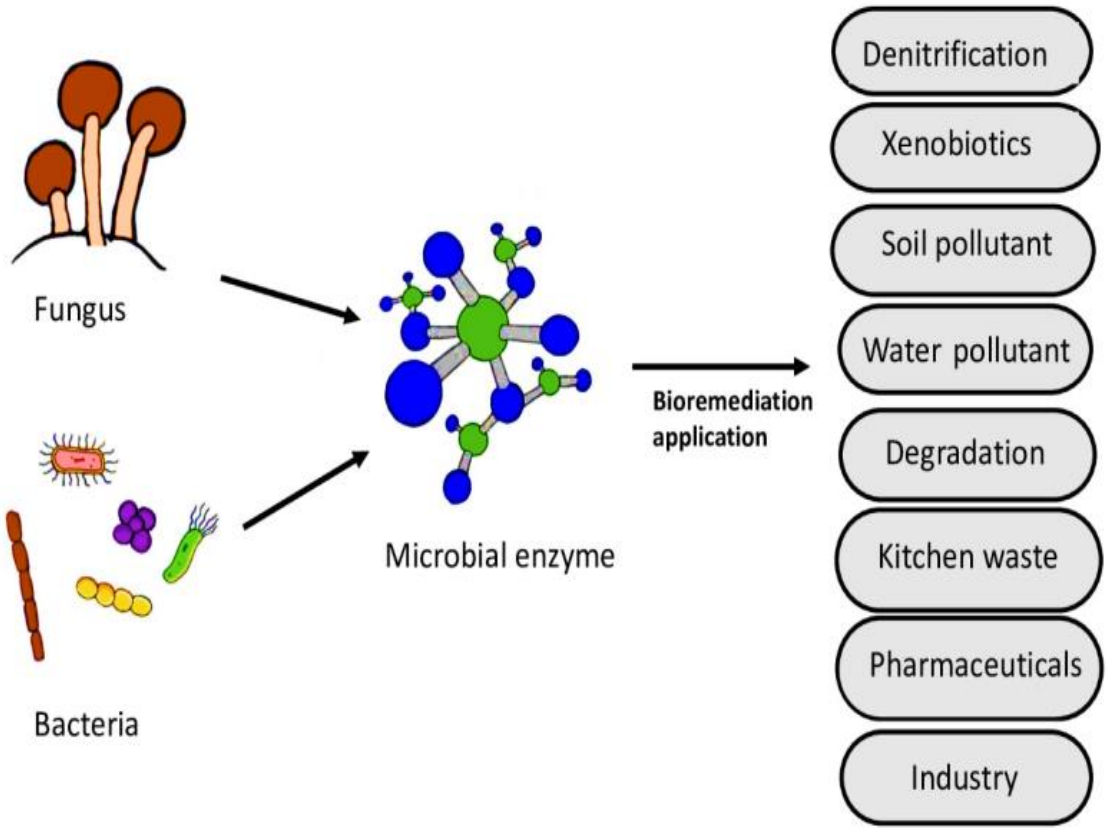

Fig 1 Microbial enzymes and their application in different aspects of bioremediation

Peroxidase

Peroxidase plays a key role in the degradation of lignin and other aromatic compounds by using hydrogen peroxide and a mediator produced by animals, plants, fungi and bacteria. They are ubiquitous in nature and can be heme or non-heme proteins (Bansal and Kanwar, 2013). The heme–containing peroxidases can be divided into two groups: one group found in animals and other group found in fungi, bacteria and plants. Peroxidases have the potential to decrease water pollution by bioremediation of phenols, cresol and chlorinated phenolic compounds in wastewater. Soybean peroxidase and chloroperoxidase have been examined for the degradation for the thiazole compounds (Sharma et al., 2018).

Among the bacterial strains, *Escherichia coli, Bacillus sp., Pseudomonas sp.* are predominant peroxidase producers. In fungi, it is found in *Thanatephorus sp, Auricularia sp, Pleurotus ostreatus.* Among peroxidases, lignin peroxidase (LiP), manganese–dependent peroxidase (MnP) and versatile peroxidase (VP) due to their high potential to degrade toxic substances in nature (Abdel-Hamid et al., 2013).

Lignin peroxidase

Lignin peroxidase (LiP) are heme proteins secreted by fungi such as *Phanerochaete chrysosporium*, *Trametes versicolor* and bacteria (Xuet al., 2014). Lignin shows a great application for the treatment of water and in the field of bioremediation (Abdel-Hamid et al., 2013). LiP degrades lignin and other phenolic compounds. It also oxidizes halogenated phenolic compounds, polycyclic aromatic compounds, and other aromatic compounds followed by a series of nonenzymatic reaction. LiP plays a central role in the biodegradation of the plant cell wall constitute lignin. Lignin degradation by bacterial peroxidase is more efficient as compared to fungal peroxidases on the basis of specificity and thermostability (Behbahani et al., 2016). It is also able to oxidize aromatic compounds with higher redox potential (Piontek et al., 2001).

Manganese peroxidase

Manganese peroxidase (MnP) is a hydrogen peroxidase dependent enzyme, but it can only oxidize organics when in the presence of MN (II). It is generally found in basidiomycetes fungus. MnP oxidizes Mn (II) to Mn (III), which acts as an obligatory oxidation intermediate for the oxidation of various compounds. The Mn (II) ions migrate away from the enzyme and start the oxidation of the lignin and other compounds. These catalyses the degradation of several phenols, amine containing aromatic compounds, and dyes (Ten Have and Teunissen, 2001). MnPTra-48424 was identified and purified from white rot fungi *Trametes sp*. 48424. This enzyme has strong capability to decolorize different kinds of dyes such as indigo, anthraquinone, azo and triphenylmethane, while other dyes such as indigo carmine and methyl green combined with heavy metal ions and organic solvent (Zhanga et al., 2016) During the degradation of anthracene, gene (pimp1) encoding manganese-dependent peroxidase was found in *P. incarnata* KUC8836. This gene was further expressed in fungi *Saccharomyces cerevisiae* to enhance the bioremediation process (Lee et al., 2016). Immobilization of MnP was also done with chitosan beads activated by glutaraldehyde

show a greater potential for decolorization of dye effluent from the textile industry (Bilal and Asgher, 2016).

Versatile peroxidase

Versatile peroxidase (VP) enzymes are able to directly oxidize MN^{2+}, methoxybenzenes, phenolic aromatic substances like MnP and LiP. Versatile peroxidase has a significant broad substrate specificity and the tendency to oxidize substrates in the absence of manganese as compared to the other phenolic substances. It is able to oxidize both phenolic and nonphenolic lignin model dimers. Because of its high productivity it is often used for bioremediation (Pinto et al., 2020).

Hydrolytic enzymes

Hydrolytic enzymes are most commonly used for bioremediation of pesticides and insecticides to reduce their toxicity. In order to reduce toxicity, hydrolytic enzymes break chemical bonds between toxic molecules. The oil spill, organophosphate, and carbamate insecticides are easily degraded by this process. The degradation of toxic organic compound through bioremediation is safe and economical compared to physico-chemical process (Karigar & Rao, 2011). It also catalyzes condensation and alcoholysis. The main advantage of this enzyme is its availability, non-selectiveness and good tolerability. Extracellular hydrolytic enzymes including lipases, DNases, amylase, protease, xylanases have many applications in food industry, chemical industries biochemical sciences and feed additive. The hemicellulose, cellulase and glycosidase are especially active in biomass degradation (Porro et al., 2003). Hydrolyzing enzyme gene was isolated from *Microbacterium sp*. djl -6F which is then cloned into *Escherichia coli* BL21 (DE3). It was observed that these enzymes able to hydrolyze carbenazim a widely used fungicide (Lei et al., 2017).

Lipases

Lipases are ubiquitous in nature and have been extracted from bacteria, plant, actinomycetes, and animal cells. Microbial lipases are more versatile because of their potent application in industries. These enzymes catalyses various reactions such as hydrolysis, inter-esterification, esterification, alcoholysis and aminolysis (Prasad & Manjunath, 2011). It helps in the drastic reduction of organic pollutants present in the contaminated soil. Lipase hydrolyses the fatty acids into triglycerol, diglycerol, nonglycerol, and glycerol. (Ghafil et al., 2016). Lipase activity is responsible for the most useful indicator parameter for testing hydrocarbon degradation in soil. An oil degrading lipase have been isolated from fungus *Pseudomonas aeruginosa* SL-72 which then further used for the bioremediation of crude oils (Verma et al., 2012). Along with its usage in bioremediation, lipase has many potential applications in food, chemicals, detergent manufacturing, cosmetics and paper industry however, its production is costly (Sharma et al., 2011).

Cellulase

Cellulases are the most abundant biopolymer found on the Earth. Cellulase enzymes are capable of degrading crystalline cellulose to glucose (Sharma et al., 2017). Cellulases are inducible enzymes synthesized by a large diversity of microorganisms including both fungi

and bacteria during their growth on cellulosic materials (Ma et al., 2013; Quintanilla et al., 2015). Cellulases produced by microorganisms can be cell-bound, associated with cell envelope or extracellular (Yang et al., 2016). These microorganisms can be aerobic, anaerobic, mesophilic, or thermophilic. Among them, the genera of *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma*, and *Aspergillus* are the most extensively studied cellulose producers (Kuhad et al., 2011). Cellulases are usually a mixture of several enzymes and three major groups of cellulases such as endoglucanase, exoglucanase or cellobiohydrolase and β-glucosidase are involved in the hydrolysis process. Some alkaline cellulases are produced by *Bacillus* strains and neutral and acidic cellulases by *Trichoderma* and *Humicola* fungi (Hmad and Gargouri, 2017). These cellulases have been employed for the bioremediation of ink in paper and pulp industry during recycling of paper (Karigar and Rao, 2011). Cellulases produced by *Humicola* species is highly adaptive for harsh environmental conditions such as high pH and temperature and can be used in detergents and washing powders industry for the breakdown of hydrogen bond (Imran et al., 2016)

Protease

Proteases constitute a very large and complex group of enzymes that hydrolyzes peptide bonds in aqueous environment and synthesize them in non-aqueous environment (Pandey et al., 2017). These are commonly used in the detergent and pharmaceutical industries, followed by the food industry (Tripathi et al., 2020). The major sources of protease enzymes are Animals, plants and microorganisms (both fungal and bacterial). Proteases are classified into two groups: endopeptidases and exopeptidases on the basis of pH, substrate specificity, similarity to well characterized enzymes, and the active site amino acid the site of action on polypeptide chains (Raveendran et al., 2018; Tavano, 2017). It has been reported that 29 *Bacillus* species and 17 fungal species produces alkaline protease (Jisha et al., 2013). Commercial producers of alkaline proteases include protein engineered *Bacillus licheniformis*, alkalophilic *Bacillus sp, and Aspergillus sp*. (Ellaiah et al., 2002).

Conclusion

Pollution of soil and water from agricultural chemicals and synthetic hydrocarbons is a major issue of concern in the World. Because of their widespread use, they have now been marked as serious environmental pollutants in a variety of marine and terrestrial ecosystems. To Bioremediation is eco-friendly for the clearance of all such harmful substances from the environment in a natural way. Enzymes present in microorganisms have proven to be the most efficient in this bioremediation process as they help nature to rejuvenate, utilizing the existing substances and manipulating it according to need. Another advantage of using microbial enzymes is that it does not create any hazardous by-products, which usually occurs while using non-biological systems. Therefore, isolation and identification of these microbial enzymes is great field of research for the biotechnologists all over the globe and will help in making the planet pollution free in a sustainable way.

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