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# Role of Microbial Enzymes in Bioremediation of Pollutants : A Review

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**Abstract**

Emerging pollution is growing more and more due to the indiscriminate and frequently deliberate release of hazardous, harmful substances. Significant destructive impacts of pollutants are perinatal disorders, mortality, respiratory disorders, allergy, cancer, cardiovascular and mental disorders, and other harmful effects. Conventional methods for removing pollutants are not efficient; instead, they lead to the secondary contamination. The significant degradation of pollutants can be upgraded by using biological treatment methods such as bioremediation which is cost effective and nature friendly technology. In the bioremediation process, fungi or bacteria and their enzymes are used to clean and purify pollution. Microbial enzymes released by these microbial bioremediator are used to neutralize pollutants into less harmful products. Some enzymes effectively used in bioremediation are hydrolyses, oxidoreductase, transferase, lyases have been extensively studied. Among all other techniques, these microbial enzymes have been found to be effective in degrading and transforming pollutants into novel useful substances. Thus, microbial enzymes serves a great role in solving the problem of pollution in environment.

**Keywords:** Bioremediation, Microbial enzyme, Pollutants, Oxidoreductse, Hydrolyase

## Introduction

Nowdays the world is facing the problem of pollution in environment like air pollution, water pollution and soil pollution, etc. These severe epidemics are occurring due to many anthropogenic activities like industrialisation, overpopulation, modern agricultural practices. The large array of pollutants causing pollution having different structure and toxicity are hazardous for our earth as well as the living beings. These pollutants have created teratogenic, carcinogenic, mutagenic and toxic effect on living beings. Environmental pollutants are of two types organic and inorganic pollutants; organic pollutants include pesticides, polycyclic hydrocarbons, DDT, hexa-chlorobenzene. Modern agricultural activities are one of the main sources of these organic pollutants (Rhind SM, 2012)

Several methodology has been applied for the remediation of pollution like electrochemical treatment, adsorption of pollutants and membrane filtration but these methods for removing pollutants are not as efficient as they end up forming secondary contaminants and also they are complex and an economical. The drawbacks in these convention methods have focused towards cost effective, natural friendly technology which detoxify and decontaminate the pollutants in effective way and making them harmless.

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Techniques are improving as greater knowledge and experience have gained and there is no doubt that bioremediation has great potential for dealing with certain types of site contamination. Bioremediation is one amongst the pollution management technology that uses some biological system like bacteria, fungi and algae to degrade noxious chemicals into less harmful forms (Karigar & Rao, 2011)

Bioremediation is also termed as biodegradation which involves the microorganism for removing dangers of many pollutants. Microorganisms like bacteria and archaea and fungi are more advantageous than any other remediation process as they restore the natural surroundings and prevent further pollution (K Mackova, 2005)

For bioremediation to be effective microorganisms must enter enzymatically attack the pollutant. Enzymes of the bioremediators play the most crucial role. Enzymes can treat different types of organic and inorganic pollutants as they have been recognised to be competent to transform pollutants at a detectable rate. This article highlights the major enzymes analogous with the bioremediation.

**Introduction to enzymes**

Enzymes are biological catalyst that help the process of converting substrate into product. It make the condition favourable by reducing the activation energy required. Enzyme is a chain of protein or maybe glycoproteins. An enzyme consists of many regions that facilitate the catalytic reaction called as active sites. The active site make either covalent or non covalent bond with the substrate to catalyse the process. The group of chains formed of proteins and glycoprotein are known as apoenzyme. While the other non protein part is the prosthetic groups which together with apoenzyme from main component holoenzyme.

Moreover enzymes have several advantages as they can catalyse large range of different compounds and may also function for those compounds for which no other technologies have been devised. It is the best part of enzymatic activity that they are not inhibited by the inhibitors and can also function in stressful conditions. They are even effective at low concentration of pollutants which can be easily detected.

Enzymes used in bioremediation could be either in isolated form or in the cell itself. The enzyme have high mobility than micro-organisms because of their small size (Gianfreda, Bollag, 2012)

But if whole microorganism is used in bioremediation, the inoculation and nutrition for them become mandatory. So the use of individual enzymes have more benefits including higher mortality, good specificity, high activity even in toxic condition and also biodegradability (Eibes, Ramos, 2015). All these advantages render enzymatic bioremediation as eco-friendly as well as environmental friendly technology.

As claimed by Alcade et.al, 2006, biocatalysis by enzyme (very often term as White biotechnology) fully participates in the "Green Chemistry" concept. Enzymes are the most efficient biodegradation tools as they facilitate all chemical changes on pollutants. The specificity of enzymes is much broad to act on different compounds having similar structure. The enzymes are much stable and efficient for extreme conditions and also recognise the particular substrate.

**Microbial enzymes useful in bioremediation**

The microbial enzymes present in the micro-organism used in bioremediation process disrupt the chemical bond of toxic harmful molecules and resulting in reduction of toxicity. Enzymes used as extracellular for biodegradation plays a crucial role than the cell itself. They efficiently utilise the organic polymers since the compound having molecular weight less than 600 daltons can pass through the cell force (Tonkova, 2003)

**Table 1: Properties and applications of various microbial enzymes from different microorganisms.**

| S. No. | Enzymes | Source of Enzyme | Application | Substrate |
|---|---|---|---|---|
| 1. | Laccase | *Pseudomonas putida $F_6$* | Degradation of Synthetic dyes | Syringaldazine (SGZ) |
| | | *Streptomyces cyaneus* | Oxidation of micropollutants as BPA, DFC, MFA | 2,2’ Azino-bis(3, ethylbenzothiazoline, 6-sulphonic acid; ABTS |
| | | *Geobacillus thermocatenulatus* | Decolourization of textile dyes | ABTS |
| 2. | Cytochrome P 450 | *Rhodococcus rhodochorous* | Degradation of RDX | Hexahydro-1,3,5, trinitro-1,3,5, triazine |
| | | *Bacillus megaterium* | Hydroxylation of PCDDs | Polychloro-dibenzo-p-dioxin (PCDD) |
| | | *Mycobacterium* | Biodegradation of Morpholine | |
| 3. | Amylase | *Bacillus/ Geobacillus* | Starch liquefaction | Diethylethanolamine (DEAE) |
| 4. | Lipase | *Bacillus subtilis* | Bioremediation of waste water | Olive oil |
| | | *Bacillus pumilus* | Degradation of palm oil containing industrial waste water | Palm oil |
| 5. | Dehydrogenase | *Pseudomonas putida* | Catabolism of 2,4-xylenol | 4- Hydroxybenzaldehyde |
| | | *S. rhizophila* | Polyvinyl alcohol degradation | Polyvinyl alcohol |
| 6. | Protease | *Bacillus subtilis* | Degradation of casein & feather | Feather culture medium |
| | | *Chryseobacterium strain Kr6* | Complete degradation of feathers | Cheicken feather |
| | | *Streptomyces thermoviolaceus* | Hydrolyze fibrin, muscle, collagen, nail & hair | Muscle, Collage, Hair Nail & Feathers |
| 7. | Dehalogenase | *Ancyclobacter aquaticus* | Degradation of halogen acid ester | Monochloroacetate |
| | | *Bacillus sp.* | Degradation of TBP | 2,4,6-Trinitrobromophenol (TBP) |
| | | *Pseudomonas sp.* | Degradation of Halogen acid | 2- Chloropropionate |
| 8. | Peroxidase | *Astromyces ramosus* | Degradation of Phenols, polyaromatics and herbicides | Phenol |
| | | Plant material | Decontamination of water | |
| 9. | Mangenese Peroxidase | *Phanerochaete* | Degradation of lignin, phenol & dyes | Pentachlorophenol |
| 10. | Hydrolase | *Pseudomonas diminuta* | Bioremediation of Organophosphorous compound | Organophosphate |

**1. Laccase:** Laccase is an enzyme containing p diphenyl: dioxygen oxidoreductase and oxidase which is produced by certain fungi, bacteria. Laccase is used as bio catalyst for degradation of many phenolic compounds like polyphenols BPA, PAH which can be easily found in wastewater of dye textile industry. They oxidize the phenolic and aromatic substrate and at the same time reduce molecular oxygen to water ( C. Mai, Mistein, 2000)

Laccase is first discovered in fungi species. It is also produced in different bacterial species like Geobacillus, Pseudomonas. Bacterial laccase have more resistivity towards extreme temperature and pH.( PS Chauhan 2017) Laccase is mainly used for bioremediation of Dyes. This enzyme can decolourize the different dyes within a small duration by 80% in the presence of acetosyringone as a mediator (M Zhao, 2012). PAH are xenobiotic pollutants present in chemical constituent of dyes. Laccase can convert the

PAH in less toxic quinine by oxidation and atlast monomerisation process.

**2.Cytochrome P450:** Cytochrome P450 are a superfamily of heme containing enzymes that catalyse different reaction which are mainly found in bacteria and archaea domain. Bacillus, Mycobacterium are among the genera from which cytochrome P450 is isolated and used for bioremediation as they are easily soluble, low cost production. It catalyse the different reaction like hydroxylation, dealkylation and bio-transform the toxic chemicals present in environment. P450 have an intrinsic capacity to degrade xenobiotics. (Azenbacher, 2001)

Similarly Chakraborty and Das (2016) have reported that several microorganisms such as Rhodococcus, Bacillus, Mycobacterium genomes expressing cytochrome P450 for degradation of POP from environment( Chakraborty and Das 2016). Dioxins, PCB (polychlorinated biphenyls), PCDD are the different pollutants that can be bioremediated by cytochrome P450. Besides transgenic plants that can produce cytochrome P450 are way towards herbicide resistant plant (S. Kumar 2010)

**3. Lipase:** Lipase is an enzyme that degrade the lipids by catalysing the process of hydrolysis of triglyceride ester bond into fatty acids and glycerol. (L. Godoy, 2012). It have been observed that lipase enzyme is closely associated with bioremediation of organic pollutants by reducing the hydrocarbon. Microbial lipase have broad application in bioremediation of oil residues, petroleum effluent, cosmetic effluents, etc.

Lipase have been extracted from bacteria, actinomycetes mainly from Bacillus and Pseudomonas genera. Lipase is used commercially in biodegradation due to their low energy requirement, maximum stability, broad specificity, etc. Lipase can enhance the bioremediation of soil which is contaminated with industrial waste oil and it also degrade palm oil and castor oil.

**4. Dehydrogenase:** Dehydrogenase is an enzyme group belongs to the family of oxidoreductase. They are mainly isolated from bacteria, yeast like Pseudomonas, Stenotrophomonas. The microbial dehydrogenase catalyse the conversion of alcohol into aldehyde and Ketone group and ultimately oxidize aldehyde to Carboxylic acid (Nickolas et al, 2003).

**5. Protease:** Protease belongs to hydrolase family which catalyse the degradation of peptide bonds. They can be isolated from bacteria, fungi etc. mainly Bacillus, Streptomyces genera. Protease hydrolyse the breakdown of protein substances released from as by-product of some industries like poultry, fisheries, leather and Pharmaceuticals. Microbial protease have high commercial application because of their high efficiency, high production and low costing

The protease enzyme, Keratinase has shown significant activity on biodegradation of chicken feathers to clear the waste biomass from Agro sector. The products released from the degradation of feathers are rich in amino acids that can be used as fertilizer for plant growth

**6. Dehalogense:** Microbial Dehalogenase enzyme has very significant importance in bioremediation process of halogenated pollutants. The halogen compounds are degraded by the dehalogenase enzyme by catalysing the cleavage of Halogen bonds by using 3 different methods like hydrolysis, reduction and oxidation. Dehalogenase enzyme help in replacement of halogen group by the hydroxyl group. (Allpress & Gowland, 1998). Mainly dehalogenase enzyme is isolated from Bacteria, the genera includes Pseudomonas, Ancyclobacter. Zu et al. have isolated a pure strain of Bacillus sp. which has an excellent capacity to decontaminate the 2,4,6- Trinitrobromophenol(TBP). The enzyme debrominate the bond of the TBP and converting it into less harmful form.

**7. Peroxidase:** Peroxidases (hydrogen peroxide oxidoreductases) are ubiquitous enzymes that catalyze the oxidation of lignin and other phenolic compounds at the expense of hydrogen peroxide (H2O2) in the presence of a mediator. In mammals, they are involved in biological processes such as immune system or hormone regulation. In plants, they are involved in auxin metabolism, lignin and suberin formation, cross-linking of cell wall components, defense against pathogens, or cell elongation (D. Koua et al., 2009). It catalyses the prosses of bioremediation of waste water by degrading the phenolic compounds and polyaromatic compounds that are contaminating the water. It also reduce the harmful effect of herbicides by oxidation process in the presence of H2O2.

**8. Mangenese Peroxidase:** Mangenese Peroxidase is an extracellular heme enzyme isolated from the basidiomycetes fungus such as Phanerochaete chrysosporium, Dichomitus squalens. etc. (H.Xu, MV Guo, et al, 2017). This enzyme manganese peroxidase catalyse the process of degrading the harmful phenolic compound like pentachlorophenol used in Dyes industries. It oxidizes Mn2+ to the oxidant Mn3+ in a multistep reaction which act as mediator for the oxidation of various phenolic compounds. MnP is capable of oxidizing nonphenolic structures (A. Hamid, J.O.Soilbaiti, 2013)

**9. Hydrolase:** Esterases,nitrilases,aminohydrolases, lipase, cutinase, and organophosphorus hydrolase are among the hydrolase enzymes used in the bioremediation of different chemicals such as herbicides, pesticides, organophosphorus compounds, nitrile compounds, and polymers. Hydrolases uses a chemical bond utilizing water and convert the alrge harmful molecules to smaller one so that their toxicity also reduces. This enzyme class's main advantages are ready availability, economical, eco-friendly, lack of cofactor stereo selectivity, and tolerance. Organophosphate (OP) compounds are highly lethal neurotoxins. They have widely used pesticides in agriculture, representing a threat in the biotic environment. The hydrolase enzyme detoxify these organophosphate compounds by hydrolysis, oxidation process and thus bioremediate the contaminated soil.

## Conclusion

Biodegradation is very fruitful and attractive option to remediating, cleaning, managing and recovering technique for solving polluted environment through microbial activity. , many pollutants degrading enzymes possess special functions and great application prospects in biocatalysis. . Enzymes as practical tools of living organisms are an ecofriendly and bio-based strategy for bioremediation. Microorganisms exposed to contaminated sites and specific pollutants are fascinating sources for the isolation of active enzymes against those pollutant Thus, many microbes and the related degrading enzymes have been successfully adapted in diverse areas, such as in the preparation of industrial biosensors, intermediates of pharmaceutical progress, medical bioremediation, etc. It is concluded that enzymes would be a promising way to reduce pollutants and make a healthier environment for humans and all other species.

## Abbreviations

ABTS: 2,2′-Azino-bis-(3-ethylbenzothiazoline-6sulfonic acid)
APAHs: Polycyclic aromatic hydrocarbons
BPA: Bisphenol
DFC: Diclofenac
MFA: Mefenamic acid
OP: Organophosphates
PCCDS: Polychlorinated di-benzo-p-dioxins
POPs: Persistent organic pollutants SGZ: Syringaldazine
TBP: Trinitro bromo phenol

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