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Review

Role of microbial enzymes in Bioremediation

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***Abstract*- Bioremediation is the branch of biotechnology that has a realistic approach towards decontamination and detoxification by utilizing the microbial colonies. Biodegradation of hazardous natural compounds is possible only by the countless microorganisms such as bacteria, fungi, algae, and plants. The purpose is to create an innovative technique in bioprocesses that will contribute to reduce the toxin hazard and thus, obtain new, useful substances. Considering a few examples of bioremediation-related compounds- oxidoreductases and hydrolyses are most widely considered. This article highlights the major enzymes associated with the biodegradation of a wide variety of poisons, applications, and a few recommendations that can be brought into research.**

Keywords- **Bioremediation, oxidoreductase, transferase, hydrolase, lyases**

I. INTRODUCTION

Industrialization, urbanization has led to an abrupt rise in water-ranging from commercial raw sewage to nuclear waste. Improper disposal of such waste has left a gap in the treatment and thus, a threat to the environment. To overcome these issues, many techniques like high-temperature incineration and poop oxidation have come into play. But they did not gain attention due to their limitations. This is where bioremediation comes into play[1], [2].

Bioremediation includes exemption of polluted water from soils, sediments, groundwater, surface water, and air. Bioremediation is processed by the microorganisms that develop due to change in the environmental conditions of the locality. They can transform highly toxic substances into non-hazardous material. The method of bioremediation is cheap comparatively as it does not require many resources. The cake formed is environmentally sustainable, eco-friendly, and decontaminated. The process of decontamination in the cake is brought about by the microorganisms, naturally[2], [3]. The major reactions involved are oxidation and reduction- changing conditions such as pH, amount of minerals, vitamins, and additional nutrients. The key players in the entire process are microbes and plants. Phytoremediation is the bioremediation brought about by plants[4]. The process of bioremediation is demonstrated in Fig 1.

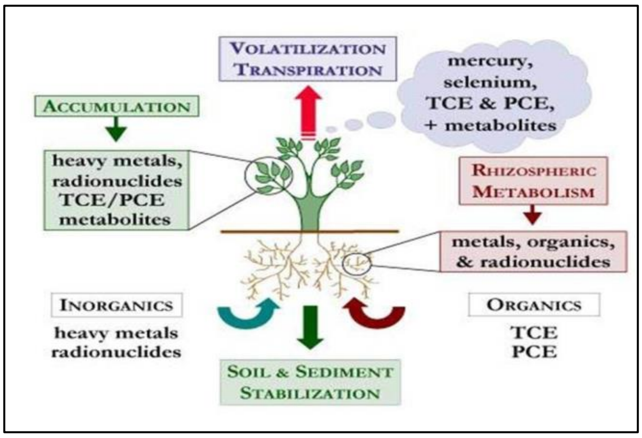

Figure 1: Bioremediation

II. BASIC REQUIREMENTS OF THE PROCESS

Bioremediation is effective in environmental conditions that facilitate microbial population and activity only. Thus, the manipulation of environmental parameters is manipulated for degradation to proceed at a faster rate. The key factors that are environmental conditions, including pH, temperature,

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oxygen, soil structure, moisture and appropriate level of nutrients, poor bioavailability of contaminants, and presence of other toxic compounds. The role of an enzyme is to breakdown complex compounds of contaminants into simple molecules that can be used as a food source by microorganisms[5], [6].

Microbes replicate repeating the cycle a desired number of times depending on the availability of the food available. To carry out their function successfully, a microorganism must be active and stable.

III. AN OUTLINE OF THE ENZYMES USED IN BIOREMEDIATION

Enzymes work by reducing the activation energy, converting substrate into product. An enzyme consists of at least one polypeptide moiety, which can be a protein or a glycoprotein. An enzyme consists of a catalytic site along with is the main component of a holoenzyme. A holoenzyme consists of an apoenzyme and prosthetic group. The substrate attaches to the enzyme via the active site by a covalent or noncovalent bond. The apoenzymes are a group of polypeptide chains-consisting of protein or glycoprotein moieties. The prosthetic group consists of the nonprotein moiety. Microbial enzymes help in making bioremediation is an effective and environmentally sustainable process[7].

A. Classification of enzymes

Enzymes are classified by a definitive E.C number, assigned by the International Union of Biochemistry. An enzyme will fall under any one of the six classes which will be fixed[8]–[10]. The six fundamental divisions are demonstrated in Figure 2.

Classification of Enzymes			
Group of Enzyme		Reaction Catalysed	Examples
1.	**Oxidoreductases**	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another.	Dehydrogenases Oxidases
2.	**Transferases**	Transfer of a specific group from one substrate to another.	Transaminase Kinases
3.	**Hydrolases**	Hydrolysis of a substrate.	Estrases Digestive enzymes
4.	**Isomerases**	Change of the molecular form of the substrate	Phospho hexo Isomerase, Fumarase
5.	**Lyases**	Nonhydrolytic removal of a group or addition of a group to a substrate	Decarboxylases Aldolases
6.	**Ligases (Synthetases)**	Joining of two molecules by the formation of new bonds.	Citric acid synthetase

Figure 2: Classification of Enzymes

B. Microbial enzymes in bioremediation

Plastics such as PVC on natural degradation result in the formation of phthalates, vinyl monomers, CFCs, and dioxins. Solid waste management relies on dumping in incineration and landfills. It is not ecologically safe as it results in the formation of harmful products and by-products[11], [12].

Thus, a huge investment is done to employ the degradation of synthetic compounds by enzymes. Microbes produce specific enzymes that catalyze the degradation of complex natural polymeric compounds to simple ones. Long-chain carbon compounds are broken down by mechanisms such as oxidation and reduction[13], [14].

1. Microbial Oxidoreductases

Oxidoreductases are responsible for the detoxification and degradation of dangerous natural mixes by different microbes and higher plants through the oxidative-reductive coupling. Microorganisms remove the vitality of toxic compounds by degrading them through biochemical responses. These biochemical enzymes are responsible to exchange electrons from a decreased natural substrate (donor) to another synthetic compound (acceptor). With such powerful reactions such as oxidation-lessening responses, the contaminants are, therefore, oxidized to safe mixes[15].

The oxidoreductases participate in the detoxification of various synthetic organics such as phenolic, azo rings, and aniline substances, which are a part of xenobiotics or soil environment. The efficiency of fungi such as *Basidiomycetes*, *P. chrysosporium*, *Trametes versicolor*, and *Pleurotus*

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ostreatus (Oyster Mushroom) to the soil is more for bacteria. Partial degradation of lignin in the pulp bleaching process results in the formation of chlorinated phenolic compounds. Thus, the paper and pulp industry require bioremediation[16].

1.1. Microbial Oxygenases

Oxygenases belong to the oxidoreductase category and are responsible for adding oxygen from sub-atomic oxygen (O_2) to the reduced substrates using FAD/NADH/NADPH as a co-substrate. Oxygenases are assembled into two classifications- the monooxygenases and dioxygenases based on the number of oxygens it has utilized for oxygenation of the reactant. Halogenated organic compounds such as pest controls are a major source of substrates for the oxygenase group of microbes. They also occupy a crucial part in the digestion of natural mixes by enhancing their reactivity or polarity or achieving cleavage of the aromatic ring[7].

2. Microbial Laccases

primary structure of laccases (p-diphenol: dioxygen oxidoreductase) has a gathering of multi-copper oxidases. They are made by explicit plants, bugs, and microorganisms and catalyzed by oxidation of many lessened phenolics and fragrant substrates with the orderly decrease of sub-atomic oxygen to water. Different genes code for different structures of laccases, creating isoenzymes[17].

Intra and extracellular laccases help in catalyzing the oxidation of ortho-diphenols, para-diphenols, aminophenols, polyphenols, polyamines, lignins, aryl diamines as well as a few inorganic ions. Lignin is converted into phenols by laccases, which is responsible for oxidation, decarboxylation demethylation, and polymerization to humic materials. They have great potential for biotechnological and bioremediation. Inhibition of action occurs through halides (excluding iodide), azide, cyanide, and hydroxide. It is sensitive to nitrogen levels[18].

3. Microbial Peroxidases

Peroxidases (contributor: hydrogen peroxide oxidoreductases) are enzymes that are high oxidizing agents. They catalyze the oxidation of coal to low molecular mass fractions, lignin, lignocellulosic materials, and other phenolic mixes to substances devoid of hydrogen. These peroxidases can either be haem and non-haem proteins associated with simple, organic procedures with a broad host range. These compounds (enzymes) yield metabolically active and non-specific free radicals got from peroxide because of the cleavage of polymeric materials into short-chain water-dissolvable particles, which, consequently, encourage their vehicle through microbial layers for intracellular degradation.

These are associated with auxin digestion, lignin and suberin arrangement in plants, cross-connecting of cell divider segments, a barrier against pathogens, or cell extension. Microbes also carry out bio-pulping, bio-bleaching, polymerization. The different fungus species contain multiple isoenzymes of lignin and manganese peroxidase[19], [20].

3.1. Microbial lignin Peroxidases

The key step for carbon recycling in land ecosystems is lignocellulose degradation. Basidiomycetes are quite stable in the process of delignification.

Lignin peroxidases are heme proteins discharged mostly by the white-rot and brown-rot basidiomycetes by optional digestion of lignocellulose as a part of their metabolizing activity. They perform the degradation of the woods very efficiently[21], [22]. Brown rot fungus is comparatively more selective. Within sight of Breakdown of lignin and other phenolic mixes occur in the presence of co-substrate H_2O_2 and veratrole liquor LiP. In this process, LiP upon getting oxidized donates its electron to H_2O_2 to reduce it to water and nascent oxygen. To return to its original state, LiP gains its electron from veratrole liquor. This results in veratraldehyde which upon gaining electrons from the substrate returns to veratrole liquor[23]. The process is represented in Fig. 3.

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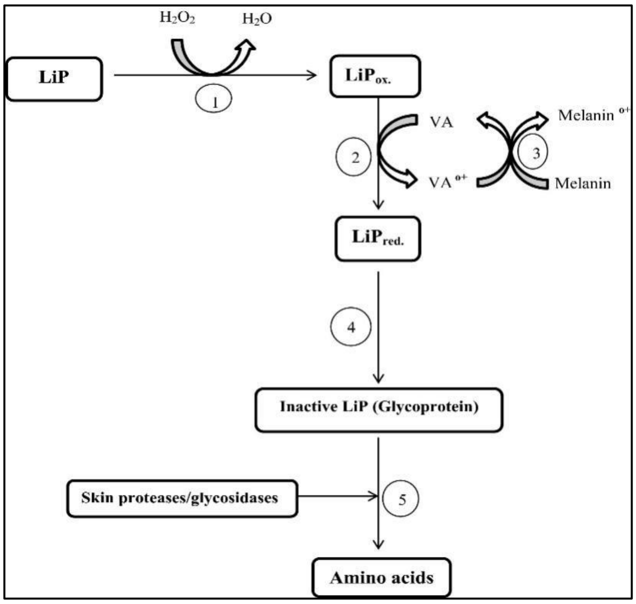

Figure 3: Catalytic reaction of lignin peroxidase[24]

3.2. Microbial Manganese Peroxidases

MnP (Manganese peroxidase) is an extracellular heme chemical present in the lignin-degrading basidiomycetes organism. The oxidation of manganate to manganite is a multistep response. Mn^{2+} acts as a substrate for MnP, initiating the reaction. This creates Mn^{3+} which is responsible for oxidizing various phenolic mixes. The subsequent Mn³+ chelate oxalate easily diffuses into regions that are inaccessible to the catalyst, such as lignin or closely resembling structures such as xenobiotic poisons covered profound inside the dirt, which is not accessible to the proteins[25]–[27].

3.3. Microbial versatile Peroxidases

V.P. chemicals, which have an exceptionally broad substrate specificity immediately oxidize Mn^{2+}, methoxybenzenes, sweet-smelling phenolic substrates like that of MnP, LiP, and HRP. V.P. is also capable of oxidizing substrates without a trace of manganese in contrast to different peroxidases. An additional feature includes the high efficiency of V.P. with both phenolic and nonphenolic lignin display dimers. Because of its high productivity, it is often desired for biotechnological industries and bioremediation[7].

4. Hydrolases

To reduce toxicity, hydrolytic enzymes break chemical bonds between toxic molecules. The oil spill, organophosphate, and carbamate insecticides are easily degraded by this process. 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, amylases, proteases, xylanases, and pullulanase have many applications such as the food industry, chemical industries, biomedical sciences, and feed additive. The hemicellulase, cellulase, and glycosidase are especially active in biomass degradation[28], [29].

4.1. Microbial Lipases

Lipase, an enzyme that assists in degrading lipids is isolated from microorganisms, animals, and plants. It helps in the drastic reduction of organic pollutants present in the contaminated soil. Lipase activity was responsible for the drastic reduction of total hydrocarbon from contaminated soil[30], [7].

IV. CONCLUSION

Bioremediation is thus the clearance of all harmful substances from the environment in a natural and eco-friendly way. The purpose of this cycle is to help nature rejuvenate, utilizing the existing substances, and manipulating it according to need. Enzymes present in microorganisms are used to clear chemicals. The advantage attached to biotransformation is that it does not create any hazardous by-products, which usually occurs while using non- biological systems. Post-treatment, the proteins are processed by indigenous microorganisms. The generation of catalysts at a larger scale, with upgraded security and additionally action and at a lower cost is attainable by utilizing recombinant-DNA innovation, where the entire culture is not required. This helps in customization.

Be that as it may, the catalyst execution for in situ bioremediation of polluted groundwater and soils might be influenced by numerous variables. They are solvency, transport, adsorption, scattering and unpredictability of toxin mixes, discovery, assurance, and checking of toxins, science, material science and microbiology of groundwater and soil, hydrogeology and hydrology of the debased site, confinements of ecological gauges for water and soil, ecological conditions, supplement sources and nearness of electron acceptors, and mostly the biodegradability of contaminants.

Fast advances in different pathways, agents in microorganisms for the corruption of contaminations, have tossed all the more light on their instruments, and pathways have been described to create maintainable bioremediation procedures for dirtying mixes. Be that as it may, the structures of proteins, administrative perspectives, and sub-atomic science still require intensive comprehension.

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