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REVIEW PAPER

Microbial Enzyme and Process Involved in the Bioremediation

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ABSTRACT

To dispose of industrially generated technologies such as land filling, high temperature drying, sludge spreading, lime added stabilization, burning, and composting have been adopted worldwide. Due to high sludge treatment costs, the bulk of textile manufacturers in India discharged their wastewater discharges into the farming fields, open dumps, fallow land, ineffectively managed sanitary landfills, and besides the railway lines. Furthermore, it contaminates the groundwater, posing significant risk on human health. Various industries and municipalities are now working on environmentally friendly and low-cost sludge treatment methods. Thus, it is vital to alleviate toxic environmental contaminants for sustainable development. Microbial enzymes can play a crucial role to degrade such pollutants with high efficiency and specificity.
Keywords: - *Enzymes, industrial effluents, microorganisms, bio stimulation.*

INTRODUCTION

Soil pollution caused by industrial or agricultural operations causes severe health risks to humans and animals and can consequently have a negative impact on ecosystems by making land unsuitable for farming and other fiscal reasons. Several industries like carpet, textile, and petrochemical production, imposes serious problems in the natural environment by disposing of toxic wastes and producing a large amount of leftover, oily sludge, and petroleum waste in soil, posing a significant challenge for hazardous waste management[1]. In order to dispose of industrially generated technologies such as landfilling, high-temperature drying, sludge spreading, lime added stabilization, burning, and composting have been adopted worldwide. Due to high sludge treatment costs, the bulk of textile manufacturers in India discharged their wastewater discharges into the farming fields, open dumps, fallow land, ineffectively managed sanitary landfills, and besides the railway lines. Furthermore, it contaminates the groundwater, posing significant risk on human health. Various industries and municipalities are now working on environmentally friendly and low-cost sludge treatment methods. Thus, it is vital to alleviate toxic environmental contaminants for sustainable development [2].

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Bioremediation is emerging as a novel technology due to its economic feasibility, increased competence, and ecofriendly behavior. Bioremediation is a term that refers to procedures that employ biological systems to repair or clean up polluted areas. To address the ever-increasing problem of environmental contamination, the technology employs a variety of eco-friendly microbiological routes. Most indigenous microorganisms are capable of successfully restoring the environment by oxidizing, immobilizing, or converting the pollutants [3].The main goal of bioremediation is to decrease or bring pollutant levels down to undetectable, nontoxic, or acceptable (i.e., within regulatory authorities' limitations) levels. Bioremediation was first employed on a wide scale in 1972 to clean up a Sun Oil pipeline spill in Ambler, Pennsylvania [4].Though, the laboratory-based experiment for this process was first carried out by George M. Robinson while performing experiments on sewage and oil treatment [5].The modern techniques of bioremediation seek for new microorganisms from polluted areas. The isolated microorganisms are believed to have a high potential for pollution remediation.

Inorder to improve the bioactivity of a bioremediant the genetically engineered strains and microbial consortiums has been utilized, either directly or indirectly. For the removal of pollutants, several processes such as bioaccumulation, biodegradation routes, and different forms of biosorption have been studied [6,7]. Extensive study is being conducted in this sector since bioremediation looks to be a viable alternative to traditional cleanup technology.

MICROORGANISMS INVOLVED IN BIOREMEDIATION

Microorganisms that are involved in biodegradation and are present in various environments are recognized as active members of microbial consortiums. These microorganisms are: Acinethobacter, Actinobacter, Acaligenes, Arthrobacter, Bacillins, Berijerinckia, Flavobacterium, Methylosinus, Mycrobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctomia, Serratio, Trametes and Xanthofacter [8]. Microorganisms are not able to mineralize various hazardous compounds individually. Therefore, for complete mineralization sequential degradation by a consortium of microorganisms are required which involves actions like synergism and co metabolism. Aerobic bacteria derived enzymes like *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium* resulted in the degradation of pesticides as well ashydrocarbons. Whereas anaerobic bacteria derived enzymes were utilized inthe bioremediation of polychlorinated biphenyls (PCBs), de-chlorination of trichloroethylene (TCE) [9].

MICROBIAL ENZYMES USED IN BIOREMEDIATION

Bacteria are found throughout the biosphere owing to their metabolic capabilities. They can thrive in a variety of environments and generate enzymes. Various enzymes from microorganisms as well as from genetically modified microbes liable for pollution breakdown in bioremediation procedures are discussed in detail given below:

CYTOCHROME P450

Cytochrome P450 is a heme enzymes and is ubiquitous in all three biological realms: Eukaryota, Bacteria, and Archaea [10], and are able to perform variety of functions in living systems, such as the production of complex natural products and in the metabolism of drugs, as well as the bioconversion of toxic chemicals present in our environment[11].P450s possess an inherent ability to degrade xenobiotics [12] by chemical conversions such as aliphatic hydroxylation's, epoxidations, dealkylations, dehalogenation, and other mechanism-based inactivation's, which are essential in the chemistry of bioremediation.CYP (101,102,1A1,1A2, and1B1) have shown metabolizing PAHs, with CYP1A1 showing significant activity against the dibenzo-p-dioxin (DD) and also towards the mono-,di, trichloro-DDs[13].They entail the use of molecular oxygen and the use of NADH or NADPH as a cofactor in the production of carbon substrate and oxidized products [14]. Ferredoxin and ferredoxin reductase are also used as electron sources for catalytic activity.

LACCASES

Laccases (benzenediol oxygen oxidoreductases, extracellular enzymes with multi-copper present in them and composed of glycoproteins are found in plants, bacteria, and fungi [15].Microbial laccases obtained from various microorganisms. They include species such as *S. cyaneus*, *S. coelicolor*, *S. bikiniensis*, and *S. ipomoea*, and among these *S. coelicolor* is most widely characterized [16]. Laccase possesses the catalytic capacity due to which the phenolic compounds , aromatic amines, and their substituted compounds with various functional groups are oxidized by producing two water molecules while simultaneously losing an electron from a singlet oxygen molecule[17] andnon-phenolic compounds which possess low solubility and high stability, and aids in the management of groundwater as well as pollution of underwater[18].

Stability of laccases are the most important biochemical features towards different physico-chemical conditions like increase ad decrease of pH, temperature in presence of different organic solvents, and towards different salt concentrations[16]. Laccase has the ability to remove substances like xenobiotic and produce polymeric compounds that can be utilized in bioremediation procedures. Laccase transforms PAHs to their quinone form, which is then more degraded to carbon dioxide, but when combined with the most efficient laccase mediator[19], 1-hydroxy benzene triazole (HBT), it changes acenaphthylene to 1,2-acenapthalenedione and 1,8-naphthalic acid [20]. Laccase can detoxify the textile dyes and phenols generated from textile industries [21].

DEHALOGENASE

Microbial dehalogenase has received a lot of interest due to its important role in the bioremediation of halogenated organic components. Dehalogenase enzyme cleaves C-X bonds found present in a number of halogenated components [22] by three mechanisms, which include hydrolytic, reductive, and oxygenolytic ones, which may result in dehalogenation by replacing the halogen atom with a hydroxyl group attained from water and a hydrogen atom from H2, respectively [23].Zu et al.,2012[24] identified a pure culture of Bacillus sp. GZT from waste recycling site sludge that possess an outstanding ability to debrominate and mineralize TBP at the same time.

DEHYDROGENASE

Dehydrogenases are oxidoreductases present in different micro-organisms, plants, animals, even in humans .Alcohol dehydrogenase derived from microbes catalyses the reversible conversion of alcohol to aldehyde or ketone and is classified as (a) NAD+ or NAD(P)+-dependent dehydrogenases and (b) NAD^+ or $NAD(P)^+$-independent enzymes that utilizes pyrroloquinoline quinone, heme, or cofactor F420 as a cofactor [25,26]. The activity of polyethylene glycol dehydrogenase was seen in bacterial cell free extract in which industrially generated xenobiotic, polyethylene glycol of different molecular weights were found degrading [27].Several Sphingomonas species utilise polyethylene glycol as an energy and carbon sources by oxidising the polymer chain's terminal alcohol groups; both crude and refined enzymes oxidise the corresponding aldehyde, although the process is sluggish [28].

HYDROLASE

Hydrolases decrease the toxicity of contaminants, by breaking the chemical bonds with the help of water which transforms the bigger molecules to smaller ones. They aid in the cleavage of C–C, C–O, C–N, S-S, S-N, S-P, C–P, and other bonds by water and catalyze a number of related processes such as condensations and alcoholics.The primary benefits of this enzyme family are its easy availability, low cost, environmentally friendly, lack of cofactor stereoselectivity, and tolerance towards the addition of water-miscible solvents [29].

Microbial hydrolytic enzymes like lipases, esterases, amylases, proteases etc. are employed for the management of waste generated at the time of food processing, plastic degradation and insecticides, treating the biofilm deposits, and soil which is contaminated with oil and so on. Hydrolytic enzymes have a wide range of possible applications, including feed additives, medicinal sciences, and chemical industries [30] . protease and lipase are the hydrolase enzymes,discussed below.

PROTEASE

Protease is an enzyme in the hydrolase family that breaks down the peptide bonds in proteins. Proteases derived from microbes possess great significance as they are cost effective with high activity. and are frequently utilised in sectors like in leather, food, and wastewater treatment [30] .Keratin which is an insoluble protein which is resistant towards degradation is found in appendages, dead animals, wastes generated from poultry and also in the horns and nails of animals . They result in environmental pollution accompanying with foul-smell. Therefore, keratinase which is a proteinase enzyme will help in the degradation of the keratin proteins and can be employed for bioremediation in poultry wastes by digesting and recycling keratinous wastes into functional byproducts. Keratinase, a protease enzyme generated by *Stenotrophomonas maltophilia* KB13, has demonstrated considerable activity in the degradation of chicken feathers [31].

LIPASE

Lipases are serine hydrolases that are known as biocatalysts for breaking down the triglyceride ester bonds into fatty acids and glycerol[32]. Lipase destroys lipids produced from different microbes, animals, and plants, lowering hydrocarbon levels in polluted soil. Microbial lipase has large number of applications for the bioremediation of oil residues, petroleum contaminants, effluents, and soil recovery [33] and in therapeutic, polymerization, pulp and paper, and cosmetic industries due to its lower requirements of energy, high substrate specificity, maximum stability, and short processing time [34,35]. Lipases can help with the bioremediation of greasy discharges comprising oils, lipids, and protein that are released from a variety of sources [36].Lipase produced by Acinetobacter sp., Mycobacterium sp., and Rhodococcus sp. are utilised to control oil spills [32]. Also Pseudomonas sp. lipase was utilised for bioremediation of soil polluted with industrial waste oil, while Pseudomonas aeruginosa derived lipase has been found to degrade castor oil [37].

PROCESS OF BIOREMEDIATION

There are many treatment technologies or procedures used in bioremediation processes. Bio-stimulation, attenuation, augmentation, venting, and heaps are the fundamental bioremediation techniques.

BIO-STIMULATION

This type of strategy involves the injection of particular nutrients to the location (soil/ground water) so as to encourage the functioning of autochthonous microorganisms. It focuses on the stimulation of indigenous or naturally occurring bacteria and fungi communities. To begin, fertilisers, growth supplements, and trace minerals are provided. Second, by meeting additional environmental needs like as pH, temperature, and oxygen, they can increase their metabolic rate and pathway [38,39]. A little quantity of pollution will also function like a stimulant by activating the operons for bioremediation enzymes. This sort of strategic approach is usually followed by adding the nutrients and oxygen to aid autochthonous microbes. These nutrients are the fundamental building blocks of life, allowing microorganisms to produce essentials such as energy, cell biomass, and enzymes to breakdown pollutants. They will all require nitrogen, phosphorus, and carbon [40].

BIO-ATTENUATION

Bio-attenuation, also known as natural attenuation, is the removal of pollutant concentrations from the environment. It is carried out in biological processes, which comprise of either aerobic or anaerobic biodegradation, plant and animal absorption,and through physical phenomena (advection, dispersion, dilution, diffusion, volatilization, sorption/desorption).Nature may clean the chemically polluted environment in four possible ways[41]: 1) Some chemicals are used as food by tiny bugs or microorganisms that dwell in soil and groundwater. When the compounds are entirely digested, they can be converted into water and harmless gases. 2)Chemicals can cling or adsorb on to the soil, which keeps them in place. This will not remove the chemicals, but it does prevent them from contaminating groundwater and leaving the site.3) Pollution can mix with pure water as it travels through soil and groundwater. This lessens or dilutes pollutants.4) Some chemicals, such as oil and solvents, have the ability to evaporate, which implies they can convert from liquids to gases inside the soil. If these gases leak into the air on the surface of ground, they may be destroyed by sunlight. If natural attenuation is insufficiently fast or complete enough, bioremediation will be aided by biostimulation or bioaugmentation.

BIOAUGMENTATION

Bioaugmentation is the technique of introducing designed microorganisms into a system that function as a bioremediators to remove complicated contaminants rapidly and completely. To accelerate the growth of indigenous microbe populations and improve degradation, which preferentially feed on pollutants at the site of contamination. Microbes are extracted from the remediation area, grown separately, genetically changed, and returned to the contaminated site. It is employed to guarantee that the in-situ microorganisms completely eliminate and convert the pollutants to non-toxic ethylene and chloride[42].Furthermore, genetically engineered microbes are demonstrating and proving that they may enhance the degradative effectiveness towards a large variety of environmental contaminants. Because of their varied metabolic profiles, they can be converted into less complicated and safe end products [43,44,45].

MODIFIED CELLS

A genetically engineered microbe is one in which genetic material has previously been altered via the use of genetic engineering techniques motivated by natural or artificial genetic exchange between microorganisms. This type of creative effort combined with a scientific method is known as recombinant DNA technology. By developing genetically engineered organisms, genetic engineering improved the use and removal of harmful undesirable wastes under laboratory settings [46]. Currently, it is possible to introduce the proper gene for the synthesis of a specific enzyme that can digest a variety of contaminants [47]. GEMs (genetically engineered microbes) have demonstrated capability for bioremediation properties in soil, groundwater, and activated sludge surroundings, displaying improved degradative capacities for a diverse spectrum of chemical pollutants.

BIOVENTING

Bioventing is the process of venting oxygen across soil to encourage the growth of naturally occurring or added bacteria and fungi in the soil by supplying oxygen to existing soil microorganisms; it is particularly useful for aerobically degradable compounds. Bioventing makes advantage of low air flow rates to supply just enough oxygen to keep microbial activity going on. The most frequent method of supplying oxygen is by direct air injection in the residual pollution in soil via wells. Adsorbed fuel residuals are biodegraded, as are volatile chemicals as they travel slowly through biologically active soil. Many researchers have demonstrated the effectiveness of bioventing in the cleanup of petroleum-contaminated soil [48,49].

CHALLENGES IN BIOREMEDIATION

The primary limitation of bioremediation is that not all components are biodegradable, thus limiting the bioremediation process. In certain situations, even though the substance may be biodegradable, its downstream processing and breakdown produce hazardous material. Because of the presence of some site-related limiting variables, a specific bacterial strain that works well at one location may not perform well at another. The microbial metabolic processes, the type of contaminants, and the availability of an adequate quantity of nutrients all contribute towards the complexity of biological activities. Because the procedure involves soil excavation, building, and modification of particular site layout, it is more time-consuming and labor-intensive. The procedure is often carried out underground and in remote locations away from residential areas. The usage of heavy machinery and pumps, on the other hand, may cause some noise and disruptions. Sometimes, ethical concerns about the use of specific bacterial strains call the procedure into question in terms of their impact on another local microflora [7].

CONCLUSION

Bioremediation is a new technique that may be used in conjunction with existing physical and chemical treatment methods to provide comprehensive control of a wide range of environmental contaminants. It appears to be a sustainable method inorder to control the pollution in environmental, therefore additional research in this field is required. Efforts should be made to create a synergistic relationship between environmental effect and the destiny and behavior of environmental pollutants as well as the selection and performance of the best bioremediation method and other suitable methods that can sustain the effective and successful operation and monitoring of a bioremediation process. Under specific optimal circumstances, vermi-biofiltration as well as genetic engineering technologies may be promoted and adopted at bigger scales for sustainable waste recycling, contaminated soil remediation, solid waste management, and so on. Future regulations will depend upon the efforts made by research and development activities,how to address bioremediation objectives, pollutant availability, and the potential harm towards natural ecosystems and human health. Furthermore, the availability of contaminants followed by their degradation in any natural or artificial system, as well as the degree of harm towards human health caused by diverse environmental contaminants, could be more easily anticipated by employing interdisciplinary technologies.

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