

BIOREMEDIATION OF BIOLOGICAL AND CHEMICAL ENVIRONMENTAL CONTAMINANTS: THE ROLE OF ENZYMES PRODUCED BY MICROBES

Abstract

The degradation of detrimental organic pollutants has been observed to involve a variety of enzymes derived from bacteria, fungi, and plants. Microbial enzymes provide the energy for the environmentally beneficial and economically viable biotechnology known as bioremediation. The research in this area will aid in the development of innovative bioprocess methods to reduce the impact of the contaminants and also yield new advantageous molecules. The fundamental process on which bioremediation is based is biodegradation, which can refer to the entire mineralization of organic pollutants into inorganic compounds, carbon dioxide, water, and cell protein or the alteration of intricate organic pollutants into other less complicated organic compounds by living organisms like microorganisms. Numerous naturally occurring bacteria in soil and water can degrade hydrocarbon contaminants.

Keywords: Bioremediation, Microbial Enzymes, Toxic Pollutants, Hydrocarbons.

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I. INTRODUCTION

The bulk of the molecules utilized in the biological remediation of organic pollutants are produced by bacteria, fungus, and plants. Microbial enzymes are the source of power for the environmentally safe, economically viable, and green biotechnology known as bioremediation. Traditionally, waste was disposed of by digging a hole and filling it with waste. Because there wasn't a new site to dump every time, it was difficult to maintain this method of garbage disposal. New methods for chemical breakdown (such base-catalyzed dechlorination and UV oxidation) and high-temperature burning of waste have advanced. Although they have a number of downsides, they can be quite successful at reducing a variety of contaminants (S.Rao n.d.). These techniques are difficult, expensive, and unpopular with the general population. Instead of just collecting the pollution and storing it, biological remediation is a microbiologically efficient systematic activity that is utilized to break down or transform pollutants into less hazardous or harmless basic and chemical forms. 2017 (Abatenh E). In order to decontaminate contaminated locations, bioremediators are used as biological agents. Restoring the recalcined environment and controlling contaminants through detoxification and mineralization are the goals of bioremediation, a biotechnological technique. Environmentally friendly, inexpensive, new, and promising are all adjectives that describe the employment of enzymes produced by microbes in the treatment of persistent organic pollutants (POPs) (Bhandari et al., 2021). The environment should support the growth of bacteria during the bioremediation process, and microbes should have access to enough nutrients. The chapter's purpose is to explain how current techniques using enzyme microorganisms to remediate contamination are used. Microbes are used in their native form as well as bioengineered form for the remediation of biotic and synthetic wastes. Enzymes constitute the molecules that can change the rate and energy of activation of reactions even when they are not present in the process. Variations in pH and temperature are two examples of environmental alterations when enzymes can still work. Enzymes have a significant role in metabolism and biochemical processes because of their catalytic role. Since they can quickly destroy poisons, digestive enzymes are among the naturally occurring substances that have an outstanding ability to eliminate and effectively transform contaminants. Enzymatic processes generally claim to eliminate pollutants through biodegradation. The focus of the current review was on using enzymes produced by microbes for the catalytic degradation of dangerous pollutants in our surroundings. the results of serious hazardous pollutants, including. Descriptions of heavy metals, pigments plastics, chemical pesticides, and hydrocarbons that are polycyclic aromatic are included in this summary. (PAHs) (A. Saravanan a n.d.) .

II. FACTORS AFFECTING MICROBIAL BIOREMEDIATION

Bioremediation is the process of removing, altering, immobilizing, or detoxifying various chemicals and physical contaminants from the environment with the aid of bacteria, fungus, and plants. Microorganisms work as biological catalysts by accelerating the biochemical reactions that eliminate the desired pollutant through their enzymatic pathways. Microorganisms can only start to combat pollution once they have the availability of a variety of products molecules that may help them manufacture energy and nutrients to develop new cells. The environment's physical and chemical properties, the kind and amount of pollutants, and their reach to microorganisms are merely a few of the aspects that influence how successful bioremediation is. (Endeshaw Abatenh* 2017) (El Fantroussi S 2005)

- 1. Biological Factors:** The breakdown of organic molecules is influenced by abiotic factors such as the rivalry amongst bacteria for limited carbon sources, aggressive interactions between microbes, or the exploitation of microbes by protozoa and bacterial pathogens. The rate of decomposition of contaminants is typically correlated with the degree of concentration of the contaminating and the amount of "catalyst" present. In this context, the quantity of "catalyst" refers to both the number of organisms capable of breaking down the contaminant and the quantity of enzymes created by each cell. The pace of contaminant breakdown can be changed by the cells' production of certain enzymes. Additionally, the degree of contaminant metabolism-specific enzyme participation and their "affinity" with the contamination as well as the contaminant's availability are both highly dependent on each other. The main biological elements are covered here: mutagenesis, Horizontal transfer of genes, activity of the enzyme, interactions (competition, succession, and predation), its own development until critical biomass is achieved, population size and composition, and its interaction with other organisms. (Madhavi GN 2012) (R 2000)
- 2. External Factors:** Potential interactions throughout the procedure are determined by the physicochemical features of the targeted pollutants and the metabolic traits of the microorganisms. However, whether the two genuinely connect depends on the local context of the contact point. Microorganism activity and growth are influenced by a variety of factors, including pH, temperature, moisture, soil composition, nutrients, site features, the potential for redox reactions, oxygen content, a lack of experts in this field, and the physico-chemical bioavailability of pollutants (pollutant concentration, type, the ability to dissolve, molecular structure, and toxicity). The aforementioned variables affect the kinetics of deterioration. (Madhavi GN 2012) (Adams GO 2015). In most aquatic and terrestrial environments, a pH that ranges from 6.5 to 8.5 is normally suitable for biodegradation, whereas biological degradation can take place in a number of pH environments. Moisture impacts the pace of contaminant metabolism because it alters the types and availability of soluble elements, and also the pressure of osmosis and pH of aquatic as well as terrestrial systems. (Cases I 2003)
- 3. Nutrient Availability:** Nutritional supplementation modifies the equilibrium of vital nutrients for microbiological development and growth, as well as the efficiency and rate of biodegradation. Balancing the nutrients, in particular the availability of crucial nutrients like N and P, might boost the effectiveness of biodegradation by increasing the bacterial C: N: P ratio. For microorganisms to thrive and perform its microbiological functions, a multitude of factors are necessary. nutrients like carbon, nitrogen, and phosphorus. Additionally, minute levels of decomposition of hydrocarbons are found. Limit. The proper amount of nutrients can speed up biological degradation in cool settings through raising the metabolic process of microorganisms. (Couto N 2014) (Phulia V 2013). Nutritional accessibility in aquatic environments constrains biological degradation (Thavasi R 2011) . Similar to all living things, oil-eating microbes additionally require nutrition for proper development and expansion. Although they are present in little amounts in the natural environment, these nutrients can be found there. (BM 2014)
- 4. Temperature:** The utmost physical component for influencing the composition of hydrocarbons and the survival of microbes is temperature (Das N, Microbial degradation

of petroleum hydrocarbon contaminants: an overview 2011). In cold climates like the North Pole, oil breakdown by biological processes is incredibly slow, placing a greater load on the microbes to absorb the spilled oil. The sub-zero temperature of water that exists in this area causes the movement systems within bacterial cells to close or even freeze the entire cytoplasm, rendering the majority of oleophilic bacteria metabolically inert. (BM 2014) (Yang S Z 2009). The optimal temperature for biological enzymes involved in the breakdown process varies with temperature, and they do not always have the same metabolic turnover. Additionally, a certain temperature is required for a specific compound's breakdown process. The biological features of bacteria are significantly influenced by temperature, which may accelerate up or reduce the process of bioremediation. The degree of microbe growth is influenced by temperature and increases with temperature, peaking at the optimal temperature. When the temperature reached a particular threshold, it rapidly began to decline as it continued to rise or fall.

5. **Concentration of Oxygen:** Various organisms might or might not require oxygen, which increases the effectiveness of biodegradation. Both aerobic and anaerobic settings can induce biological degradation since the majority of living organisms require oxygen as a gas. The majority of the time, however, oxygen can enhance hydrocarbon decomposition. (BM 2014)
6. **Moisture Content:** To develop, microorganisms need a sufficient supply of water. The amount of soil moisture has a negative impact on the biodegradation of agents. (Abatenh E 2017)
7. **pH:** The pH of a substance, which refers to its acidity, basicity, and alkalinity, affects microbial metabolic activity and might accelerate or slow down the elimination process. The capacity of microbes to develop in soil might be determined by measuring the pH there (Asira 2013). Higher or lower pH levels produced subpar results, and metabolic processes are extremely sensitive to even little pH alterations. (. Wang Q 2011)
8. **Size Characterization and Selection:** Before recommending a bioremediation solution, sufficient proper study must be carried out to accurately state the scope and amount of pollution. This work should at a minimum include the following elements: fully determining the horizontal and vertical extent of contamination; listing the parameters and locations to be sampled and the justification for their selection; and describing the procedures to be followed for sample acquisition and analysis.
9. **Toxic Compounds:** High concentrations of some pollutants, which have a poisonous character, can harm microorganisms and impede cleanup. Specific toxicants, their concentrations, and the microbes they are exposed to have a different impact on toxicity levels and processes. Certain inorganic as well as organic compounds are toxic to certain forms of life. (Madhavi GN 2012)

III. ENZYMES

The naturally occurring catalysts known as enzymes that make it easier to transform substrates into products by reducing the reaction's activation energy through the creation of enabling circumstances. An enzyme is made up of at least one polypeptide component and

can be either a protein or a glycoprotein. The active sites are the parts of the enzyme that are really catalyzing the reaction. Such an enzyme's nonprotein component is referred to as the prosthetic group, and its protein or glycoprotein moiety as the apoenzyme. The protein or glycoprotein moiety is referred to as the cofactor, and such an enzyme might consist of a number of groups connected to the active regions through covalent or noncovalent connections that are necessary for catalytic action. The holoenzyme is created when the prosthetic group and apoenzyme are combined. (Rao, Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review 2011)

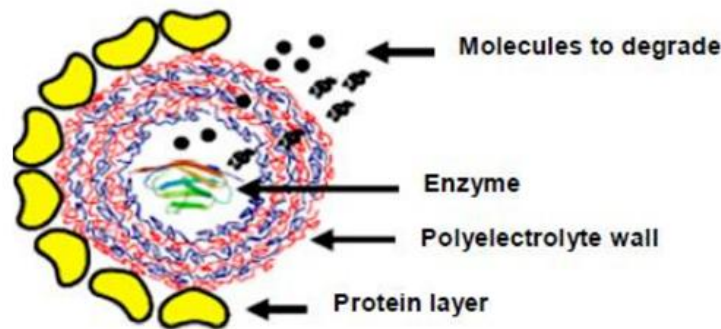


Figure 1: Degradation of molecules by enzymes (T. H. Rodríguez Couto S 2006)

According to the above figure, the molecule that has to be broken down might be exposed to the polyelectrolyte wall and permitted to infiltrate. Enzymes could participate in catalytic processes by forming covalent or noncovalent bonds, or they could participate in the biodegradation process used in the bioremediation of pollutants. The six groups of oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases might possibly comprise enzymes. It is possible to incorporate the six kinds of enzymes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Oxidoreductases are enzymes that can transport both protons and electrons from a source of transfer to a receiver. The transferases enzymes may transfer one of the functional groups from a transferor to a receptor. Hydrolases are enzymes that help water dissolve bonds. By eliminating them, the lyases enzymes may accelerate the splitting of bonds. Isomerase enzymes aid in structural or geometrical retuning. Ligases enzymes assist in connecting two molecules together (Karigar CS 2011). Enzymatic activity in various fungi was examined by Mohsenzade et al. for use in bioremediation of petroleum contamination. In this study, the activity of three enzymes—Catalase, Peroxidase, and Phenol Oxidase—was examined in a fungus. On petroleum-contaminated soil, the potential for fungal enzyme bioremediation was examined. The results demonstrate that fungal enzymes have high resistance to the removal of contaminants. They might effectively generate petroleum pollutants, in other terms. The amount of contaminants might be reduced throughout the bioremediation process. *Aspergillus terreus* fungus has the greatest level of enzymatic activity. (Mohsenzadeh F 2012).

IV. MICROBIAL ENZYMES

1. Laccases: The extracellular enzymes known as laccases (benzenediol oxygen oxidoreductases, EC 1.10.3.2) are composed of monomeric, dimeric, and tetrameric glycoproteins and are found in plants, bacteria, and fungi (R. S. Shraddha 2011). In particular, the *Streptomyces* laccase from actinomycetes has been identified, described,

and studied, making up the majority of bacterial laccase from diverse microbes. *S. cyaneus*, *S. coelicolor*, *S. bikiniensis*, and *S. ipomoea* are some of these species, the most thoroughly described of which is *S. coelicolor* (Z.-B. Guan, Bacterial laccases: promising biological green tools for industrial applications n.d.). Agricultural wastes including sawdust, banana peels, and rice bran contain lignin and phenolic chemicals that increase laccase synthesis (N. P. Muthukumarasamy 2015). The oxidative degradation of phenolic substances (ortho- and paradiphenol), aromatic amines, and its added substances with different functional groups can be catalyzed by laccase by resulting in the creation of a pair of water molecules and the simultaneous loss of an electron from only one oxygen molecule.(Chowdhary 2015) and aids to control groundwater and underwater pollution (L. Gianfreda 1999).

The stability of laccases across a range of temperature, organic solvents, salt concentrations and pH is one of their most notable biochemical characteristics (Z.-B. Guan, Bacterial laccases: promising biological green tools for industrial applications 2018). As demonstrated in CotA from *B. subtilis* at 75°, Recombinant CotA from *E. coli* having an approximate half-life of 2 hours, whereas laccase is frequently a very secure, commercially applicable thermally stable enzyme. (Chowdhary 2015). Laccase has the ability to eliminate xenobiotics and generate polymeric compounds employed in bioremediation procedures. The primary recognized contaminant, PAHs, which are made up of a benzene ring oriented linearly, are uniformly dispersed in a natural environment (J. Zeng 2011) (X. Li 2010). These pollutants, together with their intermediates, pose a serious threat to the environment because of their toxic effects, tenacity, carcinogenicity, and mutagenicity. They are created by the partial combustion of different industrial wastes and petroleum-based fuels. These aromatic hydrocarbons are xenobiotic because they are poorly degradable and have a low water solubility (J. Ihssen 2015). By doing so, laccase changes PAHs into their quinone form, whereupon it further degrades them into carbon dioxide (R. Khelifi 2010). In contrast, when used with the most efficient laccase mediator, 1-hydroxy benzene triazole (HBT), it changes acenaphthylene into 1,2-acenaphthalenedione and 1,8-naphthalic acid (Lele 2009). Textile dyes and phenols generated by the textile industry can be removed and detoxified using laccase (S. Sondhi 2008). The microbial laccase has been reported for the breakdown and decolorization of distillery effluent containing amino carbonyl complexes, as well as the purification of post-methanated distillery waste product and chlorolignin-containing pulp paper factory waste. Recombinant CotA laccase from *E. coli* has been proven in a research to be able to decolorize synthetic textile effluents (STE). Seven structurally different dyes were successfully decolorized by recombinant CotA laccase in both pure and unpurified forms. Purified and unpurified CotA laccase decolorized at greater rates when STE was buffered at a neutral pH (Zhao 2017).

Similar to this, Lu et al. discovered that reactive black and carmine, two synthetic hues, were entirely destroyed by alkaline laccase generated from a recombinant strain of *Bacillus licheniformis* within an hour. More than 93% of the investigated hues are decolorized in 4 hours by pure recombinant laccase at pH 9.0 (L. Lu 2013). The recombinant laccase made from the *B. vallismortis* strain fmb103 demonstrates its uses in aquaculture wastewater bioremediation (J. Sun 2017). On the other hand, effluent from the textile printing industry was used to decolorize recombinant CueO from *E. coli* K12 produced in *Pichia pastoris* (X. Ma 2017).

- 2. Dehalogenase:** The important role that microbial dehalogenase (EC 3.8.1.5) plays in the bioremediation of halogenated organic compounds has garnered a lot of attention. Dehalogenase is an enzyme that can break down a wide variety of halogenated compounds by cleaving C-X bonds (D. Wang 2018) (Y. Wang 2018) using one of three different mechanisms: hydrolytic, reductive, or oxygenolytic (Gowland 1998). Hydrolytic dehalogenation involves replacing the halogen atom with a hydroxyl group from water or a hydrogen atom from H₂. A pure strain of *Bacillus* sp. GZT that is good at simultaneously debrominating and mineralizing TBP was obtained by Zu et al. from the sludge of a waste recycling facility. Reductive bromination and methyl bromination are the two paths via which the debromination phase has been accomplished. To produce 2,4-DBP and 2,6-DBP, respectively, the reductive debromination continues with the debromination at the ortho and para locations. The mineralization product is CO₂, and at 148 hours, a 3 mg/L TBP solution showed the maximum mineralization efficiency of 29.3% (L. Zu 2012). The same species was cultured under optimal circumstances by Liang et al., who subsequently harvested three fractions from the lysed cells: extracellular enzyme, intracellular crude extract, and membrane protein. Only the intracellular portion of the extract of crude showed debromination activity when the dehalogenase activity of all three fractions was evaluated, proving that the dehalogenase is an intracellular enzyme. It was assumed that the degradative process of TBP would include the uncovered reading frame ORF05005, which codes for the peptide ABC carrier substrate-binding protein. The TBP dehalogenase sequence of genes from *Bacillus* sp. GZT was transformed into a competent *E. coli* BL21 (DE3) in order to confirm TBP dehalogenase activity. At 35 °C and 200 rpm, the isolated enzyme destroyed TBP in 120 minutes with an efficiency of 80%. The dehalogenase activities were boosted by H₂O₂, NADPH, Mn²⁺, and Mg²⁺ and severely inhibited by Cu²⁺, methyl viologen, EDTA, Ni²⁺, Fe²⁺, Ca²⁺ (Z. Liang 2019)

Similar to this, the unique bacterial strain *Ochrobactrum* sp. created recombinant strain *E. coli* BL21 (DE3) when it was cloned with *tbbpaA*. As a result, the recombinant strain's purified dehalogenase enzyme had a high rate of TBBPA degradation (78%) (Z. Liang 2019). By performing an epoxide assimilating reaction and degrading haloalkane, respectively, the *Rhizobium* sp. gene encoding haloalkane dehydrogenase (S. S. Cairns, Cloning, sequencing and expression in *Escherichia coli* of two *Rhizobium* sp. genes encoding haloalkanoate dehalogenases of opposite stereospecificity 1996) and halohydrin dehydrogenase (HHDH) producing strain *Pseudomonas umsongensis* YCIT1612 (F. Xue, Heterologous overexpression of *Pseudomonas umsongensis* halohydrin dehalogenase in *Escherichia coli* and its application in epoxide asymmetric ring opening reactions 2018) also aid in bioremediation.

Pseudomonas sp. (J. Q. Liu 1994), *Ancylobacter aquaticus* strain UV5 (A. Kumar 2016), *Pseudomonas umsongensis* YCIT1612 (F. Xue, Heterologous overexpression of *Pseudomonas umsongensis* halohydrin dehalogenase in *Escherichia coli* and its application in epoxide asymmetric ring opening reactions 2018), and *Rhizobium* sp. (S. S. Cairns, Cloning, sequencing and expression in *Escherichia coli* of two *Rhizobium* sp. genes encoding haloalkanoate dehalogenases of opposite stereospecificity 1996) are only a few of the additional bacterial species that have produced enzymes that can break down diverse halogenated substrates. In three different methods, Pentachlorophenol and other chlorinated phenols were converted into 3, 5-dichlorophenol (DCP) by Fricker et al. using

the Dehalococcoides mccartyi strain JNA in pure culture (A. D. Fricker 2014). Similar to this, Nelson et al. dehalogenated chlorobenzenes, dichlorotoluenes, and tetrachloroethene using the enrichment culture of three Dehalobacter sp. strains (12DCB1, 13DCB1, and 14DCB1). Strain 12DCB1 preferred to dehalogenate singly flanked chlorines. While both of these strains (pure cultures of Dehalobacter sp.) dehalogenated polychloroethene to cis-dichloroethene, 13DCB1 dehalogenated the refractory 1, 3, 5-trichlorobenzene to monochlorobenzene (MCB). All strains dehalogenate 3, 4-DCT to mono chlorotoluene, however only strain 14DCB1 dehalogenates para-substituted chlorines. These results demonstrate the adaptability of Dehalobacter sp. (J. L. Sevanan 2014).

Zhang et al. discovered that 2-haloacid dehalogenases (EC 3.8.1.2) dehalogenase 1 and dehalogenase 2 were derived from marine bacterium Pseudomonas stutzeri DEH130. After performing the dehalogenase activity assay for both the enzymes, it was concluded that dehalogenase 2 was more active towards the substrate L-2-CPA and they were unable to purify dehalogenase properly. Compared to dehalogenase 1, dehalogenase 2 was more stereospecific towards halogenated substrate (J. Zhang 2013)

- 3. Oxidoreductases:** Heme groups and flavin groups, which include flavin mononucleotide, flavin adenine dinucleotide, and metal ions, are cofactors that are transported via oxidoreductases (Martinez AT 2017). In general, oxidoreductases are more reactive than bacteria because they can create smaller molecules (Søltøft-Jensen J 2005). Enzymatic bioremediation for cashew nut shell liquid pollution was studied by Cheriyan et al. Enzymes called oxidoreductases and proteases were employed in this study to bioremediate cashew nut shell liquid (CNSL). The liquid cashew nut shell solution's colour was lightened by peroxidase enzymes, and the phenolic content of the solution was broken down by protease (Cheriyan S 2010). The following figure illustrates the many oxidoreductase types, which include oxidases, dehydrogenases, and hydroxylases.

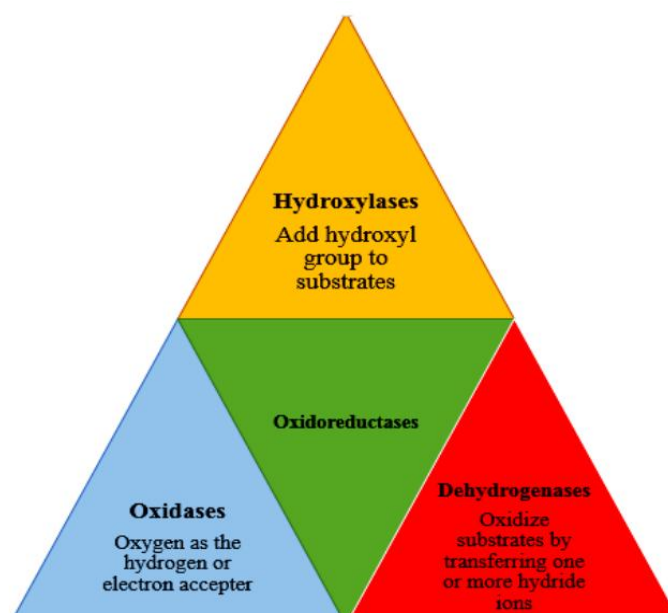


Figure 1: Types of oxidoreductase enzymes (H. 2019) (Gity Behbudi, Microbial Enzymes Based Technologies for Bioremediation of Pollutants 2021)

4. **Oxidases:** By acting as an electron acceptor and using dioxygen molecules, oxidases are able to catalyze processes (Phale PS 2019). Molecular oxygen serves as an electron acceptor in oxidases, in other words (Fetzner S 2010). Oxidases transport electrons using a variety of substances, including metals and cofactors. These substances include amine oxidases, flavin, or metals based on alcohol (Martin C 2020).

5. **Dehydrogenases:** When coenzymes such nicotinamide adenine dinucleotide (NAD), flavin mononucleotide (FMN), and nicotinamide adenine dinucleotide phosphate are present, dehydrogenase enzymes catalyze the reaction. (NADP) (Phale PS 2019). The dehydrogenase enzyme, which is present in all living microorganisms, transfers hydrogen atoms from organic transporters to electron acceptors substances (Dotaniya M 2019). Polyethylene glycol dehydrogenase activity was seen in extracts without cells from the microbes that break down xenobiotic polyethylene glycol produced commercially (Yamanaka 1989). Certain *Sphingomonas* spp. use polyethylene glycol as a source of energy and carbon by oxidation the final ethanol groups of the chain of polymer. Both raw and purified enzymes oxidize the equivalent aldehyde, however the process is slow. (M. Sugimoto 2001). The NAD⁺-dependent polypropylene glycol dehydrogenase (PPG-DH) from *Stenotrophomonas maltophilia* preferentially oxidizes di-, tri-, and polypropylene glycols, as well as those having secondary alcohol groups in their molecular structure (N. N. S. Tachibana 2008). Similar to this, *Stenotrophomonas maltophilia*'s dye-linked PPG dehydrogenase is active in the metabolism of low-molecular-weight PPG whereas the cytoplasmic enzyme is engaged in the degradation of high-molecular-weight PPG (F. K. S. Tachibana 2002). The PPG's oxidative metabolic pathway is shown by the PPG dehydrogenase activity of *Sphingobium* sp. strain PW-1 grown on 0.5% PPG (X. Hu 2008). Additionally, recombinant polyvinyl alcohol dehydrogenase is found to oxidize glycols including polypropylene glycol, 1,3-butane/cyclohexanediol, and 2,4-pentanediol but not primary or secondary alcohols (R. Hirota-Mamoto 2006). This enzyme degrades water-soluble xenobiotic polyvinyl alcohol.

6. **Hydroxylases:** Enzymes called hydroxylases have active sites that include metal and can catalyze reactions (P. 2000) (Di Gennaro P 2011). The metal acting as the activator modifies the substrate's state of oxidation. The oxidation state of iron metal is often changed, and the metal also transfers electrons along the electron transfer chain (Di Gennaro P 2011). Iron metal can create OH radicals or transmit oxygen to other materials (Massart L 1959).

Table 1: Microbial Enzymes Applications

| Enzyme | Application | References |
|-----------------|---|---|
| Oxidoreductases | the bioprocessing of lignocellulosic biomass, carbohydrate derivatives, food sector, protection of the environment, organic material creation, polymer and nanomaterial synthesis creation of pharmaceutical materials catalysts for the bioeconomy textile sector, applications for cleaning, biodecontamination, biodegradation, and biodecontamination bioreporters and biosensors, applications in medicine, Coenzyme resynthesis, Medical applications, The oxidation of organic materials | (Martinez AT 2017) (Husain Q 2019) (F 2005) (SR 2020) (SW 1999) |

- 7. Hydrolase:** Hydrolases utilize water to dissolve a chemical bond, dividing larger molecules into smaller ones, reducing the toxicity of contaminants. They aid in the water-induced cleavage of C-C, C-O, C-N, S-S, S-N, S-P, C-P, and other bonds, and they accelerate a variety of related processes, such as condensations and alcoholic reactions. The main advantages of this enzyme family include its easy accessibility, low cost, environmental friendliness, absence of cofactor stereoselectivity, and tolerance for the addition of water soluble solvents (Karigar CS 2011).

Microbial hydrolytic enzymes (lipases, esterases, amylases, proteases, cellulases, nitrilases, peroxidases, cutinase, etc.) can be used to dissolve pesticides and biofilm deposits in addition to cleaning up oil-contaminated soils and biofilm deposits. Hydrolytic enzymes have a variety of possible applications in the chemical, pharmaceutical, and feed additive sectors, among others (Sharma 2019). Esterases, amidases, and proteases can cleave ester, amide, and peptide bonds to form less hazardous or non-toxic molecules. Pollutants such as carbofuran, carbaryl or parathion, diazinon, and coumaphos have been successfully changed by using microbial hydrolase from *Achromobacter*, *Pseudomonas*, *Flavobacterium*, *Nocardia*, and *Bacillus cereus* (T. Sutherland 202).

Organophosphate-based substances (OP) are particularly lethal neurotoxins. They have a history of using a lot of pesticides in farming, which creates a concern in an environment where pyrethroids and malathion are harmful to live in. While organophosphate pesticides may be detoxified by hydrolyzing phosphodiester bonds, pyrethroids and malathion can be detoxified by hydrolyzing carboxyl ester connections. The primary chemical reactions that occur are hydrolysis, oxidation, alkylation, and dealkylation since phosphorus is frequently present in OP insecticides as a phosphate ester or phosphonate. Therefore, it is believed that microbial degradation through the hydrolysis of P-O-alkyl and P-O-aryl bonds is the most significant step in detoxification (Singh 2014). According to Rajagopal's study, "Enzymatic bioremediation of organophosphorus insecticides by recombinant organophosphorous hydrolase," considerable amounts of OP pollution were identified in the soil, drinking water, cereals, and fruit, which led to OP poisoning.

According to Littlechild (2015), malathion may be broken down by the bacteria *Alicyclobacillus tengchogenesis*, *Brevibacillus* sp., *Bacillus licheniformis*, and *Bacillus cereus*. *B. licheniformis* hydrolytic enzymes assist in the bioremediation of malathion-containing soil since it has been demonstrated that malathion serves as a carbon source for various bacteria, including *B. licheniformis* (Z. Xie 2013). Microbial enzymes such OP hydrolases, OP acid anhydrolases, or methyl parathion hydrolases (MPH) have been used to successfully remove OP contamination (G. Schenk 2016). Similar to this, the *E. coli*-produced *Pseudomonas diminuta* organophosphorus hydrolase gene (GenBank accession number M20392) revealed its potential for detoxification and methyl parathion ranges between 10-80% and 3.6-45%. Recombinant Organophosphorous Hydrolase Enzymatic Bioremediation of Organophosphorus Insecticides, Rajagopal, 2011.

In a separate investigation, Su et al. established that the OP hydrolase enzyme tightly associated with outer membrane vesicles (OMVs) of Gram-negative bacteria, resulting in the efficient degradation of parathion chemical compounds (Su 2017). The chlorinated herbicides (s-triazine), widely used as pesticides in agriculture, also pose

carcinogenic risks and contribute to various health issues. Through processes like dechlorination, deamination, and cyanuric acid breakdown, bacteria's amidohydrolase enzyme, encoded by the *atZ* genes, converts s-triazine into carbon dioxide and ammonia. *Pseudomonas* sp. ADP, a model bacterium for s-triazine degradation, is capable of breaking down the s-triazine ring found in chlorinated herbicides (M. Seeger 2010).

- 8. Lipase:** The enzyme lipase (EC 3.1.1.3), a widely recognized biocatalyst, plays a crucial role in hydrolyzing the ester bond within triglycerides, resulting in the formation of fatty acids and glycerol (L. Casas-Godoy, "Lipases: An Overview" in *Lipases and Phospholipases, Methods in Molecular Biology* 2012). Lipases belong to the category of serine hydrolases. Across a range of organisms including microbes, animals, and plants, lipase acts on lipids, aiding in the degradation process that reduces the hydrocarbon content in contaminated soil. These lipolytic reactions primarily occur at the interface between lipids and water. At this interface, lipolytic substrates often establish an equilibrium among monomeric, micellar, and emulsified forms, facilitating the lipolytic processes (Karigar CS 2011). Microbial lipase finds extensive practical uses in the business applications of remediating oil remnants, petroleum pollutants, waste products, and soil rehabilitation (S. W. M. Hwang 2018). Additionally, it plays a role in diverse sectors such as therapeutic applications, polymerization processes, pulp and paper production, and the cosmetic industry. This broad applicability is attributed to the enzyme's minimal energy demands, remarkable specificity for substrates, optimal stability, shortened processing durations, and its incapacity to augment the expenses of industrial manufacturing while utilizing various accessible raw materials (N. K. Arora 2020) (N. Gurung 2013).

Lipases offer a means to enhance the bioremediation process of fatty discharges released from a variety of locations, containing oils, fats, and proteins (S. M. Basheer 2007). Instances of oil spills, encompassing substances such as n-alkanes, aromatic hydrocarbons, and PAHs, have been effectively managed through the utilization of lipase derived from bacteria like *Acinetobacter* sp., *Mycobacterium* sp., and *Rhodococcus* sp. (L. Casas-Godoy, "Lipases: An Overview" 2012). Similarly, the lipase present in *Pseudomonas* sp. has been harnessed for remediating soil tainted by industrial waste oil. Additionally, the lipase derived from *Pseudomonas aeruginosa* has been observed to catalyze the degradation of castor oil (Salem 2009). Furthermore, there is an observable gradual decline in oil concentrations and a notable 80% reduction in waste toxicity within a span of one week (S. Verma 2012), serving as additional evidence of *Pseudomonas aeruginosa*'s efficacy in remediating wastewater tainted by crude oil. A significant environmental concern revolves around soil contamination originating from mineral oil hydrocarbons, which stem from petroleum derivatives. Through the assistance of lipase produced by bacterial strains isolated from soil that has been contaminated with motor oil, it becomes feasible to degrade the hydrocarbon, a key soil pollutant (M. H. Mahmood 2017). Furthermore, lipases find application in household laundry tasks, contributing to the mitigation of environmental pollution while enhancing detergent efficiency in tackling resilient grease or oil blemishes. In a bid to curtail the utilization of phosphate-based compounds within detergent formulations, the detergent sector employs untreated lipase sourced from the *Bacillus subtilis* strain (R. Saraswat 2017). Another prominent environmental concern involves the contamination of soil due to mineral oil hydrocarbons, which emerge from petroleum-derived sources. By harnessing lipase

generated by bacteria isolated from soil that has been polluted with motor oil, the principal soil pollutant, the hydrocarbon, can be disintegrated (M. H. Mahmood 2017). Moreover, lipases play a role in household laundering procedures, contributing to the abatement of environmental pollution while enhancing detergent efficacy in the elimination of persistent grease or oil blemishes. In efforts to diminish the presence of phosphate-based compounds within detergent compositions, the detergent industry opts for the unrefined lipase sourced from the *Bacillus subtilis* strain (R. Saraswat 2017).

Table 2: Other enzymes involved in bioremediation (Sobika Bhandari 2021)

| S. No. | Enzyme | Mechanism | Function | Reference |
|--------|-----------------|--|---|-------------------|
| 1. | Cytochrome P450 | employs the decrease or oxidation of heme iron to execute electron transfer mechanisms and catalytic reactions. generates carbon substrates and oxidized outcomes through the utilization of pyridine nucleotides as contributors of electrons. $\text{NAD(P)H} + \text{O}_2 + \text{R} \rightarrow \text{NAD(P)}^+ + \text{RO} + \text{H}_2\text{O}$ | Steroids, fatty acids, and xenobiotics undergo oxidation within cellular environments due to the formation and processing of numerous compounds and substances. | (Guengerich 2018) |
| 2. | Protease | assist in the breaking of protein peptide bonds | keratin, casein, and other protein degradation, leather dehairing | (A. Razzaq 2019) |

Table 3: Microbial Degradation of Petroleum Hydrocarbon Contaminants (Chandran 2011)

| Enzymes | Substrates | Microorganisms | References |
|------------------------------------|---|---|-----------------------|
| Soluble Methane Monooxygenases | C ₁ –C ₈ alkanes alkenes and cycloalkanes | <i>Methylococcus</i> <i>Methylosinus</i> <i>Methylocystis</i> <i>Methylomonas</i> <i>Methylocella</i> | (I. R. McDonald 2006) |
| Particulate Methane Monooxygenases | C ₁ –C ₅ (halogenated) alkanes and cycloalkanes | <i>Methylobacter</i> <i>Methylococcus</i> , <i>Methylocystis</i> | (I. R. McDonald 2006) |
| AlkB related Alkane Hydroxylases | C ₅ –C ₁₆ alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth | <i>Pseudomonas</i> <i>Burkholderia</i> <i>Rhodococcus</i> , <i>Mycobacterium</i> | (B. Jan 2003) |

| | | | |
|---------------------------------|--|---|-------------------------|
| Eukaryotic P450 | C ₁₀ –C ₁₆ alkanes, fatty acids | <i>Candida maltose</i> <i>Candida tropicalis</i> <i>Yarrowia lipolytica</i> | (T. Iida 2000) |
| Bacterial P450 oxygenase system | C ₅ –C ₁₆ alkanes, cycloalkanes | <i>Acinetobacter</i> <i>Caulobacter</i> <i>Mycobacterium</i> | (J. B. Van Beilen 2006) |
| Dioxygenases | C ₁₀ –C ₃₀ alkanes | <i>Acinetobacter sp.</i> | (J. H. O. Maeng 1996) |

V. CONCLUSION

A pressing global concern involves the pollution of water and land due to industrial chemicals and petroleum hydrocarbons. Under specific conditions of temperature, pH, contact duration, and concentration, a particular microbial enzyme recognizes a specific pollutant substrate. This recognition facilitates the efficient enzymatic conversion of the substrate into harmless products using various enzymatic reaction mechanisms like oxidation, reduction, elimination, and ring-opening. With advancements in manipulation and processing methods, enzymes with enhanced physiological characteristics can be engineered, enabling operations under extremely high or low temperatures. Moreover, during enzyme production, the focus is on minimizing nutrient, water, and energy consumption within the enzyme systems. The design should prioritize delivering optimal efficiency and yield. Nevertheless, techniques employing genetic engineering amplify the production of the enzyme. Yet, further investigation is warranted to tackle the economic aspect associated with the genetic engineering approach, even with heightened efficiency. Recently, there has been notable interest in cost-effective approaches to crafting nanoparticles and materials derived from nanoparticles, owing to their distinct attributes and wide array of potential applications. These nanoparticles possess the capacity to interact with xenobiotic substances, leading to either their complete degradation or conversion into less hazardous derivatives, thereby aiding in environmental purification. While the aforementioned technologies suffice for efficient bioremediation, the advancement of sophisticated, efficacious, and ecologically sound methodologies hinges on the imperative for impeccable mechanical intervention.

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