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Eco-Friendly Strategies for Pesticide Removal: Biotechnological and Microbial Approaches

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ABSTRACT: Population growth was very rapid and triggered significant advancements in both the industrial and agricultural sectors, leading to increased production of pharmaceuticals and pesticides. The over-utilization of chemical compounds greatly accelerated several environmental pollution problems that were highly harmful to both local ecosystems and human health. Therefore, this justified the study of the environmental fate and transport of pesticides, their effects on human health, and an overview of enzymatic decomposition as a biological means for pesticide removal. More specifically, the study focused on potential agents such as carboxylesterases and hydrolases, examining their mechanisms, advantages, and disadvantages in bioremediation applications. It discussed the environmental fate and transport of pesticides and their impact on human health. The subsequent sections addressed enzymatic degradation, with a focus on carboxylesterases and hydrolases, presenting their mechanisms along with the benefits and limitations of applying them in bioremediation. The article also examined future prospects for enzyme reactions in bioremediation and purification processes. Bioremediation was identified as a highly promising method for remediating pesticide-contaminated soil. Microorganisms removed the compounds from the environment. Among various remediation approaches, enzymatic breakdown of biocides emerged as a particularly promising method for treating biodegradable pollutants by breaking down persistent chemical compounds and eliminating waste materials through enzymatic reactions. This method demonstrated the ability to degrade most organic pollutants and was shown to be both feasible and eco-friendly, with considerable potential for treating other types of organic contamination.

KEYWORDS: Soil bioremediation; microbial enzymes; pesticides-contaminated soil; bio pesticides; enzymatic degradation

1. Introduction

The rapid growth of the global population drove significant advancements in the industrial and agricultural sectors, leading to increased production of goods such as pharmaceuticals and pesticides, which required substantial additions of aromatic compounds. The overutilization of these chemical compounds gave rise to various environmental pollution issues, resulting in detrimental effects on local ecosystems and human health. For instance, soil contamination recently emerged as a global issue, attracting significant attention and concern worldwide. According to Mirsal, pesticides, polycyclic aromatic hydrocarbons (PAHs), and heavy metals were identified as the most common contaminants found in the soil system [1]. Biological accumulation along the food chain occurred as a result of excessive and uncontrolled pesticide application, due to the limited capacity of soil to degrade and eliminate harmful contaminants [2–6]. Consequently, individuals who frequently consumed pesticide-contaminated crops or foods may have experienced health implications, particularly related to the immune system, due to long-term pesticide exposure [7, 8]. The severity of soil contamination was gradually exacerbated by increasing global population density, which encouraged continuous industrial and agricultural development. Pollutants, defined as unwanted and harmful substances, were often disposed of into the environment, where they remained and accumulated, causing various degrees of pollution [9]. For example, any alteration in the physical condition of the soil that reduced its ecological capacity could harm resident organisms and the regional environment—a phenomenon known as soil degradation [10]. Soil pollution ultimately resulted in the accumulation of various pollutants until the environmental threshold level was reached. Therefore, the employment of soil remediation techniques to treat contaminated soil was crucial in preventing further contamination and minimizing the risk of soil pollution. This study aimed to investigate the environmental fate and transport of pesticides, assess their impact on human health, and review the potential of enzymatic degradation as a biological approach for pesticide removal, focusing on the mechanisms, benefits, and challenges of using carboxylesterases and hydrolases for bioremediation.

2. Pesticides-contaminated soil.

Soil was described as the most complex ecosystem in the world due to its structure, which contained a diverse range of organisms, including organic resources, mineral deposits, and microorganisms. A mixture of organic resources and mineral deposits was formed. These appeared in aqueous and gaseous states within the soil system, which was recognized as a favorable natural condition to support plant growth [11]. However, pollutants such as heavy metals, pesticides, and PAHs tended to be absorbed by the soil, where they remained and accumulated. Consequently, the food chain became contaminated, significantly influencing the environmental cycle and human health, especially in soil systems exposed to pesticide contamination [12].

Pesticide-contaminated soil presented a complex combination of different chemical pollutants that required sophisticated modifications for effective breakdown. Hence, it was recognized as a global concern that received substantial attention, as its occurrence was known to be both disadvantageous and detrimental to local ecosystems and human health [13]. According to Kaur et al., pesticides could be classified into four major categories: organophosphorus, organochlorines, pyrethroids, and carbamates [14]. These categories served different purposes. To elaborate further, pesticides could be more extensively categorized into insecticides, herbicides, fungicides, rodenticides, garden chemicals, and domestic disinfectants [9]. The major categories of pesticides, including plant growth regulators and herbicides, were divided into respective classes, as shown in Table 1. The degree and severity of pesticide contamination in the soil system were influenced by several factors, such as soil uptake and soil half-life, interactions between the physicochemical features of pesticides and soil characteristics (e.g., moisture content, climate variability, pH level, presence of organisms, and organic/inorganic matter content). For example, research indicated that the interaction between soil and pesticide negatively affected the half-life of pesticides [15,16].

Table 1. Different classes of pesticides.

Class	Category	Description
Herbicides	Bipyridyl derivatives	Herbicides that target weeds through disruption of photosynthesis.
	Chlorophenoxy compounds	Common herbicides used to control broadleaf weeds by affecting plant growth.
Insecticides	Pyrethroids	Synthetic insecticides modeled after pyrethrins, targeting the nervous system of insects.
	Carbamate Esters	Insecticides affecting the enzyme acetylcholinesterase to disrupt insect nerve function.
	Botanical Insecticides	Naturally derived insecticides, often from plants, used for pest control.
	Organophosphates	Widely used insecticides that inhibit acetylcholinesterase, disrupting insect nervous system.
	Organochlorines	Chlorine-based insecticides that persist in the environment and affect the insect nervous system.
Fungicides	Phthalimides	Fungicides used in the prevention of fungal diseases in crops.
	Pentachlorophenol	A fungicide and preservative used in industrial and agricultural applications.
	Dithiocarbamates	Fungicides that inhibit fungal growth by interfering with cell wall synthesis.
	Hexachlorobenzene	A fungicide used to control fungal infections in plants, though toxic to humans and the environment.
	Organomercurials	Fungicides that contain mercury compounds, effective but highly toxic.
Fumigants	Ethylene dibromide	A fumigant used for controlling pests in stored products and soil.
	Phosphine	A fumigant used to control pests in stored grains and other commodities.
	Dibromochloropropane	A fumigant used to control nematodes and other pests in soil.
Rodenticides	Fluoroacetic acid and derivatives	Rodenticides that disrupt the metabolic processes of rodents, leading to their death.
	Zinc phosphide	A rodenticide that causes poisoning in rodents by interfering with cellular respiration.
	α-Napthyl Thiourea (ANTU)	A rodenticide that disrupts the metabolism of rodents, often used in the control of rats and mice.
	Anticoagulants	Rodenticides that cause internal bleeding in rodents by inhibiting blood clotting.

The effect of pesticides on the soil system could be thoroughly examined in abandoned agricultural land where excessive and intense quantities of pesticides had been applied. As a result, these abandoned areas were expected to experience soil degradation, significantly affecting the growth and activity of essential microbes within the soil environment [9]. The human practice of applying large and intense amounts of pesticides for agricultural purposes was often driven by the misinterpretation of the soil's infinite absorption capacity, which led to severe and uncontrollable soil pollution [17]. The excessive use of pesticides altered soil conditions by persisting and accumulating in the soil system for years, creating an unfavourable environment for beneficial microorganisms. It was important to acknowledge soil protection, as soil played a critical role in supporting diverse ecosystem services such as detoxification, flood mitigation, food supply, greenhouse gas regulation, provision of raw materials and infrastructure support, pollutant elimination, pest control, and cultural and aesthetic value [18]. Nevertheless, the application of pesticides also had some positive effects. Pesticides could reduce the transmission rate of diseases and enhance agricultural productivity [19, 20].

3. Fate of pesticides in the soil

It was essential to examine and analyse the fate of pesticides in the soil system to further investigate their impacts on local ecosystems and water resources. In general, the utilisation and dosage of pesticides were gradually increased to provide stronger effects in eliminating resistant pests and to meet rising agricultural demands. However, the overutilisation of pesticides resulted in higher persistence within the soil system, leading to more severe consequences such as groundwater contamination, soil degradation, and biodiversity loss.

3.1. Transport of pesticides in the environment.

Pesticides were typically capable of being transported three-dimensionally across most environmental media. Factors such as the chemical nature of the pesticides and the characteristics of the transport medium greatly influenced the extent, rate, and residual time of pesticide presence in the environment. Pesticides were found throughout the environment, including in air, water, soil, and the tissues of various organisms (Figure 1), through successive or simultaneous processes such as wash-off, emission, volatilisation, degradation, leaching, runoff, sorption/desorption, and plant uptake [21–23].

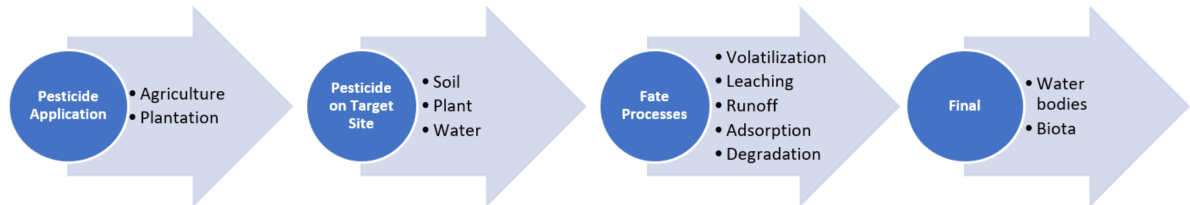

Figure 1. Environmental fate of pesticide pollutants.

These processes could be categorised based on their influence on pesticides (Figure 2). For instance, chemical degradation, photodegradation, and microbial degradation were processes that affected the persistence of pesticides. In contrast, leaching, runoff, volatilisation, sorption, wind erosion, and plant uptake were processes that impacted the mobility of pesticides within the soil system [24]. Regarding the transport of pesticides in the environment, several elements are required to be considered, which include (a) transport processes, (b) the amount of pollutants transported along the medium, (c) behaviour and fate of the pollutants during the transport, and (d) biological effects. The environmental dynamics of the pesticides are considered as existing as they involve complicated treatments that are related to a wide range of studies on meteorology, pesticide volatility and solubility, photodecomposition, leaching behaviour, chemical degradation, biodegradation, bioaccumulation, detoxification, toxicology, absorption, biomagnification, aerosol performance, impacts on the non-targeted organisms etc.

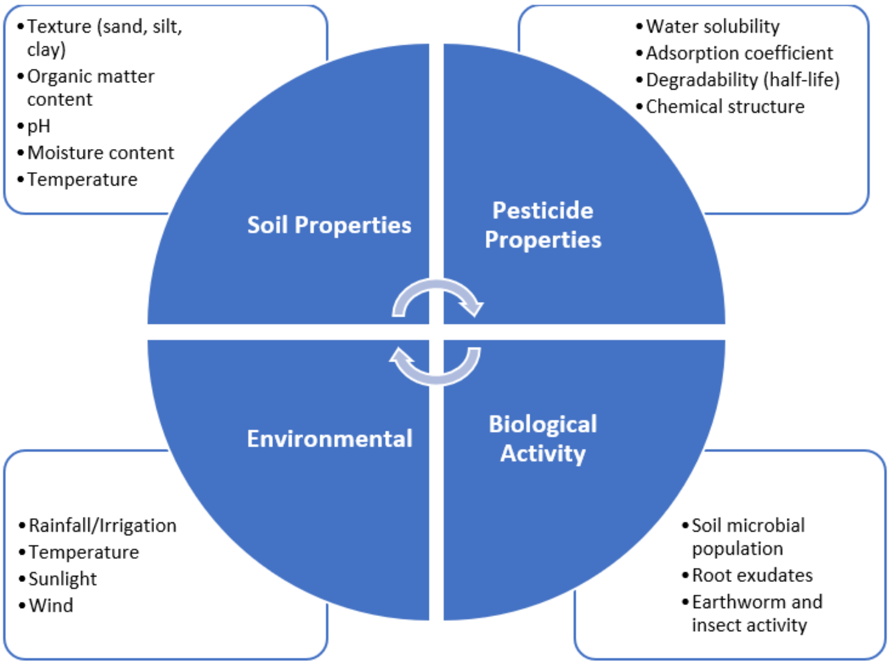

Figure 2. Factors that influence the fate of pesticides in soil.

4. Health Impacts of Pesticides

Masses of people worldwide were exposed to pesticides due to their overutilisation. It was found that most developing countries faced a higher risk of pesticide exposure, with approximately 2.2 million people residing under such health threats [25]. Health implications, such as fertility disorders, were commonly diagnosed in individuals exposed to high concentrations of pesticides through contaminated soil and water sources [26]. Previous study stated that pesticides could cause both direct and indirect health effects, including allergies [27]. Pesticides were also found to contribute to the development of asthma, leukaemia, endocrine disorders, and various cancers [28]. According to research, individuals in close contact with pesticides had a significantly higher risk of being diagnosed with intestinal cancer, ovarian cancer, stomach cancer, bladder cancer, lung cancer, leukaemia, neuroblastoma, Burkitt's lymphoma, Alzheimer's disease, Parkinson's disease, asthma, and diabetes [29]. Importantly, the health risks of pesticides should not be underestimated, even when present at low environmental concentrations, due to their high toxicity and persistence [30]. The severity of pesticide impacts on human health depended on both the toxicity of the chemical compounds present in the pesticides and the extent of exposure. As a result, accurately evaluating and assessing these impacts was challenging, given the wide variability in chemical toxicity, exposure levels, and the meteorological and geographical characteristics of different locations [31–33]. Furthermore, children, pregnant women, and the elderly were considered particularly susceptible to the adverse effects of pesticide exposure.

Pesticide toxicity was classified as productive toxicity, originating from agricultural manufacturing processes and gradually spreading throughout the environment. Organisms unintentionally ingested or inhaled contaminated materials with high pesticide residues. Therefore, modern life was fraught with risks due to the widespread, industrial-scale use of pesticides in agriculture [20]. According to the World Health Organization (2010), 86 types of pesticides—including 5 herbicides and 51 insecticides—were categorised into four major hazard classes: extremely hazardous (Ia), highly hazardous (Ib), moderately hazardous (II), and slightly hazardous (III) [34]. Additionally, 9 of the 12 most hazardous chemicals identified by the Stockholm Convention on Persistent Organic Pollutants (POPs) were recognised as pesticides [35].

5. Biological Approaches for Elimination of Pesticides

Pesticide contamination of agricultural soils can be mitigated and controlled through bioremediation, particularly via biodegradation processes that involve the application of microorganisms to carry out metabolic activities. This method is considered feasible, effective, and environmentally friendly for the removal of pesticides from the environment [36]. During the bioremediation process, pesticides are used as substrates by microorganisms in their metabolic reactions, along with other nutrients, ultimately leading to the elimination of these compounds from the soil. However, the effectiveness of bioremediation is directly influenced by pesticide properties such as bioavailability, persistence, and distribution in soil. The application of microorganisms for pesticide degradation remains essential, although it is challenged by the low water solubility of many pesticides and their strong adhesion to soil particles [36]. Moreover, environmental factors and soil properties, including moisture content, temperature, pH, and microbial diversity, also significantly affect the efficiency of the bioremediation process.

5.1. Mechanisms of microbial degradation.

Microorganisms can completely mineralise or transform pesticides into degradation products by utilising them as nutrient sources during their metabolic processes. Enzymes such as peroxidases, hydrolases, and oxygenases play critical roles in this biotransformation by catalysing various biochemical reactions. Through these enzymatic actions, pesticides are gradually broken down into less harmful byproducts. Both intracellular and extracellular enzymes produced by microorganisms, including fungi and bacteria, are fundamental to this degradation process. The time required for pesticide breakdown during bioremediation is influenced by multiple factors, such as the initial and final concentrations of pollutants, which can often be modelled using first-order kinetics [37]. Nevertheless, factors like moisture content, microbial activity, temperature, leaching potential, and pesticide availability in soil may limit the process's overall efficiency and must be considered in assessments [36].

5.2. Enzymatic degradation.

Enzymes derived from microbial metabolic activities are capable of facilitating enzymatic biodegradation, thereby playing a significant role in pesticide degradation. These enzymes function by lowering the activation energy of reactions, thereby altering reaction rates [38]. The major types of enzymatic activities involved in degradation include oxidation, hydrolysis, reduction, and conjugation. The degradation process typically begins with oxidation, during which electrons are transferred from reductants to oxidants. This step is commonly catalysed by enzymes such as laccase and oxygenase. Laccases contribute to breaking down aromatic rings in pesticide molecules by generating free radicals and reducing oxygen to water. Oxygenases incorporate oxygen atoms into pesticide molecules, facilitating their transformation. The heat or energy generated during these oxidation reactions is often utilised in the microorganisms' metabolic processes. The second step, hydrolysis, involves the addition of hydroxyl or hydrogen groups from water molecules, which stimulates the cleavage of chemical bonds in pesticide substrates. Enzymes such as esterases, lipases, and cellulases are commonly active in this pathway, leading to the breakdown of pesticide molecules into smaller chains. Subsequently, reduction is carried out by reductive enzymes such as nitroreductase, which further transforms the pesticide compounds. The degradation pathway concludes with conjugation reactions and fungal biodegradation. In these processes, pesticides are further mineralised through the addition of endogenous or exogenous natural molecules and undergo modifications such as acylation, alkylation, hydroxylation, and nitrosylation.

6. Microbial Enzymes Used in Pesticide Degradation

6.1 Carboxylesterases.

Carboxylesterases (CEs) are recognised as one of the enzyme families that are essential in carrying out hydrolysis of organophosphorus (OP) pesticides that contain a great quantity of xenobiotic and endogenous ester-containing molecules [39]. Microbial CEs are primarily utilised to break the ester bonds between the xenobiotic molecules and act as biocatalysts to enhance the synthesis of organic molecules. Table 2 summarises the utilisations of different microbial CEs in pesticide degradation. CEs are effective biocatalysts that enhance the hydrolysis of the substrates, especially those that contain thioester, amide, and ester bonds. A large substrate specificity can be found across the CEs, which is useful for allowing attachment onto a great conformable active site with a high diversity of structural substrates such as carbamate, OPs, and pyrethroid insecticides. The hydrolysis process of the phosphodiester bonds can detoxify the organophosphate pesticides. Meanwhile, the malathion and pyrethroids can be detoxified through the hydrolysis of carboxyl ester bonds [39]. Generally, phosphorus is present as phosphonate or phosphate ester in organophosphate pesticides. Hence, the main reactions involved in presenting as an ester are oxidation, hydrolysis, dealkylation and alkylation [39]. The microbial degradation that involves the hydrolysis of P-O-aryl bonds and P-O-alkyl bonds is identified as the key procedure in carrying out the detoxification process in which the bio-mineralization and co-metabolic of the organophosphorus molecules are reported by the isolated microbes [40]. Hydrolases are recognised as the most common enzyme applied in bioremediation and have been detached from the pesticide-resistant variants of eukaryotic organisms [39]. Apart from that, the hydrolysis of OPs at the ester linkage will tend to produce and yield alkyl phosphates, ignoring the low toxicity groups that the animals secrete. It is important to note that hydrolysis acts as the primary degradation reaction in alkaline aerobic environments. The final products generated from the hydrolysis of OPs are often identified as acid and alcohol, and instantaneous decomposition from them into amine and carbon dioxide will occur. Thus, the metabolites generated from the oxidation process of parent

OPs promote the intact ester bonds, which can remain susceptible to hydrolysis by applying CEs.

Table 2: Utilisation of different microbial CEs in pesticide degradation.

Microorganisms	Pesticides degradation	Reference
Pseudomonas putida, strain 32zhy	Malathion and carbaryl	[41]
Ochrobactrum anthropic, strain YZ-1	Pyrethroid	[42]
Pichia pastoris, yeast	Chlorpyrifos and organophosphates	[43]
Flavobacterium sp. *Culex pipiens*	Carbamate, organophosphorus, and pyrethroid pesticides	[44]
Ancylobacter sp. XJ-412-1	Metsufuron -methyl	[45]
Sphingobium sp. Strain JZ-1	Pyrethroid	[46]

6.2. Hydrolase.

Hydrolase can break down chemical bonds through water by converting the larger molecular compounds into smaller ones to decrease the toxicity of the pollutants (Table 3). The hydrolase facilitates the splitting of C-O, C-N, C-C, C-P, S-N, S-S and other bonds in the presence of water. It acts as a catalyst for alcoholics and condensation reactions. The advantages of the application of hydrolase include tolerance of the addition of water-miscible solvents, low cofactor stereoselectivity, high availability, economical-feasible, and environmental-friendly [47]. The common examples of microbial hydrolytic enzymes such as amylase, cellulases, esterases, proteases, nitrilases, lipases, cutinase, peroxidases, etc., have been practised in waste management, especially for oil-contaminated soil, biofilm deposits treatment, food processing, and insecticides and plastics degradations. These hydrolytic enzymes have been discovered to have broad potential applications in many fields, including chemical industries, feed additives, and biomedical sciences [48]. The breakdown process performed on the peptide, amide and ester by the protease, amidases, and esterases can result in products with minimal toxicity. Thus, there are some successful applications of microbial hydrolases including parathion hydrolase or carbamate from *Pseudomonas*, *Nocardia*, *Achromobacter*, *Bacillus cereus*, *Flavobacterium* in converting and transforming several pollutants such as parathion, coumaphos, carbaryl, carbofuran and diazinon via the hydrolysis reaction [49].

Despite the fact that OP compounds are known to be highly poisonous neurotoxins, they have been extensively practised as the content of pesticides in agriculture. The overutilization of pesticides threatens the natural ecosystem due to the toxicity of malathion and pyrethroids. Usually, sources such as drinking water, fruits, grounds, and grains are discovered to have severe OP contamination, which can result in OP poisoning [50]. The common microorganisms used for malathion degradation include *Bacillus cereus, Bacillus licheniformis, Alicyclobacillus tengchogenesis, and Brevibacillus* sp. [51]. For example, malathion was recognised as a carbon source for the microorganism, namely *Bacillus licheniformis*; hence, the hydrolytic enzymes of *Bacillus licheniformis* are effective in the bioremediation of the malathion-contaminated soil [52]. The potential of using microbial enzymes such as OP acid hydrolases, OP hydrolases, and methyl parathion hydrolases (MPH) in solving OP contamination receives substantial attention to provide an alternative in clean bioremediation approaches [53]. For instance, chlorinated herbicides, namely s-triazine, which is widely used as agricultural pesticides, are known to be carcinogenic and can have health implications. The s-triazine can be degraded into ammonia and carbon dioxide through deamination, degradation, and dichlorination of cyanuric acid by the amidohydrolase of *atZ* genes and its products. Besides, *Pseudomonas* sp. ADP also effectively degrades the s-triazine ring in chlorinated herbicides [54].

Table 3. Summary of the microbial enzymes.

Enzyme	Mechanism and Function	Advantage	Disadvantage	Reference
Carboxylesterases	Carboxylesterases catalyze the hydrolysis of carboxyl esters, breaking ester bonds in xenobiotic compounds. They act as biocatalysts in the degradation of a wide variety of ester-containing chemicals, including pesticides and other environmental pollutants.	These enzymes exhibit high biocatalytic efficiency and are eco-friendly, making them suitable for bioremediation. They also have a broad substrate specificity, allowing them to break down a range of pollutants.	Despite their effectiveness, carboxylesterases are costly to produce on an industrial scale, limiting their widespread use for environmental cleanup.	[39, 55, 56]
Hydrolase	Hydrolases catalyze the hydrolysis of triglycerides, where one mole of triglyceride reacts with three moles of water to produce one mole of glycerol and three moles of fatty acids. This enzymatic process helps in the degradation of fats and proteins, breaking down complex lipid structures.	Hydrolases are versatile, showing tolerance to water-miscible solvents and low cofactor stereoselectivity. They are highly available, cost-effective, and environmentally friendly, making them ideal for industrial applications.	Hydrolases face challenges such as high expenses related to the purification process and limited efficiency in crossing the cell membrane, which restricts their potential for large-scale application.	[47, 57]

7. Future Perspectives of Enzymatic Systems for Bioremediation and Decontamination

Scientists have continuously enhanced the application of enzymes for bioremediation and decontamination through molecular biology and protein design technologies. Hence, the researchers can significantly modify the existing enzymes to improve their respective catalytic reactions and substrate binding abilities. With this fundamental research and study, OP hydrolases will be continuously improved and evolved to overcome their restrictions and emerge as an alternative for clean bioremediation and decontamination. Generally, the application of enzymes is often restricted by expenditure, production, and stabilization issues, especially the commercial usage of enzymes in the therapeutics field. However, some companies have made efforts to shift the commercialization of enzymes towards more profitable and achievable ones, such as Novozyme. Thus, companies such as Gingko Bio Works have shown significant effort in enhancing and promoting gene optimization, fermentation models and bio-production capabilities via protein selection to advance the field of synthetic biology [58]. The rapid advancements made by private companies in the field of bioremediation and decontamination have the potential to provide substantial financial support, reflecting both the recognition and promise of these technologies. Future research can focus on several key areas, firstly, soil contamination assessment tools should be designed with high integration, precision, intelligence, and low energy consumption in mind, ensuring effective evaluations of both contamination levels and the success of remediation efforts. Secondly, the development of remediation approaches should consider not only the parent organic pollutants but also their respective metabolites. These metabolites may have longer half-lives and higher toxicity than the parent pollutants, necessitating tailored strategies for their management and removal.

8. Conclusion

The utilisation of pesticides has grown rapidly to cater the global demand. However, these pesticide compounds are poisonous and lethal to local ecosystems and human health at different levels. Thus, it is important to note the high persistency of these pesticide compounds, which are difficult to degrade and tend to remain in the environment. Bioremediations show great potential to remediate pesticide-contaminated soil, indicating microorganisms' capability to eliminate pesticides from the environment. Among all the bioremediation techniques, bio-pesticide enzymatic degradation is a promising approach to degrade persistent chemical compounds and remove pollutants through enzymatic reactions. It is deemed as a feasible and eco-friendly technique with great potential to degrade other organic pollutants.

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Author Contribution

Upeksha Gayangani Jayasekara, Nabila Benhamada, Risky Ayu Kristanti: Writing, Conceptualization, Data Collection; Rubiyatno Ferdaus Mohd Altaf Hossain, Md Abu Hanifa Jannat, Annisa Andarini Ruti, Daniel Twum-Ampofo, Lucky Caesar Direstiyani: Writing, Methodology.

Competing Interest

The authors declare that there are no competing interests or conflicts of interest regarding the publication of this review article.

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