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Bacterial-Based Technological Innovations for Pesticide Bioremediation: A Mini Review

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Abstract

Pesticide pollution in the environment is a global challenge that requires sustainable solutions, especially due to its toxicity and persistence which can disrupt the balance of ecosystems and endanger human health. Bacteria-based bioremediation offers an effective and environmentally friendly approach to the decomposition of pesticides into less toxic compounds. Various species of bacteria, such as Pseudomonas, Burkholderia, and Sphingomonas, can degrade pesticides through enzymatic mechanisms, including hydrolysis, oxidation-reduction, and dehalogenation. However, the main challenges in the implementation of bioremediation include fluctuating environmental factors, degradation efficiency at high concentrations of pesticides, and limited microbial adaptation in various ecosystem conditions.

Technological advances have improved the efficiency of bioremediation through a variety of innovative approaches. Genetic engineering, particularly CRISPR-Cas9 technology, allows for the optimization of the expression of pesticide degradation genes in bacteria, thereby increasing the effectiveness of biodegradation. In addition, bioreactor systems have helped control environmental conditions to improve microbial stability and performance. Bacterial immobilization techniques using polymer or nanoparticle matrices have also been shown to prolong the degradation activity of microorganisms under extreme conditions. The microbial consortium-based approach has shown higher efficiency compared to single strains in dealing with complex pesticide degradation.

In the future, the integration of biotechnology with artificial intelligence and nanotechnology has the potential to significantly increase the effectiveness of bioremediation. With clearer regulatory support and ongoing research, this technology can be an innovative and sustainable solution in the mitigation of pesticide pollution and the protection of global ecosystems.

Index terms *Bioremediation, environmental pollution, genetic engineering, pesticide degrading bacteria.*

INTRODUCTION

Pesticides have become an indispensable part of modern agricultural systems due to their ability to protect crops from pests and improve agricultural yields. However, the massive and uncontrolled use of pesticides poses serious environmental impacts, especially the accumulation of residues in soil and water. The toxic nature and persistence of pesticides cause environmental pollution that is difficult to tackle, thus contributing to the degradation of ecosystem quality, a decrease in soil microbial biodiversity, and contamination of the food chain [1]. The presence of pesticide residues in food products is also a threat to human health, as some pesticide compounds are known to have carcinogenic, neurotoxic effects, and can cause endocrine disorders [2].

Various methods have been developed to deal with pesticide waste, including physical and chemical methods such as adsorption, photodegradation, and oxidative reactions. However, these methods are often ineffective in removing pesticides thoroughly and can even result in more toxic byproducts [3]. In addition, high operational costs and limitations in their application in a large-scale environment are the main obstacles to conventional techniques in pesticide waste treatment [4]. Therefore, biotechnology-based approaches, especially bioremediation using microorganisms,

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are being developed as a more environmentally friendly and sustainable alternative [5].

Bioremediation is an environmental restoration strategy that utilizes microorganisms to degrade or neutralize organic pollutants, including pesticides. Among the group of microorganisms that have bioremediation capabilities, bacteria play a key role in pesticide degradation due to their high metabolic flexibility as well as their ability to grow in a wide range of environmental conditions [6]. Several bacteria have been identified to have the ability to decompose different types of pesticides through specific enzymatic mechanisms. Bacteria of the genus Pseudomonas, Burkholderia, and Flavobacterium are known to be able to degrade pesticides such as organophosphates, carbamates, and organic chlorine through the activity of the hydrolase, oxidase, and dehalogenase they produce [7].

Although bacteria-based bioremediation has great potential in pesticide waste management, several challenges still need to be overcome for this technology to be applied more widely. Environmental factors such as temperature, pH, and nutrient availability greatly affect the effectiveness of bacteria in degrading pesticides [8]. In addition, some bacteria have limitations in adapting to environments that contain high concentrations of pesticides or suboptimal conditions [9]. Some bacterial strains that are effective in laboratory studies often experience reduced efficiency when applied in real environments due to their inability to compete with other microorganisms or due to the presence of environmental conditions that do not support their growth [10]. The stability and viability of bacteria under varying environmental conditions are still major challenges in the application of in-situ and ex-situ bioremediation [11].

Along with the increasing need for more efficient environmental management technologies, various innovations have been developed to improve the effectiveness of bacteria in pesticide bioremediation. One approach that is starting to be widely applied is genetic engineering to increase the expression of pesticide- degrading enzymes in certain bacteria. CRISPR-Cas9 technology has been used to modify genes in *Pseudomonas putida* to increase the activity of hydrolase enzymes that can decompose organophosphates more efficiently [12]. In addition to genetic engineering, the development of bioreactor systems is also one of the main strategies for improving the efficiency of pesticide degradation in industrial and agricultural waste. The use of bioreactors allows for better control of environmental conditions, allowing bacteria to work at a higher level of efficiency under optimal conditions [13].

In addition, bacterial immobilization techniques through the use of nanoparticles and polymer matrices were also developed to improve the stability and durability of bacteria in the face of fluctuating environmental conditions [14]. By immobilizing bacteria on a specific matrix, pesticide degradation can be carried out more efficiently and sustainably without losing the bacterial population due to leaching or competition with other microbes [15]. In addition to a technology-based approach, the strategy of utilizing microbial consortiums, which is a combination of several bacterial species with complementary metabolic pathways, also shows more effective results in dealing with complex pesticide contamination [16].

With the development of innovations in environmental biotechnology, the potential application of bacteria for pesticide bioremediation is getting wider, especially in agricultural land management and industrial waste treatment. Therefore, a deeper understanding of the mechanisms of pesticide degradation by bacteria, as well as innovative strategies in their application, is an important aspect that needs to be continuously developed [17].

This mini-review aims to review the latest developments in the use of bacteria for pesticide bioremediation by highlighting the degradation mechanisms used by bacteria, technological innovations that have been developed to improve their efficiency, and the challenges and prospects for the future application of this technology. Through this study, it is hoped that a more in-depth insight can be obtained regarding the optimization strategy of bacteria-based pesticide bioremediation, as well as its implications for environmental sustainability and the agricultural sector [18].

MECHANISM OF PESTICIDE DEGRADATION BY BACTERIA

Pesticides are chemical compounds that are generally stable and resistant to natural degradation, so they can last for a long time in the environment. However, some groups of bacteria can metabolize pesticides through complex enzymatic mechanisms. This process allows the conversion of pesticides into simpler, less toxic compounds, or even fully decomposed into carbon dioxide, water, and other inorganic compounds through a full mineralization process [19]. Pesticide degradation by bacteria can occur through several key biochemical pathways, including hydrolysis, oxidation-reduction, and dehalogenation.

3.1. Hydrolysis as an Initial Mechanism in Pesticide Degradation

Hydrolysis is one of the main mechanisms in the degradation of pesticides by bacteria. This process involves breaking chemical bonds in pesticide molecules through a reaction with water, which is catalyzed by hydrolase enzymes [20]. These enzymes facilitate the breakdown of ester, amide, and phosphate bonds in the pesticide structure, resulting in a simpler product that is easier to metabolize by bacteria.

Bacteria of the genera Pseudomonas and Flavobacterium are known to have hydrolase enzymes that can degrade various organophosphate pesticides such as paration and diazinone [21]. Recent studies have shown that *Pseudomonas putida* can hydrolyze paration through the activity of the enzyme paration hydrolase, producing p-nitrophenol and diethyl phosphate as intermediate products which can then be further elaborated [22]. In addition, the bacterium *Burkholderia cepacia* has been reported to have a specific enzyme that can hydrolyze

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the pesticide 2,4-Dichlorophenoxyacetate (2,4-D), which is one of the most widely used herbicides in the world [23].

The mechanism of hydrolysis is very important because it is often the first step in the degradation of pesticides before the intermediate product undergoes further transformation through oxidative or reductive pathways. The effectiveness of hydrolysis by bacteria is influenced by environmental conditions such as pH and temperature, where hydrolase enzymes generally show optimal activity in the neutral to slightly alkaline pH range [24].

3.2. Oxidation and Reduction in Pesticide Transformation

In addition to hydrolysis, oxidation and reduction are the main pathways in the transformation of pesticides by bacteria. This process is catalyzed by oxidase and reductase enzymes, which work by adding or removing electrons from pesticide molecules, thereby changing their structure to a more reactive and degradable form [25].

One notable example of this mechanism is the degradation of the organophosphate insecticide malathion by *Rhodococcus erythropolis*, which uses the enzyme oxidase to convert malathion into malaoxon, an intermediate product that is more easily degraded further by the bacterial metabolic pathway [26]. In addition, the peroxidase enzyme produced by *Phanerochaete chrysosporium* is effective in oxidizing organochlorine pesticides such as pentachlorophenol (PCP), which is a highly persistent compound in the environment [27].

In some cases, a reduction reaction is also required to remove toxic functional groups in pesticides. For example, reductase produced by *Sphingomonas* spp. can transform paration into a simpler intermediate product, reducing its toxicity and facilitating further degradation by other microorganisms within the soil microbial community [28].

3.1. Dehalogenation as a Key Strategy in Chlorine-Based Pesticide Biodegradation

Organic chlorine-based pesticides, such as DDT and lindan, are well-known for their very high persistence rates and their ability to accumulate in the tissues of living organisms through bioaccumulation processes [29]. To overcome this problem, some bacteria have special metabolic pathways that allow them to dehalogenate, that is, the removal of chlorine atoms from the structure of pesticides, thereby reducing their stability and toxicity.

Bacteria of the genera Rhodococcus and Sphingomonas have been identified as microorganisms that possess dehalogenase enzymes capable of degrading organochlorine pesticides [30]. For example, *Rhodococcus erythropolis* is known to produce a dehalogenase enzyme that can convert lindane into an intermediate product that is more biologically degradable [31]. Other studies have also shown that *Sphingomonas paucimobilis* can use DDT as a carbon source through a gradual dehalogenation process, ultimately resulting in less toxic compounds that can be further mineralized by other microbial communities [32].

Dehalogenation is an important step in the detoxification of organochlorine pesticides, but its efficiency is often affected by environmental conditions. For example, studies show that the activity of the enzyme dehalogenase increases in anaerobic conditions or in environments with high organic matter content, which serve as electron donors in redox reactions [33]. Therefore, optimization of environmental conditions is essential to support the effectiveness of bacteria in dehalogenation-based bioremediation processes.

3.2. Metabolic Pathway Interactions in Microbial Consortiums

In natural ecosystems, pesticide degradation is rarely carried out by a single species of bacteria, but more often occurs through the interaction of a consortium of microbes that have complementary metabolic pathways. This consortium is made up of several species of bacteria that work together in various stages of pesticide degradation, allowing the conversion of complex compounds into non-toxic end products [34]. For example, the combination of *Pseudomonas putida* and *Rhodococcus erythropolis* is more effective in deciphering organophosphate insecticides compared to each species individually. This is due to the differences in the enzymatic pathways they have, where *Pseudomonas putida* is more effective in early hydrolysis, while *Rhodococcus erythropolis* has an oxidase enzyme capable of accelerating the transformation of intermediate products into simpler compounds [35].

Other studies have also shown that the interaction between Sphingomonas and Burkholderia improves the degradation efficiency of the herbicide atrazin. Sphingomonas plays a role in the early stages of degradation by breaking chlorine bonds, while Burkholderia continues the degradation of intermediate products until complete mineralization occurs [36].

TECHNOLOGICAL INNOVATIONS IN PESTICIDE BIOREMEDIATION

Bacteria-based pesticide bioremediation has advanced rapidly thanks to various technological innovations that improve the degradation efficiency and stability of microbes in a wide range of environmental conditions. Technologies such as genetic engineering, bioreactor systems, bacterial immobilization techniques, and the utilization of microbial consortiums have been developed to overcome the limitations that have been a challenge in bioremediation applications at large. The development of this technology not only aims to increase the capacity of bacteria to degrade pesticides more effectively but also to ensure the sustainability of bioremediation processes on an industrial and agricultural scale [37].

3.1. Genetic Engineering for Improved Efficiency of Pesticide-Degrading Bacteria

One of the main approaches to improving the ability of bacteria to bioremediate pesticides is through genetic engineering. This technology allows modification of the expression of certain genes that play a role in pesticide degradation pathways, thereby increasing the efficiency and

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resistance of bacteria to extreme environmental conditions. One of the widely used techniques is CRISPR-Cas9, which allows for high-precision genetic manipulation. Several studies have successfully increased the activity of hydrolase enzymes in *Pseudomonas putida* through directed mutation techniques, which optimize the degradation of organophosphates [38].

In addition, cloning-based approaches and heterologous expression have been undertaken to increase the production of degrading enzymes. For example, the gene encoding the enzyme paration hydrolase from Flavobacterium has been successfully expressed in *Escherichia coli*, resulting in an engineered strain that is more efficient at degrading paration compared to natural strains [39]. The use of genetic-based biosensors has also been developed to detect the presence of certain pesticides and automatically induce the expression of degrading enzymes when pesticide concentrations increase [40].

Although genetic engineering promises to improve the efficiency of bioremediation, challenges in its implementation still exist, especially related to environmental safety and regulatory aspects. The release of engineered bacteria into the environment must go through rigorous evaluation to ensure that they do not disrupt the balance of the ecosystem or potentially cause unforeseen negative impacts [41].

3.2. The Use of Bioreactors for Bioremediation Optimization

Bioreactor technology has become an innovative approach to improving the efficiency of pesticide bioremediation on an industrial scale. Bioreactors allow for better control of environmental conditions such as pH, temperature, aeration, and nutrient availability, thereby improving the stability and activity of bacteria in degrading pesticides. Batch-based and continuous bioreactor systems have been developed for the treatment of wastewater containing pesticide residues, with results showing a significant increase in the rate of degradation compared to conventional methods [42].

One type of bioreactor that is effective in pesticide bioremediation is membrane bioreactors. This technology allows the separation of bacterial cells from the liquid medium thereby improving degradation efficiency and preventing the leaching of microorganisms during the process. Biofilm-based bioreactors have also been developed, in which pesticide-degrading bacteria are attached to the surface of a special medium to improve cell retention and extend contact time with pollutants [43].

Bioreactor technology is not only applied to industrial wastewater treatment but also in agricultural systems that use in situ bioremediation strategies. Mobile bioreactors have begun to be used on farmland to reduce pesticide residues in the soil before the next planting period. The implementation of this technology has the potential to reduce dependence on more expensive and less environmentally friendly physical and chemical methods [44].

3.3. Bacterial Immobilization to Improve the Stability and Effectiveness of Bioremediation

The bacterial immobilization technique is one of the innovations that aims to improve microbial stability in harsh environments. This process is accomplished by encapsulating bacteria into a matrix of polymers, nanoparticles, or organic materials such as alginate and chitosan, thereby protecting the cell from pH fluctuations, temperature extremes, and other environmental stresses [45].

The main advantage of immobilized bacteria is their ability to maintain enzymatic activity over a longer period compared to free bacteria. Recent studies have shown that *Pseudomonas putida* immobilized in carbon-based nanoparticles can degrade the herbicide atrazin with higher efficiency compared to free cells [46]. In addition, immobilization also allows for the reuse of microbes in the bioreactor system without the need for periodic reinoculation, thereby reducing operational costs [47].

Some of the immobilization technologies that have been developed include micro capsulation, embedding in organic matrices, and the use of inorganic materials such as zeolite and silica. Although this technology offers a wide range of advantages, the main challenge is the limitation of the diffusion of the substrate into the immobilization matrix, which can slow down the degradation of pesticides in some cases [48].

3.4. Utilization of Microbial Consortiums to Improve Bioremediation Efficiency

The latest approach in pesticide bioremediation is the utilization of microbial consortiums, which are combinations of several species of bacteria that work synergistically to degrade pesticides more efficiently compared to a single strain. This combination of microbes allows for gradual degradation, in which one species of bacteria breaks down complex compounds into intermediate products, while another species continues the degradation until complete mineralization occurs [49].

For example, the combination of *Pseudomonas putida* and *Rhodococcus erythropolis* is more effective at deciphering organophosphate insecticides compared to each species individually. In addition, the consortium of Sphingomonas and Burkholderia has demonstrated high efficiency in degrading the herbicide atrazin, with the mechanism by which Sphingomonas performs the initial stage of dehalogenation, while Burkholderia is responsible for the transformation of intermediate products into simpler compounds [50].

The use of microbial consortiums not only improves degradation efficiency but also increases resistance to environmental fluctuations. However, the main challenge in this approach is to ensure population balance under real conditions, as interactions between species can change depending on different environmental parameters [51].

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DYNAMICS OF CHALLENGES AND FUTURE DIRECTIONS OF PESTICIDE BIOREMEDIATION

Although bacteria-based bioremediation technology has come a long way, its widespread application still faces challenges that need to be overcome to become a more effective and sustainable solution. Factors such as bacterial resistance in a fluctuating environment, degradation effectiveness at high pesticide concentrations, and challenges in managing microbial ecosystems at bioremediation sites remain major obstacles. In addition, the prospects of pesticide bioremediation are also highly dependent on the development of innovative technologies that can optimize degradation efficiency, as well as regulatory support and public acceptance of microbial applications in the environment [52].

4.1. Stability and Adaptation of Bacteria in Diverse Environments

One of the biggest challenges in bioremediation is maintaining the stability and activity of bacteria in changing environmental conditions. External factors such as temperature, pH, salinity, and nutrient availability greatly affect the effectiveness of bacteria in decomposing pesticides. Some studies show that although bacteria such as *Pseudomonas putida* and *Burkholderia cepacia* have a high degradation potential under laboratory conditions, their activity often decreases when applied in an open environment with more complex variability of environmental parameters [53].

To address this problem, an approach based on microbial evolutionary adaptation and ecological engineering has been proposed. Long-term laboratory adaptation (ALE) methods have been used to improve bacterial resistance to extreme environmental conditions, such as pH variations and the presence of heavy metals often found in pesticide-contaminated soils [54]. Additionally, the use of microbial biofilms has been shown to improve bacterial survival by providing protection against environmental stresses and improving substrate accessibility [55].

4.2. Degradation Effectiveness at High Concentrations of Pesticides

Although pesticide-degrading bacteria have been extensively researched, their effectiveness is often reduced at very high concentrations of pesticides. At high doses, pesticides can be toxic to microorganisms, causing decreased bacterial growth and even cell death. Some studies have shown that exposure to pesticides at levels higher than LC50 (Lethal Concentration 50%) can inhibit the activity of degrading enzymes such as hydrolase and dehalogenase required in the bioremediation process [56].

To address these challenges, microbial consortium strategies have been implemented, in which a combination of several bacterial species is used to address different stages of pesticide degradation. For example, the combination of *Sphingomonas paucimobilis* and *Rhodococcus erythropolis* is more effective in deciphering organochlorins compared to each species individually [57]. Another approach that is beginning to develop is the use of natural biosurfactants, such as ramnolipids and sophorolipids, which can increase the bioavailability of pesticides in aqueous environments and soils, thus facilitating faster degradation [58].

4.3. Regulation and Safety in Bioremediation Applications

Regulations and policies governing the use of bacteria for bioremediation are still a challenge in the widespread application of this technology. Some countries have adopted strict guidelines for the release of genetically modified microbes into the environment, due to the potential ecological impacts that are not yet fully understood. The safety of pesticide-degrading microbes is also a major concern, especially related to the possibility of horizontal gene transfer that could lead to the emergence of antibiotic resistance or undesirable changes in soil microbial communities [59].

Currently, various international environmental organizations are developing ecosystem-based risk assessment standards to evaluate the long-term impacts of microbial bioremediation on soil and water biodiversity. One of the approaches being studied is the use of genetic biomonitoring, in which the expression of pesticide degradation genes is monitored in real time to ensure that the microbes used remain within safe limits for the ecosystem [60].

In addition to the regulatory aspect, the factor of public acceptance is also a consideration in the implementation of bacteria-based bioremediation. Public perceptions of microorganisms being modified or added to the environment are still mixed, with major concerns related to the potential side effects on human health and the environment. Therefore, transparency in effective scientific research and communication is crucial in increasing public acceptance of this technology [61].

4.4. Future Prospects: Technology Integration and a Holistic Approach

In the future, the development of bioremediation technologies will not only depend on improving the efficiency of bacteria in decomposing pesticides but also on integration with other technologies to form a more holistic approach. One of the growing trends is the use of artificial intelligence (AI) and machine learning to predict microbial interactions with pesticides in various environmental conditions. Using big data and bioinformatics models, researchers can design more efficient microbial communities for specific applications [62].

In addition, innovations in the field of nanotechnology have also begun to be used to increase the effectiveness of bioremediation. For example, carbon-based nanoparticles have been used to increase the absorption of pesticides in the environment, thereby increasing their bioavailability to degrading bacteria [63]. Another promising approach is plant-based bioremediation (phytoremediation) combined with rhizosphere bacteria, where microbes that are symbiotic with plant roots can increase pesticide degradation more efficiently in agricultural soils [64].

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As the environmental challenges caused by pesticide use become increasingly complex, a multidisciplinary approach that combines genetic engineering, bioinformatics, bioreactor engineering, and ecosystem mitigation strategies will be key in ensuring the success of bioremediation in the future. With further development in this technology, bacteria-based bioremediation could become a broader and more sustainable solution in pesticide waste management as well as global ecosystem protection [65].

CONCLUSION

Bacteria-based bioremediation is a promising solution for overcoming pesticide pollution in the environment. Various bacteria such as Pseudomonas, Burkholderia, and Sphingomonas are effective in decomposition pesticides through enzymatic pathways such as hydrolysis, oxidation-reduction, and dehalogenation. However, challenges in microbial stability, degradation effectiveness at high concentrations, and environmental fluctuations still limit their application on a wide scale.

Technological advances such as genetic engineering, bioreactors, immobilization techniques, and the utilization of microbial consortiums have improved the efficiency of bioremediation. However, regulatory challenges and potential environmental impacts still need to be considered before the application of this technology can be widely applied.

In the future, the integration of biotechnology with artificial intelligence and nanotechnology has the potential to increase the effectiveness and resistance of microbes in pesticide bioremediation. With the support of ongoing research and clear regulations, this technology can be a sustainable solution for pesticide pollution mitigation and global ecosystem protection.

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