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Review

Advanced Bioremediation Strategies for Organophosphorus Compounds

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Organophosphorus (OP) pesticides, particularly malathion, parathion, diazinon, and chlorpyrifos, are widely used in both agricultural and residential contexts. This refractory quality is shared by certain organ phosphorus insecticides, and it may have unintended consequences for certain non-target soil species. Bioremediation cleans organic and inorganic contaminants using microbes and plants. Organophosphate-hydrolyzing enzymes can transform pesticide residues into non-hazardous byproducts and are increasingly being considered viable solutions to the problem of decontamination. When coupled with system analysis, the multi-omics technique produces important data for functional validation and genetic manipulation, both of which may be used to boost the efficiency of bioremediation systems. RNA-guided nucleases and RNA-guided base editors include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR), which are used to alter genes and edit genomes. The review sheds light on key knowledge gaps and suggests approaches to pesticide cleanup using a variety of microbe-assisted methods. Researches, ecologists, and decision-makers can all benefit from having a better understanding of the usefulness and application of systems biology and gene editing in bioremediation evaluations.

Keywords: **Bioremediation, organophosphates, biodegradation network, genome editing**

Introduction

Owing to the widespread pollution of water and soil from unrestrained pesticide usage, research into pesticide degradation is crucial. For decades, farmers have used pesticides to lessen the spread of disease, protect crops from damage, enhance quality, and boost output [1]. When commercial pesticides are used in excess, only a small percentage kills the pests they are designed to kill, whereas the rest contaminate the environment. Chronic and acute consequences on non-target wildlife and human life are likely to result from this hazard to the soil and water [2]. Insects, plant diseases, weeds, mollusc's, birds, and nematodes are examples of agricultural pests that may be controlled using chemical or biological pesticides. The most popular pesticides include organochlorines, organophosphates, carbamates, pyrethroids, and triazines. The biological mechanism function or their methods of application have also led to the categorization of pesticides as contact, systemic, specific, or nonspecific. Pesticides are categorized by target and help increase crop productivity and reduce pest-related agricultural losses [3]. Humans and other animals that are not intended to be exposed to pesticides can get their hands on them in several ways [4]. Direct contact (via the skin, mouth, or lungs), as well as indirect contact through contaminated water and food sources, can lead to absorption of chemical residues into the circulation.

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Approximately 45 percent of the world's pesticides are organophosphates, which are extremely harmful to the soil and water ecology. Over 200,000 fatalities per year are attributed to approximately 140 different varieties of organophosphate insecticides used worldwide.

Pesticides can be detected in the environment using several different techniques, including capillary electrophoresis, gas chromatography, liquid chromatography, bioassays, and immunoassays [5–7]. The food supply, from the farm to the table, is contaminated with pesticides. Pesticide contamination has been reported in various foods, including fruit juices, milk, and seaweeds [8]. The elimination of these chemical compounds poses an enormous ecological challenge to the sustainability of life on Earth. Long-term exposure to pesticides, either orally or topically, poses a threat to human health and can result in ailments linked to metabolic, growth, and developmental issues as well as cancer in humans. Several mechanisms, such as oxidation, reduction, isomerization, conjugation, hydrolysis, hydration, dehalogenation, methylation, and cyclization, have been reported to contribute to the physical, chemical, and biological degradation of pesticides. Bioremediation is used more often to deal with pollution because of recent developments in gene editing, systems biology, and omics technology. With the help of systems biology, we can learn more about microbial communities and how they adapt to various settings, even the most hostile ones. In addition, this review discusses gene editing methods, such as CRISPR Cas, TALEN, and ZFNs, all of which have the potential to enable the creation of a design microbe that has a functional gene of interest for the degradation of recalcitrant pesticides and further enhance bioremediation.

Overview of Organophosphorus Pesticides

In terms of market share, organophosphorus (Ops) compounds account for almost 80% of all insecticides sold worldwide. Organophosphorus (OP) pesticides (malathion, parathion, diazinon, and chlorpyrifos) are among the most extensively used pesticides in both agricultural and residential settings. Every year, the United States uses approximately 80 million pounds of organophosphate (OP) pesticides, with agriculture accounting for 75 percent of that consumption. Poisoning from organophosphorus compounds is a worldwide health concern, resulting in approximately 3 million cases of poisoning and 200,000 fatalities per year [9, 10]. The ability of OPs to inhibit acetylcholinesterase, a critical enzyme in the control of the central and peripheral nervous systems, contributes to acute toxicity. OPs interfere with neurological signalling by inactivating acetylcholinesterase (AChE) via phosphorylation and irreversible adherence to the catalytic serine, which results in an increase in acetylcholine levels and cholinergic hyperactivity [11]. Salivation, vomiting, nausea, miosis, and convulsions can occur after exposure to large doses of OPs, leading to paralysis and death [12, 13]. Low-level, long-term exposition to OPs may also be harmful, with consequences that extend to the developing human brain and the health of children [14, 15].

Agrochemicals are eventually decomposed in soil by microorganisms, and it is well documented that only a fraction of the overall quantity of agrochemicals employed goes towards the prevention and treatment of pests and diseases, and the presence of agrochemicals and their breakdown products in the environment is unavoidable. A recalcitrant chemical does not break down in the environment because microorganisms lack the mechanism for utilizing it as a carbon or energy source. Some members of organ phosphorus pesticides have this refractory property and may consequently have adverse impacts on certain non-target soil organisms [2, 16]. Soil fertility and agricultural crop productivity rely on microbial transformations such as nitrification, ammonification, and organic matter decomposition, but these processes can be inhibited, eliminated, or altered by recalcitrant pesticides and their degradation products that accumulate in the topsoil [17]. Long-term exposure to pesticide-contaminated environments results in the evolution of microbial tolerance and degradation mechanisms against a particular pesticide; furthermore, they can be employed in the breakdown of pesticides [18]. When a soil microbe uses a pesticide as a carbon and energy source, catabolism and detoxification processes occur. Finding decontamination techniques that are both effective and practical is a serious challenge in light of the severe toxicity of OPs and the extensive usage of these chemicals. OP decontamination options have considered chemical, physical, and biological methods. These treatments are severe, unsuit-

able for decontaminating individuals, and unsuitable for large-scale environmental remediation. It is imperative that immediate action be taken to reduce the amount of environmental damage that is being brought about by the careless application of these chemical compounds in the field of agriculture.

Emerging Bioremediation Strategies

Bioremediation is the technique of using microorganisms and plants for environmental cleanup of both organic and inorganic xenobiotics [19]. Since bioremediation eliminates, degrades, detoxifies, and immobilizes dangerous wastes and pollutants, microorganisms are crucial. Understanding the whole range of physiological, microbiological, ecological, biochemical, and molecular processes involved in pollutant transformation is essential for successful bioremediation employing microorganisms [20]. *Ex situ* or *in situ* bioremediation can be utilized, depending on a variety of criteria, such as cost, pollutant kinds, and concentration. Bioremediation is cheaper than incineration, and certain contaminants may be handled on site, minimizing exposure hazards for clean-up workers or broader exposure from transportation accidents. Bioremediation carried out *in situ*, including bioventing, biosparging, and bioaugmentation, decontaminates without removing soil from the site while *ex situ* treatments (such as land farming, biopiling, composting, bioreactors, and electrodialysis) treat the soil that is unearthed at the location [21].

There are a number of factors that can affect how quickly pesticides break down in the soil. These factors include the amount of pesticides that are already there, the activity level of microorganisms that break down pesticides, the bioavailability of the pesticides, and a range of soil factors such as pH, soil water content, and temperature [22]. By increasing the richness of its species or modifying its enzyme systems, a microbiome can help speed up the agrochemical's metabolism and subsequent removal from the environment. Organophosphates' severe mammalian toxicity and ubiquitous and widespread use make microbial breakdown of these substances of special interest.

Microbial degradation of organophosphorus compounds

Communities of microorganisms have developed remarkable defenses against the hazards of organophosphorus chemicals. Microorganisms are able to breakdown and detoxify organophosphorus chemicals because they possess particular genes which encode enzymes which degrade these chemicals. The bacteria ability to degrade contaminants suggests interesting directions for bioremediation technologies and the recovery of polluted ecosystems. The effectiveness and environmental fate of organophosphorus chemicals like glyphosate, chlorpyrifos, malathion and parathion have been the subject of much research. Degradation of these substances primarily occurs through hydrolysis, oxidation, alkylation, and dealkylation. Degradation by microorganisms, especially hydrolysis, is an important aspect of the detoxification process.

The bacterial genus *Pseudomonas* appears to be particularly effective when it comes to degrading pesticides, since it is able to break down approximately 90–99 percent of them [23]. *Bacillus pumilus* strain C2A1, *Bacillus aryabhattai*, *Streptomyces olivochromogenes*, *Pseudomonas resinovarans* AST2.2 and *P. indoloxydans* has been shown to effectively degrade chlorpyrifos [24–28]. Evidence suggests that microorganisms may remove chlorpyrifos from the environment, albeit the rate of removal varies by strain. Complete removal of chlorpyrifos was obtained by *Stenotrophomonas* sp. after 28 h of treatment, by *Enterobacter* strain B-14 after 24 h, and by *Sphingomonas* sp. within 24 h [29]. Even more effective were *Trichosporon* sp. and *Serratia* sp., which were able to eliminate chlorpyrifos entirely in just 18 h [30]. Researchers in their work, used six wild-type microorganisms as prospective phosphotriesterases sources: *Streptomyces phaeochromogenes*, *Streptomyces setonii*, *Nocardia corynebacterioides*, *Nocardia asteroides*, and two *Arthrobacter oxydans* and then these were evaluated to hydrolyze paraoxon, methyl paraoxon, methyl parathion, coumaphos, dichlorvos, and chlorpyrifos [1].

The breakdown of pesticides into their component parts occurs in three distinct stages. Oxidation, reduction, or hydrolysis are some of the processes that are used to convert the potentially harmful forms of these chemicals into essences that are less damaging, water-soluble, and non-toxic. In the second act, the altered elixirs are combined with sugar or amino acids in order to produce an amalgam that is compatible with the other

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ingredients. In the third stage extracellular hydrolytic enzymes of bacteria and fungi conclude the degradation [31].

Organophosphate degradative genes

Degradative genes are often grouped or placed inside operons to enable the efficient degradation of organophosphorus compounds. Microbial communities in contaminated environments may acquire more degradation genes over time as they become more suited to the pollution. It is possible to enhance biodegradability by genetic engineering by recombining genes, which plays a role in biodegradability. That's why it's crucial to identify the genes responsible for resistance. It has been determined that several different microbial species are capable of degrading chlorpyrifos. *Pseudomonas* sp. WBC-3 is notable in that it possesses the *oph* gene, which is the gene responsible for the first step in the breakdown of chlorpyrifos. This *oph* gene is responsible for encoding the enzyme known as organophosphorus hydrolase (OPH), which is responsible for catalyzing the hydrolysis of chlorpyrifos into 3,5,6-trichloro-2-pyridinol (TCP). Many different kinds of microbes contribute to the breakdown of parathion. The *phd* gene, which encodes paraoxonase, hydrolyzes parathion to p-nitrophenol and is found in *Pseudomonas aeruginosa* and *Pseudomonas* sp. WBC-3 [32].

Support for enhancing organophosphorus biodegradation has been greatly bolstered by the discovery of many genes encoding organophosphorus degrading enzymes like *opd*, *mpd*, and *oph* [33–35]. *P. diminuta's* pCMS1 plasmid included the first known organophosphorus degrading (opd) gene. The gene was found to be 1.5 Kb and had restriction sites for SalI, PstI, XhoI-restricted and BamHI. In *Flavobacterium* sp., a plasmid designated pPDL2 had additional opd gene with an analogous restriction map. Widespread degrading ability against organophosphorus pesticides has been discovered after the isolation of the *opd* gene from *Enterobacter* sp. cons002. The breakdown power of altered OPH enzymes on P-S bonds of organophosphorus insecticides can be increased by genetic mutation and screening of OPH expressing genes. Parathion and fenitrothion are two pesticides that can be broken down by an enzyme encoded by the *mpd* gene, which is separate from the opd gene. Gene cloning has been used to effectively express the *ophc2* gene, which has little similarity to other organophosphorus hydrolase genes, in *Pichia pastoris* and *Escherichia coli* [36, 37]. Table 1 summarizes the several bacterial genes that contribute to the breakdown of organophosphates. Toxic pesticides are degraded into less dangerous compounds by the enzymes produced by these genes, which aids in environmental cleanup.

Enzymes degrading organophosphate compounds

Organophosphate insecticides (OPs) can be broken down by a wide variety of enzymes that can be found in both microorganisms and mammals. A few examples of these enzymes include the organophosphorus acid anhy-

Table 1. Microbial genes playing role in organophosphorus degradation.

Sr No.	Organophosphate	Gene	Source Microorganism	References
1	Chlorpyrifos	mpd	*Plesiomonas* sp. *M6*	[38]
2	Phosmet and Fenthion	opd	*Agrobacterium radiobacter P230*	[39]
3	Chlorpyrifos	mpd	*Cupriavidus* sp. *DT-1*	[40]
4	Chlorpyrifos	opdD	*Lactobacillus sakei WCP904*	[34]
5	Chlorpyrifos	mpd	*Ochrobactrum* sp. *JAS2*	[41]
6	Parathion	phd	*Pseudomonas aeruginosa*	[38]
7	Methyl parathion	ophc2	*Stenotrophomonas* sp. *SMSP-1*	[42]
8	Methyl parathion, Phorate, Parathion	opdE	*Enterobacter* sp.	[43]
9	Dimethoate, Paraoxon, Methyl parathion	mph	*Serratia marcescens MEW06*	[44]
10	Chlorpyrifos, Coumaphos, Diazinon, Methyl parathion, and Parathion	opdB	*Lactobacillus brevis WCP902*	[45]
11	Triazophos, Methyl parathion	tpd	*Ochrobactrum* sp., *Pseudomonas putida KT2440*	[46, 47]

drolase (OPAA), which was isolated from rabbit liver; the organophosphorus degrading hydrolases (OPHs), which involved phosphotriesterase (PTE) and organophosphorus degradation enzyme (OPD); and the methyl parathion hydrolase (MPH) and glycerophosphodiesterase (GpdQ), which were isolated from soil microorganisms [48].

The toxicity of organophosphorus compounds to animals including humans is much diminished once they have been hydrolyzed or breakdown. Organophosphate hydrolyzing enzymes are gaining popularity as potential solutions to the problem of decontamination since they can break down pesticides into residues that are harmless to people and the environment. Researchers have devised techniques to make use of the enzymes for biological remediation of OP chemicals, as microbial species have swiftly evolved enzymatic systems to breakdown these toxins. Enzymatic detoxification is an effective and environmentally friendly method for removing organophosphate pesticides and pollutants from contaminated sites [49]. Phosphatases, a type of hydrolase enzyme, have been widely used to eliminate phosphate groups from compounds, such as organophosphate pesticides, proteins, alkaloids, and nucleotides. Among these enzymes, phosphotriesterase (PTE) or organophosphorus hydrolase (OPH) was the earliest discovered enzyme with the ability to hydrolyze different types of organophosphate compounds by breaking P-S and P-O linkages. Microorganisms, particularly bacteria, are the primary source of these enzymes, including OPH, OP acid anhydrolases (OPAA), and methyl parathion hydrolase (MPH), which have proven to be highly effective for pesticide elimination.

S. phaeochromogenes entire cells converted 98% of chlorpyrifos into hydrolysis products at pH 8 and 40℃. Immobilized whole cells and enzyme extracts had similar hydrolytic activities, suggesting that both can be used depending on the application. Immobilization approaches, such as the utilization of genetically altered bacteria and bacterial consortia immobilized on diverse matrices, have been shown to be effective in pesticide biodegradation [50–52]. Compared with free cell cultures, studies have demonstrated that immobilized cells degrade and mineralize poisons more effectively. Immobilized cells showed superior breakdown rates compared to free cells when used for the treatment of chlorinated phenols and chlorpyrifos. Bio stimulation approaches, when used in conjunction with immobilized cells, represent a novel and promising approach to the bioremediation of pesticide-contaminated sites. Specific catalytic activity against a range of organophosphate pesticides has been observed in phosphotriesterases isolated from *Flavobacterium* sp. Another notable enzyme, OpdA, derived from *Agrobacterium radiobacter*, is an effective organophosphate-degrading enzyme that differs from OPH in substrate specificity because of differences in its active site structure [39, 53]. Immobilized OpdA has been demonstrated to efficiently remove methyl parathions from solution. Hydrolysis plays a crucial role in the breakdown of organophosphate compounds, making them more susceptible to microbial degradation. Organophosphorus hydrolase (OPH) and organophosphorus acid anhydrolase (OPAA) are two enzymes that have garnered the most attention. OPH is an enzyme found in a variety of bacteria; it has been the subject of much research due to its broad substrate specificity.

OpdB, a member of the OPD subclass, exhibits a hydrolase domain and displays a sequence identity of 52% with OPD. OpdB enzymes, in both their crude and their purified form, have an impressive capacity for the breakdown of organophosphates [54]. The discovery of this enzyme was kicked off by research conducted on a number of organophosphates (OPs) that were discovered in eight distinct types of pesticides. The capability of the *Cupriavidus nantongensis* X1T strain to hydrolyze OPs led to the discovery of the opdB gene, which is responsible for the breakdown of Ops [55].

Genetic engineering has been used to engineer microorganisms capable of the complete mineralization of organophosphorus compounds. Mutants with enhanced activity were obtained through directed evolution, leading to increased protein expression and improved degradation properties. Mutagenesis increases enzyme activity and substrate selectivity. More research is required to decipher the catalytic processes and evolutionary history of these enzymes. Table 2 summarizes various enzymes with potential to degrade organophosphorus compounds. Organophosphorus-degrading enzymes are increasingly used for prophylactic, therapeutic, and environmental purposes. Future research may improve the enzyme activity against difficult substrates and chemical combinations. New protein sequencing and struc-

Table 2. Key enzymes in organophosphate pesticide breakdown.

S. No.	Enzyme	Source Organism	Degrading Organophosphorus Pesticide	Reference
1	Paraoxonase (PON1)	Human	Parathion, Malathion, Chlorpyrifos, Diazinon	[56]
2	Organophosphorus hydrolase (OPH)	*Pseudomonas indoloxydans*	Chlorpyrifos	[28]
3	Organophosphorus hydrolase (OPH)	*Pseudomonas resinovarans*	Chlorpyrifos	[27]
4	Hydrolase	*Cladosporium cladosporioides*	Chlorpyrifos	[57]
5	Hydrolase	*Plesiomonas* sp.	Methyl Parathion	[38]
6	Hydrolase	*Flavobacterium* sp.	Parathion	[58]
7	Phosphotriesterase	*Pseudomonas monteilii C11*	Coroxon	[39]
8	Carboxylesterase	*Pseudomonas* sp. *M-3*	Malathion	[59]
9	Organophosphorus hydrolase (OPH)	*Pseudomonas* sp. *BF1-3*	Chlorpyrifos	[60]
10	Methyl Parathion hydrolase	*Burkholderia cepacia*	Methyl Parathion	[61]
11	Esterase B1	*Bacillus strain C5*	Methyl Parathion	[62]
12	Organophosphorus phosphatases	*Bacillus thuringiensis MB497*	Chlorpyrifos, Triazophos and Dimethoate	[63]
13	Alkaline phosphatase	*Spirulina platensis*	Chlorpyrifos	[64]
14	Phosphotriesterase	*Pseudomonas diminuta*	Methyl Parathion, Diazinon, Cyanophos, Dursban, Parathion, Coumophos	[65]
15	Methyl Parathion hydrolase	*Serratia marcescens MEW06.*	Methyl Parathion, Paraoxon, Dimethoate	[44]

tural determination will improve enzymatic destruction technologies, notably for chemical warfare weapons, and our understanding of evolutionary routes.

The multi omics approach

Microorganisms are essential for pesticide degradation in both soil and water. Important natural processes, such as nutrient recycling, also rely on these bacteria. However, researchers still need more data on the interplay between genes in these bacteria to accurately anticipate how pesticides will break down. Researchers seek to better understand how these bacteria degrade pesticides by integrating information from systems biology and biogeochemistry [66]. With this information, we can develop procedures and guidelines for the safe and effective management of areas polluted by pesticides [67]. Degradation networks, computational biology, and state-of-the-art high-throughput omics tools have helped researchers to examine how bacteria degrade pesticides [68]. Researchers should investigate how various microorganisms in a community interact with one another and learn how microbial systems impact other biological systems. Many researchers have found that modern multi-omics techniques, such as genomics, metagenomics, transcriptomics, metabolomics, and proteomic analysis, were used to collect data on the gene and protein synthesis stages in whole microbial taxa in hostile environments to determine how biodegradation works and how to use it effectively [69–71]. Recent advances in sequencing methods have also helped isolate genes involved in the degradation of persistent contaminants [72]. Owing to the rapid development of gene sequencing technology, particularly high-throughput sequencing (HTS), several bacteria with biodegradation capabilities have been discovered [73]. HTS and metagenomics work together to reveal the species of bacteria that interact with one another in a given ecosystem. We learned more about the traits of biodegrading bacteria through transcriptomics, proteomics, and metabolomics research into their genotypes and phenotypes. Genome-scale models (GEMs) that help choose the most efficient microbes for bioremediation may be developed with the use of this information. Modelling pesticide breakdown can be improved by using information gained from diverse microbial omics [74]. Microbial strains from genera *Paenibacillus* sp., *Pseudomonas* sp., *Burkholderia* sp., *Rhodococcus* sp., and *Pencillium* sp. have been demonstrated to be capable of degrading chlorpyrifos, parathion, dimethoate, deltamethrin, p-nitrophenol, chlorimuron-ethyl, and nicosulfuron by using a systems

biology approach that accompanied multi-omics strategies [75]. Particularly promising for bioremediation of pesticides are bacterial strains for which the complete genome has been sequenced, such as *Pseudomonas putida* KT2440 and *Rhodococcus* sp. Integrated omics techniques, which include genomics, transcriptomics, metabolomics, and proteomics, are helpful in systems biology research on microorganisms to examine the genetic-level regulation of bioremediation processes [74]. In the study of the transcriptome, methods such as microarrays and, more recently, next-generation sequencing especially RNA-Seq have been utilized to evaluate the response of microorganisms to various types of pesticides [71]. The complex processes, metabolic activity, genetic regulation, and molecular biological variables involved in the breakdown of xenobiotics by microorganisms were investigated. To improve the efficacy of bioremediation systems, the multi-omics approach, when combined with system analysis, generates a useful output for functional validation and genetic manipulation.

Biodegradation network

An innovative strategy for pesticide bioremediation is the use of computational tools and bioinformatic resources. Information on microbial biodegradation pathways and the biocatalytic processes involved may be found in online biodegradative databases including UM-BBD, Bionemo, PTID, MBGD, OxDBase, BioCyc, and MetaCyc [76]. These resources help find info on microorganisms degrading pesticides and also identify microbial degradation intermediates. The database UM-BBD, maintained by the University of Minnesota, demonstrates how microorganisms utilise enzymes to degrade several pesticides into less toxic chemicals [77]. MetaRouter is a one-stop shop for biodegradation and bioremediation data mining [78], whereas MBGD makes it easier to do comparative genomic research [79, 80]. Biodegradative oxygenases are the main focus of OxDBases because of their importance in the degradation of persistent organic molecules. OxDBase is a powerful tool applicable to bioremediation studies, providing knowledge of oxygenases-catalyzed reactions and aiding in the understanding of degradation processes involved in bioremediation [81, 82]. Biodegradation gene sequences and data on their transcription and regulation can be found in Bionemo [76]. These records will help in the construction of reliable biodegradation systems. Utilizing various computational tools and resources from the field of bioinformatics will allow us to get an understanding of the process by which microorganisms in the environment break down harmful chemicals such as pesticides. With the provision of information on enzymes, genes, and reactions, the databases contribute to the process of bioremediation. To better clean the environment and lessen the effects of dangerous substances, scientists must understand these processes.

Way microbes interact with chemical substances and their potential use in bioremediation is being studied using state-of-the-art scientific tools and systems biology methodologies. Enzyme-based processes for enhanced bioremediation and soil health have been developed using computational approaches. Computational biology and other similar in-the-computer methods have made the study of genes, proteins, and cellular systems possible. Biodegradation and bioremediation rely on intricate metabolic pathways, which can only be fully comprehended with the aid of these computational methods. Data mining and comprehension of cellular metabolic networks, especially as they pertain to biodegradation and bioremediation, can be accomplished using a number of *in silico* methods [8]. Stoichiometric studies of metabolic networks are commonly performed using tools such as flux balance, metabolic flux, and metabolic route analyses. Increased consumption of pesticide chemicals and tinkering with the features of degrading bacteria are both possible with the use of computational techniques. To foretell how pesticide chemicals would interact with bioremediating bacteria, scientists employ quantitative structure-activity relationship (QSAR) and three-dimensional quantitative structure-activity relationship (3DQSAR) models [83]. For effective pesticide bioremediation, we can manipulate genes, introduce novel enzymes, and control metabolic pathways using tools such as OptKnock, OptStrain, and OptReg [8]. To further bioremediation studies, these computational tools will aid in the comprehension of genome-scale models, interacting genes, and genomic data.

Genome editing

Editing genes is a unique technique that allows precise manipulation of DNA through the use of designed

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nucleases. Meganucleases, also referred to as homing nucleases, were one of the earliest types of nucleases designed to target specific genomic regions for use in gene editing [84, 85]. Meganucleases have long nucleotide recognition sequences that may be present once in the genome, and these nucleases cause a double-strand break (DSB) when they reach their target. Protein engineering, structure-based design, and molecular evolution are some of the methods that may be used to reengineer meganucleases to target novel sequences, but the process is often time-consuming [86]. To edit DNA, one must first locate the target gene using a reference classification system created for that gene, then allow for a disruption that will be repaired by homologous recombination, and finally modify the desired sequence components [87]. Genome expurgation, also known as genome editing, is a form of clean gene technology that safely eliminates genetic markers that are utilized throughout the process of genetic modification; hence, plants are free of any genetic residue. This approach has the potential to lessen the risk caused by bacteria that have antibiotic resistance markers or intentionally chosen markers. Technological developments need to come in the form of more potent genome-editing technologies, which may allow for a broader range of comprehension. This includes creating novel strains that are genetically amenable to transformation and development of CRISPR-Cas-based methods for multiplex genome silencing and editing. A systems biology-based high-throughput strategy that combines the use of synthetic biology and genome modification with in silico simulation and prediction is expected to significantly accelerate the method of transformation in cells [88].

The eradication of xenobiotics, simplification of complex molecules, and breakdown of highly hazardous chemicals into less harmful compounds include all examples of biodegradation, and further genetic editing techniques can speed up the biodegradation process by changing the gene architecture of microbes [89]. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat/CRISPR-associated protein (CRISPR/Cas) are commonly used tools for genome editing [90–92]. Techniques such as genome editing and systems biology hold great promise for use in the bioremediation of heavy metals, persistent organic pollutants, gasoline, acid drainage, and xenobiotics.

Genome editing tools. The discovery of novel genes involved in the degradation of various recalcitrant pollutants has been aided by high-throughput next-generation sequencing [93]. CRISPR-Cas, ZFN, and TALEN are the main gene editing techniques that can be applied to the aforementioned promises [91, 92]. ZFNs and TALENs merge transcription factor DNA-binding domains with the nuclease domain of FokI, creating an obligatory dimer. Zinc finger DNA-binding domains and TAL effector DNA-binding domains are responsible for this cleavage. These genome-editing techniques generate double-stranded breaks (DSBs) in the target gene sequence, which are subsequently repaired by homology-directed repair (HDR) and non-homologous end joining (NHEJ) [94]. The goal of these genome-editing technologies is to produce microorganisms of the best possible quality, as well as better microorganisms with more complex genetic factors [95]. We can engineer new microorganisms with desirable traits by altering the genetic makeup of their wild ancestors [96]. Limitations of the use of the aforementioned genome editing procedures include off-target mutations, dangerous mutations, and the possibility of unintentional release of modified organisms into the natural environment [97].

Zinc finger nucleases. Zinc finger nucleases (ZFNs) are a specialized class of DNA-binding proteins created specifically for targeting and modifying particular DNA regions [98]. Zinc Finger Nucleases contain Zinc Finger Proteins (ZFPs) that are artificial restriction enzymes. Eukaryotic ZFPs are transcription factors that double the DNA-binding domains. The nucleotide cleavage domain (Folk I) from *Flavobacterium okeanokoites* is also present in the ZFNs. The cleavage domain is normally surrounded by four to six ZFPs; however, this number may vary depending on the target site. The ZFPs' 18-bp target specificity enables highly efficient targeted genome editing. ZFPs include 30 amino acids and are folded into a three-dimensional structure consisting of an alpha helix and two antiparallel beta sheets [8]. The programmable nuclease, ZFN, is composed of a sequence-specific zinc-finger protein with a nuclease domain obtained from the FokI restriction endonuclease.

DSBs of ZFNs are induced by their DNA-cleavage domain once the DNA-binding domain recognizes the target DNA sequence. DNA damage is repaired by the cell's own mechanisms, which can lead to alterations or disruptions in the genes.

Although ZFNs have received much attention for their potential use in genome editing, their direct role in the bioremediation of pesticides is not very significant. ZFNs can be employed to create microbes or plants with enhanced ability to break down or detoxify pesticides. ZFNs can improve the bioremediation capacity of these species by altering genes involved in pesticide metabolism or resistance [8]. One strategy is to employ ZFNs to induce specific genetic alterations in pesticide-degrading microbes. ZFNs may improve the efficiency and efficacy of enzymes that assist in pesticide breakdown by precisely altering the genes essential for pesticide synthesis. This may lead to microorganisms with enhanced ability to break down or eliminate pesticide chemicals. ZFNs can also be used to create pesticide-resistant or detoxifying food crops. ZFNs can modify critical genes involved in pesticide sensitivity or metabolism to confer resistance or increase the plant's pesticide breakdown.

Transcription activator-like effector nucleases. When it comes to genome editing and modification, TALENS is state-of-the-art. TALENS contain TAL proteins that originate from the pathogenic bacterial species, *Xanthomonas*. TALEN comprises a DNA-binding domain and DNA-cleavage domain. The DNA-binding domain is similar to that of transcription activator-like effectors (TALEs). Tal proteins bind to sequences as short as 1–2 nucleotides hence making them extremely efficient. As a result of the presence of thirty-four tandem repetitions of amino acids, the nucleases implicated were extremely successful in binding. The TALE protein and nuclease domain of the FokI restriction endonuclease make up TALEN, a programmable nuclease. DSBs are created when the DNA cleavage domains of TALENs attach to their target DNA sequences. Although DSBs are very harmful, all existing organisms have developed repair mechanisms to restore the original sequence and preserve genomic function. TALENs were chosen to knock out the gene of interest (NHEJ) and knock out the target gene (HDR). The use of TALENs allows for the modification of crop plant genes that are involved in the metabolism or detoxification of pesticides, the introduction of new genes or metabolic pathways into microorganisms to breakdown particular pesticide compounds, and the modification of genes in microorganisms that encode specific enzymes that are involved in the degradation of pesticides [99].

DNA-binding and DNA-cleavage domains derived from TALE and FokI endonucleases, respectively, are combined to form TALEN. TALEN was initially used to disrupt genes and regulate transcription in the fungus *Trichoderma reesei* [100], and to modify genes in *Rhizopus oryzae* [101]. TALEN have several benefits over CRISPR, including a larger variety of target locations, fewer off-target effects, and greater editing effectiveness in heterochromatin areas. The creation of zinc finger nucleases and transcription activator-like effector nucleases requires considerable time and effort, further restricting their application in genome editing. ZFNs and TALENs are less common than CRISPR/Cas because of their complexity, high efficiency, and multiplexed genome editing capabilities [102, 103].

CRISPR-Cas. To perform precise genome editing or regulation at the DNA level, a molecular machine is required which consists of two primary components: a DNA-binding domain that recognizes and binds to specific DNA sequences, and an effector domain that enables DNA cleavage or regulates transcription near the binding site. CRISPR-Cas systems can be categorized into three distinct types: I, II, and III. Each system, including the model organisms, has its own distinct Cas [104]. CRISPR/Cas 9 has been widely studied and used for gene editing. This system is made up of endonuclease Cas9, CRISPR-derived RNA (crRNA), and trans-activating CRISPR RNA (tracer RNA) and has been the subject of numerous scientific investigations and developments [105]. Cas protein and guide RNAs (usually between 18 and 20 nucleotides in length) are two components used in CRISPR CAS technology. The advent of Cas9, an RNA-guided DNA endonuclease, has greatly simplified sequence-specific gene editing because it can be developed to target new sites by modifying its guide RNA sequence. Cas9, in its nuclease-inactive state, offers a flexible RNA-guided DNA-targeting platform for sequence-specific genome regulation, imaging, and epigenetic editing. Cleavage by *Streptococcus pyogenes* Cas9

(SpCas9) leads to blunt-end double-strand breaks (DSBs), whereas cleavage by Cas12a, which requires a crRNA to identify the T-rich PAM site, leads to staggered-end DSBs [106]. The complex, which consists of Cas9 and sgRNA, causes the nucleases RuvC and HNH to cleave the target DNA, resulting in breaks in the complementary and non-complementary strands, respectively. Specific guide RNAs (SgRNAs) are constructed to target locations three base pairs upstream of the protospacer adjacent motif (PAM) [107, 108]. The two endonuclease domains (RuvC and HNH) of the Cas enzyme cleave target DNA strands and generate double-stranded breaks (DSBs), which are subsequently repaired by processes such as non-homologous end joining (NHEJ) and homology directed repair (HDR).

Single-strand annealing (SSA) and microhomology-mediated end joining (MMEJ) are two additional error-prone repair processes [109]. After a double-strand break (DSB), mutations such as indels and substitutions frequently result from NHEJ mechanisms [110]. NHEJ is recommended for gain/loss of functionality due to the mutagenic behaviour of likely insertions or deletions (indels) that result in changed reading frames and the development of mutations. In contrast, HR is a cell cycle-dependent event that takes place during the S/G2 phase and has a decreased efficiency of genome editing owing to its reliance on a template to carry out the repair process. In most cases, HR is the method of choice for gene knockout and insertion investigations. In addition to DNA, RNA can also be edited using CRISPR/Cas systems. Cas9, Cmr/Csm, and Cas13 are only a few CRISPR systems that can target and edit RNA molecules. A number of organisms, including fungi, have used these RNA-targeting tools to silence particular RNA, alter RNA in precise areas, and monitor RNA levels [111–113]. The key amino acids present in the nuclease domains of Cas9 and Cas12a have been changed to make their inactive variants [114]. These variants still have the capacity to attach to specific targets and hence allow for gene expression modification in various species. Repression of gene transcription by damaging RNA transcription factors is possible using inactive forms of Cas9 or Cas12a. Furthermore, these inactive variations can be used to stimulate gene expression via rearrangement of transcription activation regions. Cas12a, a novel endonuclease, cuts DNA without CRISPR RNA. Cas9 and Cas12a can create double-stranded breaks at several genomic target locations by using various crRNA guides.

Clustered Regularly Interspaced Short Palindromic Repeats technology has great potential to change many areas of biotechnology, and bioremediation is one area where it can make a significant difference. Conventional bioremediation methods are inefficient, slow, and difficult to target particular contaminants. Molecular biologists are warming up to CRISPR because this technique can be used with bacterial, fungal, and archaeal systems [115]. CRISPR technology, which permits genome editing, may help overcome these limitations. The bioremediation processes, such as the removal of xenobiotics, the transformation of harmful substances into less toxic substances, and the breakdown of pesticides into simple components, can all be significantly improved by the use of gene editing techniques [89, 95]. The end goal of these gene-editing methods is to develop microorganisms with the highest possible quality and to generate microbes with more complicated genes [95, 116]. Scientists can enhance the metabolic pathways, pollutant tolerance, and contaminant removal efficiency of microorganisms employed in bioremediation by modifying their genes employing CRISPR technology. This precise engineering improves the organisms' capabilities, leading to stronger and more efficient bioremediation procedures. Bioremediation and the degradation of pesticides by rhizospheric bacteria via CRISPR Cas mechanisms have attracted a lot of attention recently [117, 118].

Achieving gene editing successful is a major challenge since it depends on a wide variety of parameters, including the speed of the Cas enzyme, the design of the sgRNA, the number of copies of the gene, and many more. The form of sgRNA is a crucial aspect that is often overlooked by current sgRNA design methods. In order to better forecast the morphologies of sgRNAs and enhance the design process, we need more accurate computer models. The accessibility of the target gene and presence of additional substances in its immediate vicinity can potentially affect the success of gene editing. For instance, the Cas enzyme may have difficulty accessing the target gene if it passes through a stretch of the DNA. Improved sgRNA design requires the creation of methods for predicting the DNA structure around target gene. Off-target mutations, deadly mutations, and the potential for inadvertent or purposeful release of trans-

formed organisms into the environment, among other things, restrict the use of the aforementioned gene editing methods with functional genes of interest [97].

The benefit of CRISPR technology is that it allows multiplexed genome editing, which means that numerous genes may be targeted at once. This allows researchers to fine-tune microbes for use in bioremediation of targeted contaminants and habitats. The effectiveness and adaptability of cleaning can be increased by focusing on several genes implicated in biodegradation. To detect environmental toxins, CRISPR-based biosensors integrate the CRISPR-Cas system with a reporter system. These biosensors enable effective monitoring of polluted areas by providing quick and sensitive detection of contaminants through the construction of appropriate gRNAs. This makes it easier to implement precise bioremediation solutions to clean up certain areas. Precise delivery of genetic alterations to microbes in contaminated settings is now possible using CRISPR technology. Researchers can improve the performance of preexisting microbial communities for bioremediation without having to replace them entirely by infusing Cas9, gRNA, and other components, or by employing viral vectors. To correct errors in the DNA code that trigger disease, researchers have developed base editors as a new tool. These base editors combine enzymes that can alter specific bases in the DNA code (nCas9) with the original Cas9 protein. Cytidine and adenosine base editors are the two main types of chromatin modifications. Base editors for cytidine alter C to T and G to A [119], whereas those for adenosine switch A to G and T to C [120]. These base editors have been successfully used in living organisms to make precise changes to their DNA at specific locations. These base editors have been successfully used in living organisms to induce precise changes in their DNA at specific locations [121, 122].

Bacteria that thrive in polluted environments are considered to be promising option for metabolic engineering and genome editing because of their capability to adapt and survive in a variety of harsh environments that include poisonous, refractory, and non-degradable xenobiotics [123]. CRISPR-Cas technology has been widely adopted for use in model organisms such as *Pseudomonas* and *E. coli* [124]. Recent research has shown that non-model bacteria notably *Achromobacter* sp. HZ01 and *Comamonas testosteroni* are potentially effective when used in bioremediation [125]. Because of advancements in our understanding of CRISPR tools including the invention of gene-targeting gRNA, these species now have the ability to express particular genes that are critical for ecological restoration. Researchers have genetically manipulated the *Cupriavidus nantongensis* X1T strain using the CRISPR/Cas9 system in conjunction with the red system. Following the construction of two plasmids, pACasN and pDCRH, and their subsequent transfer to the X1T strain, targeted deletion of the opdB gene, which is responsible for the catabolism of organophosphorus pesticides, was achieved. This work shed light on gene targeting and the breakdown process of organophosphorus insecticides in the *Cupriavidus* genus, which was the subject of the study [126]. CRISPR-Cas9 was recently utilized by researchers to remove the yvmC gene from *Bacillus licheniformis*, increasing biotransformation efficiency to 100%. CRISPR-Cas9 may be used to improve organism strains in the future. CRISPR-cas9 can create bacteria that bioremediate environmental OPs [127].

Conclusion and Future Perspective

The application of CRISPR technology presents a significant opportunity to fundamentally alter bioremediation. Scientists are able to improve the metabolic capacities of microorganisms, construct contamination-sensing systems, and design synthetic microbes to target and efficiently degrade pollutants using precise and diverse genome editing capabilities. These innovations have the potential to dramatically increase the efficiency and speed of bioremediation operations, leading to cleaner and healthier ecosystems for humans.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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