

## Review

# From Chlorpyrifos Degradation to Detoxification: Bacterial Diversity, Metabolic Pathways, Microbial Consortia, and Prospects for Field-Scale Bioremediation

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## Abstract

Chlorpyrifos (CPF) is the most commonly used organophosphorus insecticide in agriculture globally. This has raised concerns due to its persistence, bioaccumulation, neurotoxicity, and environmental and human health effects. Until 2020, CPF was the most commonly used pesticide in European Union (EU) food production. CPF is also used in other parts of the world, though it has been discontinued in the EU, as national pesticide surveillance programs indicate it is found in soil, water, and food. This type of persistence is potentially harmful to farmers, consumers, and animals because CPF is toxic. Markedly, CPF has the potential to change the microbiota composition of soils, i.e., fungal, bacterial, and actinomycete communities, and inhibit the mineralization of nitrogen. The key CPF activity is associated with the inhibition of acetylcholinesterase (AChE), leading to reproductive, neurotoxic, and genotoxic effects. Microbial degradation, especially when applied by means of bacteria, has become one of the promising alternatives to the traditional physicochemical means since it is inexpensive, does not harm the environment and may possibly be fully detoxified. This review summarizes the latest developments in the study of CPF-degrading bacteria, enzyme pathways, microbial diversity, and the evaluation of the environmental impact of microbial remediation. Recent research findings, genomic research developments, and potential applications of CPF-degrading bacteria are addressed. In addition, this study reveals the current knowledge gaps, presents biotechnological challenges, and suggests future directions in the application of field-scale studies, focusing on the application of microbial solutions in sustainable agriculture.

**Keywords:** bacterial degradation; soil remediation; microbial consortia; bioremediation; sustainable agriculture; environmental health



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## 1. Introduction

Pesticides are essential for modern agriculture, as they protect crops from insects. However, their indiscriminate use can also have adverse effects on the environment and health [1]. As agriculture is being used to produce food, it becomes more intensive, without realizing it, in the use of pesticides. Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate] (CPF) is a broad-spectrum organophosphorus pesticide that

is broadly applied to agriculture, horticulture, and home gardens for pest control [1,2]. CPF is an acetylcholinesterase (AChE) inhibitor with concerns regarding reproductive toxicity, neurotoxicity, and genotoxicity in both pests and the human nervous system [3]. The widespread use of CPF in agriculture results in its dispersion across environmental compartments, posing a threat to ecosystem integrity [4]. Soil contamination poses risks to non-target organisms due to the lipophilic nature and high water solubility of CPF [5].

Although CPF was banned in the European Union in 2008 and restricted in China (prohibition of use on vegetables in 2016), CPF or CPF metabolites have been discovered in fruits, grains, vegetables, and human tissues, indicating prevalent contamination [3,6,7]. CPF, however, is still used throughout the world, especially in developing countries [8]. Exposure to humans on a daily basis, particularly in household settings, has impacts on the nervous, cardiovascular, and respiratory systems [9]. The World Health Organization (WHO) revealed that pesticide poisoning is a major global health concern, which shows that the use of pesticides in soil, food, and water poses a threat to not only workers and farmers but also to vulnerable populations, as the pesticides' toxic effects can be a serious risk to human health [10,11]. It has a negative impact on soil microbial diversity, nitrogen mineralization, and ecosystem function. Hence, its management should be sustainable [12].

The need to explore biological remediation is due to the limitations of conventional methods, and bioremediation is a safer remediation process than conventional methods. Microbial degradation, especially by bacteria, is an environmentally friendly method for the detoxification of CPF [13,14]. Although promising laboratory studies have been conducted, field-scale applications are still limited. Recent studies have identified CPF-degrading bacteria and the metabolic pathways and functional genes involved in their degradation. These bacteria are shown to be effective in CPF reduction [15–17].

Although the use of bacterial bioremediation techniques has made great strides, there are still some significant gaps in knowledge that must be addressed in the effective use of these technologies for CPF-contaminated environments. Importantly, there are no long-term, field-scale experiments to show that CPF degradation is efficient and sustainable under typical agricultural practice conditions, particularly over various soil types and climates. Currently, most research is lab-based or short-term trials, and the performance of microbes is a matter of multiple cropping cycles [3,10,18].

In addition, studies of novel enzymes, functional genes, and regulatory networks for CPF degradation based on omics technologies are limited. This review highlights the search for more efficient microbial pathways and the potential for developing targeted bioengineering approaches. Likewise, the network of interactions between the microbes within microbial consortia, including their behavior, communication, and synergistic effects under competitive soil conditions, requires further clarification. Moreover, little is known of the degradation processes of CPF in extreme or stressed environments, such as saline soils, arid areas, and heavy metal-contaminated areas, where unique microbial adaptations may play a key role. Plant–microbe synergistic systems are potential solutions that could help with CPF detoxification while improving soil health and the productivity of crops. The ability to bioremediate can be further expanded by engineered microbial consortia adapted to diverse environments.

Given the widespread use of CPF in agriculture and its associated environmental and health risks, we hypothesize that a comprehensive review of recent research on bacterial degradation of CPF in agricultural soils will reveal promising avenues for sustainable remediation. We explore CPF-degrading bacteria, their enzymatic pathways, and interactions within microbial communities to highlight effective bioremediation strategies. Furthermore, an in-depth analysis of the literature on CPF-induced toxicity will enhance our understanding of the molecular mechanisms associated with long-term exposure to CPF contamination.

This review combines recent research on CPF-degrading bacteria, enzymatic mechanisms, and microbial diversity, evaluates environmental implications, and proposes future research priorities to facilitate large-scale, sustainable CPF remediation, while safeguarding environmental and agricultural productivity.

Review framework: Following this introduction, Section 2 describes the sources of data and the literature-search approach. Section 3 summarizes the environmental fate, persistence, toxicity, and ecological implications of CPF. Section 4 reviews the diversity, rhizosphere interactions, and consortia of CPF-degrading bacteria. Section 5 discusses the enzymatic, genetic, and metabolic mechanisms underlying CPF transformation. Section 6 examines the environmental implications and field-relevant factors affecting microbial remediation. Section 7 integrates the principal challenges with future prospects, including ecological engineering, AI-assisted design, and synthetic biology. Section 8 provides the concluding remarks.

## 2. Sources of Data

This review was prepared based on information obtained from internationally recognized regulatory authorities such as the United States Environmental Protection Agency (US EPA), the European Commission (EC), and the European Food Safety Authority (EFSA) that provide scientific evaluations relevant to pesticide regulation and human health risks. In addition, a thorough literature review was conducted, which focused on CPF and its environmental and health implications. The scientific publications were retrieved from the PubMed database using keywords such as “chlorpyrifos,” “bacterial degradation,” “bioremediation,” “environmental impact,” “human health risks of chlorpyrifos,” “chlorpyrifos toxicity,” and “environmental occurrence of chlorpyrifos” from 2005 to August 2026. Over the past two decades, extensive research has explored the adverse effects and environmental persistence of CPF, and much has been documented on the topic. Original research, review, systematic review, and meta-analysis articles were selected during the search. Studies were excluded if the full-text article was unavailable or if the article was not in English. The letter to the editor, the conference abstracts, and book chapters were also excluded. In this review, 95 relevant publications were selected and critically analyzed, and the main focus was on bacterial degradation mechanisms, diversity of microorganisms involved in CPF degradation, and associated environmental implications.

## 3. Toxicity and Environmental Fate of Chlorpyrifos

### 3.1. Environmental Fate and Persistence of Chlorpyrifos

The extensive use of CPF in the agricultural field has been widely distributed throughout the terrestrial and aquatic environments. After application, CPF is subject to a series of complicated environmental processes such as adsorption, volatilization, runoff, leaching, photolysis, hydrolysis, and microbial transformation. Due to its hydrophobic nature ( $\log K_{ow} \approx 4.7\text{--}5.3$ ) and moderately persistent, and binds well to soil organic matter and sediments, which allows the long-term accumulation of CPF in agricultural soils and aquatic depositional environments [5,19]. Reported half-lives can be days to even months, depending on environmental conditions, and in some cases, the persistence is especially high for soil types with low microbial activity, low moisture levels, or low organic matter levels.

The environmental fate of CPF is further complicated by the production of transformation products, such as 3,5,6-trichloro-2-pyridinol (TCP), which is often more persistent than the parent compound. TCP exhibits greater water solubility and mobility, and it can be transported to groundwater and to other areas by surface runoff. TCP has antimicrobial activity, which needs to be further degraded by microbes for complete detoxification [19,20].

This process speeds up pesticide degradation, minimizes the potential for bioaccumulation, and avoids harmful residues from persisting in soil, water, and crops [21]. CPF and TCP have been detected in agricultural soils, rivers, groundwater, sediments, fruits and vegetables, cereals, and animal-derived food products in numerous monitoring studies. Thus, they are known to be disseminated into the environment in a broad manner, which may lead to human and wildlife exposure. The longevity of CPF and TCP in environmental matrices highlights the need for sustainable remediation technologies that will lead to the complete mineralization of CPF and TCP instead of partial transformation.

### 3.2. Human Health Effects and Neurotoxicity

Humans are exposed to CPF mainly via food, work practices, breathing in contaminated aerosols, contact with contaminated soil and water. The agricultural workers, pesticide applicators, and people living near intensive farming zones are considered to be especially vulnerable to chronic exposure. Epidemiological and toxicological data have shown that CPF is a serious health hazard, with detrimental effects on several physiological systems, such as the nervous, endocrine, reproductive, immune, and cardiovascular systems [9,22].

The major mechanism of toxicity of CPF is irreversible inhibition of acetylcholinesterase (AChE), a key enzyme that regulates cholinergic neurotransmission. Cytochrome P450 enzymes metabolize CPF to chlorpyrifos-oxon, a highly reactive metabolite with significantly higher AChE inhibitory activity than CPF [23]. Inhibition of AChE causes an increase in the concentration of acetylcholine in the synaptic gap and thus causes continuous stimulation of the neurons and causes neurological dysfunction [3].

In addition to the classical cholinergic toxicity, recent studies have shown that several non-cholinergic mechanisms contribute to the CPF-induced neurotoxicity. These involve oxidative stress, mitochondrial dysfunction, neuroinflammation, disruption of calcium homeostasis, altered neurotransmitter signaling, and epigenetic modifications. CPF exposure has been linked to the production of reactive oxygen species (ROS), lipid peroxidation, DNA damage, and inflammation pathways in neural tissues [24]. Experimental studies have shown that there are harmful effects on neuronal differentiation, synapse formation, and brain development, and epidemiological studies have revealed that exposure during pregnancy and early life is associated with cognitive impairments, lower IQ, attention deficit disorders, autism spectrum disorders, and behavioral abnormalities [25]. The results suggest that the effects of CPF toxicity may be more severe than the inhibition of AChE and can lead to long-term neurological effects at subclinical exposure doses [22].

### 3.3. Endocrine and Reproductive Disruption

Evidence suggests that CPF is known to act as an endocrine-disrupting chemical that can disrupt more than one hormone. CPF and its metabolites may affect the production, transport, metabolism and receptor-mediated actions of various hormones such as thyroid hormones, estrogens, and androgens [26,27]. Experimental studies have reported disruption of the hypothalamic–pituitary–gonadal axis and hypothalamic–pituitary–thyroid axes after exposure to CPF, with the development of hormonal imbalance and reproductive dysfunction [22].

In males, CPF exposure is known to impair sperm quality, decrease production of testosterone, impair spermatogenesis, and cause structural changes in male reproductive tissues [28]. In females, the reproductive toxicity involves dysfunction of the ovary, a change in the estrous cycle, follicular deficiency, and pregnancy defects [29]. Exposure to the developing organism may lead to lifelong sensitivity to reproductive problems. Furthermore, endocrine changes that occur due to CPF can have a secondary effect on

metabolic dysfunction, obesity, insulin resistance, and immune function [30]. Recent awareness of endocrine-related effects has greatly increased the concern about the potential long-term health effects of chronic low-dose exposure to CPF.

### 3.4. Ecotoxicological Impacts on Ecosystems

The effects of CPF contamination are not just limited to target pest species and have impacts on a large array of non-target species. The sensitivity of soil microorganisms, earthworms, insects, pollinators, amphibians, fish, birds, and aquatic invertebrates to CPF exposure varies. These organisms interact with each other and with other organisms in complex ways that affect ecosystem functioning. CPF contamination can cause cascading ecological disturbances down food webs [8,9].

In the soil ecosystem, CPF has also been demonstrated to alter the composition and diversity of soil microorganisms, decrease soil microbial populations, decrease enzyme activity, and decrease soil nutrient cycling efficiency [10]. Potential negative impacts on N<sub>2</sub> fixation, P transformation, and soil organic matter breakdown can affect soil fertility and agricultural productivity. Pollinators, especially honeybees, are highly susceptible to CPF [12]. Exposure to CPF affects navigation, foraging, colony development, and colony survival. Likewise, acute and chronic toxic effects are common in aquatic organisms resulting from the entry of pesticides into freshwater. These ecological effects provide the environmental rationale for prioritizing bacterial systems that remove both CPF and persistent transformation products such as TCP [31].

Exposure of fish and amphibians to CPF can cause behavioral abnormalities, developmental defects, oxidative stress, endocrine disruption, and impairment of reproduction. One of the most sensitive taxa is aquatic invertebrates, which are an important part of the freshwater food webs. Therefore, contamination by CPF can lead to loss of biodiversity, changes in species interactions, and disruption of ecosystem functions. The ecotoxicological effects highlight the need for appropriate remediation strategies that can minimize pesticide residues to prevent irreversible damage to ecosystems [8].

### 3.5. Bioaccumulation and Food Chain Transfer

Although CPF is not considered to be one of the most bioaccumulative persistent organic pollutants, its lipophilic nature allows it to bioaccumulate in biological tissues and to bioaccumulate through trophic transfer in food chains. Exposure to the environment can lead to the accumulation of CPF in plants, aquatic life, animals, and livestock, providing several routes of human exposure. Chronic dietary exposure has been of concern as residues have been found in fruits, vegetables, grains, milk, fish and other food products [6,19].

The bioaccumulation potential of CPF is especially relevant in aquatic environments, where residues of CPF can bind to sediments and thus be consumed by benthic organisms and then passed up trophic levels. Exposure may be enhanced in the aquatic food chain for carnivorous fish, birds, and mammals. In addition, chronic exposure to an environmentally relevant concentration can cause subtle but biologically significant effects that may not be recognized by traditional toxicity testing. CPF residues have been detected in environmental and biological matrices, thereby raising the need for remediation efforts that not only lower the concentration of parent compounds but also fully remove all the toxic metabolites like TCP. Failure to achieve complete mineralization may result in continued ecological exposure, resulting in long-term health risks [6,19,32].

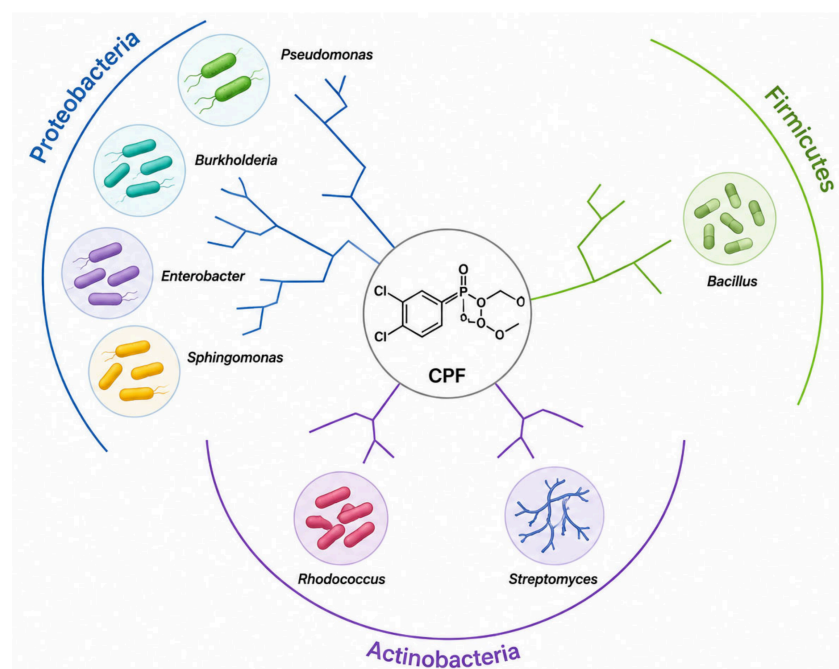
### 3.6. Implications for Sustainable Remediation

The recalcitrance, toxicity, and ecological effects of CPF collectively suggest that traditional physicochemical remediation methods might not be adequate to resolve long-term environmental contamination. Pollutant degradation by adsorption, chemical oxidation, or

incineration is effective in lowering levels, but it is often accompanied by high operation costs, secondary pollution, and incomplete pollutant degradation. One of the most important benefits of microbial degradation is that the CPF and the toxic metabolites are converted to non-toxic end-products by natural metabolic processes that do no harm to the environment [10,13]. The mechanisms of environmental fate and toxicity of CPF are essential for the development of advanced microbial remediation technologies. The increased awareness of the neurotoxicity, endocrine disruption, ecological effects, and bioaccumulation potential of CPF further reinforces the need to investigate more efficient microorganisms and engineered microbial consortia that degrade CPF as sustainable solutions that restore the environment [33,34].

#### 4. Diversity of Chlorpyrifos-Degrading Bacteria

A variety of bacterial taxa from different groups and ecological niches have been shown to break down CPF and its toxic metabolite 3,5,6-trichloro-2-pyridinol (TCP) through enzymatic transformations [33,35]. The knowledge of their taxonomic, functional, and ecological diversity is important to drive the optimization of bioremediation strategies [34,36]. Figure 1 highlights the broad taxonomic distribution of bacteria reported to participate in CPF degradation. *Pseudomonas*, *Burkholderia*, *Enterobacter*, and *Sphingomonas* are representative genera of the Proteobacteria, which are common in the rhizospheres, sediments, wastewaters, and soils of plants polluted by pesticides. Members of this group are able to transform organophosphates, and have metabolic versatility, rapid physiological adaptation and ability to carry catabolic genes on the chromosome or on mobile genetic elements. The figure also includes *Bacillus* as a representative firmicute. The advantage of *Bacillus* species for bioremediation is that they produce extracellular enzymes, and many species can form endospores, leading to increased survival in the field under varying conditions. The members of the actinobacterial group, such as *Rhodococcus* and *Streptomyces*, have large oxidative and hydrolytic metabolic capabilities and are especially important for the transformation of structurally complex or hydrophobic xenobiotics.



**Figure 1.** Taxonomic diversity of representative chlorpyrifos-degrading bacteria across Proteobacteria, Firmicutes, and Actinobacteria, illustrating the phylogenetic breadth of bacterial CPF biodegradation.

#### 4.1. Rhizoremediation and Plant–Microbe Interactions

Rhizoremediation combines plant-based remediation with rhizosphere microbial activity. Plant roots release organic compounds that can promote microbial growth, improve nutrient availability, and provide favorable conditions for biodegradation. Some bacterial genera, including *Pantoea*, *Acinetobacter*, *Kosakonia*, *Morganella*, *Enterobacter*, and *Klebsiella*, can promote plant growth through nitrogen fixation, phosphate solubilization, and phytohormone production and therefore have potential for rhizoremediation. In one reported study, the tested strains removed 100% of chlorpyrifos and diazinon (50 mg/5 mL) after 14 days of exposure [15]. CPF-degrading bacteria may therefore provide dual functions by degrading pesticides while supporting plant growth through nutrient mobilization, phytohormone production, or stress reduction. However, plant–microbe interactions are species-specific and environmentally dependent, and optimal partnerships require further ecological information and field testing [14,15].

#### 4.2. Taxonomic and Functional Diversity

Bacterial strains that can degrade CPF have been isolated from contaminated environments such as agricultural soils, industrial effluents, sediment, and wastewater [14]. Recent studies have demonstrated several different groups of bacteria that play a role in CPF degradation. The Proteobacteria phylum is the most studied group of CPF-degrading bacteria because of its metabolic versatility. *Pseudomonas syringae*, *Pseudomonas putida*, *Pseudomonas stutzeri*, and *Pseudomonas aeruginosa* efficiently degrade CPF via OPHs and dehalogenase enzyme systems, often in combination with TCP mineralization [12,37–40]. *Enterobacter cloacae* and *Enterobacter aerogenes* isolated from soil and wastewater have hydrolytic and oxidative degradation pathways [19,41,42]. The plant growth-promoting *Burkholderia* sp. plays a role in the degradation of CPF in the rhizosphere [19,43].

Spore-forming Gram-positive bacteria among the Firmicutes phylum also reveal resilience and effective pesticide degradation. These bacteria, including *Bacillus cereus*, *Bacillus toyonensis*, *Bacillus subtilis*, and *Bacillus megaterium*, are reported to hydrolyze CPF and TCP, some of them have further plant-beneficial activities [2,22,37,41]. The actinobacterial species have been found to have a variety of catabolic pathways for organophosphorus compounds [44,45]. *Rhodococcus* sp. degrades both CPF and TCP through the co-metabolic processes [41]. *Streptomyces* sp. is mainly known for its role in the production of antibiotics, and some studies have shown that it can even degrade organophosphorus [46]. Table 1 shows the CPF-degrading bacteria, containing the microorganism's name, source, reaction, condition, and degradation rate, along with the relevant references. Table 1 summarizes representative CPF-degrading bacteria, their isolation sources, major transformation routes, experimental conditions, degradation performance, and supporting references.

**Table 1.** Chlorpyrifos Insecticides Degrading Bacteria.

| Microorganism Name           | Source                                   | Reaction                             | Condition                                | Degradation Rate                                 | Reference |
|------------------------------|------------------------------------------|--------------------------------------|------------------------------------------|--------------------------------------------------|-----------|
| <i>Paracoccus</i> sp. TRP    | Activated sludge, pesticide plant        | Hydrolysis and mineralization        | TYC medium; 50 mg/L; 4 d                 | 100% in 4 d                                      | [47]      |
| <i>Bacillus pumilus</i> C2A1 | Agricultural/pesticide-contaminated soil | Hydrolysis and oxidative degradation | MSM; 50–1000 mg/L; pH 8.5; ~37 °C        | 82% (50 mg/L, 5 d); 89% (1000 mg/L, 15 d)        | [48]      |
| <i>Gordonia</i> sp. JAAS1    | Chlorpyrifos-exposed paddy soil          | Hydrolysis to TCP                    | MSM; 110 mg/L; 24 h                      | Rapid degradation; TCP accumulated               | [49]      |
| <i>Ochrobactrum</i> sp. JAS2 | Chlorpyrifos-treated paddy rhizosphere   | Hydrolysis and mineralization        | Aqueous medium; 300 mg/L; soil microcosm | CP complete in 12–24 h; TCP complete in 72–120 h | [50]      |

Table 1. Cont.

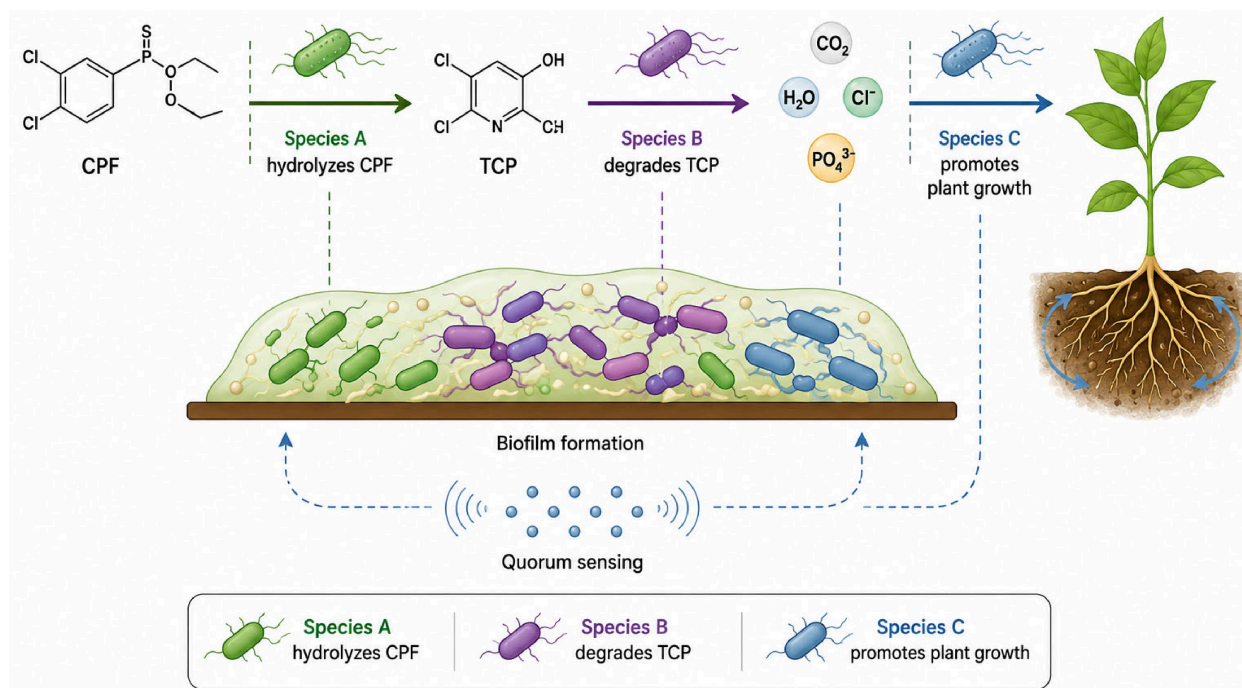
| Microorganism Name                              | Source                                   | Reaction                                                      | Condition                                    | Degradation Rate                | Reference |
|-------------------------------------------------|------------------------------------------|---------------------------------------------------------------|----------------------------------------------|---------------------------------|-----------|
| <i>Achromobacter xylosoxidans</i> JCp4          | Pesticide-contaminated agricultural soil | Hydrolysis and mineralization                                 | MSM; 100 mg/L; 10 d                          | 84.4%; 7.9 mg/L d <sup>-1</sup> | [51]      |
| <i>Ochrobactrum</i> sp. FCp1                    | Pesticide-contaminated agricultural soil | Hydrolysis and mineralization                                 | MSM; 100 mg/L; 10 d                          | 76.8%; 7.3 mg/L d <sup>-1</sup> | [51]      |
| <i>Cupriavidus nantongensis</i> X1 <sup>T</sup> | Chlorpyrifos-polluted environment        | Hydrolysis and degradation                                    | 200 mg/L; ~37 °C                             | ~100% in 48 h                   | [52]      |
| <i>Bacillus thuringiensis</i> MB497             | Agricultural soil                        | Organophosphorus-phosphatase-mediated hydrolysis              | M9 medium; ≤200 mg/L                         | 81–94.6%                        | [53]      |
| <i>Arthrobacter</i> sp. HM01                    | Pesticide-contaminated soil              | Hydrolysis → TCP → 2,6-dihydroxypyridine; further degradation | mMSM; 100 mg/L; pH 7; 32 °C                  | 99% in 10 h                     | [54]      |
| <i>Bacillus cereus</i> H27                      | Farmland soil                            | Enzymatic transformation; multiple proposed pathways          | Culture system; temperature/pH dependent     | >80%                            | [55]      |
| <i>Bacillus</i> sp. UPMB10                      | Chlorpyrifos-treated cassava-farm soil   | Biotransformation; GC–MS-detected metabolites                 | MSM; 4 g L <sup>-1</sup> ; pH 7; 37 °C; 48 h | 99% in 48 h                     | [56]      |
| <i>Bacillus cereus</i> Ct3                      | Cotton-growing agricultural soil         | Hydrolysis and ring cleavage/mineralization                   | Soil; 125 mg/L resistance; pH 8; 30–40 °C    | 88% in 8 d                      | [57]      |
| <i>Bacillus subtilis</i>                        | Contaminated soil                        | Hydrolysis and oxidative degradation                          | Ex situ, 30 °C, pH 7                         | 85% in 30 days                  | [58]      |
| <i>Pseudomonas aeruginosa</i>                   | Sewage sludge                            | Sequential hydrolysis and oxidation                           | In situ, 25 °C, pH 6.5                       | 92% in 28 days                  | [58]      |
| <i>Sphingomonas paucimobilis</i>                | Polluted water                           | NR                                                            | Ex situ, 30 °C, pH 7                         | 98% in 30 days                  | [59]      |
| <i>Lysinibacillus</i> sp.HBUM206408             | Polluted soil                            | NR                                                            | Ex situ, 30 °C, pH 7                         | 87.16% in 16 days               | [60]      |
| <i>Stenotrophomonas maltophilia</i>             | Polluted soil                            | NR                                                            | Ex situ, 30 °C, pH 7                         | 82.04% in 16 days               | [60]      |
| <i>Pseudomonas putida</i>                       | Polluted soil                            | NR                                                            | Ex situ, 30 °C, pH 7                         | 89.52% in 16 days               | [60]      |
| <i>Achromobacter insuavis</i>                   | Polluted soil                            | NR                                                            | Ex situ, 30 °C, pH 7                         | 91.08% in 16 days               | [60]      |
| <i>Ochrobactrum intermedium</i>                 | Polluted soil                            | NR                                                            | Ex situ, 30 °C, pH 6.8–7                     | 69.1% in 8 days                 | [61]      |
| <i>Flavobacterium</i> sp.                       | Activated sludge                         | Sequential hydrolysis and dephosphorylation                   | Ex situ, 30 °C, pH 7                         | 82% in 26 days                  | [62]      |
| <i>Vibrio</i> sp.                               | Marine bivalves                          | Hydrolysis and oxidation                                      | In situ, 28 °C, seawater                     | 68% in 4 days                   | [63]      |
| <i>Sphingobium</i> sp.                          | Environmental water                      | Hydrolysis and metabolic transformation                       | Aerobic conditions, 20–35 °C                 | NR                              | [64]      |
| <i>Klebsiella pneumoniae</i> CP19               | Municipal soil sediment                  | Co-culture                                                    | pH 7                                         | 94.5 ± 3.3% in 16 days          | [65]      |

Abbreviations: NR, not reported; CP, chlorpyrifos; TCP, 3,5,6-trichloro-2-pyridinol; DETP, diethylthiophosphoric acid; MSM, mineral salt medium; mMSM, modified mineral salt medium.

#### 4.3. Microbial Consortia and Synergistic Degradation

Although many bacterial species alone exhibited excellent degradation properties of CPF, microbial consortia may have complementary metabolic properties to achieve fast degradation under varying environmental conditions [15,66]. In natural environmental settings, bacterial consortia are often more effective at degrading CPFs than individual

bacteria [67]. In the synergistic action, different microbial species work together to increase the efficiency of the process [14]. In a microbial consortium, some species hydrolyze the TCP, and others break down TCP or its metabolites, resulting in complete hydrolysis [37]. A bacterium may require the presence of additional carbon sources to complete the degradation of CPF. Some bacteria may provide it under nutrient-limited conditions, and some can co-metabolize [3]. Figure 2 illustrates how metabolic cooperation among bacterial populations can overcome limitations associated with single-strain CPF degradation. In the conceptual consortium, species A starts detoxification by hydrolyzing CPF to some TCP-related intermediates. Subsequently, these intermediates are eaten by species B, so they do not build up and cause product-associated toxicity that can stifle primary degraders. Ongoing metabolism results in the creation of simpler inorganic products, which decreases the length of time pesticide-related residues persist. Species C are organisms that can provide complementary plant growth-promoting functions but may not be involved in the primary hydrolysis of the pesticides. These can range from nutrient mobilization, modulation of phytohormones, stress alleviation, or assistance of microbial activity in the rhizosphere. As indicated in the figure, the biofilm matrix creates a spatially organized microenvironment allowing cells to stay in close contact, to exchange metabolites, and to cope with variations in contaminant concentration and physicochemical conditions. Quorum-sensing signals are one of the mechanisms that may be employed to coordinate the behaviors of a population, such as biofilm formation, enzyme production, motility, and interactions between communities. This cooperation in metabolic labor offers a mechanistic explanation for the expected ability of mixed microbial consortia to effectively and persistently remediate. The synergistic consortia improve degradation under variable environmental conditions and help in bioremediation under diverse ecosystems [68]. Table 2 summarizes reported chlorpyrifos-degrading consortia, their component microorganisms, environmental sources, operating conditions, and degradation performance.



**Figure 2.** Functional division of labor in a chlorpyrifos-degrading bacterial consortium, integrating CPF hydrolysis, degradation of TCP-related intermediates, biofilm formation, quorum sensing, mineralization, and plant-growth-promoting functions.

**Table 2.** Chlorpyrifos-Degrading Microbial Consortia.

| Microbial Consortium                      | Dominant Microorganisms                                                                                                                           | Source                                                      | Reaction Condition                                                                       | Degradation Rate                                                               | Reference |
|-------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------|
| Agricultural-soil consortium              | <i>Pseudomonas putida</i> NII 1117, <i>Klebsiella</i> sp. NII 1118, <i>P. stutzeri</i> NII 1119, <i>P. aeruginosa</i> NII 1120                    | Chlorpyrifos-contaminated agricultural soil                 | MSM; chlorpyrifos as selective substrate                                                 | Efficient degradation; consortium developed from four high-performing isolates | [66]      |
| C5                                        | <i>Staphylococcus warneri</i> CPI2, <i>Pseudomonas putida</i> CPI9, <i>Stenotrophomonas maltophilia</i> CPI15                                     | Pesticide-contaminated soil                                 | 125 mg/L; pH 7; 30 °C; 8 d                                                               | 90%                                                                            | [69]      |
| ECO-M                                     | <i>Agrobacterium tumefaciens</i> ECO1, <i>Cellulosimicrobium funkei</i> ECO2, <i>Shinella zoogloeoides</i> ECO3, <i>Bacillus aryabhattai</i> ECO4 | Himalayan chlorpyrifos-contaminated soil                    | 50 mg/L; 30 °C; 10% inoculum                                                             | 100% in 6 d; 94.3% in soil microcosms                                          | [68]      |
| CON11                                     | <i>Bacillus amyloliquefaciens</i> CP28, <i>B. pumilus</i> CP30, <i>B. marisflavi</i> CP31, <i>B. subtilis</i> CP34                                | Pesticide-contaminated soil, Kerala, India                  | 100 mg/L; pH 7; 30 °C; 6 d                                                               | 91.2% ± 1.2%; t½ = 1.73 d                                                      | [70]      |
| CP6–CP19 consortium                       | <i>Bacillus cereus</i> CP6 + <i>Klebsiella pneumoniae</i> CP19                                                                                    | Municipal soil sediment                                     | 200–300 mg/L; neutral pH; liquid culture/soil                                            | 93.4% ± 2.8% liquid; 94.5% ± 3.3% soil in 16 d                                 | [65]      |
| C1                                        | Chlorpyrifos-tolerant isolates S271 + S28I                                                                                                        | Pesticide-polluted Kuzhikandam Creek, Kerala                | Chlorpyrifos as sole C source; free vs. charcoal–alginate/kaolin–alginate immobilization | Immobilized consortium > free cells; highest among tested systems              | [71]      |
| <i>Eichhornia</i> phyllosphere consortium | <i>Achromobacter xylosoxidans</i> + <i>Stutzerimonas stutzeri</i>                                                                                 | Water-hyacinth ( <i>Eichhornia crassipes</i> ) phyllosphere | Chlorpyrifos as sole C source; 7 d                                                       | 98% in 7 d                                                                     | [72]      |

Recent research underscores the significance of microbial interactions in forming biofilms and regulating cell–cell signaling via quorum sensing for the stability and functional efficiency of these consortia [73]. The formation of biofilms helps bacteria to adhere to soil particles, making the cells more resistant to environmental stressors, and promotes cooperative degradation of CPF and its metabolites [74].

Quorum sensing mechanisms in a consortium allow bacteria to coordinate gene expression, such as the increase in degradation enzymes, when the population density or environmental signals change [75]. New metagenomic initiatives have also helped to elucidate the functional potential and community dynamics of CPF-degrading consortia in situ, identifying novel genes involved in CPF degradation, regulatory elements, and interactions that impact the overall degradation efficiency [76,77]. Furthermore, the development of synthetic biology has now made it possible to design and engineer a synthetic consortium to have desired metabolic pathways, environmental resilience, and CPF detoxification capabilities [78]. These novel approaches offer great potential for improving bioremediation processes, especially in the field under challenging conditions. Many bacterial strains

have been shown to achieve removal efficiency >90% in controlled laboratory settings. However, degradation rates can significantly drop when grown in field conditions because of ecological competition, environmental heterogeneity, nutrient deficiency, variable temperature and moisture regimes in the field, and limited bioavailability of the pollutants. Interestingly, biofilm consortia, rhizoremediation systems, immobilized cells, and biochar-assisted bioremediation methods tend to be more stable in the field and more effective for removal as compared to single-strain inoculants. The observations indicate that ecological engineering and integrated remediation are essential to achieve good success in implementing microbial CPF bioremediation technologies on a large-scale. Laboratory removal efficiencies often exceed those achieved under field conditions because environmental heterogeneity, indigenous microbial competition, nutrient limitation, contaminant aging, and variable moisture can constrain activity. These constraints reinforce the need for robust consortia, rhizosphere-based systems, and immobilization or carrier-assisted approaches that improve persistence and performance in realistic soils.

#### 4.4. Ecological Distribution and Sources

The widespread discovery of CPF-degrading bacteria in various environments indicates their adaptability in different environments [62,79]. Agricultural soils are the reservoirs of pesticides [80,81]. The repeated applications of pesticides in agricultural soils increase the amount of native degraders in the rhizosphere [82]. Furthermore, industrial effluents and treatment plants can act as habitats for strong CPF-degrading communities [83]. Sediments receiving agricultural runoff harbor bacteria capable of mitigating aquatic pesticide contamination [84]. CPF-degrading strains have been identified in biostimulants and composts and can be employed for integrated remediation [83,85].

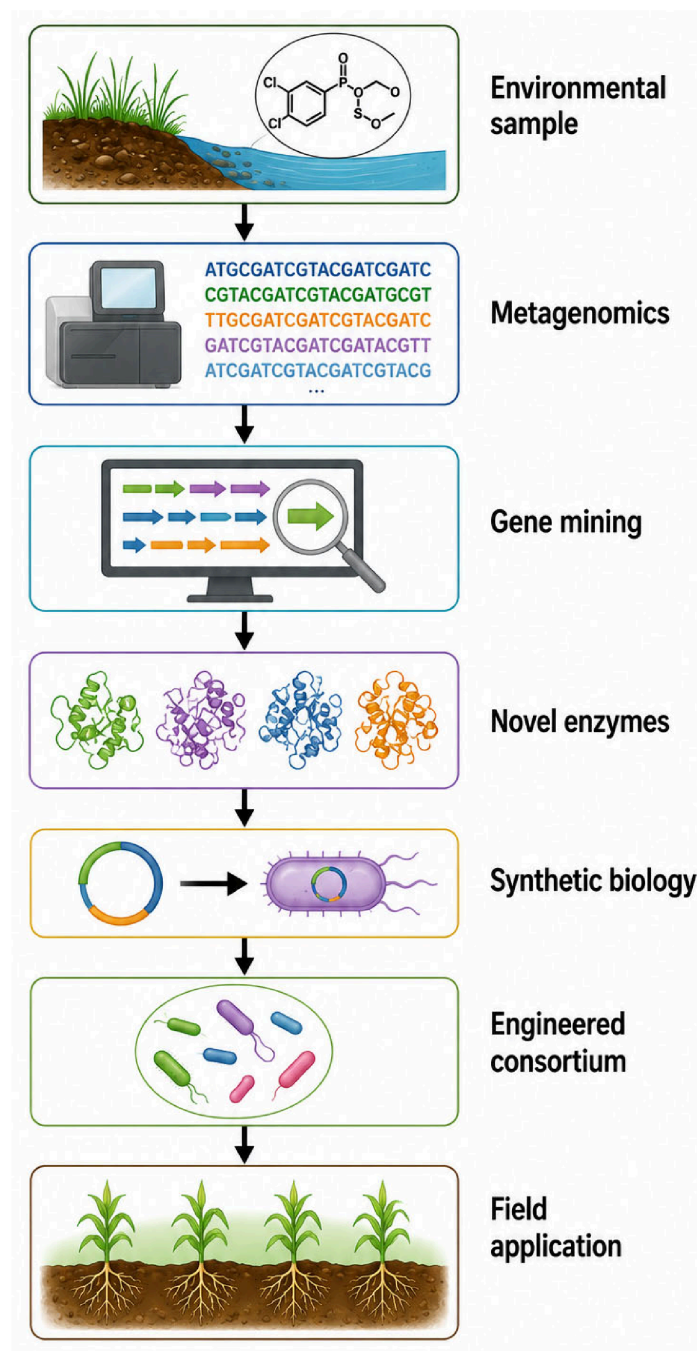
### 5. Mechanisms of Chlorpyrifos Degradation

#### 5.1. Chlorpyrifos Degradation by Microbes

Pesticide degradation studies have been revolutionized by recent breakthroughs in omics technologies and systems biology, which allow for full characterization of microbial communities, metabolic pathways, regulatory networks, and environmental interactions [13,40]. Such methods allow for the identification of important degradative genes, functional taxa, metabolic bottlenecks, and ecological relationships that all contribute to the overall remediation efficiency. The genetic determinants enable the microorganism to use CPF as a source of phosphorus, a source of carbon, or a co-metabolic substrate, which aids in the removal of its toxicity and promotion of its eventual mineralization [33,86,87]. Therefore, in recent years, the use of omics for investigations has driven the evolution of CPF bioremediation from empirical strain selection to predictive and engineering approaches [13,40,78]. Figure 3 presents a conceptual workflow linking environmental sampling and omics-based discovery to enzyme characterization, consortium design, and field application.

The initial step involves taking samples of CPF-exposed soils, sediments, rhizospheres, or waters, where extended exposure to pesticides can lead to an enrichment of microorganisms and genes capable of transforming organophosphates. Metagenomic sequencing can provide insights into the characteristics of both cultured and uncultured community members, including candidate hydrolases, dehalogenases, oxygenases, transporters, regulatory proteins, and accessory functions involved in the metabolism of CPF or TCP. Bioinformatic gene mining and functional screening can then prioritize novel enzymes according to sequence signatures, catalytic domains, genomic context, and predicted substrate interactions. Protein engineering and/or synthetic biology methods can be used to experimentally test and, where applicable, optimize candidate enzymes and pathways. The ultimate step in the process of translation is field use, and testing can be carried out under realistic

environmental heterogeneity, competition by native microbiota, constraints on pesticide bioavailability, and biosafety for field use.



**Figure 3.** Integrated discovery-to-application pipeline using environmental sampling, metagenomics, functional gene mining, enzyme discovery, synthetic biology, and engineered consortia to advance field-scale chlorpyrifos bioremediation.

A wide range of catabolic genes coding for enzymes that are capable of both metabolizing organophosphorus compounds and their transformation products are fundamental to the biodegradation of CPF. In recent years, the emergence of new knowledge on molecular biology, genomics and metagenomics, which shed new light on the degradation of CPFs, mainly consisting of hydrolytic cleavage, oxidative, reductive, and dechlorination reactions, which transform the CPF and its by-products into non-toxic end-products, including  $\text{CO}_2$  and  $\text{H}_2\text{O}$  and inorganic phosphates (IP) [33,35]. Metagenomics can be used to show the genetic potential of microbial communities, whereas metatranscriptomics can be used

to show what genes are being expressed during CPF degradation. Metatranscriptomic analyses have shown that exposure to CPF leads to coordinated changes in the expression of genes related to hydrolysis, oxidative stress response, membrane transport, metabolism of aromatics, and energy generation. At the same time, patterns of expression provide information about the ecological relationships between the microbial populations, such as metabolic cooperation, competition, stress adaptation, etc. One of the most significant observations from transcriptomic studies is that degradative genes are not always expressed and result in active degradation. Nutrient limitation, pH changes, low oxygen, build-up of toxic metabolites, etc., can lead to suppression of gene expression even if the catabolic pathway is available. This is one reason why strains of microbes with good degradation ability in the lab do not show the same performance in the field. Therefore, the genetic potential alone is not enough to consider it for future bioaugmentation techniques. Its expression stability under environmentally relevant conditions should also be taken into consideration. Table 3 highlights how genomics, transcriptomics, proteomics, metabolomics, and integrated systems approaches contribute to understanding and improving chlorpyrifos biodegradation. The combination of metagenomics, genomics, transcriptomics, proteomics, metabolomics, and systems-level analyses has greatly enhanced the understanding of degradative CPF pathways, microbial community dynamics, functional gene expression, and metabolic interactions. The technologies have enabled the identification of new degradative enzymes, the identification of key catabolic genes, the reconstruction of metabolic pathways, and the engineering of microbial consortia with a better ability to remediate. Overall, the omics investigations will drive a shift in CPF bioremediation from an empirical approach of selecting strains to a data-driven and predictive approach that involves synthetic biology for environmental restoration.

**Table 3.** Omics-based investigations and systems biology approaches applied to chlorpyrifos (CPF) degradation and bioremediation.

| Omics Approach                | Biological System/Sample                 | Major Findings                                                                                                       | Significance for CPF Bioremediation                                                | References |
|-------------------------------|------------------------------------------|----------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------|
| Metagenomics                  | CPF-contaminated agricultural soils      | Discovery of novel organophosphate hydrolase genes ( <i>opd</i> , <i>mpd</i> , <i>oph</i> ) and uncultured degraders | Expanded understanding of microbial diversity and degradation potential            | [76]       |
| Metagenomics                  | Pesticide-contaminated rhizosphere       | Identification of microbial community networks associated with CPF degradation                                       | Revealed cooperative degradation pathways within microbial consortia               | [77]       |
| Whole-Genome Sequencing (WGS) | <i>Enterobacter</i> sp. HSTU-ASH6        | Characterization of CPF-mineralizing genes, plant growth-promoting traits, and metabolic pathways                    | Demonstrated multifunctional potential for remediation and sustainable agriculture | [17]       |
| Whole-Genome Sequencing (WGS) | Organophosphate-degrading rhizobacteria  | Identification of broad-spectrum degradation enzymes and catabolic operons                                           | Facilitated gene mining and enzyme discovery                                       | [86]       |
| Comparative Genomics          | Multiple CPF-degrading bacterial strains | Analysis of conserved and strain-specific degradation genes                                                          | Revealed evolutionary adaptation to pesticide-contaminated environments            | [13]       |

Table 3. Cont.

| Omics Approach                         | Biological System/Sample            | Major Findings                                                                       | Significance for CPF Bioremediation                                       | References |
|----------------------------------------|-------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------|------------|
| Transcriptomics                        | CPF-exposed bacterial cultures      | Upregulation of hydrolytic enzymes, transport proteins, and stress-response genes    | Demonstrated active metabolic response during CPF degradation             | [33]       |
| Transcriptomics                        | Rhizosphere microbial communities   | Differential expression of genes involved in carbon metabolism and detoxification    | Explained adaptation of microbial communities under CPF stress            | [12]       |
| Proteomics                             | CPF-degrading bacterial isolates    | Identification of OPH, dehalogenases, monooxygenases, and antioxidant enzymes        | Linked gene expression with actual enzymatic degradation activity         | [13]       |
| Proteomics                             | Organophosphate-stressed bacteria   | Detection of proteins involved in oxidative stress tolerance and membrane adaptation | Explained microbial resilience in contaminated environments               | [88]       |
| Metabolomics                           | CPF degradation cultures            | Identification of TCP, DETP, chlorinated intermediates, and ring-cleavage products   | Reconstruction of complete degradation pathways                           | [19]       |
| Metabolomics                           | Microbial consortia degrading CPF   | Mapping of carbon flux into TCA cycle intermediates                                  | Confirmed complete mineralization of CPF metabolites                      | [2]        |
| Multi-omics Integration                | CPF-degrading microbial communities | Integration of genomic, transcriptomic, proteomic, and metabolomic datasets          | Improved understanding of microbial interactions and degradation networks | [40]       |
| Systems Biology                        | Synthetic microbial consortia       | Modeling of metabolic cooperation and pathway optimization                           | Enhanced degradation efficiency and ecological stability                  | [78]       |
| Genome Mining                          | Environmental microbial databases   | Discovery of previously unknown OPH variants and catabolic enzymes                   | Accelerated identification of candidate bioremediation strains            | [87]       |
| Artificial Intelligence-Assisted Omics | Large-scale microbial datasets      | Prediction of enzyme-substrate interactions and consortium performance               | Emerging tool for next-generation CPF bioremediation design               | [40]       |

### 5.2. Enzymatic and Genetic Mechanisms of Chlorpyrifos Degradation

Proteomics provides direct evidence of the biosynthesis of proteins and the activity of the enzymes produced, linking genetic potential and metabolic function. Under CPF bioremediation systems, proteomic analysis has shown a higher expression of organophosphorus hydrolases, dehalogenases, monooxygenases, proteins of stress response, membrane transporters, and enzymes of energy metabolism during the degradation of the pesticides. The recent advances in microbial genomics have revealed new genes, enzymes, and metabolic pathways involved in these degradation processes are being identified, which are helpful in developing bioremediation strategies [2,35]. The most well-identified functional elements so far associated with CPF degradation are the genes for organophosphates degrading (*opd*), methyl parathion hydrolase (*mpd*), organophosphorus hydrolase (*oph*), dehalogenase, and monooxygenase. The genes for these enzymes are necessary to induce CPF biotransformation [87,89]. Table 4 links representative functional genes and enzymes with their proposed

roles in chlorpyrifos transformation and downstream detoxification. This table shows that the CPF degradation is controlled through a well-coordinated array of hydrolytic (*opd*, *mpd*, *oph*), dehalogenating (*dhaA*, *dhlA*), oxidative (*tcpA*, *tcpX*), and aromatic ring-cleavage (*catA*, *catB*, *catC*) genes. Multiple enzymes and microbial consortia are needed for CPF to be completely mineralized, highlighting the need for multifunctional bacterial strains and engineered microbial consortia for field-scale bioremediation.

**Table 4.** Major enzymes and functional genes involved in chlorpyrifos (CPF) degradation and detoxification pathways.

| Gene             | Enzyme                           | Primary Function                             | Representative Organism(s)                                                               | Role in CPF Degradation Pathway                           | References |
|------------------|----------------------------------|----------------------------------------------|------------------------------------------------------------------------------------------|-----------------------------------------------------------|------------|
| <i>opd</i>       | Organophosphorus hydrolase (OPH) | Hydrolysis of organophosphate ester bonds    | <i>Pseudomonas diminuta</i> ,<br><i>Flavobacterium</i> sp.,<br><i>Pseudomonas putida</i> | Initial hydrolysis of CPF to TCP and DETP                 | [86,87]    |
| <i>mpd</i>       | Methyl parathion hydrolase (MPH) | Broad-spectrum organophosphate degradation   | <i>Bacillus subtilis</i> ,<br><i>Ochrobacterium</i> sp.,<br><i>Burkholderia</i> sp.      | Hydrolysis of CPF and related organophosphorus pesticides | [33]       |
| <i>oph</i>       | Organophosphorus hydrolase       | Detoxification of organophosphorus compounds | <i>Enterobacter</i> sp.,<br><i>Agrobacterium radiobacter</i>                             | CPF detoxification through phosphotriester hydrolysis     | [87,89]    |
| <i>ophC2</i>     | Organophosphorus hydrolase C2    | Enhanced hydrolysis and detoxification       | <i>Enterobacter</i> sp.,<br><i>Leuconostoc mesenteroides</i>                             | Accelerated CPF transformation and detoxification         | [89]       |
| <i>dhaA</i>      | Haloalkane dehalogenase          | Dechlorination of chlorinated metabolites    | <i>Rhodococcus</i> sp.                                                                   | TCP dechlorination and detoxification                     | [90]       |
| <i>dhlA</i>      | Dehalogenase                     | Removal of chlorine substituents             | <i>Burkholderia</i> sp.,<br><i>Pseudomonas</i> sp.                                       | Detoxification of TCP and chlorinated intermediates       | [90]       |
| <i>tcpA</i>      | Monooxygenase                    | Hydroxylation of TCP                         | <i>Cupriavidus</i> sp.,<br><i>Ralstonia</i> sp.                                          | Initiation of TCP degradation pathway                     | [19]       |
| <i>tcpX</i>      | Monooxygenase                    | Aromatic ring hydroxylation                  | <i>Cupriavidus</i> sp.                                                                   | Conversion of TCP into more biodegradable metabolites     | [19]       |
| <i>catA</i>      | Catechol 1,2-dioxygenase         | Aromatic ring cleavage                       | <i>Pseudomonas</i> sp.,<br><i>Rhodococcus</i> sp.                                        | Cleavage of hydroxylated aromatic intermediates           | [91]       |
| <i>catB</i>      | Muconate cycloisomerase          | Aromatic compound metabolism                 | <i>Pseudomonas</i> sp.                                                                   | Downstream degradation of ring-cleavage products          | [91]       |
| <i>catC</i>      | Muconolactone isomerase          | Central carbon metabolism integration        | <i>Pseudomonas</i> sp.                                                                   | Conversion to TCA cycle intermediates                     | [91]       |
| <i>pnpA/pnpB</i> | Monooxygenase system             | Oxidative degradation of aromatic compounds  | <i>Burkholderia</i> sp.,<br><i>Sphingomonas</i> sp.                                      | Oxidation of TCP-derived metabolites                      | [88]       |
| <i>todC1C2BA</i> | Toluene dioxygenase complex      | Aromatic ring oxidation                      | <i>Pseudomonas putida</i>                                                                | Oxidative degradation of chlorinated aromatic metabolites | [91]       |

#### 5.2.1. Organophosphate-Degrading Gene (*opd*)

The *opd* gene was first isolated from organophosphate-degrading bacteria and encodes for organophosphate hydrolase (OPH), a metalloenzyme that can break the phosphoester bond found in many organophosphorus pesticides [92]. OPHs are often found on plasmids or mobile genetic elements and can be horizontally transferred between microbial populations [86]. This enzymatic transformation is the key first step in the detoxification of CPF to less toxic metabolites. The hydrolysis reaction yields two major metabolites, 3,5,6-trichloro-2-pyridinol (TCP) and diethyl thiophosphoric acid (DETP) [6,22,32,33]. The presence of *opd* homologs in represented genera like *Pseudomonas*, *Flavobacterium*, *Enterobacter*, *Agrobacterium*, and *Sphingobium* suggests that the degradation of organophosphates has been highly selected for in contaminated habitats. Importantly, however, the degradation efficiency is often related to the number of gene copies, the strength of the promoter, the amount of enzyme produced, and environmental conditions affecting the regulation of transcription. But the presence of *opd* alone is not enough to ensure complete mineralization, as TCP often remains as a long-lived intermediate. Thus, organisms that are both able to degrade TCP and have downstream pathways that degrade the resulting products tend to be better remediators. The *opd* gene is also an important target in biotechnology for the development of genetically engineered microorganisms with higher degradation abilities. However, in field conditions, the advantages obtained by over-expression of *opd* in the lab do not necessarily manifest in the field because of ecological competition, metabolic burden, and environmental stressors. This emphasizes the importance of combining genetic and ecological strategies in the development of CPF systems for bioremediation.

#### 5.2.2. Methyl Parathion Hydrolase Gene (*mpd*)

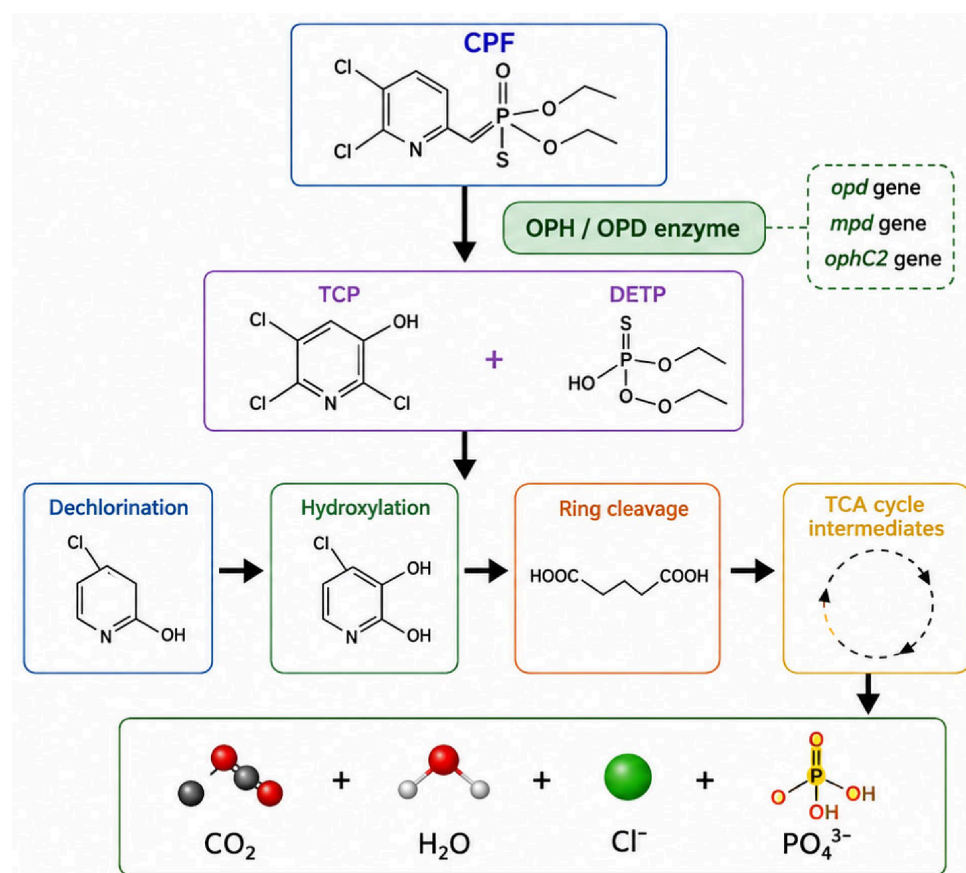
Another relevant gene determining organophosphorus pesticide degradation is methyl parathion hydrolase (*mpd*). Initially, these enzymes were described for the degradation of methyl parathion, but later it was shown that they have broad specificity and are able to degrade CPF and related compounds [93]. Because of this catalytic ability, the microorganisms containing *mpd* are even more appealing for use in cleaning up environments where mixed pesticide residues have been found. The enzymes in the MPD have been structurally characterized and show active-site structures that can accept a wide range of organophosphate substrates, which rationalizes their ability to degrade a wide range of organophosphate molecules [94]. The substrate specificity and catalytic activity of enzyme degradation by MPD could differ under varying environmental conditions. A major problem with the degradation by *mpd* is the failure to remove all the toxic intermediates. Hydrolysis significantly decreases the toxicity of CPF, but some persistent metabolites like TCP can still have ecological and toxicological effects. Thus, for complete detoxification, a set of complementary metabolic pathways must be present in microbial strains containing *mpd*.

#### 5.2.3. Organophosphorus Hydrolase (*oph*) and Related Genes

Organophosphorus hydrolase (*oph*) is a gene family that encodes enzymes that hydrolyze phosphorus-containing ester bonds. Like OPD enzymes, OPH proteins catalyze the conversion of CPF to less toxic metabolites and are an important protective mechanism against organophosphorus toxicity [95,96]. The variants, like *ophC2*, have gained great attention due to their high catalytic activity, wider range of substrates, and better environmental stability [97].

In multiple isolates of CPF-degrading bacteria, an *ophC2* gene has been linked to higher degradation rates and greater resistance to higher levels of pesticides. Like other members of the *oph* gene family, sequence differences in these genes are predicted to

have a substantial effect on substrate specificity and catalytic efficiency [97]. These results demonstrate that enzyme engineering could be used to improve the degradation properties if certain active-site residues were targeted. Figure 4 integrates the major biochemical steps proposed for bacterial chlorpyrifos transformation, from initial phosphoester cleavage through TCP conversion, ring cleavage, and mineralization. The transformation is shown as the hydrolytic cleavage reaction and is catalyzed by organophosphorus hydrolase/organophosphate-degrading (OPH/OPD) enzymes. The representative genetic determinants of organophosphate hydrolysis are shown as genes *opd*, *mpd*, and *ophC2*. This reaction involves the removal of the chlorinated pyridinol fragment from the molecule and the formation of TCP-related and dialkyl phosphate/phosphorothioate products. Progressive oxidation and dehalogenation ultimately result in mineralization ( $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , chloride, and inorganic phosphate).



**Figure 4.** Proposed bacterial degradation pathway of chlorpyrifos, highlighting initial organophosphorus hydrolase-mediated cleavage, formation and subsequent transformation of TCP-related intermediates, ring cleavage, entry into central metabolism, and eventual mineralization.

#### 5.2.4. Dehalogenases and Detoxification of TCP

CPF hydrolysis can produce TCP, a persistent and antimicrobial transformation product that may limit further biodegradation [19,90]. Consequently, effective CPF remediation requires not only initial hydrolysis but also efficient TCP transformation. Dehalogenases facilitate the removal of chlorine substituents through reductive or hydrolytic dechlorination reactions, decreasing the chemical stability of chlorinated intermediates and making them more susceptible to subsequent oxidative transformation. Dehalogenase-associated activities have therefore been proposed as important components of pathways leading from TCP toward less chlorinated intermediates and, ultimately, mineralization [19,90].

Dehalogenating microorganisms may also support community-level remediation by reducing the toxicity of TCP and thereby improving the survival and activity of other CPF-degrading populations. This complementary function provides a mechanistic rationale for using microbial consortia in which different members perform sequential steps of CPF and TCP transformation [43,90].

#### 5.2.5. Monooxygenases and Aromatic Ring Transformation

After dechlorination, aromatic-ring transformation can proceed through oxygenation and subsequent ring-cleavage reactions. Monooxygenases introduce oxygen into aromatic structures and can generate hydroxylated metabolites that are more amenable to cleavage [19,91]. These products may undergo ortho and/or meta cleavage and enter central metabolic pathways. Sequential oxidation and ring opening can yield lower-molecular-weight organic acids and intermediates connected with the tricarboxylic acid cycle; when mineralization is complete, carbon is released as CO<sub>2</sub> and hydrogen as H<sub>2</sub>O, while chlorine and phosphorus are released as inorganic ions [19,91]. Because complete transformation depends on multiple downstream reactions, monooxygenases and ring-cleavage enzymes are relevant indicators of broader biodegradation potential [91].

#### 5.3. Oxidative and Reductive Transformations of TCP

Besides hydrolysis, some bacteria can promote oxidative or reductive modifications that play an important role in the degradation of CPF to achieve complete detoxification of the environment contaminated with CPF [31]. Metabolic pathways involved in the conversion of TCP to less toxic intermediates, including dechlorination, hydroxylation, and ring cleavage [88]. Monooxygenases and dioxygenase enzyme systems introduce hydroxyl groups and facilitate ring cleavage [91]. The reactions produce chlorinated or non-chlorinated aliphatic molecules that are transformed into harmless end products such as CO<sub>2</sub>, H<sub>2</sub>O, and inorganic chloride ions [2]. Furthermore, reductive dechlorination is done under anaerobic or low-oxygen conditions [98]. Some bacterial species mediate reductive dechlorination of TCP, which makes the process faster to break down and detoxify TCP, especially by dechlorinating the chlorinated pyridinol ring [90].

These alternative pathways increase the diversity and effectiveness of bacterial degradation processes besides hydrolysis, though they are of minor importance [90,98]. Further, the metabolic pathways involved in the degradation of CPF are linked to the central metabolic pathways, which enable microbes to incorporate degradation products as carbon, nitrogen, or phosphorus sources, promoting further microbial growth and enhancing bioremediation performance [2]. Thus, the bacterial degradation of CPF is a multi-step process that includes: (a) hydrolysis of CPF to TCP and DETP, (b). dechlorination, hydroxylation, and ring cleavage of TCP, (c). oxidation/reduction reactions of CPF and its intermediates. (d). mineralization of final products to CO<sub>2</sub>, H<sub>2</sub>O, and inorganic salts. All these mechanisms help to decrease the persistence and toxicity of CPF in the environment and provide a way of thinking about bioremediation with an effective bacterial strain.

## 6. Environmental Implications of Chlorpyrifos-Degrading Bacteria

The widespread application of CPF, a long-lasting organophosphorus pesticide, has caused widespread ecological concerns, such as soil degradation, contamination of water bodies, adverse effects on non-target species, and bioaccumulation in food chains. Bacterial degradation of CPF provides an ecologically friendly solution that can help minimize the environmental impact, improve soil health, protect biodiversity, and ensure sustainable agricultural practices. The bioremediation potential can be enhanced by maintaining an effective chlorpyrifos-degrading microbial community in the contaminated site and opti-

mizing environmental conditions [99]. In terrestrial ecosystems, beneficial soil microbiota restoration and enzymatic activity restoration, such as dehydrogenase and phosphatase, improve nutrient cycling and soil fertility [100–102]. Remarkably, these beneficial soil bacteria have plant growth-promoting properties, which are vital for maintaining soil health and productivity by various mechanisms, such as nitrogen fixation and solubilization of phosphates [15,100,101].

The use of CPF-degrading bacteria in in situ and ex situ bioremediation can provide a cost-effective and eco-friendly solution to chemical or physical bioremediation [103–105]. Bioaugmentation and rhizoremediation (with natural consortia or plant-associated bacteria) improve degradation efficiency of contaminated soils and confer increased plant resilience and productivity. In addition to pesticide removal, microbial degradation also provides indirect protection to non-target organisms. Minimizing CPF residues in soils and on crops reduces exposure risk to pollinators, earthworms, and other soil fauna and assists in the maintenance of ecosystem functions and agricultural sustainability [106]. Furthermore, microbial activity mitigates pesticide levels in water bodies by minimizing leaching pesticide concentrations in water and protecting fish, amphibian, and invertebrate communities from the harmful effects of pesticides [106].

Most importantly, bacterial breakdown of CPF is also a way to reduce contamination of the food chain. Bacterial activity lowers the risk of CPF entering the food web by reducing pesticide residues in the rhizosphere and on edible plant parts, thus decreasing bioaccumulation and biomagnification in higher trophic levels, including humans [107]. In addition, the degradation of CPF restores soil microbial diversity and activity, which in turn contributes to soil ecosystem services, including carbon sequestration and soil resistance to environmental stress, in turn affecting climate change mitigation. Kiran et al. (2026) demonstrated that integrating CPF-degrading *K. pneumoniae* C78 with *R. communis* L. and biochar amendment constitutes a promising, eco-compatible strategy for enhancing CPF degradation and restoring the productivity of contaminated agricultural soils [108]. Table 5 summarizes key physicochemical and biological factors that influence chlorpyrifos biodegradation and the implications of these factors for field-scale implementation. The table lists major environmental and operational parameters that affect CPF biodegradation in both the laboratory and the field. The degradation efficiencies of CPF are under the control of the complex interaction among physicochemical parameters, microbial community structure, and ecological processes. The pH, temperature, nutrient status, oxygenation status, organic matter status, salinity, and concentration of contaminants can directly impact microbial growth, enzyme activity, and degradation kinetics. Further, microbial diversity, biofilm formation, quorum sensing, and interaction of microbes and plants are biological factors that are important in remediation stability and efficacy. Knowledge of these factors is crucial to optimizing the field-scale bioremediation strategies and long-term performance of CPF-degrading microbial systems.

To summarize, the environmental effects of CPF-degrading bacteria are not only on the detoxification of pollutants. They play a vital role in improving soil health and fertility, reducing environmental hazards, safeguarding biodiversity, and boosting agricultural output, thereby highlighting their importance in sustainable bioremediation practices. Ongoing research into optimizing microbial consortia, improving degradation in the field, and assessing long-term ecological effects is essential for the safe and effective application of these bacterial solutions.

**Table 5.** Environmental and operational factors influencing chlorpyrifos (CPF) biodegradation and field-scale bioremediation performance.

| Factor                        | Influence on CPF Biodegradation                               | Mechanism of Action                                                                          | Implications for Field Application                                                              | References |
|-------------------------------|---------------------------------------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|------------|
| pH                            | Strongly influences degradation rate and enzyme activity      | Affects organophosphorus hydrolase (OPH) activity, microbial growth, and CPF bioavailability | Neutral to slightly alkaline conditions (pH 6.5–8.0) generally favor CPF degradation            | [92]       |
| Temperature                   | Controls microbial metabolism and enzymatic reactions         | Influences membrane fluidity, growth rate, and degradation kinetics                          | Most CPF-degrading bacteria exhibit optimum activity between 25 and 35 °C                       | [37]       |
| Soil Organic Matter (SOM)     | Can enhance or inhibit degradation depending on concentration | Provides nutrients and microbial habitats but may increase CPF sorption                      | Moderate SOM promotes biodegradation; excessive sorption may reduce bioavailability             | [5]        |
| Soil Moisture                 | Regulates microbial activity and pesticide mobility           | Influences oxygen diffusion, nutrient transport, and microbial proliferation                 | Adequate moisture supports degradation; drought conditions suppress microbial activity          | [82]       |
| Salinity                      | Often reduces degradation efficiency                          | Causes osmotic stress and suppresses microbial growth                                        | Salt-tolerant degraders may be required in coastal and arid agricultural regions                | [20]       |
| Heavy Metals                  | Frequently inhibit CPF biodegradation                         | Enzyme inhibition, oxidative stress, and disruption of microbial metabolism                  | Co-contamination can significantly reduce remediation efficiency                                | [18]       |
| Nutrient Availability         | Enhances microbial growth and degradative activity            | Supplies carbon, nitrogen, phosphorus, and micronutrients required for metabolism            | Nutrient amendments may accelerate remediation but excessive inputs can alter microbial ecology | [66]       |
| Oxygen Availability           | Determines dominant degradation pathways                      | Supports aerobic oxidation and ring-cleavage reactions                                       | Aerobic conditions generally favor complete mineralization of CPF and TCP                       | [98]       |
| Initial CPF Concentration     | Influences degradation kinetics and microbial stress          | High concentrations may become toxic to degraders                                            | Adapted microbial consortia often tolerate higher CPF levels than single strains                | [68]       |
| TCP Accumulation              | Major limiting factor for complete degradation                | TCP exhibits antimicrobial activity and inhibits microbial growth                            | Efficient TCP-degrading microorganisms are essential for successful remediation                 | [19]       |
| Microbial Community Diversity | Strongly affects degradation stability and resilience         | Functional redundancy and metabolic cooperation improve degradation performance              | Diverse microbial consortia generally outperform monocultures under field conditions            | [43]       |
| Biofilm Formation             | Enhances long-term degradation efficiency                     | Improves cell adhesion, stress tolerance, and metabolic cooperation                          | Biofilm-forming consortia are often more effective in contaminated soils and sediments          | [74]       |
| Quorum Sensing                | Regulates expression of degradative enzymes                   | Coordinates microbial communication and collective metabolic activity                        | Enhances stability and efficiency of microbial consortia                                        | [75]       |
| Plant Root Exudates           | Stimulate rhizosphere biodegradation                          | Supply carbon sources and signaling molecules that support degraders                         | Important in rhizoremediation and plant–microbe remediation systems                             | [14]       |
| Biochar Amendment             | Generally enhances microbial degradation                      | Improves microbial habitat, nutrient retention, and contaminant adsorption                   | Promising strategy for integrated CPF remediation and soil restoration                          | [79]       |

Field studies have demonstrated the practical potential of bacterial remediation under real-world agricultural conditions. For instance, a bacterial consortium, ECO-M, was formed by isolating CPF-degrading bacterial strains, including *Agrobacterium tumefaciens* strain ECO1, *Cellulosimicrobium funkei* strain ECO2, *Shinella zoogloeoides* strain ECO3, and *Bacillus aryabhatai* strain ECO4, which degraded 100% of CPF within 6 days at an initial concentration of 50 mg/L [68]. Another study demonstrated that up to 96% CPF removal was observed in the bacterial–plant consortium [14]. However, there may be unintended consequences that need to be carefully considered. Incorporation of non-native strains of bacteria or genetically engineered degraders could have an unknown ecological impact on the structure of indigenous microbial communities or horizontal gene transfer of resistance or degradation-associated genes. Moreover, if they are not metabolized efficiently, accumulation of intermediate metabolites may create further environmental hazards. Therefore, long-term monitoring, ecological risk assessments, and regulatory monitoring are crucial for the safe application of bacterial remediation measures. Therefore, it is crucial to understand the genetic basis of CPF biodegradation to create improved bioaugmentation strategies and engineer stronger microbial CPF degraders. Traditional physicochemical methods like adsorption, advanced oxidation, and chemical treatment can result in rapid CPF removal, but can be expensive, generate secondary waste, and may not fully mineralize the CPF. Biological methods, such as phytoremediation, bacterial remediation, and microbial consortia, have been considered environmentally friendly and can convert the CPF to less harmful compounds or mineralize the CPF completely. Of these technologies, microbial consortia-based systems seem to be especially promising as they have a combination of high degradation efficiency, ecological resilience, low operational costs, and good field applicability.

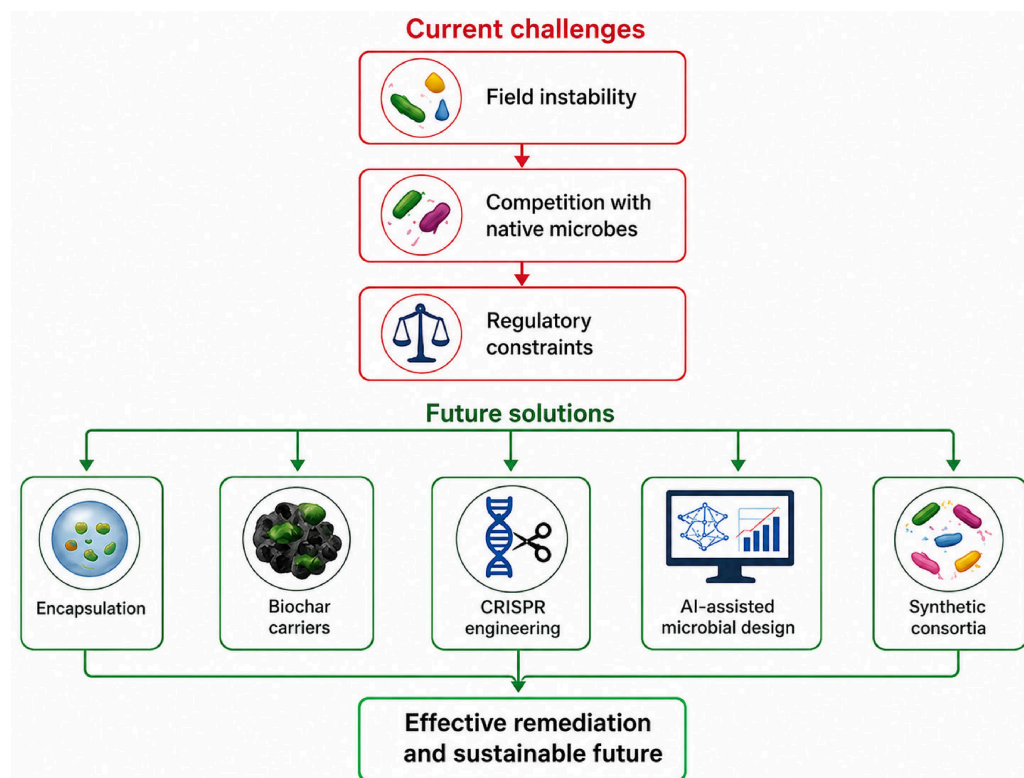
## 7. Challenges and Future Prospects

Among the limitations, perhaps the greatest challenge to the widespread use of CPF-degrading bacteria is the lack of consistency in bacterial survival and functionality in the field due to changing soil moisture, nutrient levels, soil pH, and temperature. Although extensive research has been done on microbial pesticide-remediation products, relatively few products have yet been commercially deployed. Regulatory evaluations may necessitate in-depth evaluations of the environmental safety, ecological impacts, genetic stability, and long-term efficacy of the product. The requirements make development more expensive and slow down the commercialization process [34,43].

Other challenges include microbial production on a large scale, formulation stability, maintaining shelf life, performance variability in the field, and public acceptance of microbial technologies. Additionally, there are concerns about the unintended alteration of the native soil microbial community that can affect soil nutrient cycling, soil structure, and plant health. The use of genetically modified organisms (GMOs) or consortia in the complex soil ecosystem might result in unexpected ecological effects [109].

Regulatory issues are even more significant if GMOs are used for environmental applications. In addition, regulatory frameworks governing the environmental release of GMOs for bioremediation purposes in many regions around the world are not allowed, which hinders the use of advanced microbial technologies. There are also major barriers related to public perception and acceptance, especially in the agricultural sector, where there is still a lot of doubt about GMOs. For commercialization, standardized evaluation protocols, long-term field trials, risk assessment procedures, and collaborations between microbiologists, environmental engineers, policymakers and industry partners should be focused on the future. Figure 5 summarizes the principal obstacles that currently separate laboratory demonstrations of CPF biodegradation from reliable field implemen-

tation. Field instability occurs because of differences in potentially sub-optimal field conditions such as moisture, nutrient availability, pH, soil texture, contaminant aging, and pesticide bioavailability. Introduced degraders must also compete with indigenous microorganisms, withstand predation and environmental stress, and establish sufficient population density and metabolic activity at contaminated microsites. Issues of regulatory or biosafety concern will be particularly relevant if genetically modified strains and engineered communities or deliberate environmental release are being considered. The proposed future solutions aim to overcome these limitations with complementary solutions. CRISPR-based genome engineering could be used to improve specific traits of interest such as catabolic, stress tolerance, or regulatory traits, though this would require ecological containment and regulatory approval. By combining genomic, metabolic, and ecological data, AI-assisted microbial design can streamline the process of identifying compatible strains, enzymes, pathways, and conditions for microbial growth, potentially saving time in selecting the most effective microbial systems. In combination, these strategies work to enhance persistence, predictability, biosafety, and degradation completeness in actual contaminated environments.



**Figure 5.** Major barriers to field-scale chlorpyrifos bioremediation and emerging technological solutions, including microbial encapsulation, biochar-based carriers, genome engineering, AI-assisted microbial design, and synthetic consortia.

### 7.1. Bridging the Gap Between Laboratory Success and Field Reality

Although microbial degradation research has progressed in remarkable ways, achieving good results in the lab does not necessarily mean that it will be the same in the field. Many strains of bacteria have been shown to remove more than 90% under controlled laboratory conditions, but in the field, there is often a lack of consistent results. This discrepancy may result from environmental heterogeneity, variation in climatic factors, nutrient scarcity, competition amongst microorganisms, and the accumulation of toxic metabolites. Therefore, for the commercialization of the CPF bioremediation technologies, there is a

need to shift from a “strain-based” to an “ecological engineering” approach that can work under complex environmental conditions.

Further studies should focus on the optimization of the genome and metabolic pathways of synthetic microbial consortia, which may be designed to improve degradation efficiency, environmental stability, and biosafety. The future of CPF bioremediation will be a combination of systems biology, ecological engineering, and digital technologies. Next-generation microbial remediation technologies will be multifunctional microbial consortia, biochar-aided bioaugmentation, rhizosphere engineering, and microbial design with AI. Reliable field-scale performance and regulatory acceptance will be the key to advancing innovations in the lab to solutions that can be deployed across the globe to reduce CPF contamination. Systematic ecological risk assessments and community engagement efforts will be key to developing trust in microbial bioremediation technologies and facilitating regulatory approval for field-scale use.

### *7.2. Artificial Intelligence and Predictive Bioremediation*

Artificial Intelligence (AI) and Machine Learning (ML) are new enabling technologies that are making their way into the field of environmental biotechnology. AI models can merge genomic, transcriptomic, proteomic, and environmental data to predict degradation efficiency, identify vital functional genes, and design better microbial consortiums. Machine learning algorithms are widely used to make predictions of enzyme-substrate interactions, to find new biodegradation pathways, or to model interactions between microorganisms in an ecological community [110]. These methods can significantly shorten the development time of very efficient CPF remediation systems and minimize experimental expenses.

The emerging integration of omics technologies, systems biology, and the use of AI for data analysis and analytics is predicted to shape future directions in pesticide bioremediation, conducting in a new era of environmental restoration driven by data.

### *7.3. From Functional Genes to Synthetic Biology*

New insights into CPF degradation mechanisms have been enhanced through recent progress in genomics, metagenomics, and transcriptomics, as well as through synthetic biology approaches. High-throughput sequencing technologies are still revealing new catabolic genes, new breakdown pathways, and intricate regulatory networks involved in the metabolism of pesticides. Meanwhile, synthetic biology techniques are making it possible to create new engineered microorganisms and synthetic microbial consortia with increased degradation capabilities [111].

Instead of single-gene strategies, future CPF bioremediation strategies will likely shift toward a systems-level engineering approach that incorporates several functional genes that are involved in hydrolysis, dechlorination, oxidation, and mineralization. Engineered microbial platforms can potentially enhance degradation efficacy, reduce the accumulation of toxic intermediates, and integrate the *opd*, *mpd*, *ophC2*, dehalogenase, and monooxygenase pathways. The potential of these developments to overcome the current limitations and to progress sustainable remediation of CPF-contaminated environments is very promising.

## **8. Concluding Remarks**

This review synthesizes chlorpyrifos biodegradation across four interconnected levels: bacterial diversity, molecular mechanisms, community-level metabolic cooperation, and environmental constraints, and evaluates the critical transition from laboratory-measured pesticide removal to demonstrable detoxification and field-scale remediation. Bacterial degradation of CPF is a potential environmentally friendly approach to reducing the contamination of pesticides. The metabolic adaptability of different bacterial taxa, microbial

genomics, and enzymology have extended our knowledge of the CPF detoxification pathway. Additionally, synergistic microbial consortia have been shown to be more efficient at degradation, providing opportunities for better bioremediation under field conditions. However, there are still issues to be addressed about their stability, survival, and scaling up of these biological solutions. The use of advanced genetic tools, optimized microbial formulations, and rhizosphere management may play a key role in moving from the laboratory to large-scale application for addressing these problems. Ultimately, the successful use of CPF-degrading bacteria can greatly decrease pollution of the environment, improve soil health, increase agricultural sustainability, and decrease human and ecological health risks. Further research is needed to address the knowledge gaps, improve bioremediation techniques, and play its role in the creation of a pollution-free environment.

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