# Progress in bioremediation of pesticide residues in the environment

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## ABSTRACT

The increasing use of various pesticides (e.g., organophosphate, organochlorine, carbamates, and pyrethroid) has helped to improve agricultural productivity by minimizing the potential crop losses associated with insect attacks. Owing to their highly recalcitrant nature, most pesticides and their residues often accumulate in the environment to exert deleterious effects on human health and various ecosystems. Among a variety of remediation options, biological approaches have attracted a widespread attention for the treatment of pesticide in soil/water systems due to their environmentally benign nature. In this regard, this review article was organized to highlight the recent advancements in the application of various bioremediation approaches for the degradation/removal of pesticides from soil/water matrixes along with the catabolic capacity of microorganisms. Our discussions were expanded further to emphasize identification of specific bacterial communities/strains, such as *Bacillus* sp. and *Pseudomonas* sp. This review is expected to provide an overview of the modern biotechnological methodologies along with the associated merits and hurdles for the effective abatement of pesticides.

**Keywords:** Bioreactors, Degradation factors, Microbial degradation, Organophosphate pesticides, Organochlorine insecticides, Soil remediation

## 1. Introduction

Pesticides produced as insecticides, herbicides, fungicides, and in many other forms have been used for agricultural or horticultural purposes [1]. This revolutionary development in agriculture technologies enhanced the crop yield while reducing the risk of crop loss or quality deterioration [2]. However, these substances are commonly dispersed contaminants due to their toxicity, persistence, and degradation by-products.

Pesticides have become essential features in modern agriculture for economical pest management and better crop production accompanied by the rapid growth of the global population, e.g., 1.1% increase of pesticide use in 2016 over the previous year [3]. The use of pesticides and associated environmental contaminants are expected to become worse in the foreseeable future. Approximately 2.4 million metric tons of pesticides were used worldwide (as of 2014) to control various insects, weeds, fungi, and other unwanted organisms in agricultural and urban environments [4]. Due to excessive application, pesticide residues were reported to remain in the environment longer than 10 years and are detected at a level of $\mu$ gL$^{-1}$ in water resources or $\mu$g kg$^{-1}$ in soils [5]. Human exposure to pesticides at a relatively high concentrationcan occur through soils and drinking water, thereby threatening human health and potentially causing fertility disorders due to their high carcinogenicity and neurotoxicity [6, 7].

Due to the detection of pesticides and residues in water sources,

**Table 1.** Chemistry and Physicochemical Characteristics, Production, Hazard Levels, and Health Effects of Pesticides

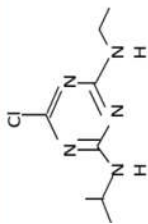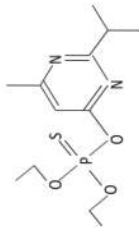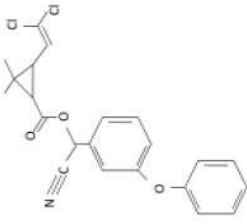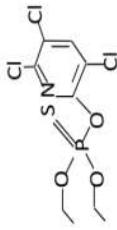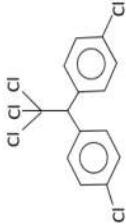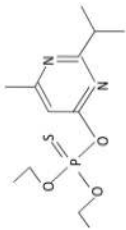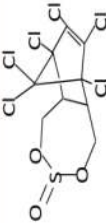| Order | Pesticide | 2D structure of pesticide | Target crops | Global production 2014-15 (MT) | Maximum permissible residual limit in food (ppm) | Class | Human health effects | References |
|---|---|---|---|---|---|---|---|---|
| 1 | Atrazine |  | Sugarcane, rice, green vegetables | 1200 | 0.2 | Moderately hazardous | Responsible for damage to liver, kidney, and heart in animals Damage of vital organs in humans has yet to be studied in detail | [77,48] |
| 2 | Carbofuran |  | Potatoes, corn, soybeans | 8590 | 0.3 | Highly hazardous | Reproductive disorders Affectsnervoussystem Cholinesterase inhibitor | [77,101,84, 47] |
| 3 | Cypermethane |  | Cotton, fruit and vegetable crops | 8590 | 0.03 | Moderately hazardous | Possible carcinogen Suspected endocrine disruptor | [77,67,48] |
| 4 | Chlorpyrifos |  | Corn, soybeans, fruit and nut trees, other row crops | 9880 | 0.05 | Moderately hazardous | Cholinesterase inhibitor | [77, 119,16] |
| 5 | DDT |  | Various food crops | 95 (2010-11) | 0.5 | Highly hazardous | Vomiting,shakiness, seizures | [77,2,48, 14] |
| 6 | Diazinon |  | Fruit, vegetable, nut and field crops | 0 | 0.1 | Moderately hazardous | Cholinesterase inhibitor | [9,48] |

**Table 1.** Continuation
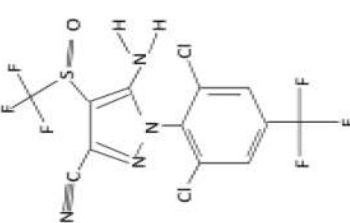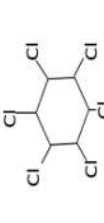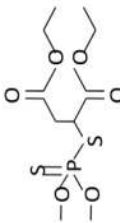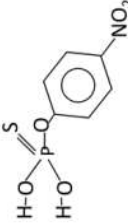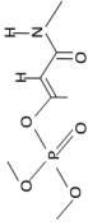
| Order | Pesticide | 2D structure of pesticide | Target crops | Global production 2014-15 (MT) | Maximum permissible residual limit in food (ppm) | Class | Human health effects | References |
|---|---|---|---|---|---|---|---|---|
| 7 | Endosulfan | | Food crops such as teas, grains, fruit, vegetables Non-food crops such as tobacco, cotton | 1350 (2011-12) | 0.5 | Moderately hazardous | Suspected endocrine disruptor | [83,63] |
| 8 | Fipronil | | Rice, cotton | 0 | 0.01 | Moderately hazardous | Sweating, dizziness | [48,120, 58,31] |
| 9 | Lindane | | Sugarbeet crops,grains, fruit, vegetables | 800 (2007-08) | 1 | Highly hazardous | Aplastic anemia, breast cancer | [77,75,63] |
| 10 | Malathion | | Strawberries, limes, cotton, cherries, garlic, greens, dates, celery | 2346 | 8 | Moderately hazardous | Carcinogenic to humans and animals Cholinergic toxicity and neurotoxicity in animals and immunity of higher vertebrates | [73,82, 121] |
| 11 | Methyl parathion/ parathion | | Fruit, cereals, vines, vegetables, cotton, field crops | 885 (2007-08) | 0.2 ppm methylparathion 0.05 ppm parathion | Highly hazardous | Cholinesterase inhibitor Possible carcinogen Suspected endocrine disruptor | [122,59,63, 123] |
| 12 | Monocrotophos | | Potato, cotton crops | 4500 | 0.05 | Moderately hazardous | Carcinogenic; Affects reproductive system | [124,125, 63] |

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**Table 2.** A Survey of Bioremediation Approaches for Pesticides at Various Environmental Conditions

| Order | Pesticide | Microorganism | Process | Experimental conditions | | | | | Pesticide removal efficiency (%) | Lab scale study for pesticide degradation in soil | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Batch study of Pesticide concentration (mg/L) | pH | Temperature (°C) | Stirring speed (RPM) | Duration (day) | | | |
| 1 | Atrazine | Arthrobacter sp. | Batch | 500 | 7 | 30 | 120 | 3 | 95 | - | [126] |
| | | Pseudomonas sp. | Batch immobilized | 100 | 7.4 | 25 | 150 | 70 | 64 | - | [13] |
| | | Pichiakudriavzevii | Batch immobilized | 500 | 7 | 30 | 120 | 5 | 94.3 | - | [37] |
| | | Microbial consortium | Site study | - | - | - | - | 160 | 48 | 160 μg kg⁻¹ soil | [41] |
| 2 | Carbofuran | Burkholderiacepacia | Batch | - | 6.9 | Room temperature | - | 35 | - | 5000μg kg⁻¹ soil | [84] |
| | | Burkholderiacepacia PCL3 | Field study | - | 6.7 | 29-32 | - | 60 | - | 1830μg kg⁻¹ soil (Field study) | [47] |
| 3 | Chlorpyrifos | Ochrobactrumsp | Batch | 100-500 | 6.8-7 | Room temperature | 100 | 5 | 50 | - | [127] |
| | | Paracoccus sp. | Batch | 50 | 7 | 30 | 100 | 5 | - | - | [14] |
| | | Pseudomonas putida | Batch | 10-100 μgL⁻¹ | 3 | 25 | 200 | 3 | 97 | - | [72] |
| | | Microbial consortium | Batch | - | 7 | 30 | 150 | 10 | 82 | 50 mg kg⁻¹ soil | [128] |
| 4 | DDT | Chryseobacterium sp. | Pilot-scale ex situ | 50 | 6.5±0.5 | Room temperature | | 45 | 80.3 | 6.97-35.37 mg kg⁻¹ | [25] |
| | | Trametesversicolor | Batch | - | 4.5 | | | 40 | 73 | 35.449 mg kg⁻¹ | [103] |
| | | Microbial consortium | Slurry | - | 7 | Room temperature | | 20 | 78.93-92.11 | 33.23 mg kg⁻¹ | [104] |
| 5 | Diazinon | Pseudomonas sp. | Batch | 50 | 7.2 | 30 | - | 14 | 80-92 | 100 mg kg⁻¹ soil | [129] |
| | | Serratiamarcescens | Batch | 50 | 7.2 | 30 | 200 | 14 | 80 | - | [129] |
| | | Microbial consortium | Batch | 10 | 7 | 28 | 150 | 4 | 35 | - | [52] |
| 6 | Endosulfan | Alcaligenesfaecalis | Batch | 100 | 7 | 40 | - | 5 | 89 | - | [7] |
| | | Pseudomonas sp. | Batch | - | 7 | 37 | 200 | | 86 | 203.465 | [106] |
| | | Aspergillusniger | Batch | 400 | 6.8 | 28±2 | 120 | 12 | 72 | - | [53] |
| | | Microbial consortium | Batch | 100 | 7 | Room temperature | - | 4 | 70 | - | [130] |

Table 2. Continuation

| Order | Pesticide | Microorganism | Process | Batch study of Pesticide concentration (mg/L) | Experimental conditions | | | | | Pesticide removal efficiency (%) | Lab scale study for pesticide degradation in soil | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | pH | Temperature (°C) | Stirring speed (RPM) | Duration (day) | | | | |
| 7 | Fipronil | Bacillus firmus | Batch | - | 7 | 25 | - | 56 | | 71 | 1.50-20.5 mg kg⁻¹ | [33] |
| | | Bacillus thuringiensis | Batch | - | 7 | 28 | - | 42 | | 73 | 1.5mg kg⁻¹ | [55] |
| | | Brassica pekinensis | Field study | - | 7±0.5 | 25±10 | - | - | | - | - | [83] |
| | | Microbial consortium | Batch | - | 5.85-8.35 | 30±1 | - | 19 | | - | 2 µg g⁻¹ soil | [76] |
| 8 | Lindane | Streptomyces consortium | Batch immobilization | 50 | - | 30 | 200 | 28 | | 94 | - | [88] |
| | | Pleurotusostreatus | Batch | 4.46 mg L⁻¹ | 7 | 28 | 90 | 12 | | - | - | [131] |
| | | Hymeniacidonperlevis | Batch | 1 µg L⁻¹ | - | Room temperature | | 8 | | 97 | - | [12] |
| | | Fusariumverticillioides | Batch | 100 | 6.8 | 30±2 | 120 | 12 | | 83.3 | - | [81] |
| 9 | Malathion | Bacillus sp.S14 | Batch | 25 | 7±0.2 | 30±1 | 120 | 10 | | 64.5 | - | [132] |
| | | Bacillus thuringiensis | Batch | 250 | - | - | - | 7 | | 50 | - | [30] |
| | | Bacillus cereus | Batch | 100 | 7±0.2 | 30 | 160 | 12 | | 49.31 | - | [28] |
| | | Bacillus licheniformis | Batch | 25 | 7.5 | 32 | 250 | 10 | | 78 | - | [116] |
| 10 | Methyl parathion | Pseudomonas sp. | Batch | 50-100 | 7 | 28 | 180 | - | | 75.95 | - | [95] |
| | | Penicilliumcitrinum | Batch | 120 | 8 | 27 | 130 | 15 | | 90 | - | [133] |
| | | Fusariumproliferatum | Batch | 360 | 8 | 30 | 130 | 15 | | 90 | - | [133] |
| | | Acinetobacterradioresistens | Batch | 130 | 5.0-8.0 | 30 | 200 | 4 | | 41.66 | - | [62] |
| 11 | Monocrotophos | Aspergillusoryzae | Batch | 100-500 | 6.8 | 30±2 | 120 | 8 | | 75 | - | [134] |
| | | Stenotrophomonas sp. | Batch | 50 | - | 40 | - | 10 | | 63 | - | [96] |
| | | Microbial consortium | Batch slurry | - | 6.5±0.5 | 28 ± 4 | - | 20 | | 96-98 | 100 µg g⁻¹ | [26] |

many studies have focused on developing physicochemical technologies for wastewater treatment to remove such residues [8-10]. However, conventional techniques suffer from critical disadvantages, such as equipment complexity, high operating costs, excessive sludge generation, and toxic wastes as byproducts. To solve these issues, many studies suggested the use of biological methods for treating a wide range of pesticides, due to their cost-effective, highly selective, and environmentally benign nature [11-14]. However, the use of biological approaches is also limited by requirements such as a need to be compatible with the environment, uncomplicated access of the microbial population to the pesticide molecules, and procurement of suitable pesticide-degrading microorganisms [15]. Despite decades of research, the scale-up of pesticide bioremediation approaches from lab-scale into field trials has been very challenging.

The term "bioremediation" is the method of pollutant biodegradation in nature based on the metabolic capacity of microbes to breakdown various organic compounds like pesticides [16, 17]. In pesticide bioremediation, microbes with specifically/genetically enhanced functionality utilize pesticide molecules for their metabolic activity through conversion into environmentally benign products/metabolites [18]. A brief summary of the literature on the general properties of pesticides and bioremediation is provided in Tables 1 and 2, respectively.

This review focuses on the impact of various factors (e.g., pesticide structure, concentration, pH, temperature, and moisture) on the biodegradation of pesticides and the major techniques that are available for pesticide assays in soils. The latest bioremediation approaches on the degradation of organophosphate, organochlorine, carbamates, and pyrethroid pesticides in soil and water are also discussed. In this review, authors sought to highlight the advantages and drawbacks of the present bioremediation approaches for pesticides through an in-depth analysis and comparison with conventional physicochemical methods. The results of this effort will help us to enhance our knowledge of this highly challenging field of research. Furthermore, we highlight the use of microorganisms to understand the catabolic ability of the target soil and to demonstrate the benefit of combining traditional bioremediation techniques with molecular techniques.

## 2. Chemistry of Pesticides

Pesticides are classified by their nature, feedstock, and pest control capability. Depending on the pesticide's origin, it is classified as a chemical pesticide or a bio-pesticide. Chemical pesticides are further divided into four main types, namely organophosphates, organochlorines, carbamates, and pyrethroids. Bio-pesticides are derived naturally from living organisms, including bacteria, fungi, and plants. They can commonly be classified into three major groups, microbial, biochemical, and plant-incorporated protectants. Further, the classification of pesticides can also be made based on their pest-controlling capabilities: insecticides (for insects), nematicides (for nematodes), fungicides (for fungi), herbicides/weedicides (for weeds), algaecides (for algae), and rodenticides (for rats) (Figure 1 and 1S; Pesticides can be applied directly to specific plant parts or above-ground to be transported into the soil and to soil-based organisms. Depending on the application method, a fraction of the pesticide, ranging from 30.0-90.0%, infiltrate directly into the soil system [21, 22]. The impacts of various pesticides on specific soil organisms, soil food chains, and biological soil functions can vary depending on the type or amount of pesticides, soil environment, and soil biota. The impacts can be expanded to the health of the entire soil community with noticeable damage to various soil functions [23]. Pesticides are degraded by both biotic and abiotic processes into intermediate or secondary products that may have even worse toxicity than the parent pesticide. Biodegradation of pesticides/herbicides is also greatly influenced by soil conditions (e.g., temperature, moisture, organic matter content, and pH) along with microbial characteristics and pesticide solubility [24].
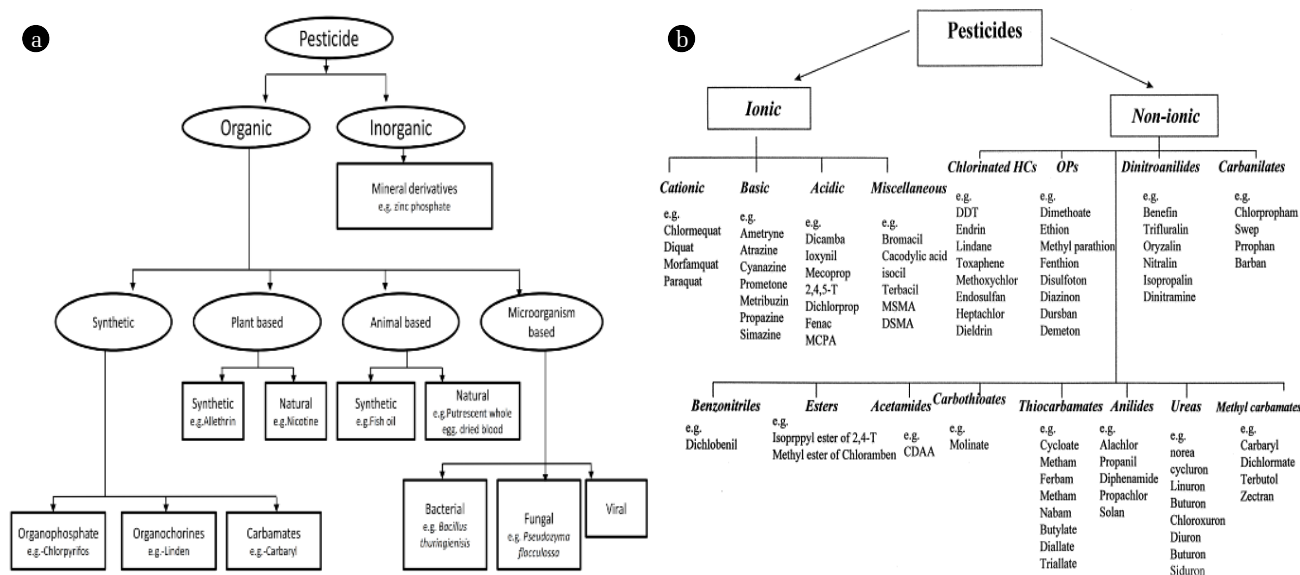


**Fig. 1.** Classification of several types of pesticides and examples: (a) on the basis of inorganic and organic pesticides, (b) on the basis of ionic forms.
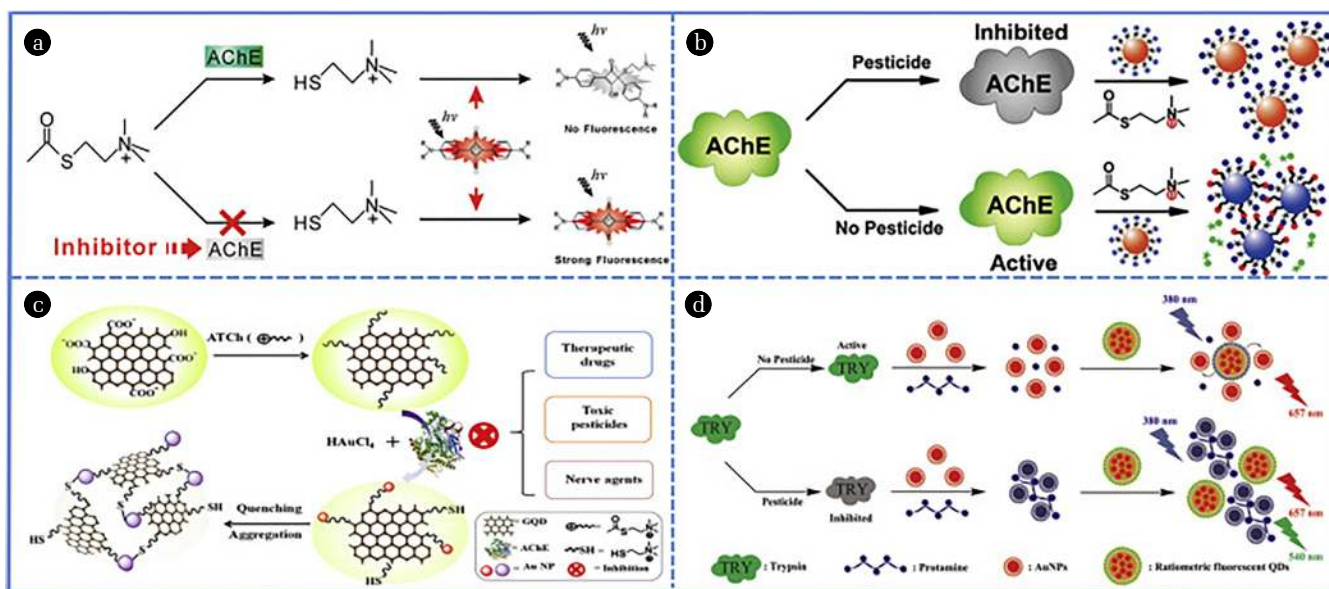
**Fig. 2.** Optical sensing strategies for pesticides: (a) description of the signal-on fluorometric strategy for cetylcholinesterase inhibitors; (b) design of the dual-readout (colorimetric and fluorometric) assay for pesticides; (c) schematic illustration of GQD-ATC/AuNPsfluorometric detection of AChE and its inhibitors; (d) illustration of the fluorescent detection of OPs through the inner-filter effect of gold nanoparticles on RF-QDs.

As the world population increases, the consumption of pesticides has dramatically increased and accelerated to maximize agricultural productivity and to satisfy food demand. However, its effects on long-term sustainability, soil degradation, water nitrification, natural resource management, and climate change are still unclear, as shown in Fig. 2 [25, 26]. The residual levels of pesticides in foods have been monitored and regulated based on the maximum residue level (MRL), as established by phyto-sanitary studies. In 2007, approximately $2.3 \times 10^6$ tons of pesticides were used worldwide, and their sales in 2014 reached 52 billion USD [27]. In the European Union, more than 800 pesticides have been authorized, although fewer than 300 pesticides are used in practice [28-30]. As of 2016, China was the largest consumer of agricultural pesticides ($1.81 \times 10^6$ ton $y^{-1}$), followed by the US ($3.86 \times 10^5$ ton $y^{-1}$), Argentina ($2.65 \times 10^5$ ton $y^{-1}$), Japan ($5.2 \times 10^4$ ton $y^{-1}$), and India ($4.0 \times 10^4$ ton $y^{-1}$). The potential crop losses by pests without any pesticides varied from ~50.0% (e.g., barley) to~80.0% (e.g., sugar, cotton, and beet) [31]. Actual losses with proper pesticides are estimated to be 26.0-30.0% for soybeans, sugar, barley, beets, cotton, and wheat, while they are 35.0% for maize, 39.0% for potatoes, and 40.0% for rice [31].

# 3. Physicochemical Methods as Pesticide Treatments

## 3.1. Extraction

Extract is a commonly used lab-scale method to remove pesticides from soils and water systems; this technique includes solid-phase extraction (SPE) and liquid-liquid extraction (LLE) [32]. For the LLE technique, chlorinated solvents (e.g., tetrachloroethane, chlor-

obenzene, and carbon tetrachloride) or $n$-hexane are widely used to determine the toxicity of pesticides. Supercritical extraction (SC-CO$_2$) was used to treat organophosphate pesticides. In SC-CO$_2$, pesticide removal depends on the solubility of the pesticide, the critical temperature, and pressure of supercritical CO$_2$ [29]. Unfortunately, there is no breakdown of pesticides into less toxic compounds. This technique requires a high operation cost to maintain the critical temperature and pressure [33]. An average removal of 90.0% was reported for organophosphate pesticides in a very short time (e.g., 20 min) at a temperature of 90.0℃ and a pressure of 235 atm (Table 3). The major disadvantages of this process are high cost and limited operational conditions (i.e., no decomposition of pesticides below the SC-CO$_2$ temperature). The nature of pollutants such as pesticides is also crucial to determine the suitability of separation methods. This may be due to the fact that the selection of a suitable solvent is very crucial to remove the pollutants such as pesticides. Pesticides in diverse forms (e.g., suspensible concentrates, granules, controlled-release formulations, and baites) require special attention and treatment. Comparisons of diverse approaches have been made for the extraction of pesticides using liquid–liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase micro-extraction (SPME) against enriched river water samples. These authors have reported that the SPE is better than LLE because of 10 times less soil sample requirements which contained the pesticides or other organic pollutants [29-33]. In general, compared to liquid-liquid extraction, solid phase extraction can be exercised with a small amount of samples.

## 3.2. Adsorption

Adsorption is a method to remove pesticides in which a certain adsorbate is transferred selectively to the surface of an insoluble immobile phase, i.e., the adsorbent. Adsorption is classified by

**Table 3.** Conventional Removal Approaches for Pesticides

| Order | Technique | Compound | Condition/Findings/Applicability | Advantages | Disadvantages | Cost | References |
|---|---|---|---|---|---|---|---|
| | | | | Physico-chemical treatment | | | |
| 1 | Extraction (Supercritical fluid extraction) | Organophosphate pesticides | SC-CO$_2$ (supercritical CO$_2$ extraction) Temperature 90°C, Pressure 325 atm, Time 20 min, Removal 99% | Removal nearly equal to 99% in a reasonably short time; Liquid-liquid extraction is a type of separation process that allows different compounds to be separated based on their solubilities | No decomposition at SC-CO$_2$ temperature; No breakdown of pesticides into less toxic compounds; Solvent required | Costly process | [135] |
| 2 | Adsorption | Lindane and malathion; Lindane; Arazine | Adsorbent dose (activated carbon) 5 mg L$^{-1}$, Particle size 200-250µm; Time 60 min, pH 6,Removal 97%-80%, Adsorbent dose 40 mg L$^{-1}$, pH4-10, Removal 90%; Rice husk straw biochars Atrazine (10 mg L$^{-1}$) Removal 93% | Simplicity of equipment needed; Required equipment relatively inexpensive to fabricate | Relatively high capital cost; Adsorbent capacity progressively deteriorateuses number of cycles increases; Adsorbent regeneration requiring a steam or vacuum source; Spent adsorbent may be considered a hazardous waste | Costly process | [138,80] |
| 3 | Coagulation/ Flocculation | Lindane; Pesticides; Pesticides | Coagulant: poly(aluminumchlorosulfate) NaOH solution pH 4-10, 200 rpm, 10µg L$^{-1}$, Lindane removal 16%; Chlorine removes 60%; Coagulant: aluminumsulfate Removal less than 30% | Fast settling of flocs; Broad pH compatibility; Easy dehydration of sludge, etc. | Disadvantage of coagulation/flocculation is the formation of trihalomethanes; -; High molecular weight polymers (coagulant aids) required; Pesticides possibly interact with colloidal particles by adsorption on the flocsduring coagulation-flocculation | Costly process | [71,94] |
| 4 | Nano-filtration/ Reverse osmosis (RO) | DDT,benzenehexachloride (BHC),lindane; Aromatic pesticides | Asymmetric cellulose acetate (CA) membrane; Membrane rejected all pesticides at >92.4% | Groundwater softening; Pesticide removal for drinking water supply; Surface water treatment for drinking water supply | Nano-filtration/RO membranes are an expensive part of the process; Repairs and replacement of membranes is dependent on total dissolved solids, flow rate, and pollutant feed load | Operating costs for a nano-filtration plant approximately € 0.33m$^{-3}$ at a permeate output of 20,000 m$^3$d$^{-1}$, which means an increase in drinking water price of approximately 9%. By blending the permeate and the conventionally treated water at a ratio to yield a dissolved organic carbon concentration in the blend of 1 mg l$^{-1}$, the additional costs for nano-filtration would come to € 0.1 lm$^{-3}$. | [137,99,13 8,6,139] |
| 5 | UV Fenton | Organophosphorus pesticides; Pesticide industrial wastewater; Pyrimethanil pesticide | Mole ratio of COD:H$_2$O$_2$ = 1:2.2, Fe(II):H$_2$O$_2$ = 1:100, pH 2-5, COD 3242-3418 mg l$^{-1}$; pH value of 4, a dosage of 2.5 g/L of FeSO$_4$·7H$_2$O, 100 ml/L of 30% H$_2$O$_2$ solution, time of 90 min, COD removal efficiency of 98.3%; 3.5 mg/L or 100 mg/L DOC | Rapid reaction rates; Potential to reduce toxicity and possibly complete mineralization of organics treated; Does not concentrate waste for further treatment with methods such as membranes | Complex reactant must be used for specific application; Quenching of excess peroxide is required; Expensive process | Cost of UV+Fenton oxidation for wastewater treatment containing the pyrimethanil pesticide needs 46 Wm$^{-2}$ irradiation and total costs were in between 0.76 € m$^{-3}$ and 1.39 € m$^{-3}$, out of which 10.5% is for iron cost, while the high cost of hydrogen peroxide is at least 83% of the reagent costs. | [82, 140, 141] |

the type of bonding between the adsorbed species and the adsorbent, i.e., physisorption (by weak van der Waals forces) or chemisorptions (by covalent bonding). Pesticide residues need to be treated physically or chemically to avoid water contamination [34]. Residual removal of pesticides by conventional methods is being applied in wastewater treatment facilities through chemical oxidation, sedimentation, flocculation, coagulation, and filtration (using traditional sorbents). Nonetheless, they may not be effective enough [35]. In contrast, the adsorption method using activated carbon appears to be the most effective because it can remove a wide range of organic compounds from water. Activated carbon is one of the most extensively used adsorbent materials because of its porous in nature and high surface area (Table 3). Similarly, a study found that rice straw biochar showed similarities to activated carbon and removed 95.0% of atrazine at 10 mg $L^{-1}$ [10]. Another researcher reported that the adsorption doses of lindane and Malathion were around 5.0 mg $L^{-1}$ and 200-250 $\mu$m, respectively, when using activated carbon as an adsorbent [36]. Removal efficiency of 95.0% was also observed for biochar derived from rice husk for the sorptive removal of atrazine (at an initial concentration of 10 mg $L^{-1}$. The disadvantages of adsorption-based methods are the relatively high capital cost and progressive deterioration in the sorption capacity as the number of cycle increases. Also, the conventional adsorbents often lack target specific functional sites on their surfaces to lower the adsorption capacities of pesticide. In this regard, future research should be directed to properly assess the performance of novel sorbents (e.g., metal-organic frameworks) towards the removal of pesticides. The surface of conventional adsorbents can also be suitably modified to enhance pesticide adsorption capacity.

## 3.3. Coagulation/flocculation

Pesticide levels in drinking water have significantly increased, and thishasbecome a major concern [37].The European Parliament & Council (EPC, 2000) [38] set a concentration limit of 0.1 mg $L^{-1}$ for pesticides in drinking and groundwater for a single pesticide and 0.5 mg $L^{-1}$ for the total content of all pesticides [39, 40]. Removal efficiencies of common pesticides methyl parathion and chlorpyrifos were 79.0% and 82.0%, respectively, using commercial coagulants alum and ferric chloride [41]. The coagulant dose, initial pH, and type of coagulant were considered to estimate the removal of chlorpyrifos and methyl parathion.

In a wastewater treatment plant, coagulation/flocculation is a general step in the physicochemical process. Coagulation is a method used to remove humic substances, heavy metals, phenols, and cyanides from industrial alkaline wastewater, landfill leachate, and drinking water [42]. The mechanisms involved in pesticide pollutant removal include a combination of entrapment, adsorption, charge neutralization, and interactions with the aggregation of insoluble precipitates/polymers, as shown in Table 3[42].

## 3.4. Nano-filtration (NF) and Reverse Osmosis (RO) Membranes

Various membrane technologies were developed in the mid-1980s, followed by progress in related factors or variables, such as the capacity to reject salt, resistance to chemicals, and pressure requirements. These developments have led to the fabrication of nano-filtration (NF) membranes, and subsequently, and the pro-

duction of ultra-low-pressure reverse osmosis (RO) membranes [43]. There are differences between the technologies mentioned above like nano-filtration and reverse osmosis membranes in terms of extraction yield, simplicity of operation, investment cost, operation time, safety, and degree of automation. All of them have comparative advantages relative to the traditional solvent extraction such as extraction, distillation method, pressing, and sublimation according to the extraction principle. Regardless of the technology selected for the extraction, subsequent steps are needed for separation, purification, and final concentration. The next section will introduce conventional and non-conventional separation methodologies.

Nanofiltration is a pressure-driven membrane process used for removing solutes with molecular weight in the range of 200–1,000 gmol$^{-1}$, typically from aqueous streams [170]. The operating pressures of reverse osmosis and nanofiltration are 100 - 300 and 50 - 150 psi [170]. A myriad of commercial NF/RO membranes have been investigated for the effective removal of a large number of pesticides (e.g., atrazine, diazinon, and dichlorovos) from various water matrixes [44]. The selection of a suitable membrane plays a pivotal role in the removal of pesticides from drinking water. The removal of pesticides by membranes is primarily governed by the physicochemical properties of the pesticide (e.g., molecular weight and size, acid disassociation constant, and hydrophilicity/hydrophobicity. In general, the sieving effect (size exclusion principal) is the prime mechanism for the membrane-based treatment of pesticides [45-47] estimated the operating cost for aNF plant to be € 0.23 $m^{-3}$ at a permeate output rate of 20,000 $m^3d^{-1}$, which implied an approximately 9.0% hike in the price of potable water. Water was treated by mixing with a NF permeate.

Membranes with an average pore size smaller than pesticide molecules may retain the target compounds depending on the physicochemical affinity of the pesticide molecules towards the membrane. Membranes are commercially used to remove pesticides from raw water for producing potable water [46] due to their easy operation. However, membrane fouling decreases the efficiency of pesticide removal from water [44].

## 3.5. Ultraviolet (UV)-Fenton

Ultraviolet (UV)-Fenton oxidation is an accelerated photoreaction in the presence of a catalyst. In the UV-Fenton reaction, UV light is absorbed by an adsorbate substrate and used for the removal of a wide range of pesticides. Hydroxyl radical-based advanced oxidation processes (AOPs) have been developed to remove pesticides, including atrazine, and hydroxyl radicals can be generated by UV photolysis of hydrogen peroxide [48]. This process is much faster than bioremediation in treating pesticides. However, it is costly and requires very high energy consumption and strong oxidizing chemical doses, which are the major drawbacks for AOPs [49]. The Fenton reaction has widely been applied in the treatment of wastewater pesticides [50]. Many organophosphate pesticides can be removed by UV-Fenton techniques. The advantages and disadvantages of various operation conditions are shown in Table 3. UV-Fenton oxidation is also a very common method for treating wastewater containing the pesticide pyrimethanil. For example, 100 mg $L^{-1}$ DOC needs 46 Wm$^{-2}$ irradiation along with a total cost of € 0.76-1.39 $m^{-3}$ (10.5% of this is for ion cost).

# 4. Bioremediation

Although a wide array of research and development has been undertaken in the area of pesticide abatement form soils, the transfer of these technologies to the field is very challenging. In the subsequent sections, authors highlight the benefits of combining conventional bioremediation methods with the molecular techniques reported. Bioremediation is a greener route to remove many pollutants from the environment [14, 51]. Microbe-assisted degradation of pesticides is governed by the access of pesticide molecules to a pesticide-consuming microbial population and the activity of this population [52]. Nature keeps the concentration of pesticides in soil in check through the consumption of toxic pesticide molecules by indigenous microbial populations, thus bringing benefits for both agriculture and ecology [21, 53, 54]. However, the natural biodegradation kinetics of pesticides is very slow because of their highly recalcitrant molecular structure. These pesticide molecules remain persistent in soil. As a result, microbiological investigations are essential for developing new and advanced biotechnological tools for the detoxification of pesticides by highly selective microbial species [22].

Bioremediation technology utilizes the natural biodegradation process of hazardous pollutants in its favor by significantly elevating the activity and development of these organisms to convert toxic compounds into environmentally benign products. Bacteria, fungi, or plants can be used to treat pesticides for various contaminated sites. These microbes play a crucial role in the breakdown of hazardous pesticide molecules. An estimate revealed that 1-g soil carries more than one hundred million bacteria (including 5,000–7,000 unique strains) and more than 10,000 colonies of fungi [55]. Natural attenuation (usage of indigenous microbial population) can be effectively utilized for the removal of toxic pollutants from the environment [56]. In recent decades, many researchers have focused on the application of *in-situ* biodegradation of hazardous compounds with naturally occurring microbial populations [54]. The strains of *Acinetobacter johnsonii*, *Lysini bacillus*, *Bacillus* sp., and *Pseudomonas* sp. have been isolated from contaminated soils and sludge generated from agricultural and industrial sites and used for degradation of pesticides [57]. Table 2 shows the degradation of pesticides using specific microorganisms.

The capability of fungal populations to convert a myriad of toxic compounds into environmentally benign species has attracted a great deal of scientific attention for bioremediation applications [58]. The uniqueness of fungi lies in the fact that they secrete diverse extracellular enzymes. Although several soil bacterial species aregenerally omnipresent in a wide array of moist soils, fungi display a higher removal tendency for pesticides, even in semi-arid and aridsoils. Highly recalcitrant pesticides such as the chlorinated triazine herbicide 2-chloro-4-ethylamine-6-isopropylamino-1,3,4-triazine (atrazine) have been transformed by the white-rot fungi *Phanerochaete chrysosporium* and *Pleurotus pulmonarius*, yielding hydroxylated and *N*-dealkylated metabolites [59].

The biodegradation of atrazine, malathion, and parathion was carried out in a two-stage integrated aerobic treatment plant (IATP) using *Bacillus* sp. (consortia) isolated from an agricultural field [22, 54]. The influent stream containing these pesticides (initial

chemical oxygen demand COD of 123 mg L$^{-1}$) was fed to the first reactor which was fed to the second reactor. The maximum removal of pesticides in IATP was greater than 90%. Further, these studies attempted the biodegradation of atrazine in synthetic wastewater by the isolated microbial *Alcaligenes* sp. S3 from an agricultural field in an alternating aerobic-anoxic lab-scale pilot plant [21]. Wastewater contaminated with atrazine at 200 mg L$^{-1}$ and a COD value of 1,356 mg L$^{-1}$ was treated across varying flow rates. Accordingly, 90.6% removal of COD was obtained at a flow rate of 300 mLh$^{-1}$ on the 122$^{nd}$ day of operation [54]. The performance of coupled system was studied with an initial atrazine concentration of 300 mg L$^{-1}$ to yield a maximum removal efficiency of 93.0% for the coupled treatment system of UV-Fenton and biological method [169]. Malathion removal has been reported around 89.0% in batch packed bioreactor [169]. In comparison, continuous packed bioreactor was also operated at various flow rates (5–30 mLh$^{-1}$) over a period of 75 days. The inlet loading rates and elimination capacities were reported in the range of 36–216 and 7.20–145.4 mgL$^{-1}$d$^{-1}$, respectively with an average removal efficiency of more than 90.0% under steady state conditions [169].

Highly efficient colonization and contaminated soil exploration can be achieved using fungal populations due to their high branching and filamentous growth mode [60]. Highly filamentous fungal species (e.g., white-rot fungi) possess great advantages over most bacterial strains in terms of the wide range of hazardous compounds that they can oxidize [58]. Moreover, many fungal species are highly resistant to high concentrations of toxic compounds (e.g., pesticides) as compared to bacterial species [61]. As such, they are considered to be mighty biotechnological tools (many genetically modified fungal species have already been patented) in the field of biodegradation of soil pollutants [62].

## 4.1. Types of Bioremediation

### 4.1.1. Bio-stimulation

In bio-stimulation, vitamins, substrates, oxygen, and other required nutrients are added to stimulate the microbial activity for enhancing pesticide degradation. The addition of stimulating nutrients brings fresh carbon sources, which results in swift depletion of the available stocks of the main inorganic nutrients (e.g., phosphorus and nitrogen) [63]. Bio-stimulation has effectively been utilized for removing pesticides from the environment. The supplementary nutrients include organic/inorganic additives such as nitrate and phosphate. These supplementary nutrients could be essential for inducing enzyme formation and as co-metabolic substrates in the biodegradation pathway of pesticides [63]. To stimulate the microbe-assisted degradation of pesticides, a variety of water-soluble nutrients (e.g., $NH_3NO_3$, $NaNO_3$, $KNO_3$, $K_2HPO_4$, and $MgNH_4PO_4$) are added to fertilizers [64].

As a general principle, the N:P ratio is maintained between 5:1 and 10:1 for 1-5% N by weight of pesticide for the abatement of pesticides. These specific quantities might be inaccurate/insufficient for sites contaminated with different types of hazardous compounds [65]. Lima et al. [64] investigated the impact of soil inoculation with *Pseudomonas* sp. and bio-stimulation with citrate ($\leq$ 4.8 mg g$^{-1}$ of soil sample) on the microbe-assisted degradation of atrazine at very high concentrations (i.e., 20 to 200 times higher

than the recommended dosage [RD]) (Table 4). Interestingly, at a very high atrazine concentration (i.e., 200 times higher than the RD value), the addition of citrate greatly boosted the removal efficiency from 79.0 to 87.0% [64]. These authors noted that very high levels of atrazine (i.e., 62 mg g$^{-1}$ soil) can efficiently be removed by subsequent bio-stimulation and inoculation of soil with *Pseudomonas* sp.

### 4.1.2. Bio-augmentation

Bio-augmentation implies the addition of exogenous microbial populations with particular catabolic activities into a polluted site or a biological reactor to promote the biodegradation process. This might be an on-site or off-site operation that involves the addition of native microbes to contaminated sites for the elimination of hazardous pollutants [16, 66] and is widely recognized as an effective biotechnological approach for improving the degradation of pesticides in polluted water and soils [63]. Bio-augmentation has effectively been engaged for degrading a wide array of hazardous pollutants (e.g., NH$_3$, H$_2$S, petroleum products, and other organic contaminants) present in water and soils (Table 4) [67, 68]. The main advantages of the bio-augmentation are the addition of pre-grown microbial cultures to enhance microbial populations at a site to improve contaminant clean up and to reduce clean up time and cost.

A few case studies have been conducted on soil bio-augmentation for pesticide abatement (Table 4). Lima et al. [64] investigated the impact of inoculation of soil samples with *Pseudomonas* sp. on the microbe-assisted degradation of atrazine in a polluted soil (the atrazine concentration was in the range of 20-200 times higher than RD) (Table 4). It was reported that 99.0% atrazine removal was achieved in the first 8 days (without citrate addition) after soil bio-augmentation for the soil having an atrazine concentration of 20 times higher than RD [64]. Under similar conditions, 79.0% removal was obtained for the soil having an atrazine concentration as high as 200 times higher than RD. Similarly, Wang et al. [69] reported a high atrazine removal efficiency for *Arthrobacter* sp. based bio-augmentation of agricultural soil samples containing 400 mg kg$^{-1}$ of atrazine (Table 4). Bio-augmentation with *Arthrobacter* sp. displayed 90.0% and 70.0% removal of atrazine after the first three days for sterile and non-sterile soil samples, respectively.

Bio-augmentation of chloropyrifos using *Alcaligenes faecalis* has been conducted. Native cabbage plants were cultivated in the soil rich in chlorpyrifos at 100 mg kg$^{-1}$ and bio-augmented with a strain of *Alcaligenes faecalis*. The study reported 100% chlorpyrifos removal after the first 12 days, whereas only 22.0% removal was attained in the control (Table 4) [70]. Similarly, Ahmad et al. [71] introduced *Bacillus pumilus* into soil samples rich in chlorpyrifos at 50 mg kg$^{-1}$ and observed a 97.5% chlorpyrifos removal as compared to only 11.0% removal in the control (Table 4). Lakshmi et al. [72] also reported that the bio-augmented chlorpyrifos-rich soil samples at 50 mg kg$^{-1}$ were individually treated with strains of *Pseudomonas fluorescens* and *Bacillus subtilis*. The average chlorpyrifos degradation was observed to be in the range of 85.0-92.0% after the first 30 days for these bacterial strains as compared to only 34.0% chlorpyrifos removal in the control (Table 4).

The agricultural use of dichloro-diphenyl-trichloroethane (DDT) has been banned in the United States since 1973, although its residues/byproducts are still found to be persistent in soils around the globe. As a result, detoxification of such contaminated sites is a crucial task, and various bioremediation approaches are being actively used for this purpose. Recent investigations have elucidated that various fungal species can effectively remove DDT from the soils. This great potential of fungi was shown by Purnomo et al. [73]. The authors studied the capability of *Gloeophyllum trabeum* and *Daedalae dickinsii* to degrade DDT in polluted sterile/non-sterile soils. They observed that the introduction of these brown-rot fungi into an artificially contaminated sterile soil resulted in 41.0 and 15.0 % degradation of DDT by *G. trabeum* and *D. dickinsii*, respectively. For non-sterile soil samples, *G. trabeum* and *D. Dickinsii* lowered the initial amount of DDT by approximately 43.0 and 32.0 %, respectively, when compared to the control (Table 4).

## 4.2. Factors Influencing the Bioremediation of Pesticides In soil

The fate of pesticide molecules in soils is very complicated in interdependent physicochemical and biological processes. These complex interactions directly govern pesticide transport within the soil as well as their transfer from soil to air, food, and water. The chemical characteristics of pesticide molecules and soil characteristics govern the extent of the influence of the processes mentioned above.

### 4.2.1. Structures of pesticides

The structures of pesticides determine their physicochemical properties and inherent biodegradability. Pesticides are more susceptible to microbial attack and biodegradation if there are polar substituent on the phenyl ring, e.g., -OH, -COOH, and -NH$_2$, whereas halogen or alkyl substituent tends to make the pesticide more resistant to biodegradation [5, 74]. Minor alteration in a structural substitute causes a drastic change in the susceptibility of a compound towards bio-transformation [22]. During the pesticide biodegradation process, the chemical structures of pesticides might drastically be changed by either oxidation or reduction of active functional groups, causing the breakdown of their complex structures into small molecules, such as carbon dioxide, nitrate, phosphate, ammonia, and water [75]. The 2D structures of selected pesticides are shown in Table 1. The toxicities of organochlorine pesticides are relatively lower as compared to organophosphate and carbamates pesticides. The toxicological properties are analogous to organochlorine pesticides that have similar structures, such as chlordane and heptachlor. The toxicity can vary depending on the position of the substituting chlorine in the molecule [76].

Chlorinated hydrocarbons (such as pentalene, dieldrin, and DDT) are unable to biodegrade because they are insoluble in water and have a high sorption affinity in soil [29]. In contrast, carbofuran and 2,4-dichlorophenoxyacetic acid (2,4-D) have different molecular structures and can be biodegraded in few days in field soils [54]. A minor difference in the position or nature of a substituent in the same class of pesticides can significantly influence the degradation rate [21, 54].

### 4.2.2 Pesticide concentration

The concentration of a pesticide in a soil (P) is a crucial parameter for determining the biodegradation rate (i.e., -d[P]/dt) in nature.

**Table 4.** Summary of Bio-augmentation for Pesticide Removal at Various Process Conditions

| Pesticide | Microorganism used | Type of soil | Dose (mg kg⁻¹) | Conditions of experiment | Findings | Reference |
|---|---|---|---|---|---|---|
| Atrazine | Arthrobacter sp. DAY1 | Loam, with pH 7.6 | 400 | In laboratory, 25°C | >95% of atrazine was removed within 3 days | [77] |
| | Pseudomonas sp. ADP | Sandy loam (sand 62%, silt 21%, clay 16%, pH 6.1) | 40 T ha⁻¹ or 400 T ha⁻¹ | In laboratory, 25°C | 99% and 79% of atrazine at recommended dose of 20× RD and 200× RD, respectively, was removed within 8 days | [142] |
| Chlorpyrifos | Alcaligenes faecalis DSP3 | Silty clay (sand 26%, silt 34%, clay 37%, pH 6.9) | 100 | In laboratory, 25°C | Almost 100% of chlorpyrifos was removed within 12 days (only 22% in control soil) | [36] |
| | Bacillus pumilus C2A1 | Agriculture soil, pH 7.8 | 25, 50 | In a green house, 25°C | Chlorpyrifos in unplanted soil was degraded 81% and 89% at concentrations 25 mgkg⁻¹ and 50 mg kg⁻¹, respectively; in non-inoculated as well as unplanted control soils, 9% and 11% of pesticide was degraded, respectively | [66] |
| | Bacillus subtilis | Sandy loam, pH 7.3 | 50 | In laboratory | The degradation of chlorpyrifos was 56% and 85% after 10 days and 30 days, respectively | [40] |
| | Pseudomonas fluorescens | Sandy loam, pH 7.3 | 50 | In laboratory | The degradation of chlorpyrifos was 43% and 89% after 10 days and 30 days, respectively | [40] |
| Carbofuran | Pichia anomala HQ-C-01 | Sandy loam (sand 65%, silt 28%, clay 7%, pH 6.9) | 50 | In laboratory, 30°C | Carbofuran degradation increased from 2.4% to 85.1% after non-sterile soil inoculation | [143] |
| Cypermethrin | Streptomyces aureus HP-S-01 | Sandy loam (sand 65%, silt 28%, clay 7%, pH 7.2) | 50 | *In situ*, 24°C-30°C | 81.1% of cypermethrin was removed in bio-augmented soil (32.1% in control) | [144] |
| DDT | Daedaleadickinsii | Andisol (sand 44%, silt 40%, clay 8%, pH 5.6) | 45 | In laboratory, 30°C | DDT was reduced by 32% within 14 days (15% in control) | [4] |
| | Gloeophyllumtrabeum | Andisol (sand 44%, silt 40%, clay 8%, pH 5.6) | 45 | In laboratory, 30°C | Bio-augmentation with bacteria increased DDT removal to 43% within 14 days (41% in control) | [4] |
| Lindane | Staphylococcus cohnii spp. urealyticus | Garden soil | 5, 50 | In laboratory, 28°C | 100% and 70% of lindane at concentrations of 5 mg kg⁻¹ and 50 mg kg⁻¹ were degraded within 45 days, respectively | [60] |
| Methyl-parathion | Pseudomonas sp. WBC-3 | Loam, pH 7.2 | 536 | In laboratory, 30°C | Complete removal of methyl parathion within 15 days | [93] |
| Parathion | Serratia marcescens | Sand (sand 91%, silt 6%, clay 3%, pH 6.5) | 100 | In laboratory, 30°C | DT50 was reduced by 9.7, 14.5, and 12.6 days in sand | [129] |

The degradation of numerous pesticides follows pseudo-first-order kinetics, where the biodegradation rate depends on the residual pesticide concentration [77]. The biodegradation rate decreases proportionally with the residual concentration of the pesticide (i.e., d[P]/dt = -$k$[P]), where d[P]/dt is the pesticide concentration gradient with respect to time, and $k$ is the biodegradation rate constant.

The half-life values of Inceptisol, Vertisol, and Ultimo are essentially independent of the initial pesticidedose, i.e., 10.1-31.0 d (1.0 μg kg$^{-1}$ soil) vs. 13.0-29.2 d (10.0 μg kg$^{-1}$ soil) [78]. In theory, the pesticide concentration for a 20-day half-life should decay to 0.2% of its initial concentration after 180 days. However, the biodegradation rate, $k$, is smaller at higher initial concentrations. The concentrations of pesticides (e.g., atrazine, carbofuran, cypermethrin, and chloropyrifos) used in experimental studies are given in Table 2. A half-life is the time required to reduce the amount of a given pesticide to a half level. This occurs as it dissipates or breaks down in the environment. After two half-lives, about 25% will remain. Several pesticides (e.g., DDT, HCH, endosulfan, BHC, and atrazine) belong to such ubiquitous compounds which persist in soil and sediments due to less bioavailability. Odukkathil and Vasudevan reported that the half life of less bio-available pesticides (e.g., DDT, HCH, endosulfan, BHC, and atrazine pesticides) ranges from 100 to 200 d [77]. Most of these residues are adsorbed on soil particles, and they are unavailable to the soil microbes for further degradation. In this review, an attempt has been made to present a brief idea on 'major limitations in pesticide biodegradation in soil' based ona few case studies.

### 4.2.3. Soil types

The soil characteristics (e.g., organic matter content, concentration of clay minerals, water content, and pH) affect pesticide biodegradation in soil [78]. Soil plays an important role in microbe-assisted pesticide degradation in the environment. Soil particles can absorb the pesticides, thereby regulating bio-availability and influencing the persistence of pesticides [77]. The activity of microorganisms towards pesticide biodegradation can be influenced by soil characteristics such as clay content and type of organic matter. A number of variables (e.g., soil type, pH, and clay content) can greatly influence the persistence of pesticidesunder field conditions including bifenthrin, chlorpyrifos, cypermethrin, fenvalerate, permethrin, and isofenphos. It has been further confirmed that the degradation rates of metalaxyl and propachlorin in soils were dependent on the soil conditions. The half-lives of metalaxyl and propachlor were 10 and 19 days for pasture soils, 36 and 2.6 days for arable soils, and 6.1 and 8.2 days for pine forest soils, respectively. Imidacloprid biodegradation and diazinon were faster in silty loam soil, followed by sandy loam and sandy soils [77].

### 4.2.4. Moisture content

Water is important for the motion and diffusion of pesticide molecules, and the presence of wateris vital for the microbe-assisted degradation of pesticides. Typically, the biodegradation of pesticides is observed to be very slow in dry (or negligible moisture content) soil samples. The transformation rate of pesticides is directly proportional to the moisture content in the soils [79]. Herbicides (e.g., atrazine and trifluralin) generally degrade at higher rates underanoxic conditions as compared to an oxygen-rich environment. DDT is fairly stable in aerobic soils but degrades very slowly with the formation of 2,3-dihydrodiol-DDT and 4-chlorobenzoic acid as metabolites in submerged soils [80].

### 4.2.5. Temperature

The molecular configurations of pesticide molecules govern the impact of temperature on their stability. Temperature impacts sorption of pesticides by changing their solubility and rate of hydrolysis in a soil sample in terms of salvation (G) and hydrolysis rate [81]. Growth of microorganisms and their activity are optimal in a well-defined physiological temperature range of 25-35℃. Hence, pesticide degradation is optimal in a mesospheric temperature range of 25-40℃ [82]. A temperature range of 15-40℃ is thought to be suitable for the biodegradation of a pesticide by isolated pesticide-consuming bacterial strains [81, 83]. The optimal degradation temperature range of pesticides is shown in Table 2. Qingyan et al. [82] reported atrazine degradation at a temperature of 30℃ and found 95.0% removal at a concentration of 500 mg L$^{-1}$. Many studies for different pesticides, including carbofuran, chlorpyrifos, and DTT, showed that they were degraded in a temperature range of 25-30℃, as shown in Table 2 [20, 83-85]. Temperature mainly affects the metabolism of bacterial species, such as *Pseudomonas* sp., *Bacillus* sp., and *Alcaligenes* sp., which perform well in a temperature range of 25-30℃ [84-87].

### 4.2.6. Soil acidity

Soil acidity is an important parameter influencing the bioremediation of pesticides [88]. Essentially, the biodegradation of a pollutant molecule depends upon the particular enzymes produced by a microbial population. These enzymes operate in narrow pH ranges, as most bacterial species prefer an optimum soil pH range of 6.5-7.5, similar to their intracellular pH. The pH of soil plays an important role in regulating pesticide adsorption and other biotic and abiotic degradation processes. It influences the adsorption tendency of a pesticide (on clay and organic matter), as well as the pesticide molecule's mobility, speciation, and bioavailability [89]. The effect of soil pH on the biodegradation of pesticide molecules largely depends upon their chemical susceptibility towards hydrolysis catalyzed by a base or an acid [19, 81]. Bacterial metabolism performs well at neutral pH; hence, bioremediation studies are typically performed at pH 7.0. For example, Das et al. [85] conducted a study of atrazine degradation at pH 7.0 by *Pichia kudriavzevii* and found an efficient removal of 94.3%.

### 4.2.7. Organic matter

The organic matter present in soil can either decrease the pesticide biodegradation (through stimulation of pesticide sorption) or elevate microbial activity (through pesticide co-metabolism) [79]. The organic matter present in soil also influences biodegradation of pesticide molecules by supplying essential nutrients for cell growth and by governing their mobility via the adsorption/desorption process [90]. The bacteria-mediated biodegradation of organochlorine insecticides (e.g., benzenehexachloride [BHC], DDT, Methoxychlor, and heptachlor) was enhanced upon the introduction of organic carbon sources to flooded soils [91]. A minimum amount of organic matter (greater than 1%) can secure an active population of autochthonous microbes capable of degrading pesticides. The introduction

of wheat residue-derived biochar liberated micronutrients stimulated the growth of the microbial population for pesticide degradation in soil [92]. Organic matter can also be a co-substrate, which increases the microbial activity (production rate and biofilm formation) in a soil. Co-metabolism of microbes enables the biodegradation of pesticides present at relatively low concentrations; hence, microorganisms consume co-substrates to meet their carbon and energy needs. Soils enriched with organic matter contain a source of potential co-substrates, which can facilitate co-metabolic biodegradation of pesticides. The addition of a carbon-rich substrate to contaminated soil is used in bioremediation to stimulate the microbial activity and facilitate co-metabolism [93]. Tan et al. [94] reported that *Bacillus sp.* could degrade 98.5% triazophos at 100 mg $L^{-1}$ from sewage sludge wastewater via co-metabolism when fed with nutrients, such as peptone, yeast extract, and glucose.

## 4.3. Bioremediation Techniques: Developments and Applications

### 4.3.1. Isolation of pesticide-biodegrading microbes and their characterization

The biodegradation rate of pesticides is typically very slow as compare to other reported techniques. To ensure sufficient degradation rate, specific microorganisms should be selected (Table 2). The isolation of naturally occurring effective microorganisms from a contaminated site is an important step in bioremediation. Therefore, many researchers have worked on the isolation, characterization, and biodegradation of pesticides [54]. Another researcher showed the degradation of 300 mg $L^{-1}$ chloropyrifos using the *Pseudomonas species* isolated from an agricultural field and observed the removal efficiency greater than 91.0 % (Table 5). Geed et al. [54] used isolated *Bacillus* sp. $S_4$ for the degradation of Malathion at 300 mg $L^{-1}$ and achieved 90.0% removal in a continuous packed bed bioreactor (Table 5).

In recent years, a wide array of microbial strains has successfully been isolated that are capable of degrading hazardous compounds that were previously thought to be non-degradable, suggesting that microorganisms are rapidly evolving under the influence of rampant environmental contamination (Darwin's theory: the survival of the fittest species for the situation)to be able to degrade pesticides more effectively. Recently, the soil-derived microbial consortium capable of degrading a mixture of pesticides was analyzed using PCR-amplified 16S rRNA fragments [57]. The analysis detected 16S rRNA sequence types that represented organisms closely related to known pesticide-degrading bacteria (e.g.,*Bacillus species)* [95]. Several researchers have isolated the bacterial species and characterized for the effective pesticides degradation which are summarized in Table 2.

Isolation of atrazine-biodegrading species from herbicides containing wastewater was characterized by 16S rRNA [96]. The thermal cycle operated at 94°C for 5 min for initial denaturation, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final incubation at 72°C for 10 min. Yang et al. [97] isolated the novel bacterial *Citricoccus* sp. strain TT3 from the wastewater outfall of a pesticide factory (Table 5). *Citricoccus* sp. strain TT3 was analyzed using 16S rRNA at an operating cycle consisting of preheating to 95°C for 2 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 90 s,

and a final extension for 5 min at 72°C. Similarly, various microorganisms, such as *Rhodococcus sp. strain*, *Bacillus sp.*, *Acinetobacter*, *Stenotrophomonas sp.*, and *Fischerella sp.*, were isolated and characterized by 16S rRNA for pesticides such as DDT, endosulfane, lindane, parathion, malathion, and methyl parathion, as given in Table 5 [29, 98-102].

### 4.3.2. Cultivation in the laboratory to develop microbial populations

Microorganisms capable of mineralizing a variety of pesticides under laboratory conditions have been isolated [5, 54]. These microorganisms were cultured in nutrient media as a co-substrate (i.e., glucose, nutrient broth, and yeast extract). The laboratory-grown microbes were commonly used for degradation experiments. Laboratory culturing of microorganisms is very important for efficient bioremediation in the field.

Isolation of an atrazine-degrading bacterial strain was done by procuring 25-mL of enriched sludge from anaerobic wastewater/municipal treatment provision in Israel [96] (Table 5). The sludge sample was incubated in a 250-mL flask under an oxygen-rich environment at 28°C in the presence of atrazine as the only source of nitrogen [96]. Stepwise incremental addition of atrazine was done from 7 to 30 mg $L^{-1}$ over a period of 14 days. Three unique microbial colonies were observed via DNA sequencing analysis.

An isolated chloropyrifos-degrading species and contaminated soil samples were procured from an agricultural field in Varanasi, India (Table 5). The sampling site was utilized for extensive agricultural operations for several years and was greatly exposed to chlorpyrifos pesticide. Enriched bacterial isolate cultures were acquired from the contaminated soils by utilizing a suitable mineral salt medium (MSM). A chlorpyrifos stock solution at 25.0 mg $L^{-1}$ was added into the MSM medium and left for a week-long incubation period. The control was based on a culture medium carrying only chlorpyrifos (e.g., without bacterial strains). The onset of turbidity can be an indicator of the growth of a bacterial population. Afterward, the vials that had a turbid appearance were placed on Luria Broth (LB) and minimal salt medium (MSM) agar culture plates holding chlorpyrifos populations; they were subsequently incubated at 30°C for 7 days.

An enrichment methodology was developed to isolate DDT- and endosulfane-degrading species using pesticide-contaminated (endosulfan and DDT) soils procured in large amounts from an insecticide facility in Cochin, India [98] (Table 5). Briefly, an enrichment medium was used to dissolve the contaminated soils along with the addition of an inoculums and minimalistic DDT as the only energy/carbon source with subsequent incubation. The enrichment process was repeated three times, and the resulting microbial culture was sequentially diluted and spread onto culture plates containing tryptic soy agar (TSA). Similarly, many researchers isolated microorganisms from pesticide-contaminated soils and wastewater using the cultivation methods in laboratories [101, 102].

### 4.3.3. Bench studies of microbes in a pesticide-contaminated soil

The simplest strategy of bioremediation is improving the biodegradation performance of a microorganism through the addition of a *'specialist'* organism. Microorganisms exhibit novel and high catabolic activity towards a target pollutant. The targeted pollutants are broken down into metabolites ina series of enzymatic reactions.

Table 5. Profile of Pesticide-Degrading Microorganisms and Their Molecular Characteristics

| Pesticide | Microbial sp. | Primers | Sequence(5'-3') | Thermal PCR cycle conditions | Reference |
|---|---|---|---|---|---|
| Atrazine | Raoultella planticola | 16S rRNA gene | - | Parameters: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final incubation at 72°C for 10 min | [45] |
|  | Citricoccus sp. strain TT3 | 16S rRNA gene | 27F-AGAGTTTGATCCTGGCTCAG 1492R-GGTTACCTTGTTACGACTT | Preheating at 95°C for 2 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 90 s, and a final extension for 5 min at 72 °C | [35] |
| chlorpyrifos | Pseudomonas | 16S rRNA gene | 27F-AGAGTTTGATCMTGGCTCAG 1492r-TACGGYTACCTTGTTACGACTT | Denaturation step for 1 min at 94°C, annealing at 60°C for 1 min, and extension at 72°C for 1 min 30 s, and final extension at 72°C for 5 min | [145] |
|  | Ochrobactrum sp. | 16S rRNA gene | forward primer-AGAGTRTGATCMTY GCTWAC reverse primer- CGYTAMCTTWTTACGRCT | - | [127] |
| DDT | Rhodococcus sp. strain | 16S rRNA | 8–27F AGAGTTTGATCCTGGCTCAG 1500r-AGAAAGGAGGTGATCCAGGC | - | [46] |
| Endosulfane | Acenetobactor | 16S rRNA | - | - | |
| Lindane | Bacillus sp. | 16S rRNA | 27F -AGAGTTTGATCMTGGCTCAG 1492 R -TACGGYTACCTTGTTACGACTT | Amplification cycles were performed at 94°C for 45 s, 55°C for 60 s, and 72°C for 60 s | [10,87] |
| Parathion, Malathion Atrazine | Bacillus sp. | 16S rRNA | Bac8F-AGA GTT TGA TCC TGG CTC AG 1492R-GGT TAC CTT GTT ACG ACT T | Denaturation step for 1 min at 94°C, annealing at 60°C for 1 min, and extension at 72°C for 1 min 30 s, and final extension at 72°C for 5 min | [135] |
| Methyl parathion, Malathion | Stenotrophomonas sp. | 16S rRNA | fD1-AGAGTTTGATCCTGGCTCAG rD1-ACGGCTACCTTGTTACGACTT | PCR conditions were as follows: 94°C denaturation for 10 min, followed by 30 cycles of 96°C for 30 s, 56°C for 30 s, and 72°C for 45 s, and a final step of 72°C for 10 min | [11] |
| Methyl parathion | Fischerella sp. | 16S rRNA | fD1- AGAGTTTGATCCTGGCTCAG and rD1-AAGGAGGTGATCCAGCC | PCR reaction was started by preheating at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min; the last cycle was a final extension at 72°C for 5 min | [24] |

Many oxidase/reductase enzymes play important roles in pesticide breakdown. Batch-scale experiments have been performed by many researchers to understand the proper functioning of microbes. Bacterial colony growth was studied to ascertain the toxicity of a pollutant. Biodegradation of a pollutant was confirmed by using the available organic load and bacterial role. The progress of bioremediation and the production of metabolites were examined using various analysis methods, e.g., GC, FT-IR, and GC-MS. The pesticide metabolites were easily identified, and their toxicity was determined with respect to their parental compound [5, 54, 95].

Generally, acatabolic process occurs in microorganisms to breakdown pesticides. This may depend on what type of pesticide degradation is involved. The breakdown of any compound by microbial cells takes place through three unique processes, namely hydrolysis, reduction or oxidation, and addition. The hydrolysis of a pesticide molecule generally takes place in a water-rich environment. The oxidation or reduction of pesticide molecules is accompanied by a change in the pesticide's redox state [103]. The occurrence of either oxidation or reduction is governed by the presence of co-substrates in the environment. Finally, microbes add a new functional group to the pesticide molecule during the addition process. The strategy of addition is employed by microbes when the conditions prevailing in its surroundings are unfavorable for the other types of reactions. Although the addition process requires energy, the addition of a functional group elevates the susceptibility of the pollutant molecule towards biodegradation [96].

### 4.4. Field Applications of Pesticide Bioremediation Techniques

There are two basic treatment options depending on the site selected for pesticide treatment: *in-situ* and *ex-situ* bioremediation. *In-situ* bioremediation techniques involve on-site treatment of the hazardous material, whereas *ex-situ* approaches involve off-site treatment of toxic pollutants.

#### 4.4.1. In-situ techniques

The *in-situ* technique involves the stimulation of microbial activity by adding microbes and nutrients and by optimizing factors associated with the environment at the polluted sites [104]. These techniques involve treating polluted substances at the selected site of the pollutant. Site excavation is not required; hence, there is little or no disruption to the soil structure. *In-situ* bioremediation involving the biological degradation of organic contaminants under naturally prevailing conditions can be described as a process whereby organic pollutants are biologically degraded to $CO_2$, water, or other minimally toxic products under natural conditions. It is an economical, low-maintenance, environmentally benign, and sustainable undertaking for the detoxification of contaminated sites, as shown in Table 6. Seech et al. [104] reported a case study on the *in-situ* treatment of dieldrin in soil using the cycled DARAMEND treatment. Nearly 2,600 tons of dieldrin-contaminated soil was removed in the coastal areas of Florida, the United States in November 2004. The total operation cost was estimated to be approximately 12.5 USD yd$^{-3}$. The most practical *in-situ* methodologies and their salient features are as shown in Table 6.

#### 4.4.2. Ex-situ techniques

*Ex-situ* techniques involve excavating/removing the polluted soil from selected sites and transporting it to another site for treatment. *Ex-situ* techniques are evaluated according to the cost of the treatment, type of pollutant, depth of pollution, geographical location, and degree of pollution. Methods include land farming and composting for off-site rehabilitation of polluted materials in specifically assigned locations. As a result of the added requirement of polluted soil excavation and transport, the operational cost of *ex-situ* approaches can be much higher than *in-situ* techniques, as shown in Table 6. Moreover, the biodegradation kinetics and consistency of the process outcomes for *in-situ* and *ex-situ* techniques is microbe dependent [105]. *In-situ* bioremediation is preferred over *ex-situ* for environmental rehabilitation of polluted soils and aqueous ecosystems [105]. The practicality of a specific biotechnological approach depends on multiple parameters, such as the condition of the contaminated site, native microbial populations, and the amount and toxicity of pesticide present. *Ex-situ* bioremediation techniques are shown in Table 6.

A case study on *ex-situ* treatment was carried out for initial concentrations of toxaphene, DDT, DDD, and DDE of 29, 94, 132, and 94 mg kg$^{-1}$, respectively [104]. These authors reported that the remediation goals ($\geq$ 90% removal) were reached on various organochlorine pesticides (OCPs) in groundwater/saturated soils in the United States. The *ex-situ* bioremediation goals were reached in the treatment cell using 3-12 treatment cycles. The number of treatment cycles required to reach the remedial goal was primarily dependent on the initial concentrations of the target pesticides. Their results indicated that the initial concentrations of toxaphene, DDT, DDD, and DDE (i.e., 189, 81, 180, and 25 mg kg$^{-1}$, respectively) were reduced to 10, 9, 52, and 6 mg kg$^{-1}$, respectively. These changes correspond to removal and destruction efficiencies (RDEs) of 95.0, 89.0, 71.0, and 76.0 %, respectively. The treatment cost per ton varied in relation to their initial concentration, ranging from 29-63 USD t$^{-1}$, and average treatment cost would be ~55 USDt$^{-1}$ for 4,500 tons of contaminated soil.

### 4.5. Hybrid Bioreactors

Pesticide treatment in bioreactors has the benefit of continuous monitoring of waste processing under controlled conditions. Bioreactor technology can be customized in wide-ranging arrangements to maximize microbial degradation [63]. Yadav et al. [5] studied the degradation of chlorpyrifos through *Pseudomonas* sp. in batch and continuous reactors using polyurethane foam as the packing media. They optimized the process parameters for maximizing the removal efficiency of chlorpyrifos through batch experiments and determined the following optimal parameters: pH 7.5, a temperature of 37$^{o}$C, a DO of 5.5 mg L$^{-1}$, and a chlorpyrifos concentration of 500 mg L$^{-1}$. Further, the bioreactor operating under continuous mode was run at different flow rates from 10-40 mLh$^{-1}$ and displayed 91.0% removal of chlorpyrifos at steady state. Geed et al. [54] employed the integrated aerobic treatment plant (IATP) to treat synthetic wastewater containing atrazine, Malathion, and parathion using isolated *Bacillus* sp. (Consortia) from an agricultural field. The maximum COD removal of synthetic wastewater in IATP was greater than 90.0%.

Multiple types of bioreactors are available all around the globe including continuous, batch, sequential, membrane, airlift, and

**Table 6.** Various Kinds of Biological Degradation Processes for Pesticide Removal

| Order | Technique | Compound | Condition/Findings/ Applicability | Advantages | Disadvantages | Cost | References |
|---|---|---|---|---|---|---|---|
| | | | | | Bioremediation: in-situ | | |
| 1 | Biosparging | Pesticides | Most pesticide-contaminated sites | Equipment is readily available; Easy to install, little disturbance to site operations; Treatment times from 6 months to 2 years | Can only be used in areas where air sparging is suitable,complex; Chemical,physical, and biological processes are not well understood, potential for the migration of contaminants | About 8100 yd³ soil treated; Six carcinogenic PAHs reduced from 100–200 mg kg⁻¹ to 23–92 mg kg⁻¹; Estimated cost $70 yd⁻³; Project completed in 8 months | [108,15,115,23,146, 69] |
| 2 | Bioventing | Pesticides | Treatment of pesticides and toxic intermediate metabolites | Equipment is readily available and easy to install; Short treatment times, from 6 months to 2 years; easy to combine with other technologies; May not require off-gas treatment | High concentrations of contaminants may be toxic to organisms; Cannot reach low cleanup limits; Effective only in unsaturated contaminated soils | Various soil warming techniques used for demonstration purposes; Site still operative; $758,077 capital cost incurred; $177,160 annual operating cost | [165,187-189,89] |
| 3 | Phytoremediation | Pesticides | Wide range of pesticide treatment | Cost-effective for large areas, no impact on the environment, formation of secondary waste is minimal; Post-treatment soil can remain in the treated area and can be used in agriculture; Remediation is accomplished with minimal environmental disturbance | Period required longer than one growing season; Climate and hydrological conditions limited-plant growth and the plant species that can be used; Can enter the food chain, requires special disposal of plants | - | [108,110,27,147,148,68] |
| | | | | | Bioremediation: ex-situ | | |
| 1 | Land-farming | Pesticides | Wide range of pesticide treatments | Relatively simple design and implementation; Short treatment times (6 months to 2 years under optimal conditions) | Reductions of concentrations greater than 95% and concentrations lower than 0.1 ppm are difficult to achieve; Required area is high; Dust and vapor generation during land-farming aeration may cause air quality problems. | - | [149,115,5] |
| 2 | Bio-pile | Pesticides | Wide range of pesticide treatments | Treatment time is 6 months to 2 years; Advantages over land-farming; requires less space; possibility of aeration, pesticide control is possible | Not efficient for the heavy components of pesticides, possibility of contamination; Migration into the environment, difficult to expect a pollutants concentration reduction > 95% | Field demonstration unit, about 20,000 t soil treated; Operational cost estimated as $921⁻¹ soil treated, based on 5 years of treatment and compliance with RCRA | [149,115,5] |
| 3 | Composting | Pesticides | Wide range of pesticide treatments | Cost effective, takes less time and money to remediate; Leads to complete destruction of pollutants; Suitable for treating large volumes of contaminated soil | Pesticides must be pre-treated, possibility of contamination migration into the environment; Composting processes to "lock up" pollutants, the long-term stability of such "stabilized" matrices is uncertain | - | [149,115,5] |
| 4 | Bio-slurry systems | Pesticides | Wide range of pesticide treatments | Control of temperature, moisture, pH, oxygen, nutrients; Pesticide contamination, addition of microorganisms, monitoring of reaction conditions | Dewatering after treatment; Disposal method is needed for wastewater extensive site and contaminant investigation | Large-scale treatment, approximately 300,000 t soil treated in 20 months. Operational cost estimated as $900t⁻¹ soil or sludge treated | [149,5] |

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fluidized bed, biofilm, and hybrid systems [106, 107]. Although such bioreactors have the great advantage of control, they suffer from the drawbacks of high operation/capital costs along with the requirement of polluted site excavation. Other off-site bio-degradation techniques include land farming, composting, and bio-piles. These methods are found to have various disadvantages, such as large space requirement, extended treatment duration, mass transfer problems, and restricted bio-availability of contaminants [108]. Table S1 shows the different types of bioreactors used for bioremediation.

Hybrid processes that are an effective combination of multiple treatment methodologies have been proposed. Some may have the ability to effectively remove organic pollutants. The synergistic effects can effectively be utilized to enhance the abatement of pesticides through the combination of multiple processes. For example, the presence of activated carbon can elevate the biodegradability of pre-viously highly recalcitrant pesticide molecules via adsorption [109].

Interestingly, combining suspended biomass with biofilm has been proposed as an innovative strategy for potential enhancements in pesticide biodegradation due to biodiversity expansion in the treatment system. The utilization of bio-filtering setups consisting of bio-films grown on fixed beds has intensively been investigated with the main emphasis on porous media biofiltration processes (e.g., sand filters) [110]. The retention time plays a crucial role in controlling the suspended biomass culture-based conventional systems for organic pollutants. Therefore, the best performance can be anticipated using biofilm processes at low loadings (resulting in a more diverse bacterial colony). Luo et al. [35] investigated the short-term removal rates of pesticides during 24-h batch experi-ments through acclimatized as well as non-acclimatized biomasses supported on a sponge. A continuous bench-scale moving bed biofilm reactor (MBBR) was also set up for a long-term assessment of 100 days to remove selected organic pollutants. In their sub-sequent study, Luo et al. [35] compared the removal of pollutants using a conventional membrane bioreactor (MBR) and a hybrid MBBR-MBR system. The observed results showed that the hybrid MBBR-MBR system was better for the abatement of recalcitrant pesticides.

The fouling of membranes was greatly lowered in the hybrid reactor due to the variation in the soluble species of the micro-organisms and the extracellular polymeric materials. Additionally, an improvement in the pesticide removal was attained with a novel configuration of a plant built on an up-flow anaerobic sludge blanket (UASB) reactor integrated with a hybrid aerobic MBR at room temperature with alow hydraulic retention time (HRT) [109]. Interestingly, significant removal of aqueous pesticide molecules was observed due to the synergistic effect of the combination of cross-linked enzyme aggregates of laccase (CLEA-laccase) and mi-crofiltration membranes [111]. The sequential treatment steps can also be used to treat pesticide-rich wastewater through aerobic or anaerobic processes [19, 112].

The levels of pesticides in water have increased due to their excessive use in the modern agricultural domain. Choosing a suit-able water treatment method for pesticide removal depends on the type of pesticide and the efficacy of the treatment process. Both single-treatment and hybrid methods are thoroughly described

and critically discussed [5, 35, 54, 108-110]. The use of hybrid removal techniques offers the potential opportunities to develop innovative options. Furthermore, the decentralization of water treat-ment was also discussed as a means to improve effluent water quality at lower prices. Many affordable techniques such as activated sludge and adsorption by agricultural adsorbents showed high effi-cacy in treating high levels of different pesticides.

### 4.6. Economic Cost and Sustainability of Bioremediation

Bioremediation technology is considered to be a highly economic approach compared to conventional pesticide abatement tech-nologies (approximate savings of 65.0-85.0 %). For instance, the incineration of contaminated soil costs approximately 250-500 USD $t^{-1}$, whereas biotechnological approaches are estimated to require an operational cost of 40-70 USD $t^{-1}$ [113]. The estimated cost of microbe-assisted treatment of contaminated soil is approximately one-third cheaper than that of conventional soil remediation methods [114]. Bio-treatment typical costs of incineration and landfill disposal are 50-130, 300-1,000, and 200-300 USDm$^{-3}$, respectively [106, 115].

Various biotechnological approaches have been applied for mi-crobe-assisted degradation of pesticides in contaminated soils (e.g., on-site subsurface techniques, land-farming/engineered oil pile methods, and fully blended soil slurry reactors for *ex-situ* abatement of contaminated excavated soils). The aim of bioremediation is to stimulate the optimum process environment to catalyze the growth of appropriate microorganisms and use them to decompose pollutants. Modern biological treatment systems have successfully been applied for the abatement of a wide range of pesticides.

However, many studies have shown that bioremediation ap-proaches are kinetically slow and have not been able to lower pesticide concentrations to environmentally accepted values. Due to its poor performance historywith the rash of *"quick-fix"* method-ologies (but without proper field trials and validation), potential users have become more unwilling to adopt biological treatment technologies [116]. The costs of analyses and sampling increases substantially in the case of non-homogeneous process conditions which resulting in highly elevated operational costs. Advent of modern biotechnological tools can help accelerate bioremediation operation and provide higher process reliability [17, 30, 117]. Timeframe may range from 5-25 years for natural processes, 0.5-3 years for *in-situ* sub-surface processes, 1-18 months for soil compost-ing processes, 1-12 months for land-farming and slurry phase sys-tems, and 15 days for accelerated slurry phase systems [118].

## 5. Conclusions and Future Work

The physicochemical processes involved in the removal of pesti-cides from various environmental matrixes were described at the beginning of this review to provide a clear contrast with the sub-sequently discussed biological approaches. The physicochemical approaches are often energy intensive and costly in nature for practical implementation for pesticide treatment. Some phys-icochemical processes often require the application of chemical compounds to worsen environmental pollution issues and to ampli-fy the overall operational costs. Among all available methods, bio-

remediation methods are the most promising, eco-friendly, and inexpensive approaches for the effective degradation of various pesticides present in the environment. Various microorganisms have been employed as biological agents for the degradation of pesticides into either non-toxic or less toxic byproducts. Bioremediation approaches combined with conventional techniques can be used to detoxify and remove hazardous pesticides in heavily contaminated soils with > 95% removal efficiency and incidental benefits.

Although bioremediation has proven to be a promising tool for the degradation/detoxification of pesticides, its sustainability in the field is still questionable. It would be difficult to achieve complete degradation/detoxification of pesticides in nature since the biochemical pathways of microbial species are strongly dependent on the physicochemical properties of soils. Therefore, further research is imperative for a better understanding of degradation pathways by microbes and their interactions with soils having various contaminants and different environmental conditions.

Bioremediation has a critical limitation for pesticide abatement in terms of maintaining optimum conditions for the growth of microbial populations. The rate of pesticide degradation is also very slow and time-consuming. Pesticide degradation, the exploration of specific microbes for specific pesticides, optimization of the process parameters, development of a highly efficient bioreactor, and verification of natural, easily available, and highly porous packing media should be further investigated.

Advanced biotechnological/microbiological tools and genetic engineering can help provide swift advancements in the area of pesticide bioremediation by developing robust and highly adaptable microbial strains and by improving the treatment facilities/technologies that already exist. Using these tools, genes may be targeted that are responsible for biodegradation, and further studies can be done to obtain better results. Further collaborations between genetic engineers, biochemists, environmental engineers, and microbiologists are required to overcome the various hurdles remaining in the present bioremediation methodologies and to further improve the research and development directions as recommended.

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## Author Contributions

B.S.G (Post Doctoral Fellow), S.R.G (Ph.D), K.V. (Ph.D) have written this review and S.S.L. (Professor), K.H.K. (Distinguish Professor), S.K.K. (Professor), M.V. (Professor), critically review the paper on various aspect while P.C. (Scientist), R.S.S. (Professor), B.N.R. (Professor) and K.H.K. (Distinguish Professor) have supervised the PDF and Ph.D Scholars.

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