



A REVIEW ON CARBOFURAN IN THE ENVIRONMENT: PERSISTENCE, TOXICOLOGICAL IMPACTS AND MICROBIAL BIOREMEDIATION STRATEGIES

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ABSTRACT

Carbofuran is one of the most toxic broad-spectrum and systemic N-methyl carbamate pesticide, which is extensively applied as insecticide, nematocide and acaricide for agricultural, domestic and industrial purposes. It is extremely lethal to mammals, birds, fish and wildlife due to its anticholinesterase activity, which inhibits acetyl-cholinesterase and butyrylcholinesterase activity. In humans, carbofuran is associated with endocrine disrupting activity, reproductive disorders, cytotoxic and genotoxic abnormalities. Therefore, cleanup of carbofuran-contaminated environments is of utmost concern and urgently needs an adequate, advanced and effective remedial technology. Several conventional technologies are used for the removal of carbofuran including physicochemical processing like photo-catalysis, ozonation/UV-irradiation, membrane filtration, adsorption and fenton degradation. However, none of these technologies is feasible and cost effective for complete mineralization of carbofuran pollution from the environment. Microbial technology is a very potent, pragmatic and ecofriendly approach for the removal of carbofuran. Microbial carbofuran degradation provides a safe and cheaper alternative compared to chemical and physical methods. Microbial enzymes and their catabolic genes exhibit an exceptional potential for bioremediation strategies. This study aimed to review the microbial pathways of the biodegradation of carbofuran in agricultural soil to sustainable mitigation of environmental and human health risk. To conduct this review, databases such as Scopus, web of science, PubMed and google scholar were searched to extract studies on the carbofuran biodegradation methods from 2010 to 2025. The role of the isolated bacteria as degrader of carbofuran make it an important instrument for biodegradation of pollutants for sustainable agricultural practices. Microbial degradation of carbofuran has been used as a promising candidate for environmentally sustainable remediation of carbofuran-contaminated sites and provides a framework for further scale-up and field-level application.

Keywords: Carbofuran, Biodegradation, Insecticide, Organocarbamate, Catabolic Genes

INTRODUCTION

Tremendous amount of pesticides is globally used for agricultural and household applications. Worldwide consumption of carbamate and organophosphate pesticides in agriculture, commercial and domestic purposes gave birth to numerous environmental problems including severe environmental toxicity to aerial, aquatic and terrestrial biota (Mishra *et al.*, 2020). Pesticides are highly toxic compounds for the environment but still are considered to be the most effective means for protecting crops and livestock from pests, insects, nematodes and weeds (Jahan *et al.*, 2025). In spite of their usefulness, pesticides pose severe threat to natural resources and poisoning to animals and plants. Humans are indirectly affected via food chain because of the chemical complexity and persistent nature of pesticides (Sun *et al.*, 2018; Kaur, and Balomajumder, 2019). Due to the widespread agricultural applications, people have been exposed to several kinds of broad-spectrum pesticides such as organophosphates, organochlorine, organosulfur, carbamates, dinitrophenols, triazines and thiocarbamates (Anjum *et al.*, 2017). Pesticide residues enter the food chain via biomagnification. Due to the non-persistence and low bioaccumulation in the environment, carbamates are frequently used in agriculture, gardening, forestry and therapeutic pharmaceuticals but their life-threatening hazards to mammals and birds are well reported (Dias *et al.*, 2015; Ariffin and Rahman, 2020).

Carbofuran is one of the most toxic broad spectrum, systemic nematocidal, insecticidal and acaricidal carbamate pesticide that belongs to class I of N-methyl carbamate pesticide with ester and amide linkage (Ariffin and Rahman, 2020). It is extensively used in agricultural practices due to its broad horizon of controlling soil dwelling and leaf feeding insects. It is recognized as fairly persistent in soil and water ecosystems in neutral or acidic conditions and is extremely toxic to mammals as an anticholinesterase compound that inhibits acetylcholinesterase and butyrylcholinesterase in vivo and in vitro (Randika *et al.*, 2022). It can pass through the placental barrier to severely effect maternal-placental fetal unit (Gupta, 2025). According to World Health Organization (WHO), it can cause neurotoxic, genotoxic, cytotoxic, mutagenic, reproductive, endocrine disrupting, embryo-toxic and dermal skin problems (WHO, 2009). Carbofuran can damage biological properties of soil such as fertility, respiration, microbial biomass and diversity, nitrogen and phosphate mineralization capacity and enzymatic activities of plants. Carbofuran can also cause acute and chronic toxicities in aquatic organisms by disrupting immune cells and biochemical and enzymatic activities (Yaseen *et al.*, 2025). Concerns about these ecotoxicological, environmental and hazardous health impacts of carbofuran have prompted the researchers to remediate this pesticide for environmental safety (Li *et al.*, 2020).

Microbial degradation has received extensive attention as the most effective and sustainable approach for the degradation and detoxification of carbofuran contaminated environments. Most of the previous carbofuran related work is based on bacterial degradation. Bacterial strains belonging to the genera *Flavobacterium*, *Pseudomonas*, *Sphingomonas*, *Archomobacter*, *Bacillus*, *Cupriavidus* *Novosphingobium*, *Sphingium* and *Enterococcus* have been isolated from carbofuran-contaminated environments and characterized for the degradation of carbofuran (Jahan *et al.*, 2025; Lee-Ann Ataikiru and Ajuzieogu, 2023; Mishra *et al.*, 2020). Due to the special features of specific enzymes and catabolic genes, genetically engineered microorganisms (GEMs) could be a potent tool for biodegradation studies to provide new information about metabolic pathways.

Carbofuran, a widely used pesticide due to its effectiveness in controlling insects and nematodes on various crops and agricultural lands thereby leading to bioaccumulation of the pollutant in soil, water and air. It has a broad-spectrum biological action with low persistence compared to organochlorine insecticides. The pollutant (carbofuran) can also undergo biomagnification as it moves along the food chain leading to serious health risks. Even accidental exposure of carbofuran can result in acute toxicities and fatalities even to human beings, thereby making it highly hazardous (Baharudin *et al.*, 2023). The use of carbofuran has been highly restricted for use in several regions worldwide. Although it lacks carcinogenic properties, its cholinesterase inhibition can lead to neuro logical overstimulation, causing symptoms ranging from nausea and dizziness to respiratory paralysis and death, with chronic and acute toxicity observed in mammals, birds, and fish, and posing acute dietary risks well above recommended levels.

Apart from the health effects of carbofuran, degradation of carbofuran is important from an environmental point of view. Carbofuran also has negative impacts on soil, since it greatly reduces soil fertility, respiration, microbial bio mass and diversity, capacity for the mineralization of phosphate and nitrogen, and plant enzyme activity (Mishra *et al.* 2020). Therefore, there is a strong need for carbofuran degradation using the biological method other than conventional techniques (Jahan *et al.*, 2025).

Conventional methods for the removal or remediation of carbofuran are not eco-friendly, sustainable, user friendly and are generally very expensive. Carbofuran removal using physicochemical procedures such as photocatalysis, ozonation/UV-irradiation, membrane filtering, adsorption and fenton degradation (Ibrahim and Solpan 2019). However, none of these options is practical or economical enough to completely clean up the environment's carbofuran pollution. For the oxidation and detoxification of environmental toxins and pollutants, biological remediation has emerged as a "boon

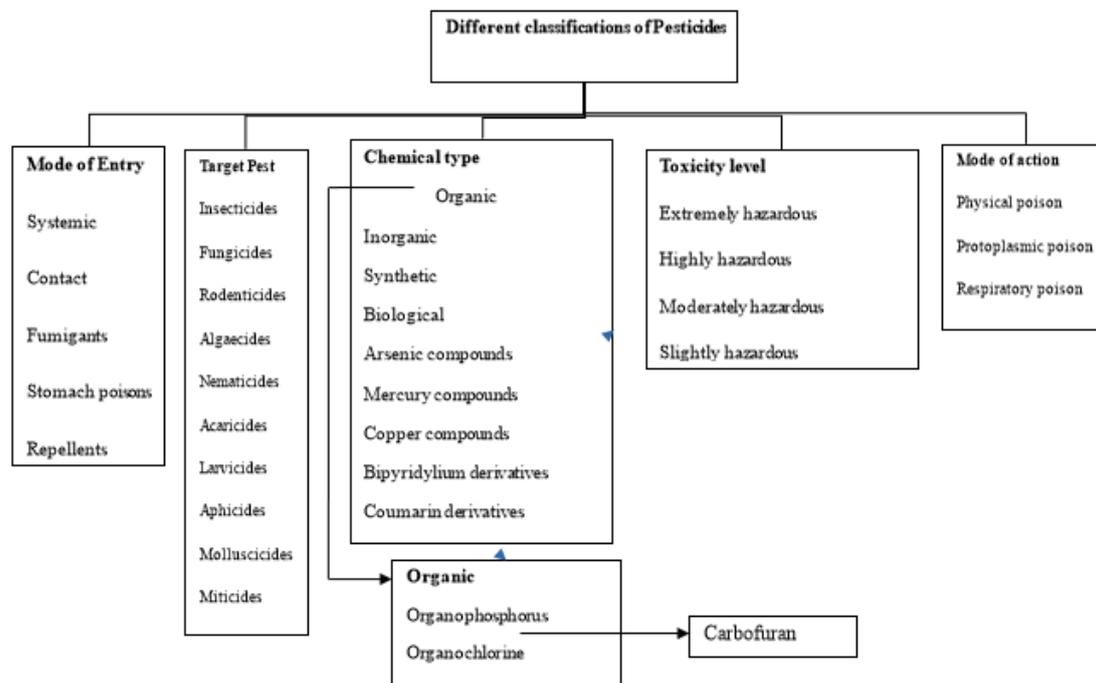
technology". The microbial degradation of dangerous contaminants and pesticides is a more economical and environmentally friendly alternative to chemical and physical cleaning techniques (Mustapha *et al.*, 2019; 2020).

Isolation of indigenous soil bacteria capable of metabolising carbofuran provides environment friendly means of detoxification. Such microbes develop as a result of the repeated use of carbofuran, leading to the adaptation of native soil bacteria capable of degrading it. Therefore, the most important route for its removal from the environment is through microbial degradation. Several native bacteria capable of degrading carbofuran have been isolated and characterized in diverse geographical locations. Carbofuran present in the soil also affects soil fertility, as the compound inhibits growth of seedlings and seed germination (Lee-Ann Ataikiru and Ajuzieogu, 2023). Therefore, there is a strong need for carbofuran degradation from products and waste streams by routes other than conventional extraction techniques.

Pesticide

Pesticide is any substance that can destroy, diminish, prevent, repel, control, attract, or even kill a pest or non-target organism (Nayak *et al.*, 2018). Pesticides are important chemicals used in agriculture to ensure maximum produce are recovered and ready to be used by humans. There are side varieties of pesticides that are used in agriculture ranging from pesticides that have a broad range of activity acting upon a wide variety of pests down to pesticides that have a specific or narrow range of impact. scientists have identified that the pesticides applied to the land can be either built up in the environment or degraded (Verasoundarapandian *et al.*, 2022). Although the prolong existence of these chemicals may be an advantage for the sustained pest control, cumulative effect of residual accumulation can be adverse on the balance of environment and all living organisms including human beings (Jeschke, 2024).

Pesticides have been classified into groups using various classification methods (Ahmad *et al.*, 2022). Pesticides has also been classified by the World Health Organization (WHO) according to their degree of danger based on the lethal dose (LD₅₀) in rats (Garud *et al.*, 2024). Exposure to the substance, either by single or multiple exposures during a short period, causes an effect on the person who handles the product. However, there are mainly three popular methods used for pesticide classification such as, classification based on the "mode of entry, target pests, and chemical type". Figure 1 shows the classification of pesticides based on their mode of entry, target pest, chemical type, toxicity level and mode of action. It shows that carbofuran is an organic carbamate pesticide.

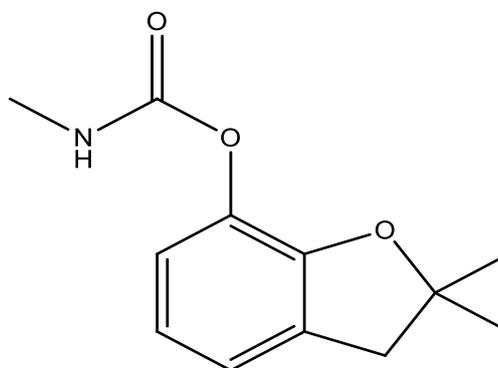
Figure 1: Different Classification of Pesticides (Baharudin *et al.*, 2024)

Environmental Fate of Carbofuran

Carbofuran is technically derived from carbamic acid that is composed of benzofuranyl component connected to a carbamate group by the reaction of methyl isocyanate with 2,3 dihydro-2,2-dimethyl-7-hydroxybenzofuran. It is represented by a chemical formula $C_{12}H_{15}NO_3$. "FMC Corporation" introduced carbofuran in 1967 under commercial trade name of "Furadan" for controlling soil dwelling and foliar feeding insects. It is a white crystalline solid chemical with slightly phenolic odor, high solubility and low adsorption in soil (Rahman and Sabiha, 2018).

Carbofuran is particularly a plant systemic pesticide, which acts through phloem sap against piercing-sucking pests of rice crop such as *Nephotettix virescens* (green leafhoppers), *Nilaparvata lugens* (brown plant hopper), stem borers and whorl maggots. Carbofuran is also applied on potatoes, corn, soybeans, strawberries, grapes, wheat and alfa-alfa for

controlling corn rootworm, aphids, white grubs, wireworms and boll weevils. After the application in soil or water, it is readily absorbed by plant roots and translocate into leaves and other plant parts. Earlier, carbofuran was used in liquid and granular form but USEPA and European Union have completely banned its use in both forms because of toxicological effects on the birds (Onunga *et al.*, 2015). The granular form of carbofuran look-alike seeds and mistaken by birds as grit or food that causes death. However, liquid formulations of carbofuran are still used in developing countries including India, Kenya, China, Thailand, Pakistan and Sri Lanka. Carbofuran is known as a powerful inhibitor of acetyl-cholinesterase and possesses serious toxicity, producing negative health effects for wild animals and humans and poisoning rivers and groundwater (Meka and Dukessa, 2021). Figure 2 shows the chemical structure of carbofuran.



Carbofuran

Chemical Formula: $C_{12}H_{15}NO_3$ Figure 2: Chemical Structure of Carbofuran (Stoyanova *et al.*, 2023)

In addition, the application of carbofuran to crops results in changes in the metabolism of plants and changes the soil microbiota and their metabolites which ultimately affects crop quality (Meena *et al.*, 2020). It has a relative solubility in water but a high solubility in acetone, acetonitrile, benzene, and cyclohexane. It degrades at temperatures above 130°C and stimulates combustion when ignited. The substance is available as a powder, granules, and flow-able formulation in a solvent (Getenga *et al.*, 2023). It has a shorter environmental

half-life than chlorinated hydrocarbon insecticides, degrades faster, and is excreted by non-target organisms. Carbofuran may reach the aquatic environment by run-off from treatment plantation fields, direct application or broadcasting of granular formulations, or drift from spray able mixtures (Baharudin *et al.*, 2024). Carbofuran residues can persist in different matrices of the environment and at varying concentrations as shown in Table 1 below.

Table 1: Concentration of Carbofuran Residues in Water from the Various Matrixes

Concentration	Matrix	References
0.03–158.5 mg/L	Freshwater	(Dutra <i>et al.</i> , 2008)
1 µg/L	Streams	(Degradadoras <i>et al.</i> , 2013)
1–30 µg/L	Groundwater	(Mustapha <i>et al.</i> , 2020)
0.18–14 µg/L	Drinking water	(Bencko and Foong, 2017)
1–5 µg/L	Water table aquifers	(Bencko and Foong, 2017)
0.1–0.5 µg/L	Rainwater	(Bencko and Foong, 2017)
0.005–0.495 mg/L	Rivers	(Otieno <i>et al.</i> , 2011)
2.301 mg/L	Ponds and dams	(Otieno <i>et al.</i> , 2011)
0.949–1.671 µg/L	Lake's water	(Chowdhury <i>et al.</i> , 2012)
0–3.395 µg/L	Paddy water	(Chowdhury <i>et al.</i> , 2012)
233 µg/L	Paddy water	(Clasen <i>et al.</i> , 2014)
16–28 µg/L	Runoff water	(Larson <i>et al.</i> , 2019)
4.3 µg/L	Ground water	(Moreira <i>et al.</i> , 2015)

History of Carbofuran

It was during World War II that a lot of new methods were developed for dealing with pests (Baharudin *et al.*, 2024). Many manufactured chemicals were tested for their insecticidal efficacy because of the pressing need to reduce human disease transmission by pests in tropical areas. Carbamates, a third class of chemically synthesized insecticides, were identified by Swiss scientists in the 1940s (Rezende-Teixeira *et al.*, 2022). In the mid-1940s, the Geigy Chemical Company aimed to create an insect repellent and began working on carbofuran. A set of tests on carbamate compounds found that these compounds were ineffective

insect repellents and poisonous to flies, mosquitoes, aphids, and other smaller pests. Geigy was prompted by this situation to explore the development of dimethyl carbamate insecticides instead of carbamate repellents (Thapa *et al.*, 2022). Figure 3 displays a timeline of carbofuran usage throughout history. In 1953, Union Carbide Corporation synthesized a new family of carbamate compounds in which the dimethyl carbamoyl moiety was substituted with a monomethyl moiety. Compared to dimethyl carbamic acids, the insecticidal properties of aryl N-methylcarbamate were superior (Malhotra *et al.*, 2021). Carbofuran was derived from these aryl N-methylcarbamate insecticides.

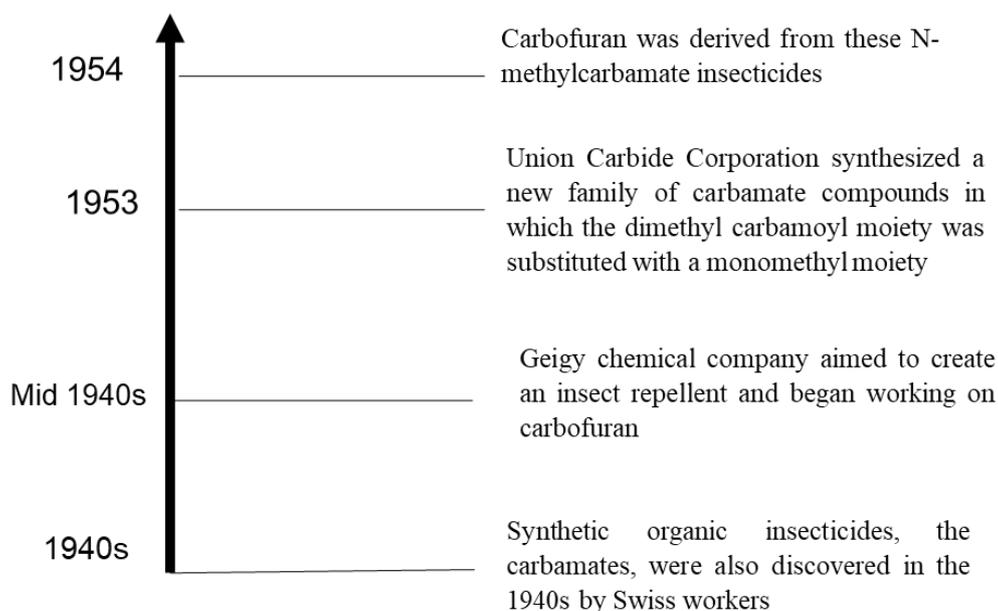


Figure 3: Timeline Showing the History of Carbofuran Production (Thapa *et al.*, 2022)

Source of Carbofuran Contamination into the Environment

The principal source of carbofuran contamination into the environment is either through direct applications in agricultural fields and industries or indirectly through drifting and volatilization from the treated fields contaminating air, soil, water-bodies, non-target plants and animals (Sun *et al.*, 2018). The exact amount of pesticide that is applied on the crops, never completely reaches to the target organism(s) and most of its amount is dispersed into the environment thus contaminating air, soil and water ecosystems. Donovan *et al.* (2012) comprehensively studied the environmental fate, chemistry and detection of carbofuran in soil and water. Carbofuran is highly water-soluble (~700 mg/L at 25 °C) and has low adsorption coefficient [$K_{oc}=30$] that strongly favors its contamination in surface and ground waters (www.epa.gov). Field studies suggested that carbofuran has a moderately persistent half-life in soil (55-115.5 days) and its

mobility in soil contaminates ground water bodies. Soil pH is one of the major determinants of pesticide persistence and carbofuran tends to be more stable in acidic soils than neutral or alkaline soils. The environmental fate of carbofuran depends upon various important biotic and abiotic factors such as pH, soil type, temperature, moisture content, organic content, microbial population and functional groups attached to the pesticide molecule (Wang *et al.* 2009). Depending upon different soil types, carbofuran mobility can contaminate aquatic resources such as ponds, lakes, pools, rivers, streams and drinking groundwater. In drinking water, it has been detected at very low concentrations (1-5ppb), which is also a matter of great concern. Carbofuran can be degraded into metabolites through chemical or microbial degradation (Figure 4). In soils with high organic content, the microbial degradation of carbofuran is more rapid than chemical degradation. However, carbofuran decomposes in water by direct photolysis and photo oxidation (Fenoll *et al.* 2013).

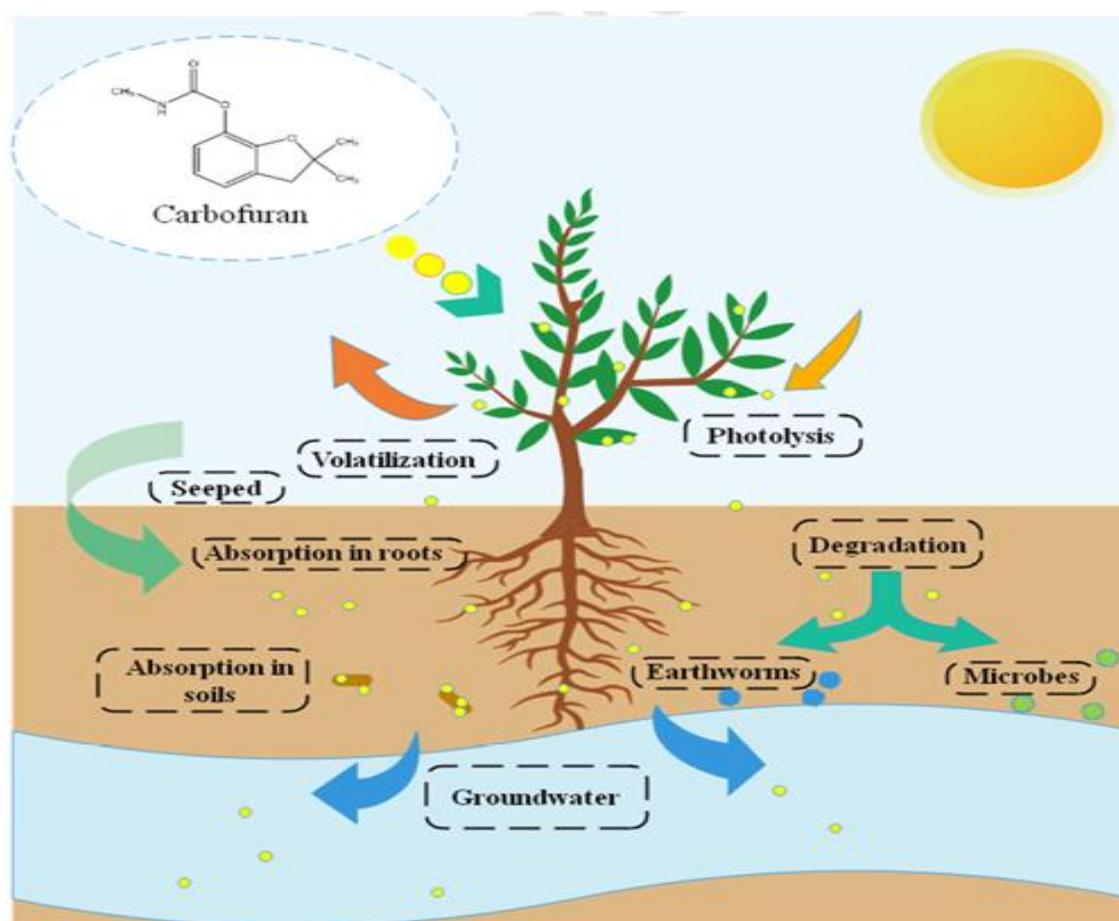


Figure 4: Environmental Fate of Carbofuran

Toxicity Mechanism of Carbofuran and its Toxicological Impact

The toxicities related to carbofuran and its derivatives have been documented. It is dangerously toxic to non-target organisms such as birds, rodents, honeybees, fishes and other water organisms through direct inhalation, ingestion or dermal absorption. It is frequently involved in malicious poisoning of animals, plants and humans leading to death (Jahan *et al.*, 2025). Though, it is not considered as carcinogen but can cause genotoxic, mutagenic, carcinogenic and teratogenic effects (WHO, 2009). Carbofuran causes acute and chronic toxicities by acetylcholinesterase inhibition in nerve synapses of central nervous and i system that is the main

toxicological characteristic of this pesticide (WHO, 2009). Carbofuran also imposes severe developmental and reproductive defects in maternal-placental-fetal unit by crossing placental barrier. It also causes significant decrease in isoenzyme I and isoenzyme II in mother and fetus, respectively. The toxic concentration of carbofuran in biological tissues ranging from 0.4-18 μ g/mL (in blood), 0.4-60 μ g/mL (in bile), 2.2 μ g/g (in liver) and 0.3-300 μ g in stomach contents/stomach results in fetal cases of suicide and homicide (Tennakoon *et al.* 2013). The acute toxicity level of carbofuran differs among organisms. The toxicity of carbofuran to mammals is higher through the oral and inhalation routes than the dermal route (Da Luz *et al.*, 2024).

Furthermore, carbofuran effects in humans are associated with reproductive disorders, endocrine-disrupting activity, and cytotoxic and genotoxic abnormalities (Mishra *et al.*, 2020). In past years, carbofuran has emerged as a global contaminant that can harm health and the environment (Meena *et al.*, 2020).

Carbofuran toxicity mechanism is based on reversible competitive inhibition of acetylcholinesterase enzyme (AChE) in the central and peripheral nervous systems. Carbofuran causes carbamylation or modification of serine residue active site within carboxylesterase and butyrylcholinesterases enzymes. The inhibition of AChE enzymes causes successive accumulation of acetylcholine (ACh) in nerve synapses that separates into choline and acetate residues leading to clinical consequences. Depending upon the stimulation ACh receptor, there are two types of acute clinical manifestations known as nicotinic and muscarinic. Nicotinic effects result in muscle weakness and tremors whereas muscarinic effects can damage cardiac, gastrointestinal and respiratory systems resulting in defecation, increased salivation, gastroenteritis, bradycardia and obstruction of airways. In Asia, Australia, Africa and South America, carbofuran is widely used. Meanwhile, Canada, Kenya, Brazil, the United States, and the European Union are among the countries that have banned or restricted the use of carbofuran (Sharma *et al.*, 2020).

Potential of Carbofuran-Degrading Microorganisms

Due to the large-scale carbofuran toxicity, development of microbial technology is urgently needed for complete bioremediation of carbofuran from the environment. A large number of pesticides and xenobiotic compounds have been reported that are rapidly degraded by microorganisms such as bacteria, fungi, algae, cyanobacteria and genetically engineered microbes (Ariffin and Rahman, 2020; Omolo *et al.*, 2012; Slaoui *et al.*, 2007). Microbial bioremediation plays an imperative role in safe and efficient removal of toxic pollutants from the environment by utilizing pollutants or contaminants as a sole source of carbon and energy. Carbofuran biodegradation has been extensively studied and potent carbofuran degrading bacteria have been isolated from carbofuran-contaminated soil, sludge and water samples by enrichment culture technique. These bacteria include *Achromobacter* sp. WM111, *Rhodococcus* TE1, *Pseudomonas* sp. 50432, *Sphingomonas* sp., strain SB5, *Enterobacter* sp., *Burkholderia* sp. PLC3, *Bacillus* sp. and *Cupriavidus* sp. ISTL7.

Bacterial strains effectively utilize carbofuran as a sole carbon or nitrogen source and reduce, degrade or mineralize it into non-toxic phenolic substrates. Microbial degradation depends on various abiotic and biotic environmental factors including pH, temperature, strain selectivity, inoculum size, microbial distribution, soil nutrient bioavailability and chemical complexity of the pollutant (Lukman *et al.*, 2024). Studies showed that soils with a long history of pesticide application result in enhanced biodegradation as compared to soils with no history of pesticide application. Researchers have reported that microbes develop themselves to survive better under pesticide stress. Soil bacteria play a vital role in enhanced biodegradation of pesticides from contaminated soils. Repeated application of carbofuran in agricultural fields results in a more potent carbofuran degrading native microbial communities due to the continuous expression of specific enzymes. These native soil microbial communities perform a decisive role in the degradation of carbofuran by evolving novel biodegrading genes, metabolic pathways and degradation mechanism because of inherent horizontal gene

transfer resistance property (Slaoui *et al.*, 2007). Longer exposure of pollutants to microbes produces effective bioremediation. He investigated three carbofuran-contaminated fields with different exposure times of 8 years, 3 years and 1 year and isolated high number of carbofuran degrading bacteria i.e. 42 isolates from 8 year exposed soil field in comparison to 3 and 1 year with only 21 and 12 number of bacterial isolates, respectively. Therefore, this finding may conclude that prior exposure of pesticides to microbes may result in increased population to accelerate microbial degradation of carbofuran due to selective build up and adaptation mechanisms (Ariffin and Rahman, 2020).

Among various microorganism, Sphingomonads (including *Sphingomonas*, *Novosphingobium*, *Sphingopyxis* and *Sphingobium*) might be considered as the most effective carbofuran-degrading bacteria. These bacteria are highly potent to degrade carbofuran and carbofuran phenol as compared to other bacterial genera. *Pseudomonas* sp. NJ-101 was isolated from agricultural soil, which degraded carbofuran from contaminated soil samples at the rate of 0.035 mg/kg/day until complete degradation in 40 days. This strain also possesses plant growth promoting properties such as siderophore, hydrogen cyanide (HCN) and indole 3-acetic acid (IAA) production, solubilization of inorganic phosphate and antagonistic activities against *Fusarium* sp., *Pseudomonas* sp. and *Pseudomonas* sp. 50342 strains efficiently converted highly toxic carbofuran to less toxic form of 4-hydroxycarbofuran (Mishra *et al.*, 2020).

Tondon *et al.* (2018) identified four carbofuran degrading bacterial strains, *Arthrobacter globiformis*, *Bhargavaea indica*, *Bacillus beijingensis*, and *Streptomyces* sp., from contaminated soil samples, which degraded 43, 55, 35 and 44% carbofuran, respectively. These isolates grew at a high concentration (10 mg/L) of carbofuran by utilizing it as carbon and nitrogen source in minimal salt medium. Furthermore, the reduction rate of carbofuran varied for every single bacterium but the combination of *Bhargavaea indica* and *Streptomyces* sp., significantly increased (60%) carbofuran degradation. Study suggested that application of combination or mixed bacterial population is more effective in fast biodegradation of pesticide. Enriched mixed bacterial cultures behave differently with different pesticides and can degrade pesticides of various groups (Krishna and Philip, 2008). Mixed bacterial cultures or microbial consortia have been reported to possess better survival ability in non-sterile conditions that enables microbes to act successfully on a variety of toxic pollutants than pure cultures. Microbial consortiums are better equipped for environmental remediation due to continuous exposure of contaminants and possible synergistic mechanism with other strains. Microbial consortiums or mixed cultures are famous for large-scale field applications for the complete removal of carbofuran residues (Tondon *et al.* 2018). Most of the carbofuran biodegradation studies explored the use of free bacterial cells, but sometimes free bacterial cell cannot easily survive in natural environments and their growth is very slow. Immobilization of bacterial cells is a good solution to regenerate and reuse bacterial cell for a long time to degrade xenobiotic compounds. Immobilized bacterial cells are more defensive under stressful environment (abiotic and biotic) and improve bacterial growth. The degradation of carbofuran residues in synthetic medium and soil was studied by using immobilized cells of *Burkholderia cepacia* PCL3. Study showed that survival period of immobilized cells (10–6–10–7), adsorbed on corncob and sugarcane bagasse was much longer (30 days) than free bacterial cells, which started reducing after 10 days.

Microbial Metabolic Pathway of Carbofuran Degradation

Due to the overuse of carbofuran in agricultural and commercial practices, microorganisms have evolved resistance and various catabolic pathways to survive under pesticide contaminations and participate in its successful mineralization from the environment. Microbial degradation results in the complete mineralization of carbofuran into non-toxic substrates and CO₂. Therefore, microbial degradation of carbofuran is a highly significant and effective approach. To eliminate carbofuran, microbes mainly follow oxidative and hydrolytic pathways (Figure 5 and 6) (Mishra et al., 2020). Hydrolytic pathway is more convenient and produces less toxic metabolites in comparison to oxidative pathway. Hydrolytic pathway uses hydrolase enzyme to break ester bond of carbonyl group of N-methylcarbamic acid attached to the phenol and amide bond of methylcarbamic acid to produce carbofuran 7-phenol (2,3-dihydro-2,2-dimethyl-7-benzofuranol) metabolite, CO₂ and methylamine. Carbofuran-7 phenol is the initial major metabolite, which is lesser toxic than parent compound. The methylamine is used as a source of carbon or nitrogen by a variety of bacteria to degrade carbofuran but they cannot degrade aromatic ring. Among carbofuran degrading bacteria, *Sphingomonads* are potent and well documented to completely mineralize the carbofuran. The first demonstrated the degradation mechanism of carbofuran to carbofuran-7-phenol and subsequently to 2-hydroxy 3-phenol (3-methylpropane-2-ol) by *Sphingomonas* sp. which utilizes carbofuran as a sole carbon source. *Sphingomonas* sp. CF06 was further identified to mineralize both carbonyl and aromatic rings of carbofuran producing carbofuran-7-phenol and methylamine. A new degradation pathway of carbofuran by a *Sphingomonas* sp. SB5, which hydrolyzed carbofuran to a new metabolite at the furanyl ring. Strain SB5 first degraded carbofuran into carbofuran-7-phenol by utilizing CO₂ and methylamine for their growth. Carbofuran-7-phenol was further degraded into 2-hydroxy-3-(3-methylpropan 2-ol) phenol and 3-(2-hydroxy-2-methylpropyl) benzene-1,2-diol (DP-1) as a hydrolytic product of carbofuran by the addition of water molecule.

A novel metabolic pathway was reported in *Novosphingobium* sp. FND-3, where ether bond of furanyl

ring was hydrolyzed into a new hydrolytic product 2-hydroxy-3-(3 methylpropane-2-ol) benzene-N-methyl carbamate instead of labile methyl carbamate linkage. This bacterium also showed common hydrolytic cleavage of carbamate group and furanyl ring cleavage to produce carbofuran-7-phenol, 2-hydroxy-3-(methylpropane-2-ol) phenol and 5-hydroxy carbofuran, as reported earlier.

Bacterial degradation can benefit the environment by using microorganisms to break down and transform pollutants into less harmful substances (Ibrahim et al., 2016b). Microbes play key roles in biodegradation, which recycles biologically essential elements within the earth's biogeochemical cycles (Miglani and Bisht, 2020). Microbial degradation is a possible and environmentally friendly way to degrade carbofuran. However, most prior studies on carbofuran degradation have focused on bacterial degradation, and the degradation rates remain slow (Mishra et al., 2020).

In general, the use of bacterial degradation for environmental remediation can have several advantages over other methods, such as physical or chemical treatments. For example, bacterial degradation can be a cost-effective and environmentally friendly approach, as it can occur naturally and may not require additional chemicals or energy (Xu et al., 2023, Ibrahim et al., 2014; 2015). Continuous exposure to pollutants and synergy with other strains make microbial consortium more suitable for environmental remediation. Microbial consortium or mixed cultures degrade carbofuran residues on a large scale. Singh et al. (1993) discovered carbofuran-degrading bacterial strains from contaminated soil samples: *Arthrobacter* sp. Fareed et al. (2017) isolated and identified *Enterobacter cloacae* capable of degrading carbofuran.

Bacterial degradation completely mineralizes carbofuran into non-toxic substances and CO₂. As a result, microbial carbofuran degradation is an effective and practical method. Microbes mostly use oxidative and hydrolytic mechanisms to eliminate carbofuran. Carbofuran phenol is further degraded into novel metabolites by carbofuran-degrading microorganisms and their hydrolase enzymes (Park et al., 2022). Bacterial species capable of degrading carbofuran has been extensively isolated from various sources and are outlined in Table 2.

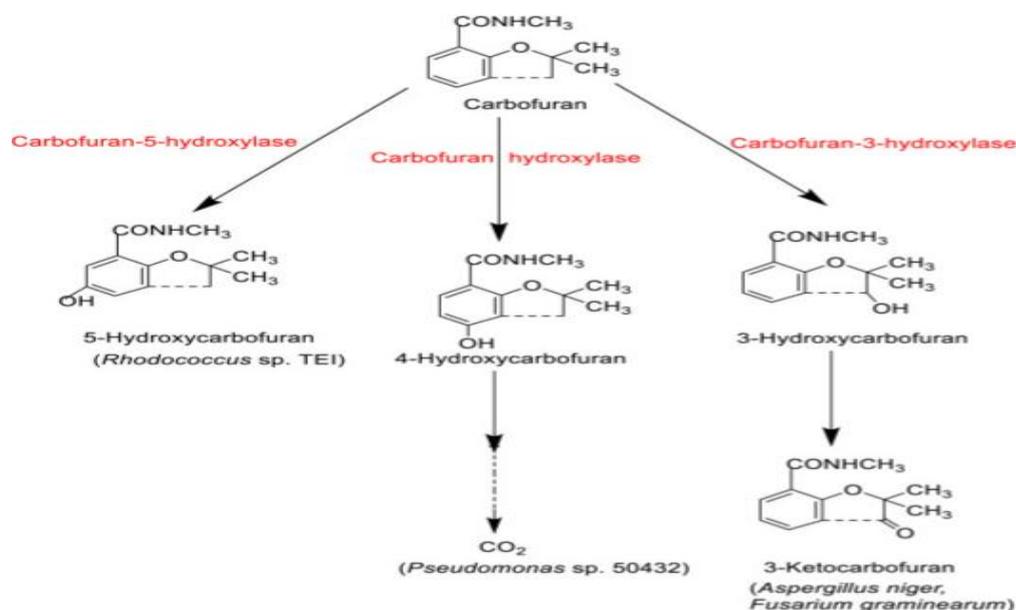


Figure 5: Proposed Oxidative Microbial Metabolic Pathway for the Degradation of Carbofuran

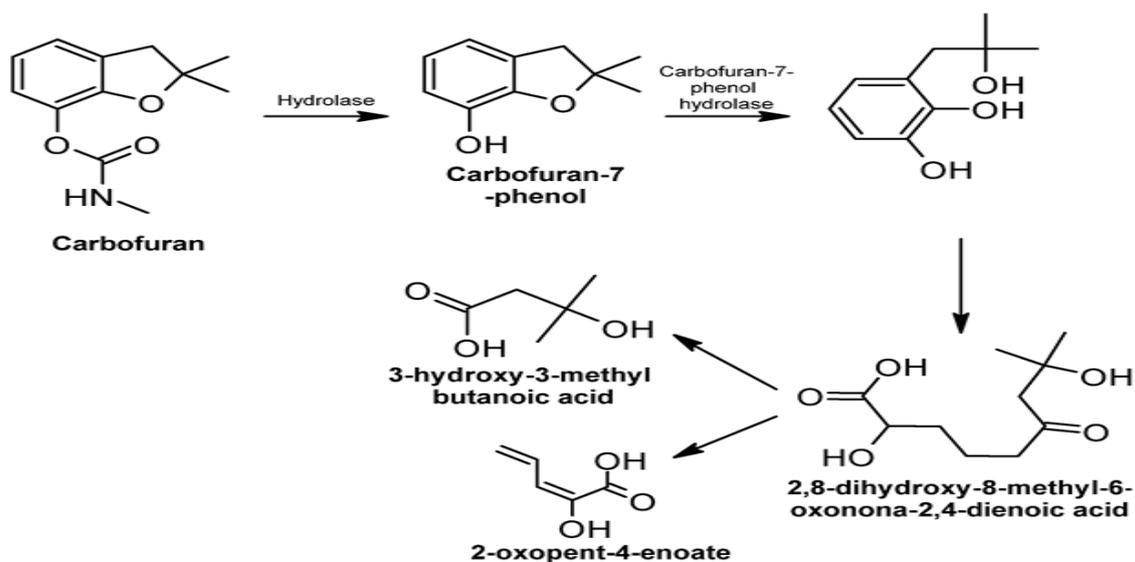
Figure 6: Hydrolytic Microbial Metabolic Pathway for the Degradation of Carbofuran (Park *et al.*, 2022)

Table 2: Carbofuran Degrading Bacteria and Source of Isolation

Organism	Sample Source	References
<i>Sphingomonas</i> sp.	Soil	(Ataikiru <i>et al.</i> , 2020)
<i>Mucor ramannianus</i>	Soil	(Zhang <i>et al.</i> , 2020)
<i>Novosphingobium</i> sp. FND-3	Sludge	(Yang <i>et al.</i> , 2024)
<i>Paracoccus</i> sp. YM3	Sludge	(Peng <i>et al.</i> , 2008)
<i>Enterobacter</i> sp.	Soil	(Ataikiru <i>et al.</i> , 2020)
<i>Burkholderia</i> sp. PLC3	Soil	(Parte <i>et al.</i> , 2017)
<i>Novosphingobium</i> sp.	Soil	(Nguyen <i>et al.</i> , 2014)
<i>Bacillus</i> sp	Soil	(Ataikiru <i>et al.</i> , 2020)
Consortia	Soil	(Degradadoras <i>et al.</i> , 2013)
<i>Enterobacter cloacae</i> strain TA7	Soil	(Fareed <i>et al.</i> , 2017)
<i>Streptomyces</i> sp.	Soil	(Sethi <i>et al.</i> , 2024)
<i>Sphingium</i> sp. CFD-1	Soil	(Cheng <i>et al.</i> , 2022)

Mechanism of Action of Carbofuran

Carbofuran's mechanism of action, which makes it an effective insecticide, acaricide, and nematicide, primarily involves the inhibition of the enzyme acetylcholinesterase (AChE) in the nervous system of target organisms (Zhang *et al.*, 2024). Carbofuran, being a carbamate insecticide, acts by binding to acetylcholinesterase (Jain *et al.*, 2022). This binding prevents the enzyme from hydrolyzing acetylcholine. When AChE is inhibited by carbofuran, acetylcholine is not broken down effectively and accumulates at the nerve synapses and neuromuscular junctions. The excessive build-up of acetylcholine leads to continuous and uncontrolled stimulation of the nervous system (Sidhu *et al.*, 2019; Zhang *et al.*, 2024). This results in a cascade of neurological and physiological effects, including:

- i. Uncontrolled muscle twitching and tremors.
- ii. Spasms and convulsions.
- iii. Paralysis.
- iv. Respiratory failure.
- v. Ultimately, death of the organism.

While carbofuran inhibits acetylcholinesterase, the inhibition caused by carbamates is generally reversible, although it can still be lethal, especially at high doses or prolonged exposure. This reversibility distinguishes them from organophosphate insecticides, which typically cause irreversible or very slowly reversible inhibition of AChE. The carbamylated enzyme undergoes spontaneous hydrolysis, albeit at a slower rate than the hydrolysis of acetylcholine itself. In essence, carbofuran

disrupts the normal functioning of the nervous system by preventing the breakdown of acetylcholine, leading to overstimulation and eventual death in susceptible organisms (Fukuto, 1990). This mechanism of action is highly effective against a broad range of pests, but it also poses significant toxicity risks to non-target organisms, including humans and wildlife, due to the fundamental similarity in their nervous systems (Sidhu *et al.*, 2019).

Application of Carbofuran-Degrading Bacteria

The potential applications of carbofuran-degrading bacteria in bioremediation are significant, offering innovative solutions for addressing environmental pollution caused by carbofuran residues (Malhotra *et al.*, 2021). The unique capabilities of these microorganisms enable them to effectively degrade carbofuran, which is a widely used pesticide that poses considerable risks to ecosystems and human health due to its persistence in the environment. Since this field is still a developing area, it has taken the attention of most worldwide scientists. Hence, new improvements blend with new technological approaches are often observed. Most recently, scientists are focusing on advancements such as gene editing and system biology tools for pesticide bioremediation (Nabil *et al.*, 2023). Scientists are attempting to understand the genetics and biochemistry of the biodegradation process performed by natural microbes and using these data, try to develop a biodegradation network consists of all the datasets which aid in assisting the degradation and deterioration

potential of microorganisms for bioremediation processes (Randika *et al.*, 2022). This approach makes a path to develop remunerative systems by compiling the knowledge obtained by individual researchers. Moreover, worldwide, scientists are taking attempts in using recombinant DNA technology and gene-editing tools like CRISPR Cas, TALEN and ZFNs which can design genetically modified microbes having functional genes of interest for degradation of pollutants that are important for improved bioremediation (Nag *et al.*, 2024).

Bioremediation

Bioremediation is a sustainable biological method used for the restoration of contaminated environment. This concept was initiated in 1952 that when there are harmful organic compounds in the environment, there will be microbes that are able to decompose the pollutant so it does not pollute the environment (Ibrahim *et al.*, 2016a). Bioremediation has many advantages. This method is widely accepted and applied by communities worldwide due to it being considered inexpensive and environmentally friendly. The bioremediation process utilizes biological agents that act as bioremediators (Lukman *et al.*, 2024; 2023). The biological agents in the form of microbes i.e., bacteria, fungi, actinomycetes plants etc. perform a function in decomposing waste substances. Bioremediation is considered a method that is safe, easy, and affordable in helping to restore polluted environmental conditions (Ibrahim *et al.*, 2020; 2014).

Much research on bioremediation has been carried out, such as research by (Hossain *et al.*, 2023) who isolated the microbes: *Acinetobacter*, *Klebsiella* and *Pseudoxanthomonas* for bioremediation of soil contaminated with carbofuran. Duc (2022) isolated *Bacillus sp.* strain DT1 for carbofuran-contaminated soil bioremediation. Thongmee and Sukplang, (2024) utilized the microbial species *Pseudomonas sp* and *Stenotrophomonas sp.* for bioremediation of carbofuran-contaminated soil. Although there have been many studies, most of the application of bioremediation focuses on the bioremediation of carbofuran in developed countries.

Bioremediation is a solution that combines both agricultural advantages and the avoidance of hazardous pesticide bioaccumulation (Hossain *et al.*, 2023). Although other techniques have been developed to lessen the effects of pesticides on the environment and human health, these include physical treatments like adsorption and percolator filters; chemical treatments like advanced oxidation that use potent transient species, primarily the hydroxyl radical but, they are vulnerable to problems like high costs, time demands, unpredictable outcomes, and others. Hence, it is becoming clear that the best course of action is to make use of the natural capacity of local microorganisms, particularly bacteria, to convert pesticides into safe, environmentally beneficial, and non-toxic residues (Reddy *et al.*, 2024).

Agents of Bioremediation

Natural organisms, either indigenous or extraneous, are the important agents used for bioremediation (Ataikiru and Ajuzieogu, 2023). The organisms vary, depending on the chemical properties of the polluting substances, and are to be chosen cautiously as they only sustain within a stipulated limit of chemical contaminants (Ataikiru and Ajuzieogu, 2023). The first patent for a biological remediation substance was recorded in 1974, was a strain of *Pseudomonas putida* capable of degrading petroleum (Ataikiru and Odesiri-Eruteyan, 2021).

Bacterial Bioremediation

The microbes have often been reported for the degradation of pesticides and hydrocarbons. A large number of bacteria utilize the contaminant as the sole carbon and energy sources. They are; *Bacillus sp* and *Pseudomonas sp* that can degrade aromatics and hydrocarbons (Leung *et al.*, 2019), *Flavobacterium sp* and *Azotobacter sp* that can degrade aromatics (Sidhu *et al.*, 2019) and *Nocardia sp* that can degrade hydrocarbons (Kim *et al.*, 2017). Metals play important role in the life processes of microbes. Some metals such as chromium (Cr), calcium (Ca), magnesium (Mg), manganese (Mn), copper (Cu), sodium (Na), nickel (Ni) and zinc (Zn) are essential as micronutrients for various metabolic functions and for redox functions (Alhassan *et al.*, 2020; Patowary *et al.*, 2023).

Fungal Bioremediation

Fungi represent the promising group of microbes for biodegradation. The ability of fungi, both yeasts and moulds, to convert a broad variety of hazardous chemical substances has developed interest to use them in bioremediation (Wang *et al.*, 2020). Fungi can mineralize xenobiotic compounds to CO₂ and H₂O through their non-specific ligninolytic and highly oxidative enzyme system, which is also responsible for the degradation and decolorization of a wide range of dyes (Reddy *et al.*, 2024).

Effects of Carbofuran on Microorganisms

Carbofuran can affect microorganisms either positively or negatively depending on factors like concentration, exposure level or environmental conditions. The effects are as follows:

- i. Toxic effects on beneficial soil microorganisms caused by inhibition of microbial activity or reduction in enzymatic activity.
- ii. Stimulation of pesticide-degrading microbes as only microorganisms that can metabolize carbofuran will be able to survive and thrive in the presence of carbofuran.
- iii. Impact on symbiotic relationships such that beneficial soil microbes like Mycorrhizal fungi and Nitrogen fixing bacteria which aid in plants nutrient uptake can be disrupted due to the toxicity of carbofuran.
- iv. Effect on aquatic microorganisms like planktonic bacteria and algae.
- v. Development of resistance due to prolonged exposure to carbofuran.
- vi. Disruption of soil Health arising from disruption of beneficial microbial communities by carbofuran.

Carbofuran's effects on microorganisms are numerous and complex. While it can be toxic to some species on one hand, it can also be a nutrient source for others on another hand and can play a crucial role in bioremediation process (Duc, 2022). The specific effect of carbofuran ranges from but not limited to: the type of microorganism, the concentration of the pesticide, and the environmental conditions (Ariffin and Rahman, 2020).

Factors Affecting Carbofuran Degradation

Understanding the factors affecting degradation is essential to understand the interactions among different bacterial strains and their responses to various environmental factors, such as nutrient availability and the presence of co-contaminants (Malhotra *et al.*, 2021). Optimization and control of bioremediation is a complex procedure driven by many factors (Nag *et al.*, 2024). There are various factors affecting bioremediation potential such as:

Initial Substrate Concentration

Availability of sufficient concentration of pollutant or toxic compounds that can be utilized by microorganisms for their nutrients or energy needs is one of the most important factor that affects bioremediation potential (Abatenh *et al.*, 2017). Even though microorganisms are present, if the toxicity of the pollutant is not tolerable by microorganisms, it will reduce their bioremediation potential by being toxic and inhibit their growth (Lukman *et al.*, 2024; 2023). Chemical structure of contaminants is also an important factor. Sometimes, the compounds in pollutants may be non-biodegradable compounds such as, polymers, plastics etc.

Temperature

Temperature is an important environmental factor that has a huge impact on the bacterial biodegradation rate due to the fact that bacterial species tend to have an optimum temperature in which their metabolic activity is at optimum hence the need to determine the appropriate temperature where bacteria can degrade the contaminant optimally. Temperature control is absolutely essential for a good biodegradation process as lower temperature causes reduced degradation rate while higher temperature adversely affects the growth of microorganisms (Ibrahim *et al.*, 2020).

pH

Most bacterial isolates employed in biodegradation process tend to have an optimal operating pH as it could significantly affect the activity of the bacterial enzymes during the degradation process (Ibrahim *et al.*, 2020). It is worth noting that extreme pH either acidic or basic can significantly degrade the enzymes of the bacteria therefore usually the pH requirement for bacteria is around the neutral pH of 7.

Inoculum Size

The bacterial population otherwise known as inoculum size can considerably affect the biodegradation process where very low population density might take a very long time to degrade the pollutant while a very high population density can lead to high competitiveness among the bacterial strains therefore being detrimental and consequently leading to lower degradation (Ibrahim *et al.*, 2020).

Nitrogen Source

Nitrogen source is an important factor that affect the biodegradation process. Nitrogen is important building block for bacterial metabolism and as such it is required for growth. Bacterial strains tend to prefer certain nitrogen sources than others probably due to the presence of certain enzymes that enable them to utilize certain nitrogen sources than others. (Ibrahim *et al.*, 2020; Randika *et al.*, 2022). The effectiveness of the remediation process can vary depending on such different environmental conditions.

Co-contaminant

The environment is at any point in time contains multiple pollutants as it is an open system and therefore no contaminant is occurring singly. Presence of multiple contaminants in a particular environment can hinder the biodegrading capability of bacteria significantly. Some examples might include: heavy metals, pesticides and herbicides etc. (Sharma *et al.*, 2019; Ibrahim *et al.*, 2016c).

CONCLUSION

Carbofuran is a persistent, highly toxic pesticide requiring sustainable remediation strategies. This review confirms

microbial biodegradation as a superior, eco-friendly alternative to conventional methods. Key bacterial genera like *Sphingomonas*, *Pseudomonas*, *Bacillus*, and *Novosphingobium* efficiently degrade carbofuran via hydrolytic and oxidative pathways, often initiating breakdown with carbofuran hydrolase. Degradation efficiency depends critically on optimized environmental factors such as pH, temperature, and inoculum size. Advancements in microbial consortia, cell immobilization, and genetic engineering offer promising pathways for enhancing bioremediation efficacy. Future efforts should prioritize field-scale applications and integrated approaches to translate this microbial potential into practical, sustainable solutions for mitigating carbofuran pollution and protecting ecosystem health.

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