Microbial Remediation of Persistent Agrochemicals

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Priyanka Priyadarshinee, Sophia Subhadarsini Pradhan, Ritesh Mishra, S. Aravindan, P. C. Rath, Pradipta Kumar Mohapatra, and Totan Adak

Abstract

Agrochemicals are an integral part of the agricultural ecosystem as it contributes significantly to improving the crop yield through pest management. The chemically synthesized products such as insecticides, herbicides, and fungicides exhibit harmful effects on living organisms and few of them are characterized as resistant to degradation. Besides being persistent in nature, they may leach into groundwater and run off to surface water. Thus, to degrade the persistent agrochemicals, bioremediation with the help of microbes is one of the best options. This approach is environmentally friendly, effective, and less expensive with the least adverse effects. Microbes such as bacteria, actinobacteria, fungi, and cyanobacteria are reported of having the exclusive trait of degradation. The microbial world consumes persistent toxic chemicals as the source of their growth by facilitating the mineralization of those chemicals. This detoxification process is carried out with the help of microbial enzymes. Some efficient and potential bioremediation agents are *Bacillus* sp., *Pseudomonas* sp., *Arthrobacter* sp., *Anabaena* sp.,

P. Priyadarshinee · S. S. Pradhan
Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha, India

Department of Botany, Ravenshaw University, Cuttack, Odisha, India

R. Mishra
Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha, India

Department of Entomology, Orissa University of Agriculture & Technology, Bhubaneswar, Odisha, India

S. Aravindan · P. C. Rath · T. Adak (✉)
Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha, India
e-mail: Totan.Adak@icar.gov.in

P. K. Mohapatra
Department of Botany, Ravenshaw University, Cuttack, Odisha, India

S. K. Nayak et al. (eds.), *Advances in Agricultural and Industrial Microbiology*,
https://doi.org/10.1007/978-981-16-9682-4_8

Leptolyngbya sp., *Nostoc* sp., *Spirulina* sp., etc. This chapter discusses the extent of the use of persistent agrochemicals and key biodegradation pathways. The chapter also discusses on the advantages and disadvantages of microbial remediation and the scope of commercial utilization of microbes for agrochemical degradation.

Keywords

Pesticides · Bioremediation · Enzymes · Persistent agrochemicals (PAs) · Bioconcentration factor (BCF)

8.1 Introduction

Use of agrochemicals has increased manyfold from the period of "Green Revolution." Agrochemicals, more particularly pesticides, are applied to improve crop yield with better quality of product through the management of pest (insecticides), disease (fungicides), and weed (herbicides). Synthetic pesticides are semi-volatile, toxic, and persistent in nature and trigger harmful effects on humans, environment, and wildlife. These chemicals take decades of time to degrade significantly in natural environment. During such longer period of time they may get transported to groundwater, surface water, surface, and core part of soil. From soil and water, they are accumulated in food crops and enter into the food cycle (El-Bestawy et al. 2007). Apart from this, beneficial microbes and nontarget organisms are affected by the indiscriminate use of synthetic chemicals in the agroecosystem. Aquatic flora, fauna, and microorganisms are affected by the discharges of agrochemical manufacturing factories as well as by unintended spills. Persistent organic pollutants in agriculture (persistent agrochemicals, PAs) can be degraded by various mechanisms such as photodegradation (Bustos et al. 2019), bioadsorption (Mishaqa 2017), bioaccumulation (Xu and Huang 2017), and biodegradation (Bhadouria et al. 2020).

The term bioremediation comprises two words, i.e., "bios" (Greek) means life and "remedium" (a Latin term) means to take out an evil. So, bioremediation is a process that eradicates, degrades, and detoxifies the persistent pollutants by living beings. The two highlighted classes of bioremediation are phytoremediation and microbial remediation. In ex situ bioremediation, the contaminants are removed from its native place to another place and treated with microbes. In in situ bioremediation, the microbes are directly inoculated at the contamination site. For certain microorganisms, PAs are the source of nutrients and act as electron donors. Hence, they can be used to manage the PAs in polluted areas.

Some of the important microbial genera efficient in bioremediating agrochemicals are described here. Bacterial strains having degrading capacity of PAs belong to the genera of *Bacillus* sp., *Arthrobacter* sp., *Rhodococcus* sp., *Alcaligenes* sp., *Flavobacterium* sp., *Yersinia* sp., *Pseudomonas* sp., *Acetobacter* sp., *Burkholderia* sp., *Weeksella virosa* sp., *Stenotrophomonas* sp., etc. (Padmanabhan et al. 2003). Among the actinobacteria group, reports suggest that

Streptomycetes can significantly detoxify PAs. Cyanobacteria with pesticide degradation prospective include *Nostoc* sp., *Anabaena* sp., *Phormidium* sp., *Oscillatoria* sp., and *Spirulina* sp. Among fungi, *Fusarium* sp., *Aspergillus niger*, *Penicillium* sp., *Lecanicillium* sp., and *Oxysporum* sp. are known to be the most potent degraders of agrochemicals. Enzymes released by these microorganisms, namely, oxygenase, phosphotriesterase, hydrolases, peroxidases, dehydrogenase, dehalogenase, lignin-modifying enzymes, organophosphorus acid anhydrolase, and laccase, play a crucial role in PA degradation.

This chapter discusses the extent of use of persistent agrochemicals and key biodegradation pathways. It also focuses on the pros and cons of microbial remediation of persistent agrochemicals, and successful and commercial level utilization of microbes for agrochemical degradation. Mechanisms, genes, and enzymes involved in the metabolism of agrochemicals are also discussed in this chapter.

8.2 Persistent Agrochemicals

An ideal agrochemical/pesticide may be defined as a noxious compound that is only harmful to targeted organisms. Unfortunately, this is not true; pesticides also have a negative effect on non-targeted organisms and human beings. Thus, persistent agrochemicals (PAs) can be defined as groups of synthetic and nonvolatile chemicals exposed intentionally or non-intentionally to targeted or non-targeted organisms and having toxic/adverse impacts on humans, environment, and wildlife. According to the sources only 0.1% of applied pesticides reach the targeted organism, whereas the remaining pesticides are deposited on non-targeted environmental compartments such as soil, water, and sediments. Thus, pesticides and their metabolites are the main factors for environmental pollution posing serious threat to the health of non-target organisms like humans and wildlife (Rani et al. 2020).

Nowadays, in international market more than 1000 pesticide compounds and their metabolites have been registered. Popp (2011) reported that the international market capital of agrochemical/pesticide per annum is valued at about USD 40 billion and the total consumption is three million tonnes. Recently, the practical usage of agrochemical covers 25% of the total cultivated land. In India, use of agrochemicals has immensely increased after independence. On international platform, India has become the fourth largest manufacturer of agrochemicals after the USA, Japan, and China (Nayak et al. 2018). The most common agrochemicals of India are organophosphate, neonicotinoids, organochlorines, etc. Among the total pesticide consumption, India has accounted for 50% of insecticides, 35% of fungicides, and 15% of herbicides.

Depending on the chemical structure and mode of action, PA can be divided into several forms such as organochlorine, organophosphate, carbamates, pyrethroids, nicotinic, pyrazole, phenolics, trizines, benzoics, sulfonylureas, bipyridilium, chloroacetamide, glycine, dinitroaniline, phenylpyrazoline, methyl benzimidazole carbamate, demethylation inhibitor, phenylamide, anilopyrimidine, quinone outside inhibitor, and phenylpyrrole. In organochlorine group, on the basis of chlorination

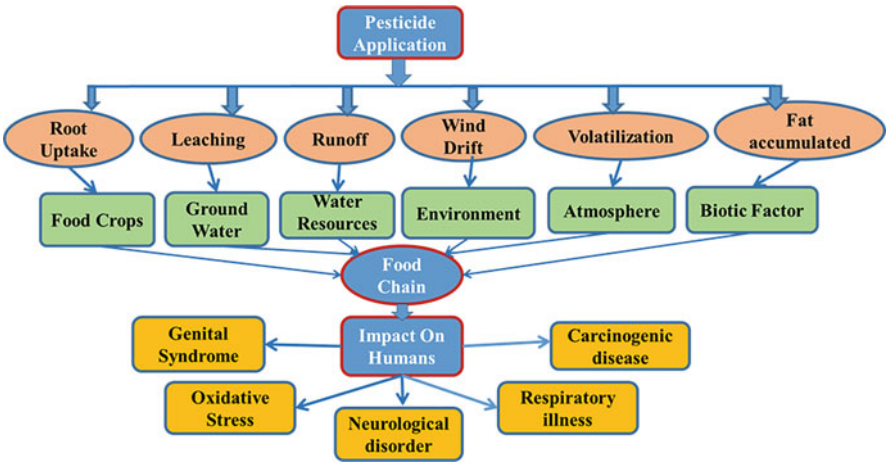

Fig. 8.1 Entry of pesticides into food chain through different ecological factors

number and substitution position, there may be 209 different polychlorinated biphenyls. In aromatics, the most persistent chlorine- and bromine-containing compounds are polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, polybrominated diphenyl ethers, and organochloride pesticides (e.g., dichlorodiphenyl trichloroethane (DDT), toxaphene, chlordane) (Nayak et al. 2018). In Stockholm Convention, the United Nations Environment Programme (UNEP) motivates countries to get rid of 12 persistent organic pollutants that are termed as "dirty dozen" that constitutes 8 agrochemicals, 2 commercial enterprise chemical substances, and 2 accidental industrial intermediate products. However, these persistent virulent eight agrochemicals are prohibited by most of the developed countries, while in case of developing countries, it is being used till today due to their low cost.

The fate of PAs depends on three basic processes of transport, transfer, and transformation (Fig. 8.1). Throughout the mechanism of transport, the PA is departed from its original area of application to the surroundings, and thereafter dispersed throughout the surface water. In the process of transfer, various factors are involved in the distribution and dispersion of PAs in the environment. Last one is the transformation activity, which indicates the natural process along with chemical mechanisms that alters the PA into less complex form of chemicals or degrades it entirely. These persistent agrochemicals are considered as tolerant to degradation or it may take decades or even centuries to eradicate them successfully. These chemicals may get dispersed into extended areas that lead to environmental pollution and some of them get transported into food cycles and immensely affect humans.

8.2.1 Impact of Persistent Agrochemicals on Agriculture and Environment

According to the WHO report, more than three million people are suffering severely each year from exposure of pesticides. In India, the first agrochemical poisoning incident happened in 1958 in Kerala, where the death of over 100 people occurred by the consumption of wheat flour which was contaminated with parathion (Karunakaran 1958). Another terrible case was Bhopal Gas Tragedy of 1984, where the leakage of methyl isocyanate killed about 2259 people. Some studies also revealed that the accumulation of minute amount of persistent agrochemicals by a person can induce combined harmful effects on health conditions like induction of breast cancer, decrease in the number of sperms which results in male sterility, and suppression of immune response with hypersensitive response to some other agrochemicals/chemical antigens (Carvalho 2006). Pesticide application results in decrements in cell development, increment in mutagenesis condition, and nuclear anomalies (Iqbal Lone et al. 2013).

Moreover, agricultural step-up and excessively widespread usage of agrochemicals are responsible for the extinction of various indigenous flora and fauna, which causes a functional disorder in the agroecosystems. Through the long-term use, such persistent agrochemicals are either deposited in soils or leached into the groundwater, thereby dispersing to and polluting different land, marine, and fresh aquatic ecosystems (Nayak and Mishra 2020). These chemicals are decreasing the microbial population of soil and water. For example, the earthworm populations are negatively affected by PAs (Mahmood et al. 2016). By the excessive use of persistent agrochemicals, minor pests are turning into major pests. Natural predators and competitors are being eliminated by excessive use of insecticides. The agrochemical residues decrease the quality of groundwater. Another harmful effect is the leaf interception of agrochemicals, which causes several damages to the non-target plants. The air and other organisms may also be polluted by the excessive use of volatile agrochemicals.

8.3 Mechanism of Microbial Bioremediation of Persistent Agrochemicals

In the ecosystem, various mechanisms are put forward to make it pollutant/contaminant free. Bioremediation can be defined as a process to eradicate, degrade, and detoxify the persistent pollutants by using living beings. Bioremediation may be active or passive based upon the supply of energy, and various mechanisms of bioremediation are as follows:

8.3.1 Bioadsorption

Bioadsorption of PAs is categorized under passive process. Bioadsorption involves a number of mechanisms, i.e., electrostatic interaction, complexation of surface, exchange of ions, absorption, and precipitation (Bilal et al. 2018). Microbes like microalgae are more efficiently used as adsorbents. Mishaqa (2017) found that the cultured algae were able to get rid of 87–96% of pesticides (i.e., alachlor, atrazine, pendimethalin, propanil, simazine, isoproturon, molinate, and carbofuran) in aqueous phase. The efficiency of removal of pesticides was different depending upon the kinds of surface groups present in algae (Ata et al. 2012). The cell wall composition of microalgae plays an important role in PA biodegradation as it facilitates the adsorption of contaminants from polluted water (Qiu et al. 2017). *Gracilaria verrucosa* having hydroxyl, amine, and carboxyl as the surface groups was found to adsorb 2,4-dichlorophenoxyacetic acid (Ata et al. 2012). Several factors, i.e., optimal conditions of the biome, chemical composition and structure of organisms and pesticides, density of organism, pH, temperature, quality and strength of light, salinity, nutrients, water availability, organism (biological) and pesticide (substrate) contact, their surface bonding, redox potential, alternative substrates of carbon, oxygen tension, and electron accepter along with donor, are responsible for the completion of a suitable bioadsorption process.

8.3.2 Bioaccumulation

Bioaccumulation requires externally driven energy and is based on the bio-concentration factor (BCF). BCF reflects the concentration quotient of a contaminant of a certain organism with regard to its surroundings. The variation depends on different factors, i.e., bio-concentration activity differences, bioavailability of chemicals, physical barriers, dissolved organic matter, variation in interspecies, metabolism, and ionization of ionizable chemicals with certain ecological parameters. Reports suggested that the exposure of microbes to the pesticides (PA) produces reactive oxygen species (ROS) within the cell (Pérezgarcía et al. 2013) which is lethal as that causes functional damage leading to cell death. However, some microalgae manage to produce several antioxidants of the group, polyphenols, carotenoids, and sterols. These antioxidants are able to minimize the ROS effect on the matter of cell damage. In this way agrochemicals can induce the process of detoxification activity in microalgae and this reflects the possibility of biodegradation of PAs through bioaccumulation process. The combination of bioaccumulation and biodegradation process to detoxify agrochemicals rapidly is seen in microalgae group (Xu and Huang 2017). Biodegradation of triadimefon by green algae *Scenedesmus obliquus* through bioaccumulation has been successfully reported (Xu et al. 2007).

8.3.3 Biodegradation

The mechanism of catabolic activity to form simpler, nonhazardous, and smaller form of toxic PAs is termed as biodegradation. PA degradation can be done both by aerobic and anaerobic conditions. Under aerobic circumstances, the use of oxygenase enzyme on aromatic compound is generally initiated by electrophilic attack; however, it is delayed with the occurrence of various electron-withdrawing substituents like azo, nitro, and chloro groups. In anaerobic conditions the degradation is initiated via nucleophilic attack and these groups will favor preliminary reductive attack. For the agrochemicals like DDT and heptachlor, anaerobic degradation works better than aerobic degradation. The biodegradation of PAs refers to the chemical activities like reduction, ring cleavage dehydrogenation, dealkylation, oxidation, alkylation, and dehalogenation (Bhadouria et al. 2020).

Hatzios (1991) reported that pesticide degradation process is concluded under three stages. In stage I, the agrochemicals transform to less poisonous by-products by oxidation or hydrolysis. Oxidation, the essential step in the process of degradation, is controlled through the oxidative enzymes, e.g., peroxidase, dioxygenase, polyphenol oxidases, and cytochrome P450 polyphenol oxidases. The hydrolytic reaction plays a key role in some degradation processes. In the next stage, conjugation of PA metabolites occurs to amino acid, glutathione, or sugar. Pesticide or agrochemical conjugation can be defined as "a metabolic procedure where a natural compound is joined to an agrochemical or to its metabolite(s)/intermediate products facilitating sequestration, compartmentalization, detoxification, and/or mineralization."

8.4 Persistent Agrochemical-Degrading Microbes

8.4.1 Bacteria as PA-Degrading Agents

Bacteria can degrade diverse groups of pesticides (Table 8.1). A huge puddle of bacterial strains with degrading capacity include *Bacillus* sp., *Arthrobacter* sp., *Ralstonia* sp., *Rhodococcus* sp., *Yersinia* sp., and *Pseudomonas* sp. (Padmanabhan et al. 2003). The detoxification of PA is achieved by co-metabolism and it is further amplified through root fluids excreted in rhizosphere, because of the gross microbial interaction. In bacteria, PAs are generally taken as carbon and energy sources and get degraded to minerals (Fritsche and Hofrichter 2008). This degradation ability is influenced by several physiochemical factors like soil texture and water-holding capacity, pH, temperature, and availability of nutrients (Singh 2008).

Bacterial multiplication and growth are affected by pesticides because of the proficient absorption of PA in soil organic particles. Apart from this limitation, bacteria have exceptional significance to detoxify the PAs. Reports suggest that aerobic remediation is much faster than anaerobic; however, some exceptions are there such as DDT degradation that occurs ten times faster in anaerobic condition than in aerobic remediation. The most active prokaryotic genus for remediation

Table 8.1 Biodegradation of persistent agrochemicals by bacteria

Sl. No.	Pesticides	Bacteria	References
1.	Acetamiprid	*Ochrobactrum* sp. D-12	Wang et al. (2013)
2.	Alachlor	*Pseudomonas* sp. ADP, *Ancylobacter* sp. S15, *Agrobacterium* sp. CZBSA1	Katz et al. (2001); Ewida (2014)
3.	Aldrin	*Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp.	Sharma et al. (2016)
4.	Atrazine	*Arthrobacter* sp., *Clavibacter* sp.	Sharma et al. (2016)
5.	Cadusafos	*Pseudomonas putida*, *Flavobacterium* sp.	Karpouzas et al. (2005)
6.	Carbaryl	*Pseudomonas* sp., *Achromobacter* sp., *Arthrobacter* sp., *Xanthomonas* sp., *Pseudomonas cepacia*	Chapalamadugu and Chaudhry (1991); Gunasekara et al. (2008)
7.	Carbendazim	*Pseudomonas* sp., *Brevibacillus borstelensis*	Arya et al. (2017)
8.	Carbofuran	*Flavobacterium* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Achromobacterium* sp., *Sphingomonas* sp., *Arthrobacter* sp.	Sharma et al. (2016)
9.	Chlorpyrifos	*Achromobacter xylosoxidans* (JCp4), *Ochrobactrum* sp. (FCp1)	Akbar and Sultan (2016)
10.	Cyhalothrin	*Klebsiella* sp., *Pseudomonas oleovorans*	Thatheyus and Selvam (2013)
11.	Cypermethrin	*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter asburiae*, *Pseudomonas stutzeri*	Thatheyus and Selvam (2013)
12.	DDT (dichlorodiphenyltrichloroethane)	*Klebsiella pneumonia*, *Bacillus* sp., *Pseudomonas putida*, *E. coli*, *Hydrogenomonas* sp.	Sharma et al. (2016)
13.	Diazinon	*Pseudomonas cepacia*	Tewari and Saini (2012)
14.	Dieldrin	*Pseudomonas* sp.	Sharma et al. (2016)
15.	Dimethoate	*Bacillus cereus*, *Bacillus subtilis*, *Bacillus safensis*	Ishag et al. (2016)

(continued)

Table 8.1 (continued)

Sl. No.	Pesticides	Bacteria	References
16.	Endosulfan (α- and β-endosulfan)	*Pseudomonas* sp., *Bacillus* sp., *Flavobacterium* sp.	Karpouzas et al. (2005)
17.	Ethoprophos	*Sphingomonas paucimobilis*	Karpouzas et al. (2005)
18.	Fenvalerate	*Bacillus cereus*, *Pseudomonas viridiflava*	Thatheyus and Selvam (2013)
19.	Glyphosate	*Clostridium* sp., *Arthrobacter* sp.	Tewari and Saini (2012)
20.	Imidacloprid	*Achromobacter* sp., *Pseudoxanthomonas* sp., *Sinorhizobium* sp., *Mesorhizobium* sp., *Microbacterium* sp.	Sharma et al. (2016)
21.	Iprodione	*Pseudomonas fluorescens*, *P. paucimobilis*, *Arthrobacter* sp. C1, *Achromobacter* sp. C2	Mercadier et al. (1997); Campos et al. (2015)
22.	Lindane	*Bosea thiooxidans*, *Sphingomonas paucimobilis*	Karpouzas et al. (2005)
23.	Malathion	*Pseudomonas aeruginosa* AA112	Abo-Amer (2007)
24.	Molinate	*Achromobacter xylosoxidans* subsp. *denitrificans*, *Stenotrophomonas maltophilia*, *Pseudomonas chlororaphis* IFO3904, *Pseudomonas nitroreducens* IAM 143, *Curtobacterium flaccumfaciens var*, *Flaccumfaciens* LMG 3645	Barreiros et al. (2003)
25.	Monocrotophos	*Rhodococcus* sp.	Tewari and Saini (2012)
26.	Pendimethalin	*Pseudomonas aeruginosa*, *Bacillus mycoides*, *Bacillus cereus*	Sharef Ibrahim et al. (2013)
27.	Pentachloronitrobenzene	*Cupriavidus* sp. BIS7	Teng et al. (2017)
28.	Pyridine	*Paracoccus* sp.	Qiao and Wang (2010)
29.	Rizolex	*Bradyrhizobium* sp.	Moawad et al. (2014)
30.	Strobilurin	*Stenotrophomonas maltophilia*, *Bacillus amyloliquefaciens*, *Bacillus flexus*, *Arthrobacter oxydans*	Clinton et al. (2011)

(continued)

Table 8.1 (continued)

Sl. No.	Pesticides	Bacteria	References
31.	Tetrachlorvinphos	*Stenotrophomonas maltophilia*, *Proteus vulgaris*, *Vibrio metschnikovii*, *Serratia ficaria*, *Serratia* sp., *Yersinia enterocolitica*	Ortiz-Hernández and Sánchez-Salinas (2010)
32.	Thiamethoxam, clothianidin, dinotefuran	*Leifsonia* sp.	Sabourmoghaddam et al. (2015)
33.	Tetramethylthiuram disulfide	*Pseudomonas aeruginosa*	Ray and Mondal (2017)
34.	Triclosan	*Aspergillus versicolor*	Taştan and Dönmez (2015)
35.	Triazine (s) methylthio-s-triazines	*Rhodococcus* sp. strain FJ1117YT	Fujii et al. (2007)
36.	Tributyltin chloride (TBTCl)	*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*	Ebah et al. (2016)
37.	Vitavax (37.5% thiram)	*Rhizobium leguminosarum*	Moawad et al. (2014)

purpose is *Pseudomonas* sp. and they are found universally. *Pseudomonas putida* is able to degenerate organophosphates (fenamiphos) and carbamate compounds (carbofuran) (Chanika et al. 2011). *Bacillus* sp. and *Pseudomonas* sp. have the capacity to degrade highly persistent substituents such as pyridine and their metabolites, triclopyridine, picloram, nitrapyrin, and fluridone aerobically (Sims and O'Loughlin 1989). Atrazine, a herbicide, breaks down by the excreted hydrolytic enzyme of *Pseudomonas* sp. and *Klebsiella pneumoniae* (Baishya and Sarma 2015).

8.4.1.1 Mechanism and Pathways of Remediation Process

The biodegradation or detoxification of PAs is a very complex process, involving numerous enzyme-controlled biochemical pathways. The thorough understanding of PA biodegradation pathway within bacteria enhances the capacity to modify microbes for bioremediation. PA biodegradation is based on various classes of enzymes, such as transferase, hydrolase, isomerase involved in redox reactions, conversion of amino to nitro group by oxidation, nitro group reduction, dehalogenation hydrolysis, insertion of O_2 to a double bond and a ^{-}OH group in benzene ring, and sulfur replacement (Megharaj et al. 2011). In aerobic conditions *Pseudomonas* species is able to degrade organochlorides as it has initially dechlorinated them and then converted to other forms by various reactions. For example, DDT is initially converted to less toxic dichlorodiphenyldichloroethane (DDD) which is then transformed to dihydroxy metabolites by dioxygenase enzyme (Nadeau et al. 1994).

The degradation process for 2,4-dichlorophenoxyacetic acid is carried out aerobically by an ortho-cleavage pathway by *Flavobacterium* sp., *Alcaligenes* sp., and *Pseudomonas* sp. finally yielding chloromaleylacetic acid along with its derivatives 2,4-dichlorophenol and 3,5-dichlorocatechol (Gibson and Sulflita 1990). Similarly, *Flavobacterium* sp. (ATCC 27551) is able to satisfy the need of carbon by breaking down the organophosphate compounds through phosphotriesterase enzyme. Atrazine (S-triazine group member) is degraded by dechlorination and hydrolysis. The bacteria (specially found in soil) have the ability to degrade atrazine moderately or completely with carbon dioxide and ammonia as the final yields (Singh et al. 2004).

Sims and O'Loughlin (1989) suggested that *Bacillus* sp. and *Pseudomonas* sp. carry out the metabolism of pyridine to produce hydropyridine and successive split into saturated aliphatic compounds. Association of *Burkholderia* sp. and *Ralstonia* sp. in the remediation of aromatic (unsaturated) hydrocarbons and degradation of n-hexadecanoic acid through intracellular β-oxidation (Yuan et al. 2013) was suggested. Glyphosate metabolism by the bacterium *Streptomyces lusitanus* was done by Lipok et al. (2009).

8.4.1.2 Bacterial Enzymes/Genes Involved in PA Degradation

Bacteria possess remediating genes in both chromosome and plasmid. Suenaga et al. (2001) reported that enzymes degrade PAs by considering them as their substrates. Evolution of microbial biodegrading gene gives a huge opportunity to use them as a bioremediating tool and also raise a ray of hope to deal with the challenge of agrochemical pollution. *Pseudomonas* sp., *Actinobacteria* sp., and *Klebsiella* sp. (Sayler et al. 1990) possess genes encoded for pesticide degradation and pollutant degradation within anticatabolic plasmids and transposons (Laemmli et al. 2000), respectively. The genes, i.e., *atzA*, *atzC* (*trzC*), and *atzB* (*trzB*), produce carbamate-degrading enzymes such as atrazine chlorohydrolase, N-isopropyl-ammelide isopropyl-amino-hydrolase, and hydroxy-atrazine ethylamino-hydrolase, respectively (Sadowski et al. 1998). These clusters of genes manage the successive conversion of atrazine to cyanuric acid after which it completely mineralizes into carbon dioxide and ammonia (Sene et al. 2010).

Degradation of organophosphate compounds by *Plesiomonas* sp. strain M6 is carried out through the enzyme methyl parathion hydrolase (MPH), encoded by mpd gene. Likewise, some cluster of genes have been identified from diverse bacterial species such as *Rhodococcus* sp. strain NI86/21 (Nagy et al. 1995) and *Achromobacter* sp. WMll (Tomasek and Karns 1989), which are able to degrade EPTC by the enzymes of aldehyde dehydrogenase, and P450 was responsible for the degradation of thiocarbamate. 2,4-D (2,4-dichlorophenoxyacetic acid) biodegradation is carried out via the plasmid pJP4 (entitled as *tfd* gene) of *Alcaligenes eutrophus* JMP134, 2,4-dichlorophenoxyacetate monooxygenase encoded by tfdA (Streber et al. 1987), 2,4-dichlorophenol hydroxylase encoded by *tfdB* (Kaphammer and Olsen 1990), and chlorocatechol-1,2-dioxygenase, chloromuconate cycloisomerase, chlorodienelactone isomerase, and chlorodienelactone hydrolase encoded by *tfdCDEF* (Kaphammer and Olsen 1990). Some transposons of *Ralstonia eutropha* (Tn4371), *Burkholderia cepacia* (Tn5530), *Alcaligenes* sp. (Tn5271), and

Pseudomonas putida (Tn4654) enable the degradation of biphenyl 4-chlorobi-phenyl molecules, 2,4-D, toluene, carbofuran, and 3-chlorobenzoate, respectively (Verma et al. 2014). Nagata et al. (1999) reported that *Sphingobium japonicum* UT26 has dechlorinase enzyme, LinA (γ-hexachlorocyclohexane dehydrochlorinase, EC 4.5.1), encoded by *linA* gene, which catalyzes a dehydrochlorination of two steps: γ-HCH to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) via γ-pentachlorocyclohexene (γ-PCCH).

8.4.2 Cyanobacteria as PA-Degrading Agents

Cyanobacteria are the largest group of gram-negative, oxygen-evolving photoautotrophic prokaryotes which belongs to the kingdom Eubacteria. The other well-known name of cyanobacteria is blue-green algae (BGA), named because of its diverse morphology (unicellular, filamentous, and colonial) and pigmentation (pigments like chlorophyll a, phycocyanin, allophycocyanin, phycoerythrin, carotenoids, and xanthophylls). They can easily accommodate in diverse ecosystems. Nowadays, cyanoremediation is a new term evolved to define the use of cyanobacteria to fulfil the purpose of degradation or detoxification of contaminants like PAs, heavy metal, and dye. There are frequent instances of successful bioremediation of PAs by cyanobacteria (Table 8.2).

8.4.2.1 Mechanism and Pathways of Remediation Process

Nostoc ellipsosporum and *Anabaena* sp. PCC7120 are able to degrade hexachlorocyclohexane to a combination of 1,2,3- and 1,2,4-trichlorobenzenes. According to some reports, cyanobacteria, namely, *Nostoc*, *Phormidium*, and *Oscillatoria*, can utilize methyl parathion by considering it as the solitary source of nitrate and organic phosphorus (Megharaj et al. 1994) for their growth and metabolism. In aerobic conditions, *Anabaena* sp. strain PCC 7120 is able to reduce the nitro group of methyl parathion to an amino group (Barton et al. 2004). One of the intermediate products of OP decomposition is para-nitrophenol which is more lethal than OP. Report suggests that cyanobacteria oxidize nitro group of para-nitrophenol and release nitrite. However, the biological mechanism of this process is still unknown. Nevertheless, further metabolism of released nitrite is carried out by "nir" operon which encodes nitrite reductase enzyme (Megharaj et al. 1994). *Phormidium valderianum* BDU 20041 is able to tolerate the exposure of chlorpyrifos by showing the enhancement activity of oxidoreductase enzymes for chlorpyrifos degradation (Palanisami et al. 2009). Thengodkar and Sivakami (2010) reported that *Spirulina platensis* is tolerant up to high concentration (80 ppm) of chlorpyrifos treatment by converting it to 3,5,6-trichloro-2-pyridinol by utilizing its alkaline phosphatase enzyme. Report suggests that *Nostoc muscorum*, *Spirulina platensis*, and *Anabaena oryzae* facilitate the degradation of malathion and high-concentration pesticides enhance the protein, carbohydrate, and biomass content in these cyanobacterial cells (Ibrahim et al. 2014). Forlani et al. (2008) reported that *Nostoc punctiforme*, *Anabaena* sp., and *Microcystis aeruginosa* have the potential of

Table 8.2 Biodegradation of persistent agrochemicals by cyanobacteria

Sl. No.	Pesticides	Cyanobacteria	References
1.	2,4-d (Dichlorophenoxyacetic acid)	*Anabaena fertilissima*, *Aulosira fertilissima*, *Westiellopsis prolifica*	Kumar et al. (2013)
2.	2,4-DNP (dinitrophenol)	*Anabaena variabilis*, *Anabaena cylindrica*	Hirooka et al. (2006)
3.	Anilofos	*Synechocystis* sp. PUPCCC 64	Singh et al. (2013)
4.	Acetochlor	Cyanobacterial mat consisting of *Phormidium* and *Oscillatoria*	El-Nahhal et al. (2013)
5.	Carbaryl	*Calothrix brevissima*	Habib et al. (2011)
6.	Carbendazim	*Oscillatoria* sp.	Ravindran et al. (2000)
7.	Carbofuran	*Anabaena sphaerica*, *Nostoc hatei*, *Westiellopsis prolifica*	Jha and Mishra (2005)
8.	Chlorpyrifos	*Phormidium valderianum*, *Spirulina platensis*, *Synechocystis* sp. PUPCCC64	Palanisami et al. (2009)
9.	Cypermethrin	*Oscillatoria* sp.	Thengodkar and Sivakami (2010)
10.	Endosulfan (α- and β-endosulfan)	*Anabaena* sp. PCC 7120, *Anabaena flosaquae*, *Aulosira fertilissima*	Singh et al. (2011a, b, c); Ravindran et al. (2000); Lee et al. (2003)
11.	Fenamiphos	*Nostoc muscorum*, *Anabaena* sp.	Cáceres et al. (2008)
12.	Glyphosate	*Spirulina platensis*, *Nostoc punctiforme*, *Microcystis aeruginosa*, *Leptolyngbya boryana*	Kumar et al. (2012); Cáceres et al. (2008); Forlani et al. (2008); Lipok et al. (2009)
13.	Isoproturon	*Anabaena inaequalis*	Arunakumara et al. (2013)
14.	Lindane	*Anabaena* sp. PCC7120, *Nostoc ellipsosporum*	González et al. (2012)
15.	Malathion	*Anabaena oryzae*, *Nostoc muscorum*, *Spirulina platensis*	Ibrahim et al. (2014)
		Anabaena sp. PCC7120	El-Bestawy et al. (2007)
16.	Methyl parathion	*Anabaena fertilissima*, *Aulosira fertilissima*, *Westiellopsis prolifica*, *Fischerella* sp., *Scytonema* sp. BHUS-5	Ibrahim et al. (2014); Tiwari et al. (2017)

glyphosate degradation and consume it as a prime source of phosphorus. *Trichodesmium erythraeum* has been reported to carry out the glyphosate transformation process for the utilization of phosphorus (Dyhrman et al. 2006).

8.4.2.2 Cyanobacterial Genes Involved in PA Degradation

The genetically manipulated cyanobacterial strains such as *Anabaena*, *Nostoc* sp. PCC7120 (Masukawa et al. 2007), *Anabaena variabilis* ATCC 29413 (Roessler et al. 2009), *Synechococcus elongatus* PCC 7942 (Kaczmarzyk and Fulda 2010), and *Synechococcus* sp. PCC 6301 (McNeely et al. 2010) have been tested for their bioremediating capacity. *Anabaena* sp. PCC 7120 and *Nostoc muscorum* FACHB244 were genetically modified by introducing a plasmid which contains opd (organophosphorus degradation) gene through conjugation gene transfer system. By the process of genetic engineering, fcABC was introduced into *Anabaena* sp. and *Nostoc ellipsosporum* for dechlorination of 4-chlorobenzoate. For the degradation of lindane, *linA* gene was introduced into *Anabaena* sp. and *Nostoc ellipsosporum* (Kuritz and Wolk 1995).

8.4.3 Fungus as PA-Degrading Agents

In the biogeochemical cycle, fungi play a significant role as they are responsible for degrading different kinds of xenobiotics including agrochemicals (Diez et al. 2012) (Table 8.3). Various fungal species are able to mineralize different groups of substances (Esterhuizen-Londt et al. 2016). Gianfreda and Rao (2004) reported that fungi are able to alter the structures of agrochemicals and other fractious compounds releasing biotransformed products. These biotransformed products are further broken down by other potential microbial strains.

Another strain, *Penicillium oxalicum*, showed 99.9% biodegradation of methamidophos within the incubation period of 12th day (Zhao et al. 2010). The phenylurea agrochemicals such as linuron, chlortoluron, isoproturon, and diuron were found to be degraded by *Mortierella* sp., *Bjerkandera adusta*, and *Rhizoctonia solani* (Khadrani et al. 1999). Several reports suggested that soil fungi such as *Penicillium* sp., *Eurotium* sp., and *Aspergillus* sp. have the capacity to degrade chlorpyrifos and its by-product TCP after 7 days of incubation (Maya et al. 2012). *Mucor racemosus* can degrade dieldrin (93%), DDE (79%), endosulfan sulfate (95%), heptachlor (94%), endosulfan (80%), heptachlor epoxide (67.5%), and DDT (49.3%) (Kataoka et al. 2010). Several white-rot fungal isolates including *Phanerochaete sordida*, *Trametes hirsutus*, and *Pleurotus ostreatus* have also revealed their potential to degrade diuron, lindane, and other fractious agrochemicals (Sagar and Singh 2011). Purnomo et al. (2014) suggested that *P. ostreatus*, a white-rot fungus, had the ability to eliminate around 89% of heptachlor and 32% of heptachlor epoxide after the incubation period of 28 days.

Current scenario of pesticide biodegradation is the utilization of fungal-bacterial co-culture because they frequently share the same niche (Warmink et al. 2009). Reports (Ellegaard-Jensen et al. 2014) suggested that the consortium of fungi (*Mortierella* sp. LEJ703 and LEJ702) and bacteria (*Arthrobacter globiformis*, *Sphingomonas* sp., and *Variovorax* sp.) has the ability of fast mineralization of the agrochemical diuron. Barathidasan et al. (2014) recorded a consortium of *Cellulomonas fimi* (bacteria) and *Phanerochaete chrysosporium* (fungi) able to

Table 8.3 Biodegradation of persistent agrochemicals by fungi

Sl. No.	Pesticides	Fungi	References
1.	Aldrin	*Phlebia acanthocystis*, *Phlebia brevispora*, *Phlebia aurea*, *Mucor racemosus*	Bhosle and Nasreen (2013); León-Santiesteban and Rodríguez-Vázquez (2017)
2.	Atrazine	*P. ostreatus* INCQS 40310, *Rhizopus stolonifer*, *Penicillium purpurogenum*	Pereira et al. (2013); Gonçalves et al. (2012)
3.	Bensulfuron-methyl	*Penicillium pinophilum*	Peng et al. (2012)
4.	Chlordane	*Boletus edulis*	Bhandari (2017)
5.	Chlorothalonil	*Pleurotus* ECS-0190	Camacho-Morales and Sanchez (2016)
6.	Chlorfenvinphos	*Trichoderma harzianum*	Oliveira et al. (2015)
7.	Chlorpyrifos	*Verticillium* sp.	Yu et al. (2006)
		Aspergillus sp., *Penicillium* sp., *Eurotium* sp., *Emericella* sp.	Maya et al. (2012)
		Cladosporium cladosporioides	Chen et al. (2012)
		Ganoderma sp.	Silambarasan and Abraham (2014)
		Acremonium sp. strain GFRC-1	Kulshrestha and Kumari (2011)
		Streptomyces sp. M7	Fuentes et al. (2013)
		Verticillium sp.	Fang et al. (2008)
8.	Cypermethrin	*Pseudomonas aeruginosa*	Bhosle and Nasreen (2013)
9.	β-Cypermethrin	*Aspergillus niger* YAT	Deng et al. (2015)
10.	DDT (dichlorodiphenyltrichloroethane)	*Laccaria bicolor*, *Boletus edulis*, *L. scabrum*, *Gymnopilus viscidus*, *P. ostreatus*, *G. trabeum*, *Daedalea dickinsii*, *Fomitopsis pinicola*, *Gomphidius viscidus*	Purnomo et al. (2010); Purnomo et al. (2011); Bhandari (2017)
11.	Dimethoate	*Phlebia acanthocystis*, *P. brevispora*, *Phlebia aurea*	Xiao et al. (2011)
12.	Diuron	*Phanerochaete chrysosporium*, *Cunninghamella elegans*, *Mortirella*	Fratila-Apachitei et al. (1999); Tixier et al. (2000, 2001); Badawi

(continued)

Table 8.3 (continued)

Sl. No.	Pesticides	Fungi	References
		isabellina, *Beauveria bassiana*, *Aspergillus niger*, *Mortierella isabellina*, *Mortierella* sp., *Aspergillus fumigatus*	et al. (2009); Oliveira et al. (2015)
13.	Endosulfan (α- and β-endosulfan)	*Aspergillus terricola*, *Aspergillus terreus*, *Trametes hirsute*, *Aspergillus niger*, *Aspergillus niger* ARIFCC 1053, *Mortierella* sp. Cm1–45, *Mortierella* sp. W8, *Aspergillus sydoni*, *Gloeophyllum trabeum*	Hussain et al. (2007); Kamei et al. (2011); Bhalerao and Puranik (2007); Bhalerao (2012); Kataoka et al. (2010); Goswami et al. (2009); Spina et al. (2018)
14.	Endrin	*Leccinum scabrum*	Bhandari (2017)
15.	Heptachlor	*Pleurotus ostreatus*	Purnomo et al. (2014)
		Phlebia acanthocystis, *Phlebia tremellosa*, *Phlebia brevispora*	Xiao et al. (2011)
16.	Isoproturon	*Mortierella* sp., *Mucor* sp., *Alternaria* sp., *Phoma eupyrena*, *Basidiomycete* Gr177, *Cunninghamella elegans*, *Penicillium melanoconidium*	Rønhede et al. (2005); Oliveira et al. (2015)
17.	Lindane	*Fusarium solani*, *Fusarium poae*, *Fusarium verticillioides*, *Irpex lacteus*, *Phanerochaete chrysosporium*, *Phanerochaete sordida*, *Phlebia radiata*, *Stereum hirsutum*, *Gloeophyllum trabeum*	Dritsa et al. (2009); Sagar and Singh (2011); Guillen-Jimenez et al. (2012); Quintero et al. (2008); Spina et al. (2018)
18.	Malathion	*Fusarium oxysporum* JASA1	Peter et al. (2015)
19.	Methamidophos	*Penicillium oxalicum*	Zhao et al. (2010)
20.	Monocrotophos	*Aspergillus flavus*, *Fusarium pallidoroseum*, *Macrophomina* sp.	Jain et al. (2014)
21.	Parathion	*Bjerkandera adusta* 8258, *P. ostreatus* 7989, *Phanerochaete chrysosporium* 3641	Jauregui et al. (2003)

(continued)

Table 8.3 (continued)

Sl. No.	Pesticides	Fungi	References
22.	Pendimethalin	*Aspergillus terreus*, *A. versicolor*	Caihong et al. (2011)
		Lentinula edodes	Pinto et al. (2016)
23.	Pentachlorophenol (PCP)	*T. harzianum* CBMAI 1677	Vacondio et al. (2015)
		Anthracophyllum discolor	Rubilar et al. (2007)
24.	Pyrene	*Pseudotrametes gibbosa*	Wen et al. (2011)
25.	Vydate	*Trichoderma viride*	Helal and Abo-El-Seoud (2015)

mineralize chlorpyrifos completely within 16 h. Abraham and Silambarasan (2014) reported a co-culture of bacterial strains such as *Enterobacter cloacae* JAS7 and *Klebsiella pneumoniae* JAS8 and fungal strains such as *Lasiodiplodia* sp. JAS12, *Aspergillus tamarii* JAS9, and *Botryosphaeria laricina* JAS6 which had the ability to degrade endosulfan completely in both aqueous and solid media.

8.4.3.1 Mechanism and Pathways of Remediation Process

The fungal strains follow various pathways during the degradation process. For example, during the biodegradation process of isoproturon (IPU) by *Mortierella* sp. Gr4, it was found that IPU undergoes two successive demethylation activities on urea chain and results in generating monodemethyl isoproturon and didemethyl isoproturon and then hydroxylation of isopropyl ring takes place which leads to the formation of 1-OH-IPU, 1-OH-monodemethyl isoproturon, and 1-OH-didemethyl isoproturon (Hussain et al. 2007). Another agrochemical β-cypermethrin, a pyrethroid insecticide, was esterified into two intermediates, i.e., permethric acid and α-cyano-3-phenoxy benzyl alcohol by *Aspergillus niger* (Deng et al. 2015). Kadimaliev et al. (2011) observed phenol degradation by *Lentinus tigrinus* in liquid medium via peroxidase and laccase enzymes.

León-Santiesteban and Rodríguez-Vázquez (2017) found that *Rhizopus oryzae* CDBB-H-1877 has the efficiency of pentachlorophenol biosorption. However, it has been notified that this agrochemical can be degraded through the process of methylation along with dechlorination. Two dark septate endophytes (DSEs) as *Alternaria alternata* and *Cochliobolus* sp. are able to degrade glyphosate, cypermethrin, and carbendazim by their intracellular enzymes (Spagnoletti and Chiocchio 2020).

8.4.3.2 Fungal Genes/Enzymes Involved in PA Degradation

Recently, few studies have revealed different fungal enzymes involved in the biodegradation of different types of agrochemicals (Jain et al. 2014). *Conidiobolus*

sp. (a fungal strain) was found to be capable of removing lindane from liquid medium by using its extracellular oxidative enzymes (lignin peroxidase and lignin-modifying enzymes). Similar enzymatic activity was observed during the degradation process of dieldrin, trifluralin, and simazine by *Trametes versicolor* and *Phanerochaete chrysosporium*, and the production of extracellular enzymes such as dehydrogenase/cellulase was enhanced in inoculated soil (Fragoeiro and Magan 2008). Likewise, Jain et al. (2014) documented the degradation of monocrotophos by three fungal isolates *Macrophomina* sp., *Fusarium pallidoroseum*, and *Aspergillus flavus* that were coupled with the release of extracellular enzymes like alkaline phosphatase, ammonia, and inorganic phosphates.

Nguyen et al. (2014) tested the efficiency of crude form of laccase extracted from *Trametes versicolor* to degrade the various agrochemicals such as fenoprop, ametryn, and atrazine. The genotype and growth conditions permit certain fungi to release specific enzymes such as manganese-dependent peroxidase (MnP) and lignin peroxidase (LiP) that play a significant role in pesticide degradation (Purnomo et al. 2010). Nowadays, the genetically transformed fungal strains are playing a vital role as they enhance the efficiency of pesticide degradation (Zhou et al. 2007; He et al. 2014).

8.5 Factors Affecting Biodegradation of PA

Bioremediation of PAs is affected by numerous chemical, physical, and environmental factors like chemical structure and concentration of PAs, soil moisture, soil pH, temperature, salinity, sustainable microbial population, aeration, and medium composition.

8.5.1 Chemical Structure and Concentration of PA

Chemical structure of PAs is a crucial factor in the biodegradation of PAs. The physiochemical properties of agrochemicals are varying from compound to compound. It was revealed that the polar group such as NH_2 and OH, of an agrochemical, is an easier site of attack by the microbial system (Cork and Krueger 1991). However, the presence of any substituent of alkyl or halogen in a pesticide makes it resistant to degradation. It was stated that minor differences in the structure and nature of substituent groups of same class can affect the rate of degradation. The amount of agrochemical significantly affects the biodegradation of agrochemicals. Reports suggested that some microbes can be able to degrade PA rapidly at high concentrations, whereas some can carry out degradation at low concentrations.

8.5.2 pH

pH is always an important factor in the environment and affects degradation of PAs by fungi and other microbes (Fang et al. 2008). It also affects the bioavailability, chemical speciation, and mobility of the chemical compounds. Racke et al. (1997) stated that biodegradation of an agrochemical depends on the soil pH. Any variation in pH from the optimum value adversely affects the biodegradation capacity of specific fungi. pH range of 4.0–8.0 showed good degradation rate for dieldrin by *Mucor racemosus*, a potential fungal strain (Kataoka et al. 2010). Yang et al. (2011) found that pH 7.5 was the optimum pH for the highest degradation rate of carbofuran by *Pichia anomala*. An optimal pH for the highest degradation rate of chlorpyrifos by a consortium of *Serratia* sp. and *Trichosporon* sp. was found to be 8 (Xu et al. 2007). However, several investigations also suggest that somewhat acidic pH is comparatively more desirable to carry out optimal fungal degradation of agrochemicals (Hussain et al. 2007). Caihong et al. (2011) observed that the maximum biodegradation of an agrochemical pendimethalin (belongs to dinitroaniline class) by *Aspergillus versicolor* was achieved at pH 6.5.

8.5.3 Temperature

Besides pH, temperature also has significant effects on the pesticide degradation. The optimum temperature is not fixed; it can be variable in certain conditions. For example, reports found that the optimal chlorpyrifos degradation by a bacterial strain, *Verticillium* sp., was attained at 35 °C (Fang et al. 2008). Derbalah and Belal (2008) reported the optimal degradation of cymoxanil by microbes to be 30 °C. A reliable temperature of 30 °C was reported in the degradation process of various pesticides—endosulfan, carbofuran, and pendimethalin—by the isolates of *Aspergillus terreus*, *Pichia anomala*, and *Aspergillus versicolor*, respectively (Hussain et al. 2007; Yang et al. 2011). Reports of Dritsa et al. (2009) suggested the optimal temperature to degrade lindane by *Ganoderma australe* to be 18 °C.

8.5.4 Moisture and Water Availability

Moisture is a considerable factor that affects the biodegradation rate by facilitating water as the medium for mobility and diffusion of agrochemicals as well as essential for making agrochemicals available for microbes. For a blooming degradation process, the moisture content of soil should be in a range of 25–85% of the water-holding capacity. However, the optimum range varies between 50 and 80%. Water availability has an impact on oxygen supply that later impacts the growth of fungus and production of enzymes (Philippoussis et al. 2001). It also impacts the agrochemical binding patterns and its dispersion in soil by affecting the accessibility of compounds to the soil microbiota. Bastos and Magan (2009) investigated the biodegradation of atrazine in a period of 24 weeks by *Trametes versicolor* and

indicated that 98% and 85% degradation took place at water potential of −0.7 MPa and −2.8 MPa, respectively.

8.5.5 Salinity

There is less information on the effect of salinity on the rate of biodegradation process. But salinity is a hurdle in varied regions like coastal, arid, and semiarid. Thus, it may influence the degradation rate of PAs. In nonsaline soil condition, the rate of parathion degradation is much faster than saline condition. The stability of agrochemicals is affected by the degree of salinity. For example, high salinity may cause an obstacle for biodegradation of agrochemicals as it inhibits degradation process.

8.5.6 Nutrients

The optimal biodegradation takes place by the occurrence of high nitrogen content along with 1% of glucose. Zhao et al. (2010) reported that only 1% of glucose is needed for the biodegradation of methamidophos by *Penicillium oxalicum* ZHJ6. Likewise, Kataoka et al. (2010) found *Mucor racemosus* to have better efficiency in degrading dieldrin in the presence of nitrogen and glucose. Dritsa et al. (2009) reported the media composition of 1.28 g/L of nitrogen to be the optimal condition for lindane biodegradation by the fungus *Ganoderma australe*. Hussain et al. (2007) reported that the rate of endosulfan degradation by fungi *Aspergillus terreus*, *Aspergillus terricola*, and *Chaetosartorya stromatoides* is considerably higher in agitation incubation than in static incubation. Similarly, Xu et al. (2007) suggested that the addition of sucrose with a little higher concentration was able to enhance chlorpyrifos mineralization by a consortium of *Trichosporon* sp. and *Serratia* sp. Likewise, Caihong et al. (2011) reported that *Aspergillus versicolor* showed enhancement in the rate of pendimethalin degradation by adding 1–2% of sucrose. In case of soil, oxyfluorfen degradation by fungus is affected by both temperature and mineral fertilizers.

8.6 Advantages and Limitations of Bioremediation

For a successful bioremediation process, microbes with specific quantity and correct timing under correct place and environment are required. The definition of a perfect microbe regarding this context is having the potential to degrade, detoxify, or remove contaminants including agrochemicals and other persistent pollutants. Thus, bioremediation is such a process that helps in keeping the environment clean by removing contaminants through biological aspects such as microorganisms and plants. In nature, every aspect has its own pros and cons; however, bioremediation technique offers numerous benefits with little limitations. Bioremediation can be

carried out in contamination sites, termed as in situ bioremediation. In this context, the substrates or nutrients are added to that particular contaminated site which stimulates the growth of indigenous microbes to enhance the degradation rate. This process is often less expensive as it minimizes the site disruption which leads to non-disturbance of soil and ensures soil fertility and integrity. Additionally, it helps to get rid of waste permanently and eradicates long-term liability. After the destruction of pollutants, the land is allowed to use. In some cases, the contaminated or polluted material is collected from the site and supplied with essential microbes or microbial consortium at an organized site, called as ex situ bioremediation. This process is found successful in wastewater management. The latter technique is more controlled than the former one. The bioremediation method can easily be coupled with other treatment (chemical and physical) methods. It has more public acceptance with proper regulatory encouragement. In the context of performance efficiency, there is no such kind of universal guidelines to define degradation efficiency as standard. Thus, there is always a variation in performance. The bioremediation process needs microorganisms along with a suitable environmental condition to keep them growing, which might not be always possible in in situ bioremediation. In case of certain uncontrolled remediation processes, the partial destruction might produce more poisonous and ambulant products than its native form. Uninterrupted observations are essential to check the status and know the speed of degradation of the persistent agrochemicals. In ex situ process, the organic compounds which have volatile property are challengeable to control. As the process is dependent on biological and physiological activity of microbes, its duration is slightly longer than that of chemical and physical processes. The genetically modified microbes are hard to take away from application sites and there is always a frightened possibility of causing more potential damage by these microbes than the original pollutants. Apart from all these limitations, bioremediation is considered as a significant tool in mitigating today's environmental issues.

8.7 Strategies to Enhance the Efficacy of PA Degradation

As contamination of environment is rising rapidly, to cope up this situation certain strategies are needed which enhance the efficacy of biodegradation. These processes are described below:

8.7.1 Immobilization

The immobilization concept may be defined as the act of restricting the movement of molecules/cellular organelles/enzymes/cells/microbes in a matrix. This concept is developed from the attachment nature of microbes onto a surface, thereafter growing on them (Robinson et al. 1986). In this method the accurate positioning of microbes takes place in such a manner that they are active in biodegradation (Mohamad et al. 2015). This process needs high biomass (mass culture) of microbes with proper

catalytic activity, simple separation, and reusability. This technique can be divided into four types, i.e., surface adsorption, embedding, covalent bonding, and cross-linking (Vasilieva et al. 2018). The surface adsorbent is the most affordable and simple process. The adsorbent materials/carriers play vital roles with reversible route as it convenes the prospect of sustaining the catalytic activity for a longer duration (Chen and Georgiou 2002). A suitable carrier should have the following criteria: (1) affordable price, (2) nonhazardous, (3) non-pollutant, (4) easy physical structure, and (5) lightweight. For passive immobilization, natural carrier and polyvinyl and polyurethane (synthetic carrier) are mostly used. In active immobilization the carriers are flocculent agents (chitosan), chemical attachment (glutaraldehyde), and gel entrapment (natural polysaccharides and synthetic polymers, i.e., acrylamide; proteins, i.e., collagen) (Taha and Khateb 2013). Reports suggest that the immobilization technique has been widely employed in the bioremediation of pesticides and wastewater treatment (Cassidy et al. 1996). Immobilized microalgae like *Chlorella* were used for the removal of lindane (Kuritz and Wolk 1995). The combination of algae and bacteria can be used for the enhancement of pollutant removal. Thus, immobilization techniques are considered as an admirable way for removal of pesticides.

8.7.2 Acclimation

This process is defined as the continuous association of a population of microbes to a particular chemical, leading to quick degradation of that chemical. In this association, microbes produce enzymes that can provide them tolerance or degrading capacity against that particular chemical (Guo et al. 2017). In stress conditions, organisms always tend to retain their internal mechanism by altering gene expression (Borowitzka 2018). Reports suggest that lindane concentration between 5 and 120 mg/L could be tolerated by *Staphylococcus intermedius* under this acclimation process with 99% lindane-degrading efficiency (González et al. 2012). The extended acclimation period is the major obstacle on the way of achieving a potential microbial strain.

8.7.3 Co-cultivation

Co-cultivation is a process where the existence of more than one microbial group is found. Cyanobacteria and bacteria (Patel et al. 2017), bacteria and microalgae co-cultures remediate organic pollutants more efficiently. Cyanobacterium offers growth substrates and oxygen along with suitable environmental condition to bacteria for promoting their growth (Subashchandrabose et al. 2013). The bacteria produce carbon dioxide which is used as a carbon source by cyanobacteria and microalgae (Kumar and Singh 2017; Kumari et al. 2016). In certain cases, *Bacillus pumilus*, for example, promotes the growth of *Chlorella vulgaris* in a medium without nitrogen and inhibits the growth of nannochloropsis species (Fulbright

et al. 2016). The research on co-cultivation concept should be more focused to be a promising bioremediation tool.

8.7.4 Genetic Modification and Enzyme Application

The genetic alteration of microbes is an innovative strategy that inserts certain target genes into the chromosome of host cell or erases a particular chromosomal fragment, which can undergo successive screening and acclimation activity to instantly express the preferred form and intensify the metabolism of the cell (Poliner et al. 2018). A wild fungal strain with hygromycin B phosphotransferase (hph) gene insertion showed improved quality to decadent pesticide. In fungal species, cytochrome P450 monooxygenases induce the gene clusters to express deferentially, based on the availability of nutrients and xenobiotic compounds (Yadav et al. 2015). The CCA gene cluster consisting of copied carbonic anhydrase and cyanase in *Fusarium oxysporum* has the efficiency to detoxify an agrochemical, cyanate (Elmore et al. 2015).

8.8 Conclusion

The hazardous chemicals need a promising tool for detoxification and remediation of its toxicity from our environment. The environmental consciousness resulted in improved regulatory measures to remediate environmental pollution and defend our ecosystem from upcoming pollution and exploitation. Because of this rational motive, it is essential to make strategies for the bioremediation of contaminated environment. Nowadays bioremediation is a most active, innovative, fascinating, and multidisciplinary area of research. In developing countries, the microbial remediation can enhance soil quality by detoxifying the hazardous chemicals from soil. However, more research programs are required to improve the potential of bioremediation and restore the soil quality by applying microbes. Economically, the use of PAs is beneficial as it improves the crop production and controls the diseases and pests, while in the environmental context PAs are considered as the most harmful factor for the environment. Thus, from both the environmental and economic standpoints, biodegradation is a fruitful technology upon PA application. Currently, the usefulness of indigenous as well as genetically modified organisms in removing or detoxifying persistent agrochemicals has emerged as a potential in situ remediation method. Numerous research reports have been collected and presented here on various organisms like bacteria, blue-green algae, and fungus, used for the bioremediation of environmental pollutants. However, the large-scale utilization of microbes for the degradation of PA pollutants is still to be explored.

Acknowledgement Authors sincerely thank the Director, ICAR-National Rice Research Institute, Cuttack, for providing technical and financial support. The authors declare that they have no competing interests.

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