Chapter 13
Microbial Remediation of Persistent Agro-chemicals by Soil Bacteria: An Overview

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13.1 Introduction

Agricultural innovations during 'Green Revolution' (GR) period of 1966–1985 had transformed agricultural practice and productivity. By virtue of 'Green Revolution' the world has witnessed a significant growth of crop productivity over the past five decades, which is primarily due to rapid increase in the use of fertilizer, pesticides, new crop strains as well as developments in irrigation and other technologies. This improved modern agriculture feeds around six billion people globally. Despite the success, rising world population and change in diets demands to increase food production by 70% (FAO 2009), which is also challenged by limited provisions for additional agricultural lands and protection of crops from various pests. During pre- and post-harvest period an average 35% of global yield is decreased due to diverse pests (Oerke 2006). In the wake of these new and old challenges, use of agrochemicals for sustainable production of food or feed on existing land is by far became the better choice.

13.2 Prevalence of Agro-chemicals in Agriculture and Environment

The beneficial outcome of pesticide usage provides an easy and economic way of maintaining and improving living standards of the people. Mostly in many developing countries, there has been rigorous practice of employing various agrochemicals as fertilizers and pesticides (Carvalho 2006). More specifically the production of

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various pesticides dramatically increased after the 1960s that resulted in doubling of the average yield of wheat, rice and maize per year. Per annum the worldwide market capital of chemical-pesticide is worth of USD 40 billion with total consumption 3 million tones (Popp 2011), of which 24% is consumed alone in the USA and 45% in Europe. Currently, the application of chemical pesticides covers 25% of the cultivated area. The estimated consumption of pesticides is about 5–7 kg/ha in UK, 13 kg/ha in China, 6.6 kg/ha in Korea and 12.0 kg/ha in Japan (Gurusubramanian et al. 2008). Moreover, the three common pesticides namely DDT, HCH and malathion shared 70% of the total pesticide consumption in developing countries.

Similarly the dependence of India on agro-chemicals has increased immensely since independence. This is evident from the fact that India had produced over 5000 mt of basic pesticides in the year 1958, mainly DDT and benzene hexachloride (BHC), whose productions were subsequently increased approximately to 85,000 mt in mid-1990s (Gupta 2004). Since then India has become the number one manufacturer of basic pesticides in Asia and fourth largest global manufacturer after USA, Japan and China. Approximately the demand for pesticides by the domestic consumers is accounted to be 50% which is expected to increase from 6.5% per annum in 2015 to 9% by 2020. Among these agrochemicals, 75% of India's total pesticide consumption is accounted by insecticides followed by 12% of fungicides and 10% of herbicides.

Various entomologists termed the vicious cycle of dependence on chemical pesticides as "*pesticide treadmill*" (Bosch 1989). The "pesticide treadmill" has two major aspects. The first one is the enhancement of the dosage and frequency of less effective pesticide which is due to the development of resistance in pests as well as destruction of the natural enemies of pests and the second one is the formulation and commercialization of new pesticide. Despite the popularity of various agrochemicals or pesticides, the harmful effects of pesticides during occupational exposure of farmers have raised worldwide concern. To that extent, the general population is also affected from the residues in food and drinking water (van der Werf 1996; Pimentel 2005).

13.3 Impact of Agro-chemicals

13.3.1 Direct and Indirect Effects on Humans and Environment

The worldwide human morbidity and mortality is the result of considerable acute human poisonings due to various pesticides. In developing countries, about 25 million farm workers are exposed to synthetic pesticides (Jeyaratnam 1990). The World Health Organization (WHO) indicated that at least in a year three million people are affected severely by pesticide poisoning. Even though developing countries use only 20% of the World's agrochemicals, 99% of 3 lakh deaths from pesticide

poisoning are from low and middle income countries (Gunnell and Eddleston 2003). Various incidences of accidental poisoning by pesticides have been reported in India since its production. In 1958, the first pesticide poisoning case was reported in Kerala state of India where over 100 people died after ingesting wheat flour contaminated with parathion pesticide, introduced by Bayer (Karunakaran 1958). Similar poisoning cases of consuming contaminated wheat with aldrin dust and gammexane in Madhya Pradesh of India, hexachlorocyclohexane (HCH) in Uttar Pradesh of India (1977) and deaths due to aluminum phosphide (1992) have been reported (Gupta 2004). Not to forget the painful tragedy of '*Bhopal gas leakage*' of methyl isocyanate pesticide. Most of these poisoning cases had developed symptoms like myoclonic jerks, generalized colonic convulsions, weakness in the extremities and grand mal seizures (Nag et al. 1977).

In addition, agricultural intensification and widespread use of pesticides have caused the extinction of many local wild plant and animal species and this has a profound effect on functioning the agro-ecosystems. Long after their use, these persistent and recalcitrant organic compounds such as heterocyclics, polynuclear aromatics, chlorinated aromatics and nitroaromatics, either remain in soils-sediments or percolate down to ground water. Thereby contaminating and dispersing to other land or aquatic ecosystem. So, indirect accumulation or biomagnifications has serious impact on the health of higher tropic level organisms and biodiversity of the ecosystem. Even when organic pollutants are present in minute quantities, their variety, toxicity and persistence capability have an adverse effect on the equilibrium of ecosystems to which welfare of humans, birds, fish and trees are associated (Gupta 1986).

The revised classification of pesticides based on their toxicity introduced by the World Health Organisation (WHO) in 2009 has five groups. The following groups (i) extremely hazardous (Ia), (ii) highly hazardous (Ib), (iii) moderately hazardous (II), (iv) slightly hazardous (III), (v) unlikely to present acute hazard (U) are categorized on the basis of LD_{50} for the rat (mg/kg body weight). Food and Agriculture Organization and WHO recommends of pesticides based on the toxicity Ia and Ib pesticides should be avoided in developing countries (PAN-UK 2003) and that of class II pesticides be avoided. However, aggressive marketing strategies of large chemical industries and ignorance of farmers allows the use of these toxic agrochemicals extensively.

It has been reported that organophosphorus based pesticides such as malathion, parathion, dimethoate, chloropyrophos and monochrotophos are the prime cause of deaths in certain developing countries. The mortality rate observed following poisoning by organophosphate (OP) pesticides varies between 4% and 30% (Yamashita et al. 1997). Organophosphate and lipophilic OPs compounds get easily absorbed by the mucosa linings of respiratory or gastrointestinal tracts and through the skin, respectively (Karalliedde 1999). Similarly, accumulation of non-degradable, chlorinated pesticides in various living systems for extended period induces a variety of toxic symptoms (Gupta and Salunkhe 1985). Scientific studies reported that even if persons are exposed to little amount of persistent

chemicals, the combined effect these chemicals manifests various clinical conditions like suppression of immune response and hypersensitivity to other chemical antigens, reduction in sperm count or male sterility and development of breast cancer (Carvalho 2006).

13.3.2 Persistent Organic Pollutants (POPs)

Persistent organic pollutants (POPs) are groups of semi-volatile, synthetic chemicals produced/released intentionally or non-intentionally that has harmful effects on humans and wildlife. These toxic chemicals are characterized as resistant to degradation as they may take decennia or centuries to degrade substantially. This enables them either to get transported to a long distance leading to global pollution or to enter into food cycles affecting mostly to humans. Being fat-loving chemicals, they particularly partitioned into solid organic tissues or matter avoiding the aqueous phases in the ecosystems or sediments. Thus, these chemicals eventually accumulated in fatty tissue without entering into an aqueous milieu of cells and the persistence in biota is more augmentation by slow metabolism of cells. Though, it is most difficult to establish a direct relationship between the illness and exposure to specific persistent organic pollutants, sharp effects after a high-level of exposure have been described for some of the organochloride and organophosphate pesticides. For example, pregnant women exposed to persistent pesticides may have no or minor disease manifestations, but their children acquired developmental disorders due to disruption in the endocrine system.

There are many thousands of POP chemicals that are grouped into series or families of chemicals. For instance, there are theoretically 209 different polychlorinated biphenyls, which is grouped under ogranochlorides, differing from each other by the number of chlorination and position of substitution. Most of the major POPs fall under families of chlorinated (and brominated) aromatics that includes polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs), polybrominated diphenyl ethers (PBDEs) and organochloride pesticides (eg. DDT and its metabolites, toxaphene, chlordane etc.). At Stockholm Convention, a global treaty was ratified under the United Nations Environment Programme (UNEP) that motivates to eliminate 12 designated POPs, called the "*dirty dozen*". Interestingly the "dirty dozen" comprise of 8 pesticides, two industrial chemicals and two unintended industrial by-products. Although these eight persistent toxic pesticides are banned by many developed countries but are still in use in many developing countries because of its production cheapness.

13.3.3 Fate of Agro-chemicals in Agricultural Soil

Long after their use, these agrochemicals stay behind in soil and sediments. Once the pesticide finds its way into the soil environment, many and varied of factors act upon it. They either enter into food chain or subjected to diverse natural degradation process. This can happen in several ways like photo-decomposition, volatilization, adsorption, leaching, and diverse microbial activity. Each pesticide reacts differently to each of these factors and often several of these processes are occurring simultaneously.

Photo-decomposition (light breakdown) and volatility are influenced by the climatic conditions where as soil texture and quality has larger impact on adsorption, leaching and microbial activity. Adsorption is the accumulation of a component of a mixture at an interface. The Leaching ability of a pesticide depends on (1) the entrance of the compound into solution, (2) adsorption pops to soil particles and (3) evapotranspiration. More likely the pesticides leach or follow the free water, when heavy rain or instant irrigation is occurred after the pesticide application. If the soil surface is dry, the pesticide will most likely move in with the water i.e. percolated to ground water. If there is a high evapo-transpiration ratio, there is more water being utilized by the plant, thus more pesticide is being moved into the plant via the roots. Broadly the fate of pesticides depends on three major process, i.e. transport, transfer and transformation. During transport process the POPs move away from its point of application into the environment and spread throughout surface-water system. The transfer process is more of controlling phenomena involving various factors in distribution in soil and environment. Finally the transformation process refers to the biological and chemical processes that converts the POPs into simpler compounds or completely degrade it. In general, the fates of pesticides in surface water system are affected by the structural, chemical and biological attributes of the pesticides.

13.4 Soil Microbes and Pesticide Degradation

Biologically catalytic curtailment of complex, noxious organic molecules such as pesticides or agrochemicals to its simpler, smaller and non-toxic (iso)forms coined as biodegradation. Soil microbes possess some inherent mechanisms to carry out the biodegradation process. So, to understand the process of biodegradation by the microbes needs a better understanding about the microbes (Alexander 1994). However, microbial degradation of the majority of recalcitrant compounds is restricted due to anionic species in the structure. The anions like Cl^{-}, SO_4^{2-} etc. are strongly bonded to the close-ring and prevents the common microbes from attacking the compound and this may be due to increased toxicity of anionic group (Julia et al. 2001). Apart from nutrient recycling (Van Der Heijden et al. 2008), plant growth promotion, biological nitrification and denitrification occurring in dynamic soil ecosystems, soil microbes collectively involved in extensive degradation of pesticides

or agrochemicals (xenobiotics) (Schneider et al. 2010; Pino and Penuela 2011; Zhao et al. 2009).

Bacteria exhibit diverse physiological metabolism of degrading many varieties of natural and synthetic organic compounds, including pesticides in soils. Reports suggest that consortia soil microbes can more efficiently degrade pesticides than single non-indigenous strains. Even if the degrading metabolic processes are longer, it's been more viable and suitable option for eliminating the xenobiotic compounds from soil (Finley et al. 2010). The continued exposure of contaminated environment makes the soil bacteria to evolve and develop a modified genetic system against various toxicants (Parsek et al. 1995). Bacterial degradation of pesticides is basically higher on the surface than subsurface soils which has been influenced by spatial variability and heterogeneity soil (Linn et al. 1993). In rhizosphere, the efficient degradation of these xenobiotic compounds results from co-metabolism which is further augmented by roots exudates generated due to gross microbial activity (Fang et al. 2001).

Pesticides have reduced bioavailability due to efficient absorption of pesticides in soil organic matter. Consequently pesticide absorption affects the soil microbial community structures, metabolisms and more specifically bacterial multiplication and growth (Johnsen et al. 2001). In situ removal of and detoxification of agrochemicals from the soil ecosystem by the soil microbes, more specifically by the bacteria, have exceptional significance (Wang et al. 2005). A large pool of soil bacterial isolates including *Bacillus* sp., *Pseudomonas* sp., *Arthobacter* sp., *Ralstonia* sp. (erstwhile in *Pseudomonas*), *Rhodococcus* sp., *Acetobacter* sp., *Alcaligenes* sp., *Flavobacterium* sp., *Burkholderia* sp., *Yersinia* sp., *Stenotrophomonas* sp., and *Weeksella virosa* (Padmanabhan et al. 2003) from different soils environments have been discovered with pesticide degrading ability.

13.4.1 Microbes Involved in Degradation of Persistent Agro-chemicals

As the name bioremediation suggests, it is the detoxification or reclamation of the POP contaminated environment by the biological system, more specifically with the indigenous or genetically modified bacteria. This inclusive potential of bacteria facilitates efficient and economical viable way of removing contaminants. Wide varieties of bacteria are involved in degradation of different persistent pesticides (Table 13.1).

In most instances aerobic remediation favours much faster than anaerobic decomposition. On the contrary, anaerobic conditions favour the degradation of DDT (dichloro-diphenyl-trichloro-ethane) ten times faster than aerobic conditions (Scott 2000). DDT and HCH (Hexachlorohexane) degrading potential of the species of *Micrococcus* and *Lactobacillus* (Azizi 2011) have been established. Different strains of ubiquitously available *Pseudomonas* genus are actively involved pesticide

Table 13.1 Soil bacteria involved in degradation of the most persistent agrochemicals/pesticides

Sl.	Pesticides group/ classes	Representatives	Degrading bacteria	References
High persistence agrochemicals				
1.	Organochlorates or chlorinated hydrocarbons	Alachlor	*Pseudomonas* sp. strain ADP, *Ancylobacter* sp. S15, *Agrobacterium* sp. CZBSA1	Katz et al. (2001) and Ewida (2014)
		Aldrin	*Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp	Sharma et al. (2016)
		DDT	*Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumonia*, *Bacillus* sp., *Pseudomonas putida*, *E. coli*, *Hydrogenomonas* sp.	Patil et al. (1970) and Sharma et al. (2016)
		Dieldrin	*Pseudomonas* sp.	Sharma et al. (2016)
		1,4- dichlorobenzene	*Pseudomonas* sp.	Spain and Nishino (1987)
		Endosulfan	*Pseudomonas* sp., *Bacillus* sp., *Flavobacterium* sp.	Karpouzas et al. (2005)
		Heptachlor	*Clostridium* sp.	Sethunathan (1973)
		Lindane (γ-HCCH, α-BHC, β-BHC)	*Bosea thiooxidans*, *Sphingomonas paucimobilis*	Karpouzas et al. (2005)
		Methoxychlor	*Enterobacter aerogenes*	Fogel et al. (1982)
		Pentachloronitrobenzene	*Cupriavidus* sp. strain BIS7	Teng et al. (2017)
		Pentachlorophenol (PCP)	*Arthrobacter* sp., *Flavobacterium* sp., *Sphingobium chlorophenolicum*	Sharma et al. (2016)

(continued)

Table 13.1 (continued)

Sl.	Pesticides group/ classes	Representatives	Degrading bacteria	References
2.	Organo-phosphate	Cadusafos	*Pseudomonas putida*, *Flavobacterium* sp.	Karpouzas et al. (2005)
		Chlorpyrifos	*Achromobacter xylosoxidans* (JCp4), *Ochrobactrum* sp. (FCp1)	Akbar and Sultan (2016)
		Diazinon	*Pseudomonas capaciea*	Tewari et al. (2012)
		Dimethoate	*Bacillus cereus*, *B. subtilis*, *B. safensis*	Ishag et al. (2016)
		Ethoprophos	*Sphingomonas paucimoblis*	Karpouzas (2005)
		Glyphosate, acephate	*Clostridium* sp., *Arthrobacter* sp	Tewari et al. (2012)
		Malathion	*Pseudomonas aeruginosa* AA112	Abo-Amer (2007)
		Monocrotophos	*Rhodococcus* sp.	Tewari et al. (2012)
		Tetrachlorvinphos	*Stenotrophomonas malthophilia, Proteus vulgaris, Vibrio metschinkouii, Serratia ficaria, Serratia* sp., *Yersinia enterocolitica*	Ortiz-Hernández and Sánchez-Salinas (2010)
3.	Carbamates	Aldicarb	*Arthrobacter* sp., *Rhodococcus* sp.	Behki and Khan (1994)
		Carbayl (1-naphthalenyl methyl carbamate)	*Pseudomonas* sp., *Achromobacter* sp., *Arthrobacter* sp., *Xanthomonas* sp. and *P. cepacia*	Chapalamadugu (1991) and Gunasekara et al. (2008)
		Carbofuran	*Achromobacter* sp., *Pseudomonas* sp., *Flavobacterium* sp.; Pseudomonas sp., *Flavobacterium* sp. *Achromobacterium*, *Sphingomonas* sp. Arthrobacter sp.,	Chaudhry and Ali (1988) and Sharma et al. (2016)

(continued)

Table 13.1 (continued)

Sl.	Pesticides group/ classes	Representatives	Degrading bacteria	References
4.	Thiocarbamates	Butylate (S-ethyldiiso butylthio carbamate)	*Rhodococcus* TE1	Behki and Khan (1991)
		Dietholate	*Pseudomanas putida*, *Azotobacter chroococcum* (NRC 18002), *B. cereus Rhodococcus erythropolis,*	Tam et al. (1988)
		EPTC (S-ethyl dipropyl carbamothioate)	*Rhodococcus* sp., *Arthrobacter* sp.,	Nagy et al. (1995) and Tam et al. (1987)
		Molinate (S-ethyl-N-hexahydro-1 H-azepine thiocarbamate)	*Achromobacter xylosoxidans* ssp. *denitrificans*, *Stenotrophomonas maltophilia*, *P. chlororaphis* IFO 3904, *P. nitroreducens* IAM 143, *Curtobacterium flaccumfaciens* var. *flaccumfaciens* LMG 3645	Barreiros et al. (2003)
5.	Organotins	Tributyltin (IV) TBT	*Desulfovibrio* sp., *Pseudomonas fluorescens*	Gajda and Jancso (2010)
		Tributyltin chloride (TBTCl)	*Pseudomonas aeruginosa*, *P. fluorescens*	Ebah et al. (2016)
		Triphenyltin acetate	*Nitrosomonas* sp., *Nirrosococcus* sp., *Nitrospira* sp., *Nitrosolobus* sp., *Nitrobacter* sp.	Kuthubutheen et al. (1989)
6.	Organosulfur	Rizolex	*Bradyrhizobium* sp.	Moawad et al. (2014)
		TMTD	*Pseudomonas aeruginosa*	Ray and Mondal (2017)
		Thiram (tetramethyl thiuram disulfide)	*Pseudomonas aeruginosa*	Shirkot and Gupta (1985)
		VitavaX (37.5% thiram)	*Rhizobium leguminosarum*	Moawad et al. (2014)

(continued)

Table 13.1 (continued)

Sl.	Pesticides group/ classes	Representatives	Degrading bacteria	References
7.	Dinitrophenols	Nitrobenzene	*Pseudomonas pseudoalcaligenes* JS45, *P.* putida HS12, *P.* mendocina KR-1, *P. pickettii* PKO1,*Comamonas* sp. JS765, *Arthrobacter* sp. NB1, *Serratia* sp. NB2, *Stenotrophomonas* sp. NB3, *Clostridium* sp. and *Comamonadaceae* sp.	Sharma et al. (2016)
		Pendimethalin	*P. aeruginosa*, *B. mycoides, B. cereus*	Sharef Ibrahim et al. (2013)
8.	Urea derivatives	Chlorsulfuron, rimsulfuro, imazosulfuron,	Aerobic cellulolytic bacteria	Gigliotti et al. (1998)
		Isouron	*Pseudomanas putida*	Ozaki et al. (1986)
		Sulfometuron, thifensulfuro, metsulfuron, bensulfuron-methyl	*Pseudomonas striata* Chester	Kaufman and Blake (1973)
9.	Triazine (s)	Atrazine	*Arthrobacter* sp., *Clavibacter* sp.	Sharma et al. (2016)
		Methylthio-s-triazines	*Rhodococcus* sp. strain FJ1117YT	Fujii et al. (2007)
10.	Trichloro-picolinic acid complexes or Pyridine family	Isonicotinic acid (4-pyridine carboxylic acid)	*Acinetobacter* sp. and *Bacillus brevis*	Sims and O'Loughlin (1989)
		Monohydroxy pyridines, 2,3-dihydroxy pyridine, amino- and methyl pyridines	*Micrococcus luteus, Brevibacterium* sp. and *Corynebacterium* sp.	Sims et al. (1986)
		Pyridine	*Paracoccus* sp.	Qiao and Wang (2010)
		2-pyridine carboxylic acid	*Bacillus* sp.	Sims and O'Loughlin (1989)
		2,6-pyridinedicarboxylic acid (2,6-dipicolinate)	*Bacillus* sp. and *Agrobacterium* sp.	Sims and O'Loughlin (1989)
		2-pyridone	*Arthrobacter crystallopoietes*	Sims and O'Loughlin (1989)
		Strobilurin	*Stenotrophomonas maltophilia*, *Bacillus amyloliquefaciens*, *B. flexus*, *Arthrobacter oxydans*	Clinton et al. (2011)

(continued)

Table 13.1 (continued)

Sl.	Pesticides group/ classes	Representatives	Degrading bacteria	References
11.	Naphthoquinones	Juglone	*Pseudomonas* sp.	Schmidt (1988)
		1,4-naphthoquinone	*Bacillus* sp.	Sethunathan (1989)
Moderate persistence agrochemicals				
1.	Imidazolinone	Carbendazim	*Pseudomonas* sp., *Brevibacillus borstelensis*	Arya et al. (2017)
		Iprodione	*Pseudomonas fluorescens*, *P. paucimobilis, Arthrobacter* sp. (strain C1), *Achromobacter* sp. (strain C2)	Mercadier et al. (1997) and Campos et al. (2015)
2.	(Nitro) guanidines	Thiamethoxam, clothianidin, dinotefuran	*Leifsonia* sp.	Sabourmoghaddm et al. (2015)
Less persistence agrochemicals				
1.	Neonicotinoids	Acetamiprid	*Ochrobactrum* sp. strain D-12	Wang et al. (2013)
		Imidacloprid	*Achromobacter* sp., *Pseudoxanthomonas* sp., *Sinorhizobium* sp., *Mesorhizobium* sp., *Microbacterium* sp.,	Sharma et al. (2016)
2.	Pyrethroids	Cyhalothrin	*Klebsiella* sp., *Pseudomonas oleovorans*	Thatheyus and Selvam (2013)
		Cypermethrin	*E. coli*, *S. aureus*, *P. aeruginosa, B. subtilis, Enterobacter asuburiae*, *Pseudomonas stutzeri*	Thatheyus and Selvam (2013)
		Fenvalerate	*Bacillus cereus* and *Pseudomonas viridiflava*	Thatheyus and Selvam (2013)

degradation. Such as, soil isolates of *Pseudomonas putida* degrade organophosphates, including fenamiphos, oxamyl and carbofuran carbamates (Chanika et al. 2011). Gigliotti et al. (1998) reported efficient endurance of soil bacteria under sulfonylureas pesticides in alkaline conditions. Even highly persistent ring substituents like pyridine and pyridine derivatives, including picloram, nitrapyrin, flouridone, 4-amino pyridine and triclopyridine are degraded aerobically by *Bacillus* sp. and *Pseudomonas* sp. in soil sediments (Sims and O'Loughlin 1989). Degradation of polychlorinated biphenyls (PCBs) by *Pseudomonas* sp. are guided by chromosomal genes and naphthalene, salicylate, toluene, xylene, parathion, and quinoline through plasmid-associated genes (Chapalamadugu and Chaudhry 1991). *Klebsiella pneu-*

moniae and *Pseudomonas* sp. excretes excellular hydrolytic enzymes and breaks down atrazine, a member of s-triazine herbicides (Baishya and Sarma 2015). Broad spectrum phenylurea herbicides, including N-methoxy-N-methyl and N,N-dimethyl-substituted phenylureas are degraded by *Arthrobacter globiformis* (Tixier et al. 2001; Cullington and Walker 1999).

The bacterial degradation of pesticides is fundamentally based on two processes i.e. growth and co-metabolism. Organic pollutants are consumed as the source of carbon and energy for bacterial growth and metabolism and thereby facilitating conversion or mineralization of organic pollutants (Fritsche and Hofrichter 2008). Degradation ability of the soil bacteria depends on various physio-chemical parameters such as surrounding temperature, environmental pH, water holding potential (Ψ) of soil, available nutrients, soil texture and porosity (Singh 2008). Chlorpyrifos, malathion, parathion, fonofos, diazinon, ethion, and gusathion are some of the persistent organophosphorous insecticides that serve as carbon sources for the growth of *Pseudomonas* sp., *Flavobacterium* sp. and *Arthrobacter* sp. either in pure or in mixed cultures (Digrak et al. 1995; Ghisalba et al. 1987).

Species of *Rhodococcus* isolated from soil degrade metamitron (Parekh et al. 1994) while chlorpyrifos is degraded by *Flavobacterium* sp. (ATCC 27551), *Pseudomonas diminuta* strain (Gm) and *P. putida* (Rani and Kumari 1994). *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and *Klebsiella oxytoca* are able to degrade phenylephrine in mixed consortia in cultivable land, enriched with PAHs and other metals (Kim et al. 2009). Few species of *Sphingomonas* (Ye et al. 1996; Van Herwijnen et al. 2003), *Burkholderia* (Kang et al. 2003), *Stenotrophomonas*, *Mycobacterium*, and *Rhodococcus* are also characterized as biodegrading microbes for various PAHs starting from bi-ring to penta-ring structures (Beate et al. 1993; Moody et al. 2004). Group bacteria able to degrade collectively and sequentially the carbamate pesticides such as carbofuran (a member of *N*-methylcarbamate class) are *Arthrobacter* sp., Achromobacterium sp., Flavobacterium sp., *Pseudomonas* sp. and *Sphingomonas* sp. (Porto et al. 2011).

In ideal environmental condition, the microorganisms degrade or modify persistent compounds by the help of a variety of enzymes. Extracellular enzymes predigest poorly transportable pesticides to facilitate transportation these compounds into the cell. Once inside of an organism, organic compounds are metabolized by internal enzyme systems. Maximal degradation of the pesticides are achieved with the interaction between pollutant and the enzymes (intracellular or extracellular) and sufficient biomass (Baishya and Sarma 2015).

13.4.2 Basic Mechanism of Degradation

The biodegradation of persistent organic compounds like synthetic agrochemicals is often complex, involving vast array of biochemical reactions. The organic compound ranges from simple carbon compounds to more complex structures which may be broken down by enzymes (both extracellular and intracellular) before

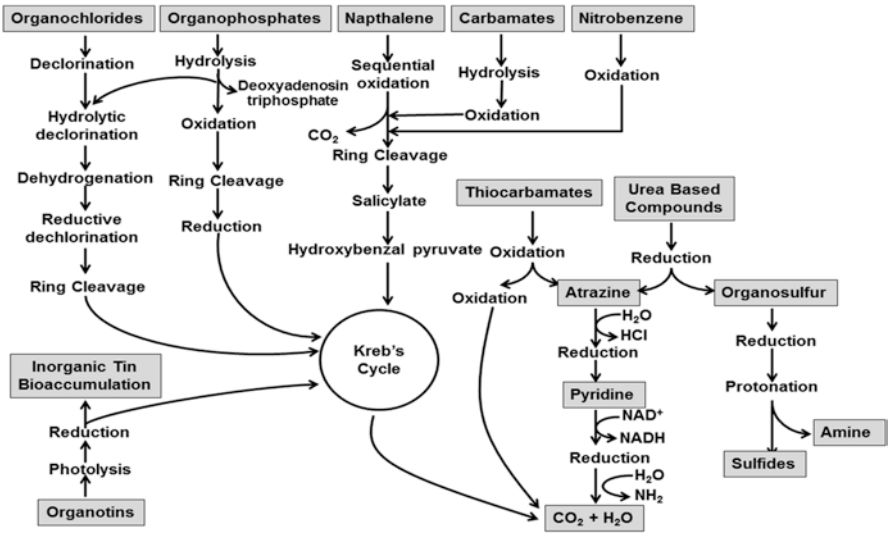

Fig. 13.1 Schematic representation of various biochemical pathways followed by microbes in remediation of complex and persistent agro-chemicals into simpler utilizable forms

cellular uptake and metabolism (Gadd 2010). Thus detoxification or degradation of pesticides is co-ordinated by various enzyme-controlled pathways (Fig. 13.1). According to Abraham, metabolic cooperation can be interpreted as interchanging of the organic molecules, substrates and products of the enzymatic reactions, within a microbial community (Abraham et al. 2002). Understanding the metabolic pathways involved in bacterial degradation of the agrochemicals (Somasundaram and Coats 1990) will widen our capability enhance the remediation process by modifying soil microbes.

Enzyme based pesticide biodegradation is a timely advanced treatment method for removal of pesticide pollutants from environment. Various classes of transferase, isomerase, hydrolase and other enzymes catalyze reactions, including hydrolysis, oxidation/reduction, addition of oxygen to a double bound, oxidation of an amino group (-NH_2) to a nitro group, addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2), replacement of a sulfur with oxygen, metabolism of side chains, ring cleavage etc. (Megharaj et al. 2011). For example, the degradation of carbofuran is carried out via single-step hydrolysis which is sufficient enough to inactivate carbamate group of insecticides. The initial step of dechlorination of organochlorides like DDT to DDD was carried out by *Pseudomonas* species under aerobic condition. Then it is further oxidized by enzyme dioxygenases to produce a dihydroxy derivative for meta-cleavage reaction, finally yielding a degraded product of 4-chlorobenzoic acid (Nadeau et al. 1994).

In aerobic condition *Alcaligenes* sp., *Arthrobacter* sp., *Flavobacterium* sp. and *Pseudomonas* sp. metabolize 2, 4-D (2,4-dichlorophenoxyacetic acid) through a ortho-cleavage pathway, converting ultimately into chloromaleylacetic acid with the

intermediates of 2,4-dichlorophenol and 3,5-dichlorocatechol (Gibson and Sulflita 1990). Likewise *Flavobacterium* sp. (ATCC 27551) isolated from paddy field (Sethunathan and Yoshida 1973) are able to fulfil the carbon requirement by metabolizing the organophosphate compounds, parathion, diazinon. Enzyme parathion hydrolase (elsewise phosphotriesterase) inactivates parathion along with diazinon and other organophosphates by a single catalysed reaction (Brown 1980). Some soil bacteria biodegrade atrazine through N-dealkylation of the side ethyl and isopropyl chains to de-ethylatrazine and de-isopropyl atrazine. Even if, the methylthioether and N-alkyl substituents on the s-triazine ring in herbicides deter the direct metabolism by bacteria (Wackett et al. 2002), but few researchers have reported that soil bacteria are capable of degrading atrazine partially or fully with end products as CO_2 and NH_3 (Rousseaux et al. 2003; Singh et al. 2004). *Pseudomonas sp.* ADP isolated from atrazine contaminated soil was able to biomineralize the triazinic ring completely (Mandelbaum et al. 1995).

In the case of pyridine carboxylic acids biodegradation, the ring is usually hydroxylated via nucleophilic attack of OH^- group. But in case of hydroxyl pyridines, a second hydroxyl is generally introduced by nucleophilic addition at electron deficient sites. However, the fission of pyridine and some alkyl pyridines can occur by a mechanism which apparently does not involve any hydroxylated intermediates. The suggested mechanism of initial reduction to form dihydropyridine and subsequent break down into saturated aliphatic intermediate compounds is observed in soil *Bacillus* sp. and *Pseudomonas* sp. (Sims and O'Loughlin 1989). Bacterial species like *Burkholderia* sp., *Ralstonia* sp. and *Rhodanobacter* sp. involvement in degradation of aromatic hydrocarbons (Bacosa et al. 2010), remediation of n-hexadecanoic acid by intracellular β-oxidation pathways (Yuan et al. 2013), has also been reported.

13.4.3 Microbial Genes Involved in Degradation

Soil microorganisms are highly adaptable to polluted environment and produce various enzymes for the mineralisation of persistent agrochemicals. The biochemical reactions of degrading persistent chemicals are carried out by variety of microbial enzymes. The productions of such enzymes are controlled by a special kind of genes which gives them the ability to consume these compounds as their growth substrates (Suenaga et al. 2001). Moreover, studies on the distribution of catabolic genes in different bacteria can significantly contribute to our understanding of how bacteria evolve new metabolic functions. The evolution of microbial genes guiding biodegradation of xenobiotic molecules is a powerful development in mitigating challenges of environmental pollution. Anti-catabolic plasmids with pesticide degrading genes and transposons encoding the pollutant-degrading enzymes (Laemmli et al. 2000) are present in soil bacteria *viz. Pseudomonas sp.*, *Alcaligenes sp.*, *Actinobacter sp*., *Cytophaga sp*., *Moraxella sp*. and *Klebsiella* sp. (Sayler et al. 1990).

In polluted or contaminated environments bacteria carry out genetic events, such as transformation, transduction or conjugation, which equip them in acquiring improved abilities to degrade contaminants (Stoodley et al. 2002). The atrazine degrading soil bacteria generally initiate degradation through dechlorination and hydrolysis. *Pseudomonas* sp. ADP was studied extensively to comprehend the response and interaction between the catabolic genes and stress response of atrazine. The carbamate degradation by soil microbes is carried out by enzymes atrazine chlorohydrolase, hydroxy-atrazine ethylamino-hydrolase and N-isopropyl-ammelide isopropyl-amino-hydrolase, encoded by *atzA, atzB (trzB) and atzC (trzC)* gene respectively (De Souza et al. 1996; Sadowski et al. 1998). These gene clusters convert atrazine successively to cyanuric acid and then mineralizing completely into CO_2 and NH_3 (Sene et al. 2010).

Plesiomonas sp. strain M6 carry gene *mpd* which encodes methyl parathion hydrolase (MPH) enzyme, therby hydrolyzing series of organophosphorus compounds. The encoded enzymes from *mcd* (methyl carbamate degradation) gene of these species have broad spectrum activity against carbamates. Similarly, the cluster of genes managing the degradation of EPTC by aldehyde dehydrogenase enzymes and thiocarbamate degradation by P-450 have been identified and cloned from different bacteria like *Rhodococcus* sp. NI86/21, *Achromobacter* sp. WMll (Tomasek and Karns 1989) and *Rhodococcus* sp. strain NI86/21 (Nagy et al. 1995). The degradation of 2,4-D (2,4-dichlorophenoxyacetic acid) by *Alcaligenes eutrophus* JMP134 is controlled by the plasmid pJP4. The genes involved in degradation of 2,4-D are designated as *tfd* that are arranged in three operons as *tfdA* encoding 2,4-dichlorophenoxyacetate monooxygenase (Streber et al. 1987), *tfdB* encoding 2,4-dichlorophenol hydroxylase (Kaphammer and Olsen 1990) and *tfdCDEF* encoding chlorocatechol-1,2-dioxygenase, chloromuconate cycloisomerase, chlorodiene lactone isomerise and chlorodienelactone hydrolase, respectively (Kaphammer et al. 1990). In addition to this *Alcaligenes* sp. (Tn5271), *Burkholderia cepacia* (Tn5530), *P. putida* (Tn4654) and *Ralstonia eutropha* (Tn4371) bears various transposons that facilitates the degradation of carbofuran, 2,4-DToluene, 3-chlorobenzoate and biphenyl 4-chlorobi-phenyl molecules, respectively (Verma et al. 2014). An enzyme, carbofuran hydrolase, capable of hydrolyzing several N-methyl carbamate insecticides, including carbaryl, carbofuran, and aldicarb was purified (Derbyshire et al. 1987), and the gene *mcd* responsible for expression these has been cloned (Tomasek and Karns 1989).

13.5 Strategies to Enhance the Efficiency of Pesticide Degradation

Pesticide degradation in soils provides an important type of information for predicting the end result of pesticides in the ecosystem. For enhanced biodegradation, direct application of bacterial decontaminants without proper knowledge on

application area, may result in minimal success. These may be due to several reasons such as overgrowth of autochthonic microflora (Slater et al. 1983) and sometimes because of the inoculated microorganisms have been partly degenerated by in vitro cultivation, shifting of metabolism for degradation. Not adapted to the conditions in the soil and need a quite long time for adaptation. To overcome such types of limitations few biotechnological tools and techniques are discussed.

13.5.1 Whole Cell Immobilization

Cell immobilization has been a favourable choice of biodegradation strategy for persistent pesticides due to the ease of maintaining catalytic activities for a long period of time (Richins et al. 2000; Martin et al. 2000; Chen and Georgiou 2002). The enhanced degradation through immobilized cells is primarily because of restriction of direct contact with inhibitory substances in the surroundings. The advantages of whole-cell immobilization technique over traditional biological methods of employing free cells directly for efficient biodegradation of agro-pollutants, are involvement of large number of cell, limited cell washout, smooth separation of cells from the reaction system, repeatability in cell use and better protection of cells from adverse environmental conditions. Moreover, immobilized cells are much more tolerant and resistant to fluctuations during the reaction and less susceptible to toxins, which makes immobilized cells, potentially suitable for the treatment of pesticides toxic substances (Ha et al. 2008). The degradation rates for repeated operations in batch cultures were successfully increased for successive batches, indicating that cells became efficiently adapted to the reaction conditions over the time period (Ha et al. 2009).

Based on methods of physical processes involved, the cell immobilization can be grouped into two categories; one as retention type (entrapment and inclusion membrane) and other involving chemical bond formation as in biofilms (Kennedy and Cabral 1983). In cell immobilization methods may use various inorganic and organic materials or substrates such as clays, silicates, glass and ceramics (inorganics) and cellulose, starch, dextran, agarose, alginate, chitin, collagen, keratin, etc. (organics) (Arroyo 1998). Mostly, the immobilized cells entrapped in polymeric gels were particularly successful against xeniobiotic comunds (Lusta et al. 1990). Furthermore, several reports suggested the use of variety of materials for immobilization of microorganisms such as plant fibres used as the supporting material for immobilizing bacterial consortium are more efficient in degrading persistent or recalcitrant pesticides. The use of materials such as petiolar felt sheath of *Aracaceae* sp. (palm tree) for entrapment method is an addendum to natural matrices for immobilization. The advantages accrued from likewise bioforms are repeatability in use, toxin free structures, support through mechanical strength and ample spaces for entrapped cell for growth thus eliminating cell rupture and diffusion. The *Luffa cylindrica* (the loofa sponge) have been used as natural support for immobilization of *Porphyridium cruentrum,* for chlorinated substances treatment (Iqbal and Edyvean 2004; Mazmanci and Unyayara 2005).

Removal of chlorpyrifos increased from 40% to 71% with the application of immobilized cells of *Streptomyces* sp., while 14% increased from 5% in case of pentachlorophenol. These in situ remediation results confirm the successful cleaning of mixtures of xenobiotics through free or immobilized cell cultures (Fuentes et al. 2013). Profenofos, an organophosphate pesticide efficiently degraded by *Pseudoxanthomonas suwonensis* strain HNM isolated from pesticide-contaminated soil. Common immobilization matrices are sodium alginates (SA), sodium alginate-polyvinyl alcohol (SA-PVA) and SA-bentonite clay (Talwar and Ninnekar 2015). Propachlor an organochlorine persistent herbicide degraded through the immobilized cells of *Pseudomonas* strain GCH1. The cells use ceramic support to be used as an immobilized cell system which reaches upto 98% with increased in cell viability (Martin et al. 2000). Immobilized cells of *Pseudomonas putida* on calcium alginate beads or on granular activated carbon have been used successfully in vitro to degrade phenols with concentrations ranging from 100 to 1200 ppm (Mordocco et al. 1999; El-Naas et al. 2009). Few *Rhodococcus* species P1 are also involved in phenol degradation via suspended cells of immobilization (Basha et al. 2010).

13.5.2 Enzyme Application

Microorganisms have elaborate enzymatic systems for the breakdown of xenobiotic chemicals. Biodegradation of various organic pollutants involves diverse enzymes such as phosphatase, esterase, hydrolase and oxygenase etc. Solubility of the enzymes influences their performance. The xenobiotics are commonly catalysed by extracellular enzymes, which are deliberately released by the cells into their nearby environment.

The utilisation of cell free enzymes extracted from the cells has been always an advantageous one. The cell-free enzymes have unique catalytic sites for the specific reaction and have the ability to bio-remediate recalcitrants or substances toxic to active bacterial cells. Extracellular enzymes from the class of oxidoreductases and hydrolases may exhibit degradation of complex structures to simpler forms or oxidizing products which can easily be absorbed by bacteria. Incomplete oxidation of PAHs by extra cellular oxidative enzymes gives rise to ionic products with increased polarity and solubility (Meulenberg et al. 1997). Various iso-forms of oxidoreductases and hydrolases, extracted from cells of soil *Pseudomonas* sp. and *Bacillus* sp. have significant role in the biotransformation of xenobiotic molecules (Tabatabai and Fu 1992). Modern molecular techniques are helpful in monitoring the health of environment, reducing various pollutants into nonhazardous forms and establishing protocols for disposal of contaminants from industries. Genetic modification of indigenous bacteria or engineering catalytic proteins facilitates removal of contaminants (Fig. 13.2).

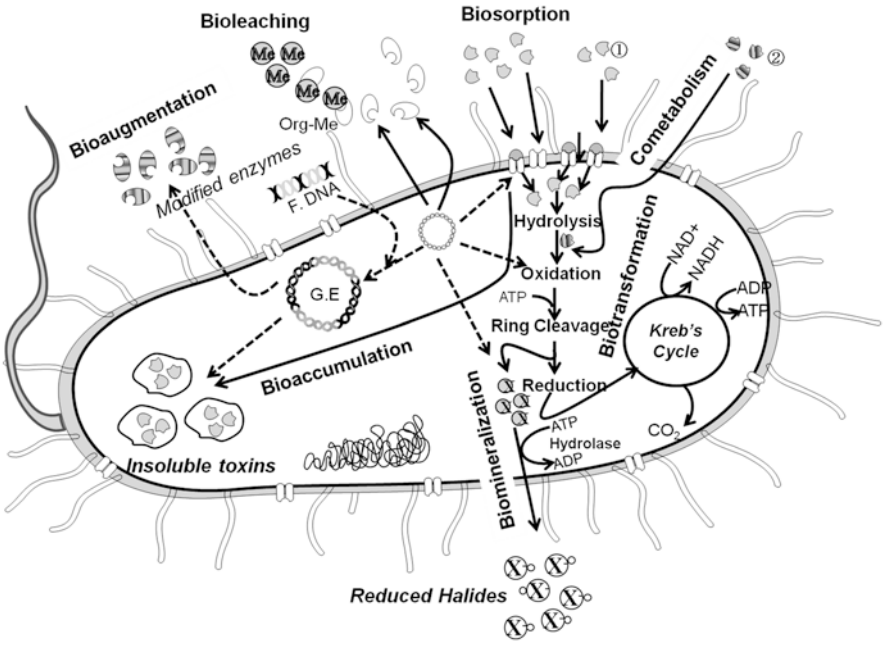

Fig. 13.2 Different processes exhibited by diverse soil bacteria in remediating persistent agrochemicals. (Insoluble Metal, Organo Metal, Geneticaly engineered plasmid, Pesticide 1, Pesticide 2, Non reduced Halides, Reduced Halides, Cell surface proteins, Modified enzymes, Foreign DNA)

13.5.3 Genetically Modified Organisms

Microbial biodegradation is an ecofriendly, nontoxic, economical method of removing pollutants (Amarger 2002). Due to developments in microbial genetics, construction of new strains with pollutant eradiating capabilities have been made possible and are grouped as Genetically Modified Microorganisms (GMMs). In comparison to indigenous or natural strains these genetically modified organisms are more efficient in bioremediating in-situ and can mineralize various organic pollutants (Cases and de Lorenzo 2005). The *E. Coli* bacteria are genetically modified for degrading organophosphate pesticides by successfully inserting genes for secretory organophosphorus hydrolase enzyme (Mulbry and Kearney 1991; Chen and Mulchandani 1998). Similarly, consortia of *E. coli* microbes with plasmids carrying 2,4-D degrading genes accelerates degradation of 2,4-D by native soil bacteria (Top et al. 1998).

The *P. fluorescens* HK44 with a combined gene of *lux* to naphthalene-degradative proteins were the first GMMs that are approved for field trials in the United States (Ripp et al. 2000; Strong et al. 2000). Even the *P. putida* KT2442 is genetically modified to carry three different properties; *E. coli* S17-1 (pTn*Mod*-OTc) provides tetracycline resistance gene, *Pseudomonas* sp. 142NF (pNF142) enables it to degrade naphthalene and *P. putida* KT2442 provides *gfp* gene acting as a bioreporter. In 2001, Lipthay et al. (2001) reported the degradation of 2,4-D by *Ralstonia eutropha* and *E. coli* HB101 carrying pRO103 plasmid which bear genes for 2,4-dichlorophenoxyacetic acid/2-oxoglutaric dioxygenase. Rodrigues et al. (2006) have successfully developed two GMMs strains, *Rhodococcus* sp. RHA1 (pRHD34: *fcb*) and *Burkholderia xenovorans* LB400, to degrade mixture of PCBs and Aroclor 1242 in soil. In some cases the GMMs can also be applied in promoting plant growth. Thus, these GMMs are enabled with multiple functions from degrading soil pollutants to promoting agriculture production.

13.6 Benefits and Limitations of Microbial Remediation

Microbial degradation is the major process involved in the disappearance or detoxification of pesticides. During *in situ* bioremediation, the nutrients or substrates added to the contaminated site or environment modify or stimulate the growth of indigenous or GMMs to increase the rate of degradation. Most of the cases *in situ* bioremediation being most cost effective as it minimizes disruption of remediation site. Non-disturbance of the soil will insure soil integrity and fertility. In addition, the *in situ* bioremediation has other advantages like contaminants are usually converted to innocuous products or destroyed i.e. not just simply transferred to different environmental site, nonintrusive thereby potentially allowing continued use of site and relatively easy to implement. The technology required to multiply microorganisms may be ideally suited for small business development. While in *ex situ* bioremediation, the polluted materials are collected from polluted sites and treated with requisite microorganisms (a consortium of microorganisms) at a designed place/site. This has been most successful in bio-remediating water. Most instances, it is more controlled compared to *in situ*, and the process can be improved with enrichment of microbes.

Bio-remediation, although considered advantageous in mitigating present day environmental pollution situations, has some limitations and can be considered problematic to use. As the bioremediation is carried out by microbes, the conditions must be ambient for microbial activities which make the process venerable to physiochemical changes. If the process is not controlled, there is possibility that the partially broken down contaminants may become more toxic and mobile than the original pollutants. Continuous site monitoring is needed to track the speed and status of biodegradation of the organic contaminants. In ex situ process, volatile organic compounds (VOCs) are difficult to control. Being dependent on biological activity the treatment time is generally longer than other less effective physical pro-

cess. Evaluating performance efficiency is difficult because there are no universal guidelines defining the level of a "clean" site and therefore there is variation in performance regulations. Even after certain time recombinant microbes are difficult to remove from the site of application and always there is apprehension about potential damage may caused by the modified organism than the pollutant itself.

13.7 Future Prospects

The bioremediation biochemistry of detoxifying agrochemicals or pesticides is more fascinating, multi disciplinary, active and challenging area of scientific research. The microbial remediation to improve the fertility of soil as well as removing the soil contaminants extensively used in developed countries. India is also progressing towards the application of microorganisms for the restoration of POP contaminated soil. Moreover, developing countries need extensive research programs to increase the capabilities of bioremediation. The present chapter has discussed and highlighted key bacteria that are involved in degrading key groups of pesticides or agrochemicals and underline the role of biotechnology in remediation process. However, much of the research has to be done to improve microbial inventories and biochemical pathways involved in degrading persistent agrochemicals. A key aspect, in bioremediation process is to understand and use of chiral compounds in these techniques. In addition, developments of sophisticated physico-chemical property based models and creation of database for physicochemical property will be highly beneficial. Explicit studies are needed to quantify the effects of POPs on humans and bigger animals of the food chain. Apart from this research on molecular modeling of biodegradation, transformation and mechanisms of toxicity have to hasten. The on-going microbial genomics studies will guide development of newer technologies for the remediation of contaminated water and soil.

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