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Soil Microorganisms and Their Potential in Pesticide Biodegradation: A Review

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PESTICIDES play an important role in preventing insect pests and weeds in crops. However, excessive use of pesticides has been known to be unsafe, due to their toxicity to non-target organisms and the ecosystem. Biodegradation is an innovative approach for decontaminating pesticide pollution. However, compared with the list of extensively used pesticides there are few well-characterized strains of microbes that transform pesticides into less-toxic or more labile products at environmentally useful rates. Fortunately, the technology required to isolate and characterize such microbial strains has improved immensely in the recent years. Furthermore, recent experimental developments have made practical the modification of potentially beneficial biodegradation genes so that they may be optimally expressed in a wide range of microbial species. This reviews article explore the recent studies that have focused on biodegradation of pesticide residues, the mechanism of microbial degradation of pesticides, the factors that affect the degradation of pesticides and the new application of microbial degradation of pesticides.

Key words: Biodegradation, Pesticides, Fungi, Bacteria, Immobilization, Molecular.

Introduction

Pesticides are chemical substances used to control, prevent or destroy pests including insects, nematodes, microorganisms and weeds (Anderson *et al.*, 2011 and Porto et al. 2011). The world human population continues to grow at about two percent each year which resulted in high demand for resources. The consumption of pesticides has advanced modern societies by increasing the amount of agricultural yield and production which reduce the cost of agricultural related materials. Obviously, the use of pesticide has turn out to be an essential part of modern society due to constant evils of pests, therefore pesticide usage is difficult to be stopped. Pollution of water, air and soil by pesticides has become a worldwide problem (Sultan & Kertesz, 2015 and Sukul & Spiteller, 2001). True accurate data about the effects of pesticides is still scanty (Chanika *et al.*, 2011). Several developed nations have already banned or restricted the use of most these toxic compounds but yet they are still manufactured and are available in the markets (Almeida-Gonzalez *et al.*, 2012). The consumption of pesticides in current farming system has become inevitable. While many local farmers are still not fully aware of the hazards that may arise as a result of using such toxic chemicals (Boada *et al.*, 2016). Several information are available on the existence of insecticide residues in soil and water bodies and the atmosphere (Banerjee & Mukherjee, 2017 and Singh & Singh, 2017). Studies are also available on the level of pesticide residues present in agricultural products (Barriada-Pereira *et al.*, 2005). Pesticide residue affects the atmosphere through its volatilization as a result of wind action. Pesticides residues further transport from the site used to other places or leach through the soil by means of percolation to contaminate ground water (Aamand and Smets, 2014). Pesticides used in agricultural fields directly or indirectly are in contact with non-target organisms. The persistence of pesticide in the environment is of major concern due to their imminent toxicity, carcinogenicity and possibility to bio accumulate in food chain and food web (Almeida-Gonzalez *et al.*, 2012). Detailed chemical composition and characteristics pesticides are presented in Table 1

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TABLE 1. General Characteristics of some classes of Pesticides (Adapted from Badii and Landeros, 2007; Biju and Krishnamurthy, 2017)

Pesticides	Characteristics	Main composition
Organochlorines	Soluble in lipids, they accumulate in fatty tissue of animals, are transferred through the food chain; toxic to a variety of animals, long-term persistent	Carbon atoms, chlorine, hydrogen and occasionally oxygen. They are nonpolar and lipophilic
Organophosphates	Soluble in organic solvents but also in water. They infiltrate reaching groundwater, less persistent that chlorinated hydrocarbons; some affect the central nervous system. They are absorbed by plants and then transferred to leaves and stems, which are the supply of leaf-eating insects or feed on wise	Possess central phosphorus atom in the molecule. In relation whit organochlorines, these compounds are more stable and less toxic in the environment. The organophosphate pesticides can be aliphatic, cyclic and heterocyclic.
Carbamate	Carbamate acid derivatives; kill a limited spectrum of insects, but are highly toxic to vertebrates. Relatively low persistence	Chemical structure based on a plant alkaloid Physostigmavenenosum
Pyrethroid	Affect the nervous system; are less persistent than other pesticides; are the safest in terms of their use, some are used as household insecticides.	Compounds similar to the synthetic pyrethrins (alkaloids obtained from petals of Chysanthemuncinerariefolium).
Biological	Only the Bacillus thuringiensis (Bt) and its subspecies are used with some frequency; are applied against forest pests and crops, particularly against butterflies. Also affect other caterpillars.	Viruses, microorganisms or their metabolic products

Classifications of pesticides

The World Health Organization anticipated to categorize the pesticides based on their toxicity ranking order from lowest to highest toxicity in numbers I through IV, being extremely toxic, highly toxic, moderately toxic and slightly toxic, respectively (WHO, 2010). Approximately five hundred different pesticide formulations are certified worldwide to handle various kind of pests in agriculture (Arias-Estévez *et al.*, 2008). The most synthetic pesticides consumed were categories into this four group's organophosphate, organochlorines, carbamates, and pyrethroids.

Fate and transportation of pesticides in the environment

Introduction of pesticide to the environment is mainly influenced by many processes and these processes explain their fate, persistence as well as their movement in the environment. It is very essential to know the fate as well as behavior of pesticides as it can aid in warranting effective and ecologically harmless application of pesticide in the environment (Menzie, 1972). Run-off from aquatic habitats may transport pesticide away from intended sites to non-target specie (Wong, 2006). Several factors influencing the fate of pesticides include, their adsorption, absorption, Run-off, microbial degradation, volatilization, photochemical decomposition uptake by plant and chemical degradation. Pesticides application to soil may disappear to the atmosphere, taken up by plants or consumed by living organisms in the soil; descend underground, degradation by solar energy ,*i.e.* photochemical degradation or breakdown through microbial action. The difference in pesticides chemical structures plays an important role in defining the movement of pesticides in the environment. Some pesticides are water soluble and can move with water. Some volatize easily meaning they can transform from liquid to gas making them move easily in the atmosphere. Some pesticides may be transformed to nontoxic to both their target species and the environment while others may degrade into more toxic than their parent compound. Pesticides classification is summarized in Table 2

TABLE 2. Pesticides classification according to their target species, chemical state and mode of action proposed by(adapted from Arias-Estevez and Fernandez-Calvino, 2013)

By target		By mode or time of action		By chemical structure
Type	**Target**	**Type**	**Action**	
Bactericide (sanitizers organic or disinfectants)	Bacteria	Contact	Kills by contact with pest	Pesticides can be either organic or inorganic. Most of today's chemicals are organic.
Defoliant	Crop foliage	Eradicant	Effective after infection by pathogen	
Desiccant	Crop foliage	Fumigants	Enters pest as a gas	
Fungicide	Fungi	Nonselective	Toxic to both crop and weed	
Herbicide	Weeds	Post-emergence	Effective when applied after crop or weed emergence	
Insecticide	Insects	Pre-emergence	Effective when applied after planting and before crop or weed emergence	
Miticide (acaricide)	Mites and ticks	Preplant	Effective when applied prior to planting	Commonly used inorganic pesticides include copper-based fungicides, lime-sulfur used to control fungi and mites, boric acid used for cockroach control, and ammonium sulfamate herbicides
Molluscicide	Slugs and snails	Protectants	Effective when applied before pathogen infects plant	
Nematicide	Nematodes	Selective	Toxic only to weed	
Plant growth regulator[3]	Crop growth processes	Soil sterilant	Toxic to all vegetation	
Rodenticide	Rodents	Stomach poison	Kills animal pests after ingestion	
Wood preservative	Wood-destroying organisms	Systemic	Transported through crop or pest following absorption	
				Organic insecticides can either be natural (usually extracted from plants or bacteria) or synthetic. Most pesticides used today are synthetic organic chemicals. They can be grouped into chemical families based on their structure

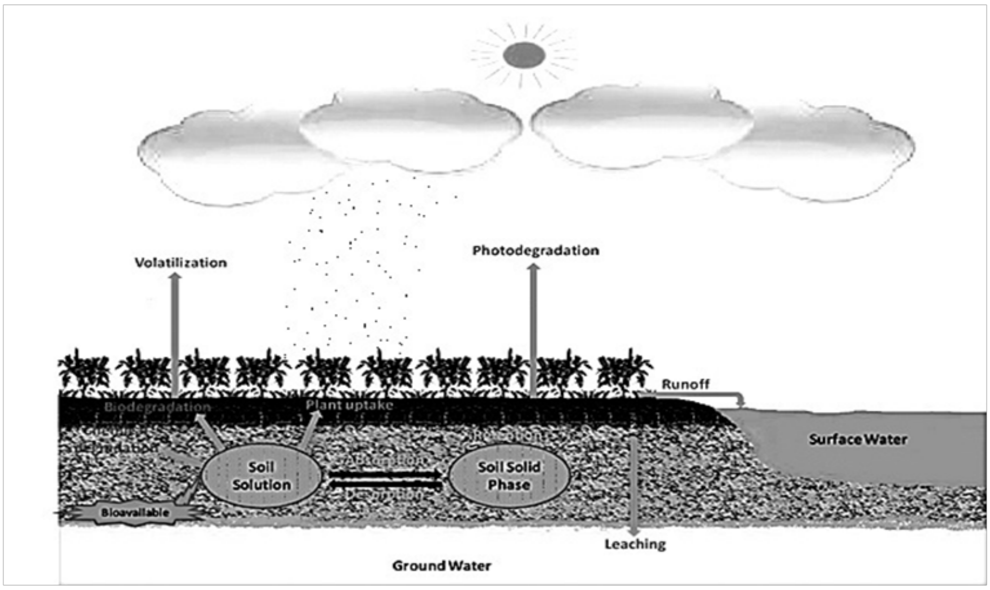

Fig.1. Fate and Transportation of Pesticides in the Environment (D. K. Singh, 2008)

Absorption

These deals with the ability of plants, animals, humans or microorganisms absorb or take-up chemicals (Cobb and Anderson, 2009). Pesticides absorption by a target and non-target organisms is usually affected by environmental circumstances as well as the physical and chemical properties of the pesticide and the soil.

Adsorption

This is reversible process of bonding of gas, liquid molecules or dissolved solids to a particular surface. Adsorption of pesticide in soil occurs through soil components (organic matter and clay particles). It has been established that a large portion of pesticide used in agricultural field is retained by soil segment as a result of adsorption (Blume and Ahlsdorf, 1993). This usual phenomenon thus affects the rest of the processes such as volatilization leaching and uptake by plants. Many factors may effect pesticides adsorption and desorption in soil such as soil texture, soil moisture and soil acidity. The adsorbed portion may differ from little quantity up to 90% of the pesticide used depending on the physicochemical properties of the soil as well as the pesticide (Ye, 2003). Usually, clay soils which contain higher content of organic matter are highly adsorptive than sandy soils, due to presence of supplementary particle surface area on which the insecticides are bind. Pesticide adsorbed by soil are unlikely to leach, volatilize, or undergo microbial degradation. Once a pesticides are bind by soil components, they would not be accessible for absorption by plants or animals (Forano and Besse-Hoggan, 2011).

Volatilization

Volatilization is the conversion of pesticides into gas from solid or liquid and plant surfaces. It is the movement of pesticide vapors through the air. Farm workers and bystanders may be at risk of pesticide exposure through inhalation of these vapors. Volatilization is considered differently than pesticide movement either by spray drift, erosion, or windblown particles. Volatilization of pesticide is influence by environmental conditions such as physicochemical properties of soil such as soil moisture, organic matter and soil texture.

Run-off

It is the horizontal movement of water over a sloping surface. These transports of insecticides may amalgamate in aqueous solution or bind to eroding soil. Depending on the climatic situations of soil such as rainfall, the pesticide and their derivatives in soil are transported to the other areas by runoff (Chikere and Okpokwasili, 2016). Environmental factors like soil texture, soil moisture and amount of rainfall and irrigation determine the level of pesticide runoff. The runoff of pesticides generally occurs when the pesticide application is followed by continuous rainfall. Also physical and chemical parameters of the pesticide such as adsorption to soil influence run-off or absorption by plant. Figure 1 explained the fate and transport of pesticides in the environment.

Leaching through soil profile

Leaching is the vertical movement through the soil which aid the transports of pesticides to groundwater under certain circumstance. Contrary to runoff, which is the movement of water on the soil surface, leaching is the movement downward through the soil profile(Sethunathan and Johri, 2002). Insecticide in the soil solution might leached downwardto the underground level over an extended period of time which result in delayed pollution of aquifers.The shallower the depth to ground water, the less soil there is to perform as a filter. When pesticides are exposed to soil layers with higher depths, more chance exists for the chemicals to dilute, degrade or sorb before going downward(Nejidat and Ronen, 2011). Neighboring surface water is more liable to contamination when pesticides are applied to highly erodible soils or to over-irrigated or rain-soaked fields. Studies examining the leaching of pesticide were conducted both in field and laboratory scale using packed soil column and the result from the studies revealed that pesticides leach out through the root zone and the leaching process is control by may processes like physical chemical and biological parameters of the pesticide and soil and volume of rainfall events after the use of the pesticide(Nousiainen et al., 2015 and Mingorance & Pena, 2014) (Figure 1).

Effect of pesticide on soil microbes and plants

The extensive use of pesticides causes their buildup in soils and directly or indirectly affects the functional activities of non-target soil micro-biota. Microorganisms and plants species are among the most essential biological agents that help in the degradation toxic constituents or allow their recycling in the environment. Soil micro flora, essentially fungi, protozoa and bacteria significantly contribute in making the soil fertile through their primary catabolic role in the degradation of plants and animal residues in the cycling of the organic, inorganic nutrients content of soil (Moorman and Koskinen, 2007). Pesticide that disrupt the activities of the soil microorganisms could be expected to affect the nutritional quality of soils and would therefore, have serious ecological consequences. Pesticide applied to soils transformed by biological and non-biological processes into different transformation products. These transformations happen through different mechanisms physical, chemical and biological agents in which microbes play an important role. The transformation mechanism includes oxidation hydrolysis reduction conjugation which are catalyzed by various types of enzymes resulting in less bio active products (Brogan and Relyea, 2017).

Effect of pesticides on humans

The extensive use of pesticides has led to serious health effects to humans and the ecosystem. Most acute pesticides poisonings to humans are caused by organophosphate. Pesticides sprayers, loaders and agricultural farm workers are at high risk of pesticides exposure. Many previous literatures shows that pollution as a result of pesticides application may be linked to several illnesses such as neurological problems, immune system disorders, human cancers, mental as well as reproductive effects in mammals. Children may be at high risk as a result of pesticides exposure through breast milk and food (Wohlfahrt-Veje *et al.*, 2011). Consumption of pesticide polluted crops causes life-threatening illnesses in humans and animals.

Effect of pesticides on aquatic environment

Exposure to pesticides result in possible severe health problems. Approximately 1 million deaths and prolonged sicknesses are reported each year as a result of pesticide poisoning globally. Mortality and loss of zooplankton due to pesticide exposure is an example of direct consequence of pesticides on marine and sea animals. Surface movement of pesticides into waterways and rivers is extremely dangerous to aquatic life, sometimes it may kill all the fish in a particular river. The pesticides used are mostly organic molecules with hydrophobic components triggering prompt sorption of the pesticides residue to the soil constituent and then washed into the aquatic environments in dissolved forms (de Moreno and Moreno, 2004) Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata 7600, Argentina. kmilglior@mdp.edu.ar</auth-address><titles><title>Organochlorine pesticides sequestered in the aquatic macrophyte Schoenoplectus californicus (C.A. Meyer. Sorbed pesticide residues have a tendency to be less degradable compared with dissolved ones subsequently they are less available to the degrading action of microbes, ultraviolet light and photochemical degradation (Qu and Yang, 2009). Hence widespread consumption of pesticides would lead to accumulation of these compounds in sediments of stream and river.

Persistence of pesticides in the environment

The perseverance of pesticides is generally determine using half-life, which is, the time taking for half of the original concentration used to breakdown (Fantke and Trapp, 2015). Generally, the longer the half-life, the more the likely is for pesticide transport, a pesticide will breakdown to 50% of the original volume after a single half-life and 25% will remain after two half-lives and nearly 12% will remain after three half-lives. This continues until the volume left over is nearly zero (Jacobsen *et al.*, 2015). Pesticide persistence is among the most essential aspects in determining the fate of pesticide as well as their effects in the environment. The persistence of pesticides may be classify as persistent, non-persistence, moderately persistent, and permanently persistent (Walker and Wright, 2002). Organohlorines pesticides used in agriculture such as Aldrin, Dieldrin and DDT are highly persistent (Mansouri *et al.*, 2017).

Methods of pesticide remediation

Considerable amounts of pesticides are applied to agricultural field globally. Some pesticides degrade quickly within few days whereas, others are complex and may take several years to degrade due to their persistence. Complete fate and pathways of several insecticides degradation in the environment are not fully understood. Several insecticides do not reach their targets rather contaminate plants, animals and soils. Remediation of polluted areas usually takes a very long time depending on nature of pollutant. The soil remediation technology is currently evolving as novel scientific area for multi-disciplinary researches. The remediation approaches for the management of pesticide polluted areas includes;

Low temperature thermal desorption

This is an ex situ remediation approach, often applied to remediate areas polluted with pesticides. Low temperature thermal desorption usually treat volatile and semi-volatile compounds, such as pesticides the method remediate pesticides from sediments and sludge. The pollutant is usually heated at about 300 to 1000 °F, which causes volatilization, instead of removal of the compounds. While subsequent organics pollutants in the polluted gas stream are depolluted by moving in the condenser or seized by carbon adsorption beds. The condenser transforms the gas into aqueous form for advance treatment whereas the carbon adsorption beds immobilize, but never rescind the pollutants. The method needs advance services and are relatively not cost effective. The method do not treats inorganic compounds or limited to few concentrations.

Incineration techniques

This is another technology that has commonly used to treats pesticide polluted areas. The incineration approach is greatly applicable for the treatment of soil, sediments and sludge contaminated with organic chemicals. In this method oxygen and heat are applied to the polluted areas while the organic substances are consecutively oxidized. Initial phase of incineration, polluted media pass through a very high temperature usually from 1,000 to 1,700 °F which also cause oxidation as well as volatilization of the pollutants. The second phase of incineration technology runs by heating from 1,800 to 2,000 °F which would completely eliminate the pollutants. Incineration technology remove almost all the contaminants however, constraint like high operation costs. Small amounts of polluted media can be treated off-site by centralized facilities however; transporting polluted and hazardous material increases the liability.

TABLE 3. Factors affecting Pesticides Persistence in the environment

Environmental Factors	Role in Chemical Degradation
Sunlight	Radiation from the sun breaks certain chemical bonds, creating break down products.
Microbes	Bacteria and fungi can break down chemicals, creating biodegradation products.
Animals/Plants Metabolism	Plants and animals can change chemicals into forms that dissolve better in water (metabolites). This makes removal from the body easier.
Water	Water breaks chemicals apart to make pieces that dissolve better in water (hydrolysis). This is typically a very slow process.
Dissociation	Chemicals can break apart into smaller pieces (dissociation products).
Sorption	Chemicals that stick tightly to particles can become inaccessible and/or move away with those particles.
Bioaccumulation	Some chemicals can be absorbed by plants/animals from the soil, water, food, and air. When the plant/animal is exposed again before it can remove the chemical(s), accumulation can occur.

Bioremediation techniques

These is an innovative technology that is often being chosen for the cleanup of areas polluted with pesticides and other toxic chemicals (Azubuike *et al.*, 2016). Due to cost-effectiveness and environmental friendly nature, bioremediation is becoming more eye-catching cleanup technology. Bioremediation process influences the rate of the natural pollutants degradation by microorganisms by providing nutrients and energy sources (Azubuike *et al.*, 2016). Bioremediation methods lead to complete mineralization of pollutants into water and carbon dioxide without evolving any intermediates. Bioremediation processes can be generally classified into two groups: ex-situ and in-situ. Ex-situ bioremediation technologies include bioreactors, bio filters, land farming and some composting methods. In-situ bioremediation technologies include bioventing, liquid delivery systems, biosparging, biostimulation, and some composting methods. In-situ bioremediation tend to be more attractive, as less equipment is required in the process, generally have a lower cost and generate fewer disturbances to the environment. However, the difficulties linked with implementing in-situ bioremediation have limited their application in the field conditions (Vogt and Richnow, 2014). The technologies of bioremediation based on the principles of bio-augmentation and bio-stimulation comprise of land farming, bioreactor, bioventing and composting.

Phytoremediation techniques

Phytoremediation is an environmental friendly tools for remediation of contaminants using plant species. The fact that herbicides are intended to destroy plants, the application of phytoremediation technology to remove them might be very complex (Zhao and Coats, 2006). Several researches were conducted to determine the efficiency of removing persistent environmental contaminants using many plant species and more findings were already being reported. Plants have the capability of take-up and storage of high toxic compound concentrations in their roots, shoots and leaves, known as phyto-extraction. The plants are then harvested and disposed of in an appropriate way, for instance in waste landfill. This method results in at least 90% reduction in waste volume over the equivalent concentration of contaminated soil (Henderson *et al.*, 2006). The plants involves in this form of remediation are called the hyper-accumulators. The plants specie mostly used in phytoremediation remediation include; cabbage, mustard plant, alpine pennycress and broccoli etc. Phyto-transformation is a situation in which the pollutants are transform by the plants into less toxic, less mobile or more stable form. These includes the following; phyto-degradation, which is the metabolism of the organic pollutant by the plant enzymes and phyto-volatilization, the volatilization of pollutants as they pass through the plant leaves (Alkorta and Becerril, 2002). Phyto-stabilization immobilizes the pollutants and decreases their movement over the soil by absorbing and binding leachable elements to the plant structure. This process efficiently decreases the bioavailability of the harmful pollutants. Almost all vegetation present at contaminated areas will contribute to phyto-stabilization (Ramírez-Sandoval and Domínguez-Ojeda, 2012). While between the soil and root boundaries, known as the rhizosphere, there is bulky and very active communities of microorganisms. The rhizosphere environment is rich in microorganisms with high metabolic activity, which enhance the biodegradation rate of pollutant (Singh and Singh, 2017). Generally, the plant species are not directly involved in the biodegradation process, but serves as a catalyst for increasing microbial growth and activity, which then increases the biodegradation potential. However, the rhizosphere has limitation in its remediation ability because it does not extend far from the root. This process is often called the phyto-stimulation or plant-aided bioremediation (Henderson *et al.*, 2006) (Figure 2).

Biodegradation of pesticides

Biodegradation is an effective and environmentally friendly method that involve the use of natural materials to neutralize environmental pollutant (Porto *et al.*, 2011). Microbes like bacteria, algae and fungi play an important role in pesticide dissipation in soil and other environmental samples. The interaction between pesticides and microbes has received great deal of attention over the years. Previous approaches of pesticide decontamination includes the use of incineration and landfills which produce secondary pollution complications because of leaching of pesticides into the nearby soil and underground water table as well as possible releases toxic by-product (Ashiq and Tahir, 2016). Many researchers have reported pesticides biodegradation using different microbial populations in different environmental matrix (Akbar & Sultan, 2016, Natália Alvarenga *et al.*, 2018, Mobasherizadeh & Naser, 2017and Zhao *et al.*, 2015). The effectiveness of pollutants degradation differs significantly among diverse microbial communities and even among species belonging to the same populations. The degradation of pesticides by microbes may be characterized base on two major categories as metabolic degradation and co-metabolic degradation.

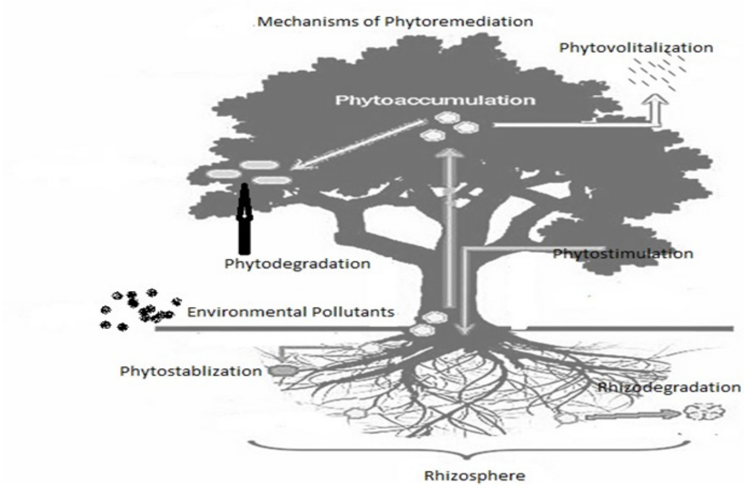

Fig. 2. Mechanism Involves in Pesticide Phytoremediation adapted from (Velázquez-Fernández et al., 2012)

The metabolic pesticide degradation

This comprises the biodegradation of pesticides which perform by the microorganisms present in soil authorizing particular catabolic enzymes to perform full mineralization of pesticides. However, the metabolic degradation may sometimes result to incomplete degradation causing the emergence of other byproducts (Badawi *et al.*, 2009) The enzymes needed for pesticides metabolic degradation are either produced by a distinct microbial specie or by several microorganisms operated as microbial consortium (Fournier *et al.*, 2004).

The co-metabolic pesticide degradation

These relates to the broad-spectrum degradation of pesticides by microbes. Normally, the method is not harbored by any phenomenon it is taking place due to existence of enzymes that can degrade toxic substances. In this situation, the target compound does not support the growth of the microorganisms. Therefore, the proportion of pesticide dissipation is associated to the extent of the microbial biomass and never increase even after frequent introduction to pesticide treatment (Akoijam and Singh, 2014). There are many evidence and laboratory data showing the environmental significance of co-metabolism. It has been mentioned that co-metabolism may be responsible for the degradation of many organic compounds like pesticides which do not tolerate the growth of microbes. Chemicals such as DDT, di-phenyl aliphatic, and polychloroaromatics have been defined as recalcitrant compounds as a result of recurrent failures to isolate microorganisms with ability of using them as sole sources of carbon and energy for growth. Nevertheless, co-metabolism of these elements has been revealed to occur in laboratory settings and may possibly be a significant process for remediation of these pesticides in the environment. For instance, chlorobenzilate, a diphenyl aliphatic having similar structure with Dichlorodiphenyltrichloroethane, was exposed to oxidation by unicellular pigmented yeasts called Rhodotorula gracilis in a culture media supplemented with yeast extract and mannitol salt agar.

Principles of pesticide biodegradation

The complete pesticide biodegradation comprises of parent compound oxidation to produce CO_2 and H_2O which as a result offers carbon and energy for the microbial growth as well as reproduction. Every stage in degradation process is usually catalyzed by a particular enzyme formed by enzymes located in the microbial cell (Jacquet *et al.*, 2016). The degradation of organic compounds by both external and internal enzyme may cease at some stage if a suitable enzyme does not exist. The nonexistence of suitable enzyme is mostly the reasons for pesticides persistence in the environment (Van Dyk and Pletschke, 2011). In some instance if the suitable degradative microbes are absent, or if the capability of the degradative microbes has been reduced as a result of the pesticide toxicity then specific microbial community may be introduced so as to increase the metabolic action of the already present microbes. The microbes might be natural microorganism or genetically modified microbes (Shah and Chellapandi, 2015). The microbial populations in soil environments are diverse populations rather than a single microbial strain. Microorganisms may develop the mechanisms to interact with organic and inorganic constituents either chemically or physically, leading to complete degradation of the target molecule (Sethunathan and Naidu, 2011).

Bacteria involvement in biodegradation of pesticides

Microbial populations in soil are one of the most complex and diverse groups on earth. The diversity of microorganisms symbolizes their metabolic, genetic and morphological features. Studies on molecular basis using small-subunit rRNA and rDNA sequencing systems displayed an unbelievable diversity and distribution among microbes in the environment (Hu and Li, 2013). Microorganisms are virtually adapted to all environments present on the earth. Studies have recurrently established that minor changes in the environmental conditions significantly affect the community of the microbial species. The fate of pesticides in the environment is mostly influenced by the activity of microbes. Several classes of pesticides are readily degraded by microbes, while, some were confirmed to be recalcitrant. Biodegradation by microbes depends not only on the availability of degradative microbes with suitable degradative enzymes, but also on a varied degree of ecological factors. Different groups of bacteria species such as *Pseudomonas, Alcaligenes, Rhodococcus Bacillus and Flavobacterium*, metabolize pesticides as shown in Table 4. Microbes carry-out a different kind of metabolisms, using everything from solar energy to organic carbon as well as inorganic substance as energy sources, which also have the ability to change between these metabolic modes (Fang *et al.*, 2014). Some microbes may exist in several ranged of physicochemical settings, from boiling thermal springs to acid mine drainage. Several microbes have developed catabolic degradative mechanisms to different organic compounds i.e. majority of degradative microbes can utilize external compounds as an energy and nutrient source (Alvarenga *et al.*, 2018, Azadi *et al.*, 2017, Liu *et al.*, 2016 and Wang & Xie, 2014).

Fungi involvement in biodegradation of pesticides

Myco-remediation or application of fungal specie as bioremediation agents dated back to some few decades ago (Seleghim and Porto, 2014). Researchers have tested many fungal species for their ability to degrade different organic compounds. For instance the discovery of white-rot specie of fungi as bioremediation agent has inspired and open way to many researchers. Recently, bioremediation technologies using fungus has attract significant attention. Fungus are widespread in the environment while the literature on the ecology of fungal species is enormous. In spite of this, the reports on potential of fungi as bioremediation agent are scares (Mariana *et al.*, 2010). Furthermore, the biology and ecology of mycoremediation have not often been studied (Aust & Benson, 1993 and Mori *et al.*, 2017). Fungal species have strong enzymatic action that makes them grow well on many natural and synthetic constituents. Fungal specie release greater amounts of many extracellular enzymes into their exterior surroundings and can remediate several compounds to small fragments (Jda *et al.*, 2013). Though fungi are static they can react more rapidly to varying environmental circumstances for their survival. They produce unique extracellular enzyme. Furthermore, they are well suited for bioremediation due to specificity of their catabolic enzymes and ability to form mycelial networks as well as independence of using compounds as a growth substrate (Friloux & Walker, 2002 and Kiyota & Sato, 2010). Fungal species can escape negative circumstances in heterogeneous environments which offer them an advantage. Majorities of fungi are multicellular eukaryotic specie and have noble extracellular enzyme, and peroxidase removing many groups of organic compounds. Several organic chemicals including PAHs, pesticides and PCBs were effectively degraded by common fungal species. An aerobic fungal specie *Chrysosporium* strain was found to be effective for remediation of soil contaminated with organochlorines pesticides the specie was revealed to have degraded the organochlorine using several pathways. *Fusarium verticillioides* strain was isolated from the leaves of tequila agave plants using enrichment methods was able to degrade lindane under aerobic conditions by using it as carbon and energy source (Feijoo and Lema, 2008). The white rot fungus *Phanerochaete chrysosporium* is one of the best agent of bioremediation, as it is more effective than other species of fungus in the degradation of poisonous or insoluble compounds (Mori *et al.*, 2017). Several other white-rot fungal species were tested for the degradation of persistent organic substances, *e.g., Pleurotus tuberregium, Pleurotus ostreatus, Bjerkandera adusta, Trametes versicolor, Agaricus bisporus, Pleurotus pulmonarius and Lentinula edodes* (Khardenavis & Purohit, 2016 and Shimizu & Kamei, 2017) (Table 5).

TABLE 4. Bacterial Species Used For Pesticides Biodegradation

Pesticides		Bacteria used	Source	References
Carbamate	Carbofuran	*Sphingomonas sp.*	Soil	(I. S. Kim *et al.*, 2004; Park *et al.*, 2006)
		Micrococci sp.	Soil	(Krishna and Philip, 2008)
		Enterobacter sp.	Soil	(Mohanta *et al.*, 2012)
		Paracoccus sp.		(X. Peng *et al.*, 2008)2008
		Pseudomonas sp.	Soil	(Bano and Musarrat, 2004; Karpouzas, Morgan and Walker, 2000; Kevin Mbogo, 2012)
		Bacillus thuringiensis sp.	Soil	(Onunga *et al.*, 2015)
		Flavobacterlum sp.	Soil	(Edwards, Kremer and Keaster, 1992)
		Alcaligenes		(Kevin Mbogo, 2012)
	Cabaryl	*Corynebacteriumsp*	Soil	(Hamada, Matar and Bashir, 2015)
		Morganella	Soil	(Hamada *et al.*, 2015)
		Pseudomonas sp	Soil	(M. Larkin and Day, 1986; M. J. Larkin and Day, 1985; Trivedi *et al.*, 2017)
		Micrococcus sp.	Soil	(Doddamani and Ninnekar, 2001)
		Rhodococcus sp. *Arthrobacter sp.*		(Ren, Zhang, Zhao and Sun, 2016)
		Sphingobiumsp	Water	(Yan, Wang, Li, Li and Hong, 2010)
	Propuxur	*Pseudainobacter sp.* *Nocardioides sp.*	Soil	(H. Kim, Kim, Lee, Yun and Ka, 2017)
		Pseudomonas sp.	Soil	(Kamanavalli and Ninnekar, 2000)
		Staphylococcus aureus	MSW	(Anusha, Kavitha, Louella, Chetan and Rao, 2009)
		Neisseria subflava	Soil	(Anusha *et al.*, 2009)
Organochlorines	DDT	*Aerobacter sp.*	Soil	(Neerja *et al.*, 2016)
		Stenotrophomonas **sp.**	Soil	(Pan *et al.*, 2016)
		Pseudomonas sp.	Soil	(Chauhan and Singh, 2015)
		Enterobacter aerogens.	Soil	(Chauhan and Singh, 2015; Neerja *et al.*, 2016; Pathak, Chauhan, Ewida and Stothard, 2016)
		Enterobacter cloacae.	Soil	(Neerja *et al.*, 2016)
		Alcaligeneseutrophussp	Soil	(Erdem and Cutright, 2016)
		Bacillus species	Soil	(Sariwati, Purnomo and Kamei, 2017)
	Lindane	*Staphylococcus sp*	Soil	(D. Kumar, Kumar and Sharma, 2016)
		Kocuriasp	Soil	(D. Kumar *et al.*, 2016)
		Chromohalobacter sp.	Soil	(Bajaj, Sagar, Khare and Singh, 2017)
		Bacillus sp.	Soil	(Pannu and Kumar, 2017)
		Sphingobiumbideri sp.		(Kaur *et al.*, 2013)
		Acinobacter sp.		(Aparicio, Sola, Benimeli, Amoroso and Polti, 2015)
	Endosulfan	*Bordetellapetrii*	Soil	(Supreeth and Raju, 2017)
		Alcaligenesfaecalis	Soil	(Y. Zhang *et al.*, 2016)
		Acinetobacter species	Soil	(maheswari Sepperumal, Palanimanickam and Sivalingam, 2017)
		Bacillus sp.	Soil	
		Klebsiella **sp.**	Soil	(M. Singh and Singh, 2014)

TABLE 4. Cont.

Pesticides		Bacteria used	Source	References
		Pseudomonas sp.	Soil	(Jesitha, Nimisha, Manjusha and Harikumar, 2015)
Organophosphate	Methyl Parathion	*Bacillus sp*	Mangrove peat	(Natália Alvarenga *et al.*, 2018)
		Kosakonia sp.	Mangrove peat	(Natália Alvarenga *et al.*, 2018)
		Burkholderia sp.		(Fernández-López *et al.*, 2017; Popoca-Ursino, Martinez-Ocampo, Dantan-Gonzalez, Sanchez-Salinas and Ortiz-Hernandez, 2017)
		Klebsiella sp.		(Pattanayak, Chakraborty, Biswas, Chattopadhyay and Chakraborty, 2018)
		Fischerella sp.	Soil	(Tiwari, Chakraborty, Srivastava and Mishra, 2017)
	Malathion	*Acinetobacter sp.*		(Azmy, Saafan, Essam, Amin and Ahmed, 2015)
		Bacillus sp.		(Ishag *et al.*, 2016; Khan *et al.*, 2016)
		Staphylococcus vitulinus		(Kadhim, Rabee and Abdalraheem, 2015)
		Pseudomonas putida		(Kadhim *et al.*, 2015)
Pyrethroid	Cypermethrin	*Bacillus sp*	Soil	(Murugesan, Jeyasanthi and Maheswari, 2010; Pankaj *et al.*, 2016; Xiao *et al.*, 2015)
		Pseudomonas sp		(Chen *et al.*, 2015; Malik, Singh and Bhatia, 2009)
		Corynebacterium		(Shaohua Chen, Meiying Hu, *et al.*, 2011; Murugesan, Jeyasanthi and Maheswari, 2010)
		Ochrobactrum sp.		(Shaohua Chen, Meiying Hu, *et al.*, 2011)
	Deltamethrin	*Acinetobacter sp.*		(Jin, Guo, Zhang and Yan, 2014)
		Streptomyces aureus **sp.**	Soil	(Shaohua Chen, Kaiping Lai, *et al.*, 2011)
		Bacillus cereus sp.		(H. Zhang *et al.*, 2016)
		Serratiamarcescenssp	Soil	(Cycoń, Żmijowska and Piotrowska-Seget, 2014)

Degradative pathways of some pesticides by microbes

Degradation of pesticides occurs through various mechanisms. Generally, the degradation of pesticides results in the production of carbon dioxide (CO_2) and water (H_2O) by the oxidation of parent compounds. Many studies were conducted and showed that degradation happens under both aerobic and anaerobic conditions, under the influence of gram positive and negative bacteria as well as some fungi. Biological physical and chemical agents play a vital part in the transformation process of different pesticides into several metabolites. The mechanisms of pesticides transformation comprise of hydrolysis, oxidation reduction, conjugation hydration mineralization and isomerization. The subsequent metabolites or derivatives are commonly less bioactive than the parent compound, however, some of the derivatives were reported to be of higher bioactivity. The physicochemical parameters of the degradation product are also different from the parent pesticide molecules, their fate and significance are also altered with the structural changes. Example of degradative pathways of some pesticides by microbes can by explained as follows (Fig. 3 and 4).

Cell immobilization for efficient pesticide degradation

Immobilized microbial cell means a living microbial cell that is entrapped from moving freely from its original position to other parts or a localization of microbial cells to a certain area or space by maintaining their metabolic as well as catabolic activity. Immobilization of microbial cell may increases the efficacy of the cultures by optimizing the degradation rate of pollutants (Dominguez-Velez and Merino-Castro, 2011). Cell immobilization techniques can be categorized as either organic or inorganic based on their chemical composition, it can also be subdivided into natural or synthetic polymers example starch, agarose, agar, cellulose, alginate, clay, aluminum oxide, hydroxyapatite, ceramic, activated carbon, chitin, collagen, activated pumice polyacrylamide, dextran and keratin. For pesticides degradation, it is essential to explore a for immobilization matrices with appropriate characteristics and physical structure as well as easiness of washing and the likelihoods of using it repeatedly (Talwar and Ninnekar, 2015). Cell immobilization is one the effective biological method for removal of pollutants due to

TABLE 5. Fungal specie used for degradation of pesticides

Compound	Fungal Specie used	Nature of Study	References
Dieldrin	PenicilliumMiczynskii	Transformation	(Birolli *et al.*, 2015)
Methyl Parathion	AspergillusSydowii	Degradation, Metabolites	(N. Alvarenga *et al.*, 2014)
Methyl Parathion	PenicilliumDecaturense	Degradation, Metabolites	(N. Alvarenga *et al.*, 2014)
Clothianidin	PhanerochaeteSordida	Degradation, Metabolites	(Mori *et al.*, 2017)
(Alpha + Beta) Endosulfan	*BjerkanderaAdusta*	Dissipation/ Metabolites	(Rivero, Niell, Cesio, Cerdeiras and Heinzen, 2012)
Lindane, Chlordane And Methoxychlor	Streptomyces Consortium	Degradation	(Fuentes, Raimondo, Amoroso and Benimeli, 2017)
Imiprothrin, Cypermethrin, Carbofuran	TrametesVersicolor	Transformation Metabolite	(Mir-Tutusaus *et al.*, 2014)
DDT	GloeophyllumTrabeum, FomitopsisPinicola	Degradation, Metabolites	(Purnomo, Mori, Takagi and Kondo, 2011)
β-Cypermethrin, Deltamethrin	Cladosporium	Degradation, Metabolite, Kinetics	(S. Chen *et al.*, 2011)2011
Aldrin, Dieldrin	Phlebia sp.	Degradation, Metabolic Pathways	(Xiao *et al.*, 2011)
DDT	Gloeophyllum,Trabeum	Transformation	(Purnomo, Kamei and Kondo, 2008)
DDT	PhanerochaeteChrysosporium	Degradation	(Zheng, Selvam and Wong, 2012)
DDT	XerocomusChrysenteron	Degradation, Mineralization And Metabolite	(Huang and Wang, 2013)
Aldrin,Dieldrin	PleurotusOstreatus	Transformation, Metabolite	(Purnomo *et al.*, 2017)
Heptachlor Heptachlor Epoxide	PleurotusOstreatus	Transformation, Metabolite	(Purnomo, Putra, Shimizu and Kondo, 2014)
Ensulphuron-Methyl (BSM)	PenicilliumPinophilum	Degradation, Metabolites	(Xingxing Peng *et al.*, 2012)
Penoxsulam	*Aspergius sp.*	Degradation, Metabolites	(Sondhia, Rajput, Varma and Kumar, 2016)
Metribuzin	PleurotusMutilus	Adsorption	(Behloul, Lounici, Abdi, Drouiche and Mameri, 2017)
β –Cypermethrin	*Aspergillus Niger*	Degradation, Metabolites	(Deng *et al.*, 2015)
Chlorotoluron,Diuron, Isoproturon and Linuron	Mortierella s*p.*	Transformation Metabolites	(Badawi *et al.*, 2009)

acceleration of metabolic activity of the microbial cells by the immobilization matrix (Shah and Naqvi, 2017). The immobilized cells have several advantages over free cells like higher metabolic action, higher mechanical capability as well as tolerant to toxic chemicals due to higher biomass concentrations. Cell immobilization may escalate the survival and metabolic activity of the microbial cells in bioremediation approaches (Tao and Yang, 2011). Previous literatures have advocated that the higher efficiency of immobilized cells results from cellular or genetic variations induced by the immobilization. Immobilized cells are unaffected by distresses in the environment and are less disposed to poisonous materials, which makes immobilized cell attractive for remediation of organic compounds such as pesticides (Engler and Wild, 2009). The biological approaches for the treatments of pollutants have attracted increase attention to be a promising alternative for the removal of pesticides and other pollutants present in the environment (Es *et al.*, 2016; Wang and Wu, 2017). Recently, the entrapment of the cells in gellan gum, calcium alginate, polyacrylonitrilehas agarose, polyamide, polyvinyl alcohol, polyurethane as well as polypropylene were recommended as promising method for microbial degradation of contaminants (Paisio *et al.*, 2016; Talwar and Ninnekar, 2015). The immobilized mediums were non-toxic to the viable cells and low-cost as well as better stability. The approach for effective immobilization is dependent on the concentration of the cell (Gaikwad and Pundle, 2017) Table 6 and 7 shows different mobilized matrix used for the degradation of pesticides and other chemicals.

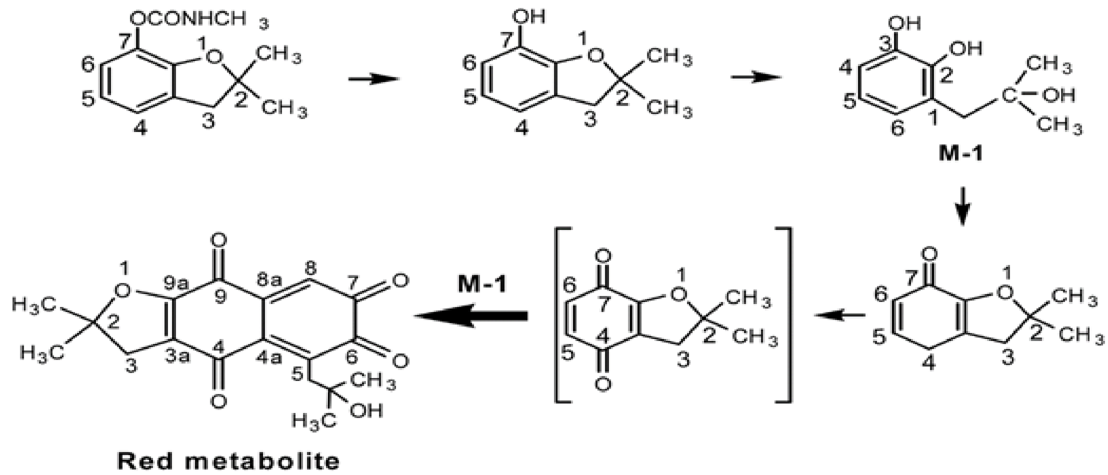

Fig.3. Proposed degradation pathway of carbofuran by Sphingomonas sp. strain SB5 (Park et al., 2006)

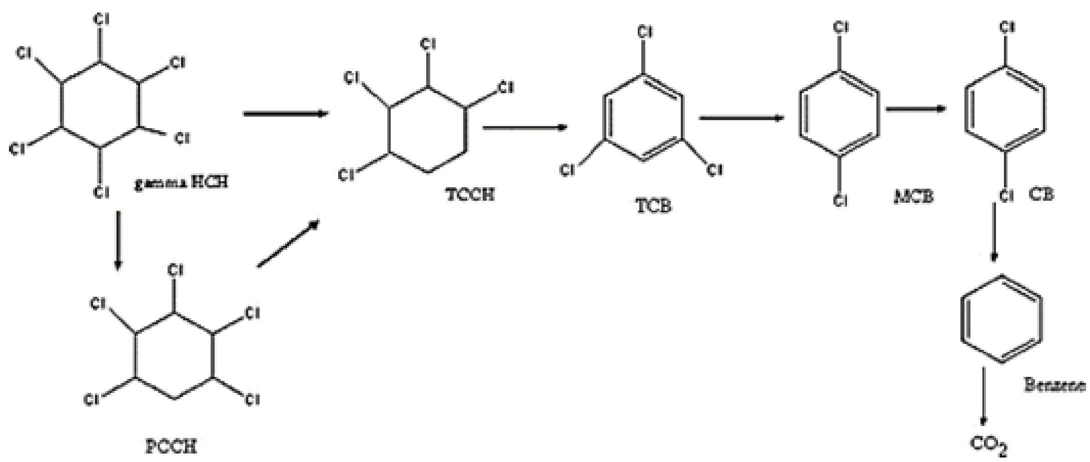

Fig. 4. Proposed pathway for lindane biodegradation by various enriched bacterial isolates (Krishna and Philip, 2008)

Molecular methods for the degradation of pesticides

Microbes have the capability to degrade multiplicity of environmental pollutants, including pesticides. It is highly significant to understand the biochemical bases for the development of new degaradative capacities of microorganisms involve in pesticide degradation (Quintero-Hernandez and Munoz-Rojas, 2017). Genetic components like the plasmid and transposons were ascertain to have encode enzymes responsible for several pesticides degradation. The isolation and characterization of gene encoding pesticides degradative enzymes as well as novel procedures for isolating and analysis of nucleic acid from microorganisms, would display some distinctive insight into the molecular actions that lead to advance of pesticides degradation phenomena (Chakraborty and Das, 2016). Genetic manipulation offers a technique of engineering microbes to depollute many compounds, including pesticides that may be present in the polluted areas. The main approach is to prolong the degradative abilities of existing metabolic pathways in the organism either by introducing additional enzymes from other organisms or by modifying the already present catabolic genes. Degradation of pesticide by microorganism involves several forms of enzymes. These approaches are based on several kinds of genes coded with plasmid or chromosomal DNA (Chakraborty and Das, 2016). Pesticide degrading genes in microorganisms have were described to be located on plasmids, transposons or chromosomes of the organism. Recent researches have shown the way that lead to development of degradative pathways and the organization of catabolic genes, hence making it simpler to develop genetically modified organisms (GMO) for pesticide degradation. The genetically engineered microbes have substantial capacity to degrade the pesticide as they discharge several specific enzymes that have the specific catabolic gene into plasmids (Chen and Huang, 2016). The studies of recombinant DNA provide the means to develop DNA and RNA examinations for the purpose of identifying microorganisms from diverse populations with exceptional capacity to degrade pesticides

TABLE 6. Immobilized microbial cells used for degradation of pesticides

Pesticides	Microbes used	Immobilization matrix	References
Carbazole	*Sphingomonas sp.*	Gellin gum	(Ahmad, 2010)
Chlorpyrifos	*Streptomyces Sp.*		(Fuentes *et al.*, 2013)
Cypermethrin	*Micrococcus sp.*	Polyurethanefoam (PUF)	(Tallur *et al.*, 2015)
Carbofuran	*Pseudomonas fluorescens*	Ca-alginate	(X. Wang, Liu, Yao, Zhang and Bao, 2017)
Methyl parathion	Consortium	Loofa sponge	(Ahmad, 2010)
Coumaphos	Consortium	Loofa sponge	(Ahmad, 2010)
2,4-D(2,4-dichloro-phenoxy acetic acid)	*Pseudomonas fluorescens*	Alginate beads	(Ahmad, 2010; Boivin, Amellal, Schiavon and van Genuchten, 2005)
Aldicarb	*Enterobactr cloacae*	Agar entrapment	(Fareed *et al.*, 2017)
Carbofuran	*Enterobacter cloacae*	Agar entrapment	(Fareed *et al.*, 2017)
Carbaryl	*Enterobacter cloacae*	Agar entrapment	(Fareed *et al.*, 2017)
Profenofos	Pseudoxanthomonas	Sodium alginate	(Talwar and Ninnekar, 2015)
Endosulfan	*Pseudomonas aeruginosa*	Ca-alginate	(Pradeep and Subbaiah, 2016)
Atrazine	*Pseudomonas sp.*	Sodium alginate	(A. Kumar, Nain and Singh, 2017)
Atrazine	*Burkholderia* sp.	Sodium alginate	(A. Kumar *et al.*, 2017)
Endosulfan	*Pseudomonas sp.*	Ca-alginate	(Pradeep and Subbaiah, 2016)
Quinalphos,	*Staphylococcus sp.*	Ca-alginate	(Punitha and Rose, 2018)
Monocrotophos	*Staphylococcus sp.*	Ca-alginate	(Punitha and Rose, 2018)
Chlorpyriphos	*Staphylococcus sp.*	Ca-alginate	(Punitha and Rose, 2018)
Pendimethalin	Bacillus lehensis	Ca-alginate	(More, Tallur, Niyonzima and More, 2015)

TABLE 7. Immobilized Microbial Cells Used For Degradation of other Pollutants

Pollutants	Microorganism	Immobilized bead	References
Acrylamide	Pseudomonas aeruginosa	Ca-alginate	Prabu and Thatheyu, 2007
Azo dye	Pseudomonas fluorescens	PVA-alginate	Caiet al., 2011
TextileEffluent (dyes)	Phanerochaete chrysosporium	PVA-alginate	Nasiret al., 2011
Cd	Pseudomonas fluorescens	Ca-alginate	Sarin and Sarin, 2010
Cd	Pseudomonas sp.	Ca-alginate	Rani et al., 2010
Hydrocarbon	Consortium	Ca-alginate	Rahmanet al., 2006
Pesticide	Consortium	Ca-alginate	Ha, 2005
^PAH	Sphingomonas sp.	Gellan gum	Wang et al., 2007a.
SDS	Pseudomonas aeruginosa sp.	Gellan gum	Mahmoodet al., 2010

Conclusion

The extensive use of pesticides leads to contamination of the environment. The advances in biochemical and genetic techniques has expanded the potential for new developments in biodegradation in that it has become practical to isolate and modify potentially useful genes responsible for biodegradation from a wide range of microbes. Plentiful microbes with novel pesticide degradation activities are characterized, biological degradation by microbes can efficiently remediate pesticides from the environment. The ability of these organisms to remove organic pollutants such as pesticides is directly related to their long-term adaptation to the areas where these compounds exist therefore, these microbes that occupy polluted areas are armed with resistance mechanisms to remove the toxic compounds. Furthermore, genetic engineering may be used to enhance the performance of such microbes that have the properties, essential for bioremediation. Microorganisms contribute considerably for the removal of toxic pesticides applied to agricultural field effective microbial consortia need to be grown in large quantity for large scale field application.

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