1. Introduction

1.1 History of bioremediation

In recent decades, significant progress has been achieved in the agricultural industry such as the breeding of high yield cultivars which leads to considerable improvement in both yield and quality of crops (Ju *et al*., 2007). Revolutionary development in pesticide technologies helped enhance the crop yield and reduce the risk of crop loss or quality deterioration (Robinson and Sutherland, 2002; Pimentel, 2005; Aktar *et al*., 2009). Pesticides are synthetic chemical substances that are intended for preventing, repelling, destroying, or mitigating any pests e.g., insects, mites, nematodes, weeds, and rats (Damalas and Eleftherohorinos, 2011). These substances, often accumulated into the environment after the use, are considered as commonly dispersed contaminants. The surplus use and disposal of outdated pesticide storage have also resulted in severe contamination (Felsot, 1996; Eqani *et al*., 2012).

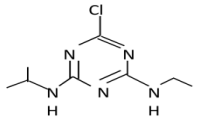
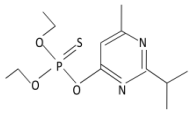
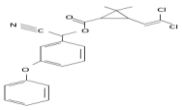
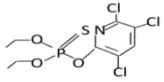
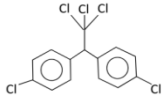
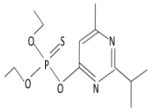
A large proportion of productive agricultural land has been lost due to urbanization (Deng *et al*., 2006; Imhoff *et al*., 2004), biofuel production (Fargion *et al*., 2008; Pimentel *et al*., 1992), and climate change (Bonan, 2008). Since 1960s, the pesticides have become essential features in the modern agriculture for economical pest management and better crop production accompanied by the rapid growth of the global population, e.g., an increase by 1.1% in 2016 from the year before (Mandal and Singh, 2017). Pesticide use and environmental contamination are expected to be worsened in the foreseeable future.

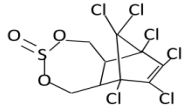
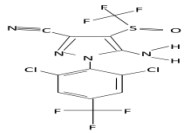
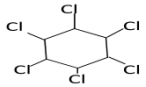
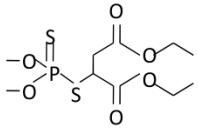
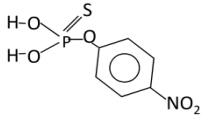
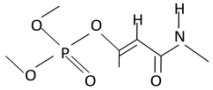
Approximately 2.4 million metric tons of pesticides were applied worldwide (as of 2014) to control various insects, weeds, fungi, and other unwanted organisms in the agricultural and urban environments (Helbling, 2015). Pesticide residue can exist in the environment longer than ten years (Hamscher *et al*., 2002; Yadav *et al.*, 2015). The human exposure to pesticides at a relatively high concentration can occur through soils and drinking water and potentially threaten human health because of their toxicity such as carcinogenicity, neurotoxicity, and fertility disorder (Alavanja *et al*., 2004; Sharpe and Irvine, 2004). Production, permissible limit, hazardous level, and health effects of pesticides are shown in **Table 1**.

The treatment of pesticides is necessary to avoid the harmful effects on human and animals and to prevent the environmental health. Many literatures have shown the development of physicochemical technologies for treating wastewater (Ormad *et al*., 2008; Plakas and Karabelas, 2012; Mandal and Singh, 2017). However, the conventional techniques have critical disadvantages such as equipment complexity, high operating cost, excessive generation of sludge, and hazardous wastes as by-product (Plakas and Karabelas, 2012). To solve these, various studies have suggested the biological treatments for treating a wide range of pesticides (Cycon *et al*., 2009; Mandal et al., 2013; Aresta *et al*., 2015; Saez *et al*., 2015; Gupta *et al*., 2016).

As the biological treatment, the bioremediation is commonly used. The "bioremediation" is the process in which contaminant are biologically degraded in an innocuous state. The series of enzymatic reactions of microbes are involved in the breakdown of various organic compounds like pesticides (Perelo, 2010). In bioremediation, microbes are used to ingest and convert hazardous pollutants into less harmful compounds or metabolites (Hussaini *et al.*, 2013).

Table 1: Production, 2D structure, permissible limit, hazardous level, and health effects of pesticides

Order	Pesticides	2D-Structure of pesticide	Target Crops	Global production 2014-15 (MT)	Maximum permissible residual limit in food (ppm)	Class	Human health effects	References
1	Atrazine		Sugarcane, rice, green vegetables	1200	0.2	Moderately hazardous	Responsible for damage to liver, kidney, and heart in animals. Damage of vital organs in human has yet to be studies in details	(Abhilash and Singh, 2009; Brusick, 1994; Colborn et al., 1993; Jardim and Caldas, 2012)
2	Carbofuran		Potatoes, corn and soybeans.		0.3	Highly hazardous	Reproductive Disorders. Affect nerve system Cholinesterase inhibitor	(Abhilash and Singh, 2009; Bretaud et al., 2000; Plangklang and Reungsang, 2009, 2010)
3	Cypermethane		Cotton, fruit and vegetable crops	8590	0.03	Moderately hazardous	Possible carcinogen Suspected endocrine disruptor	(Abhilash and Singh, 2009; Grewal et al., 2010; Jardim and Caldas, 2012; Mcdaniel and Moser, 1993; Yousef et al., 2003)
4	Chlorpyrifos		Corn, soybeans, fruit and nut trees, other row crops	9880	0.05	Moderately hazardous	Cholinesterase inhibitor	(Abhilash and Singh, 2009; Kmellar et al., 2008; Ngowi et al., 2007)
5	DDT		Variety of food crops	95 (2010-11)	0.5	Highly hazardous	Vomiting, Shakiness, Seizures	Beard and Collaboration, 2006; Jardim and Caldas, 2012; Longnecker et al., 1997; Xu et al., 2008)
6	Diazinon		Fruit, vegetable, nut and field crops	0	0.1	Moderately hazardous	Cholinesterase inhibitor	(Freeman et al., 2005; Jardim and Caldas, 2012)

7	Endosulfan		Food crops	1350 (2011-12)	0.5	Moderately hazardous	Suspected endocrine disruptor	(Saiyed et al., 2003; Song et al., 2011; Soto et al., 1994)
8	Fipronil		Rice, cotton	0	0.01	Moderately hazardous	Sweating, dizziness	(Jardim and Caldas, 2012; Ohi et al., 2004; Tingle et al., 2000; Tingle et al., 2003)
9	Lindane		Sugar beet crops, grains, fruit, vegetables	800 (2007-08)	1	Highly hazardous	Aplastic anaemia, Breast cancer	(Abhilash and Singh, 2009; Colborn et al., 1993; Song et al., 2011; Sujatha et al., 2001)
10	Malathion		Strawberries, limes, cotton, cherries	2346	8	Moderately hazardous	Carcinogenic to humans and animals 2.cholinergic toxicity and neurotoxicity in animals and immunity of higher vertebrates	(Abdollahi et al., 2004; Bonner et al., 2007; Lewis and Elvin-Lewis, 2003; Sujatha et al., 2001)
11	Methyl parathion/parathion		Fruit, cereals, vines, vegetables **cotton** an d field crops	885 (2007-08)	0.2 ppm methyl parathion 0.05 ppm parathion	Highly hazardous	Cholinesterase inhibitor carcinogen Possible carcinogen Suspected endocrine disruptor	(Eskenazi et al., 1999; Rao and Kaliwal, 2002; Song et al., 2011; Uzunhisarcikli et al., 2007)
12	Monocrotophos		Potato and, cotton crops	4500	0.05	Moderately hazardous	Carcinogenic Affect reproductive system	(Azmi et al., 2006; Kavitha and Rao, 2007; Rao and Kaliwal, 2002; Santhakumar et al., 1999; Song et al., 2011)

1.2 Types of the pesticides and its uses

Pesticides are classified according to their nature, feedstock, and pest control capability. Depending on pesticides' origins, they are classified as chemical-and bio-pesticides. Chemical-pesticides are further divided into four main types, namely organophosphate, organochlorine, carbamates, and pyrethroid. Bio-pesticides are derived naturally from living organism's including bacteria, fungi, and plants. They can commonly be classified into three major groups such as microbial, biochemical, and plant incorporated protectants. Further classification of pesticides can also be made on the basis of their pest controlling capabilities whether they are used for insecticides (insects), nematicides (nematodes), fungicides (fungi), herbicides/weedicides (weeds), algaecides (algae), and rodenticides (rats) (**Figure 1**; available on https://www.biotechnologyforums.com) (Wang *et al.*, 2014a).

Pesticides are directly applied to specific plant part or above-ground to possibliy be transported into the soil and soil organisms. Depending on the application method, the fraction of pesticide can be infiltrated directly into the soil system, ranging from 30%-90% (Fuhr *et al*., 1991). The impacts of various pesticides on specific soil organisms, soil food chains, and biological soil function are varied depending on the type and amount of pesticides, soil environment, and soil biota. The impacts can be expanded to the entire soil community with noticeable damage on various soil functions (Wagschal *et al*., 2007). Pesticides can be degraded by both biotic and abiotic processes into intermediate or secondary products that may have even worse toxicity than the parent pesticide (Mansour and Feicht, 1994). Biodegradation of pesticides/herbicides is also greatly influenced by the soil conditions (e.g., moisture content, organic matter temperature, and pH) along with bacterial strain characteristics and pesticide solubility (Manolov, 2014).

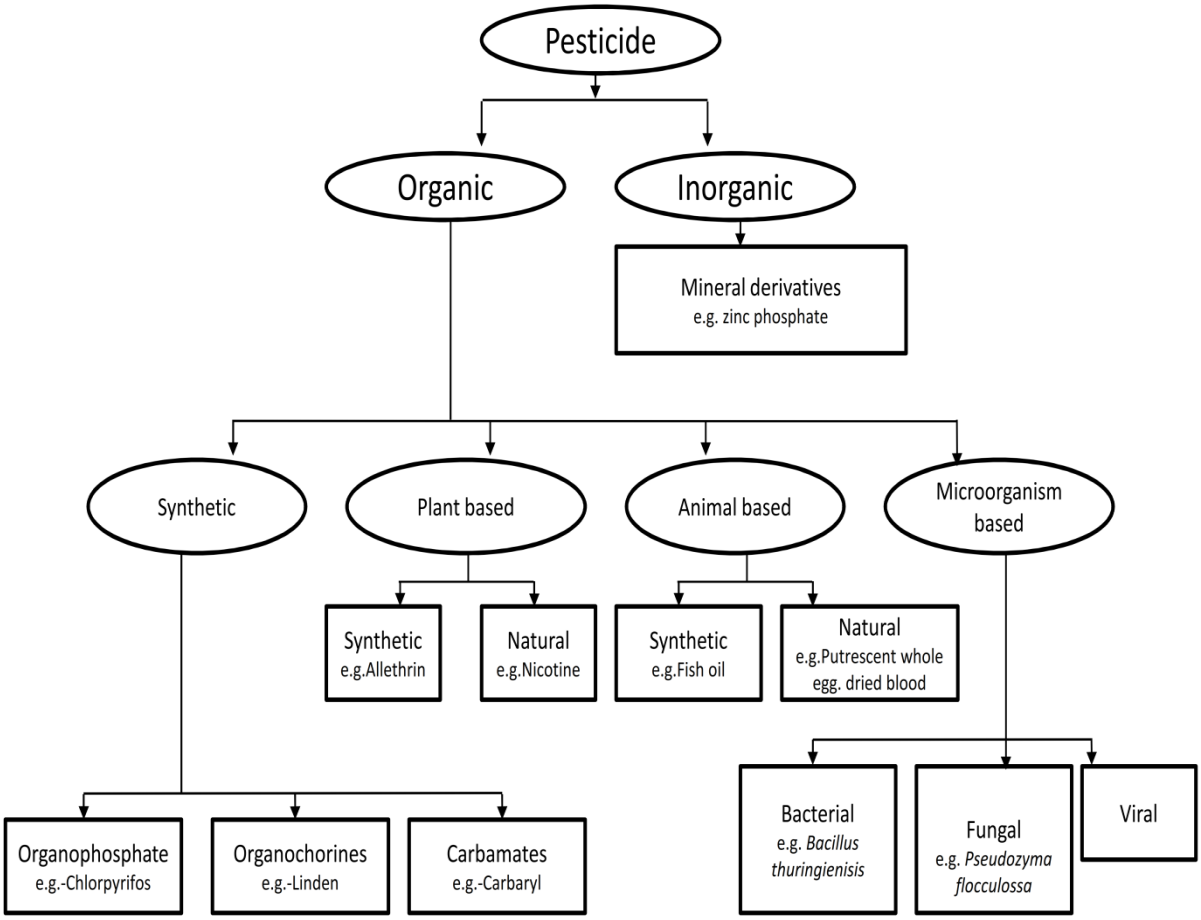


Figure 1: Types of the pesticides used in agricultural field

As increasing world population, the use of pesticides has dramatically been accelerated to maximize agricultural productivity and satisfy food demand. However, its effects on long-term sustainability, soil degradation, water nitrification, natural resources management, and climate change are questionable (Follett, 2001; Szulejko *et al*., 2017). The residual levels of pesticide in foods have been monitored and regulated as the maximum residue level (MRL) established by phyto-sanitary studies.

In Europen Union, more than 800 pesticides had been authorized although less than 300 pesticides are used in practice (Jess *et al*., 2014; Renwick, 2002). In 2007, about 2.3×10^6 tons of pesticides were used world-wide and their sales in 2014 reached 52 billion USD (Watson,

2014). As of 2016, China is the largest consumer of agricultural pesticides (1.806×10^6 ton y^{-1}), followed by US (3.86×10^5 ton y^{-1}), Argentina (2.65×10^5 ton y^{-1}), Japan (5.2×10^4 ton y^{-1}), and India (4.0×10^4 ton y^{-1}) (Available on http://www.worldatlas.com). The potential crop losses by pests without any pesticide varied from ~50% (barley) to ~80% (sugar, cotton and beet) (Oerke and Dehne, 2004; Popp *et al.*, 2013). Actual losses with proper pesticides are estimated to be 26~30% for soybean, sugar, barley, beet, cotton, and wheat while 35% for maize, 39% for potatoes, and 40% for rice. The use of chemical pesticides is one of the most effective ways to enhance the crop production and achieve valuable pest controls.

1.3 Bioremediation

Bioremediation is a greener route to remove many environmental pollutants (Deng *et al*., 2006; Gupta *et. al*., 2016). Despite decades of research, scaling up from the lab-scale to field trials has been very challenging. Biodegradation of toxic pollutant is prescribed by bioavailability to degrad pesticides by specific microorganism (Seeger *et al.,* 2011). Bacterial degradation is an important mechanism to control a level of toxicants in soils (Cheung and Gu, 2007). Here is a brief account of different microorganism used for bioremediation, their target pesticides, catabolic pathway and benefits of combined conservative bioremediation techniques.

1.4 Types of bioremediation

1.4.1 Bio-stimulation

Bio-stimulation refers to augmentation of pesticide degrading capabilities of microorganism by addition of nutrients like nitrogen, phosphates, potassium, vitamins etc. The stimulus of microbes by the adding up supplimentary nutrients which brought a huge quantity of carbon sources which have a tendency to result in a rapid reduction of the

available pool of major inorganic nutrients such as nitogen and phosphate (Trindade *et al*., 2005; Plangklang and Reungsang, 2010). Bio-stimulation has accustomed to eliminate pesticides from atmosphere. The supplementary nutrients include organic or inorganic additives such as nitrate and phosphate, representatively. The supplementary nutrients are essential as co-metabolic substrates and enzyme inducers in the pesticide biodegradation pathways (Plangklang and Reungsang, 2010; Robles-Gonzalez *et al*., 2008). In order to stimulate the microbial biodegradation by adding NPK (fertilizers) (water soluble, e.g., NH_3NO_3, $NaNO_3$, KNO_3, K_2HPO_4, and $MgNH_4PO_4$) as a nutrients (Lima *et al*., 2009). As a thumb rule for toxic pesticide bioremediation, approx 1- 5% N_2 by weight of pesticide with Nitrogen: Phosphate between 5:1 and 10:1 is applied. The supplements additions may be varied with the pollutant to treat at respective sites (Yang *et al.,* 2011). Lima *et al*. (2009) have reported the soil incubation effect with *Pseudomonas* species and citrate bio-stimulation (4.8 mg/g of soil) of atrazine degradation which has a concentration of 20- to 200-fold greater than RD (recommended doses). At a 200 times the recommended dose, the atrazine was biodegraded by 79% at a same period; though, citrate utilization enhanced atrazine mineralization to 87%. They have successfully degraded atrazine at higher concentration (62 mg/g soil) by inoculation of soil using *Pseudomonas* species with bio-stimulation.

1.4.2 Bio-augmentation

Bio-augmentation is a process in which exogenous microbes with definite catabolic abilities are introduced in certain polluted environment. The techniqe maybe an *ex-situ/in situ* bioremediation method in which as expected microorganism are added into the polluted environment for the detoxification (Perelo, 2010). Plangklang and Reungsang (2010) have studied the efficient bioremediation of polluted environment. Bio-augmentation is used to

degrade a wide variety of toxic pollutant like pesticides petroleum compounds, ammonia, hydrogen sulfide, and a rising number of toxic chemicals found in the contaminated site (Khan *et al*., 2005; Park *et al*., 2008; Xu *et al*., 2008).

Few case studies reported on soil bio-augmentation for the pesticides elimination as shown in **Table 2**. Lima *et al*. (2009) have reported soil inoculation effect on *Pseudomonas* sp. for the biodegradation of atrazine pesticide in a soil polluted with a concentration of 20-to 200-times greater than recommended dose. The bio-augmentation of soil 20 times the recommended dose shows the faster removal (99%) of atrazine within 8 days without citrate concentration. At a 200 concentration of 200 times the recommended dose, the atrazine was biodegraded by 79% with the same time. Similarly, Wang *et al.* (2013) also reported the better removal efficiency of atrazine from agricultural soil with 400 mg/Kg by *Arthrobacter* species. The potential strain was inoculate in sterile/non-sterile/ soils, to remove 70- 90% of atrazine.

Bio-augmentation of chloropyrifos using Alcaligenes faecalis in planted soil, achieved 100% mineralization in 12 days whereas only 22% of initial dose (100 mg/kg of soil) of chloropyrifos was removed in the control group. (Yang *et al.*, 2005). Similarly, Ahmad *et al.* (2012) have introduced *Bacillus pumilus* in a planted soil and observed 97.5% degradation of the added chlorpyrifos pesticide (50 mg/kg of soil), whereas, from the control soil 11% of the initial dose was eliminated. Bio-augmentation of *Bacillus subtilis* and *Pseudomonas fluorescens* in a soil, was capable to degrade chlorpyrifos (50 mg/kg) (Lakshmi *et al*., 2008). After 30 days, the removal of chlorpyrifos by *Bacillus subtilis* and *Pseudomonas fluorescens* individually inoculated in soil was found 85-92% as compared to control soil (34%) (Lakshmi *et al*., 2008).

Table 2: Bio-augmentation of pesticides

Pesticide	Microorganism used	Type of soil	Dose (mg/kg)	Condition of Experiment	Findings	Reference
Atrazine	*Arthrobacter sp. DAT1*	Loam, with pH 7.6	400	In laboratory, 25 ^{0}C	>95% of atrazine was removed within 3 days	Wang et al. (2013)
	Pseudomonas sp. ADP	Sandy loam (sand 62%, silt 21%, clay 16%, pH 6.1)	40 or 400 L/ha	In laboratory, 25 ^{0}C	99% and 79% of atrazine at recommended dose of 20 to 200× RD	Lima et al. (2009)
Chlorpyrifos	*Alcaligenes faecalis DSP3*	Silty clay (sand 26%, silt 34%, clay 37%, pH 6.9)	100	In laboratory, 25 ^{0}C	Almost 100% of Chlorpyrifos was removed	Yang et al. (2005)
	Bacillus pumilus C2A1	Agriculture soil, pH 7.8	25, 50	In a green house, 25 ^{0}C	Chlorpyrifos in unplanted soil was degraded 81-89% 25 - 50 mg/kg,	Ahmad et al. (2012)
	Bacillus subtilis	Sandy loam, pH 7.3	50	In laboratory	The degradation of chlorpyrifos was 56% and 85% after 10 and 30 days,	Lakshmi et al. (2008)
	Pseudomonas Fluorescens	Sandy loam, pH 7.3	50	In laboratory	The degradation of chlorpyrifos was 43% and 89% after 10 and 30 days,	Lakshmi et al. (2008)
Carbofuran	*Pichia anomala*	Composition of Sandy loam (sand 65%, silt 28%, clay 7%, pH 6.9)	50	In lab scale, 30 ^{0}C	Carbofuran degradation increased from 2.4-85.1%	Yang et al. (2011)

Cypermethrin	*Streptomyces aureus*	Composition of Sandy loam (sand 65%, silt 28%, clay 7%	50	In situ, 24e30 ^{0}C	81.1% of cypermethrin was removed in bio-augmented soil (in control 32.1%)	Chen et al. (2012)
DDT	*Daedaleadickinsii*	Andisol composition (sand 44%, silt 40%, clay 8%, pH 5.6)	45	In lab scale, 30 ^{0}C	In 14 days 32%DDT was removed	Purnomo et al. (2011)
	Gloeophyllumtrabeum	Andisol composition (sand 44%, silt 40%, clay 8%, pH 5.6)	45	In lab scale, 30 ^{0}C	43% removal of DDT by the bioaugmentation in 14 days (in control 41%)	Purnomo et al. (2011)
Lindane	*Staphylococcus cohnii spp. Urealyticus*	Garden soil	5, 50	In lab scale, 28 ^{0}C	70-100% of Lindane was removed within 45 days at a concentration of 5-50 mg/Kg	Abhilash et al. (2011)
Methyl-parathion	*Pseudomonas sp.*	Loam,	536	In lab scale, 30 ^{0}C	In 15 days c100 % mineralization of complete methyl parathion	Wang et al. (2014b)
Parathion	*Serratia Marcescens*	Composition of Sand (sand 91%, silt 6%, clay 3%, pH 6.5)	100	In lab scale, 30 ^{0}C	Parathion was 50 was consummated from 50 to 9.7	Cycon et al. (2013)

Many years ago DDT (Dichlorodiphenyltrichloroethane) was banned around the world but their residue are still found in contaminated site. Thus, the various biological techniques are involved to continuously clean up the contaminated site. The fungi are also potential microbes for treatment of DDT from the contaminated site. The potential fungal species *Gloeophyllum trabeum* and *Daedalae dickinsii* were investigated to degrade DDT in artificially and historically contaminated sterile/ non-sterile soil (Purnomo *et al*., 2011). Purnomo *et al*. (2011) has reported that the *Gloeophyllum trabeum* and *Daedalae dickinsii* used to degrade DDT contaminated synthetic soil and found 41% and 15% removal respectively. As compared to the control *Gloeophyllum trabeum* and *Daedalae dickinsii* strain in a non-sterile soil is capable to reduced 43% and 32%, of the initial concentration DDT.

1.5 Influencing factors for the bioremediation of pesticides in soil

Removal of pesticide from contaminated soil depends on physical, chemical, and biological processes of the soil. The diffusion of pesticides in soil is controlled by microorganism, the transfer phase of pesticide from the soil to food, air and water.

1.5.1 Pesticides Structures

The structure of pesticides determines its physicochemical properties and inherent biodegradability. Pesticides are more susceptible to microbial attack and biodegradation if there are polar substituent on the phenyl ring, e.g., -OH, -COOH, and -NH_2, whereas pesticides become more resistive from alkyl or halogen group towards the bioremediation (Pal *et al*., 2006; Yadav *et al*., 2014; Geed *et al*., 2016). A minor alteration in structural substitute causes a sudden change in the vulnerability of a pollutant towards bio-transformation (Pal *et al*., 2006). During the pesticides biodegradation process, the chemical

structures of pesticides were drastically changed either by oxidation or reduction of active functional groups, causing the breakdown of their complex structures into small molecules such as carbon dioxide, nitrate, phosphate, ammonia, and water (Porto *et al*., 2011). The 2D-structures of selected pesticides are shown in **Table 1**. The toxic properties of organochlorine pesticides are not similar to organophosphate and carbamates pesticides. Toxicological profile of organochlorine pesticides can be varied by the substituting position of chlorine in the molecule (Pal *et al.*, 2006; Singh, 2012).

The chlorinated hydrocarbons such as pentalene, dieldrin, and DDT residues are unavailable for biodegradation because of their insoluble nature in water and high sorption affinity to a soil (Pal *et al.*, 2006). In contrast, the soils contaminated with chlorinated hydrocarbons, have diverse chemical structure and are biodegraded in short period. The rate of degradation is significantly affected by minor difference in the position of substituent in same class pesticides (Topp *et al.*, 1997; Pal *et al.*, 2006).

1.5.2 Pesticide concentration

Applied pesticide concentration is one of the most important parameters to determine the biodegradation rate. The degradation of numerous pesticides follows the pseudo-first-order kinetics where the biodegradation rate depends on residual pesticide concentration (Topp *et al.*, 1997; Pal *et al.*, 2006). The concentration of pesticide in soil [P], the rate of biodegradation (i.e., -d[P]/dt) was determined from the appliance of important parameter. The biodegradation rate decreases approximately in proportion with the remaining pesticide concentration (i.e., d[P] /dt = -*k*[P]), where d[P]/dt is pesticide concentration gradient with respective time; *k* is a biodegradation rate constant, and P is a pesticide concentration.

The half-life values of inceptisol, vertisol, and ultimo were essentially independent of the initial pesticide dose, i.e., 10.1~31.0 days (1.0 μg kg^{-1} soil) vs. 13.0~29.2 days (10.0 μg kg^{-1} soil) (Gupta and Gajbhiye, 2002). In theory, for a 20-day half-life, the pesticide concentration should decay to 0.2% of its initial concentration after 180 days. However, the biodegradation rate, *k*, is smaller at higher initial concentrations. The concentrations of pesticides (e.g., atrazine, carbofuron, cypermethane, and chloropyrifos) used in experimental studies are given in **Table 3**.

1.5.3 Types of Soil

Properties of the soil such as clay content, organic matter content, water content, pH do influence the biodegradation of pesticides. (Gupta and Gajbhiye, 2002; Pal *et al.*, 2006). The microorganisms play an important role in biodegradation of pesticides contaminated soil in the environment. The soil particles can absorb the pesticides, thereby regulating bioavailability and influencing persistence of pesticides (Pal *et al*., 2006). The activity of microorganisms towards pesticide biodegradation is influenced from soil properties like clay content and organic matter. Gold and Rangarajan (1996) reported that a number of variables (e.g. soil type, clay and content, pH) can highly influence the persistent pesticides including bifenthrin, chlorpyrifos, cypermethrin, fenvalerate, permethrin, isofenphosunder in field circumstances. Jones and Ananyeva (2001) further confirmed the biodegradation rates of metalaxyl and propachlor in the soils were depending on soil conditions. The half-lives for metalaxyl and propachlor applied into arable, pasture and pine forest soil were 19, 10, and 36 days and 6.1, 2.6, and 8.2 days, respectively. The biodegradations of diazinon was quicker in the silty followed by sandy loam and sandy soils (Monkiedje *et al*., 2003; Pal *et al*., 2006).

1.5.4 Moisture content

For the movement and diffusion of pesticide molecules, the water plays the role of solvent and it is important for bacterial degradation of pesticide. Typically, in dry soils the biodegradation of pesticide is sluggish. Transformation of pesticides increases proportionally with the water content of the soil (Chowdhury *et al*., 2008; Gillett, 1989). There was slow degradation of atrazine and trifluralin herbicides in aerobic conditions than anaerobic condition (Chowdhury *et al*., 2008).

1.5.5 Temperature

The degradation of pesticide is influenced by temperature and depends on the pesticides structure. Pesticide adsorption is affected by temperature and changing its hydrolysis rate and solubility in a soil, in terms of hydrolysis rate and solvation (ΔG) (Burns, 1975b; Racke *et al*., 1999). The activity of bacterial growth is optimal in a well-defined physiological temperature range, 25~35 °C (Alexander *et al*., 1979). Hence, the mesospheric range of temperature 25~40°C is optimum for degradation of pesticide (Topp *et al*., 1997). The 15~40°C range of temperature is favourable for isolated pesticide-degrading bacterial species (Singh *et al*., 2006a; Singh *et al*., 2006b). Optimal degradation temperature range of pesticides is shown in **Table 3**. Qingyan *et al.* (2008) reported that atrazine degradation is maximum at a temperature of 30°C and found 95% removal at a 500 mg/L concentration. Literature survey reveals that the optimum temperature range is 25~30°C for degradation of carbofuran, chlorpyrifos, and DTT as shown in **Table 3** (Plangklang and Reungsang, 2009; Kong *et al*., 2013; Das, 2015; Liu *et al*., 2016). Temperature mainly affects metabolism of bacterial species like *Bacillus* species, *Pseudomonas* species, and *Alcaligenes* species, which

performed well at a temperature range of 25~30°C (Cycon *et al*., 2009; Kong *et al*., 2013; Mandal *et al*., 2014).

1.5.6 pH

The pH of soil is an important parameter to influence the pesticide degradation (Arshad *et al*., 2007; Sikdar *et al*., 1998). Microorganisms produced the enzymes and involved in the biodegradation of pesticide. The produced enzymes are pH-dependent and have optimum range from 6.5 to 7.5 (Sikdar *et al*., 1998). Pesticide degradation is affected by pH of soil (Burns, 1975a). The alkaline/acid catalyzed hydrolysis reaction liable to biodegradation of pesticide which is depends on pH (Peer *et al*., 2007; Singh *et al*., 2006b; Xu *et al*., 2008). The bacterial metabolism performs well at neutral pH hence the bioremediation studies were typically performed at pH 7. For example, Das *et al*. (2015) conducted the atrazine degradation study at pH 7 by *Pichia kudriavzevii* and found efficient removal by 94.3% at neutral pH.

1.6 Field applications of pesticides bioremediation techniques

According to the site selected for pesticide treatment, there are two basic treatment options such as *in situ* and *ex-situ* bioremediations. In case of *in-situ* bioremediation the treatment of pollutant is performed at the contaminated site, but in *ex-situ* bioremediation the treatment of pollutant is done elsewhere.

1.6.1 *In-situ* bioremediation techniques

In-situ bioremediation technique has the bacterial community stimulated with the additions of nutrients, microbes and optimized environmental factors (Seech *et al*., 2008). These techniques engage treating polluted substances at the selected site of pollutant. Site excavation is not required and hence little or no disruption to the soil structure. *In-situ*

technique explained the biodegradation of organic pollutants under the natural conditions and mineralized product obtained as water, carbon dioxide or other minimally toxic products. It is cost effective, eco-friendly and cleanup the contaminated sites by the sustainable approach. Seech *et al*. (2008) reported a case study on the treatment of dieldrin in a soil by daramend cycled using *in-situ* bioremediation. In United States at coastal Florida in November 2004 experiment was conducted for the removal of Dieldrin from 2,600 tons of soil. The total cost by *in-situ* bioremediation technique was found approximately US$12.50 yd^{-3} (Seech *et al*., 2008).

1.6.2 *Ex-situ* bioremediation techniques

Ex-situ techniques involve excavating/removal of the polluted soil from selected sites and are transported to another site for treatment. *Ex-situ* techniques are ranked according to the cost of treatment, type of pollutant, depth of pollution, geographical location, and degree of pollution. The *ex-situ* bioremediation technique is more expensive than *in-situ*, because in case of *ex-situ* technique there is need to design the recator, transportation and treatment of contaminated soil etc. (Jorgensen, 2007). *In-situ* technique is favored more than *ex-situ* technique for environmental reinstatement of polluted soils and water (Jorgensen, 2007). Several factors like indigenous population of microorganism, site conditions, and the quantity, type, and toxicity of pollutant etc. are important for selection of appropriate bioremediation technology.

A case study on *ex-situ* treatment was carried out for the initial concentrations of Toxaphene 29 mg/kg, DDT94 mg/kg, DDD 132 mg/kg, and DDE 94 mg/kg (Seech *et al*., 2008; Plangklang and Reungsang, 2010). These researchers reported that the remediation goals were reached on various organochlorine pesticides (OCPs) in ground water/saturated

soils in the United States. The *ex-situ* bioremediation objective (i.e., Toxaphene 29 mg/kg, DDT 94 mg/kg, DDD132 mg/kg, and DDE 94 mg/kg) were reached with the appliance of 3 to 12 cycles. The initial concentrations has important role to achieve the remediation goal and required number of treatment cycles (Seech *et al*., 2008; Plangklang and Reungsang, 2010). The results indicate that the initial concentrations of DDE, DDD, DDT and Toxaphene, were reduced from 180, 81, 25 and 189 and to 52, 9, 6 and 10, mg/kg, respectively. The variation cost for treatment of above pesticides were reported to be (US$ 29~63 t^{-1}) per ton related to initial concentration (180, 81, 25 and 189) and the average cost was US$ 55t^{-1} for the treatment of approximately 4,500 tons of soils (Seech *et al*., 2008).

1.7 Bioreactors

Bioreactors are used for the continuous monitoring of waste processing under controlled conditions. Bioreactor technology can be customized in different configurations to take full advantage of bacterial capability (Plangklang and Reungsang, 2010). Yadav *et al*. (2014) have investigated the performance of batch and continuous packed bed bioreactors for the degradation Chlorpyrifos by *Pseudomonas* species. Several types of bioreactors are available worldwide: batch, sequential batch continuous, membrane, fluidized bed, and hybrid reactor systems the details of the bioreactor were provided in Asadi *et al*., (2012) and Neoh *et al*., (2016).

The advantages of bioreactor are to treat the environment pollutant under control condition. Bioreactors have several limitations like high operation and capital costs as well as dig of polluted soil. The various *ex-situ* bioremediation techniques are available including land-farming, composting and bio-pile. These methods are found to have various disadvantages such as

required huge area, extensive treatment period, and restricted bio-availability of pollutants (Vidali, 2001; Asadi *et al.*, 2012).