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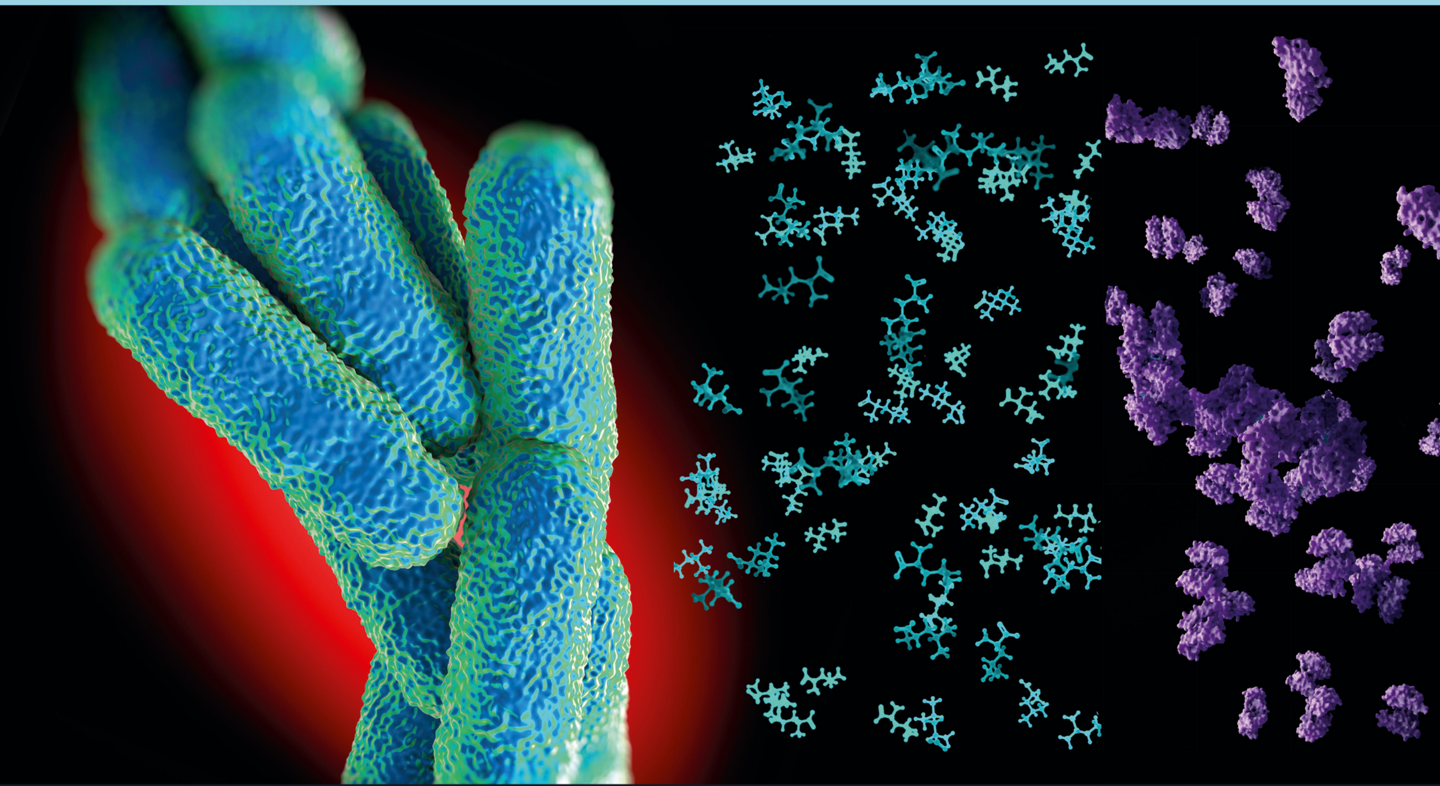

Microbial Syntrophy-mediated Eco-enterprising

Edited by

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CHAPTER 2

The bioremediation of agricultural soils polluted with pesticides

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Abstract

The use of pesticides in agriculture is dramatically increased. They are indispensable elements in protecting the crops from pests, weeds, and diseases while enhancing the harvest. Imprudent high-intensity application of pesticides in agriculture for prolonged durations has imposed serious crises viz., barren soils, polluted waterways, biodiversity obliteration, severe health concerns, etc. Ambitious efforts are being made to reverse these impacts. Among various technologies available today for remediation of pesticide-contaminated soils, bioremediation seems to be environmentally safe and cost-effective. The present chapter provides an overview of pesticides, their role in agriculture, and the negative impacts of over-application. The chapter mainly discusses the potential use of microorganisms in rectifying pesticide-contaminated agroecosystems and the influence of biotic and abiotic factors on the process. Finally, the chapter provides an update of recent research outputs which monitored and evaluated the efficiency of the technique, its advantages and disadvantages, and indications for future studies.

Keywords: Agricultural soils, Agrochemicals, Bioaugmentation, Bioattenuation, Soil pollution, Soil bioremediation, Pesticides, Microbial bioremediation, Indigenous microorganisms

1 Introduction

Many countries in the world where the main role in their economy is played by agriculture are currently facing an alarming situation due to various adverse impacts imposed on the environment and public health by pesticides. A recent study conducted on estimating the global scale risk of agrochemical-related pollution revealed that 64% of agricultural land around the world is under the threat of getting polluted by these chemicals (Tang et al., 2021). Contamination of groundwater resources, soil microbial population imbalances, and accumulation in food chains are among the fundamental impacts of extensive pesticide over-use in agriculture which attributes to long-term deleterious impacts such as environmental pollution, biodiversity destruction, global warming, and severe risks on human and animal health.

With the ever-growing global population, the requirement of a sufficient food supply to meet the increasing demand is unavoidable. In this challenging process of securing food for all, the use of agrochemicals has become a part and parcel of enhancing the crop yield quality and quantity (Sharma et al., 2019). However, the adverse effects emanated by the extensive over-use of these chemicals emphasize the importance of their limited use and the significance of discovering efficient, ecofriendly techniques to remediate the contaminated sites (Singh et al., 2017).

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Bioremediation is one such emerging technology which is currently recognized as an environmentally friendly approach to clean the agrochemical contaminated soil. The technique utilizes biological systems to decontaminate organic pollutants in the environment into harmless or less concentrated forms (Vishwakarma et al., 2020; Zouboulis et al., 2019). The current chapter mainly discusses the applications of bioremediation in rectifying pesticide-associated pollution of agricultural soils with a special focus on the potential use of various microorganisms in the process.

2 An overview of pesticides; their role in agriculture and the adverse impacts associated with the over-use

2.1 Overview of pesticides and their roles in agriculture

The invention of agriculture dates back to about 10,000 years ago into the era of Mesopotamian civilization (Kislev et al., 2004). Since then the losses of harvest due to infectious diseases, animals or insect attacks, and competitive growth of other plants became a great incentive to find efficient modes of preventing the losses. From ancient times, attention was paid to develop highly effective and time-saving techniques to protect the food crops (Abubakar et al., 2020). Subsequently, the elimination of pests and diseases using chemical compounds emerged. The earliest documented application of a chemical compound as a pesticide is the use of sulfur about 4500 years ago by Sumerians. It was the dawn of highly sophisticated agrochemicals available for sale in today's markets.

In 2014, the Food and Agricultural Organization of the United Nations in collaboration with the World Health Organization endorsed "The International Code of Conduct on Pesticide Management". This particular document provided overall guidance to everybody from pesticide manufacturers to the common general public on safe handling of pesticides from production to disposal. The same document broadly defined a pest as "any species, strain or biotype of plant, animal or pathogenic agent injurious to plants and plant products, materials or environments, vectors of parasites or pathogens of human and animal disease and animals causing public health issues". The document further described pesticides to be "any substance, or a mixture of substances composed of chemical or biological ingredients intended for repelling, destroying or controlling any pest, or regulating plant growth".

Currently, pesticides are categorized into several groups based on various criteria. Their toxicity levels, organisms inhibited, chemical composition, mode of entry into the host, working mechanism, sources of origin (synthetic chemical pesticides or biopesticides) are among the major principles of classifying pesticides (Akashe et al., 2018). Pesticides are divided into primary categories on the basis of pest organism they inhibit and cover a wide range of chemicals used for multiple purposes such as insecticides, herbicides (weedicide), fungicides, rodenticides, nematicides, biocides, algicides, acaricides, molluscicides, etc. (Pandey et al., 2018; Brandt et al., 2017; Rani et al., 2017; Yadav and Devi, 2017; Gavrilescu, 2005). The major chemical classes of organic pesticides are comprised of organochlorines, organophosphates, organometallic compounds acetamides, carbamates, triazoles and triazines, neonicotinoids, and pyrethroids (Gilden et al., 2010) while organochlorines are extremely toxic as they are nonbiodegradable and have a tendency of bioaccumulation (Ortiz-Hernandez et al., 2013). There are also heavy metal-containing inorganic pesticides, viz., lead arsenate, chromated copper arsenate, copper acetoarsenite, borax, and boric acid complexes, etc. (Tarla et al., 2020).

Herbicides are a broad group of phytotoxic pesticides that are used to kill unwanted plants in agricultural fields. They are grasses or weeds with a higher growth rate that compete with crop plants for nutrients, water, space for growth and act as bearers of pests (Gupta, 2011). Other pesticides target macro and microorganisms that are attracted to the fields such as insects, worms or nematodes, rodents, bacteria, fungi, algae, etc. that compromise the growth and yield of desired crops. In addition, "biopesticides" are extracted from biological systems such as animals, plants, and microorganisms that include microbial pesticides, plant-incorporated protectants, and biochemical pesticides.

2.2 Adverse impacts of pesticides over-use in agriculture

Soil is the basis of agriculture that renders space for the growth of food crops (Ashraf et al., 2014). Although the large-scale application of pesticides is currently an integral part of boosting crop yields, their long-term unsustainable addition into the soil contributes greatly to soil pollution. When these agrochemicals are added into the soils, they are not completely utilized for the intended purpose. Instead, around 99% become persistent as contaminants and distribute in the agricultural soils in a multiphasic manner. For instance, they may accumulate in the fields by attachment with the soil particles, dissolving in soil water, or by suspension in the soil atmosphere (Mishra et al., 2021; Boopathy, 2000). The problem intensifies when used in high quantities because their natural degradation becomes arduous. As a result, the amounts that persist in the soil drastically increase leading to pollution of soil and water, damage the microflora, microfauna, and hinder the mineral absorption of plants (Van der Werf, 1996).

The serious environmental concerns associated with the widespread application of pesticides initiate the deterioration of soil health. The soil functions, soil quality, soil productivity, and soil biodiversity are extremely threatened by extensive agrochemical usage. The indigenous microbial communities in soil that carry out essential agricultural-ecosystem processes are directly exposed to the deposited agrochemicals. Those chemicals interact with soil microorganisms and change their biochemical and physiological behavior leading to pesticides associated inhibition of microorganisms (Singh and Walker, 2006). Ultimately, the soil becomes infertile because the main soil microbial functions, viz., increasing the bioavailability of nutrients by nutrient cycling (nitrogen fixation, phosphate solubilization), production of plant growth stimulation hormones, plant debris or cellulose degradation, inhibition of plant pathogens, get highly restricted (Hayat et al., 2010). Another common risk linked with pesticide usage is the development of resistance in the targeted pests and weeds that make them uncontrollable and continue to destroy the crops (De Bon et al., 2014).

Moreover, the displacement of pesticides from agricultural fields through wind and rain creates additional agrochemical-enriched sites such as natural surface water bodies and groundwater resources. They act as extra exposure routes of agrochemicals for humans and animals (Pérez and Eugenio, 2018). Especially, the runoff of pesticides into the lakes and coastal waters consequently leads to oxygen depletion, eutrophication, kills of fish and other aquatic organisms, stinking water, and release of offensive odors into the environment while degrading water quality (Chen et al., 2017). Nitrates from agricultural runoff or leachate are recognized as the most common groundwater pollutant that contaminates drinking water (The United Nations World Water Development Report, 2013). Thereby, pollution of potable water has currently become a major concern of pesticides over-use (Rasmussen et al., 2015). Eventually, these chemical compounds end up entering food chains in the ecosystem in a persistent manner and become toxic to non-target species including humans, flora and fauna, and soil microbial communities (Lozowicka et al., 2016).

The emergence of health hazards on humans due to injudicious use of pesticides and other persistent organic pollutants in agriculture is drastically increased with time. Acute pesticide poisoning (APP) is a phenomenon that might cause chronic health issues in humans (Boedeker et al., 2020). The 48-h occupational or unintentional exposures to pesticides may develop certain respiratory, neurotoxic, cardiovascular, endocrine, gastrointestinal, nephrotoxic disorders, and allergic reactions in humans. These conditions may be fatal or non-fatal (Henao and Arbelaez, 2002). In addition, the bioaccumulation of agrochemicals in living beings causes malfunctions of the endocrine and reproductive systems (Vos et al., 2000). Another well-known example is methemoglobinemia (blue-baby syndrome), a deadly illness among infants which is caused by polluted water. Humans, especially farmers, when exposed to lower doses of agrochemicals for an extended period, experience reduced immunity and intelligence, cancer, and allergic or non-allergic asthma (Margni et al., 2002; Hoppin et al., 2009; Yadav et al., 2015; Alavanja et al., 2003).

It is evident that the adverse impacts associated with improper application of pesticides are extremely diverse and complicated. Currently, the problem is severe that urgent steps must be followed to minimize the intensity of the impacts. Most of the farmers are not aware of the safe handling of pesticides and the importance of using personal protective equipment. In addition, their lack of knowledge on hidden threats behind the intensive use of harmful chemicals further complicates the situation. Therefore, conducting awareness programs to make them knowledgeable is important. Moreover, developing ecofriendly alternatives such as biopesticides and publicizing them might play a significant role in keeping adverse impacts of pesticides over-use to a minimum level.

3 Conventional methods of remediating agricultural soils polluted with pesticides

Excavation of polluted land and transporting its soil into a landfilling site is one of the conventional methods of remediating soils contaminated with various pesticides. Another approach is a containment of the highly polluted areas on-site by setting boundaries to the area and covering up (Zouboulis and Moussas, 2011). However, these methods are not capable of providing a sustainable solution for the issue. Instead, they may add more pressure to the prevailing situation. For instance, landfilling creates another polluted scrap of land. In addition, excavation and transportation of contaminated soils demand a large amount of capital and labor investment. On-site containment of pollutants could worsen the problem as it is not helpful in the complete removal of pollutants from the site. Therefore, these methods are neither ecofriendly nor cost-effective.

Furthermore, incineration of pollutants at elevated temperature, dechlorination, and UV radiation are some of the physical and chemical methods utilized for destroying soil pollutants or for their conversion into less/or non-toxic forms (Zouboulis and Moussas, 2011). Unfortunately, these methods are far more complicated and not user-friendly or environmentally friendly. As a consequence, novel technologies that apply biological methods of detoxifying pollutants emerged.

4 Bioremediation of agricultural soils polluted with pesticides

The long-term environmental and public health concerns that occurred in the aftermath of irresponsible anthropogenic activities are usually dreadful and not easily reversible. Cleaning up pesticides polluted agricultural soils also shares the same severity. Although an ideal pesticide should have the ability to cause rapid destruction of the target pest and easily degrade into non-toxic substances, most of the synthetic compounds that are currently utilized are not readily degradable (Doolotkeldieva et al., 2018). However, untiring efforts have been made by scientists to overcome this problem through inventing ecofriendly and low-cost modes of removing environmental pollutants. Bioremediation is one such emerging technology that carries out the decontamination of polluted sites including agricultural lands using biological systems namely, green plants, microorganisms, or their enzymes, in order to restore the original condition of the site (Glazer and Nikaido, 1995).

The bioremediation process aims to bring considerable decrease in the agrochemical persistence in the environment (Velázquez-Fernández et al., 2012; Lal et al., 2010). It involves a series of biologically mediated reactions that modify the chemical structure of the particular xenobiotic compound into harmless states including carbon dioxide, water, minerals, and non-toxic biomass (Vaish and Pathak, 2020; Azubuike et al., 2016). Among the available bioremediation techniques, microbial bioremediation is considered to be more effective, inexpensive as well as ecofriendly. Moreover, there are in situ and ex situ approaches to accomplishing bioremediation (Ying, 2018). In in situ bioremediation, pesticides are biologically degraded onsite under natural environmental conditions using bioattenuation, biostimulation, or bioaugmentation whereas ex situ bioremediation needs excavation and movement of contaminated soils to another site for treatment such as using bioreactors or composting (Megharaj et al., 2011).

4.1 Microbial bioremediation of agricultural soils contaminated with pesticides

The diversity of microorganisms is incredible that the majority of them contribute greatly to ecologically important functions. Among all, the maintenance of environmental balance rests crucially upon microbial activities involved in nutrient cycling between soil, water, living organisms, and the atmosphere (Tortora et al., 2010). Microbial role in scavenging pollutants, a part of the nutrient cycling process, is known for decades. Usually, bacteria, fungi, protists, and other microbial types in any soil, including agricultural soils, are constantly employed in a process of organic matter degradation (Speight, 2018). Although the soil-inhabited microbial population degrades various soil pollutants to fulfill their energy and carbon needs, they are not capable of efficient removal when the pollutants are added on large scale. Biotechnological approaches such as bioremediation could be utilized to enhance these microbial activities (Yang et al., 2020). Microbial bioremediation is a process that uses different microbial species to clean up the pollutants in soil. Bacteria, archaea, and fungi are considered to be typical key bioremediating agents (Strong and Burgess, 2008).

4.1.1 In situ bioremediation

In situ methods are mostly preferred over ex situ methods for bioremediation. It is usually comprised of three different strategies that the use of particular technology is decided by various factors, such as site conditions, types of the microorganisms present and their specific functions, quality and quantity of the chemical pollutant present in the site (Ying, 2018). These strategies could be explained as the underlined fundamental theories applied in microbial bioremediation technologies.

4.1.1.1 Bioattenuation

This particular remediation technique is also called natural attenuation which is a process that completely depends on the natural microbial degradation. There is no anthropogenic involvement in the process while its productivity is determined by the natural microbial metabolic potential in degradation, removal, alternation, immobilization, or detoxification of various pesticides molecules (Abatenh et al., 2017). In this process, soil microorganisms have to adapt and interact with the polluted environment. The degradation process could be either anaerobic or aerobic depending on the type of microorganisms present. However, the process is less advantageous in terms of time consumption due to the lack of pertinent microbial species in sufficient population densities. If the agrochemical type used is a persistent organic pollutant, the duration required for remediation is extended. In addition, continuous screening or assessment of pollutants removal is needed to confirm the efficient performance of bioattenuation.

4.1.1.2 Biostimulation

This process brings in human activities for stimulating the natural microbial pesticides degradation. The intentional addition of water, fertilizers, nutrients (growth supplements and trace metals), electron donors, and electron acceptors into the polluted agricultural soil stimulates the indigenous microbial activities (Owsianiak et al., 2010). A stepwise approach is usually recommended to stimulate naturally existing bacterial and fungal communities. The initial step is the addition of fertilizers, growth supplements, and trace minerals to stimulate microbial growth. Second, the adjustment of environmental parameters viz., pH, temperature, moisture, redox conditions, and oxygen of soil in favor of microbial communities is done (Kumar et al., 2011; Adams et al., 2015; Hussain et al., 2009). The fortification of soil with these physicochemical parameters is extremely important to accelerate the microbial metabolism of agrochemicals in the soil. One of the key determinants of a successful biostimulation process is the availability of properly balanced carbon, nitrogen, and phosphorus ratio (C: N: P ratio) in the targeted agricultural site. These are macronutrients necessary for microbial growth (Wolicka et al., 2009; Madhavi and Mohini, 2012).

Bioventing is also an in situ bioremediation method which uses a biostimulation strategy. The pesticide degrading aerobic microbial growth is stimulated by flushing oxygen through the contaminated soil (Hinchee, 1993).

4.1.1.3 Bioaugmentation

During bioaugmentation, individual microorganisms or syntrophic microbial consortia with a confirmed potential of degrading pesticides are added into the polluted agricultural soils. Choosing the correct microbial strains for the process is extremely important. The selected species must be able to degrade the maximum amount of pollutants to a minimum toxic level within a limited time period. However, a thorough knowledge on the population dynamics of exotic and indigenous microorganisms that reside in the same habitat is extremely important to get a favorable outcome of the process (Cameotra and Singh, 2008).

The bioaugmentation process aims to expand the remediation capacity of existing microbial populations in the site with the assistance of microbial supplements. The preparation of microbial inocula for bioaugmentation is done using general microbiological techniques and advanced genetic engineering techniques. After the isolation of microorganisms from the contaminated agricultural fields, they can be enriched in pesticides containing broth media to increase the population of pesticide degrading microorganisms. If the site is contaminated with a specific pesticide, the enrichment can be done using that particular agrochemical in the medium.

Recently, during bioaugmentation, genetically modified microorganisms (GMMs) with enhanced pesticides degrading capacity are added to the polluted agricultural sites. Using GMMs as bioremediators might be faster in completely removing pollutants than the soil inhabited indigenous microbial species (Sayler and Ripp, 2000). However, their competitive growth with the natural microbial population in the soil acts as a limitation of using GMMs in the process. Moreover, their release into the agroecosystems is quite challenging due to the insufficiency of knowledge on their interactive adaptability to the fluctuations of nutrients, water, temperature, and other biotic and abiotic factors in the targeted environment. Therefore, the addition of GMMs must be done under continuous surveillance.

By genetic modification, the rates of existing pesticides degradation pathways of microorganisms could be enhanced. Moreover, easily culturable microorganisms with fast growth rates could be incorporated with genes responsible for degrading recalcitrant pesticides molecules.

Furthermore, bacterial plasmids which carry specific genes encoding essential enzymes for the catabolism of pesticides and related molecules can be transferred into indigenous bacterial species. Therefore, GMMs can be used effectively for bioremediation although further research is needed for broadening the applications (Kulshreshtha, 2013).

4.1.2 Ex situ bioremediation

4.1.2.1 Composting

There are multiple strategies of using composting for decontamination of agricultural soil viz., on-site direct composting of polluted agricultural soils, the addition of compost into the soil as a fertilizer/ nutrient supplement or surfactant to stimulate the indigenous microbial growth, and addition of compost as a source of pollutant degrading microbes (bioaugmentation). Composting uses an aerobic, thermophilic microbial treatment process in which the contaminated soil is piled up, covered and periodically aerated to facilitate microbial activities. It is also an ecofriendly, less expensive method of bioremediating agricultural soil pollutants. Moreover, adding chemical fertilizers into the soils could be replaced by compost. As an organic nutritional supplement, compost can improve the soil quality, microbial balance, and subsequent crop growth (Lim et al., 2016; Xia Guo et al., 2019: Chen et al., 2015). With proper management, composting can be practiced as an in situ bioremediation technique.

4.1.2.2 Land farming

In land farming, pesticides-containing soil is strewn over a large plot of land which is allocated for scavenging a certain pesticide. Spreading polluted soil over a large surface area tends to dilute the pesticides present and the aerobic indigenous microbial activity targets the biodegradable proportions of these pesticides (Castelo-Grande et al., 2010). Periodic soil tillage is carried out to improve the aeration and facilitate the complete degradation of pesticide molecules. Mainly, nutrient amendment and proper irrigation could stimulate the indigenous microbial functions to enhance land farming-mediated bioremediation. This process can be conducted in situ as well.

4.1.2.3 Slurry phase biological treatment

The excavated soil is mixed with nutrients and water inside a series of tanks. The contents mixed into aqueous slurry are then provided with proper reaction temperature, oxygen, and pH in order to facilitate aerobic microbial pollutant degradation (Gavrilescu, 2005).

4.1.2.4 Biopiles

Biopiles is an ex situ bioventing technique that the polluted soil that must be excavated and transported to another site. Soil is then piled up in heaps and aerated to facilitate aerobic microbial activities. It is also a combination of composting and land farming. In this strategy, bioremediation is accelerated by providing optimal temperature, pH, moisture, and essential nutrients for the rapid growth of indigenous microbial species (Gavrilescu, 2005).

All these strategies indicate the enormous potential of using microorganisms in remediating polluted soil ecosystems including agricultural soils. However, the microbial species involved as well as the physicochemical conditions of the contaminated site should work conjointly to attain the full productivity of the processes.

4.2 Factors affecting the microbial bioremediation

Microorganisms show great potential in degrading diverse groups of organic pollutants due to their metabolic mechanisms and inherent adaptability to environmental fluctuations. Therefore, successful bioremediation demands a healthy population of microorganisms in the environment and favorable environmental parameters that facilitate their growth. The biotic factors that influence the bioremediation process are the microorganisms involved and their nutritional requirements while abiotic factors are environmental factors.

4.2.1 Biotic factors affecting the microbial bioremediation

The biotic factors that have a severe impact on the bioremediation process simply include the richness of microbial population and their functional diversity (enzyme production, toxic metabolite production, tendency of mutation and horizontal gene transfer, microbial interactions such as competition for limited carbon sources, symbiosis, antagonism, predation, etc.) in the contaminated soil and other flora and fauna which directly influence the productivity of the bioremediation.

The biochemical activities of microorganisms such as bacteria fungi, protists, and algae are vital for the remediation of pesticides. The potential of microorganisms to carry out bioremediation by degradation, removal, alteration, immobilization, or detoxification of environmental pollutants depends on their enzymes-mediated biochemical pathways (Abatenh et al., 2017). Microorganisms use pesticides and related compounds as carbon and energy sources for their rapid growth in the natural environment. The breaking down of particular pesticides into their less toxic molecular states is usually achieved by the interactive action of microbial communities instead of individual microorganisms.

The microbial species suitable for the bioremediation process must exhibit a set of unique characteristics. Most importantly, they must be capable of adapting to constant fluctuations in the environmental conditions such as temperature, pH, moisture, and aeration while being a resistant genotype for the particular pollutant considered. Moreover, they must have potential in metal-processing in the contaminated site (Stelting et al., 2010). In addition, microbial strategies of adapting to harsh conditions in the environment include making changes in the growth rate, gene expression, physiological or enzymatic activities, and undergoing changes in intimate or symbiotic associations with other organisms. Furthermore, they synthesize bioactive compounds, participate in biofilm formation, and produce biosurfactants when they are exposed to extremes in temperature, salinity, or depletion of micronutrients (Mangwani et al., 2014).

The fundamental mechanism of microbial bioremediation of pesticides is the ability of microorganisms to break down xenobiotic compounds by producing a wide range of enzymes. Microbial oxidoreductases, oxygenases, monooxygenases, dioxygenases, laccases, manganese peroxidases, lignin peroxidases, microbial versatile peroxidases, and other extracellular hydrolytic enzymes viz., amylases, proteases, lipases, DNases, pullulanases, and xylanases are important groups of enzymes in catalyzing this process (Karigar and Rao, 2011). The type of microorganisms involved in the bioremediation process is also an extremely important rate-limiting biotic factor of the process.

4.2.1.1 Bacteria in bioremediation

Bacteria have been the most prominent group of bioremediating microorganisms that have been applied so far. Bacterial genera included in gamma-proteobacteria (*Pseudomonas*, *Aerobacter*, *Acinetobacter*, *Moraxella*, and *Plesiomonas*), beta-proteobacteria (*Burkholderia*, *Neisseria*), alpha-proteobacteria (*Sphingomonas*), Actinobacteria (*Micrococcus*), and Flavobacteria (*Flavobacterium*) are recognized as efficient pesticides bioremediating bacterial groups (Mamta and Khursheed, 2015). According to a recent study conducted on bacterial endosulfan pesticide degradation, five bacterial genera including *Klebsiella*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, and *Bacillus*, were found to be endosulfan degraders (Kafilzadeh et al., 2015). It proves that different bacterial genera participate in co-metabolizing the same pesticides. Moreover, enzymatic mineralization of certain pesticides by bacterial species needs both aerobic and anaerobic conditions (Langenhoff et al., 2002). For instance, organochlorines which are considered as a major highly persistent group of agrochemicals need anaerobic conditions for dechlorination and aerobic conditions for breaking down organic or aromatic constituents of the compound (Baczynski et al., 2010). In addition, some anaerobic bacteria including *Dehalobium chlorocoercia* DF1 and *Dehalococcoides mccartyi* have been successfully utilized for the bioremediation of polychlorinated biphenyls (Payne et al., 2013).

4.2.1.2 Fungi in bioremediation

The potential of fungi to degrade pesticides including different insecticides, fungicides, and herbicides has been well-recognized. Fungi which show a saprotrophic nutritional mode are highly efficient in degrading the soil pollutants such as agrochemicals. They use diverse enzymatic pathways for hydrolyzing organic compounds. In addition, fungi transform agrochemicals into nontoxic molecules by making slight alternations in their molecular structure and release them into the soil for subsequent easy degradation by other soil microflora (Hai et al., 2012). When choosing fungal isolates for bioremediation of agricultural soils with pesticides and related compounds, these characteristics could be considered as a baseline.

The process which utilizes fungi for bioremediation is called “Mycoremediation”. There are previous reports of using filamentous fungi and members of the fungal class basidiomycetes (e.g., white-rot fungi and brown-rot fungi) for this purpose. *Aspergillus flavus*, *A. niger*, and *Trichoderma harzianum* have been found capable of degrading chlorpyrifos and endosulfan (George et al., 2014). The efficiency of white-rot fungi; *Lentinus subnudus* and *Trametes hirsute* to degrade pesticides and herbicides such as Dichloro diphenyl trichloroethane (DDT) and endosulfan has been previously reported (Nwachukwu and Osuji, 2007; Singh and Singh, 2016; Singh and Kuhad, 1999). Moreover, *Pleurotus ostreatus*, *Trametes versicolor*, *Lentinula edodes*, and *Bjerkandera adusta* are some other fungal species that are capable of carrying out bioremediation of pesticides and herbicides contaminated soils (Singh, 2006).

The commonly used herbicides include atrazine, metolachlor, clodinafop propargyl (CF), 2, 4-dichlorophenoxyacetic acid (2, 4-D), diuron, paraquat, and glyphosate (GP) (Pandey et al., 2018). *Mucor genevensis*, *Phoma glomerata*, *Chrysosporium pannorum*, *Aspergillus penicillioides*, *A. niger*, and *Fusarium oxysporum* are useful in degrading 2, 4-D and its derivatives (Vroumsia et al., 2005).

Diuron is used in cotton cultivations. *Rhizoctonia solani*, *Pestalotiopsis versicolor*, *Sporothrix cyanescens* and *Cunninghamella echinulata* have shown higher potential in breaking down diuron (Ellegaard-Jensen et al., 2013). Many fungi including *Aspergillus*, *Rhizopus*, *Fusarium*, *Penicillium*, *Trichoderma*, and *Phanerochaete* are proficient to degrade atrazine which is a selective herbicide (Mougin et al., 1994).

Most of the above fungi are recognized as lignocellulolytic fungi. They are considered the most promising fungi in bioremediation applications as they are capable of producing unique sets of extracellular enzyme complexes to degrade recalcitrant compounds such as lignin (Anastasi et al., 2013). In a recent study conducted by the authors (Jayasekara et al., 2020), several filamentous fungi; *Trichoderma* spp., *Aspergillus* spp., and *Penicillium* spp., and 18 basidiomycetes isolates including *Earliella scabrosa*, *Trametes hirsuta*, *Schizophyllum commune*, *Annulohypoxylon stygium*, *Lentinus sajor-caju* isolates recorded to be highly lignocellulolytic with higher total cellulase, lignin peroxidase, manganese peroxidase, and laccase enzyme activities. These oxidative enzymes target cellulose polymer and highly recalcitrant lignin in allochthonous organic matter. Therefore, these fungal isolates may have the potential of degrading various environmental pollutants including pesticides.

The authors further studied the potential use of lignocellulolytic microorganisms in the degradation of sugarcane bagasse which is a highly recalcitrant abundant cellulosic biomass in many countries in the world. A combination of *E. scabrosa* and *Aspergillus niger* was found to be highly efficient in degrading this recalcitrant biomass (Madusanka et al., 2019). Therefore, adding lignocellulosic biomass such as straw, decaying litter, bagasse, sawdust, etc., and lignocellulolytic fungi into the agricultural soils polluted with pesticides could be a promising way of simultaneous cleaning up of soil pollutants and adding nutrients for the plants and beneficial microbial growth.

4.2.1.3 Synthetic microbial communities in bioremediation of pesticides

Although microbial bioremediation is practiced as a promising method of neutralizing the agrochemicals in soil, incomplete degradation of target pesticide molecules is recognized as a drawback in the process. On the other hand, the resulted by-products might be greater in toxicity than the parent molecule creating a severe impact on living beings. The development of synthetic microbial communities is focused on overcoming these issues. In a synthetic microbial population, two or more defined microbial populations are assembled in a well-characterized and controlled environment while retaining their key metabolic functions. Therefore, a synthetic community with a known, simple, and easily controllable microbial structure could be capable of efficiently carrying out targeted agrochemical degradation. On the contrary, natural monocultures or microbial communities might contain various unknown microbial species with numerous unknown functions. Another advantage of using a synthetic microbial population is their resistance to fluctuating environmental conditions and adverse interactions imposed by invasive species such as antagonism, predation, etc. (Brenner et al., 2008; De Roy et al. 2014).

Designing a cooperative and stable microbial community which is focused on the successful completion of an expected biochemical function is a sequential process. The community must be initiated using harmless microorganisms. Next, it is allowed to degrade, detoxify or transform toxic waste material into target end products. Then the overall bioremediation process of the microbial community is optimized by continuous monitoring of its growth and function (Liu et al., 2017). Several synthetic microbial consortia are reported to be efficient in degrading certain pesticides and herbicides. For instance, a three-member consortium of two fungal strains and a bacterium (*Mortierella* LEJ702, *Variovorax* SRS16, and *Arthrobacter globiformis* D47) was found to be breaking down Diuron (Ellegaard-Jensen et al., 2013). A fungal-bacterial consortium consisted of *Mortierella* sp. LEJ702 and

Aminobacter sp. MSH1 was found to be successfully degrading 2, 6-dichlorobenzamide (Šašek et al., 1993). Therefore, using synthetic microbial consortia could contribute in an extremely efficient way to bioremediation of pesticides contaminated agricultural soils. However, the process requires the stable initial establishment of the community and continuous routine monitoring of its progress.

4.2.2 Abiotic factors affecting the microbial bioremediation

The abiotic factors that determine the process output are mainly environmental conditions in the polluted site such as pH, water holding capacity, soil texture, permeability, soil temperature, nutrient status, dissolved oxygen content, presence of electron donors and acceptors, contaminant load, and the nature of the pollutants accumulated in the site (chemical structure and composition, hydrophobicity, hydrophilicity, toxicity, biodegradability, etc.) (Mohan et al., 2006; Wang et al., 2007; Sihag et al., 2014).

4.2.2.1 Characteristics of the pesticide contaminant in the soil

The structure of the pesticide molecule and its concentration in the soil directly impact the microbial bioremediation process. Especially, the structure determines the chemical and physical features of the pesticide which eventually decide its overall microbial biodegradability. For instance, the pesticide molecules composed of phenyl rings with non-polar alkyl or halogen groups are highly resistant to microbial activities as they are insoluble in water. The solubility of a contaminant in an aqueous medium is also playing a major role in bioremediation steps. When a pesticide molecule contains hydroxyl or carboxylic groups attached to phenyl rings, they are highly bioavailable for microbial degradation as they are polar molecules with hydrophilic nature (Cork and Krueger, 1991; Chowdhury et al., 2008).

Pesticides concentration in the soil ecosystem is an important rate-limiting factor of a microbial bioremediation reaction. Higher pesticides concentration in soil inhibits the microorganisms and their enzymes leading to a reduced bioremediation rate. However, the presence of appropriate pesticides concentration in soil is essential for the induction of microbial pesticides degrading enzymes (Adams et al., 2015; Prakash and Suseela, 2000).

4.2.2.2 Nutrient availability in the soil for microbial growth

Microbial bioremediation is highly dependent on the availability of macro and micronutrients for microbial growth. Carbon, nitrogen, phosphorus, potassium, and calcium are essential nutrients for microbial cell growth and development. Although excess concentrations in soil cause microbial toxicity, metal ions are indispensable in microbial metabolism. The function of essential enzymes depends on the presence or absence of metal ions (Mg^{2+}, Co^{2+}, Mn^{2+}, etc.) in the soil. Especially, trace elements such as Fe, Zn, Cu, Mn, Mo, and Ni are also important due to their role in metalloenzymes. These metals play an important role in microbial cell membrane stability, nucleic acid structure, function and metabolism, and protein synthesis (Dedyukhina and Eroshin, 1991). Therefore, the application of bioremediation strategies into the soil with a lack of essential nutrients for microbial growth is not a better approach to remediating the pesticide contaminants.

The addition of soil amendments like compost, manure, and decaying plant debris is a better option for enriching the polluted soil with favorable nutrients as well as desired microbial populations. This is not a novelty to traditional agriculture. For instance, Sri Lankan farmers still practice this method to augment their arable lands including paddy fields and home gardens. They usually add cattle dung, paddy straw, sawdust, rice husks, and *Gliricidia sepium* leaves into the soil to improve the nutrients in the soil.

4.2.2.3 pH of the soil

An optimal pH of about 6–8 in range must be maintained in the pesticides contaminated soils in order to induce the bioremediation process (Adams et al., 2015). When the soil pH goes down the bioremediation activities of fungi and acidophilic bacteria are enhanced while other microbial activities are suppressed (Stapleton et al., 1998). Soil pH affects the pesticides solubility and bioavailability for soil microorganisms. Maintenance of a proper soil pH could therefore accelerate the microbial bioremediation process (Racke et al., 1997).

4.2.2.4 Temperature of the soil

Temperature is a crucial factor which determines the rate of any biochemical reaction. Soil temperature level starting from 30°C to about 40°C, which also falls under the mesophilic category of microbial growth temperature requirements, enhances and optimizes the microbial bioremediation of pesticides. Moreover, the molecular structure of the pesticide molecules is altered by the temperature fluctuations in the soil and at higher temperatures, increases their solubility (Margesin and Schinner, 2001; Topp et al., 1997). Maintaining the soil temperature at which the microbial activities are optimal is important to carry out an efficient and optimal bioremediation process.

4.2.2.5 Oxygen availability in the soil

The level of oxygen in the pesticide-contaminated soil determines the predominant microbial species inhabiting the soil based on their oxygen requirement. Accordingly, the bioremediation process could be aerobic or anaerobic. The complete removal of certain pesticides needs both aerobic and anaerobic conditions in the environment. Some pesticides are completely removed from the environment when the soil is anaerobic. As an example, rapid removal of herbicides atrazine and trifluralin could be observed under anaerobic conditions. The steps of the bioremediation of a compound may be altered according to the availability of oxygen. For instance, the initial steps of biodegrading polycyclic aromatic hydrocarbons (PAH) need oxygen for the oxidation of benzene rings by monooxygenase and dioxygenase enzymes activities (Sihag et al., 2014). The anaerobic oxidation reaction needs ferrous, nitrate, and sulfate ions as electron acceptors. In addition, aerobic reactions are more eco-friendlier than the anaerobic remediation processes. For instance, the anaerobic conditions tend to release greenhouse gases, like methane, into the atmosphere thereby leading to global warming (Bamforth and Singleton, 2005). Apparently, providing proper aeration for in situ or ex situ bioremediation processes is more advantageous in terms of achieving an ecofriendly pollutants remediation process.

4.2.2.6 Moisture content of the soil

Moisture or water is an essential element in biochemical reactions. Water acts as the reaction medium which dissolves and disperses all the reactants. Water-soluble pesticides are easily contacted with the hydrolytic microbial enzymes in the environment. Therefore, wet soil is capable of accelerating the bioremediation process than dry soil. However, plenty of water or water-clogging conditions in the agricultural fields could adversely impact oxygen diffusion in soils (Chowdhury et al., 2008).

4.2.2.7 Organic matter content in the soil

Agricultural practices heavily disturb the natural microbial diversity, population density, and decomposing reactions in the soil (Adriaens and Hickey, 1993). Crop harvesting also removes a considerable share of organic matter from agricultural soils. Therefore, the organic matter content in agricultural soil is lower than that of natural surface soil. When the soil is contaminated with pesticides and related agrochemicals, indigenous microbial activities get disturbed and result in subsequent organic matter decrease. However, soil organic matter may interfere in microbial pesticides removal processes by facilitating the adsorption of pesticides molecules to organic matter. Although the availability of organic matter in the soil diversely impacts the microbial pesticide bioremediation process, the initial presence of organic matter is essential for the development of active pesticides biodegradable, indigenous microbial population (Perucci et al., 2000).

In view of the above-mentioned facts, the complete removal of an agrochemical from the polluted agricultural site using an effective microbial bioremediation technology demands the proper environmental conditions and a group of most suitable microorganisms. In other words, an efficient bioremediation process is always an output of a strong correlation between biotic and abiotic factors in the polluted agricultural site. Most importantly, it is a complex interrelationship developed by the co-existence of biodegradable pollutants, microbial population capable of degrading those pollutants, and the environmental factors facilitating the process. However, it is obvious that the setting up of all the required factors in perfect order to enhance the effectiveness of the process is extremely complicated. It must be accomplished by continuous monitoring and gradual optimization of the process parameters.

5 Research on bioremediation of agricultural soils polluted with pesticides

5.1 Current areas of research on microbial bioremediation

The topic “bioremediation” is very popular among researchers. A number of researches have already been conducted on different areas related to bioremediation. The current chapter focuses on research activities carried out on microbial bioremediation of pesticides. Isolation and identification of pesticides degrading individual microorganisms and microbial consortia and evaluating their pesticides bioremediation potential are one of the main study topics in bioremediation research. Several admirable works have been conducted so far in this area of research (Pan et al., 2016; Leitao, 2009; Aust, 1995). Carrillo-Pérez et al. (2004) isolated several bacterial species of *Pseudomonas*, *Neisseria*, *Moraxella*, and *Acinetobacter* that completely degrade DDT. Recently, pesticides are developed in a composite manner, in particular, to improve their functional spectrum. For instance, Velpar K is a hybrid herbicide composed of hexazinone and diuron which is used in sugarcane cultivation. Degradation of such complex compounds using efficient microbial consortia has also been investigated (Ramos and Yoshioka, 2012).

Currently, researches focus on genetic engineering approaches of developing genetically modified, efficient microorganisms and microbial consortia for bioaugmentation of pesticides and related residues. There are reports on using genetically modified bacteria in the bioremediation of pollutants (Hrywna et al., 1999; Menn et al., 2008). Research on microbial growth kinetics in pesticides contaminated soils, patterns of metabolism, and genetic diversity have earned enormous interest from researchers (Wirsching et al., 2020). Moreover, a number of researches have been conducted to determine the impact of certain physicochemical factors such as incubation temperature, pH, and nitrogenic source on the bioremediation process (Odukkathil, and Vasudevan, 2013).

Studies on the evaluation of the efficacy of bioremediation as a pollutant removal technology have also earned immense importance. For instance, a recent study observed that bioaugmentation and biostimulation are effective in the removal of polyaromatic hydrocarbons containing pollutants. However, the efficiency of the two techniques varied on the molecular weight of the pollutant (Sun et al., 2012). A study conducted to evaluate the pesticides removal potential of composting found that immature compost has very lower levels of organic pesticide pollutants than in the initial substrates utilized in the composting process suggesting the apparent removal of those xenobiotic compounds during composting (Vogtmann and Fricke, 1992; Strom, 1998).

Studying the impact of pesticides on soil microbiological factors is also quite important in establishing a stable bioremediation strategy. The output of these studies could provide momentous insights about the impact of pesticide molecules on different microorganisms and thereby could modify the composition of microbial types utilized in the remediation strategies. For instance, a field study conducted to observe the influence of herbicides 2, 4-D, and glyphosate on microbial biomass revealed that the herbicides reduced the microbial biomass in the soil (Wardle and Parkinson, 1992).

Microbial biomass which is composed of live microorganisms is an integral proportion of soil organic matter content. The reduction of microbial biomass indicates the decline of live microbial count in the soil. Another investigation showed the impact of imazethapyr herbicide on microbial biomass (Perucci and Scarponi, 1994). In the laboratory-scale experiment, the soil was treated with the herbicide at the recommended rate for the field crop, 10-fold of recommended rate, and 100-fold of the recommended rate. Although the recommended rate did not impact on soil microbial biomass carbon content, over-application of the herbicide drastically decreased the soil microbial biomass carbon contents. This observation suggests that pesticide over-use above the recommended rate could adversely affect the microbial populations in soil.

Soil respiration (production of carbon dioxide) measurements are effective indicators of soil health. When the soil is polluted with pesticides, their inhibitory effects on the soil microbial population would make fluctuations in the release of CO_2 from soil. A study conducted using a series of the isopropyl amine salt of glyphosate (47, 94, 140, and 234 $\mu g\, g^{-1}$ soil) inoculated soil showed that the glyphosate is capable of vitalizing the microbial functions in soil (Haney et al., 2000). An increase in C and N mineralization further confirmed the rapid microbial removal of glyphosate. Therefore, designing laboratory and field trials to understand the effect of different pesticides on soil microbial parameters is extremely important to confirm their inhibitory effects against microbial population. Then the bioremediation strategies could be altered to meet the utmost efficiency.

Many researchers have emphasized the importance of scaling up the laboratory experiments up to on-site field experiments because; some theories which show the potential of practical application in the laboratory may exhibit inefficiency at the field trials. Bioremediation strategies which perform well in the field trials could be considered as efficient pollutant removal processes. Many field trials have been currently conducted on remediating agrochemicals (Antonious, 2012; Pussemier et al., 1998).

Above mentioned examples provide only a mere glimpse of the various researches that have been conducted so far on bioremediation. Bioremediation is a complex microbial process and its complexity drastically increases with the excessive addition of agrochemicals with higher functional and structural diversity. Therefore, research activities on the subject should be broadened purposefully.

5.2 Novel trends in microbial bioremediation technology

Bioremediation technologies are rapidly evolving. The modifications added into the basic process target optimizing it. This section aims to summarize the emerging trends of bioremediation process that lead to technological advancements in the overall process. Modification of microorganisms and designing site-specific bioremediation schemes with process alternations are key areas of attention in terms of adding novelties to the process (Vishwakarma et al., 2020).

The use of proficient microbial consortia is an essential component of a progressing bioremediation technique. However, adding microbial cells into the contaminated site has certain limitations such as rapid cell viability loss and unequal dispersal in the field. Instead of adding a microbial consortium into the soil, the current trend is to incorporate plasmids that harbor genes encoding for specific pesticides hydrolyzing enzymes. These plasmids are uptaken by a competent indigenous microbial population in the field through conjugation. Microbial cell division increases the number of plasmid copies in the field. This is a novel approach in bioremediation that replaces conventional cell-mediated bioaugmentation (Garbisu et al., 2017).

The bioavailability of pesticides is a dire necessity of microbial bioremediation. The use of biosurfactants; surface-active compounds are introduced as an ecofriendly approach to enhancing hydrophilicity of pesticides molecules. Instead of using synthetic chemicals like Tween 80, surfactants of plant or microbial origin are more beneficial for this process due to their biodegradability and lower toxicity. Humic acid is a plant-based surfactant (Roy et al., 1997). Some microbial-derived surfactants viz., lipopolysaccharides, rhamnolipids, glycolipids, phospholipids, etc., are extracted from *Pseudomonas*, *Bacillus*, yeasts, and yeast-like fungi including *Starmerella bombicola* (Naughton et al., 2019).

Rhizoremediation is recognized as an efficient approach to treating pesticides contaminated agricultural soils. The process basically involves the mutual interactions between the plant and the plant's rhizosphere-inhabiting microorganisms. Microbes increase in their population density by feeding on plant root exudates. The enhancement of microbial activities in the vicinity of plant roots facilitates the microbial breakdown of pollutants (Saravanan et al., 2019).

Using microbial biofilms for pollutant removal and improving soil health is another novelty in agriculture. Some researchers in Sri Lanka currently have proved that deteriorated agricultural soils could be reinstated using microbial biofilms developed under laboratory conditions (Seneviratne et al., 2011; Seneviratne and Kulasooriya, 2013). They have observed that the direct application of bio-filmed biofertilisers (BFBFs) to depleted agricultural soils could promote better yield while restoring the soil shortly after the application. Currently, they have upgraded their BFBFs to the commercial level targeting various crops including tea, rice, maize, and vegetables.

Another most important trend that emerged in current bioremediation technology is the application of "–omics" for understanding the microbial agroecosystems functions. This is a collective of technologies which includes genomics, proteomics, metabolomics, transcriptomics, glycomics, lypomics, systems biology, bioinformatics, and computational biology tools for studying the behavior of microorganisms at the molecular level. These techniques enable the exploration of the overall biochemical activities of microorganisms through unraveling important information viz., specific genes, proteins, factors affecting cellular metabolism and catabolism, nucleic acids, etc. (Aardema and MacGregor, 2002).

Especially, recently developed metagenomics approaches enable the culture-independent assessment of microbial populations for application in bioremediation. Enrichment of microbial target genes using stable isotope probing (SIP) technology, metagenome extraction, and library construction, using function-based and sequence-based metagenomics approaches for accessing microbial biodegradative genes, and microbial community profiling using direct sequencing are recognized as sequential steps of applying metagenomics in bioremediation (Pushpanathan et al., 2014). Incorporation of novel technologies into the conventional bioremediation strategies, under a well-planned upstream process, continuous monitoring, and downstream process would contribute greatly to the overall optimization of microbial bioremediation.

5.3 Advantages and disadvantages of microbial bioremediation process

5.3.1 Advantages of microbial bioremediation process

It is compulsory to understand the positive and negative impacts associated with the bioremediation process and enacting strategies to reduce the drawbacks. One of the main advantages of bioremediation is its ecofriendly mechanism of action. Bioremediation is carried out using an appropriate microbial population for degrading the pesticide pollutants into simpler nontoxic forms such as water and carbon dioxide. Thus, the emission of toxic byproducts during the process is minimized.

Bioremediation is economically feasible and cost-effective which could be practiced on-site. Therefore, wasting labor costs on polluted soil excavation and transportation into the ex situ soil treatment plants is not required. It has minimal remediating site destruction as the microbial activities carried out on-site are less harmful. On the contrary, the use of conventional methods such as landfills demands large plots of extra lands that are eventually get polluted due to the dumping of pollutants.

Bioremediation process permanently removes the toxic pesticides contaminants from the agricultural soils. Therefore, the risk of long-term persistence of pesticides in the food chains is restricted. It will reduce the emergence of severe health concerns in the future.

5.3.2 Disadvantages of microbial bioremediation process

Bioremediation technology has certain disadvantages. Particularly, the process is limited to the removal of biodegradable agrochemicals. Other non-biodegradable pesticides including chlorinated compounds could remain intact in the soils that will accumulate in food chains. In addition, microbial pesticides metabolism also may produce poisonous compounds. For instance, under anaerobic conditions in the agricultural field, greenhouse gasses such as methane and carbon dioxide emission into the atmosphere may occur which will account for intense global warming in the future.

There are various types of synthetic agrochemicals that are used in modern agricultural practices. Those artificial compounds might have complex chemical structures that the bioremediation end products may be extremely hazardous and highly persistent in the natural environment. A common example is the production of vinyl chloride by the microbial remediation of trichloroethylene; this vinyl chloride is a carcinogenic compound.

Moreover, bioremediation is a time-consuming process. Before starting the cleaning up of the polluted site, a sequential evaluation and optimization of the process must be assessed under laboratory conditions. Especially, for bioaugmentation, a suitable microbial consortium must be developed under intensive laboratory procedures. Before the addition of the consortium into polluted agricultural soil, they must be assessed for their physicochemical growth characteristics because the effectiveness of the whole bioremediation process is determined by the efficient growth of the appropriate microbial population and the environmental parameters of the site (Zouboulis and Moussas, 2011).

5.4 Indications for future research

As previously mentioned, a more productive bioremediation process depends on the microbial capacity to degrade pollutants. Currently, the phenomenon called “aging” of pesticides changes the bioavailability of pollutants with the time that the microorganisms are being challenged due to their lack of adaptability to the aging process. Therefore, studies must be conducted to understand the methods of making these pesticides and related chemical molecules bioavailable for microbial activities (De Lorenzo et al., 2018).

Microorganisms, although they are primitive in the organization, their metabolic pathways are extremely complicated and not fully explained in modern science. Having an in-depth understanding of the metabolic pathways of indigenous and exogenous microorganisms employed in bioremediation is essential for designing an efficient bioremediation process (Dvořák et al., 2017). Therefore, an extensive number of research is required for unraveling the underlying mechanisms of microbial metabolisms related to bioremediation. A combined approach of modern molecular biology and genetics, microbiology, and biochemistry could be followed to achieve this goal.

Most of the bioremediation technologies are based on the hydrolytic potential of diverse groups of microbial enzymes. Having an in-detailed understanding of the pesticides degrading enzymes including their molecular structure, functional properties, rate-limiting factors, and enzyme inhibitory factors is essential especially in optimizing the bioremediation process. Therefore, microbial enzymatic studies can be further extended to unfold the theories behind the enzymatic activities (Vishwakarma et al., 2020).

Adding different types of pesticides into the same agricultural field might magnify the complexity of bioremediation process due to the lack of a clear view of interactions in-between pesticides. If any chemical reaction occurs among the pesticides molecules, it could produce unidentified end products insensitive to microbial activities. An end product which is naturally susceptible to microbial attack might be more beneficial for the bioremediating process. However, this information must be confirmed with further research. Another concern on end products released after bioremediation of pesticides is their toxicity. It is compulsory to guarantee that the bioremediation does not pollute the agricultural soils by releasing toxic end products. Therefore, it is necessary to evaluate the characteristics of end products and if found toxic, strategies must be established to remove them. In order to do that the downstream process of bioremediation must be comprised of proper methods of neutralizing these chemicals. Research activities on these particular concepts might be intensely beneficial for organizing a well-intended bioremediation process.

Moreover, microorganisms may develop genetic modifications during the process. These genetic modifications being unpredictable and not intended for the productivity of the bioremediation process may cause serious adverse impacts on humans, animals, and plants. Therefore, research activities must be designed to understand the post-bioremediation impacts on microbial population and remedies for rectifying them if their prevalence in the environment is not ecofriendly.

Furthermore, discovering greener alternatives for agrochemicals could also be a more desirable approach to reducing pesticides usage and consequent mitigation of the adverse impacts associated with their over application.

6 Conclusion

The application of pesticides is an integral part of modern agriculture. The excessive use of pesticides could impose adverse impacts on the natural environment and its living beings. Bioremediation is emerged as a promising technique for rectifying pesticide-associated pollution of agricultural soils. However, the beneficial output of the process basically relies upon the biodegradability of the pesticide molecules, availability of an efficient microbial population with the potential of degrading targeted pesticides as well as the environmental conditions in favor of the process. In addition, progress monitoring criteria should be established to periodically confirm that the aforementioned three factors are in optimal interconnection to achieve effective bioremediation. However, the application of bioremediation techniques without sufficient knowledge about the behavior of pesticides in the field, their interactions in-between and the environment, microbial population utilized, mechanisms of enzymatic functions on pesticides, bioremediation-mediated fate of pesticides, and influence of environmental parameters on the process, factors limiting the bioremediation and the nature of end products formed, could make the bioremediation process a failure. Therefore, it is necessary to design further research to fully understanding the working mechanisms of bioremediation. Bioremediation technology has currently reached new ventures by means of advances in scientific disciplines such as microbiology, biochemistry, molecular biology, genetic engineering, and analytical instrumentation. Development of genetically engineered microorganisms with enhanced pesticides degradation potential for bioaugmentation, application of metagenomics approaches for studying the beneficial microbial communities in agroecosystems, and inventing analytical instruments for studying the process parameters are some of the recently evolved technological advancements in bioremediation. Yet, it is essential to develop novel bioremediation technologies specifically designed for removing pesticide pollutants in agricultural ecosystems. Another important step is properly optimizing the effective laboratory-scale experiments on bioremediation up to the field-scale application. Microbial bioremediation is already accepted as an ecofriendly and cost-effective strategy for efficiently cleaning up the pesticide pollutants in agricultural soils. Focusing on discovering novelties to improve the prevailing technologies while minimizing the process-associated drawbacks would assist in making bioremediation a greener approach to reversing pesticides-associated soil pollution.

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