REVIEW

Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments

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Abstract Biodegradation is a natural process, where the degradation of a xenobiotic chemical or pesticide by an organism is primarily a strategy for their own survival. Most of these microbes work in natural environment but some modifications can be brought about to encourage the organisms to degrade the pesticide at a faster rate in a limited time frame. This capability of microbe is some times utilized as technology for removal of contaminant from actual site. Knowledge of physiology, biochemistry and genetics of the desired microbe may further enhance the microbial process to achieve bioremediation with precision and with limited or no scope for uncertainty and variability in microbe functioning. Gene encoding for enzyme has been identified for several pesticides, which will provide a new inputs in understanding the microbial capability to degrade a pesticide and develop a super strain to achieve the desired result of bioremediation in a short time.

Keywords Biodegradation • Bioremediation • Pesticide

Introduction

Biodegradation and Bioremediation of pesticide

Biodegradation and bioremediation are matching processes to an extent that both of these are based on the conversion or metabolism of pesticides by microorganisms. The difference between these two is that, the biodegradation is a natural process whereas bioremediation is a technology. In bioremediation, we use microbes to degrade the pesticides *in situ*. A successful bioremediation technique requires an efficient bacterial strain that can degrade largest pollutant to minimum level. Adequate rate of biodegradation is required to attain the acceptable level of pesticide residues or its metabolites at contaminated site in a limited time frame. The rate of biodegradation in soil depends on four variables i.e. (i) availability of pesticide or metabolite to the microorganisms, (ii) physiological status of the microorganisms, (iii) survival and/or proliferation of pesticide degrading microorganisms at contaminated site and (iv) sustainable population of these microorganisms. Therefore, to attain an achievable bioremediation, it requires the creation of unique niche/or microhabitats for desired microbes, so they can be successfully exploited. Here, the only difficulty is our limited knowledge about the population dynamics of pesticide degrading microorganism in relation to other microbes present in the same habitat. Temperature, pH, water potential, nutrients and the amount of pesticide or metabolite in soil may also act as limiting factor for pesticide degrading microorganisms, which requires further exploration in relation to total microbial population and their biochemical activities. In consideration of suitable bioremediation technique, the understanding of pesticide dynamics in soil

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environment is also required because soil has unique binding potential for variety of pesticides or metabolites. It is also necessary to understand the physiology and genetics of degradation of a particular pollutant before using any bacterium for its biodegradation.

There are three possible regions where pesticide contamination can occur in terrestrial ecosystem (i) surface soil, (ii) vadose zone and (iii) ground water or saturated zone. The biodegradation in surface is primarily aerobic and rapid because the surface soils have large number of aerobic microorganisms and their number usually decreases with the depth. Biodegradation is slow in other two zones i.e. vadose and ground water. Therefore, different bioremediation technologies are required to deal with different regions of terrestrial ecosystem.

Method

Soil sampling, storage and residue analysis

To study, the microbial degradation of pesticide, random soil sampling with utmost precision is required. Depending on experimental design or analysis, the soil sampling requires a precision to reduce the uncertainty and variability in results. ISO Geneva, Switzerland has developed standards for adequate soil sampling, which has been described in a series of ISO norms. Soil samples should be processed and analysed immediately or stored only for a very limited period. Microbial analysis must be performed as soon as possible to minimize the effects of storage on microbial population and their biochemical activities. Reduced microbial activity has been reported when soil samples were stored in field moist condition at 4°C for three months [1]. Similarly, pesticide residue analysis needs to be performed in a short time to avoid the changes during the storage. Sampling, sample processing, sampling constant, matrix effect, extraction and cleanup are very important steps in pesticide residue analysis. Residue analyses are influenced by uncertainty and variability during sample processing. Uncertainty of sample processing depends on the degree of inhomogeneity of the pesticide residues in the sample, which significantly influences the results. Other parameters need to be known before analysis can be carried out i.e. (i) limit of detection of the compound, (ii) repeatability of method (s) and (iii) stability of compound once the sample has been taken. These all points must be taken care during the quantification of residues. Residues can be quantified by using GLC, HPLC and GCMS. Validation of pesticide residue method is required to quantify the biodegradation of pesticide by microbes.

An alternative method for analysis with high precision and accuracy requires the use of radiolabelled pesticide. The use of ^{14}C -radiolabelled/specific radiolabelled pesticide allows very sensitive and specific measurements i.e. the rate of degradation of pesticide, metabolites or mineralization as $^{14}\text{CO}_2$ evolution. The technique provides the actual image of microbial degradation of pesticide.

Different approaches for biodegradation

Complete biodegradation of a pesticide involves the oxidation of parent compound to form carbon dioxide and water. This process provides both carbon and energy for the growth and reproduction of microbes. Each degradation step is catalyzed by specific enzyme produced by a degrading cell or enzyme found external to the cell. Degradation of pesticide by either external or internal enzyme will stop at any step if an appropriate enzyme is not present. Absence of an appropriate enzyme is one of the common reason for persistence of any pesticide. If an appropriate microorganism is absent in soil or if biodegrading microbial population has been reduced due to toxicity of pesticide in that case a specific microorganism can be added or introduced in soil to enhance the activity of the existing population. The microorganisms could be either natural or genetically engineered. "Super strains" are also developed that can degrade the pesticide at a fast rate. The problem is that the introduction of microorganism to contaminated site may fail because they are unable to survive in a new environment more than a few days or weeks. Another strategy is to add a specific genes that can confer specific degradation capability to indigenous microorganism. The addition of degradative genes relies on delivery and uptake of genetic material by a indigenous microorganism. There are two possible approaches that can be taken i.e. (i) the use of microbial cells to deliver gene via conjugation, (ii) to add naked gene in soil and allow its uptake via transformation. However, a good deal of work has not been done on these aspects and requires more studies to develop it as a part of the technology.

Microcosm and field trials

The soil is heterogeneous with respect to biological niche, spatial distribution and availability of contaminant. Here, a variety of complex biodegradation patterns will appear if the microbe of interest is present or introduced in soil, because of physical and biological interaction between contaminant and the microbe of interest. To understand the contaminant and microbial interactions, a closed system has been adopted as microcosm. Burn [2] reviewed the literature on microcosm studies. These are used by several

workers to understand how the soil system works in the field. Hong *et al* [3] have reported the microcosm study on bioremediation of fenitrothion using *Bukholderia* sp. FDS-1. Results indicated that the strain FDS-1 has potential for use in bioremediation of fenitrothion at contaminated field.

Development of bioremediation technology

Bioremediation works either by transforming or degrading the contaminant or by both ways in the field. The goal of the process technologist in these projects is to create most favourable growth condition for the microorganisms. Bioremediation treatment includes land farming, bioreactors, biologically enhanced soil washing, composting and solid – phase bioremediation. In situ bioremediation requires manipulation of aqueous constituents and bioventing. Genetically modified microbes are used to enhance the capability of degradation. Yet, the use of genetically – engineering technology for the use in environment is still controversial because an adverse genotype can be readily mobilized in the environment.

In a development of technology following points should be taken care i.e. (i) heterogeneity of contaminant, (ii) concentration of contaminant and its effect on biodegradative microbe, (iii) persistence and toxicity of contaminant, (iv) behaviour of contaminant in soil environment and (v) conditions favourable for biodegradative microbe or microbial population.

Use of technology at actual site

The use of technology at actual site requires (i) the knowledge of natural bioprocess at contaminated site, (ii) detail and valid data of microbial biodegradation developed in laboratory, (iii) monitoring onsite biodegradation process. Studies on microbial population, activities and soil enzymes will provide the mirror image about the functional status of the soil.

Most of the bioremediation technologies for the field are designed to remove the pollutant once it is generated or released into environment. Usually, these technologies includes, bioaugmentation (addition of organism or enzyme to the contaminant), biostimulation (use of nutrients to stimulate to naturally occurring organisms), biofilters (removal of organic gasses by passing air through compost or soil containing microorganism), bioreactors (treatment of contaminant in a large tank containing organism or enzyme), bioventing (involves the venting of oxygen through soil to stimulate the growth of natural microorganisms capable of degrading contaminant), composting (involves mixing of contaminant with compost containing bioreme-

diation organisms) and landfarming (use of farming, tilling and soil amendment techniques to encourage the growth of bioremediation organism at contaminated site).

The degradation of endosulfan [4] by mixed bacterial culture was studied in aerobic and facultative anaerobic conditions via batch experiments with an initial endosulfan concentration of 50 mgL^{-1} . After 3 weeks of incubation, mixed bacterial culture was able to degrade $71.58 \pm 0.2\%$ and $75.88 \pm 0.2\%$ of endosulfan in aerobic and facultative anaerobic conditions, respectively. Endosulfan biodegradation in soil was evaluated by miniature and bench scale soil reactors. The soils used for the biodegradation experiments were identified as clayey soil (CL, lean clay with sand), red soil (GM, silty gravel with sand), sandy soil (SM, silty sand with gravel) and composted soil (PT, peat). Endosulfan degradation efficiency in miniature soil reactors were in the order of sandy soil followed by red soil, composted soil and clayey soil in both aerobic and anaerobic conditions. In bench scale soil reactors, endosulfan degradation was observed more in the bottom layers. After 4 weeks, maximum endosulfan degradation efficiency of $95.48 \pm 0.17\%$ was observed in red soil reactor where as in composted soil-I (moisture $38 \pm 1\%$) and composted soil-II (moisture $45 \pm 1\%$) it was $96.03 \pm 0.23\%$ and $94.84 \pm 0.19\%$, respectively. The high moisture content in compost soil reactor-II increased the endosulfan concentration in the leachate. Known intermediate metabolites of endosulfan were absent in all the above degradation studies. In another study by Hernandez-Rodriguez *et al* [5] the rate and extent of endosulfan reduction from a grass substrate that was either composted or sterilized by autoclaving was observed. The removal of endosulfan from substrate colonized with *Pleurotus pulmonary* was also determined. During composting in the presence of Ca(OH)_2 for 120 h, the concentrations of α and β endosulfan were reduced by 61.4% and 45.5% respectively, significantly higher compared with the control (without Ca(OH)_2) in which the reduction was 38.5%. After sterilization the concentration of α and β endosulfan were reduced by 84.8% and 87.5% respectively. After colonization of the substrate with *Pleurotus pulmonarius* α and β endosulfan were reduced by 99% (35 days after spawning).

Kumar and Philip [6] studied the degradation of endosulfan by three bacterial species namely *Staphylococcus* sp., *Bacillus circulans*-I, and *Bacillus circulans*-II, both in mixed culture and pure culture. In mixed culture, after four weeks of incubation degradation of $71.82 \pm 0.2\%$ and $76.04 \pm 0.2\%$ of endosulfan in aerobic and facultative anaerobic conditions, respectively was observed. In pure culture a degradation potential of $93.3 \pm 0.15\%$, $93.4 \pm 0.15\%$ and 89.95% of α endosulfan and $75.96 \pm 0.05\%$, $76.73 \pm 0.05\%$ and $82.9 \pm 0.05\%$ of β endosulfan

by strains *Bacillus circulans*-I, *Bacillus circulans*-II and *Staphylococcus* sp respectively was observed after 14 days of incubation. Addition of dextrose to the system amplified the endosulfan degradation efficiency by $13.36 \pm 0.6\%$ in aerobic system and $12.33 \pm 0.6\%$ in facultative anaerobic system. They also reported the degradation of endosulfan metabolites i.e., endosulfan sulfate, endosulfan ether and endosulfan lactone in aerobic conditions. Degradation of endosulfan ether and endosulfan lactone was promising with *Bacillus circulans* I and II whereas no endosulfan sulfate was degraded by any of these strains. Endosulfan ether was reported to be converted to endosulfan lactone and some other intermediate compounds [7]. In another study using mixed culture isolated from a pesticide contaminated soil, a degradation of about 73% and 81% of α and β endosulfan respectively was observed. Endosulfan diol was identified as the degradation intermediate. Two cultures identified by 16S rRNA as *Stenotrophomonas maltophilia* and *Rhodococcus erythropolis* were found to be responsible for majority of the degradation by the mixed culture⁸.

Recent developments in pesticide biodegradation

Microbes, their catabolic gene and respective enzymes have been isolated and identified by several workers capable of degrading the pesticides. Several bacteria competent of degrading either carbofuran, carbarly, baygon or aldicarb have been isolated and characterized [9, 10, 11, 12, 13, 14, 15, 16, 17]. In another example i.e. lindane [18], endosulfan [19, 20], DDT [21] and monocrotophos [22, 23], the microbes and/or enzymes were isolated and identified. Genetic studies of microbial degradation indicates that the plasmids are the main place for the gene of interest usually spread throughout the microbial community. Sutherland et al [19] had reported Esd gene that had same sequence homology to monooxygenase family that use reduced flavin, provided by a separate flavin reductase enzyme, as cosubstrates in *Mycobacterium smegmatis*. Esd catalyzes the oxygenation of β -endosulfan to endosulfan monoaldehyde to endosulfan hydroxyether. Esd did not degrade either α -endosulfan or the metabolites of endosulfan and endosulfan sulphate. Wier et al [24] have reported the gene Ese encoding enzyme from monooxygenase family capable of degrading both endosulfan α and β from *Arthrobacter* sp. After, understanding the gene of interest and enzyme involved, the Superbugs can be created to achieve the desired result at fast rate in short time frame. Lal et al [25] has reviewed the degradation of HCH and distribution of *lin* gene in Sphingomonads. *S. indicum* B90A was found to contain two non-identical *linA* genes (designated as *linA1* and *linA2*). The *linA*-encoded HCH dehydrochlorinase (*LinA*) mediates the first two steps of

dehydrochlorination of γ -HCH. To develop bioremediation technology with precision for HCH, understanding of molecular genetics, diversity and distribution of *lin* gene will help.

Limitations of Bioremediation

There are several limitations on the way to bioremediation technology. One major limitation is the nature of the organisms. The removal of pollutants by organisms is not a benevolent gesture. Rather, it is a strategy for survival. Most bioremediation organisms do their job under environmental conditions that suit their needs. Consequently, some type of environmental modification is needed to encourage the organisms to degrade or take up the pollutant at an acceptable rate. In many instances the organism must be presented with low levels of the pollutant over a period of time. This induces the organism to produce the metabolic pathways needed to digest the pollutant. When using bacteria and fungi, it is usually necessary to add fertilizer or oxygen to the material containing the pollutant. This can be disruptive to other organisms when done in situ. In situations where simple compounds and metals are being taken up it is likely that these pollutants are at toxic levels for the organisms. Overall, the organisms do not always live as well on the pollutant diet as on other nutrients found more commonly in their environment. This causes problem when doing in situ remediation.

Other limitations of immediate concern are cost/benefit ratios i.e. cost versus overall environmental impact. Neither the government nor industry wants to spend large amounts of money to clean up pollution. Industry in particular always likes to keep their costs of products and services down for gain in the market. The petroleum industries are embroiled currently in a battle with the EPA about the added costs of maintaining new Clean Air Act standards. Bioremediation is generally very costly, labor intensive, and can take several months for the remediation to achieve acceptable levels. Air bioremediation in particular is very inefficient, considering the volume of polluted air generated by industry. Another problem is that both *ex situ* and *in situ* technologies can cause environmental disruption beyond the damage done by the pollution. The long-term effects of introducing naturally occurring non-native bioremediation organisms into an area are not fully understood. The impact of genetically altered bioremediation organisms is even less understood.

Bioremediation has been proven to work effectively under laboratory conditions. Short-term studies show that it also works under several field conditions. Like many technologies with good scientific foundations its merits are marred by over-optimistic speculations and fraudulent claims. In spite

of its limitations, bioremediation is benefiting from the rush to use biotechnology to solve public health problems. The EPA and the DOE are even investigating the feasibility of bioremediation to remove radioactive wastes from contaminated soil and water. Bioremediation's popularity is further enhanced because it is perceived as being more "green" than other remediation technologies. Companies and individuals are investing in biotechnology futures in spite of the high risks. As a result bioremediation companies have a viable future regardless of its long-term effectiveness.

Conclusion

Bioremediation is based on the idea that different organisms will work together to remove (biodegrade) the waste substances or pollutants (pesticide) from environment. Bacteria and fungi are very good degrader of complex molecules and use these for their own metabolism and growth. Bioremediation does not only involve the degradation of wastes or pollutants. Yet, some times the process is sufficient to remove the waste or pollutant without degrading. Bioremediation will work fine with some molecules but there are several limitations to it also. It depends on the nature of organisms, the enzyme involved, its concentration and availability and finally survival of microorganisms. Therefore, sometimes the use of microbial enzyme directly to the pollutant may bypass these constraints associated with live cells. The use of enzymes for degradation of pesticides can be developed as technology for bioremediations. Another limiting concern about bioremediation is cost/benefit ratio i.e. cost versus overall environmental impact. It is utmost important, when we want to use the live organism or product for removal of contaminants. To some extent these limitations can be solved by understanding the genetics and biochemistry of desired microbe. The Super strain can be created to achieve the required result in short time frame. In field, multimember microbial consortia via either elective enrichment or chemostat enrichment can be used for mineralization of specific pesticide at faster rate. In comparison to other remediation processes i.e. incineration, thermal disposition, landfarming etc., the bioremediation has a better future in development of technology for removal of contaminants from actual site.

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