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Enhanced bioremediation techniques for agricultural soils

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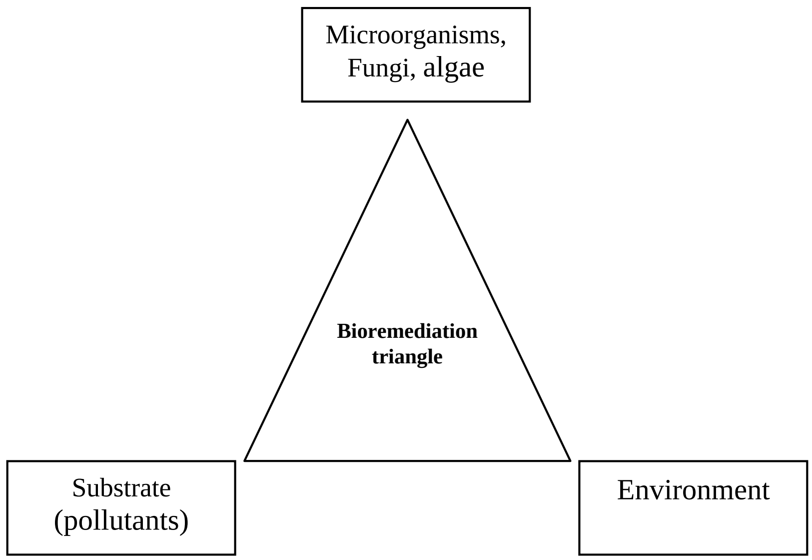
A B S T R A C T

Bioremediation strategies have potential for eliminating or reducing the xenobiotics from environment. One such strategy would to use enzymes to degrade the hazardous chemical compound from environment. Micro-organisms are now known to be the principal agents, which can clean and modify the complex lipophilic organic molecules, once considered recalcitrant, to simple water soluble products. They first attack these organic chemicals by the enzymatic apparatus acquired during the course of enrichment, when they are exposed to these specific or structurally related compounds. Presence of these contaminants in the environment either induces or depresses the enzymatic function of microorganisms. This capability largely depends upon the selective microbial community as well as on the structural and functional groups of toxic compounds. These water soluble intermediates are usually attacked by primary or secondary groups of organisms to form inorganic end products, resulting in complete biodegradation of hazardous chemicals. Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. Now day's relative new technologies are present which increase the bioremediation process faster and more fruitful in short time period. Such technologies act on either on three components of bioremediation which are important for this process to occur. These three levels are explained in this paper.

Introduction

Human is every day evolving with immense pace. With intensified agriculture and enhanced manufacturing capacity our natural resources such as soil and water are polluted by various hazardous compounds. Agriculture which is one of most important livelihood in India is the prime component for economic growth and per capita income. After green revolution, the production of crop became an intensive input task. High yielding varieties with require high dose fertilizers with mainly imbalanced use, caused a very unpleasant impact on Indian soils. Currently according to estimate of CSSRI (Central Soil Salinity Research Institute) Karnal (2010-11) 6.73 m ha of soil in India is salt affected. Not only fertilizers, pesticides which are soil drenched or applied as aerial sprays also remain to be active for a long time which ultimately effect soil microclimate and animals too for a long time. These chemical not only pollute the soil but its undesirable effect is also observed in nearby water bodies of agriculture land. The famous example is of DDT and currently Endosulphan in India which affected thousand of human life and there generations. An investigation by the FAO/UNEP/WHO suggested that 1-5 million patients develop pesticide poisoning every year, and several thousand patients die. These all constraint leads an urgent urge of amendments of the soil and only promising solution appears to be "bioremediation".

Bioremediation uses biological agents, main ly microorganism's i.e. Yeast, Fungi or bacteria to clean up contaminated soil and water (Strong and Burgess, 2008). It is naturally occurring process, by which natural organism convert or immobilize the contaminants into less harmful products. According to the EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances. Bioremediation efficiency depends on environmental conditions which permit optimal microbial growth and activity; it involves the manipulation of environmental conditions to enhance microbial growth and faster degradation. Now day's relative new technologies are present which increase the bioremediation process faster and more fruitful in short time period. Such technologies act on either on three components of bioremediation which are important for this process to occur.



Component of bioremediation- Bioremediation triangle

Bioremediation require three basic components by which whole process can be operative. These components are: a) microorganism, b) substrate (pollutants) c) environment. Advanced technologies based on either optimizing these components or by improving interaction between them.

The interaction of microorganism component with environment defines the time taken to proliferate the microorganism in particular environment (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients) while interaction between the substrate and environment defines the affinity of pollutant towards soil and water. The substrate and microorganism interaction is important for the time requires neutralizing the hazardous compound. Thus the three component and there interaction is important for optimization of bioremediation process. The current advance methods are utilizing these above interaction for formulating new consortia and their application. Some of recent advance techniques are discussed here which are used in agriculture and there near future applications.

Technique based on manipulation at microorganism level

GMMs (Genetically Modified Microorganisms) - With the advancement of biotechnology various bacteria are now manipulated at gene level. At the beginning of the 1980s the development of genetic engineering techniques and intensive studying of metabolic potential of microorganisms allowed to design genetically modified microorganisms (GMMs) (Wasilkowski D. et.al, 2012).The GMMs are able to degrade toxic compounds by manipulating metabolic pathways in such a manner so that wild strain can enhance the production of degradative enzyme which was previously very slow or around nil. In 1981 in the USA the first two genetically modified strains of *Pseudomonas aeruginosa* (NRRL B-5472) and *Pseudomonas putida* (NRRL B-5473) were patented. They were constructed by Chakrabarty in the early 70's and contained genes for naphthalene, salicylate and camphor degradation.The genetic engineering is modern technology where the gene are so designed with their specific promoter sequence to degrade specific contaminants. It provides an opportunity to make artificial combinations which are not present in nature. It includes manipulation at operon level, RNA level or protein also. The bacteria from genus *Pseudomonas* are mostly used for genetic manipulation as it has capacity to degrade many types of contaminants and also it can withstand many environments. The genetic engineering is a very promising solution for in situ bioremediation. Many reports suggested that GMMs had higher affinity to decay of various pollutants in comparison with natural strains (Cases I., de Lorenzo V. 2005). Application of genetic manipulations in bacteria for bioremediation includes modification of enzyme specificity; designing of a new metabolic pathway and its regulation etc. various examples of GMMs with their substrate are given in the table below:

A genetically modified microorganism not only helps in degradation of toxic compounds but also in promotion of plant growth. Generally, plant growth-promoting bacteria (PGPB) are not able to stimulate plant growth in the presence of various toxic compounds. GMMs have a great potential in bioremediation process and it is used frequently in agriculture soils remediation. This is best solution for *insitu* remediation.

Some important group of microbes for GMMs

Aerobic: Aerobic bacteria use the contaminant as the sole source of carbon and energy and degrade toxic compounds into less toxic products such as *Pseudomonas*, *Rhodococcus*, and *Mycobacterium.* These microbes degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds.

Anaerobic. Anaerobic bacteria require no oxygen for their function and not as commomnly used as aerobic bacteria. There is are now used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform. (Kumar.A, Bisht.B.S, Joshi.V.D, Dhewa.T, 2010)

Many different types of organisms such as plants can be used for bioremediation but microorganisms show the greatest potential. Microorganisms primarily bacteria and fungi are nature's original recyclers. Their capability to transform natural and synthetic chemicals into sources of energy and raw materials for their own growth suggests that expensive chemical or physical remediation processes might be replaced with biological processes that are lower in cost and more environmentally friendly. Therefore, microorganisms represent a promising, largely untapped resource for new environmental biotechnologies.

To boost the world's food production rate to compensate for the increasing population, pesticides are being used. The extensive use of these artificial boosters has lead to the accumulation of artificial complex compounds called xenobiotics. By introducing genetically altered microbes, it is possible to degrade these compounds.

Technique based on manipulation at environment level

This type of method involves manipulating environment in such a way that Population of microbes upto threshold level can be achieved which leads to more degradation. This inclue several methods as given below:

ORC (Oxygen Release Compounds) method and Bioventing- It is used of *insitu* bioremediation in which mostly aerobic organisms are used. But anaerobic bacteria also could be activated by injecting vegetable oil as a carbon source. The ORC method was developed by the REGENESIS Corp. This ORC method helps to proliferate the consortia required for degradation. In the ORC method, oxygen release compounds (ORC) are injected into the deeper layer by using high-pressure pumps. The supply of oxygen activates the microorganisms present in the polluted soil, enhancing the degradation rate. Bioventing is also one of prominent method which is suitable for the remediation of contaminants at deeper layer of soil over wider area.However, this method require large scale equipment, repetation of treartment, digging of area is difficult sometimes, and should be cost effective. The bioventing and ORC methods don't conform to these conditions and are therefore not applicable most of the times.

Immobilized cell carriers (BSPs)

In bioremediation the contact between microbes and substrate is necessary, which occur by either movement of bacteria by chemotaxis movement, but sometimes fixing of microbes in concentrated contaminated medium is necessary, this process is called immobilization. Immobilization is done some by biomass-supported particles (BSPs). These particles support the microbes for the long term application. Many studies have reported that the immobilization of microorganisms and its application is more useful than other approaches. Yeast and bacteria showing cohesiveness can be immobilized by BSPs. The excessive growth of cells in BSPs can be easily remedied by their flowing out from the carrier, and the high degrading activity can be maintained by these released cells. Many BSPs are reported in literature in which Charcoal is cheap and easy to use. Immobilization with charcoal has been examined previously in the treatment of waste water. For example, Takagi *et al.* examined the degradation of simazine in an experimental plot of a golf course.

Self immobilized systems- This system is based on the idea that microorganism secretes some compound which act as polymer and can be utilized instead of an immobilization carrier, which itself make it immobilized and will be more cost effective and appears to be efficient method for the bioremediation. For example The *Bacillus natto* (*Bacillus subtilis*) strain, which is used for the production of "Natto" (a traditional fermented food in*n*Japan), secretes a poly-glutamate (PGA) polymer. The PGA polymer shows high viscosity and can be used as a humectant, and was used in an attempted greening of a desert.

Bioremediations by Nano-particles

Nanotechnology is the emerging science which is having great potential for the development new innovative technologies. it is science of manipulation of particle at atomic level which operate on principle of quantum effect. The massive surface area and unique properties of nanoparticles have led to much research on their application to environmental remediation. Across the broad array of environmental concerns, research ranges from the use of TiO2 nanoparticles for photo-catalytic treatment of nitrous oxides in plant emissions, to the use of naturally occurring metal oxide nanoparticles for the treatment of organic contaminants in groundwater. This use of nanoparticles in bioremediation is sometimes also called Nanoremediation. Actually nanoparticles are either act as carrier for microorganisms or directly act in remediation.

Nanoremediation

The use of nanotechnology in the improvement of a contaminated site to prevent, minimize or mitigate damage to human health or the environment. An environmental technology, to protect the environment with, pollution prevention, treatment, and clean-up. Nanoremediation has the potential not only to reduce the overall costs of cleaning up on large scale contaminated sites, but it also can reduce clean-up time, eliminate the need for treatment and disposal of contaminated soil, reduce the contaminant concentrations to near zero.

Soil enhancers- Soil enhancers are mixture of certain of compounds particularly organic material which enhance the microbial growth particularly bacteria and fungi which are helpful in remediation of toxic compound. The basic principle shows that the carrier with inoculum has greater impact on soil remediation, for example the fungi *Trichoderma longibrachiatum* removed higher removal of Cr, Cu and Ni from industrial effluents by microbial consortium in combination with press mud. Soil enhancers are applied in very low quantity and act as the conditioners for soil. These actually improve the soil fertility and health by reducing toxic load in the soil. These soil enhancers are can be customized according to soil structure and their type. These kinds of soil enhancers are now commercialized by various companies and found to helpful to the farmers.

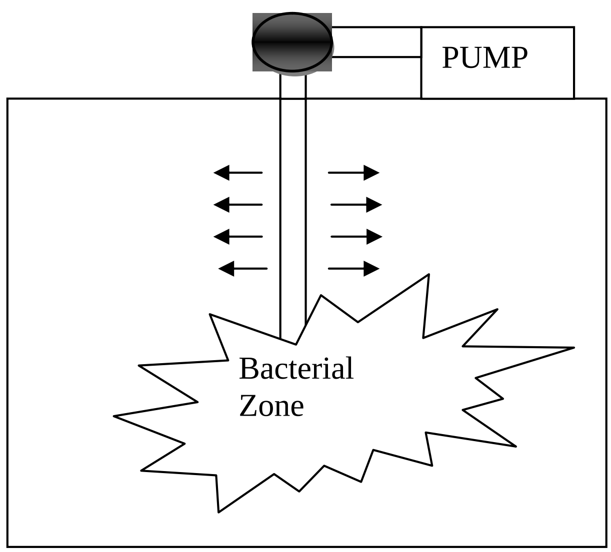
Technique based on manipulation at substrate level

Techniques till date used on substrate level of bioremediation are for sensing level of contaminants or degrade products, so that efficiency of consortia can be worked out.

Table.1

GMMs	Introduced gene/s	Organic compound	References
Pseudomonas fluorescens HK44	luxCDABE	naphthalene	Sayler G.S., Ripp S
Burkholderia cepacia L.S.2.4	pTOD plasmid	Toluene	Barac T., Taghavi S., Borremans B., Provoost A., Oeyen L., Colpaert J.V., Vangronsveld J., van der Lelie D
Escherichia coli AtzA	atrazine chlorohydrolase	Atrazine	Strong L.C., McTavish H., Sadowsky M.J., Wackett L.P
Pseudomonas putida PaW85	pWW0 plasmid	Petroleum	Jussila M.M., Zhao J., Suominen L., Lindstrom K.

Fig.1 Representing ORC method



These are at juvenile stage; few of them are method below:

Bacterial biosensors- Biosensors are analytical tools, which are biologically specific to some of target molecule which ultimately acts as molecular sensors for detection during bioremediation. These biosensors are constructed via. marker, dyes which are present in enzymes, antibodies, tissues, or living microbes as the biological recognition element. The presence of toxic compounds and the potential associated ecological risks can be determined by using bacterial biosensor and toxicity tests. Several biosensors have been developed for the detection of many petrochemical waste compounds including PAH. constructed a biosensor for detecting the toxicity of PAHs in contaminated soils with an immobilized recombinant bioluminescent bacterium, GC2 (lac::luxCDABE), which constitutively produces bioluminescence.

Remote sensing- Remote sensing is latest known technique to detect aberrant change in environment form remote place basically by satellite images. The GIS image helps in fine resolving images for finding the concentration of toxic contaminant in soil and there degradation by bioremediation. This detection technology is more advantageous than previous one as development cost of markers for identification is cheaper than and above method and also it is universal to apply in any part of world for that particular contaminant.

Conclusion

Bioremediation provides a technique which removes the pollution from our nature by enhancing the natural biodegradation processes. The current research is based on identifying the new strains and plant suitable for bioremediation. So by understanding the response of microbes for pollutants and expanding the knowledge of the genetics of the microbes to increase capabilities to degrade pollutants. There are many such techniques which ultimately enhanced the bioremediation process to its full efficiency. All this techniques are still at juvenile phase but they all of them are very promising. Moreover, these techniques have major advantages then pervious existing techniques that it can be applied in situ and exsitu environment with greater efficiency. In recent era the pollution of soil and groundwater by the use of pesticides has become a serious condition all over the world. The soil pollution is not only present in huge areas, but also its intensity is increasing day by day, leading to permanent contamination. Therefore, the conventional methods can't meet these challenges which can only overcome by new innovative techniques, making their set-up and maintenance is impractical.

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