The Scientific Temper
July, 2010; No.1: pp221-226

Academy of Innovative Research
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BIOREMEDIATION: A BIOTECHNOLOGICAL APPROACH TOWARD ENVIRONMENTAL MANAGEMENT

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Biotechnology has emerged as an applied discipline of biological research with its application in agriculture, forestry and industry as well as in medicine. An equally important area of global concern that has emerged only recently is the environmental Biotechnology. It is concerned both with the implications and applications of Biotechnology in the wider context of environment. Due to rapid industrialization, urbanization and other developments, there is a constant threat to the environment and to the depleting natural resources. Both these problems are receiving constant attention of environmentalists. The efforts are being made to use Biotechnology to protect the environment from pollution and to conserve natural resource. At a time, when the gap between those who have plenty and those who do not have even the minimum is widening, both end of this spectrum i.e. plenty and poverty is contributing to the environmental degradation. It is, therefore, necessary that the developing and developed countries jointly find a path of development, which "meets the needs of the present without compromising the ability of future generation to meet their needs. Efforts are being made to achieve these objectives through a variety of approaches and Biotechnology is certainly one of them.

In recent years, we have witnessed a debate on the environmental implication of Biotechnology. In this debate, risks involved in the use of Biotechnological approaches have often been emphasized and the adequate guidelines for safety have been suggested and enforced by law. However, there have also been rapid developments in the application of biotechnology, which may help in controlling environment at pollution, thus giving a cleaner and sustainable environment in future. It has only been recognized quite recently that biological systems, primarily of microbial origin could prove potential means of degrading some such complex environmental pollutants as well as preventing pollution through waste treatment. Such recent environmental application of biotechnology emerged into new area of research and development popularly known as ***Bioremediation***.

Bioremediation for environmental management: Bioremediation is the use of living organisms (primarily micro-organisms) to degrade environmental pollutants or to prevent pollution through waste treatment. It is emerging as most ideal alternative technology for removing pollutants from the environment, restoring contaminated sites and preventing further pollution. This environment friendly technology is expanding the range of organisms to be used to clean up pollution and form a vital component so called green movement of maintaining the nature's overall ecological balance.

Application of living organisms to degrade environmental pollutants or to treat waste to control pollution is expanding worldwide. The OECD (organization for Economic to cooperation and development) in a report entitled "*Biotechnology for a clean environment*" issued in 1994 estimate that world market potential for all

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environmental biotechnologies nearly double by 2000.

Bioremediation extends the natural processes by which microorganism consumes organic molecules including hydrocarbons. The micro organisms convert organic molecule such as carbon dioxide and water that can be readily accommodated in the environment (Atlas and Pramer 1990).

Diverse microorganisms, including many species of bacteria and fungi, have evolved the metabolic capacity to degrade the hydrocarbons (Atlas and Cerniglia, 1995). The most prevalent bacterial hydrocarbon degraders belongs to genera *Pseudomonas, Achromobacter, Flavobacterium, Nocardia, Vibrio, Bacillius, Actinomyces, Alkalygens, Corynebacterium, Klebsiella, Sarcina, Sphaerotilus and Xanthomonas*. Fungi tend to have to more significanct role than do bacteria in the biodegradation of organic compounds (Cerniglia et. al., 1992). The most prevalent fungal biodegraders are *Candida, Rhodotorula, Penicillium, Aspergillus, Cladosporium, Botrytis, Cephalosporium, Cunninghamella, Mucor, Torulopsis, Trichoderma and Trichosporon*. There are significant difference in the mechanism of the hydrocarbon metabolism used by fungi and Bacteria (Gibson and Subramanian 1984).

Bioremediation is primarily based on principle of biodegradation, bio-mineralization of complex pollutants. Microorganisms are capable of breakdown of these recalcitrant by disruption of covalent bonds by their enzymatic activity. The microorganisms may be photoautotrophs, chemoautotrophs or chemoheterotrophs. This biodegradation activity of microorganisms can be further enhanced by redesigning of these enzymes and by incorporating these bioremediation actively into new microorganisms, tested for desired result. Environmental biotechnology is not a new field but it is based on old environmental biotechnology of composting and waste water treatment by modifying microorganism or by using genetically engineered organisms.

Biodegradation of pollutants: Biological treatment of effluents is a long established practice in many countries, but some constituents of these effluents are recalcitrant and thus are not amenable to conventional treatment. Biotechnological research projects are being modified to expand the range of microorganism used for bioremediation. There is search for naturally occurring microbes that have better pollutant degradation kinetics, attack a wider range of pollutant compounds, and do so over a wider range of microbial growth conditions.

Researchers have also using genetic engineering to develop new microbial strains with novel biodegradative capabilities. For instance, modified microbes may be produced through genetic coding to attach the complex chlorinated hydrocarbons such as dioxins, which are non degradable by naturally occurring microorganisms. Adding genes that code for enzyme which breaks down toxic chemicals into microbes so that they could be able to survive and grow in much disturbed and harsh environment. These efforts would greatly extend the range of compounds that might be treated with bioremediation (Chakrabarty, 1974).

Bioremediation for upgrading waste and water treatment system: The dry anaerobic composting (Dranco) process converts the organic fraction of biodegradable organic solid wastes and refuses into energy in the form of bio gas (Methane and carbon dioxide and hydrogen) and humus like material. The biogas is produced by a consortium of anaerobic bacteria that includes Methanogens (methane producing archaebacteria).

Fluidized bed reactors had been used to remove nitrate from water at municipal water treatment. The reactors contain the methylotrophic bacteria such as *Methylotrophilus* *methylotrophus* that carry out nitrification. The bacteria convert nitrate to nitrite then to molecular nitrogen, which is released in the atmosphere. The removal of nitrate from waste water helps in prevention of eutrophication of the waterways receiving the treated water (Scheidegger, 1991).

Certain mircro-organisms can biodegrade toxic compounds like hydrocarbons and chlorinated solvents found in industrial plant waste water. Bacteria like *Pseudomonas cepacia* are able to biodegrade chlorinated hydrocarbons present in the effluents of pesticide industries manufacturing DDT, heptachlor chloradane etc.

Azo and reactive dyes constitute the largest class of dyes used commercially in textile industries. Dyes are recalcitrant molecules difficult to be degraded biologically. The extensive use of textile dyes causes environmental pollution as they are carcinogenic, mutagenic, allergenic and toxic. A large number of methods are available to treat waste water containing dyes. Chemical processes are costly, less efficient and produces large amount of sludge. Biological processes are getting more and more since they are cost effective and eco friendly. Now a days textile and dye industries are using the bacterium *Acetobacter liquefaciens* and *Pseudomonas fluorescens* to treat their waste water. These bacteria are able to consume bright coloured azo dyes. The waste effluents of several modern industries also contain heavy metal like Mercury, Lead and Cadmium, which causes poisoning. Biotechnical approaches are recommended where metal extracting forms of algae can be grown in ponds where factory effluents (rich in heavy metals) are discharged. The microbes will extract the heavy metals and sequester them inside their cell membrane. The metal can be subsequently recovered from this microbar.

Traditional water treatment system, particularly aerobic trickling filters, has been modified to treat air pollutants. In bioscrubbers and biotrickling filters, multiple microbial communities are grown on solid surfaces to produce multilayered complexes called biofilms, when gas streams coming out of water treatment plant containing organic pollutants are passed through these systems, the pollutants are degraded. Some fungi like *Candida tropicalis* are able to assimilate styrene, a fragrant liquid unsaturated hydrocarbons used chiefly in making synthetic rubber, resins and plastics. The mycelium of the fungi gives the biofilters a large surface area and greater capacity to eliminate pollutants than conventional compost biofilters. Bioreactors consist of filamentous white rot fungi *Phanerochacete chrysosporum* is used to treat effluents of paper industry. They contain lignin degrading and modifying enzyme, which is useful since it may reduce energy cost and corrosion thus increasing the life of the system and also reduce environmental hazards associated with bleach plants effluents.

The plastic industries use alkenes like ethylene and propylene which is then converted into alkenes oxides which are then polymerized to form plastics such as polypropylene and polyethylene. The raw materials have inherent danger of escaping into atmosphere thus causing pollution. *Methylococcus capsulate* has been successfully used for converting alkene into alkene oxides (Gibson and Subramanian, 1984).

Removal of oil spills: Oil spills on ocean waters caused alarming threat to biodiversity and human health. The wreck of the tanker Torrey Canyon in 1969 focus the environmental concern on the fate of hydrocarbon pollutant on the marine habitat, flora and fauna. Oil spills from oil tankers on beaches have been recognized as a major environmental hazard. This spilled oil is believed to not only destroy the habitats of aquatic animal and fish, but also create health problems for local residents and causes long-term damage to the environment. The remediation efforts using chemical or physical dispersants either cause pollution or they are expensive and time consuming. Biotechnological efforts using the process of microbial degradation has been proved efficient and cost effective method of oil removal from the environment.

Oil spills have contaminated marine shorelines and causes several environmental damages. Hydrocarbons degrading microorganisms are ubiquitously distributed in marine ecosystem and the rates of hydrocarbon biodegradation are limited by abiotic environmental factors. Low level of phosphate and fixed form of nitrogen in marine environment limits rate of hydrocarbon degradation and molecular oxygen is required for rapid hydrocarbons degradation (Atlas and Bartha, 1992). The hydrocarbons biodegradation can be accelerated by overcoming the rate limiting factors in order to remove contaminating pollutants (Atlas, 1991). The two approaches taken for bioremediation of petroleum that is able to degrade hydrocarbons and the modification of the environment by adding fertilizers or aerating the contaminated sites (Atlas 1991, 1993). Since hydrocarbons degrading bacteria and fungi are naturally present in marine habitat hence adding fertilizers and ensuring adequate aeration is more promising for treating oil spills.

In early years, application of oleophilic (oil loving) fertilizers as food for oil utilizing microbes was considered useful since this would allow rapid growth and multiplication of indigenous microbes thus speeding up the biodegradation process for removal of oil. In recent years, however, using genetic engineering, oil utilizing micro organism have been successfully produce, which would rapidly grow on oil. These genetically modified strains could not be tested in the field for removing the oils due to the debate concerning the release of genetically engineered microorganism in an open environment. Professor Ananda M. Chakrabarty, a hydrocarbon biotechnologist, working on the strain of oil eating bacteria had developed a very efficient oil eating "Superbug" using species of *Pseudomonas* through recombinant DNA technology. Many oil-utilizing microorganisms may also produce surface-active compounds that can emulsify oil in water and thus facilitate removal of oil. A strain of *Pseudomonas aeruginosa* has been developed by Chakrabarty, which produces a glycolipid emulsifier which reduces the surface tension of an oil water interface and thus helps in removal of oil from water. This microbial emulsifier is non toxic and biodegradable thus being ecologically sound. A hydrocarbon degrading bacterium was the first patented microorganism (Chakrabarty, 1974), but it has never been used in the field and it is poor competitor with reference to indigenous hydrocarbons degraders.

Bioremediation through simple addition of nitrogen containing fertilizers to the contaminated shore lines, stimulate the metabolism of indigenous hydrocarbon degradation in microorganisms and degrade both surface and subsurface oil, three to five time faster then occurred at untreated test sites. However marine water has low concentrations of nitrogen, phosphorus and other mineral nutrients that needed for biomass production. The availability of these nutrients is critical for hydrocarbon degradation (Atlas & Cerniglia, 1995). The oleophilic fertilizers are designed to concentrate these nutrients at the oil water biodegradation interface.

Bioremediation of heavy metal polluted sites: Besides organic compounds, bioremediation can be used to treat sites contaminated with heavy metals of radionuclides. Microbes' algae, bacteria and fungi as well as higher plants have capabilities to uptake these pollutants. After uptake, these are either accumulated or are assimilated by them. Accumulated heavy metals are recovered by recycling or disposal. *Zoolgea remigera* adsorbs Copper and Cadmium. Radioactive metals as Uranium and thorium are removed by *Geobacter metallireducans Rhizopus arrhizus* and *Penillium chrysogenum* from the industrial effluents. *Pseudomonas putida*, *Arthrobacter viscosa* and *Citrobacter* species removes several toxic heavy metals from industrial effluents. The bacteria like *Thiobacillus thiooxidans* bring about bioleaching of Zinc, Cobalt and Nickel from sulphide rocks. Fungi belonging to the genera. *Trichoderma, Aspergillus, Ophiostoma* and *Rhodotorula* are shown to have biosorption ability of heavy metals and these seem to play important role in detoxification of industrial effluents.

Bioremediation of weed and pest control sites: Majority of chemical herbicides, pesticides and fertilizers that are used commercially cause environmental hazards.

Due to increasing concern about contamination of environment by herbicides, new herbicides are being developed that are safer and biodegradable. Herbicides, normally affects process like photosynthesis or biosynthesis of essential amino acids. Herbicides are being developed which will be environmentally safe. To allow the use of these herbicides for crop protection programme, genetically engineered herbicide resistant plants have been produced in a number of crops, which should eventually allow the use of environmentally safer herbicides. In this approach a pathway is introduced that will de-toxify the herbicide, so that the latter will kill the weeds and not the crop. A number of detoxifying enzymes have been identified in plants as well as in microbes. Some of these emzymes including (i) Glutathione S transferase or GST (in maize and other plants) which detoxifies the herbicide atrazine; (ii) Nitrilase (coded by gene *bxn* in *Klebsiella pneumoniae* which detoxifies the herbicide bromoxynil (iii) Phosphinothricin acetyl transferase or PAT (coded by *bar* gene in *Streptomyces* spp.), which detoxifies the herbicide PPT (L Phophinothrin). Similarly popular weedicide 2-4 D which kills broad leaf plants can be effectively biodegraded by *Akalygens eutrophis*, *Burkholderia cepacia* and *Halomonas* etc.

Bacterial pesticides are being developed, though they are slow in action and are expensive. *Heliothrix* complex, which lives in close association with plant root consist of two major crop pests normally budworm and bollworm. Biological insecticides against both these insets are being prepared by transfer of gene from *Bacillus thuringiensis* (Bt), into either naturally occurring soil bacterium or into a strain of *Pseudomonas*.

Management of environmental degradation through plants

The process of recovery of hazardous substances from soil or groundwater contaminated with municipal or industrial water etc. by using plants is called phytoremediation. Green plants are able to remove heavy metals from contaminated soil ground water. Some strains of *Brassica juncea* accumulate heavy metal like Chromium growing in metal contaminated soil. Modified strains of these plants have been shown to accumulate up to 40% of their biomass as heavy metals such as Lead and Chromium. A variety of tree, *Sebertia acuminata* (Sapotaceae) a native of New Caledonia acuminate an astonishing 20-25% of their bodies dry weight of Nickel. Aquatic weeds such as species of *Salvinia, Lemna, Azolla* and *Eichhomia* are also known to tolerate up take and even accumulate heavy and other toxicants and they can provide opportunity for the removal of these metals.

Plants as such may not be able to sequester toxic pollutants from wastes. Roots and rhizomes of some plants provide an ideal habitat for the growth of different kinds of microorganism chiefly bacteria and fungi. These microbes are very effective in remediation of contaminated waste water and industrial waste containing chlorides, bromides, sulphate etc. These microbes remain active even under extreme conditions. The marshy plants are the heart of biotreatment process of phytoremediation. The plants bulrush (*Sciirpus* Spp) and cattails (Typha Spp) create the environment for the clean up process whereas the microorganisms, chiefly bacteria living roots and rhizomes of these sedges breakdown the contaminants (chloride, Sulpahte, bromide etc.).

Future outlook and conclusions: Bioremediation have has promising future with several potential applications to clean up the polluted environment and treat wastes compared to physical cleanup methods. In-situ bioremediation is much less expensive and causes less environmental perturbation. It may alter manufacturing practices, fuel production and composition, the way of individuals' disposal of wastes, cost of products and environmental quality. Diverse microorganism have the capacity of degrading a wide spectrum of pollutant by using diverse metabolic path way. Some of these path way result in detoxification and destruction of pollutants where as other inactivate potentially harmful compounds. These metabolic path way provide the basis for bioremediation.

However employing bioremediation effectively in many applications requires both further R&D and clarification of government policies particularly in release of genetically engineered organisms a controversial issue scientifically as well as politically. However, it may be possible to use them in contained bioreactor.

Our efforts to preserve the global environment and treat wastes and conserve natural resources by utilizing the sophisticated function of microorganisms to make them friendly to the environment. Once our project to develop biotechnological system to combat global environmental change will be successful, India will become leader of environment protection along with Netherlands and Germany to fulfill the phrase 'Act locally and think globally'. They implication of bioremediation for the environmental management is emerging as an important cost effective strategies to combat with environmental degradation an conservation of natural resources and ecosystem.

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