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Bioremediation technology: A new horizon for environmental clean-up

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Abstract
The hazardous wastes generated from the chemical processes/operations are being treated using physico-chemical and biological methods by the respective industries to meet the prescribed standard as per the Environmental Protection Act, 1986. The wastes treated by the respective industries are collected at Common Effluent Treatment Plant, before discharge into the environment. After the treatment of collected waste at Common Effluent Treatment Plant, the solid and treated effluents are segregated and disposed of into the soil-water environment. In spite of the present treatment technology, the organic pollutants are found persisting in the soil-water environment above their acceptable level. Hence, bioremediation is an innovative technology that has the potential to alleviate the toxic contamination. The processes of bioremediation usually occur in soil/water environment, whereby compounds are broken down into less toxic compounds and/or environmental friendly compounds by microorganisms. In this paper, the bioremediation techniques proved effective and efficient for remediation of pollutants using designed and developed laboratory bioreactors have been highlighted so as to take-up the bioremediation technology from laboratory to field to clean up the environment.

Keywords: Hazardous waste; Common Effluent Treatment Plant; Bioremediation; Microorganism; Bioreactor; Laboratory technology.

Introduction

The present treatment technology involving physic-chemical and biological methods are not efficient and are not efficient and /or effective to treat the contaminants to acceptable level. Today, biotechnology is being considered as emerging science for environmental protection. The technology involves the use of microorganisms for biological treatment of air, water and soil pollutants. Biotechnological treatment is carried out at lower temperature and pressure which requires less energy than the conventional physico-chemical treatment technology. The industries generating hazardous wastes have found beneficial measures from the emerging trend of biotechnological treatment. Biotechnological innovations for treatment for hazardous waste under controlled environmental conditions have been found cost–effective means of reducing the pollution potential of waste water, leading to enhanced public acceptance and compliance with environmental legislation (Fulekar, 2010). Environmental pollution such as contaminated soil or surface / ground water can be solved by bioremediation and / or phytoremediation by use of biological living organisms and green plants.

Bioremediation is defined as the process by which microorganisms are stimulated to rapidly degrade hazardous organic pollutants to environmentally safe levels in soils, sediments, substances, materials and ground water. Recently, biological remediation process have also been devised to either precipitate effectively immobilize inorganic pollutants such as heavy metals. Stimulation of microorganisms is achieved by the addition of growth substances, nutrients, terminal electron acceptor/donors or some combination thereby resulting in an increase in organic pollutant degradation and bio-transformation. The energy and carbon are obtained through the metabolism of organic compounds by the microbes involved in bioremediation processes (Fulekar *et al.*, 2009).

Bioremediation technology uses micro-organisms to reduce, eliminate or transform contaminants present in soils, sediments or water. Bioremediation depends on the presence of specific microorganisms in the correct amounts and combination and in the appropriate environmental conditions. Microorganisms living already living in contaminated environments are often well adapted to survive in the presence of existing contaminants and to the temperature, pH and oxidation/ reduction potential of the site. These indigenous microbes tend to utilize the nutrients and electron acceptors that are available, provided liquid water is present. Water also acts as a vehicle to transport both microorganism and dissolved substances including contaminants and their breakdown products. Bioremediation process involves biotransformation and biodegradation by

transforming contaminants to non–hazardous or less hazardous chemicals. Often, the micro-organisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass. Biotransformation is any alteration of the molecule or structure of a compound by micro-organisms. Biodegradation is the breaking down of organic or bioaccumulation and biotransformation of inorganic compounds into environmental friendly compounds.

Bioremediation Process

The process of bioremediation enhances the rate of the natural microbial degradation of contaminants by supplementing there microorganisms with nutrients, carbon sources or electron donors. This can be done by using indigenous micro-organisms or by adding an enriched culture of micro-organisms that have specific characteristics that allow them to degrade the desired contaminant at a quicker rate. Ideally, bioremediation results in the complete mineralization of contaminants to H_2O and CO_2 without the build-up of intermediates (Sharma and Fulekar, 2009).

Bioremediation processes can be broadly categorized into two groups: ex situ and in situ. Ex situ bioremediation technologies include bioreactors, bio-filters, land farming and some composting methods. In situ bioremediation technologies include bioventing, bio-sparging, bio-stimulation liquid delivery system and some composting methods. In situ treatments tend to be more attractive to vendors and responsible parties because they require less equipment, generally have a lower cost and generate fewer disturbances to the environment. However, the difficulties associated with implementing in situ processes have limited their application in the field. Bioremediation using white rot fungi to inoculate contaminated media is a promising technology that is currently being researched. This technology can be used in an ex situ or in situ manner. Generally this fungus is used to inoculate a composting process, but it does have other bioremediation applications.

Metabolic Process

The control and optimization of bioremediation processes is a complex system of many factors. The metabolism of organic contaminants can be broadly disseminated by the ability of the organisms to gain energy for cell growth from the process. These include:

Primary substrates: If the metabolism of a compound provides energy for cell maintenance and division, the contaminant is referred to as a primary substrate.

Secondary substrates: In some cases, a compound is metabolized and provides the cell with energy but does not support growth. Contaminants of this type are referred to as secondary substrates.

Co-metabolisms: If a compound is transformed with benefit of the cell (no energy or carbon provided for use by the organism) while the cell is obtaining energy from another transformable compound, the biotransformation is referred to as co-metabolic.

Energy biotransformation: Finally, an additional classification has been recently identified in which some contaminants are capable of serving as the terminal electron acceptor in the respiratory chain of certain anaerobic (without oxygen) bacteria. In this case, energy is not obtained from the contaminant itself, but its transformation is a component of metabolic processes that provide energy to the cell for growth.

Growth Requirement

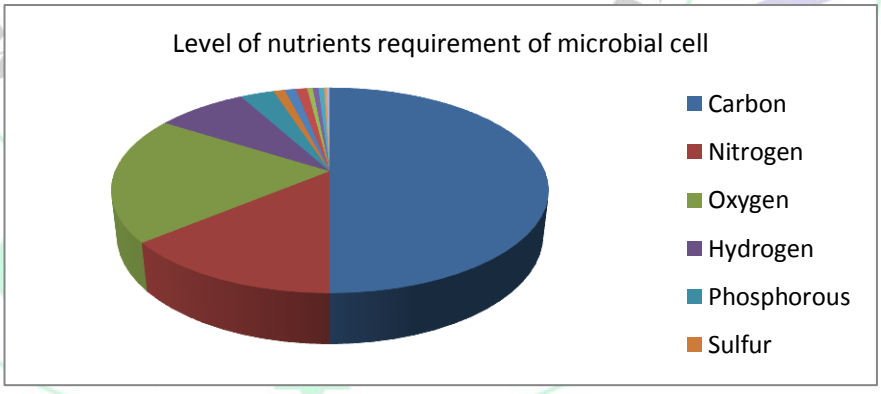
Microorganisms can be isolated from almost any environmental conditions. The potential of microorganisms for bioremediation process depends on the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population and the environmental factors. As essential element of bioremediation process is the ability to sustain enhanced levels of metabolic activity for extended period of time. The studies on assessment of conditions of contaminated sites demonstrate the following factors essential for bioremediation.

Microbial Bioremediation

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. Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacterial and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The micro-organisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminated compounds are transferred by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation, the process is called as "Bio-augmentation". The microorganisms with the genetic capacity to transform compounds of interest must be present in contaminant metabolism to occur in a bioremediation process. In certain cases, the addition of organisms acclimated to specific contaminants, or bio-augmentation, may decrease the duration of lag phases. The ability to effectively bio-augment bioremediation system is a function of the process used.

Requirements for microbial growth in bioremediation process

Requirement	Description
Carbon source	Carbon is the most basic element of living forms and is needed in greater quantities than other elements. Carbon contained in many organic contaminants may serve as a carbon source for cell growth. If the organism involved is an autotroph CO_2 or HCO_3 in solution is required. In some cases, contaminant levels may be too low to supply adequate levels of cell carbon, or the contaminant is metabolized via co-metabolism. In these cases the addition of carbon sources may be required.
Nutrients	The growth and activity of the microorganisms must be estimated by adequate maintenance and supply of nutrients. Bio-stimulation usually involves the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. Nutrients: Nitrogen (ammonic, nitrate, or organic nitrogen) and phosphorous (ortho-phosphate or organic phosphorous) are generally the limiting nutrients. In certain anaerobic systems, the availability of trace metals (e.g. iron, nickel, cobalt, molybdenum and zinc) can be of concern.  **Source:** From Stainer *et al.* (1986), Microbial World, 5th Ed., Prentice Hall, NJ.
Energy source	In the case of primary metabolism, the organic contaminant supplies energy required for growth. This is not the case when the contaminant is metabolized via secondary metabolism or co-metabolism or as a terminal electron acceptor. If the contaminant does not serve as a source of energy, the addition of a primary substrate(s) is required.
Electron acceptor	All respiring bacteria require a terminal electron acceptor. In some cases, the organic contaminant may serve in this capacity. Dissolves oxygen is a common electron acceptor in aerobic bioremediation processes. Under anaerobic conditions, NO_3^-, SO_4^{3-}, Fe^{3+}, and CO_2 may serve as terminal electron acceptors. Certain co-metabolic transformations are carried out by fermentative and other anaerobic organisms, in which terminal electron acceptors are not required.

<table>
<tr><td>Temperature</td><td>Rates of growth and metabolic activity are strongly influenced by temperature. Surface soils are particularly prone to wide fluctuations in temperature. Generally, mesophilic conditions are best suited for most applications (with composting being a notable exception).</td></tr>
<tr><td>pH</td><td>A pH is another important factor that influences bioremediation process. If the soil is acidic, it is possible to raise pH by adding lime. A pH ranging between 6.5 and 7.5 is generally considered optimal. The pH of most ground water (8.0–8.5) is not considered inhibitory.</td></tr>
<tr><td>Absence of toxic metals</td><td>Many contaminated sites contain a mixture of chemicals, organic and inorganic, which may be inhibitory or toxic to microorganisms. Heavy metals and phemelic compounds are of particular concerns.</td></tr>
<tr><td>Soil moisture</td><td>Moisture content affects the microbial growth and activity. The water-holding capacity recommended for bioremediation process may range from 25% – 28%.</td></tr>
<tr><td>Adequate contact between micro-organisms and substrates</td><td>For contaminants to be available for microbial uptake it must be present in aqueous phase. Thus contaminants that exist as non-aqueous phase liquids or are sequestered within a solid phase may not be readily metabolized. For degradation it is necessary that bacteria and the contaminants be in contact. This is not easily achieved, as neither the microbes nor contaminants are uniformly spread in the soil. Some bacteria are mobile and exhibit a chemotactic response, sensing the contaminant and moving towards it. Other microbes such as fungi grow in a filamentous, form towards the contaminant. It is possible to enhance the mobilization of the contaminant utilizing some surfactants such as sodium dodecyl sulphate (SDS).</td></tr>
<tr><td>Time</td><td>Time is an important factor in the start-up of bioremediation systems. Even the above mentioned considerations are met, lag phases are often observed prior to the onset of activity. In some cases, the dramatic bacterial population shifts that are required for bioremediation will lengthen periods of slow activity.</td></tr>
<tr><td>Environmental requirements</td><td>The optimum environmental conditions that requires for bioremediation of contaminant sites are presented in table given below.
<table>
<tr><th>Parameters</th><th>Conditions required for microbial activity</th><th>Optimum value for organic pollutant degradation</th></tr>
<tr><td>Soil moisture</td><td>25 - 28% of water holding capacity</td><td>30 – 90%</td></tr>
<tr><td>Soil pH</td><td>5.5 – 8.8</td><td>6.5 to 8.0</td></tr>
<tr><td>Oxygen content</td><td>Aerobic, minimum air filled pore space of 10%</td><td>10 – 40%</td></tr>
<tr><td>Nutrient content</td><td>N and P for microbial growth</td><td>C:N:P=100:10:1</td></tr>
<tr><td>Temperature (^{o}C)</td><td>15 – 45</td><td>20 – 30</td></tr>
<tr><td>Contaminants</td><td>Not too toxic</td><td>Hydrocarbon 5 – 10% of dry weight of soil</td></tr>
<tr><td>Heavy metals</td><td>Total content – 2000 ppm</td><td>700 ppm</td></tr>
<tr><td>Type of soil</td><td>Low clay or sill content</td><td>700</td></tr>
</table>
Source: Vidali (2001)</td></tr>
</table>

Bioremediation Organisms

Microorganisms that carry out biodegradation in many different environments are identified as active members of microbial consortiums. These microorganisms include: *Acinethobacter, Actinobacter, Acaligenes, Arthrobacter, Bacillins, Berijerinckia, Flavobacterium, Methylosinus, Mycrobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctomia, Serratio, Trametes and Xanthofacter.*

Microorganisms individually cannot mineralize most hazardous compounds. Complete mineralization results in a sequential degradation by a consortium of microorganisms and involves synergism and co metabolism actions. Natural communities of microorganisms in various habitats have an amazing physiological versatility, they are able to metabolize and often mineralize an enormous number of organic molecules. Certain communities of bacteria and fungi metabolize a multitude molecules that can be degraded is not known but thousands are known to be destroyed as a result of microbial activity in one environment or another.

Enzymatic-Biodegradative Pathways

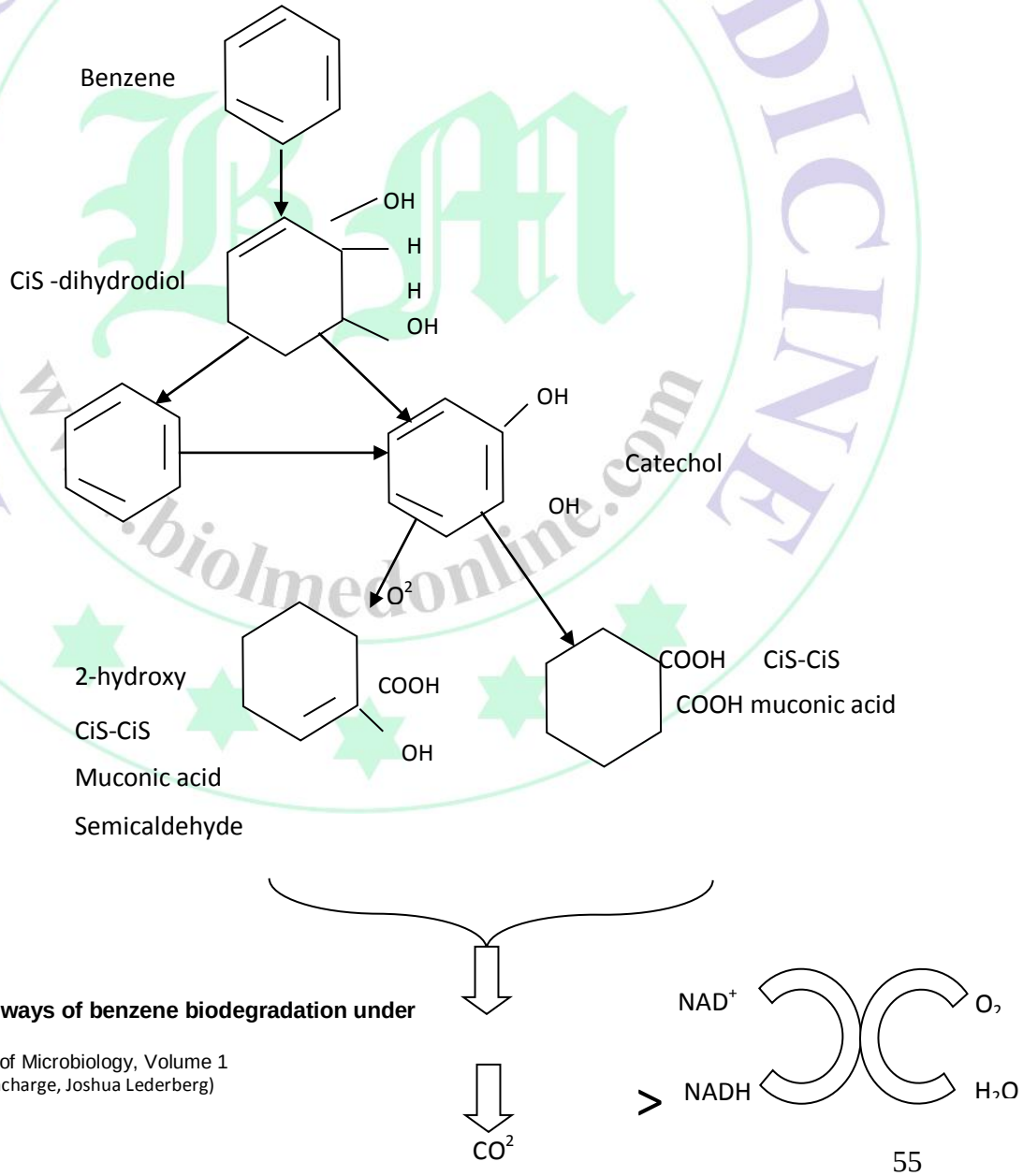
Microbial enzymes are responsible for the degradation of various organic pollutants. The enzymes that mediate biodegradative reactions are often those that initiate essential metabolic pathways to obtain energy for microbial growth. Certain biological transformation mechanisms are unique to

particular microorganisms. For example, some microbial species are capable of proliferating under both aerobic and anaerobic conditions. These microorganisms possess the enzymatic machinery to use either molecular oxygen or nitrate as final electron acceptor. In some case no electron acceptor is needed for growth of microorganism, when they are able to derive the necessary energy by a fermentative process. In addition, some microorganisms can produce enzymes that they secrete and that are active extra-cellularly. It is difficult to distinguish between enzyme–catalyzed biotransformation reactions and those that occur as a result of purely physico-chemical effects. The existence of chemical enzymes that exhibit activity outside the organisms has contributed to a recurrent problem to investigate enzymatic biodegradative pathways.

Thus, transformed products may be generated in a number of ways including:

- Enzyme catalysis occurring in the microorganisms,
- Enzyme catalysis occurring extra cellularly in the environment, or
- Physico-chemical catalysis.

Transformation may be the result of a combination of these mechanisms. It is generally assumed that xenobiotic–degrading enzymes have induced by mutation and natural selection from those constitutive or inducible enzymes responsible for general microbial metabolism. Some of the genes encoding pollutant–degrading enzymes can be found on autonomously replicating plasmids that in certain cases are transmissible among different microbial strains. Plasmid exchange within a population could result in the production of novel microbial strains with a large number of degradative capabilities.



The metabolic pathways of benzene biodegradation under aerobic conditions

Source: Encyclopedia of Microbiology, Volume 1 (Second Edition, Editor-Incharge, Joshua Lederberg)

BMID: RV6-BM11

Enzymatic Mechanism

Enzymes are central to the mode of action of many hazardous wastes: some hazardous wastes are activated in situ bioremediation by enzymatic action, and many hazardous wastes function by targeting particular enzymes with essential physiological roles. Enzymes are also involved in degradation of hazardous waste compounds, both in the target organisms, through intrinsic detoxification mechanisms and evolved metabolic resistance, and in the wider environment, via biodegradation by soil and water microorganisms. Whether involved in the mode of action or their degradation, the enzymes involved in hazardous waste biology have been subjected to heavy selection pressures over the past 50 years, and some novel enzyme activities and pathways (for example, s-triazine and lindane catabolic pathways) (Jorgenson, 2007) have evolved as a consequence. Some of the degradative activities that have evolved not only constitute remarkable case studies of the influence of human activities upon natural evolutionary processes, but also form a source of significant biotechnological potential, and the basis of strategies to reduce the environmental impacts of hazardous wastes residues by bioremediation. It is largely, throughout exclusively, the enzymes which have evolved in response to non-natural compounds that are the foundation for the hazardous waste bioremediation technologies that are currently available or in development. Bioremediation of hazardous waste is becoming an important component of integrated environmental management practices, helping to ensure that the principles of good stewardship are maintained. Enzymes can be extracted and then employed for the bioremediation. The enzymes extracted are purified or partially purified to catalyse contaminant detoxification. Unlike other technologies free enzyme bioremediation is not dependent upon the growth of intact organisms, and so the rate of detoxification is directly linked to the catalytic properties employed and concentration of the enzymes applied. Lewis *et al.* (1984) have reported increase in biotransformation of toxic metals: methane, parathion along with other agrochemicals using culture filtrates of bacteria and mixed cultures, etc. Lewis *et al.* have also reported increase in cell biomass stimulated increase in the biotransformation rates. Hence it can be concluded that each bioremediation strategy forms a frame work in which the hazardous waste detoxifying enzyme must operate, and that the required biochemical and physical characteristics of those enzymes are determined by the bioremediation strategy in which they are employed. The success of any bioremediation strategy is ultimately dependent upon the presence of appropriate enzymes.

Microbial enzymes, which detoxify hazardous compounds, could also serve as bioremediation agents (Wainwright, 1999). Enzymatic biodegradation is potentially an improved and quick method for removing hazardous wastes compounds.

Environmental Variations in Field

The research studies documented that the microorganisms possess a high biodegradative potential that can degrade any toxic organic pollutants under appropriate conditions in a laboratory set up, but may differ the degradative potential in natural environment. The main factors that play roles in natural environment include:

- Competition among other microorganisms for survival and growth,
- Favourable growth conditions may change/alter or differ,
- The availability of nutrients such as nitrogen, sulphur, phosphorus, calcium, magnesium as well as trace elements may differ or alter,
- The changes in aerobic to anaerobic conditions or anaerobic to aerobic conditions,
- The variation in pH, temperature and mixing rate,
- The close interaction of micro organic with contaminants may vary,
- The environmental factors such as soil, the presence of reactive surfaces and particulate matter may interfere with microbial decomposition of a substance,
- The rate of metabolism of a substance may vary with changes in the environmental parameters,
- The concentrations of substances present in the natural environment may be toxic or many be too low to obtain carbon source from organic contaminants,
- Sometimes, the intermediate may be more toxic than that of the parent compounds,
- The activity of the heterogeneous community, rather than that of the single species, determines a chemical's biodegradation in the natural environment.

Thus, the interaction of a large number of factors determines the ultimate fate of a particular compound in natural environment.

The innovative techniques for bioremediation of hazardous waste have been developed. The pilot scale research studies incorporating use of a novel source of

microbial consortium for bioremediation of hazardous waste compounds, both organics and inorganics in the designed and developed bioreactors have been carried out.

Bioremediation Research Studies Using Designed and Developed Laboratory Bioreactors

1. Bioremediation of pesticide in surface soil treatment unit using microbial consortia

The manufacturing and use of pesticides has been rising tremendously in India. The waste generated by the pesticide industry has become an environmental problem due to the present insufficient and ineffective waste treatment technology involving physico-chemical and biological treatment. The available data indicates that pesticide residues remain in surface soil, leading to toxicity in the soil-water environment. The recent advances in bioremediation technology using microbial consortium has been found effective for treatment of pesticides in soil. In the present study, a Surface Soil Treatment Unit has been designed wherein bioremediation of commonly used pesticides namely chlorpyrifos, cypermethrin, fenvalerate, and trichlopyr butoxyethyl ester at varying concentration viz. 25, 50 and 100 mg/kg have been carried out using cow-dung microbial consortia under simulated environmental conditions. The bioremediation conditions have been monitored and maintained during the study. The investigation has been extended till the parent compound was converted into intermediates and/or less harmful compounds. These then will further mineralize, from part of the microbial food chain and/or become integrated into the humic fractions. The results presented here highlight the potential of cow-dung slurry consortia for bioremediation of soil contaminated with pesticides in surface soil treatment unit (Geetha and Fulekar, 2008).

2. Bioremediation of pesticides using scale up process bioreactors

To assess the bioremediation potential of *Pseudomonas aeruginosa* (NCIM 2074) by improving its adaptability to increasing concentration of chlorpyrifos using scale up process. *Pseudomonas aeruginosa* isolate NCIM 2074 was adapted by subjecting to varying concentrations of chlorpyrifos, i.e. 10, 20, 50, 75 and 100 mg/l in incubator shaker at 37°C and 150 rpm. An initial 10 mg/l concentration of chlorpyrifos was supplied in minimal salt medium (MSM) under controlled environmental conditions for 14 days. The culture was subsequently scaled up to higher concentrations of chlorpyrifos by transferring one milliliter from the medium with 10mg/L to 25 mg/l of the compound. After every 14 days this process was repeated, each time using medium with higher chlorpyrifos concentration. The entire scale up process continued for a period of 70 days. *Pseudomonas aeruginosa* (NCIM 2074) was adapted to increasing chlorpyrifos up to 50 mg/l, but 75 and 100 mg/l was inhibitory to the organism. The biodegradation of chlorpyrifos, as assessed by GC-MS, showed that chlorpyrifos at 10, 25, 50 mg/l degraded completely over a period of 1, 5 and 7 days, respectively. The intermediate 3, 5, 6 trichloro-2-pyridion, 2, 4-bis (1, 1 dimethyiethyl) phenol and 1, 2 zenedicarboxylic acid persisted during bioremediation, but in the long run these convert to CO_2, biomass and nutrients. *Pseudomonas aeruginosa* (NCIM 2074) has been of potential use in bioremediation of chlorpyrifos at concentrations up to 50 mg/l, but the organism is inhibited by higher concentrations (Fulekar and Geetha, 2008).

3. Bioremediation of benzene using a designed and developed partitioning bioreactor

A bioreactor has been designed and developed for partitioning of aqueous and organic phases with a provision for aeration and stirring, a cooling system and a sampling port. The potential of a cow dung microbial consortium has been assessed for bioremediation of phenol in a single-phase bioreactor and a two-phase partitioning bioreactor. The advantages of the two-phase partitioning bioreactor are discussed. The *Pseudomonas putida* IFO 14671 has been isolated, cultured and identified from the cow dung microbial consortium as a high-potential phenol degrader. The methods developed in this study present an advance in bioremediation techniques for the biodegradation of organic compound such as phenol using a bioreactor. We have also demonstrated the potential of microorganisms from cow dung as a source of biomass (Singh and Fulekar, 2009).

4. Bioremediation of benzene using cow dung microflora in two phase partitioning bioreactor

Bioremediation of benzene has been carried out using cow dung microflora in a bioreactor**.** The bioremediation of benzene under the influence of cow dung microflora was found to be 100% and 67.5%, at initial concentrations of 100 mg/l and 250 mg/l within 72 h and 168 h

respectively; whereas at higher concentration (500 mg/l), benzene was found to be inhibitory. Hence the two phase partitioning bioreactor (TPPB) has been designed and developed to carryout biodegradation at higher concentration. In TPPB the contaminant found to be biodegraded at 5000 mg/l concentration up to 50.17% over a period Q1 of 168 h. Further the *Pseudomonas putida* MHF 7109 was isolated from cow dung microflora as potential benzene degrader and its ability to degrade benzene at various concentrations was evaluated. The data indicates 100%, 81% and 65% degradation at the concentrations of 50 mg/l, 100 mg/l, 250 mg/l within the time period of 24 h, 96 h and 168 h respectively. The GC-MS data also shows the presence of catechol and 2-hydroxymuconic semialdehyde, which confirms the established pathway of benzene biodegradation. The present research proves the potential of cow dung microflora as a source of biomass for benzene biodegradation in TPPB (Singh and Fulekar, 2009).

5. Bioremediation of pesticide chlorpyrifos in mycorrhizosphere Ecological Remediation Unit using ryegrass

The potential of ryegrass for rhizosphere bioremediation of chlorpyrifos in mycorrhizal soil was investigated by the green house pot culture experiments. The pot cultured soil amended at initial chlorpyrifos concentration of 10 mg/kg was observed to be degraded completely within 7 days where the rest amended concentrations (25–100 mg/kg) decreased rapidly under the influence of ryegrass mycorrhizosphere as the incubation progressed till 28 days. This bioremediation of chlorpyrifos in soil is attributed to the microorganisms associated with the roots in the ryegrass rhizosphere, therefore the microorganisms surviving in the rhizospheric soil spiked at highest concentration (100 mg/kg) was assessed and used for isolation of chlorpyrifos degrading microorganisms. The potential degrader identified by 16S rDNA analysis using BLAST technique was *Pseudomonas nitroreducens* PS-2. Further, bio-augmentation for the enhanced chlorpyrifos biodegradation was performed using PS-2 as an inoculum in the experimental set up similar to the earlier. The heterotrophic bacteria and fungi were also enumerated from the inoculated and non-inoculated rhizospheric soils. In bio-augmentation experiments, the percentage dissipation of chlorpyrifos was 100% in the inoculated rhizospheric soil as compared to 76.24, 90.36 and 90.80% in the non-inoculated soil for initial concentrations of 25, 50 and 100 mg/kg at the 14th, 21st and 28th day intervals respectively (Korade and Fulekar, 2009).

6. Biodegradation of petroleum hydrocarbon compounds toluene and o-xylene (BTX) by *Pseudomonas putida* strain MHF 7109

Pseudomonas putida MHF 7109 has been isolated and identified from cow dung microbial consortium for biodegradation of selected petroleum hydrocarbon compounds – benzene, toluene, and o-xylene (BTX). Each compound was applied separately at concentrations of 50, 100, 250, and 500mgL^{-1} in minimal salt medium to evaluate degradation activity of the identified microbial strain. The results indicated that the strain used has high potential to degrade BTX at a concentration of 50mgL^{-1} within a period of 48, 96, and 168 h, respectively; whereas the concentration of 100mgL^{-1} of benzene and toluene was found to be completely degraded within 120 and 168 h, respectively. Sixty-two percent of o-xylene was degraded within 168 h at the 100mgL^{-1} concentration level. The maximum degradation rates for BTX were 1.35, 1.04, and 0.51mgL^{-1} h^{-1}, respectively. At higher concentrations (250 and 500mgL^{-1}) BTX inhibited the activity of microorganisms. The mass spectrometry analysis identified the intermediates as catechol, 2-hydroxymuconic semialdehyde, 3-methylcatechol, cis-2-hydroxypenta-2,4-dienoate, 2-methylbenzyl alcohol, and 1,2-dihydroxy-6-methylcyclohexa-3,5-dienecarboxylate, for BTX, respectively. *P. putida* MHF 7109 has been found to have high potential for biodegradation of volatile petroleum hydrocarbons (Singh and Fulekar, 2010).

Genetic Engineering Approach

Scientists are currently looking into certain genetically engineered microorganisms to increase their ability to metabolize specific chemicals such as hydrocarbons and pesticides. The possibilities of using genetic engineering for improvement of bioremediation process had an early boost in the late 1980's. Recombinant DNA techniques have been studied intensively to improve the degradation of hazardous waste under laboratory condition. The genetically engineered microorganisms have higher degradative capacity and have been demonstrated successfully for the degradation of various pollutants under defined conditions. Genetic modification technology has resulted often in a wide variety of current and potential applications for use in the process of bioremediation. Bioremediation

explores gene diversity and metabolic versatility of microorganisms (Hansa and Fulekar, 2009). The genetic architecture of these organisms makes them valuable in biodegradation, biotransformation, biosorption and bioaccumulation. The necessary blue print of gene encoding for biodegradative enzymes is present in chromosomal and extra-chromosomal DNA of such microbes. Recombinant DNA techniques facilitate to evolve the ability of an organism to metabolize a xenobiotic by detection of such degradative genes and transforming them into appropriate host via suitable vector under the tight control of appropriate promoters. It depends on susceptibility to alteration and exchange of genetic information. The recombinant DNA technology explores PCR, anti-sense RNA technique, site directed mutagenesis, electroporation and particle bombardment techniques. The biotechnology armed with recombinant DNA technology is now fine tuning the bioremediation technology by improving pollutant–degrading microbes through strain improvement and genetic modification of specific regulatory and metabolic genes that are crucial in developing effective, safe and economical techniques for bioremediation.

Conflict of Interests

Authors do not have any conflicting interests.

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