Article

Microorganism as a tool of bioremediation technology for cleaning environment: A review

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Abstract

The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment. Bioremediation is the most effective management tool to manage the polluted environment and recover contaminated soil. 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.

Keywords hazardous waste; bioremediation; microorganism; bioreactor.

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1 Introduction

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 uses biological agents, mainly microorganisms i.e. yeast, fungi or bacteria to clean up contaminated soil and water (Strong and Burgess, 2008). This technology relies on promoting the growth of specific microflora or microbial consortia that are indigenous to the contaminated sites that are able to perform desired activities (Agarwal, 1998). Establishment of such microbial consortia can be done in several ways e.g. by promoting growth through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions (Hess et al., 1997; Agarwal, 1998; Smith et al., 1998). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Hess et al., 1997; Agarwal, 1998; Tang et al., 2007).

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 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.

2 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 bacteria 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. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity (Gupta, 2003).

3 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. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions (Colberg and Young, 1995) may permit microbial organisms to degrade otherwise recalcitrant molecules.

4 Bioremediation Research Studies Using Designed and Developed Laboratory Bioreactors

4.1 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).

4.2 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 CO2, 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).

4.3 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).

4.4 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).

4.5 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).

4.6 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).

5 Genetic Engineering

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 (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. Bioremediation is not effective only for the degradation of pollutants but it can also be used to clean unwanted substances from air, soil, water and raw materials form industrial waste. Bioremediation is not effective only for the degradation of pollutants but it can also be used to clean unwanted substances from air, soil, water and raw materials form industrial waste.

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