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Bioremediation: a tool for cleaning polluted environments

[Review Paper]

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ABSTRACT
Modern science has brought about hitherto unimagined progress and developments in human civilization. Although it is desirable, this progress is gradually making the world inhospitable due to adulteration and pollution of the environment by numerous products and byproducts of civilization. The key pollutants include heavy metals, chemical wastes and oil spills, among others. Scientists are aware of the impending danger, and they are making efforts to find ways of mitigating the environmental pollution, while keeping pace with civilization. Use of microbial resources, coupled to other modern techniques is one of the most promising and economical strategies for removing environmental pollutants. This paper presents a review of the various approaches to bioremediation, their advantages and disadvantages and potential areas of application.

Key words: Bioremediation, microorganism, environmental pollutants, xenobiotics, phytoremediation.

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INTRODUCTION
Indiscriminate and uncontrolled discharge of industrial and urban wastes into the environmental sink has become an issue of major global concern (Hernandez *et al*., 1998; Gupta & Mahapatra, 2003; Strong & Burgess, 2008). Intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds to the environment. Excess loading of hazardous waste has led to scarcity of clean water and disturbances of soil thus limiting crop production (Kamaludeen *et al*., 2003). Although enactment of stringent regulation has led to less indiscriminate disposal of organic and inorganic wastes (Kamaludeen *et al*., 2003), challenges remain that require other interventions.

Compared to other methods, bioremediation is a more promising and less expensive way for cleaning up contaminated soil and water (Eccles & Hunt, 1986; Kamaludeen *et al*., 2003). Bioremediation uses biological agents, mainly microorganisms, e.g. yeast, fungi or bacteria to clean up contaminated soil and water (Strong & 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, among others (Hess *et al*., 1997; Agarwal, 1998; Smith *et al*., 1998). Establishment and maintenance of favourable conditions for microbial growth and process control are basic prerequisites (Agarwal, 1998). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Hess *et al*., 1997; Agarwal, 1998; Tang *et al*., 2007).

There are two approaches to bioremediation: (1) *in situ* bioremediation involves the treatment of contaminants where they are located. In this case the microorganisms come into direct contact with the dissolved and sorbed contaminants and use them as substrates for transformation (Bouwer & Zehnder, 1993). Since the *in situ* process is slow, it is not the best approach when immediate site clean up is desired (Iwamoto & Nasu, 2001). (2) *Ex situ* bioremediation is a different approach that utilizes specially constructed treatment facility. It is more expensive than *in situ* bioremediation.

BIOREMEDIATION AGENTS

Natural organisms, either indigenous or extraneous (introduced), are the prime agents used for bioremediation (Prescott *et al*., 2002). The organisms that are utilized vary, depending on the chemical nature of the polluting agents, and are to be selected carefully as they only survive within a limited range of chemical contaminants (Prescott *et al*., 2002; Dubey, 2004). Since numerous types of pollutants are to be encountered in a contaminated site, diverse types of microorganisms are likely to be required for effective mediation (Table 1 & 2) (Watanabe *et al*., 2001). The first patent for a biological remediation agent was registered in 1974, being a strain of *Pseudomonas putida* (Prescott *et al*., 2002) that was able to degrade petroleum. In 1991, about 70 microbial genera were reported to degrade petroleum compounds (U.S Congress, 1991) and almost an equal number has been added to the list in the successive two decades. These organisms belong to at least 11 different prokaryotic divisions (Glazer & Nikaido, 2007).

Table 1: Microorganisms having biodegradation potential for xenobiotics.

Organism	Toxic chemicals	Reference
Pseudomonas spp	Benzene, anthracene, hydrocarbons, PCBs	Kapley *et al*., 1999; Cybulski *et al*, 2003
Alcaligenes spp	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs	Lal & Khanna, 1996
Arthrobacter spp	Benzene, hydrocarbons,pentachlorophe-nol, phenoxyacetate, polycyclic aromatic	Jogdand, 1995
Bacillus spp	Aromatics, long chain alkanes, phenol, cresol	Cybulski *et al*., 2003
Corynebacterium spp	Halogenated hydrocarbons, phenoxyacetates	Jogdand, 1995
Flavobacterium spp	Aromatics	Jogdand, 1995
Azotobacter spp	Aromatics	Jogdand, 1995
Rhodococcus spp	Naphthalene, biphenyl	Dean-Ross *et al*., 2002
Mycobacterium spp	Aromatics, branched hydrocarbons benzene, cycloparaffins	Sunggyu, 1995
Nocardia spp	Hydrocarbons	Park *et al*., 1998
Methosinus sp	Aromatics	Jogdand, 1995
Methanogens	Aromatics	Jogdand, 1995
Xanthomonas spp	Hydrocarbons, polycyclic hydrocarbons	Jogdand, 1995; Ijah, 1998
Streptomyces spp	Phenoxyacetate, halogenated hydrocarbon diazinon	Jogdand, 1995
Candida tropicalis	PCBs, formaldehyde	Ljah, 1998
Cunniughamela elegans	PCBs, polycyclic aromatics, biphenyls	Jogdand, 1995

Bioremediation can occur naturally or through intervention processes (Agarwal, 1998). Natural degradation of pollutants relies on indigenous microflora that are effective against specific contaminants, and it usually occurs at a slow rate. With intervention processes, the rate of biodegradation is aided by encouraging growth of microorganisms (Table 3), under optimized physico-chemical conditions (Blackburn & Hafker, 1993; Bouwer *et al*., 1998; Smith *et al*., 1998). Microbial activity is stimulated by supplementing nutrients (nitrogen and phosphorus), electron acceptors (oxygen), and substrates (methane, phenol, and toluene), or by introducing microorganisms with desired catalytic capabilities (Ma *et al*., 2007; Baldwin *et al*., 2008). Numerous methods to remediate contaminated soil and water are presented in Table 3 (Thassitou & Arvanitoyannis, 2001; Soccol *et al*., 2003) & Table 4 (Watanabe *et al*., 2001; Gupta & Mahapatra, 2003; Nataraj *et al*., 2007), respectively.

Table 2: Microorganisms.that utilize heavy metals.

Microorganism	Elements	References
Bacillus spp.	Cu, Zn	Philip *et al*., 2000; Gunasekaran *et al*., 2003
Pseudomonas aeruginosa	U, Cu, Ni	Sar *et al*., 1999; Sar & D'Souza, 2001
Zooglea spp.	Co, Ni, Cd	Gunasekaran *et al*., 2003
Citrobacter spp.	Cd, U, Pb	Yan & Viraraghavan, 2001; Gunasekaran *et al*., 2003
Chlorella vulgaris	Au, Cu, Ni, U, Pb, Hg,Zn	Pearson, 1969; Gunasekaran *et al*., 2003
Aspergillus niger	Cd, Zn Zn, Ag, Th, U	Guibal *et al*., 1995; Gunasekaran *et al*., 2003
Pleurotus ostreatus	Cd, Cu, Zn	Favero *et al*., 1991
Rhizopus arrhizus	Ag, Hg, P	Gunasekaran *et al*., 2003
Stereum hirsutum	Cd, Pb, Ca	Gabriel *et al*., 1994 &1996
Phormidium valderium	Cd, Co, Cu, Ni	Gabriel *et al*., 1994 & 1996
Ganoderma applantus	Cd, Pb	Gabriel *et al*., 1994 & 1996
Volvariella volvacea	Cu, Hg, Pb	Purkayastha & Mitra, 1992; Jagadevan & Mukherji, 2004
Daedalea quercina	Zn, Pb, Cu	Sanglimsuwan *et al*., 1993; Gabriel *et al*., 1994 & 1996

Table 3: Methods applied in soil bioremediation.

Technology	Principles	Advantages	Disadvantages	Applications
Land farming	Solid-phase treatment system	Simple procedure, Inexpensive, self-heating	Slow degradation rates, Long incubation periods	Surface application, aerobic process
Composting	Anaerobic, convert's solid organic wastes into humus-like material	Rapid reaction rate, Inexpensive, self-heating	Requires nitrogen supplementation, incubation periods months to years	Surface application, agricultural to municipal waste
Intrinsic bioremediation	Relies on natural assimilative activity	Relatively inexpensive, excavation not required	Low degradation rates, incubation periods months to years	Oils, gasoline, chlorinated aromatics, chlorinated hydrocarbons
Slurry bioreactor	Soil and water agitated together in bioreactor	Good parameters control, good microbe/compound contact, fast degradation rates, incubation periods days to weeks	High capital outlay, high exposure risks	Surface contamination, recalcitrant compounds

Table 4: Methods applied in water bioremediation.

Technology	Principles	Advantages	Disadvantages	Applications
Precipitation or Flocculation	Non-directed physico-chemical complexation reaction between dissolved contaminants and charged cellular components (dead biomass)	Cost-effective	Yet to be exploited commercially	Removal of heavy metals
Ion exchange	Removes ions from the aqueous phase by the exchange of cations or anions between the contaminants and the exchange medium	Short duration	pH and oxidants in ground water may affect the exchange	Remove dissolved metals and radionuclide from aqueous solutions
Reverse osmosis	An applied pressure forces the flow of water from a more concentrated solution to a more dilute one	Eliminates brine discharge of RO desalination. Use of ammonia-carbon dioxide as a recyclable draw solute	High energy costs	Desalination of sea water; remove pollutants and microorganisms
Microfiltration	Microfiltration membranes are used at a constant pressure	Remove dissolved solids rapidly	Yet to be exploited commercially	Waste water treatment; recovery and reuse of more than 90% of original waste water
Electrodialysis	Uses cation and anion exchange membrane pairs	Withstand high temperature and can be reused	Yet to be exploited commercially	Removal of dissolved solids efficiently

Factors limiting bioremediation: The bioremediation process is based on the activities of the aerobic, heterotrophic microorganisms. For faster degradation the substrate specific microbes must be present with favourable environmental factors (Smith *et al*., 1998; Boopathy, 2000). Microbes that have the physiological and metabolic capabilities to degrade the pollutants may include bacteria or fungi. Among the factors having a direct impact on bioremediation (Table 5) are energy sources (electron donors), electron acceptors, nutrients, pH, temperature and inhibitory substrates or metabolites (Blackburn & Hafker, 1993; Boopathy, 2000; Soccol *et al*., 2003; Jagadevan & Mukherji, 2004).

Phytoremediation: Sometimes, plants are also used to accelerate the rate of degradation or to remove contaminants, either on their own or alongside microorganisms (Prescott *et al*., 2002). Success of any plant based remediation system depends on the interaction of root exudates and in-situ microorganisms. Plant and soil microbes, including bacteria, actinomycetes, molds, algae and protozoa, evolve highly complex symbiotic and synergistic relationships.

Amongst themselves, microorganisms play a crucial role to determine the fate of contaminants. During rhizoremediation, exudates from plants can help to enhance the growth, survival and microbial action of these organisms, which results in more efficient degradation of pollutants (Wenzel, 1992). On the other hand microorganisms provide protection to the plant by restricting contact with potentially toxic chemicals. The types of phytoremediation and plants involved are presented in Table 6.

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Table 5: Factors affecting bioremediation.

Factor	Consideration
Microbial	Growth for critical biomass production, enzyme induction, enrichment of the capable microbial populations and production of toxic metabolites
Environmental	Depletion of preferential substrates and inhibitory environmental conditions
Substrate	Too low concentration of contaminants, Chemical structure, toxicity and solubility of contaminants
Aerobic vs anaerobic process	Oxidation/reduction potential and availability of electron acceptors
Growth substrate vs co-metabolism	Type of contaminants, availability of alternate carbon source Microbial interaction (competition, succession and predation)
Physico-chemical bioavailability of pollutants	Equilibrium sorption, Irreversible sorption, Incorporation into humic matters
Mass transfer limitations	Oxygen diffusion, solubility and diffusion of nutrients, solubility/miscibility with water

Table 6: Types of phytoremediation.

Process	Function	Pollutant	Plants	References
Phytoextraction	Remove metals pollutants that accumulate in plants. Remove organics from soil by concentrating them in plant parts.	Cd, Pb, Zn, As	*Viola baoshanensis* *Sedum alfredii* *Rumex crispus* *Helianthus annus*	Macek *et al*., 2000, Prescott *et al*., 2002, Zhuang *et al*. 2007
Phytodegradation	Plants and associated microorganisms degrade organic pollutants	DDT	*Elodea canadensis* *Pueraria thunbergiana*	Garrison *et al*. 2000, Prescott *et al*., 2002, Newman & Reynolds, 2004
Rhizofiltration	Roots absorb and adsorb pollutants, mainly metals, from water and aqueous waste streams	Zn, Pb, Cd, As	*Brassica juncea* *Helianthus annus*	Dushenkov *et al*., 1995, Prescott *et al*., 2002, Verma *et al*., 2006
Phytostabilization	Use of plants to reduce the bioavailability of pollutants in the environment	Cu, Cd, Cr, Ni, Pb, Zn	*Anthyllis vulneraria* *Festuca arvernensis* *Koeleria vallesiana* *Armeria arenaria* *Lupinus albus*	Prescott *et al*., 2002, Frerot *et al*., 2006 Vazquez *et al*., 2006
Phytovolatilization	Use of plants to volatilize pollutants	Se, CCl_4, EDB, TCE	*Stanleya pinnata* *Zea mays* *Brassica sp.*	Prescott *et al*., 2002, Ayotamuno & Kogbara, 2007

Although bioremediation technology is promising and has been proven to be effective, further research is needed to understand the microbial mechanisms underlying the degradation process. If used properly, bioremediation has minimal adverse effects since it can be applied with little or no disruption to contaminated sites. Although the technology may require site specific planning and design of interventions, it is the most promising and low-cost technology for cleaning up environmental pollutants. Bioremediation should be improved through biotechnology tools to enhance its exploitation for managing environmental pollution in a sustainable manner.

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