Bioremediation: A Simple Method to Clean-Up Polluted Environment

Jackson, O. I.*; Adeyemi, A. A. and Odeleye, O. A.
Department of Environmental Management and Modelling, Forestry Research Institute of Nigeria, Ibadan, Nigeria.
**Corresponding author: mail2oyeen@yahoo.com*

Accepted on July 25, 2013

Abstract
An increasingly industrialized global economy has resulted in elevated discharges of pollutants into the environment thus posing great risks to environmental and human health. The main environmental pollutants include petroleum hydrocarbons, pesticides, inorganic fertilizers, heavy metals, polycyclic aromatic hydrocarbons and solvents. Some of these contaminants are readily biodegradable while others are recalcitrant, persisting in the environment. This has led to researches on ways to mitigate environmental pollution. Bioremediation is the use of biodegradative agents or processes to remove or transform pollutants to less or non-toxic forms. This can be employed in situ or ex situ depending on the degree of saturation and aeration of the contaminated environment. This paper discusses the principle of bioremediation, the different bioremediation technologies as well as their effectiveness and limitations in the elimination of several environmental contaminants.

Key words: *Bioremediation, phytoremediation, environmental contaminants, microorganisms, degradation*

Introduction

Environmental pollution from industrialization and agricultural practices has been an issue of great concern in recent years. As global population increases by the day, there is increasing pressure on our natural resources i.e. air, water and land resources and rapid expansion of industries, food, health care, vehicles etc. in order to meet the demand of the people. The prevalent environmental pollutants include petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), pesticides, inorganic fertilizers, heavy metals and solvents. The fate of these pollutants in the soil depends on several factors such as the nature of the contaminant and characteristics of the soil. Some organic contaminants undergo degradation into less or more toxic products. Some metals also undergo changes so that they can be more or less taken up by plants and animals. Important soil characteristics that determine the behaviour of pollutants include the soil texture, soil pH, soil organic matter (SOM) content, soil moisture, temperature and the presence of other chemicals. Pollutants can reach water bodies either through point or non point sources of pollution. Point sources include discharges from various commercial and industrial activities as well as wastewater treatment plants. Non point pollution occurs when water moves across the land or through the ground and carries along with it contaminants which are then deposited into water bodies and even groundwater. Nonpoint source pollution has been stated as the leading cause of water quality problems thereby posing major threat to the environment and hence human health (USEPA, 2012).

Several efforts have been made over the years to mitigate as well eliminate environmental pollution. The conventional method involves excavation of the contaminated soil, capping and containment of the polluted area of a site. However, these methods are difficult and expensive as maintenance and monitoring are required (Vidali, 2001). Bioremediation is the use of micro-organisms metabolism to remove contaminants from the environment. This process can be done *in-situ* or *ex-situ*.

In-situ bioremediation is the treatment of polluted material (soil and water in this case) at the site of contamination, while *ex-situ* involves the removal of the contaminated material from the site to be treated elsewhere. There are different bioremediation technologies which include biostimulation, bioventing, biosparging, landfarming, bioreactor, compositing, bioaugmentation, rhizofiltration and phytoremediation. Studies by Ayotamuno *et al.* (2006) showed 50-95% efficiency in the bioremediation of crude-oil polluted agricultural soil in Nigeria through the application of fertilizers and oxygen exposure. Lebkowska *et al.*, (2011) conducted field-scale research on the bioremediation of fuel-polluted soils by multiple injection of microbial consortia and recorded about 80-95% efficiency. Atagana (2008) investigated the effect of sewage sludge on soil heavily contaminated with a complex mixture of hydrocarbons on the removal of hydrocarbons in a compost system. He observed 99% reduction of total petroleum hydrocarbons (TPH) in the sewage sludge compost. Palmroth *et al.* (2002) looked at the effects of several plant species and different soil amendments on the removal of diesel fuel. They observed that legume species were most effective in the removal of diesel fuels; pine and poplar species also enhanced diesel fuel removal hence suitable candidates for diesel fuel phytoremediation under subartic conditions. Adesodun *et al.* (2008) evaluated the use of organic wastes (cow dung, poultry manure and pig wastes) for the bioremediation of soils spiked with spent oil. They found marked reduction in TPH content in the contaminated soils treated with organic wastes with poultry manure being the best option at all oil pollution levels. Bento *et al.* (2004) compared different technologies of bioremediation of soils and found that soil properties and the indigenous soil microbial population affect the degree of biodegradation; hence decision on the proper bioremediation method requires prior detailed site specific characterization studies.

This paper reviews the principle of bioremediation, different bioremediation technologies and the advantages and limitations of bioremediation which are not well known and understood by site owners, regulators and other stakeholders.

Principle of Bioremediation

Most organic chemicals are subject to enzymatic attack by living organisms to different end products, a process referred to as biodegradation (Lehmann, 1998). Bioremediation uses biological agents primarily bacteria, fungi or plants to clean up contaminated soil and water (Table 1). These organisms may either be indigenous to the contaminated site or introduced as primary agents of bioremediation (Chatterjee *et al.*, 2008). In bioremediation processes, microorganisms use the contaminants as carbon and energy sources thus the organism used depends on the chemical nature of the contaminant as they usually survive within a limited range of contaminants. A wide range of contaminants can be cleaned up by bioremediation (Table 2). Bacteria, fungi and protista degrade complex molecules incorporating the breakdown products into their metabolism. The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms (Zeyaullah *et al.*, 2009).

Table 1. Microorganisms with biodegradative potential for contaminants.

Microorganism	Toxic chemical	Elements
Pseudomonas spp	Benzene, anthracene, hydrocarbons, PCBs	U, Cu, Ni
Alcaligenes spp	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs	
Arthrobacter spp	Benzene, hydrocarbons, pentachlorophenol, phenoxyacetate, polycyclic aromatic	
Bacillus spp	Aromatics, long chain alkanes, phenol, cresol	Cu, Zn
Corynebacterium spp	Halogenated hydrocarbons, phenoxyacetates	
Flavobacterium spp.	Aromatics	
Azotobacter spp	Aromatics	
Rhodococcus spp	Naphthalene, biphenyl	
Mycobacterium spp	Aromatics, branched hydrocarbons benzene, cycloparaffins	
Nocardia spp	Hydrocarbons	
Methosinus spp	Aromatics	
Methanogens	Aromatics	
Xanthomonas spp	Hydrocarbons, polycyclic hydrocarbons	
Streptomyces spp	Phenoxyacetate, halogenated hydrocarbon diazinon	
Candida tropicalis	PCBs, formaldehyde	
Cunniughamela elegans	PCBs, polycyclic aromatics, biphenyls	
Zooglea spp	Co, Ni, Cd	
Citrobacter spp	Cd, U, Pb	
Chlorella vulgaris	Au, Cu, Ni, U, Pb, Hg,Zn	
Aspergillus niger	Cd, Zn, Ag, Th, U	
Pleurotus ostreatus	Cd, Cu, Zn	
Rhizopus arrhizus	Ag, Hg, P	
Stereum hirsutum	Cd, Pb, Ca	
Phormidium valderium	Cd, Co, Cu, Ni	
Ganoderma applantus	Cd, Pb	
Volvariella volvacea	Cu, Hg, Pb	
Daedalea quercina	Zn, Pb, Cu	

Source : Chatterjee *et al.*, 2008

Bioremediation does not only involve the degradation of pollutants by microorganisms, it also involves the removal of pollutants from the environment without degradation. For instance, bacteria take up large amounts of metals and minerals for binary fission; algae and plants also take up metals and toxic chemicals and metals from the environment.

Table 2. Some contaminants suitable for bioremediation.

Class of contaminants	Specific examples	Aerobic	Anaerobic	More potential sources
Chlorinated solvents	Trichloroethylene		+	Drycleaners
	Perchloroethylene			Chemical manufacture
Polychlorinated biphenyls	4-Chlorobiphenyl		+	Electrical manufacturing
	4,4-Dichlorobiphenyl			Power station
				Railway yards
Chlorinated phenol	Pentachlorophenol		+	Timber treatment
				Landfills
"BTEX"	Benzene	+	+	Oil production & storage
	Toluene			Gas work sites
	Ethylbenzene			Airports
	Xylene			
				Paint manufacture
				Port facilities
				Railway yards
				Chemical manufacture
Polyaromatic hydrocarbons (PAHs)	Naphthalene	+		Oil production & storage
	Antracene			Gas work sites
	Fluorene			Coke plants
	Pyrene			Engine works
	Benzo(a)pyrene			Landfills
				Tar production &storage
				Boiler ash dump sites
				Power stations
Pesticides	Atrazine	+	+	Agriculture
	Carbaryl			Timber treatment plants
	Carbofuran			Pesticide manufacture
	Coumphos			Recreational areas
	Diazinon			Landfills
	Glycophosphate			
	Parathion			
	Propham			
	2,4-D			

Source: Vidali, 2001

For effective bioremediation to occur, microorganisms must attack enzymatically the contaminants and convert them to less toxic products. This can only occur when the environmental conditions are conducive for microbial growth and activity thus the manipulation of the environmental parameters to allow the microbial growth and degradation to proceed at a faster rate. This can be referred to as enhanced bioremediation. Factors that stimulate bioremediation include nutrients, oxygen, pH, temperature and moisture (Chatterjee *et al*., 2008; Vidali, 2001; Thapa *et al.*, 2012).

Microorganisms are present in contaminated soils but not necessarily in the numbers required for bioremediation of the site. Their growth and activity must therefore be stimulated. Carbon (C) is the basic element of living organism and is needed in large amounts than other elements (Vidali, 2001). Other nutrients required for cell growth are nitrogen (N), phosphorus (P), potassium (K), sulphur (S), magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu). These nutrients are required to enable microorganisms produce the necessary enzymes required for metabolism. In the contaminated environment, N and P are most likely the limiting nutrients therefore are added as ammonium and phosphate respectively to the bioremediation system in forms that can be readily utilized.

The amount of oxygen determines whether the system is aerobic or anaerobic. Under aerobic conditions, hydrocarbons are readily degraded. Microorganisms utilize two primary enzymes (dioxygenases and monooxygenases) in the presence of oxygen for the mineralization or transformation of the contaminants (Boopathy, 2000). Anaerobic microorganisms on the other hand use other electron acceptors like nitrate, iron, manganese, sulphate and carbon dioxide, but the energy yield is lower than oxygen used as electron acceptor hence lower degradation and longer period of time required for remediation (Thapa *et al.*, 2012).

To ensure that oxygen is supplied to the system, hydrogen peroxide can be introduced into the environment (Cauwenberghe and Roote, 1998; Vidali, 2001).

The pH affects the solubility therefore the availability of many soil constituents which can affect biological activities. Many metals that are potentially toxic to microorganisms are insoluble at high pH, thus elevating the pH of the system can reduce risk of the metals poisoning the microorganisms (Cauwenberghe and Roote, 1998).

Microbial growth and activity in the environment are greatly affected by temperature, slowing with decreasing temperature. Biochemical rates of reactions are also affected by temperature, doubling with each 10°C rise in temperature. The cells however die above a certain temperature (Vidali, 2001). Temperature also affects non biological losses of pollutants mainly through evaporation at high temperature. The solubility of pollutants typically increases with increasing temperature. Also, oxygen solubility decreases with increasing temperature (Cauwenberghe and Roote, 1998).

Bioremediation Technologies

Biostimulation

Naturally, some microorganisms are present in contaminated soils degrading organic contaminants, but for effective remediation, the growth and activity of these microorganisms can be stimulated. Biostimulation is the process of optimizing the environmental conditions of the remediation site by adding nutrient, oxygen and electron acceptor to stimulate the naturally-occurring microorganisms to degrade the contaminants (Thapa *et al.*, 2012).

Bioaugmentation

This involves the addition of indigenous or exogenous microorganisms with specific metabolic capabilities to the contaminated site to degrade organic contaminants (Cauwenberghe and Roote, 1998; Vidali, 2001). Specialized populations for degrading specific compounds are selected by enrichment culturing and genetic manipulations. Enrichment cultures expose the microorganisms to increasing concentrations of the pollutant or mixture of pollutants. However, the introduction of specialized microbial population into the polluted site may not produce the desired degree of degradation due to rare ability of non indigenous cultures to compete well with the indigenous population to develop and sustain the desired population levels (Vidali, 2001).

Bioventing

This involves the injection of atmospheric air and nutrients to the contaminated soil to stimulate and enhance the growth of indigenous microorganisms. Bioventing employs low air flow rates providing only the amount of oxygen required for the biodegradation while minimizing volatilization and release of the pollutants into the atmosphere (Cauwenberghe and Roote, 1998; Vidali, 2001). This technology uses air pump attached to one of a series of air injection probes; some also use air injection wells with air recovery wells. Bioventing is most applicable where the contaminant is deep under the surface though shallower soils and sites can be treated if the surface is capped. Several pollutants that are biodegradable under aerobic conditions can be treated using this technology.

Biosparging

This involves injection of air under pressure into the saturated zone to transfer volatile compounds to the unsaturated zone for biodegradation. Here, biodegradation is the dominant remedial process while volatilization plays a secondary role (Cauwenberghe and Roote, 1998). The air injected under pressure below the water table increases groundwater oxygen concentrations thereby enhancing the rate of biological degradation of organic contaminants by naturally occurring microorganisms (Vidali, 2001). This process is more favourable for the remediation of less volatile pollutants like diesel fuels and waste oils.

Landfarming

This technology involves the excavation of contaminated soil and spreading over a prepared bed which is periodically tilled until all the pollutants are degraded. This facilitates aerobic degradation of pollutants by indigenous microorganisms (Vidali, 2001). The conditions of the soil are controlled by monitoring the moisture and nutrient content, soil pH and the frequency of aeration so as to optimize pollutant degradation (Gan *et al.*, 2009).

Composting

This involves working into contaminated soil compost, which is a non-hazardous organic amendment prepared from animal manure and crop wastes or other plant materials. The stimulation of microbial growth by the added nutrients results in effective bioremediation in a relatively short period of time.

Biopiles
This technology involves heaping contaminated material into piles and stimulating aerobic microbial activity through the aeration and/or addition of nutrients and moisture. This is a refined version of landfarming that tends to control physical losses of the contaminants by leaching and volatilization (Vidali, 2001). While landfarms are aerated by tilling, biopiles are most often aerated by forcing air to move by injection or extracted through slotted or perforated piping placed through the piles (USEPA, 2011).

Bioreactors
These are used for *ex situ* treatment of polluted soil and water. Slurry bioreactor is a containment vessel and apparatus used to create a three-phase (solid, liquid and gas) mixing condition to increase the bioremediation rate of soil-bound and water-soluble pollutants as water slurry of the contaminated soil and microorganisms capable of degrading the target contaminants (Vidali, 2001). The biodegradation rates are greater in bioreactors because the contained environments are more manageable, controllable and nutrients and oxygen are also added to create the optimum conditions.

Anaerobic bioremediation
In cases when oxygen is absent or limited, anaerobic degradation can occur whereby anaerobic microorganisms use other electron acceptors to break down organic contaminants into smaller components, often producing carbon dioxide and methane as the final products. Some anaerobic microorganisms can also degrade organic contaminants by fermentation whereby the organic contaminants act as the electron acceptors (Gan *et al*., 2009).

This technology is suitable for the bioremediation of environment with very high degree of contamination such as cases of accidental oil spills as well as remediation of water submerged soil (e.g. paddy field), deep underground soil and swamps. In these cases, oxygen flow is limited due to soil pore saturation or clogging of aggregates.

Phytoremediation
Phytoremediation is an emerging bioremediation technology that uses plants to extract, sequester and detoxify existing environmental pollutants (Vidali, 2001; Gan *et al*., 2009). There are five types of phytoremediation techniques based on the fate of the contaminant. These include: phytoextraction, phytotransformation, phytostabilization, phytodegradation and rhizofiltration.

Phytoextraction is the process whereby plants accumulate environmental pollutants into their roots and above ground shoots or leaves.

Phytotransformation is the process whereby plants transform pollutants from the environment into more stable, less toxic, or less mobile form through their metabolism. For instance metal chromium can be reduced from hexavalent to trivalent chromium, which is less mobile and noncarcinogenic.

Phytostabilization is the technique in which plants reduce the mobility and migration of environmental pollutants. Plants structure bind and absorb leachable constituents of the pollutants so that they form a stable mass of plants hence removing them from the environment.

Phytodegradation or rhizodegradation occurs as a result of a symbiotic relationship between plants and microorganisms existing in the rhizosphere. Plants provide nutrients that enhance the growth, survival and microbial action of these organisms, thereby resulting in more efficient degradation of pollutants. Microorganisms on the other hand provide plants with a healthier soil environment by restricting contact with potentially toxic chemicals.

Rhizofiltration is mainly used to reduce pollution in natural wetlands and estuary areas as plants roots are employed to take up the pollutants.

Advantages and Disadvantages of Bioremediation

Bioremediation is a natural process therefore accepted by the public as a waste treatment method. Microorganisms that have physiological and metabolic capabilities to degrade pollutants increase in numbers when the pollutant is present; decreasing as the pollutant is being degraded hence the residues for bioremediation are usually harmless including carbon dioxide, water and cell biomass. Bioremediation can remove hazardous pollutants completely from the environment hence eliminating the chance of future liability from treatment. Bioremediation is also advantageous than conventional remediation technologies as there is less risk of transferring pollutants from hence potential threats to human health and the environment during transportation as remediation can be carried out on site with minimal disruption. Also, it reduces the cost of remediation accruing from transportation.

Despite the potentials of bioremediation as a tool for cleaning up polluted environment, there are however some challenges and limitations. For bioremediation to be successful, the methods depend on the right site factors such as the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants. Some pollutants are not susceptible to rapid and complete biodegradation hence bioremediation often takes longer than convention remediation techniques like excavation and removal of polluted material or incineration. There are also concerns that the products of bioremediation may be more persistent or toxic than the parent compounds.

Conclusion

Bioremediation is usually a simple and less labour intensive technology for cleaning up polluted environment. Studies have shown success in the bioremediation of pollutants particularly petroleum hydrocarbons, polycyclic aromatic hydrocarbons, solvents, pesticides and metals. Further researches are however required in this field to develop effective technologies for the treatment of complex mixtures of pollutants that are not evenly distributed in the environment. An understanding of the principles and the several technologies of bioremediation create opportunities for bioremediation to be used as an environmental management tool.

References

Adesodun, J. K. and Mbagwu, J. S. C. (2008). Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. *Bioresource Technology*, 99 (13): 5659 - 5665.

Atagana, H. I. (2008). Compost bioremediation of hydrocarbon-contaminated soil inoculated with organic manure. *African Journal of Biotechnology*, 7 (10): 1516 - 1525.

Ayotamuno, M. J.; Kogbara, R. B.; Ogaji, S. O. T. and Probert, S. D. (2006). Bioremediation of a crude-oil polluted agricultural-soil at Port Harcourt, Nigeria. *Applied Energy*, 83 (11): 1249 - 1257.

Bento, F. M.; Camargo, F. A. O.; Okeke, B. C. and Frankenberger, W. T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology*, 96 (9): 1049 - 1055.

Boopathy, R. (2000). Factors limiting bioremediation technologies. *Bioresource Technology*, 74: 63 - 67.

Cauwenberghe, L. and Roote, D. S. (1998). *In Situ* Bioremediation. Ground-Water Remediation Technologies Analysis Center (GWRTAC), Pittsburgh, P. A., available at http://www.gwrtac.org

Chatterjee, S.; Chattopadhyay, P.; Roy, S. and Sen, S. K. (2008). Bioremediation: a tool for cleaning polluted environments. *Journal of Applied Biosciences*, 11: 594 – 601.

Gan, S.; Lau, E. V. and Ng, H. K. (2009). Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). *Journal of Hazardous Materials*, 172 (2-3): 532 - 549.

Lebkowska, M.; Zborowska, E.; Karwowska, E.; Miaskiewicz-Peska, E.; Muszynski, A.; Tabernacka, A; Naumczyk, J. and Jeczalik, M. (2011). Bioremediation of soil polluted with fuels by sequential multiple injection of native microorganisms: Field-scale processes in Poland. *Ecological Engineering*, 37 (11): 1895 - 1900.

Lehmann, V. (1998). Bioremediation: A solution for polluted soils in the South? *Biotechnology and Development Monitor*, 34: 12 - 17.

Palmroth, M. R. T.; Pichtel, J. and Puhakka, J. A. (2002). Phytoremediation of subartic soil contaminated with diesel fuel. *Bioresource Technology*, 84: 221 - 228.

Thapa, B. Kumar, A. K. C. and Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Kathmandu University Journal of Science, Engineering and Technology*, 8 (1): 164 – 170.

USEPA (2011). Biopiles. http://www.epa.gov/oust/cat/biopiles.htm

USEPA (2012) What is nonpoint source pollution? http://water.epa.gov/polwaste/nps/whatis.cfm

Vidali, M. (2001). Bioremediation. An overview. *Pure Applied Chemistry*, 73 (7): 1163 – 1172.

Zeyaullah, M.; Atif, M.; Islam, B.; Abdelkafe, A. S.; Sultan, P.; ElSaady, M. A. and Ali, A. (2009). Bioremediation: A tool for environmental cleaning. *African Journal of Microbiology Research*, 3 (6): 310 - 314.