



Degradation of Environmental Contaminants through Bioremediation: A Review



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ABSTRACT

Cleaning up hazardous and toxic wastes at polluted sites is a serious challenge for the foreseeable future. Microorganisms are used to change harmful substances to nontoxic substances. This is a new technology for treating chemical spills and hazardous wastes. Bioremediation emerged as an essential biotechnology tool in this endeavor by providing new approaches for managing, preserving and restoring the environment. The process involves not only the use of microorganisms, in addition fungi, green plants or their enzymes are used to return natural environment altered by contaminants to its safe condition. In an attempt to review the clean-up strategies employed by scientists in getting rid of environmental contaminants, this review paper examines the potentials of bioremediation technologies in accelerating the degradation of these contaminants.

Keywords: Bioremediation, Contaminants, Nontoxic, An environment, Biotechnology.

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1. INTRODUCTION

Microbes are capable of breaking down many organic compounds, ultimately to environmentally benign compounds [1]. Certain enzymes produced by microbes attack hydrocarbons molecules, causing degradation [2]. Hydrocarbon consuming bacteria and fungi are used to clean up soil beneath leaky gasoline tanks that threaten drinking water supplies. Other kinds of microbes are used to degrade pesticide residues in soil. Plants such as wheat, tobacco, water hyacinth, and cattails have been employed to clean up toxic waste sites by letting them draw up heavy metals, such as lead and cadmium, through their roots [3]. Often living organisms can clean contaminated water effectively and inexpensively. We call this bioremediation [4]. Bioremediation is a managed or spontaneous process in which microbiological processes are used to degrade or transform contaminants to less toxic or non toxic forms, thereby mitigating or eliminating environmental contamination [5]. In bioremediation, the contaminated site is exposed to an 'army' of microorganisms which gobble up the poison and leave behind harmless substances such as carbon dioxide and water [6]. Oxygen and organisms are injected into the contaminated zones. The organisms feed on and eliminate the pollutants (as in secondary sewage treatment) and then die when the pollutants are gone. Often the microorganisms metabolize the chemicals

to produce carbon dioxide and methane, water and biomass. Alternatively, the contaminants may be enzymatically transformed to metabolites that are less toxic [7]. Considerable research is been done to find and develop microbes that will break down certain kinds of wastes more readily. Bioremediation, which may be used in place of detergents to decontaminate soil, is a rapidly expanding and developing technology that is applicable to leaking storage tanks and spills, as well as to waste-disposal sites [8]. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses low - cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scaled involved are relatively long, and the residual contaminants levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result [9].

This paper reviews the processes of bioremediation with emphasis on the potentials of Biotechnology in improving bioremediation technologies, and the current state of research in the area, and also highlights areas of further research.

2. BACKGROUND

Waste products resulting from human life have always been a serious problem. Today these waste products range from raw sewage to nuclear waste. In the past disposal of the wastes meant digging a hole, dumping the waste materials in, then filling it all in, out of sight, out of mind. But lately this method has become insufficient. The toxic materials from these “dig and dump” sites have begun to leak into water sources and into areas that sustain human life. This problem has lead to modern-day bioremediation [10].

2.1. Basic Principles of Bioremediation

Recent studies in Environmental biotechnology provide an effective and efficient biological processes for clean –up of polluted water and land areas. The microorganisms use for bioremediation may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. The contaminants are transported by living organisms through certain reactions as a form of their metabolism.

To stimulate and maintain the activity of microorganisms for bioremediation, a mechanism is required usually in form of delivery system. One or more of the following is provided; an electron acceptor in the form of oxygen or nitrate, nutrients in the form of nitrogen and phosphorous and an energy source in the form of carbon.

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate [9].

2.2. Requirements for Bioremediation

Certain factors are required for successful bioremediation processes. These factors according to Vidali [9] includes: the existence of microbial population capable of degrading the pollutants; the availability of

contaminants to the microbial population. Table 2 shows the Potential for bioremediation of selected contaminants.

2.3. Microbial Population Required for Bioremediation

Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or any waste stream. The main requirements are an energy source and a carbon source. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards [9]. If microbes are not present in a system they can be added to help promote bioremediation. The added microbes can be cultures grown from other contaminated areas or they can be microbes genetically engineered to degrade oil. However, even when these microbes are present, degradation of hydrocarbons can take place only if all other basic requirements of the microbes are met [2]. Microorganisms are usually manipulated to provide a natural method for cleaning up some of the environmental contaminants. These microbes often use contaminants as a food source, thereby completely eliminating toxic compounds by changing them into basic elements such as carbon dioxide and water.

3. ENVIRONMENTAL FACTORS

Soil structure controls the effective delivery of air, water and nutrients. To improve soil structure, materials such as gypsum or organic matter can be applied. Microbial growth and activity are readily affected by pH, temperature and moisture. Although micro-organisms have been also isolated in extreme condition, and most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions. If the soil has too much acid it is possible to rinse the pH adding lime. Temperature affects biochemical reactions, and the rates of many of them double for each 10⁰C rise in temperature. Above certain temperature, however, the cells die. Plastic covering can be used to enhance solar warming in late spring, summer, and autumn. Available water is essential for all the living organisms, and irrigation is needed to achieve the optimal moisture level. Hydrocarbons are readily degraded under aerobic conditions, whereas chlorurate compounds are degraded only in anaerobic ones. To increase the oxygen amount in the soil it is possible to till or sparge air. In some cases, hydrogen peroxide or magnesium peroxide can be introduced in the environment [9].

3.1. The Design of Bioremediation Strategies

Ideally bioremediation strategies according to Lovley [11] would be designed based on knowledge of the following:

- a. The organisms those are present in the contaminated environments;
- b. Their metabolic capabilities; and
- c. How they respond to changes in environmental conditions.

3.2. Monitoring Bioremediation

The process of bioremediation can be monitored indirectly by measuring the *Oxidation Reduction Potential* or redox in soil and groundwater, together with pH, temperature, oxygen content, electron

acceptor/donor concentrations, and concentration of breakdown products (e.g. carbon dioxide) [12] Table 3 shows the biological breakdown rate as function of the redox potential.

Table-1. Decreasing biological breakdown rate as function of redox potential [13]

Process	Reaction	Redox potential (Eh in mV)
aerobic:	$O_2 + 4e^- + 4H^+ \rightarrow H_2O$	600 ~ 400
anaerobic:		
Denitrification	$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$	500 ~ 200
manganese IV reduction	$MnO_2 + 2e^- + 4H^+ \rightarrow Mn^{2+} + 2H_2O$	400 ~ 200
iron III reduction	$Fe(OH)_3 + e^- + 3H^+ \rightarrow Fe^{2+} + 3H_2O$	300 ~ 100
Sulfate reduction	$SO_4^{2-} + 8e^- + 10 H^+ \rightarrow H_2S + 4H_2O$	0 ~ 150
Fermentation	$2CH_2O \rightarrow CO_2 + CH_4$	- 150 ~ - 220

3.3. Classifications of Bioremediation

There are three major classification of bioremediation:

- Biotransformation**- alteration of contaminant molecules into less or non-hazardous molecules
- Biodegradation**- the breakdown of organic substances in smaller organic or inorganic molecules.
- Mineralization** – the complete biodegradation of organic materials into inorganic constituents such as CO_2 or H_2O

These three classifications of bioremediation can occur *in situ* (at the site of contamination) or *ex situ* (contaminant taken out of site of contamination and treated elsewhere) [10].

3.4. In Situ and Ex Situ Bioremediation

In situ bioremediation is applied to bioremediation without removal of the contaminants from the contaminated site. Two main strategies are involved namely; biostimulation and bioaugmentation.

Biostimulation is the addition of nutrients .oxygen and other electron donors and acceptors to the coordinated site in order to increase the population or activity of naturally occurring microorganisms available for bioremediation. In the other hand *bioaugmentation* is the addition of microorganisms that can biotransform or biodegrade contaminants. The organisms added can be a completely new species or more members of a species that already exists at the site. For example, a bacterium has been identified that can detoxify perchloroethene(PCE), a common, highly toxic groundwater pollutant suspected of causing cancer. The bacteria's enzymes remove the four chlorines from the PCE producing ethylene,a naturally occurring and relatively harmless gas [14]. The advantage of in situ bioremediation is that there is no need to extract the contaminants, so there is less exposure to workers, and it is also less costly. However there are also disadvantages to this strategy. The site of bioremediation is not contained, therefore, it is harder to control conditions and monitor progress [10]. *In situ* bioremediation is possible at sites where:(a)aerobic conditions are present or can be engineered; (b)appropriate organisms are present or can be introduced effectively; (c)the potential for nitrite or nitrate accumulation can be managed [15]. Generally speaking, *in situ* methods are suited to instances where the contamination is widespread throughout, and often at some depth within, a site, and of low to medium concentration. Additionally, since they are relatively slow to act, they are of most use when the available time for treatment is not restricted [16].

Ex situ bioremediation(aka "pump and treat") removes the contaminants and places them in a contained environment [17]. This contained environment allows for easier monitoring and maintaining of

conditions and progress, thus making the actual bioremediation process faster. However, the removal of the contaminant from the contaminated site is time consuming, costly and potentially dangerous. By bringing contaminants to the surface, the workers and the general public have increased exposure to the toxic material. There are several extraction strategies to facilitate *ex situ* bioremediation. The soil can actually be dug and transported to a bioreactor. Soil washing is another method that can be used, where water is flushed through the contaminated region and then transferred to a bioreactor for treatment [18]. Similarly soil venting can be used, where air is flushed through the contaminated region and the air containing the contaminant is transferred to a bioreactor for treatment [10]. However some limitations are reported for *ex situ* methods. According to Okoh and Trejo-Hernandez [19] because *ex situ* methods require excavation, they disrupt the landscape, expose the contaminants, and require replacement of soils. For these reasons, *ex situ* methods are sometimes impracticable.

4. PHYTOREMEDIATION

Plants have many properties that make them ideally suited to clean up polluted soil, water, and air, in a process called phytoremediation [20]. Plants can be used to extract, detoxify, and/or sequester toxic pollutants from soil, water and air. Al-Najar, et al. [21] reported the Phytoremediation of Thallium Contaminated Soils by *Brassicaceae*. Phytoremediation may become an essential tool in cleaning the environment and reducing human and animal exposure to potential carcinogens and other toxins [22].

Phytoremediation techniques can be classified into five types. Vidali [9] classified these techniques based on the contaminant fate as follows:

(a) **Phytoextraction or phytoaccumulation** which is the process use by the plants to accumulate contaminants into the roots and above ground shoots or leaves. This technique saves tremendous remediation cost by accumulating low levels of contaminants from a wide spread area.

This process produces a mass of plants and contaminants (usually metals) that can be transported for disposal or recycling.

(b) **Phytotransformation or phytodegradation** which is refers to the uptake of organic contaminants from soil, sediments, or water and, subsequently, their transformation to more stable, less toxic, or less mobile and noncarcinogenic form.

(c) **Phytostabilization** where plants reduce the mobility and migration of contaminated soil. Leachable constituents are absorbed and bound into the plant structure so that they form a stable mass of plant from which the contaminants will not reenter the environment.

(d) **Phytodegradation or rhizodegradation** which is the breakdown of contaminants through the activity existing in the rhizosphere. Rhizodegradation is a symbiotic relationship that has evolved between plants and microbes, plants provides the nutrients necessary for the microbes to thrive, while microbes provide a healthier environment.

(e) **Rhizofiltration** which is a water remediation technique that evolves the uptake of contaminants by plants roots.

4.1. Approaches to Bioremediation

(1) Oil spill Bioremediation

(a) *Biodegradation of oil*

After oil spill, highly soluble and volatile components, such as benzene, toluene, and xylene, dissolve into the water or evaporate rapidly into the atmosphere. Other constituents are eventually degraded by bacteria, yeast or fungi, which may use hydrocarbons as a sole source of carbon and energy for their growth [Yinying \[23\]](#). Remediation of crude oil can be accomplished in soil by selection and optimization of known conditions that prompt the growth of natural flora and fauna capable of degrading crude oil [\[24\]](#).

The use of agro technical methods and environmental parameters [\[25\]](#) also proved to be effective. Generally speaking, the enhancement of the degradation can be achieved by stimulating the microorganisms of interest by adding electron donors (substrates) and /or nutrients to the subsurface to increase bacterial growth yielding faster degradation rates.

Bioremediation can also be enhanced using surfactants, soap-like cleaning agents that generally emulsify petroleum products, which in turn enhances bioremediation [\[26\]](#).

(b) *Marine oil spill Bioremediation*

For marine oil spill, potential bioremediation approaches fall into three categories: (i) stimulation of indigenous microorganisms through addition of nutrients (fertilization), (ii) introduction of special assemblages of naturally occurring oil-degrading microorganisms (seeding), and (iii) introduction of genetically engineered microorganisms (GMEs) with special oil-degrading properties [\[27\]](#).

To date, no significant environmental or health problems have been associated with the testing or application of bioremediation technologies to marine oil spills [\[28\]](#).

(2) Metal Bioremediation

Metal contaminants are commonly found in soils, sediments, and water. Metal pollutants can be produced through industrial processes such as mining, refining and electroplating. A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation and complexation, and changes in the valence state. These transformations affect the mobility and bioavailability of metals. At low concentrations, metals can serve as important components of life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify some metals and prevent further metal contamination [\[29\]](#). Synthetic and natural zeolites have been used as chelators for rapid mobility and uptake of metals from contaminated soils by plants. Use of synthetic chelators significantly increased Pb and Cd uptake and translocation from roots to shoots facilitating phytoextraction of the metals from low grade ores [\[30\]](#).

Bioremediation of chromium (VI) is becoming an attractive bioremediation strategy. Elemental Chromium (Cr) does not occur in nature, but is present in ores, primarily in the form of chromite ($\text{FeO} \cdot \text{Cr}_2\text{O}_3$). The two oxidation states of chromium, Cr (III) and Cr (VI) are stable and predominant in the environment. In soils, chromium occurs in two oxidative states having very different behavior. Hexavalent chromium is generally more soluble, mobile, and toxic. The reduced Cr (III) forms are generally much less mobile and less hazardous. Acceleration of Cr (VI) reduction in soils by addition of organic carbon was tested in columns pretreated with solutions containing 1000 and 10000 mg/l Cr (VI) to evaluate potential in situ remediation of highly contaminated soils [\[31\]](#) facilitating in situ reduction of toxic Cr(VI) to non toxic

Cr(III). Extensive use of hexavalent chromium in various industrial applications has caused substantial environmental contamination. Chromium plays an important role in glucose and cholesterol metabolism and is an essential element to man and animals but at higher levels is toxic to both [32]. Non-occupational exposure to the metal occurs via ingestion of chromium-containing food and water, whereas occupational exposure occurs via inhalation. Cr (III) is poorly absorbed, regardless of the route of exposure, whereas Cr (VI) is more readily absorbed. Humans and animals localize chromium in the lung, liver, kidney, spleen, adrenals, plasma, bone marrow and red blood cells. Workers exposed to chromium have developed nasal irritation (at <0.01mg/m³, acute exposure), nasal ulcers, perforation of the nasal septum (at 2 g/m³, sub-chronic or chronic exposure) and hypersensitivity reactions and “chrome holes” of the skin [33].

Respiratory and dermal toxicity of chromium is well documented [33]. Many neural defects, malformation and fetal deaths have been caused by Cr (VI). The mechanism of Cr-tolerance or resistance of selected microbes is of particular importance in both bioremediation and waste water treatment technology [34]. Gomes *et al* found the applicability of the charophyte, *Nitella pseudoflabellate* in the remediation of Cr (VI) contaminated water at different calcifying potentials [35]. In 2008, Rehman *et al* found that *Bacillus* sp had the ability to reduce hexavalent chromium into its trivalent form. These bacteria could reduce 91% of Chromium from the medium after 96 hours and was also capable of reducing 84% chromium from the industrial effluents in Lahore after 144 hours Rehman, et al. [36]. Morales, et al. [37] wanted to isolate and analyze chromium-resistant microorganisms suitable for bioremediation. They found that *Streptomyces* sp tolerated heavy metals and elevated levels of chromium despite its negative effect on growth and development was efficient at removing Cr (VI) by promoting reduction to Cr(III). Hence chromium-resistant microorganisms are a promising candidate for detoxification of sites containing heavy metals Morales, et al. [37]. Camargo, et al. [38] found that chromium resistant bacteria can tolerate 2500mg/l Cr (VI). Maximal Cr (VI) reduction was observed at the optimum pH (7.0-9.0) and temperature (30 degrees centigrade) of growth. One bacterial isolate (*Bacillus* sp. Es 29) was able to aerobically reduce 90% of Cr (VI) in six hours. The application of Electrokinetics has also shown a positive impact on nutrient amendment for the bioremediation of a chromium-contaminated soil. Reddy, et al. [39] presented the results of a preliminary investigation wherein electrokinetics was used for the delivery of nutrients to metal-reducing micro-organisms in a low permeability clay soil. In particular, the micro-organisms were used to reduce a toxic and mobile heavy metal (hexavalent chromium or Cr(VI)) to a less toxic and immobile form (trivalent chromium or Cr(III)).

4.2. Case Studies of some of the Benefits of Bioremediation

Bioremediation was used on about 74 miles of shoreline by far the largest use of this technology to date-and most shorelines were oil-free within 3 years instead of predicted decades [3]. One of the oil bioremediation projects to date has been the cleanup of the March 1989 spill of 11million gallon of crude oil by the Exxon Valdez [40]. The Exxon Valdez oil tanker hit a reef in Prince William Sound, Alaska and releases over 40 million liters of crude oil into the sound within five hours [41]. A total of 1700km of shoreline was contaminated by oil from Exxon Valdez. A number of time-consuming and labour-intensive techniques had to be employed to physically clean up the beaches. During the clean-up operation, a bioremediation project was carried out with promising result [42]. Almost 50 tonnes of nitrogen in form of slow-release and oleophilic fertilizers were applied to the oiled shorelines in Prince William Sound and the Gulf of Alaska in 1989 to 1991.No adverse effects were noted [43]. The work of Atagana [44] on compost

bioremediation of hydrocarbon-contaminated soil inoculated with organic manure shows that under control conditions inoculation of compost containing hydrocarbon-contaminated soil with sewage sludge can effectively accelerate the removal of such contaminants from the soil matrix. The *Torrey Canyon* spill in the English Channel off Land's End, in the United Kingdom in 1967 provided general incentive for research and development, as did the *Amoco Cadiz* spill off the coast of Brittany, France in 1978. Other oil spills, such as the 1968 Santa Barnara Channel, California spill, or the *Braer* spill off the Shetlands in 1993, have also stimulated specific areas of research and development on the basis of issues that arose in their particular spill scenario [45]. In one of the largest oil producing areas in Egbema/Ogba/Ndomi local government councils of Rivers State (Nigeria), the Ogbogu community started a pilot project using plants and micro-organisms to clean up oil spill from farmlands and fishing areas. Of the two plants, kenaf (*Hibiscus cannabinus*) and Vetiver (*Vetivera zizanioides*) used for the cleanup, the pulp of kenaf is flattened and placed over oil spill which they readily soak up. These bags are then taken to special cleanup sites where the bags are subjected to microbial treatment. This method involves the participation of the rural citizenry of the area who invariably protect their land by the act of do-it yourself, thereby also providing for themselves economic and opportunities [24].

4.3. The use of Biotechnology to Improve Bioremediation

The Recombinant DNA Technology is of great potential for Bioremediation. It allows the modification of specific organisms suitable for bioremediation at specific sites. The use of DNA –base procedures for the detection of biodegrading organisms or genes that code for pollutant-degrading enzymes constitutes a critical technology for following biochemical transformation and substantiating the impact of bioremediation. DNA –based technology has been demonstrated to be a sensitive technique for tracking microorganism activity at the molecular level [46]. The most resistant organism *Deinococcus radiodurans* has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste [47]. The U.S. Department of Energy (DOE) has sponsored studies on the use of *D.radiodurans* as a bioremediation agent to remove heavy metals and organic solvents such that the subsequent radionuclide isolation is easier and safer [47]. Recently, the *merA* gene from *E.coli* was successfully introduced into the genome of *D.radiodurans*. This gene encodes the enzyme that reduces highly toxic Hg (II) to the inert and much less toxic form, Hg (0). Further genetically strains may hold potential for degradation or detoxification of other contaminants as well [48]. According to Sylvia, et al. [41] if bioremediation is to be successful scientists must find ways to make it cost-effective and timely. Strategies such as selecting or genetically engineering microorganisms with superior remediation properties or altering inherent site conditions to allow indigenous or introduced organisms to function efficiently must be evaluated.

4.4. The use of Biotechnology to Enhance Phytoremediation

Microbes are not the only species that can be enhanced by genetic modification for bioremediation purposes. Plants have also been studied and used. Arsenic is one of the target of phytoremediation [49]. The health effect of Arsenic includes liver, lung, kidney and bladder cancers. One plant, *Arabidopsis thaliana* has been genetically modified to over express two bacterial genes(*arsC* and g-ECS). The gene *arsC* codes for arsenate reductase, which allows the plant to modify arsenate (AsO_4^{3-}) into arsenite (AsO_2) [50]. Essentially this genetically modified plants can take up arsenate detoxify it and store it Hooker and Skeen [51]. Tobacco plants have been genetically modified to express bacterial pentaerythritol tetranite

(PETN) reductase allowing these plants to take up high-energy compound and reduce them to non explosives substances [51].

5. CONCLUSION

Bioremediation has offered wide range potentialities for restoring contaminated sites. And as scientists learn more about its capabilities, it is likely to become one of the biotechnologies used to clean up and protect our environment [52]. Exploitation of Biotechnological tools to remediate contaminated environments appears to be a successful promising remedy. The use of genetically engineered microorganisms suited for remediation purposes has to be adopted as an alternative for better degradation of contaminants at polluted sites

REFERENCES

- [1] J. I. Drever, *The geochemistry of natural waters: Surface and groundwater environments*, 3rd ed. New Jersey: Prentice-Hall, Inc, 2002.
- [2] M. Zeyallah, M. Atif, B. Islam, A. S. Abdelkaf, P. Sultan, M. A. ElSaady, and A. Ali, "Bioremediation: A tool for environmental cleaning," *African Journal of Microbiology Research*, vol. 3, pp. 310-314, 2009.
- [3] J. Withgot and S. Brennan, *Environment: The science behind the stories*, 2nd ed. San Francisco: Pearson Benjamin Cummings, 2007.
- [4] W. P. Cunningham and M. A. Cunningham, *Principles of environmental science: Inquiry and applications*, 3rd ed. New York: McGraw-Hill Companies, Inc, 2006.
- [5] L. V. Cauwenberghe and D. S. Roote, "In situ bioremediation," *Technical Overview Report Prepared for Groundwater Remediation Technologies Center*, Pittsburg, 1998.
- [6] J. T. K. Akoachere, T. N. Akenji, F. N. Yongabi, G. Nkwelang, and R. N. Ndip, "Lubricating oil-degrading bacteria in soils from filling stations and auto-mechanic workshops in Buea, Cameroon: Occurance and characteristics of isolates," *African Journal of Biotechnology*, vol. 7, pp. 1700-1706, 2008.
- [7] Environmental Response Division, "Fundamental principles of bioremediation an aid to the development of bioremediation proposals." Retrieved from http://www.deq.state.ms.us/MDEQ.nsf/Pdf/GARD_Bioremediation/SFile/Bioremediation.pdf. [Accessed 7/10/2009], 1998.
- [8] R. T. Wright, *Environmental science*, 10th ed. Upper Saddle River, New Jersey: Pearson Prentice Hall. Pearson Education, Inc, 2008.
- [9] M. Vidali, "Bioremediation. An overview," *Pure and Applied Chemistry*, vol. 73, pp. 1163-1172 2001.
- [10] M. Leug, "Bioremediation: Techniques for cleaning up a mess," *BioTeach Journal*, vol. 2, pp. 18-21, 2004.
- [11] R. Lovley, "Cleaning up with genomics: Applying molecular biology to Bioremediation," *Nature Reviews Microbiology*, vol. 1, pp. 35-44, 2003.
- [12] Wikipedia the Free Encyclopedia, "Bioremediation." Retrieved from <http://en.wikipedia.org/wiki/Bioremediation>. [Accessed 27/6/2010], 2010.
- [13] WFE, "Bioremediation." Retrieved from <http://en.wikipedia.org/wiki/Bioremediation>. [Accessed 27/6/2010], 2010.
- [14] N. C. Brady and R. R. Weil, *Elements of nature and properties of soils*, 2nd ed. New Jersey: Pearson Education. Inc, 2004.

- [15] S. F. Nishino and J. C. Spain, Technology status review: Bioremediation of dinitrotoluene (DNT): Air Force Research Lab Tyndall Afb FL, 2001.
- [16] G. Evans and J. C. Furlong, Environmental biotechnology: Theory and application. Chichester, West Sussex PO19 8SQ, England: John Wiley & Sons Ltd, The Atrium, Southern Gate, 2003.
- [17] OLQ Geological Services, "A general outline of bioremediation. Retrieved from <http://www.in.gov/idem/land/geology/pdf/bioremediation.pdf>. [Accessed 21/11/2009]," 2009.
- [18] National and Accelerated Bioremediation Research NABIR, "Bioremediation of metals and radionuclides what it is and how it works. Retrieved from http://doesbr.org/documents/03_NABIR_primer.pdf. [Accessed 21/11/2015]," 2003.
- [19] A. I. Okoh and M. R. Trejo-Hernandez, "Remediation of petroleum hydrocarbon polluted systems: Exploiting the bioremediation strategies," African Journal of Biotechnology, vol. 5, pp. 2520-2525, 2006.
- [20] R. B. Meager and A. C. P. Heaton, "Strategies for the engineered phytoremediation of toxic element pollution: Mercury and arsenic," Journal of Industrial Microbiology and Biotechnology, vol. 32, pp. 502-513, 2005.
- [21] H. Al-Najar, R. Schulz, and V. Römheld, Phytoremediation of thallium contaminated soils by Brassicaceae (Ed) in environmental chemistry: Green chemistry and pollutants in ecosystems. Berlin Heidelberg: Springer-Verlag, 2005.
- [22] C. S. Cobbett and R. B. Meagher, "Arabidopsis and the genetic potential for the phyto- remediation of toxic elemental and organic pollutants." Retrieved from <http://www.aspb.org/publications/arabidopsis>. [Accessed 7/10/2009], 2002.
- [23] X. Yinying, "Biodegradation of high concentrations of crude oil in Microcosms," Master of Science in Environmental Engineering Thesis Submitted to the Division of Research and Advanced studies of the University of Cincinnati, 2002.
- [24] C. N. Ngobiri, A. A. Ayuk, and C. I. Anunuso, "Differential degradation of hydrocarbon fractions during bioremediation of crude oil polluted sites in the Niger Delta area," Journal of Chemical Society of Nigeria, vol. 32, pp. 151-158, 2007.
- [25] R. B. Kogbara, "Ranking agro-technical methods and environmental parameters in the biodegradation of petroleum-contaminated soils in Nigeria," Electronic Journal of Biotechnology, vol. 11, pp. 113-125, 2008.
- [26] State of Oregon Department of Environmental Quality, "UST cleanup program- approval of bioremediation technologies." Retrieved from <http://www.deq.state.or.us/lq/pubs/factsheets/tanks/BioremediationTechnologies.pdf>. [Accessed 27/10/2009], 2009.
- [27] U.S.Congress Office of Technology Assessment, Bioremediation for Marine oil spills-background paper,OTA-BP-O-070. Washigton, D,C: U.S. Government Printing Office, 1991.
- [28] U.S.COTA, Bioremediation for Marine oil spills-background paper,OTA-BP-O-070. Washigton, D,C: U.S. Government Printing Office, 1991.
- [29] A. Adeniji, Bioremediation of arsenic, chromium, lead and mercury. Prepared for U.S. environmental protection agency. Washington, DC: Office of the Solid Waste and Emergency Response Technology Innovation Office, 2004.
- [30] M. N. V. Prasad and H. M. D. Freitas, "Feasible biotechnological and bioremediation strategies for serpentine soils and mine spoils," Electronic Journal of Biotechnology, vol. 2, pp. 20-34, 1999.

- [31] T. K. Tokunaga, J. Wan, M. K. Firestone, T. C. Hazen, K. R. Olson, D. J. Herman, S. R. Sutton, and A. Lanzarotti, "Bioremediation and biodegradation: In situ reduction of chromium (vi) in heavily contaminated soils through organic carbon amendment," *Journal of Environmental Quality*, vol. 32, pp. 1641-1649 2003.
- [32] S. Ray and M. K. Ray, "Bioremediation of heavy metal toxicity-with special reference to chromium," *Al Ame En Journal of Medical Science*, vol. 2, pp. 57-63, 2009.
- [33] A. L. Holmes, S. S. Wise, and S. J. P. Wise, "Carcinogenicity of hexavalent chromium," *Indian Journal of Medical Research*, vol. 28, pp. 353-372, 2008.
- [34] B. Polissak, I. Pocs, P. Raspor, and M. Pesti, "Interference of chromium with biological systems in yeasts and fungi: A review," *Journal of Basic Microbiology*, vol. 50, pp. 21-36, 2009.
- [35] P. L. Gomes and T. Asaeda, "Phytoremediation of chromium (VI) by *Nitella* and impact of calcium encrustation," *Journal of Hazardous Materials*, vol. 166, pp. 1332-1338, 2009.
- [36] A. Rehman, A. Zahoor, B. Muneer, and S. Hasnain, "Chromium tolerance and reduction potential a bacillus sp.ev3 isolated from metal contaminated wastewater," *Bulleting of Environmental Contaminants Toxicology*, vol. 81, pp. 25-29, 2008.
- [37] D. K. Morales, W. Ocampo, and M. M. Zambrano, "Efficient removal of hexavalent chromium by a tolerant streptomyces sp. Affected by the toxic effect of metal exposure," *Journal of Applied Microbiology*, vol. 103, pp. 2704-2712, 2007.
- [38] F. A. O. Camargo, F. M. Bento, B. C. Okeke, and W. T. Frankenberger, "Chromate reduction by chromium-resistant bacteria Isolated from soils contaminated with Dichromate," *Journal of Environmental Quality*, vol. 32, pp. 1228-1233, 2003.
- [39] K. R. Reddy, S. Chinthamreddy, and R. E. Saichek, "Nutrient amendment for the bioremediation of a chromium- contaminated soil by eletrokinetics," *Energy Sources*, vol. 25, pp. 931-943, 2003.
- [40] M. D. Joesten, J. L. Wood, and M. E. Castellion, *World of chemistry essentials*, 3rd ed. Belmont, U.S.A: Thomson Brooks/Cole, 2004.
- [41] D. M. Sylvia, J. J. Fuhrmann, P. G. Hartel, and Zuberer, *Principles and applications of soil microbiology*, 2nd ed. New Jersey: Pearson Printice Hall, 2005.
- [42] A. R. W. Jackson and J. M. Jackson, *Environmental science: The natural environment and human impact*, 2nd ed. England: Pearson Education Limited, 2000.
- [43] R. C. Prince, "Bioremediation effectiveness: Removing hydrocarbons while minimizing environ-mental impact. ExxonMobil Research and Engineering." Retrieved from http://ipec.utulsa.edu/Conf2002/prince_clark_lee_109.pdf. [Accessed 15/10/ 2015], 2009.
- [44] H. I. Atagana, "Compost bioremediation of hydrocarbon –contaminated soil inoculated with organic Manure," *African Journal of Biotechnology*, vol. 7, pp. 1516-1525, 2008.
- [45] F. R. Engelhardt, "A perspective on the application of chemistry to oil spill response," *Pure Applied Chemistry*, vol. 71, pp. 1-4, 1999.
- [46] W. H. Benner and J. Hunter-Cavera, "Rapid mass spectrometric DNA diagnostics for assessing microbial community activity during bioremediation." Retrieved from http://www.osti.gov/bridge/Servlets/purl/13428_lakJpY/webviewable/13428.pdf. [Accessed 4/11/2009], 2009.
- [47] H. Brim, S. C. McFarlan, J. K. Fredrickson, K. W. Minton, M. Zhai, L. P. Wavckett, and M. J. Daly, "Engineering deinococcus radiodurans for metal remediation in radioactive mixed waste Environment," *Nature Biotechnology*, vol. 18, pp. 85-90, 2000.

- [48] R. Seipp, "Deinococcus radiodurans: Does this bug wear a lead vest or what?," BioTeach Journal, vol. 1, pp. 57-61, 2003.
- [49] M. Doucleff and N. Terry, "Pumping out the arsenic," Nature Biotechnology, vol. 20, pp. 1094 – 1095, 2002.
- [50] NABIR, "Bioremediation of metals and radionuclides what it is and how it works." Retrieved from http://doesbr.org/documents/03_NABIR_primer.pdf. [Accessed 21/11/2015], 2003.
- [51] B. S. Hooker and R. S. Skeen, "Intrinsic bioremediation: An environmental restoration technology," Current Opinion in Biotechnology, vol. 7, pp. 317-320, 1996.
- [52] J. Brandsgaard, B. Granada, A. Myers, and D. V. Hoosen, "Bioremediation." Retrieved from http://www.woodrow.org/teachers/help/temp_presentations/kim/angelabio.htm. [Accessed 29/9/2009], 2009.

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