

Bioremediation: A Management Tool

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Abstract

Bioremediation means using biological agents to clean environment. Increase in the pollution has lead to increase in toxic substances in the environment and being referred to as most effective management tool bioremediation has tremendous future to be called as "Eco bio technology". Hence we can infer that bioremediation is a attractive tool used at number of sites which were degraded and attained their original position with onset of this technology. Bioremediation technology uses the microbes to remediate contaminated environment and brings back it to original position. Bioremediation has also been a solution for various emerging problems.

Keywords: Bioremediation; Biotechnology; Microbes; Pollution; Remediation

Introduction

Bioremediation is concerned with the biological restoration and rehabilitation of contaminated sites and with the cleanup of contaminated areas in more recent times, accidentally or incidentally, as a result of the manufacture, storage, transport, and use of inorganic and organic chemicals [1,2]. Bioremediation offers the possibility of degrading, removing, altering, immobilizing, or otherwise detoxifying various chemicals from the environment through the action of bacteria [3-5], Plants and fungi [6]. The advances in bioremediation have been realized through the help of the various areas of microbiology, molecular biology biochemistry, analytical chemistry, chemical and environmental engineering, among others.

With the increase in population the food demand has also increased forced our farmers to go for intensive agriculture and use more and more pesticides. The use of these pesticides degrades the quality of soil. As the population increases the pressure on natural resources increases hence due to this expansion it's impossible to maintain quality of environment in which humans live. As every sphere of the earth is being polluted so a proper management is needed. Biotechnology offers a suitable answer for managing these degraded environments. Many contaminations have been expertise by environmental biotechnology investigators' that include chlorinated solvents, hydrocarbons, PAHS, heavy metals etc. Bioremediation is not a magic formula, but it is natural process that is alternative to incineration, catalytic destruction, the use of absorbents etc. and this technique is cost effective.

With intensive agriculture practices and with onset of industries has led to production of variety of pollutants which are being added into our environment. Excess input of hazardous wastes had led to shortage of clean soil and waters thus decreasing crop yield [7]. In Bioremediation biological agents mainly fungi, yeast or bacteria are used to clean contaminated environment [8]. This technology promotes growth of microbes that are native to degraded sites and perform desired activities [9]. Such growth of microbes can achieved in several ways e.g. by addition of nutrients, by terminal electron receptor or by controlling temperature and moisture conditions [10,11]. The microbes need the nutrients or energy source for their body metabolism this is provided by contaminants that are present in degraded environment [12].

Principles of Bioremediation

Bioremediation in its meaning means using life to remediate contaminants by the way of getting used as nutrients or energy sources

by micro-organisms.

Microbial populations for bioremediation

Microorganisms used to perform the function of bioremediation are known as bioremediators. Isolation of micro-organisms can be from any environmental conditions, microbes can grow and adapt subzero temperatures as well as extreme temperatures, microbes can dwell aerobic as well as anaerobic conditions. The main requirement of microbes is energy source and carbon source [13]. The adaptability and biological systems shown by microbes make them perfect to be used for remediation of environmental hazards. Micro -Organisms present in nature either indigenous or extraneous are the prime needs for the process of Bioremediation [14]. The use of microorganisms for the process of bioremediation process depends upon on the chemical nature of the polluting agents and micro- organisms selection is to be very careful as they survive within a limited range of chemical contamination [14,15]. In the degraded environment numerous types of pollutions are present hence diverse types of microorganisms need to handle the situation (Tables 1 and 2). Watanabe et al. [16] in 1991 about 70 microbial agents were reported to degrade petroleum compounds [17]. And equal number has been added to the list in successive decades [18]. For degradation the basic requirement is that the bacteria and contaminates must be in contact. This association is not easily achieved as contaminates and microbes are not uniformly spread in the soil. But there are bacteria's which show chemo tactic response, i.e. sensing the contamination and moves towards it (Tables 3 and 4). The activity and growth of microbes is readily affected by moisture pH, temperature, and. Although Microorganisms have been also isolated from extreme conditions, most of them have shown growth optimally over a narrow Range, so that it is important to achieve optimal conditions. If the soil is acidic it is possible to rinse the pH by adding lime. Temperature affects biochemical reactions rates, and the rates double for each 10°C rise in temperature. Above a

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Organisms	Toxic chemicals	References
<i>Pseudomonas</i> spp	Benzene, anthracene, PCBs	[34]
<i>Alcaligenes</i> spp	Caromatics, PCBs	[35]
<i>Arthrobacter</i> spp	Benzene, Polycyclic aromatic, long chain alkanes, phenols	[36,37]
<i>Bacillus</i> spp	Halogenated hydrocarbons	[38]
<i>Azotobacter</i> spp	Benzene, Cyloparaffins	[37]
<i>Rhodococcus</i> spp	Aromatics	[39]
<i>Mycobacterium</i> spp	Hydrocarbons, Polycyclic hydrocarbons,	[36]
<i>Methosinus</i> sp	PCBs, formaldehyde	[40]
<i>Xanthomonas</i>	PCBs, biphenyls	[36]

Table 1: Microorganisms with biodegradation potential for xenobiotic.

Organisms	Toxic chemicals	References
<i>Pseudomonas</i> spp	Benzene, anthracene, PCBs	[34]
<i>Alcaligenes</i> spp	caromatics, PCBs	[35]
<i>Arthrobacter</i> spp	Benzene, Polycyclic aromatic, long chain alkanes, phenols	[36]
<i>Bacillus</i> spp	Halogenated hydrocarbons	[38]
<i>Corynebacterium</i> spp	Aromatics	[36]
<i>Flavobacterium</i> spp	Naphthalene, biphenyl Aromatics, branched hydrocarbons	[36]
<i>Azotobacter</i> spp	Benzene, cyloparaffins	[37]
<i>Rhodococcus</i> spp	Aromatics	[39]
<i>Mycobacterium</i> spp	Hydrocarbons, Polycyclic hydrocarbons	[36]
<i>Nocardia</i> spp	Hydrocarbon diazinon	[36]
<i>Methosinus</i> sp	PCBs, formaldehyde	[40]
<i>Xanthomonas</i>	PCBs, biphenyls	[36]

Table 2: Microbes utilize the heavy metals.

Factor	Conditions required
Micro organisms	Aerobic or Anaerobic
Natural biological processes of micro organisms	Catabolism and Anabolism
Environmental factors	Oxygen content Temperature, pH, Electron acceptor/ donor
Nutrients	Carbon , Nitrogen , Oxygen etc
Soil moisture	25-28 % of water holding capacity
Type of soil	Low clay or slit content

Table 3: Factors for Bioremediation [13], Bioremediation is a complex system of many factors.

Environmental factor	Optimum conditions	Conditions required for microbial activity
Available soil moisture	25-28% water holding capacity	25-28% water holding capacity
Oxygen	>0.2 mg/ L DO, > 10% air filled pore space for aerobic degradation	Aerobic , minimum air filled pore space of 10%
Redox potential	Eh > 50 mill volts	
Nutrients	C:N:P= 120:10:1 molar ratio	N and P for microbial growth
pH	6.5-8.0	5.5 to 8.5
Temperature	20-30 degree Celsius	15-45 degree Celsius
Contaminants	Hydrocarbon 5-10% of dry weight of soil	Not too toxic
Heavy metals	700 ppm	Total content 2000 ppm.

Table 4: Showing environmental conditions [57].

certain temperature, however, the cells die. Plastic cover can be used to improve solar warming in late spring, summer, and autumn. Water availability is essential for all the living organisms, and irrigation

is necessary to achieve the optimal moisture level. The amount of available oxygen will determine whether the system is aerobic or anaerobic. Aerobic conditions are best suited for degradation of hydrocarbons, while as anaerobic conditions are best suited for chlorinated compounds. Oxygen content in the soil can be increased through process of tillage or sparge air, but hydrogen peroxide and magnesium peroxide can also be used. Effective delivery of air water and nutrients are controlled by soil structures. Soil structures can be improved through materials like gypsum or organic matter. Low permeability of soil can hinder movement of nutrients, water and oxygen hence these soils are not appropriate for in situ cleanup.

Environmental constrains

Temperature: Microbial metabolism is substantially affected by temperature [19]. Most microorganisms grow well in the range of 10 to 38°C. Technically it is extremely difficult to control the temperature of *in-situ* processes, and the temperature of *ex-situ* processes can only be moderately influenced, sometimes with great expense. Although temperatures within the top 10 m of the subsurface may fluctuate seasonally, subsurface temperatures down to 100 m typically remain within 1°C to 2°C of the mean annual surface temperature suggesting that bioremediation within the subsurface would occur more quickly in temperate climates [20,21].

pH: The pH range in which most bioremediation processes works most efficiently is nearly 5.5 to 8. It is no coincidence that this is also the apt pH range for many heterotrophic bacteria, the major microorganisms in most bioremediation technologies. The suitable pH range for a particular situation, however, is site-specific. The pH is influenced by a complex relationship between organisms, contaminant chemistry, and physical and chemical properties of the local environment. Additionally, as biological processes proceed in the contaminated media, the pH may shift and therefore must be monitored regularly. The pH can be adjusted to the suitable range by the addition of acidic or basic substances (i.e., mineral acids or limestone, respectively). However changes in soil pH will influence dissolution or precipitation of soil metals and may increase the mobility of hazardous materials. Therefore, the soil buffering capacity should be evaluated prior to application of amendments. The effect of pH on permeability of soils and sediments is not fully understood but it seems that soil pH has also significant effect. Soils have a negative permanent charge and a pH-dependent variable charge. Therefore, pH affects soil dispersion and its permeability. A typical volcanic ash soil has a large amount of pH-dependent charge. Its saturated hydraulic conductivity decreases under low and high pH conditions. When the predominant anion is sulphate, hydraulic conductivity does not decrease even at low pH. However, the saturated hydraulic conductivity of soils with montmorillonite and kaolinite at pH 9 is smaller than that at pH 6 [22].

Moisture content - water activity: Moisture is a very important variable relative to bioremediation. Moisture content of soil alters the bioavailability of contaminants, the transfer of gases, the effective toxicity level of contaminants, the movement and growth stage of microorganisms, and species distribution. During bioremediation, if the water content is too high, it will be difficult for atmospheric oxygen to penetrate the soil, and this can be a factor of limiting growth efficiency and determine the types of organisms that can flourish. Various workers in the field have reported that the water content of the soil should be between 20 and 80%. In cases where no extra source of oxygen is being provided (for example, bioremediation of surface contamination), 20% moisture may be adequate; however, if a continuous recirculation system (pipe networks) is being used for deeper contamination, 80%

water content would be more appropriate Soil moisture is frequently measured as a gravimetric percentage or reported as field capacity. Evaluating moisture by these methods provides little information on the “water availability” for microbial metabolism. Water availability is defined by biologists in terms of a parameter called water activity (*aw*). In simple terms, water activity is the ratio of the system’s vapor pressure to that of pure water (at the same temperature) [23].

Redox potential: The redox potential of the soil (oxidation-reduction potential, *Eh*) is directly related to the concentration of O₂ in the gas and liquid phases. The O₂ concentration is a function of the rate of gas exchange with the atmosphere, and the rate of respiration by soil microorganisms and plant roots. Respiration may deplete O₂, lowering the redox potential and creating anaerobic (i.e., reducing) conditions. These conditions will restrict aerobic reactions and may encourage anaerobic processes such as denitrification, sulfate reduction, and fermentation. Reduced forms of polyvalent metal cations are more soluble (and thus more mobile) than their oxidized forms. Well-aerated soils have an *Eh* of about 0.8 to 0.4 V; moderately reduced soils are about 0.4 to 0.1 V; reduced soils measure about 0.1 to -0.1 V; and highly reduced soils are about 0.1 to -0.3 V. Redox potentials are difficult to be measured in the soil or groundwater and are not widely used in the field [21].

Mass transfer characteristics: Mass transport characteristics are used to calculate potential rates of movement of liquids or gases through soil and include: Soil texture, unsaturated hydraulic conductivity, dispersivity, moisture content vs. soil moisture tension, bulk density, porosity, hydraulic conductivity and infiltration rate [24,25]. Site hydro geologic characteristics Hydro geologic factors for consideration include aquifer type, hydraulic conductivity, hydro geologic gradient, permeability, recharge capability, depth to groundwater, moisture content/field capacity, thickness of the saturated zone, homogeneity, depth to contamination, extent of contamination, and plume stability.

These are only some parameters that should be factored into the design of any bioremediation system [23-25]

Strategies of bioremediation: Bioremediation makes use of the natural role of microorganisms in transformation, mineralization or complexation by directing those capabilities towards organic and inorganic environmental pollutants. The primary technique that has been used in bioremediation to enhance natural detoxification of contaminated environments is stimulation of the activity of indigenous microorganisms by the adding nutrients, regulation of redox conditions, and controlling of pH conditions, etc. (Table 5).

Phytoremediation: Phytoremediation is use of plants and their linked microbes for environmental cleanup [26,27]. The technology uses of the naturally occurring phenomenon by which plants and their microbial rhizosphere flora detoxify organic and inorganic pollutants. Phytoremediation well planned cleanup technology for a variety of organic and inorganic pollutants. Organic pollutants in the environment are mostly artificial and xenobiotic to organisms. Many of them are toxic, some carcinogenic. Organic pollutants are released into the environment through spills (fuel, solvents), military activities (explosives, chemical weapons), agriculture (pesticides, herbicides), industry (chemical, petrochemical), wood treatment, etc., Organics may be degraded in the root zone depending on their properties of plants or taken up, followed by degradation, sequestration, or volatilization. Successfully phytoremediated organic pollutants include organic solvents such as TCE (the most common pollutant of groundwater) [28-33], herbicides such as atrazine [29]. Explosives such as TNT [30], petroleum hydrocarbons, and PAHs the fuel additive MTBE [31], and polychlorinated biphenyls (PCBs). Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water [32-60] (Table 6).

Technique	Examples	Benefits	Applications	Reference
<i>In situ</i>	Biosparging Bio venting Bio augmentation	Most effective Non invasive Relative passive Treats soils and waters	Biodegradative abilities of indigenous micro organisms Distribution of pollutants	[41,42]
<i>Ex situ</i>	Land forming Composting Bio piles	Cost effective, simple Low cost ,rapid reaction rate Done on site	Surface application, application of organic material to natural soils, surface application, agriculture to municipal wastes	[43]
Bioreactors	Slurry reactors Aqueous reactor	Rapid degradation Enhances mass transfer	Toxic concentration of contaminants	[44]
Precipitation	Non directed physico-chemical complex action reaction between contaminant and charged particles	Cost effective	Removal of heavy metals	[45]
Microfiltration	Microfiltration membranes are used at a constant pressure	Removes dissolved solid rapidly	Waste water treatment	-----
Electrodialysis	Uses cation anion exchange membrane pairs	Withstands with high temperatures and can be reused	Removal of dissolved solids efficiently	-----

Table 5: Developmental methods applied in bioremediation.

Process	Function	Pollution	Medium	Plants	Reference
Phytoextraction	Removes metal pollutants that accumulate in plants, Removes organics from soil by concentrating them in plant parts	Cd, Pb, Zn, petroleum, hydrocarbons and radionuclide's	Soil & ground water	<i>Viola baoshanensis</i> , <i>Sedum alfredii</i> , <i>Rumex crispus</i>	[46]
Phytotransformation	Plants uptake and degrade organic compounds	Xenobiotic substances	Soil	<i>Cannas</i>	[47]
Phytodegradation	Plants and associated microorganisms degrade organic pollutants	DDT, Explosives and nitrates	Ground water	<i>Elodea Canadensis</i> , <i>Pueraria</i>	[48]
Rhizofiltration	Absorbs mainly metals from water and waste streams	Cd, As, Pb, Zn	Ground water	<i>Brassica juncea</i>	[4]
Phytostabilization	Uses plants to reduce the bioavailability of pollutants in the environment	Cu, Cd, Cr, Ni, Pb, Zn	Soil	<i>Anthyllis vulneraria</i> , <i>Festuca arvensis</i>	[49]

Table 6: Showing types of phytoremediation, functions, plant species which remove the pollutants.

Conclusion

Bioremediation enhances the natural biodegradation and cleans the polluted environment. Understanding the microbial communities and their response towards natural environment and pollution, enhancing the knowledge of genetics of microbes to increase their capabilities to degrade the pollutants, conduct field trials of new bioremediation techniques is the need of hour. Bioremediation is paving a way to greener pastures. Regardless of which aspect of bioremediation that is used, this technology offers an efficient and cost effective way to treat contaminated ground water and soil. The advantages of this technology have out classed its disadvantages and the evident example of this is increased demand of this technique. Hence it proves that bioremediation is a management tool.

References

1. Baker KH, Herson DS (1994) Introduction and overview of bioremediation. In: Baker KH and Herson DS Bioremediation, McGraw-Hill, New York.
2. Hamer G (1993) Integrated Circuit-Based Biofabrication with Common Biomaterials for Probing Cellular Biomechanics. Trends of Biotech 34: 171-186.
3. Gadd GM (2001) Fungi in Bioremediation, Cambridge University Press.
4. Verma P, George K, Singh S, Juwarkar A, Singh R (2006) Modeling rhizofiltration: heavy metal uptake by plant roots. Environmental Modeling and Assessment 11: 387-394.
5. Morel JL, Echevarria G, Goncharova N (2002) Phytoremediation of Metal-Contaminated Soils. IOS Press, Amsterdam, and Springer in conjunction with the NATO Public Diplomacy Division, pp: 1-11.
6. Kvesitadze G, Khatisashvili G, Sadunishvili T, Ramsden JJ (2006) Biochemical Mechanisms of Detoxification in Higher Plants. Springer.
7. Kamaludeen SP, Arunkumar KR, Avudainayagam S, Ramasamy K (2003) Bioremediation of chromium contaminated environments. Indian J Exp Biol 41: 972-985.
8. Strong PJ, Burgess JE (2008) Treatment methods for wine related distillery wastewaters: a review. Biorem Jou 12: 7087-7085.
9. Agarwal SK (1998) Environmental Biotechnology. APH Publishing Corporation, New Delhi, India, pp: 267-289.
10. Hess A, Zarda B, Hahn D, Haner A, Stax D, et al. (1997) In situ analysis of denitrifying toluene- and m-xylene-degrading bacteria in a diesel fuel-contaminated laboratory aquifer column. Appl Environ Microbiol 63: 2136-2141.
11. Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49: 643-668.
12. Tang CY, Criddle QS, Fu CS, Leckie JO (2007) Effect of flux (transmembrane pressure) and membranes properties on fouling and rejection of reverse osmosis and nanofiltration membranes treating perfluorooctane sulfonate containing waste water. Jou Environ Sci Tech 41: 2008-2014.
13. Vidali M (2001) Bioremediation an overview. Pure Appl Chem 73: 1163-1172.
14. Prescott LM, Harley JP, Klein DA (2002) Microbiology. (5th edn), McGrawHill, New York, pp: 10-14.
15. Dubey RC (2004) A text book of Biotechnology. 3rd edn, S. Chand and Company Ltd. New Delhi, India, pp: 365-375.
16. Watanabe K, Kodoma Y, Stutsubo K, Harayama S (2001) Molecular characterization of bacterial populations in petroleum contaminated groundwater discharge from undergoing crude oil storage cavities. Applied and Environmental Microbiology 66: 4803-4809.
17. US Congress (1991) Office of Technology Assessment, Bioremediation for Marine Oil Spills—Background Paper, OTABPO70.
18. Glazer AN, Nikaido H (2007) Microbial biotechnology: Fundamentals of applied Microbiology. Cambridge University Press, Cambridge, New York, pp: 510-528.
19. Rike AG, Schiewer S, Filler DM (2008) Bioremediation of Petroleum Hydrocarbons in Cold Regions. Cambridge University Press, Cambridge, New York, pp: 510-528.
20. Freeze RA, Cherry JA (1979) Groundwater. Englewood Cliffs, NJ Prentice-Hall.
21. Pichtel J (2007) Fundamentals of Site Remediation for Metal and Hydrocarbon-Contaminated Soils. Government Institutes.
22. Fukue M, Kita K, Ohtsubo M, Chaney R (2006) Contaminated Sediments: Evaluation and Remediation Techniques. ASTM Stock Number, pp: 14-82.
23. Suthersan SS (1999) Remediation Engineering Design Concepts, CRC Press Lewis Publishers.
24. Sara MN (2003) Site assessment and remediation handbook. Lewis Publishers, CRC Press.
25. Hillel D (1998) Environmental Soil Physics. Academic Press.
26. Raskin I, Kumar PBAN, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5: 285-290.
27. Salt DE, Blaylock M, Kumar NP, Dushenkov V, Ensley BD, et al. (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology (NY) 13: 468-474.
28. Newman LA, Strand SE, Choe N, Duffy J, Ekuan G, et al. (1997) Uptake and biotransformation of trichloroethylene by hybrid poplars. Environ Sci Technol 31: 1062-1067.
29. Burken JG, Schnoor JL (1997) Uptake and metabolism of atrazine by poplar trees. Environ Sci Technol 31: 1399-1406.
30. Hughes JB, Shanks J, Vanderford M, Lauritzen J, Bhadra R (1997) Transformation of TNT by aquatic plants and plant tissue cultures. Environ Sci Technol 31: 266-271.
31. Davis LC, Erickson LE, Narayanan N, Zhang Q (2004) Modeling and design of phyto remediation. In Phytoremediation: Transformation and Control of Contaminants, JL Schnoor, pp: 663-694.
32. Bhadra R, Wayment DG, Hughes JB, Shanks JV (1999) Confirmation of conjugation processes during TNT metabolism by axenic plant roots. Environ Sci Technol 33: 446-452.
33. Bowen GC, Rovira AD (1999) The rhizosphere—the hidden half of the hidden half. In Plant Roots—The Hidden Half, pp: 641-669.
34. Von Fahnstock FM, Wickramanayake GB, Kratzke KJ, Major WR (1998) Biopile Design, Operation, and Maintenance Handbook for Treating Hydrocarbon Contaminated Soil. Battelle Press, Columbus, OH.
35. Lal B, Khanna S (1996) Degradation of crude oil by *Acinetobacter calcoaceticus* and *Alcaligenes odorans*. Appl Bacteriol 81: 355-362.
36. Jogdand SN (1995) Environmental biotechnology. 1st edn, Himalaya Publishing House, Bombay, India, pp: 104-111.
37. Dean-Ross D, Moody J, Cerniglia CE (2002) Utilization of mixtures of polycyclic aromatic hydrocarbons by bacteria isolated from contaminated sediment. FEMS Microbiol Ecol 41: 1-7.
38. Cybulski Z, Dzuirla E, Kaczorek E, Olszanowski A (2003) The influence of emulsifiers on hydrocarbon biodegradation by *Pseudomonadacea* and *Bacillaceae* strains. Spill Science and Technology Bulletin 8: 503-507.
39. Park AJ, Cha DK, Holsen TM (1998) Enhancing solubilization of sparingly soluble organic compounds by biosurfactants produced by *Nocardia erythropolis*. Water Environment Research 70: 351-355.
40. Ijah UJJ (2002) Accelerated crude oil biodegradation in soil by inoculation with bacterial slurry. Journal of Environmental Sciences 6: 38-47.
41. Bouwer EJ, Zehnder AJ (1993) Bioremediation of organic compounds—putting microbial metabolism to work. Trends Biotechnol 11: 360-367.
42. Niu GL, Zhang JJ, Zhao S, Liu H, Boon N, et al. (2009) Bioaugmentation of a 4-chloronitrobenzene contaminated soil with *Pseudomonas putida* ZWL73. Environ Pollut 157: 763-771.
43. Blaylock MJ, Huang JW (2000) Phytoextraction of metals. In Phytoremediation of Toxic Metals. Using Plants to Clean up the Environment, ed. I Raskin, BD Ensley, pp: 53-70.
44. Allen HE, Huang CP, Bailey GW, Bowers AR (1995) Metal speciation and contamination of soil. Lewis Publishers.
45. Natrajan KA (2008) Microbial aspects of acid mine drainage and its bioremediation, transaction of non ferrous metals society of china. Ind Acd of Sci 18: 1352-1360.

46. Macek T, Mackova M, Kás J (2000) Exploitation of plants for the removal of organics in environmental remediation. *Biotechnol Adv* 18: 23-34.
47. Balanca Antizar L, Angus Beck J, Katarina S, Lopez-Real J, Nicholas Russell J (2007) The influence of different temperature programmes on the bioremediation of polycyclic aromatic hydrocarbons (PAHs) in a coal tar contaminated soil by in-vessel composting. *Journal of hazardous materials* 14: 340-347.
48. Newman LA, Reynolds CM (2004) Phytodegradation of organic compounds. *Curr Opin Biotechnol* 15: 225-230.
49. Vazquez S, Agha A, Granado A, Sarro M, Esteban E, et al. (2006) Use of white Lupin plant for phytostabilization of Cd and As polluted acid soil. *Air and Soil pollution* 177: 349-365.
50. EPA (2006) In Situ and Ex Situ Biodegradation Technologies for Remediation of Contaminated Sites. USEPA.
51. EPA (2007) Treatment Technologies for Site Cleanup: Annual Status Report. EPA-542-R-07-012.
52. Fitts CR (2002) *Groundwater Science*. Academic Press.
53. Hong MS, Farmayan WF, Dortch IJ, Chiang CY, McMillan SK, et al. (2001) Phytoremediation of MTBE from a groundwater plume. *Environ Sci Technol* 35: 1231-1239.
54. Mueller JG, Cerniglia CE, Pritchard PH (1996) Bioremediation of Environments Contaminated by Polycyclic Aromatic Hydrocarbons. In *Bioremediation: Principles and Applications*, Cambridge University Press, Cambridge, pp: 125-194.
55. Lowe DF, Oubre CL, Ward CH (1999) *Surfactants and Cosolvents for NAPL Remediation: A Technology Practices Manual*. Lewis Publishers, Boca Raton FL.
56. Reddi LN, Inyang HI (2000) *Geoenvironmental Engineering: Principles and Application*. Marcel Dekker Inc., pp: 506.
57. Shanahan P (2004) *Bioremediation Waste Containment and Remediation Technology*. Spring 2004, Massachusetts Institute of Technology, MIT Open Course Ware.
58. Tungtitiplakorn W, Lion LW, Cohen C, Kim JY (2004) Engineered polymeric nanoparticles for soil remediation. *Environ Sci Technol* 38: 1605-1610.
59. T Cairney (1993) *Contaminated Land*. Blackie, London, pp: 4.
60. Winnike-McMillan SK, Zhang Q, Davis LC, Erickson LE, Schnoor JL (2003) Phyto remediation of methyl tertiary-butyl ether. In *Phyto remediation: Transformation and Control of Contaminants*, ed. SC McCutcheon, JL Schnoor, pp: 805-828.