Factors Affecting Bioremediation

Uqab Ali, Syeed Mudasir, Saleemm Farooq and Ruqeya Nazir*

Department of Environmental Sciences and Centre of Research for Development, University of Kashmir, Srinagar – 190 006, J & K, India
***Corresponding author: ruqiaya.du@gmail.com**

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 biotechnology". 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. Several factors affect the process of bioremediation hence these factors play a vital role in the process of Bioremediation.
Key words: Bioremediation, biotechnology, microbes, pollution, remediation factors

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 (Baker *et al.*, 1994). Bioremediation offers the possibility of degrading, removing, altering, immobilizing, or otherwise detoxifying various chemicals from the environment through the action of bacteria (Sung *et al.,* 2016; Verma *et al.,* 2006 and Boruvka and Vacha, 2006), plants and fungi (Kvesitadze et al., 2006). 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.

Factors effecting bioremediation

The principle of bioremediation is that microorganisms (mostly bacteria or fungi) are used to degrade hazardous contaminants or covert them to less harmful forms. Thus, bioremediation of contaminants is an application of the microbial metabolic activity. Microorganisms, with their enzymatic pathways, act as biocatalysts and facilitate the progress of biochemical reactions that detoxify the targeted contaminants. As a result, bioremediation processes are only applicable in environments that can sustain life. The microbes act upon the contaminants only when they have access to a variety of materials-compounds to help them extract nutrients and energy to build more cells. In very few cases the natural conditions that exist at the contaminated site provide all the essential materials in large enough amount that bioremediation can occur without human intervention - a process called *intrinsic bioremediation*.Frequently, bioremediation needs the construction of engineered systems to supply microbe stimulating materials - a process called *engineered bioremediation*. Engineered bioremediation purely depends on accelerating the desired biodegradation reactions by encouraging the growth of more organisms, as well as by optimizing the environment in which the organisms must carry out the detoxification reactions**.**

The metabolic characteristics of the microorganisms in association with the physicochemical properties of the object contaminants determine whether a specific microorganism - contaminant interaction is possible. The actual successful interaction between the two, however, depends on the environmental conditions of the site of the interaction. Specific constrains should therefore be fulfilled for a successful bioremediation attempt. These constrains encompass the microbial, chemical and environmental characteristics of the targeted site**.**

Microbial constrains

A lucrativel bioremediation effort relies on the utilization of the appropriate microorganisms (Neilson and Allard, 2008). Such microbial populations can in theory be consortia of naturally existing species or genetically engineered microorganisms. Most applications rely on the use of naturally existing microbial populations which often are not well characterized. That is to say the microbial populations are effective in their desired application but the complete characterization of the population is not well known. This knowledge gap is not necessarily the result of a scientific inability but rather of the continuous dynamic adaptation of the microbial species to their environments. An example of this ability of microbial populations to adapt to the presence of man-made chemicals comes from the field of medicine, where the rapid adaptation of pathogenic organisms and their resulting immunity to specific classes of antibiotics as a result of the excessive use of these antibiotics has been well documented. These adaptational mechanisms advance through selection processes in which variant species with a specific survival advantage for the given environment take over and survive successfully. The survival advantage often relies on the ability of an organism to metabolise as substrate organic molecules (pollutants) existing in a given site. Contemporary microbiological techniques allow the identification of such transconjugants that originate from a background microbial population confirming that such processes are active in bioremediation practice (Berkey *et al.,* 1990). Horizontal transfer of catabolic plasmids among different species existing within a site may also result into species that possess enhanced catabolic or resistance potential.Such plasmid containing bacteria have been separated from polluted sites (Hardman *et al.,* 1986). Species that can through such plasmid transfer catabolise as single carbon source hazardous xenobiotics (as for example 3-chlorobenzoate) have been reported **(**Pertsova *et al.,* 1984)**.**

The ultimate impact, however, of such plasmid transfer processes on the field application potential of bioremediation will have to pass through the previously described path of Principles of bioremediation processes natural selection. A newly acquired metabolic advantage will be assessed, through the mechanism of natural selection, and may allow the ultimate successful establishment of a transconjugants species in a contaminated site. Genetically modified microorganisms (GMOs) have often been presented as offering a major potential advantage for bioremediation. The development of recombinant DNA and other genetic engineering technologies, in the late 1970s, was believed that could be widely applied for environmentally-beneficial purposes, including the clean-up of contaminated soil and water (Romantschuk *et al.,* 2000, Singh *et al.,* 2008 and Sayler and Ripp, 2000). The continuously growing knowledge on catabolic pathways and critical enzymes provides the basis for the rational genetic design of new and improved enzymes and pathways for the development effective processes. Many researchers had expected that genetically modified organisms having novel biochemical traits or enzymatic activities would quickly find broad applicability in bioremediation of hazardous chemicals from the environment (Glass, 2005). However the practical impact of GMOs is likely to remain low for many key reasons. Public, economic and technical issues associated with the let go of genetically engineered, or recombinant, microbial species into an open environment usually arise. Many site owners, consultants and regulators are more comfortable choosing technologies and methods with which they are familiar, have a long track record of success and thus a greater predictability. Legislative reasons are associated with the strict control on the release of such organisms into the environment. There is significant concern about the long term survival of genetically engineered species into a natural environment where they would have to compete with the naturally existing consortia that had ample time to adapt to the prevailing environmental conditions. Thus, difficulties in obtaining permission to use genetically engineered microorganisms from government regulatory agencies as well as public controversies have made companies reluctant to develop bioremediation strategies based on GMOs (Glass, 2005 and Wilson, 2005).

Finally, their use is considered costly. Technically speaking, it seems more plausible to use GMOs in *ex-situ* bioremediation treatment schemes in bioreactors, designed for use with defined soil slurries or water streams in tightly controlled environments. Not only does this limit the widespread release of the GMOs in the environment and avoids the problem of competition with indigenous microflora, but also allows the microorganism to be maintained at controlled temperatures and other growth conditions, to be used with relatively well-defined waste streams containing one or a small number of specific contaminants.
The application of the genetically engineered microorganisms in industrial scale bioremediation is not yet prominent. Until today GMOs have not been used in commercial site remediation projects, with few only exceptions (Strong and Wackett, 2005). Most bioaugmentation projects have used naturally-occurring bacteria for which obtaining regulatory approval is relatively easy. However, recently transgenic plants begin to find applicability in commercial phytoremediation projects**.**

Chemical constrains

Bioavailability of contaminants

In order for the pollutants to be amenable to biological degradation they must be bioavailable (Naidu, 2008). Bioavailability is associated to the physical state of the contaminant and the possibility of efficient contact between the microorganism and the contaminant. This contact is best when the microorganism-contaminant interface is maximised. Regarding physical state, microorganisms generally assimilate pollutants from the liquid phase and cannot effectively degrade a pollutant until it desorbs from aquifer solids, diffuses out of nanopores, or dissolves from nonaqueous phase liquids (NAPLs) into the bulk solution. In such cases, the rate of biodegradation can be controlled by the diffusion, desorption, or dissolution rates. Polar, water soluble contaminants are more easily bioavailable. The increase of the contaminant - microorganisms contact surface for hydrophobic contaminants may require the addition of surface active agents. Knowledge of partitioning and rates of transfer of a chemical between its disolved-sorbed-volatile states becomes important in defining its bioavailability. Bioavailability comprises the effects of all the physical and chemical parameters that eventually dictate the potential for the microbial utilisation of a compound and thus its biodegradation potential **(**Alvarez *et al.*, 2005**).**

Biodegradability of contaminants

The success of any bioremediation project depends mainly on the chemical structure of the organic molecules present in the degraded site (Neilson and Allard, 2008**)**. Some structural features of organic compounds that are not common in nature, called ‘‘xenophores’’ (e.g., substitutions of H with Cl, NO2, CN, and SO3 groups), make such molecules difficult to be metabolized by microorganisms. Thus, contaminants that contain such xenophores tend to be recalcitrant to microbial degradation (Alexander, 1999). Table 1, presents the experienced biodegradability potential of different target organic molecules. Numerous mechanisms and pathways have been elucidated for the biodegradation of a wide variety of organic compounds (Neilson and Allard, 2008). All metabolic reactions are mediated by enzymes. These belong to the groups of *oxidoreductases*, *hydrolases*, *lyases*, *transferases*, *isomerases* and *ligases*. Many of the oxygenase enzymes that attack aromatic hydrocarbons have a remarkably wide degradation capacity due to their non specific substrate affinity. For example, toluene dioxygenase is capable of degrading more than 100 different compounds, including TCE, nitrobenzene, and chlorobenzene. Other examples are esterases, which break down ester bonds by the addition of water; depolymerases, which hydrolyze polymers; dehalogenases, which remove halogen atoms such as chlorine and replace them with —OH groups; decarboxylases which remove CO2 groups (i.e., decarboxylation), hydratases which add water to alkenes converting them into secondary alcohols; glutathione S-transferase which transfers the thiol group tochlorinated compounds with concomitant dechlorination; racemases which catalyze L and D-amino acid interconversions and finally CoA-ligase, which adds -S-CoA to fatty acids during beta-oxidation.

Table 1: Biodegradability of various compounds (Suthersan, 1999 and E. P. A., 2006)

Simple hydrocarbons, C1- C15	Very easy
Alcohols, phenols, amines	Very easy
Acids , esters, amides	Very easy
Hydrocarbons, C12- C20	Moderately easy
Ethers, monochlorinated hydrocarbons	Moderately easy
Halogenated and non halogenated volatile organic compounds (Voc`s)	Moderately easy
Halogenated and non halogenated semi volatile organic compounds(Svov`s)	Moderately easy
Hydrocarbons, greater than C20	Moderately difficult
Multichlorinated hydrocarbons	Moderately difficult
PAHS, PCBS, Pesticides and herbicides	Moderately difficult

Properties of other contaminant

Contaminant properties are critical to contaminant-soil interactions, contaminant mobility and to the ability of treatment technologies to remove, destroy or immobilize contaminants. Important contaminant properties include: Solubility in water, dielectric constant, diffusion coefficient, molecular weight, vapor pressure, density and aqueous solution chemistry **(**Sara, 2003**).**

Nutrients: Most in-situ bioremediation methods practiced today rely on the stimulation of indigenous microbial populations at the site of contamination, by addition of appropriate nutrients, principally carbon, oxygen, nitrogen and phosphorus, and by maintaining optimum conditions of pH, moisture and other factors, to trigger increased growth and activity of indigenous biodegradative microorganisms (Fingerman *et al.*, 2005). Nitrogen and phosphorus requirements are often estimated by calculating a carbon to nitrogen to phosphorus ratio C/N/P close to 100/(10 to 5)/1. Many authors report optimum experimental results C/N/P ~70/3/0.6, , 8/1/0.07 (Atlas, 1981), for crude oil bioremediation of different origin. Fertilizers such as paraffinized urea and octylophosphate in C/N/P 100/10/1 respectively have been suggested for optimal growth. Dibble and Bartha (Dibble *et al.* suggest ratio of C/N/P 800/13/1, illustrating that the nutrient requirement is specific to oil-in-water mixtures and needs individual consideration for any case. Suggested C/N values for composting are between 30-40 (Naidu *et al.*, 2008). A detailed excellent review for nitrogen and phosphorous requirements for bioremediation as well as the deterimental effects of excess nutrients can be found in the literature (Walworth *et al.*, 2008). By controlling ground water flow using injection wells or burred perforated pipes (infiltration gallery) nutrients are delivered . In common settings, ground water that is withdrawn from production wells down gradient from the biostimulation zone is amended with the nutrients required for biostimulation, treated if necessary to remove contaminants, and reintroduced to the aquifer up gradient of the biostimulation zone using the injection wells or infiltration galleries. External source of water is required if the flow of withdrawn water is insufficient to control the subsurface flow The rate of nutrient delivery to the biostimulation zone, hence, is often limited by the solubility of the nutrients in water and the reinjection flow rate**.**

Oxygen, air, hydrogen peroxide

In the most of applications, bioremediation is an oxidation process. During oxidation of contaminants, microorganisms extract energy via electron transfer. Electrons are removed from the contaminant and shifted to a terminal electron acceptor which, during aerobic biodegradation, is oxygen. Oxygen concentrations during decomposition of the organic substrate in the subsurface may become reduced (Pichtel, 2007). The availability of oxygen is the major kinetic limitation on aerobic bioremediation due to the low solubility of oxygen in water. This is more intense in the cases of organic molecules with high oxygen demand such as petroleum hydrocarbons. Air, oxygen, or other oxygen sources (e.g., hydrogen peroxide, ozone) may be added to the infiltration water to promote aerobic biodegradation. Air sparging of water can supply 8 mg/L dissolved oxygen, sparging with pure oxygen can deliver 40 mg/L, while application of hydrogen peroxide can provide more than 100 mg/L oxygen. Therefore, while air sparging is the simplest and most common oxygen delivery technique, the use of oxygen or hydrogen peroxide may speed the bioremediation process and decrease the pumping required. However, in some cases the increased cost and potential explosion hazard associated with pure oxygen supply may limit the applicability of direct oxygen use. On the other hand, application of hydrogen peroxide to *in-situ* bioremediation is limited by its toxicity to microorganisms, its potential for causing aquifer plugging due to the highly reactive nature of hydrogen peroxide resulting in chemical oxidations of organic and inorganic compounds, producing precipitates (Spain *et al., 1989*).

Alternative electron acceptors: In the absence of molecular oxygen, anaerobic microorganisms use other forms of combined oxygen. For example, denitrifying bacteria use nitrate (NO3 -), nitrite (NO2 -), or nitrous oxide (N2O); dissimilatory metal-reducing bacteria use manganese or ferric iron oxides (e.g., MnO2, Fe(OH)3, or FeOO-); sulfate-reducing bacteria use sulfate (SO42-); and methanogens use carbon dioxide (CO2) or bicarbonate (HCO3) as electron acceptors (Fenchel *et al.,* 1995). In cases where oxygen is progressively depleted, electron acceptors are generally used up in a set sequence determined by the appropriate redox potentials of the oxidation reactions under consideration (Remoundaki *et al., 2003)*. Thermodynamic concepts imply the following sequence of electron acceptor utilization:

$$O_2 \rightarrow NO_{3-} \rightarrow Mn^{4+} \rightarrow Fe^{3+} \rightarrow SO^{2-}{}_4 - \rightarrow HCO_{-3}$$

The implication of this thermodynamic analysis is that when the electron acceptor demand is relatively high (e.g., near the source zone), microbial degradation would sequentially deplete the available oxygen, then nitrate, manganese, ferric iron, and sulfate before methanogenesis becomes predominant. Thermodynamic considerations also imply that heterotrophic microorganisms capable of deriving the maximum amount of energy per unit of carbon oxidized would have a competitive advantage over other species, and their respiration mode would become dominant until their specific electron acceptor is used up.

Metal ions: Although some metals are essential in trace quantities for microbial growth, heavily contaminated sites with high concentrations of metal ions in contaminated soil or water usually inhibit the metabolic activity of the cells, thus affecting directly any bioremediation process (Talley, 2005).

Toxic compounds: High aqueous phase concentrations of some contaminants can create toxic effects to microorganisms, even if the same chemicals are readily degraded at lower concentrations. Toxicity prevents or slows down microbial metabolic activity and often prevents the growth of new biomass needed to stimulate rapid contaminant removal. The degree and mechanisms of toxicity vary with specific toxicants, their concentration, and the exposed microorganisms. Some organic compounds are toxic to targeted life forms such as insects and plants and may also be toxic to microbes. These compounds include herbicides, pesticides, rodenticides, fungicides, and insecticides. In addition, some classes of inorganic compounds such as cyanides and azides are toxic to many microbes; however, these compounds may be degraded following a period of microbial adaption (Talley, 2005).

Biogeochemical parameters

Measurements of various biogeochemical parameters such as dissolved oxygen (DO), redox potential, CO2, and other parameters such as NH^{4+}, NO_{3-}, NO_{2-}, $SO_4{}^{2-}$, S^2-and Fe^{2+} will give an indication of the existing (natural or intrinsic) microbial metabolic activity at the site (Suthersan, 1999).

Environmental constrains

Temperature: Microbial metabolism is substantially affected by temperature (Rike, 2008). Most microorganisms grows 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° to 2°C of the mean annual surface temperature suggesting that bioremediation within the subsurface would occur more quickly in temperate climates (Freeze and Cherry, 1979)

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 (Pichtel, 2007). 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 (Fukue *et al.*, 2006).

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 (Talley, 2005). 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) (Suthersan, 1999 and Talley, 2005).

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 (Pichtel, 2007)

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 (Sara, 2003; Hillel, 1998 and Hillel, 1998). 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 (Suthersan, 1999; Sara, 2003; Hillel, 1998).

Conclusion

Bioremediation is a multidisciplinary technology and successful application requires deep understanding of all the relevant scientific fields and attenuation processes. It seems that now a days we have entered in the most interesting and intense phase of process development. Potentials and limitations of the technology are well documented in many resources from the web, books and research papers. Generic and technical information are given in details. The experience accumulated over the years is promising to design cost effective successful remediation projects.

References

Alexander, M. 1999, ***Biodegradation and Bioremediation***, Academic Press, San Diego, C. A. 453 pp.

Alvarez, P. J. J., and Illman, W. A. 2005. ***Bioremediation and Natural Attenuation: Process Fundamentals and Mathematical Models,*** John Wiley and Sons. 609 pp.

Atlas, R. M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. ***Microbiol. Rev.,*** **45:** 180-209.

Baker K. H., Herson D. S. 1994. Comprehensive guide to bioremediation-introduction to the basic technique and application of bioremediation. p. 1-7. In: ***Bioremediation*** **(**K. H. Baker and D. S. Herson, eds.), McGraw-Hill, New York.

Beek, B. 2001. ***Biodegradation and Persistence***. Springer-Verlag Berlin Heidelberg. 320 pp.

Boruvka, L., Vacha, R. (2006) Litavka river alluvium as a model area heavily polluted with potentially risk elements. p. 267-298. In: ***Phytoremediation of Metal-Contaminated Soils*** (vol. 68; NATO Science Series: IV Earth and Environmental Sciences). (J. L. Morel, G. Echevarria, N. Goncharova, eds.), Springer.

E. P. A. 2006 ***In Situ and Ex Situ Biodegradation Technologies for Remediation of Contaminated Sites*** EPA/625/R-06/015 U.S. Environmental Protection Agency, Office of Research and Development, National Risk Management Research Laboratory, Cincinnati, Ohio. 22pp.

Fenchel, T. and Finlay, B. J. 1995. ***Ecology and Evolution In Anoxic Worlds***, Oxford University Press. 288 pp.

Fingerman, M. and Nagabhushanam, R. 2005, ***Bioremediation of Aquatic and Terrestrial Ecosystem***, Science Publishers Inc. Enfield (NH), USA. 622 pp.

Freeze, R. A. and Cherry, J. A. 1979. ***Groundwater.*** Prentice-Hall, Englewood. 604 pp.

Fukue, M., Kita, K., Ohtsubo, M., and Chaney, R. 2006. ***Contaminated Sediments: Evaluation and Remediation Techniques***, ASTM Stock Number: STP1482. 384 pp.

Furthorpe, R. R. and Wyndham, R.C. 1992. ***Appl. Environ. Microbiol.*: 58 (1):** 314-325.

Glass, D. J. 2005. Commercial use of genetically modified organisms (GMOs) in Bioremediation and Phytoremediation, pp. 41-96. In: ***Bioremediation of Aquatic and Terrestrial Ecosystems*** **(**M. Fingerman and R. Nagabhushanam, eds.) Science Publishers, Inc., Enfield, New Hampshire.

Hardisty, P. E., and Özdemiroglu, E. 2005. ***The Economics of Groundwater Remediation and Protection***. CRC Press, New York, 336 pp.

Hardman, D. J., Gowland, P. C., and Slater, J. H. 1986. ***App. Environ. Microbiol***., **51(1):** 44-51.

Hillel, D. 1998. ***Environmental Soil Physics***, Academic Press, New York, 771 pp.

Kvesitadze, G., Khatisashvili, G., Sadunishvili, T. and Ramsden J. J. 2006. ***Biochemical Mechanisms of Detoxification in Higher Plants,*** Springer. 256 pp.

Mirsal, I.A. 2008. ***Soil Pollution Origin, Monitoring and Remediation, Springer***. pp 114

Naidu, R., Semple, K.T., Megharaj, M., Juhasz, A. L., Bolan, N. S., Gupta, S., Clothier, B., Schulin, R. and Chaney, R. 2008. Bioavailability, definition, assessment and implications for risk assessment. P. 39-52. In: ***Chemical Bioavailability in Terrestrial Environment*** **(32nd edition) (**A. E. Hartemink, A. B. McBratney and Ravendra Naidu **eds.).** Elsevier, Amsterdam, The Netherlands.

Neilson, A. H., and Allard., A. S. 2008**,** ***Degradation and Transformation of Organic Chemicals***, CRC Press, Taylor and Francis Group. 94 pp

Pertsova, R.N., Kune, F., and Golovleva, I.A. 1984, *Folia Microbiol*., **29**: 242-247.

Pichtel, J. 2007, ***Fundamentals of Site Remediation for Metal and Hydrocarbon-Contaminated Soils*** (2nd edition) Government Institutes, Inc., Rockville, MD. 460 pp.

Pieper, D.H., and Reineke, W. 2000. Engineering bacteria for bioremediation. ***Curr. Opinion Biotechnol*., 11(3):** 262-270.

Remoundaki, E., Hatzikioseyian, A., Kousi, P., and Tsezos, M. 2003. The mechanism of metals precipitation by biologically generated alkalinity in biofilm reactors. ***Water Res*., 37 (16):** 3843-3854.

Rike, A. G., Schiewer, S., and Filler, D. M. 2008. Temperature effects on biodegrdation of petroleum contaminants in cold soils. P. 84-108. In: ***Bioremediation of Petroleum Hydrocarbons in Cold Regions***, (D. M. Filler, I. Snape and D. L. Barnes, eds.), Cambridge University Press.

Rittmann, B. E., and McCarty, P. L. 2001. ***Environmental Biotechnology: Principles and Applications***, McGraw Hill. 754 pp.

Romantschuk, M., Sarand, I., Petonen, T., Peltola, R., Jonsson-Vihanne, M., Koivula, T., Yrjolo, K., and Haahtela, K. 2000. Means to improve the effect of in situ bioremediation of contaminated soil: an overview of novel approaches. ***Environ. Poll*., 107(2)**: 179-185.

Sara, M. N. 2003, ***Site Assessment and Rremediation Handbook***, Lewis Publishers, CRC Press. 1160 pp.

Sayler, G.S., and Ripp, S. 2000. Field applications of genetically engineered microorganisms for bioremediation processes. ***Curr. Opinion Biotechnol*. 11(3)**, 286-289.

Singh, S., Kang, S.H., Mulchandani, A., and Chen, W. 2008, Bioremediation: environmental clean-up through pathway engineering. ***Curr. Opinion. Biotechnol*., 19(5):** 437-44.

Spain, J. C., Milligan, J. D., Downey, D. C., and Slaughter, J. K. 1989. Excessive Bacterial Decomposition of H2O2 During Enhanced Biodegradation. ***Groundwater*. 27:** 163-167.

Strong, L. C., and Wackett, L. P. 2005. Engineering of Bioremdiation Processes: A Critical Review. p. 379-396. In. ***Bioremediation of Aquatic and Terrestrial Ecosystem*** (M. Fingerman and R. Nagabhushanam, eds.). Science Publishers Inc.

Sung, C. Y., Yang C. Y., Ye J. A. and Cheng C. M. 2016. Integrated circuit-based biofabrication with common biomaterials for probing cellular biomechanics. ***Trends in Biotechnology***. **34 (2):** 171-186.

Suthersan, S.S. 1999, ***Remediation Engineering Design Concepts,*** Boca Raton, CRC Press, LLC, Lewis Publishers. 384 pp.

Talley, J.W. 2005, ***Bioremediation of Recalcitrant Compounds*** (Chapter 4). The Federal Integrated Biotreatment Research Consortium (Flask to Field), CRC Press, Taylor and Frances Group, Boca Raton, FL. 324 pp.

Verma, P., George, K., Singh, S., Juwarkar, A. and Singh, R. N. 2006. Modeling rhizofiltration: heavy metal uptake by plant roots. Environmental Modeling and Assessment. ***Environmental Modeling and Assessment*. 11:** 387-394.

Walworth, J., Pond, A., Snape, I., Rayner, J. L., Harvey, P. M., 2007. Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil. ***Cold Regions Science and Technology*.** 48: 84–91.