Biotechnology – applications to environmental remediation in resource exploitation

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This article describes biotechnological modes, updated in terms of environmental remediation while exploiting natural resources. Modern biotechnology embraces not only the principles of using various organisms but also the techniques of doing so. Both the aspects are dealt with in detail. Removal of arsenic from aquifer is also discussed as it is a pressing problem in the shallow aquifers in the Bengal basin seriously affecting agricultural products and drinking water.

Treatment of tannery effluents is a complex problem in India; more so, when reverse osmosis involves unaffordable cost. In such a situation, an integrated approach combining mineral processing techniques, nanotechnology and biotechnology is recommended.

Keywords: Arsenic, biotechnology, effluents, environmental remediation, nanotechnology, tannery.

Environmental protection and rational use of natural resources and other industrial raw materials have become an important sphere of mankind’s advancement in the second half of the 20th century. They are closely related to man’s economic activities and for this reason considered an integral part of the scientific and technological progress. Mankind’s demand for resources and raw materials has intensified the ecological and economic contradictions in the industries. Thus, the present trends in the industries call for revised criteria in solving the ecological and economic problems. Not a single engineering problem can be solved now or in future without taking into account the environmental protection problems.

Natural resources, environmental protection and the rotational use of the former is closely related to ecological, technological, economic and socio-political problems. Coalescence of ecological and economic problems has given rise to econology; it deals with the ecological and economic aspects of the natural resource extraction and the industrial processing. Econology also discusses the ways for optimization and intensification.

Actuality of ecological problems is emphasized by mankind’s growing concern for the damage caused to the environment. The main aspect of this concern is linked with the preservation of living being on our planet. The technological and economic aspects deal with the utilization of natural resources and raw materials and their sound exploitation. Advanced technologies are essential for an integrated scheme for utilization of these resources. The socio-political aspects emerge with the necessity of solving the environmental issues. However, the approach to the environmental protection programme has to be evolved on an integrated basis. A unified technology should be the key to solving the econological problems. An integrated technology should harmonize with development of the ecosystem. It should be based on the laws of the living substance development and laws of the biosphere functioning characterized by stability and self-regulation in time. A strategy in attaining this goal is not to restore the disrupted ecological balance, but rather to dynamically transform the industry ecosystem so that it would combine the technogenic approaches with the laws of the ecosystems evolution. An integrated technology is based on the concepts of an interconnected system reflecting (i) resource/raw material, (ii) production and (iii) environment with due account of the necessary and sufficient conditions for natural ecosystem functioning. Each antecedent element of the technology should be a source of mass/energy for the consequent unit. Biotechnological developments in the production stage offer new possibilities of such an integrated system utilizing microorganisms. The consequent unit operation involving the environmental engineering in most such cases effectively becomes less complicated and the load is also much reduced however toxic/damaging be the characteristics. Any kind of toxic damaging characteristics which are left out, call for an intervention in the ‘environment’ – it can be remedied through bioremediation technology (also known as environmental biotechnology).

Bioremediation is an ecologically sound natural process where natural strains of bacteria breakdown organic wastes most effectively. Because of their appearance in nature itself, the population of the strains explodes which drives the process of breakdown of hazardous wastes or
pushes the bioremediation process forward. These bacteria increase in number when a food source, i.e., the waste is present. When the contaminant is degraded, the microbial population naturally declines. Residues from the biological treatment are usually harmless products; the process is less expensive than the other technologies. Finally, bioremediation can often be accomplished where the problem is located. This eliminates the need to transport large quantities of contaminated waste off-site and the potential threats to health, and the environment that can arise during such transport.

Bioremediation is one of the most promising new technologies for treating industrial wastes (solid and liquid), municipal/urban wastes (sewage), mining wastes (including effluents containing heavy metals, etc.), chemical spills and hazardous wastes, etc. This list is nearly all embracing indeed. It can be used as an in situ remediation technology with indigenous microorganisms or can be used in an ex situ mode in either composite piles or bioreactors. By altering the types of microorganisms present, nutrients and climatic conditions (pH, moisture, temperature, oxygen levels), microbial degradation can be enhanced. In situ bioremediation can be implemented in many treatment modes— including aerobic (oxygen, respiration), anoxic (nitrate respiration) and anaerobic (non-oxygen respiration) and co-metabolic. The aerobic mode has proved to be the most effective mode in reducing contaminant levels of aliphatic and aromatic petroleum hydrocarbons found in gasoline (petrol) and diesel fuels. However, a technology known as biofiltration, recently developed is best suited for air-streams containing volatile organic compounds (VOCs).

**Basics of bioremediation**

We are aware that microorganisms are involved in the destruction of contaminants. The process thus calls for the growth and reproduction of the organisms and to encourage that, a source of energy (electron donor) and a means of extracting this energy via an appropriate electron acceptor are required.

Microorganisms require carbon as an energy source to sustain the metabolic functions which include growth and reproduction. The metabolic processes used by bacteria to produce energy require a terminal electron acceptor (TEA) to enzymatically oxidize the carbon source (organic matter to carbon dioxide).

\[
\text{Organic matter + oxygen + biomass} \rightarrow \text{carbon dioxide + water + } \Delta H_f
\]

where \(\Delta H_f\) is the energy generated by the reaction to fuel other metabolic processes including growth and reproduction. In this example, oxygen serves as TEA.

Microbes and electron donor (energy and carbon source) + nutrients + electron acceptor → more microbes + oxidized end products (destruction complete).

Relative energy yield from various terminal electron acceptors:

<table>
<thead>
<tr>
<th>Electron acceptor</th>
<th>Relative energy yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>50</td>
</tr>
<tr>
<td>Nitrate</td>
<td>45</td>
</tr>
<tr>
<td>Sulphate</td>
<td>2</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>1</td>
</tr>
<tr>
<td>Organic carbon</td>
<td>1</td>
</tr>
</tbody>
</table>

It is relevant here to note that microbial populations, oxygen, soil water, pH, nutrients and temperature are important microbial and environmental elements which affect the bioremediation processes.

Microbial and environmental factors which affect soil and groundwater bioremediation are: (a) microbial populations, (b) oxygen, (c) soil water, (d) pH, (e) nutrients and (f) temperature. An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode. It is pertinent, however, to mention here that for a successful bioremediation mode, an acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site under investigation.

**Groundwater bioremediation**

For the decision making, whether bioremediation is feasible or not, a preliminary screening of in situ groundwater is essential which embraces the following determinations: (a) nature and extent of contamination and (b) nature of soil. If both the aspects are favourable, as discussed earlier, a detailed appraisal covering the broader range of site and constituent characteristics must be made. The aspects related to soil characteristics that call for evaluation are: hydraulic conductivity, soil structure and stratification, groundwater mineral content (possible inhibitory effects or adverse reactions affecting the in situ bioremediation system operation), groundwater pH, groundwater temperature, microorganisms at the site, terminal electron acceptors (organic matter + \(O_2\) + biomass → \(CO_2 + H_2O + \Delta H_f\), where \(\Delta H_f\) is the energy generated by reaction to fuel other metabolic processes including growth and reproduction), and nutrient concentrations (C-source + N-source + \(O_2\) + minerals + nutrients → cell mass + \(CO_2 + H_2O + \) other metabolic byproducts), etc. However, for constituent characteristics, chemical structure of the constituents (chemical structure of the target materials for determining the possible rate of
The components needed for the above typical in situ groundwater bioremediation system design are as follows.

(a) Extraction wells – orientation, placement and construction details;
(b) Injection wells or infiltration galleries – orientation, placement and construction details;
(c) Filtration system to remove biomass and particulates that could clog the injection system;
(d) Extraction groundwater treatment system and methods for disposal or re-use of treated groundwater (suggest re-use by the present authors);
(e) Nutrient solution preparation system storage tanks (if required when the groundwater concerned does not contain adequate nutrients like nitrogen and phosphorus);
(f) Microbes addition system (if necessary);
(g) Electron acceptor system (e.g. air, oxygen, hydrogen peroxide);
(h) Orientation, placement and construction details of the monitoring wells;
(i) System controls and alarms.

It may be pointed out that the extraction wells are necessary to obtain hydraulic control over the plume to ensure that it does not spread contaminants into non-contaminated areas, or accelerate the movement towards receptors. Placement of extraction wells is thus critical, particularly in systems which use nutrient injection wells or infiltration galleries. If the groundwater systems contain appropriate concentrations of nutrients like N and P, nutrient injection system may not be required at all.

Oil bioremediation (biocatalysis)

The use of fossil fuel as energy and raw materials is the origin of widespread environmental pollution. Individual microorganisms (bacteria, yeast, fungi and algae) can metabolize only a limited range of hydrocarbons; so mixed microbial populations are needed to degrade complex mixtures of hydrocarbons, such as crude oil. Hydrocarbon degradation depends on temperature, concentration, and chemical nature of nutrients; phosphorus and nitrogen, iron availability, oil composition and the presence of other organic substances. Hence, each ecosystem and each contaminated site should be considered as a particular case.

A combination of inter-related biotechnological mechanisms is necessary for adaptation of microbes to the hydrocarbons: selective enrichment of organisms which are able to transform the specific substrate induction or depression of specific enzymes and genetic changes which result in new metabolic capabilities. Genetic mechanisms of adaptation can be carried out by the gene transfer, random mutation and genetic recombination and transposition.
Oil biodegradation is, in fact, a catalytic process with the involvement of several enzymes. However, the presence of the degradative enzymes is the final limiting factor of bioremediation. If, in the polluted site, there are appropriate biocatalytic systems, i.e. microorganisms containing genetic information for the production of degradative enzymes, the biodegradation processes will be determined by the environmental condition.

**Biosorption**

Development of the biotechnology of metals as mentioned earlier, also offers new possibilities in the utilization of microorganisms for recovering metals from solutions and treating industrial waste waters. Owing to their ability to accumulate metal contains from dilute solutions, microorganisms can be used (Figure 1) in those cases when conventional technologies fail to secure the desired purification of waste waters. The ability of microorganisms to bind metals from aqueous solution in some cases selectively is known as biosorption and microorganisms responsible for this process are called biosorbents. Various kinds of bacteria, fungi and algae have accordingly been identified. Mechanisms of biosorption are complex as shown in Figure 1.

The biomass contact with a solution of metals is realized by different techniques – in flasks on shakers, in columns, tanks, reactors, fermentors and other vessels.

The modes by which the microorganisms remove metal ions from solution are: (i) extracellular accumulation/precipitation, (ii) cell surface sorption or complexation and (iii) intracellular accumulation. Among these, process (ii) can occur, whether the organism is living or dead; process (i) may be facilitated by microbial viability while process (iii) requires microbial activity.

Biosorbents which are used for biosorption of metals from solutions could be classified into three broad categories: (1) exopolysaccharides; (2) living cultures; (3) non-living biomass and preparations.

Both living and non-living microbial cells are active in metal sorption with non-living cells, as a rule, sorbing more metal as compared to living cells. Figure 2 represents data on cadmium sorption by *Chlorella regularis* showing the high loading capacity of heat-killed cells.

Non-living microbial biomass is usually preferable for the biosorption technology as this precludes the necessity of adding nutrients required for microbial development, maintaining sterility of the process and adjusting parameters to values favourable for microbial growth.

Removal of metals by extracellular polysaccharides has been extensively studied. In this process, precipitated metals are physically adsorbed by polymer matrix produced by microorganisms. However, for removing toxic metal ions from the waste water, microorganisms need to be well adapted or they should generate separately the exopolysaccharides for utilizing the same for the metallic toxic ion removal. Biosorption of metals by yeast biomass indicates the significant roles of functional groups like amines, carboxylic acids, phosphates, sulphhydril groups and lipids. The yeast *Saccharomyces cerevisiae* is known to distinguish different metal species based on their toxicity like Hg, Sb and Se. According to Goksun-

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**Figure 1.** Schematic representation of possible centres of metal cations binding by microbial cells.

**Figure 2.** Effect of external cadmium concentration on the uptake of cadmium by *Chlorella regularis*.
GUR et al.2, any metal uptake by biosorption depends on the interactions of the functional groups native to the cell wall. Mechanism of adsorption is also observed to be probably due to electrostatic adsorption as in the case of lead by S. cervisiae2. In fact, the mechanism is primarily controlled by the specific metal, its complexation, the specific biomass and concentration of the biosorbent. At low biosorbent concentration (in case of Spirulina maxima, for example), metal uptake is higher when the intercellular distance is more. In this condition, optimality in electrostatic interaction between cells is achieved in terms of biosorption1. Such differential characteristics of biosorbents suggest that biosorption experiments at laboratory are a pre-requisite to any large scale field operation where signatures of contaminant metals have been recognized.

We have noted during the last 50 years of working in the field with laboratory support at the different mining operations at home and abroad, that Arthrobacter, Pseudomonas, Neurospora and Bacillus release a number of polysaccharides to the medium, relevant for complexing with metal ions in a stable manner. The above organisms can adsorb Pb, Cu, Cd and U. Depending on the composition of the waste water, the nature of microorganism could be fixed. Ectothiorhodospira sp. produces bio-polymers with good metal adsorption capacities. Swollen chitosan beads are similarly suitable for Mn, Co, Ni, Cu and Zn, whereas chitosan treated with ketamine shows higher loading capacity for U. Alginic acids (gel particles) generated by brown seaweeds are capable of removing rare earths from waste water. Biopolymer gel particles of alginate acid can also remove heavy metal ions. A number of microbes like Bacillus, Escherichia, Enterobacter, Micrococcus, Arthrobacter and Pseudomonas have also been used to remove metal ions like U, Th, Cd, Cr, Pb, Cu, Zn and Ni from metallurgical waste liquid effluents. The adsorption of U is, however, affected by Cu, Zn, Mn, Cd, Ca and K, as observed in the Ghabila region. Micrococcus, on the other hand, is more specific towards U. Bacillus sp. when attached to inert materials shows strong adsorption capacity for U, Cu, Cd and Zn. The bioaccumulation ability of the specific Bacillus sp., the silicate bacteria, i.e., B. circulans for Cu and Cd has been a surprise. Pseudomonas sp. can adsorb Se while Pseudomonas aeruginosa biosorbs Cr. Metal ions like Cr, Ni and Zn influence the Cu adsorption rate of P. putida and the presence of Pb, CO3, Cl and SO4 affect the kinetics. Neurospora crassa, a fungi, biosorbs chromium VI. Metal adsorption on different bacterial species such as Citrobacter, Azotobacter, Enterobacter are observed at different times. Filamentous fungi like Aspergillus, Rhizopus, Mucor, Penicillium, Actinomyces, Streptomycetes have high metal uptake capacity. The well-known yeast Saccharomyces too has a significant metal uptake capacity. Algal biomasses like Chlorella is widely used to adsorb metal ions in solution. Chlorella salina immobilized in Ca-alginate bead removes Co, Zn and Mn from plant waste water. The metal-algae biomass binding may be explained due to the formation of electrostatic bond between cationic metal ions and anionic biomass sites. Microbial cells do intracellularly accumulate both metabolically essential metals as Ca, K, Na, Fe and Mg as well as non-metabolic metals like Ni, Cd and Co. To minimize the problems of solid–liquid separation, the biomasses like Rhizopus arrhizus is immobilized in polymeric membrane – the matrix for immobilization could be alginate-polymeric bead, polyurethane foam and carbon. It has been observed that metal binding capacities and kinetics are facilitated in immobilized matrix.

We thus find that so many microorganisms as biosorbents can be utilized to absorb metal. To evaluate their stability as biosorbents, it is necessary to find out the maximum sorption capacity, kinetics of sorption, recovery of bound metal, regeneration and physical nature of the biosorbent. Metallothionein molecules, isolated from almost every variety of organism, can accumulate one particular metal to the nearly complete exclusion of all others and can be used for environmental remediation. Metallothionein molecules have large number of atoms that readily bond onto metals such as zinc, copper, lead, nickel, tin, cadmium, bismuth, mercury, silver and gold. If this protein can be synthesized, it can be used for mining or processing the metals.

The following criteria may be used for selection of the biosorbent: (a) the biosorbent should be cheap and reusable, (b) separation from the solution should be quite and efficient, (c) uptake and release of metal, i.e. kinetics should be fast, and (4) the biosorbent must be sufficiently stable to reuse.

Biosorption of metals (Figures 2–5) by microorganisms is substantially influenced by the following parameters: nature of biosorbent, age of culture and origins of biomass, the nature of interactions of metals with functional groups native to the biomass cell wall, concentration of biosorbent, properties of metals and their concentrations, solution pH, temperature and presence of other cations. The role of these parameters in biosorption is discussed below in greater detail with reference to the properties of specific biosorbents.

Biosorption reactions which are metabolism-independent and proceeds rapidly within several minutes by any one or a combination of the metal binding mechanisms like coordination, complexation, ion-exchange, physical adsorption (e.g. electrostatic) or inorganic microprecipitation. It is also plausible to speculate from the available information on metal–microbe interactions that the inherent characteristics of the fatty acid compositions (lipid compositions) of the concerned bacteria determine the differential susceptibility of microbes to various toxic heavy metals. Genes are also involved in metal accumulation.
The properties of a biosorbent are characterized by the kinetics of the biosorption process and its loading capacity, i.e. amount of metal absorbed per 1 g or 1 kg of biosorbent.

Sorption of Cu by biosorbent grains is reported, whereas Cu, Zn and other base metals from water can be removed by a treatment with high grade limestone (op. cit).

One of the authors has worked on the filamentous fungi and its environmental impact, if any, during his association with the copper mines of Chile and with Hindustan Copper Limited in India. While working, it was observed that the mycelia of filamentous fungi (waste of microbiological industry) are capable of accumulating many metals. Depending on the treatment of mycelium and the temperature during reaction, as much as 100% of Rb, Hg, Zn, Cu, Ni, Co, Mn and Cr were extracted from solutions whose volumes were 50 ml and the concentrations of the relevant metals were 0.5 mM. The chelating ability of the mycelium of fungi is connected with the presence of chitin and chitosan. With the help of the mycelia of microscopic fungi, up to 96–98% Ag and Au, up to 84% Pt, and 92% Pd may be extracted from gold and silver raffinage solution. After washing, the precipitates contain up to 20% of the total metals (Bioresource Technology, 2001–08). In this context, it may be relevant to add that researchers at the University of Torino, Italy, reported that they found some fungi which were capable of eating up the asbestos fibre from the environment (Angewandte Chemie International edition, 2002). The fungi trap the asbestos in their strands, known as hyphae. The most effective asbestos eaters are (a) *Fusarium oxysporum*, (b) *Nortiella hyalina* and (c) *Oidiodendron malus*, which are well-known for attaching plants and shrubs.

It is interesting to observe that *Azolla filiculoides* can adsorb Cs and Sr to their cell wall\(^6\). The authors suggest...
that the response is best when *A. filiculoides* is pre-
treated (at pH 7.0 by MgCl₂, H₂O₂; followed by a NaOH
wash) to modify surface characteristics.

**Removal of arsenic from groundwater resource**

Arsenic is a hazard over vast areas of West Bengal
(India) and Bangladesh. It is observed in harmful concen-
trations in aquifers contaminating the groundwater qua-
lity and in the process rendering it unsafe for human use
(i.e. for agriculture, drinking purpose, etc). The *in situ*
technology involves injection of sulphur-based polymer
compounds (patented in USA), a redox reaction compound
into the concerned aquifer. This immobilizes the arsenic
(As⁵⁺), transforms it into the insoluble arsenate complex
(As⁷⁺) which is absorbed in the soil. This method has
been used in USA successfully (where limit for As in
potable water is zero) and found to be effective. The
alternate filtration technology (patented in India by ARI,
Pune) uses *Microbacterium lacticum* that enables bio-
logical oxidation of As⁵⁺ to its easily removable form
As⁷⁺. The said plant essentially consists of three chum-
bers in the series - the first one containing the active
*M. lacticum* culture on brick pieces which biologically ox-
dizes As(III) to its removable form As(V). The water (to
be transformed to a potable type) then passes through an
alumina absorption chamber where the arsenic is remo-
vied. The water then moves through a charcoal filtration
chamber before it undergoes UV ray treatment and is
released for consumption. The pilot campaigns as reported
by ARI, Pune have shown that it is highly cost effective,
about 20–30 paisa per litre of contaminated water. The
plant requires only 250 g of *M. lacticum*, the microbe per
1000 l of water. Water pumps can be used for channel-
izing the toxic water through the reaction chambers. If the
principle of gravitation is used, the cost goes down sub-
stantially.

Anawar *et al.*⁷ report that the plant species *Spargula*
*arvensis*, *Phalacis coerulea* and *Junusus effusus* can be
used to partly remove the bioavailable toxic arsenic or
transgenic plants may be transplanted via *Escherichia
coli* genes for the phytoremediation of As-contaminated
soils. These hyperaccumulating fern species available in
the tropics may be commercially used in south-east Asia
as a form of bioremediation technology. If attempts are
successful, it may be the most cost-effective treatment suit-
able for a developing country like India.

Arsenic removal from groundwater by nanotechno-
logy⁸,⁹ is an alternative means of treatment, where the
nanoparticulate Fe⁰ with zero valency serves as the oxida-
der of arsenic.

The process, i.e. nZVI (zero valent iron) removal
mechanism may be illustrated as:

Fe⁰ + O₂ + 2H⁺ → Fe²⁺ + H₂O₂

Fe²⁺ + H₂O₂ → Fe³⁺OH⁻ + OH⁻

2OH⁻ + H₂AsO₃ → H₂AsO₄⁻ + H₂O + H⁺.

Thus As⁵⁺ is transformed to As⁴⁺ and separated from
water.

Various publications till date on As contamination in
the groundwater in the Ganges, Mekong and Red River
basins indicate that there is no unanimity as to the rela-
tive significance of biological and inorganic processes in
controlling arsenic concentrations in these systems or to
the organic carbon source, said to be responsible for the
microbial processes involved. Islam *et al.*¹⁰ and Horne-
man *et al.*¹¹ noted a decoupling of iron mineral dissolu-
tion and arsenic mobilization in the sediments of Bengal
Basin. Such a decoupling may be due to inorganic sorp-
tion processes or controlled by redox potential step
decrease. It is felt, however, that in the Bengal Basin, the
sorption process is controlled by step-wise changes in
nature and specific surface area of secondary reduced
iron phases (ferrhydrite, i.e. hydrated iron oxide). It is
suggested in conformity with observation of Charlet *et al.*¹²
that the mobilized As is localized at a step on the
mica mineral surface preferably on (0 0 1) which hosts the
nanoferrihydrite¹³,¹⁴ at these steps. Atomic force micro-
scopic images (op. cit) of these nanograins bear witness
to these postulations.

**Tannery effluents and their treatment**

The environment, we know, is under increasing pressure
from solid and liquid effluents from the leather industry,
an offshoot of the natural resources (animals).

They are categorized as:

(a) Solids: Suspended solids; Settleable solids; Gross
solids.

(b) Sulphides (S²⁻): They result from the use of sodium
sulphide and sodium hydrosulphide and the break-
down of hair and in the unhairing process. In the
alkaline condition, they remain largely in solution
and when the pH drops below 9.5, hydrogen sul-
phide, the obnoxious gas evolves.

(c) Neutral salts: Sulphates (SO₄²⁻) and Chlorides (Cl⁻).

(d) Oils and grease.

(e) Chromium compounds: Chrome (³⁺) and Chrome
(⁶⁺).

(f) Other metals: Al, Zr, etc.

(g) Solvents.
The tannery effluents damage the normal functioning of a river, destroy its ecosystem and are the main causes of imminent systems collapse of the Ganges. They are responsible for polluting the aquifers to such a significant extent that it becomes a serious environmental hazard (water becomes non-potable).

Pollution remediation of the tannery effluents is very complex. A multiprong treatment is thus called for—a combination of nanotechnology and microbial technology with prior processing by cycloning, flotation, microflootation or electroflotation. The suspended solids can be separated by an initial filtration process followed by flotation, microflootation or electroflotation or a combination depending on their surface properties and their electrostatic behaviour whereas the semi-colloidal solids, i.e., the protein residues can be separated by cycloning. During cycloning, the protein material will be separated out at the outlet (at the top of the cyclone).

Injection of black iron (Fe) nanoparticles (ZVI) or Ni/Fe bimetallic nanes produced in the laboratory into the contaminant in an effluent treatment basin (where oxygen is available) with non-porous boundary zones will immobilize the metals, particularly the hazardous chromium, detoxify and dechlorinate the other pollutants available. Biodegradation by microorganisms (discussed earlier under biosorption) will follow the nanoremediation process if it still contains partial degradation products that are considered hazardous.

The flow of the nanotreated material to a second basin is therefore imperative. When the engineering design is carried out, the material after treatment can be flowed out into the open system. Microorganisms like Bacillus, Escherichia, Enterobacter, Micrococcus, Arthrobacter, Pseudomonas, etc. and fungi like Neurospora can be utilized for biodegradation of the metals available in these effluents, after they have been exposed to ZVI remediation technology.

The heap soil washing technology using leaching microbes is receiving much attention these days for the remediation of large volumes of heavy metals and radioactive elements in contaminated soil. From another perspective, such bioremediation is gaining recognition as a metallurgical process for recovery of metals.