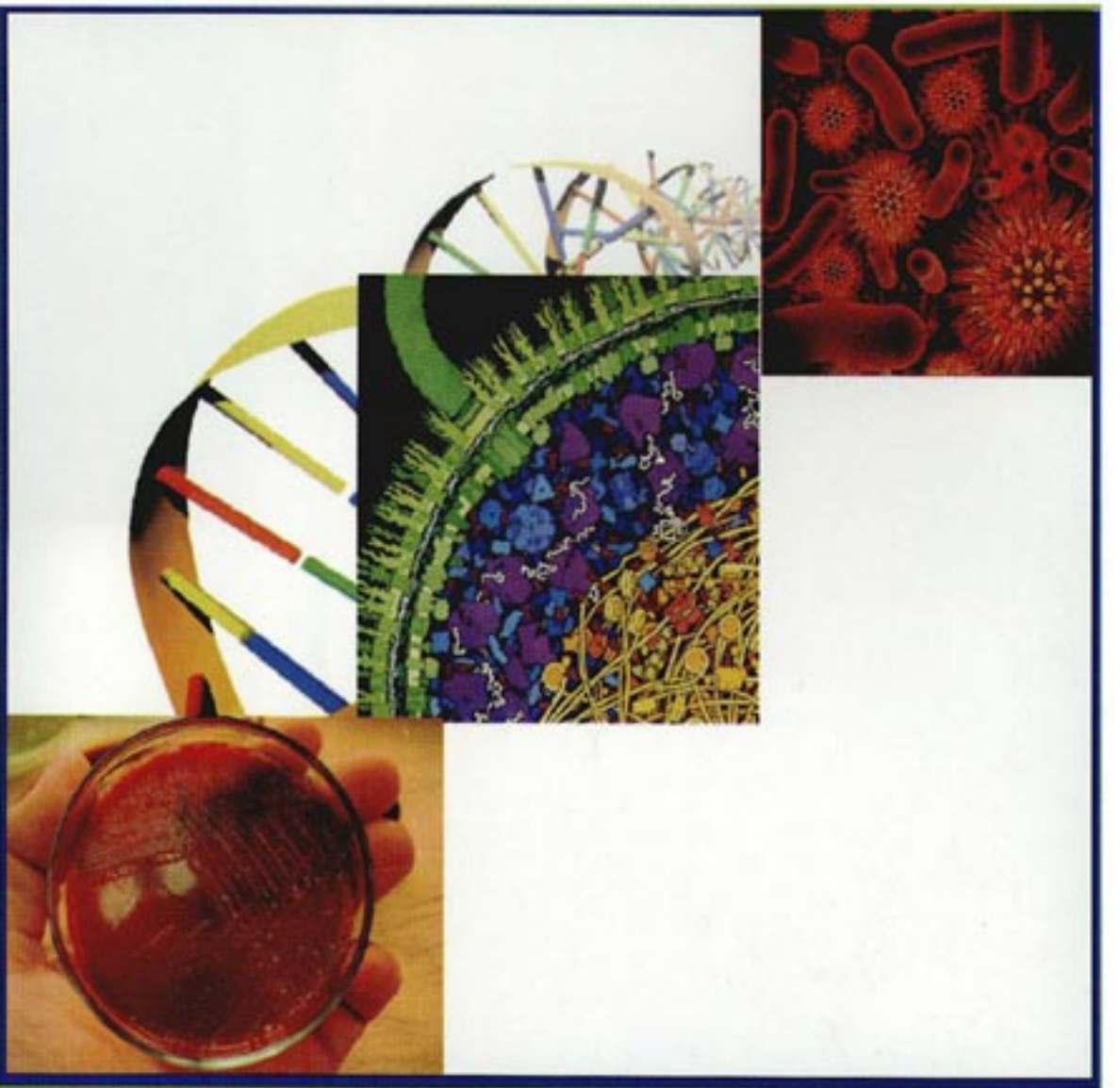


# MICROBIAL BIOTECHNOLOGY

DR. B.B.MISHRA
DR. H.N. THATOI

# MICROBIAL BIOTECHNOLOGY

**Dr. B. B. MISHRA**
Professor & Head
P.G. Department of Microbiology
Orissa University of Agriculture & Technology
Bhubaneswar

**Dr. H. N. THATOI**
Reader & Head
Department of Biotechnology
College of Engineering & Technology
Biju Pattnaik University of Technology
Bhubaneswar

**A.P.H. PUBLISHING CORPORATION**
**4435-36/7, ANSARI ROAD, DARYA GANJ**
**NEW DELHI-110 002**

*Published by*
**S. B. Nangia**
**A.P.H. Publishing Corporation**
4435-36/7, Ansari Road, Darya Ganj
New Delhi-110 002
☎ 23274050
email : aphbooks@vsnl.net

2010



*Typesetting at*
**NEW APCON**

*Printed at*
Balaji offset
Navin Shahdara, Delhi-32

# MICROBIAL BIOREMEDIATION OF CHROMIUM: A PROMISING APPROACH OF ENVIRONMENTAL MICROBIOLOGY

**B. Dhal, Abhilash, K.D. Mehta, B.D. Pandey***

**ABSTRACT**

Heavy-metal pollution represents an important environmental problem due to the toxic effects of metals, and their accumulation throughout the food chain leading to serious ecological and health problems. Metal remediation through common physico-chemical techniques is expensive and unsuitable in case of voluminous effluents containing complexing organic matter and low metal contamination. Biotechnological approaches that are designed to address to such problems have, therefore, received great deal of attention in the recent years. Micro-organisms are intimately involved in metal biogeochemistry with a variety of processes determining mobility, and therefore, bioavailability and bioremediation. The balance between mobilization and immobilization varies depending on the organisms involved, their environment and physico-chemical conditions. Metal mobilization can arise from a

National Metallurgical Laboratory, Jamshedpur – 831 007, Jharkhand, India.
Email: *bd_pandey@yahoo.co.uk / bdpnml@gmail.com*

variety of leaching mechanisms, complexation by metabolites and siderophores, and methylation, where this results in volatilization. Immobilization can result from sorption to biomass or exopolymers, transport and intracellular sequestration or precipitation as organic and inorganic compounds, e.g., oxalates (fungi) and sulfides.This chapter discusses the principle of microbial-metal interaction and mechanisms of the bioremediation process and also includes the chromium bioremediation using native isolates. Some details on bio-reduction of chromium (VI) and chromium (III) from their specific sources are described to exemplify the role of metal tolerant native microbial isolates.

**Keywords:** Microbial, Bioremediation, Chromium, Heavy metal pollution

## INTRODUCTION

The quality of life on Earth is linked inextricably to the overall quality of the environment. In early times, we believed that we had an unlimited abundance of land and resources; today, however, the resources in the world show, in greater or lesser degree, our carelessness and negligence in using them. The problems associated with contaminated sites now assume increasing prominence in many countries. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less well recognized than today. The problem is worldwide, and the estimated number of contaminated sites is significant (Carney, 1993). It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remediate many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use.

Industrialization has long been accepted as a hallmark of civilization. However, the fact remains that industrial emanations have been adversely affecting the environment.

Industrial effluents containing toxics and heavy metals drain into the river, which is often the source of drinking water for another town downstream. The main sources of heavy-metal pollutions are mining, milling and surface finishing industries, discharging a variety of toxic metals such as Cr, Cd, Cu, Ni, Co, Zn and Pb into the environment. In the last few decades, concentration of these heavy metals in river water/sediments has been clearly noticed and demonstrated. It is well-known that heavy metals can be extremely toxic as they damage nerves, liver and bones, and also block functional groups of vital enzymes (Moore, 1990; Ewan and Pamphlett, 1996). Some of the metals like Ni are also listed as a possible human carcinogen (group 2B) and associated with reproductive problems and birth defects. Besides, a range of detrimental effects on fauna and flora are also well documented. Often, these contaminants inhibit biological remediation processes due to metal sensitivity of the strain and necessitate additional combat strategies for efficient operation (Malik, 2000; Malik *et al.*, 2001).

Since these heavy metals are a valuable resource for different industrial applications, their recovery and recycle assumes even greater significance. Further, strict environmental regulations compel industries to shift to cleaner production methods, demanding the development of environmental friendly, low-cost and efficient treatment technique for metal rich effluents. Although the removal of toxic heavy metals from industrial wastewaters has been practiced for several decades, the cost-effectiveness of the most common physico-chemical processes is very limited. These are:

- Oxidation and reduction
- Chemical precipitation
- Filtration
- Electrochemical treatment
- Evaporation
- Ion-exchange and reverse osmosis

Disadvantages associated with such techniques are:

- High reagent requirement
- Unpredictable metal ion removal
- Use of strong and contaminating reagents are used for desorption, resulting in toxic sludge and secondary environmental pollution.

These disadvantages can become more pronounced and further aggravate the process cost in case of contaminated ground waters, mine tailings effluent and other industrial wastewaters due to voluminous effluents containing complexing organic matter and low metal contamination. Biotechnological approaches can succeed in those areas. Microorganisms have evolved various measures to respond to heavy-metal stress (Rai *et al.*, 1981; Macaskie and Dean, 1989; Huang *et al.*, 1990; Avery and Tobin, 1993; Brady and Duncan, 1994; Brady *et al.*, 1994; Krauter *et al.*, 1996; Veglio *et al.*, 1997) via processes such as:

- Transport across the cell membrane
- Biosorption to cell walls and entrapment in extracellular capsules
- Precipitation
- Complexation
- Oxidation– reduction reactions.

They have proven capability to take up heavy metals from aqueous solutions, especially when the metal concentrations in the effluent range from less than 1 to about 20 mg/L (Brierley, 1990).

Besides, some added advantages of biological metal clean-up techniques are:

- Flexibility to handle the range of physico-chemical parameters in effluents
- Selectivity to remove only the desired metals and
- Cost-effectiveness

These factors have promoted extensive research on the biological methods of metal removal. High metal-binding capacities of several biological materials have already been identified in part. Among the biosorbents, there are marine algae (Volesky and Holan, 1995), bacteria (Hartmeier and Berends, 1995), yeasts (Sugawara *et al.*, 1997), fungi and waste mycelia from the fermentation (Luef *et al.*, 1991) and food industry (Senthilkumar *et al.*, 2000). Further, the capacities of these microorganisms to accumulate an ample range of metal species have also been described (Volesky, 1994; Fourest *et al.*, 1994; Volesky and Holan, 1995).

**Table 7.1: Toxic effects of some heavy metals in effluents on human and their maximum permissible limits (WHO/USEPA)**

| Metal | Toxic effect | Permissible limits (mg/L)* |
|---|---|---|
| As | Skin and nasal septum cancer, Jaundice | 0.2 |
| B | Innocuous for human consumption | 2.0 |
| Cd | Shortness of breath, Anaemia, Narcosis, Hepatic and Renal disorder | 1.0 - 2.0 |
| Cr | Dermatitis, Ulceration, Cancer | 0.1 - 0.5 |
| Cu | Uremia, Thalassemia, Hemachromatoses | 2.0 - 3.0 |
| Hg | Tremors, Gingivitis, Renal disorder, Asphyxiation, Nervous failure | 0.01-0.05 |
| Mg | Cathatic and diuretic | 150.0 |
| Ni | Lung cancer and Respiratory systosis | 3.0 |
| Os | Bronchitis, Halo around eyes | - |
| Pb | Obesity, Colic, Anaemia, Pneumosis | 0.1-1.0 |
| Se | Gastrointestinal disturbance, Skin and eye irritation | 0.05 |
| Te | Garlic smell to sweat and breath | - |
| V | Catarrh, Cough, Wheezing, Sore throat, Dyspnoea, Dermatitis | - |
| Zn | Bitter astringent test, cancer | 15.0 |

Metallic pollutants cause direct toxicity both to eukaryotic and prokaryotic life forms. Heavy metals are known to have hazardous effects on human beings (Table 7.1). Several past episodes of metal toxicity have led to awareness regarding metal contamination. In the last few decades, industrialized nations have emphasized on restoring the environment and have forced environmental engineers and scientists to focus their attention on remediation of heavy metal pollution. Thus, metals which gave us the bronze age, the industrial revolution and now the "new" economy, is like a matchstick, which light up a candle to give light and at the same time to create disasters, which makes life dark. Therefore, it is a must to lessen metallic pollutants from the environment.

## PRINCIPLES OF BIOREMEDIATION

Environmental biotechnology is not a new field; composting and wastewater treatments are familiar examples of old environmental biotechnologies. However, recent studies in molecular biology and ecology offer opportunities for more efficient biological processes. Notable accomplishments of these studies include the clean-up of polluted water and land areas. *Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities* (Mueller *et al.*, 1996). By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms.

When micro-organisms are imported to a contaminated site to enhance degradation we have a process known as bioaugmentation.

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

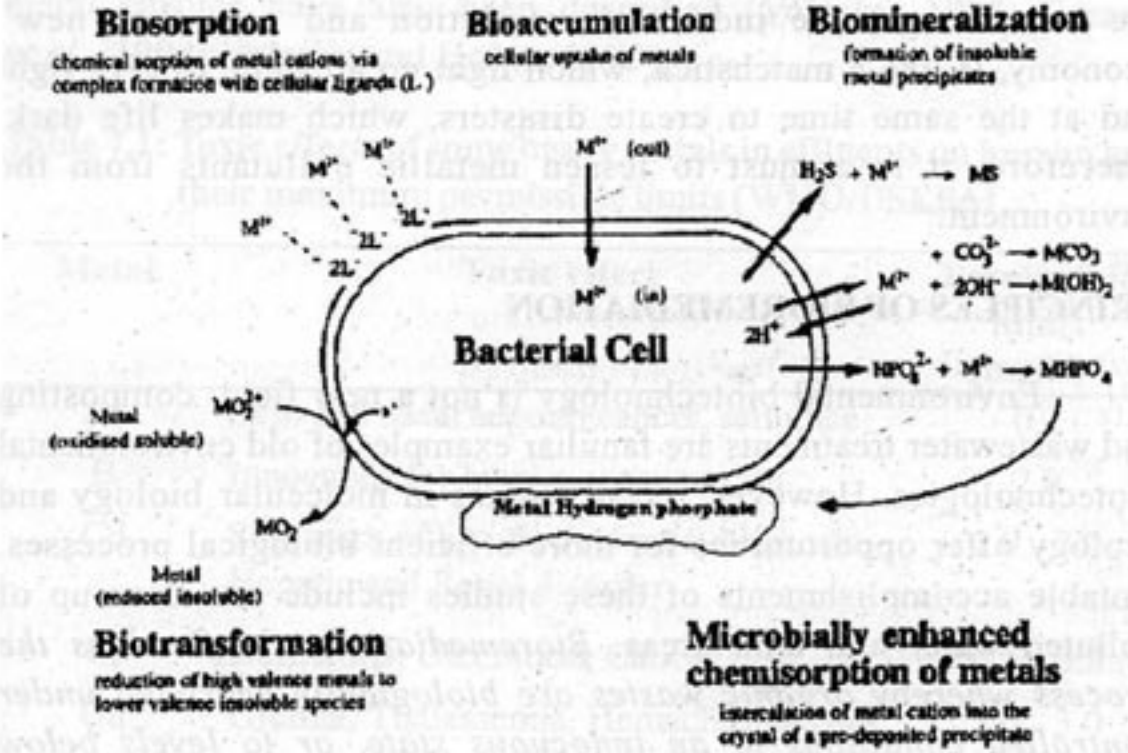



**Fig. 7.1: Different interactions of microbes with metal**

## MECHANISMS OF BIOREMEDIATION

### Microbe-metal-interactions

With $10^6$–$10^9$ viable cells $cm^{-3}$, bacteria are usually the most numerous organisms in soil (Lynch, 1988). Due to their small size, bacteria have a high surface to volume ratio and therefore provide a large contact area for interactions with the surrounding environment. Besides their occurrence in high numbers and their high surface to volume ratio, it is the negative net charge of the cell envelope that makes these organisms prone to accumulate metal cations from the environment (Collins and Stotzky, 1992). Microbes can potentially accumulate metals either by a metabolism- independent, passive, or

a metabolism dependent, active process. Thus, overall accumulation is determined by two characteristics of the cell: sorptivity of the cell envelope and capacity for taking up metals into the cytosol. Active uptake into the cytosol is usually slower than passive adsorption and is dependent on element-specific transport systems (Gadd, 1988).

Passive adsorption is likely to be the dominant mechanism in metal accumulation, since scarcity of nutrients in the ground state for many natural environments in soils, and active uptake requires energy. Additionally, microbes probably lack highly specific uptake systems for most metals. The surface characteristics of the bacteria determine their metal-adsorption properties. The differences in cell wall construction of Gram-positive and Gram-negative bacteria have minor influence on the sorption behaviour of different metals (Jiang *et al.*, 2004). The bulk functional group chemistry of both classes of bacterial surfaces is similar, but particular single constituents of the cell envelope can have great importance for metal binding. For example, phosphoryl groups of lipopolysaccharides, carboxylic groups of teichoic and teichuronic acids, or capsule forming extracellular polymers influence the metal sorption on the cell envelope (Table 7.2).

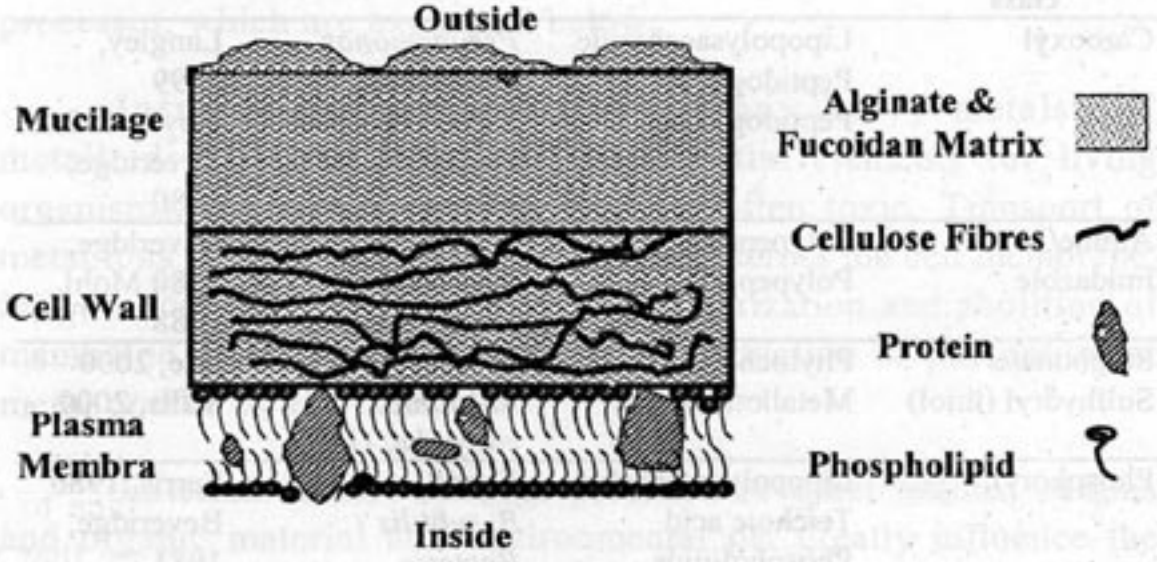



**Fig. 7.2: Cell wall structure in algae (e.g., Brown algae)**

Metals without biological function are in general tolerated only in minute concentrations, whereas essential metals with biological functions are usually tolerated in higher concentrations.

They accomplish either metabolic functions as constituents of enzymes or meet structural demands as, e.g., in supporting the cell envelope. The concentration and the speciation of the metal determine whether it is useful or harmful to the bacterial cell.

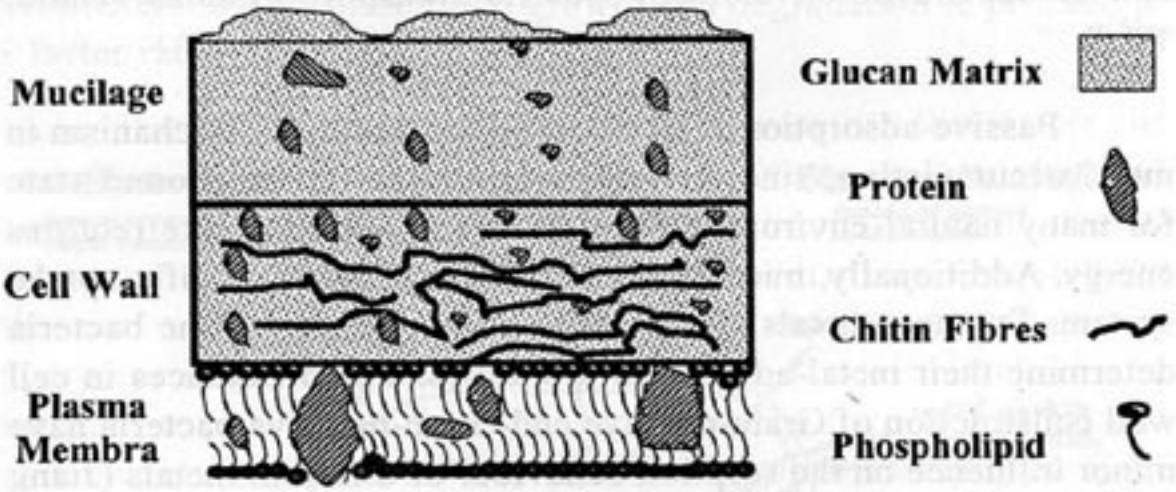



**Fig. 7.3: Cell wall structure in fungi (e.g., Euascomycetes)**

**Table 7.2: Metal-binding functional groups in bacterial surface components**

| Functional group/ Compound class | Location | Organism | Reference |
|---|---|---|---|
| Carboxyl | Lipopolysaccharide | *Pseudomonas aeruginosa* | Langley, 1999 |
| | Peptidogycan | *Escherichia coli* | Hoyle, 1984 |
| | Peptidogycan | *Bacillus subtilis* | Beveridge, 1980 |
| Amine/ imidazole | Polypeptide | *B. subtilis* | Beveridge; |
| | Polypeptide | *Klebsiella pneumoniae* | 1980 Mohl, 1988 |
| Sulphonate/ Sulfhydryl (thiol) | Phytochelatins | *E. coli, GMO* | Bae, 2000 |
| | Metallothioneins | *Ralstonia eutropha, GMO* | Valls, 2000 |
| Phosphoryl | Lipopolysaccharide | *E. coli* | Ferris, 1986 |
| | Teichoic acid | *B. subtilis* | Beveridge, 1981 |
| | Phospholipids | *Bacteria* | Beveridge, 1989 |

Homoeostasis is, therefore, essential and bacteria have developed a fine-tuned regulatory system of incorporation and

excretion. Adverse effects of metals on the microbial cell are decreased decomposition of soil organic matter, reduced soil respiration, lower diversity, and decreased activity of several soil enzymes (Ruhling, 1973; Tyler, 1974). Depending on the external conditions, microbial cells have developed mechanisms to cope with high concentrations of metals (Silver, 1988).

Microbial processes for metal remediation are now becoming important components in the combined efforts for treatment of contaminated land, solid wastes and aqueous effluents. Bioremediation of metals is mainly divided in two groups.

1. Removal of metals from solid waste - Bioleaching

2. Removal of metals from aqueous waste - Biosorption

The extraction of metal ions from solid wastes is due to acid, ferric iron and chelating agent formation by the metabolic activity of microorganisms. Metallic pollutants from aqueous phase, which is mainly, removed due to metabolism dependent or metabolism independent mechanisms by microbial biomass. The biomass used can be live or dead freely suspended or immobilized; even it may be the biomass derived products. The physico-chemical reactions between micro-organisms and metals can be divided into six distinct processes, which are explained below:

**Intracellular accumulation:** Many heavy metals and metalloids in small quantities are essential elements for living organisms, the higher concentration are often toxic. Transport of metal ions into the cell occurs by diffusion across the cell membrane, it is an energy dependent process. Depolarization and abolition of membrane give potential results in the reduction or prevention of metal uptake by the cells.

External factors such as presence of other anions, cations and organic material and environmental pH greatly influence the intracellular metal uptake. During the intracellular metal uptake, the release of 2 moles of $K^+$ for 1 mole of $Co^{2+}$, $Cu^{2+}$ and $Mn^{2+}$ uptake has been reported in order to maintain the electro neutrality. On the other hand, such relationship is not established for $Ca^{2+}$ and $Zn^{2+}$

uptake. *Sacchromyces cerevisiae* and *Neurospora crassa* have been shown to synthesize low molecular weight cysteine rich metal binding protein known as metallothionein, which binds high amount of silver and copper. Algae, plants, yeasts and some fungi produce r-glutamyl peptides, which can be used for metal detoxification. Many bacteria and fungi also release siderophores, which chelate $Fe^{3+}$, and are subsequently taken up by the cells. In many instances the taken up metals ions are converted to non-toxic forms by precipitation or binding inside the cells.

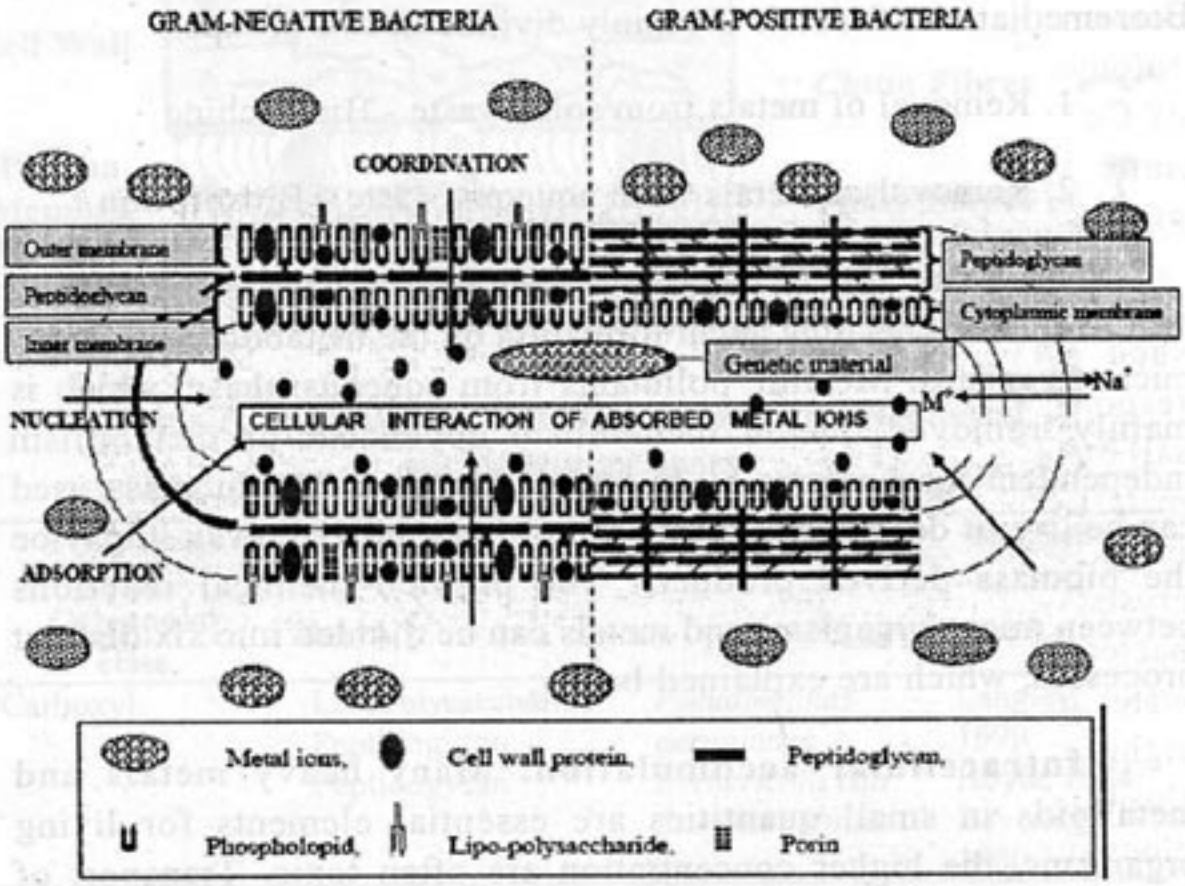


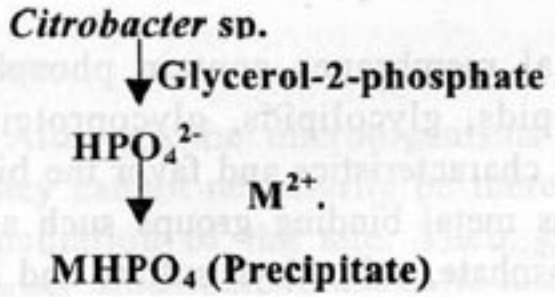

**Fig. 7.4: Schematic representation of gram-positive and gram-negative bacteria showing metal remediation sites**

**Oxidation-reduction reactions:** Microorganisms are responsible for oxidation–reduction, methylation and demethylation processes. Microbial oxidation of $As^{3+}$ to $As^{5+}$ and $Fe^{2+}$ to $Fe^{3+}$ helps in the removal of arsenic and ferric iron by precipitation. Similarly, reduction of $Cr^{6+}$ to $Cr^{3+}$ and $Se^{6+}$ to $Se^{4+}$ by microorganisms makes them less toxic and facilitate their precipitation. Microbial extracellular enzymatic transformation of $Cr^{6+}$, $Mn^{6+}$, $Pb^{3+}$, $Se^{4+}$, $Tc^{6+}$ and $V^{6+}$ to their less soluble precipitating forms has been reported.

*Pseudomonas aeruginosa* is known to volatilize $Hg^{2+}$ and thus its removal from the contaminated aquatic and terrestrial ecosystems.

**Extracellular precipitation:** Microbial activity is responsible for precipitation of metal in the form of hydroxides, carbonates, phosphates, sulphide and oxalates. Some of the typical examples are given here. Sulphate reducing bacteria *Desulfovibiro* and *Desulfotomaculum* are known to produce hydrogen sulfide as a by-product of the metabolism, which reacts with soluble metal ions and convert them as insoluble metal sulfides. *Sacchromyces cerevisiae* colonies turn dark in colour in presence of copper due to formation of CuS. *Rhodotorula sp.* and *Trichosporon sp.* isolated from acid mine water were reported to precipitate copper due to $H_2S$ production.

**Extracellular complexing:** Extracellular polymeric materials such as capsules and slime produced by microorganisms are responsible for sequestering significant amounts of metals due to salt bridging, metal hydrolysis, colloidal binding and aggregation of metal ions. Citric acid is a good metal ion chelator produced by fungi, which interacts with metal ions to form insoluble oxalate crystals around cell wall in the external medium. The application of bacterial polysaccharide emulsion when sonicated and dispersed in water hexadecane, the emulsanosol produced is reported to gather significant amounts of metals, e.g., more than 800 mg uranium sorption per gram product. *Citrobacter* species are reported for cleavage of glycerol–1–phosphate in the $HPO_4^-$, which forms complexes with metal ions and converts them into insoluble metal precipitates. Resting cell of *Citrobacter* species have surface located phosphatase enzyme that releases $HPO_4^{2-}$ from glycerol–2–phosphate. The released $HPO_4^{2-}$ reacts



precipitate at cell surface . This mechanism is very significant in the removal of metal or radionuclide from effluents having phosphate containing organic substrates.

**Adsorption to cell surface:** Heavy metals are inhibitory or even lethal to the organisms. Hence, microorganisms have developed strategies to control or adapt to the metal concentrations around them. One such mechanism is the ability of the cell surface to bind metals. The anionic nature of the bacterial cell surface acts like a sponge in which it can soak up metal ions from the surrounding environments. It is through its surface that the cell first encounters the environment. Transport of materials in or out of the microbial cell is controlled by a number of active and passive systems based mainly on the structure and chemistry of the cell surface.

Cell envelope of gram-positive cell is characterized by the presence of peptidoglycan, teichoic acids, teichuronic acids and lipoteichoic acid. The carboxyl and phosphate groups of these compounds give an anionic character to the cell wall. This anionic cell wall attacks cationic metal ions and sorption of metal ions takes place on surface of cell wall. The anionic character changes from organism to organism and within organisms also from growth phase to growth phase and the type of nutrients in which they are cultivated.

- Presence of phospholipids, lipopolysaccharide and peptidoglycan of Gram-negative bacteria is chiefly responsible for its metal binding ability.
- Mannans, glucans, phosphomannans, melanins, chitin and chitosan found in fungal walls are found to adsorb sufficient amount of many metals.
- Archaebacterial membranes contain phospholipids, phospoglycolipids, glycolipids, glycoprotein which impart anionic characteristics and favor the binding of metals. Various metal binding groups such as amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl are present in the polymers. Amount of these metal

binding groups and their alignment in the cell wall determine the metal loading capacity of the material.

- Algal cells contain various poly-functional metal binding sites for metals. Ionic charges and covalent binding have been reported in *Chlorella, Ulothrix, Chlamydomonas, Spirulina* and *Sargassum.*

**Volatilization:** Volatilization of arsenic and mercury due to microbial activity reduces these metals from the solid or liquid wastes, but if proper care is not taken the volatile compounds contaminate the atmosphere. It is, therefore, essential to trap the volatile compounds in liquid sorbants, otherwise the released methyl mercury and trimethyl arsine have severe lethal effects. The microbes such as *Pseudomonas scopulariopsis, Candida gliocladium, Clostridum* and *Neurospora* are known to methylate arsenic and mercury.

## FACTORS INFLUENCING METAL BIOREMEDIATION

The control and optimization of bioremediation processes is a complex system of many factors. Factors intrinsically related to the biosorbents, biosorbates and environmental conditions are the deciding factors for metal remediation rate, amount and specificity. The major factors contributing in the process are: type of biomass, concentration of biomass, type of metals, initial concentration of the metal, anions, presence of competing cations, pH of the reaction mixture, temperature of the reaction and pretreatment given to the biomass, over and above these factors, the type of immobilizing material used and its concentration, size of beads and configuration of reactions also play an important role, when immobilized biomass is used in the process. Influence of some of these factors is described below:

### Nutrients

Although the microorganisms are present in contaminated soil, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated. Biostimulation usually involves the addition of nutrients and oxygen to help indigenous micro-organisms. These nutrients

are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorous, and carbon (e.g., see Table-7.3). Carbon is the most basic element of living forms and is needed in greater quantities than other elements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95 percent of the weight of cells. Phosphorous and sulfur contribute with 70 percent of the remainders. The nutritional requirement of carbon to nitrogen ratio is 10:1, and carbon to phosphorous is 30:1.

**Table 7.3: Composition of a microbial cell**

| Element | Percentage | Element | Percentage |
|---|---|---|---|
| Carbon | 50 | Sodium | 1 |
| Nitrogen | 14 | Calcium | 0.5 |
| Oxygen | 20 | Magnesium | 0.5 |
| Hydrogen | 8 | Chloride | 0.5 |
| Phosphorous | 3 | Iron | 0.2 |
| Sulfur | 1 | All others | 0.3 |
| Potassium | 1 | | |

Microbial growth and activity are readily affected by pH, temperature, and moisture. Although microorganisms have also been isolated in extreme conditions, most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions. If the soil has too much acid it is possible to rinse the pH by adding lime. Temperature affects biochemical reaction rates, and the rates of many of them double for each 10 °C rise in temperature. Above a certain temperature, however, the cells die. Plastic covering can be used to enhance solar warming in late spring, summer, and autumn. Available water is essential for all the living organisms, and irrigation is needed to achieve the optimal moisture level.

The amount of available oxygen will determine whether the system is aerobic or anaerobic. Hydrocarbons are readily degraded under aerobic conditions, whereas chloraurate compounds are degraded only in anaerobic ones. To increase the oxygen amount in the soil it is possible to till or sparge air. In some cases, hydrogen peroxide or magnesium peroxide can be introduced in the environment. Soil structure controls the effective delivery of air, water, and nutrients. To improve soil structure, materials such as

gypsum or organic matter can be applied. Low soil permeability can impede movement of water, nutrients, and oxygen; hence, soils with low permeability may not be appropriate for *in situ* clean-up techniques.

**Biomass**

Microorganisms exhibit a very high diversity, and thus they differ in their cell wall structure and composition. Growth of the microorganisms is associated with change in metabolic rate, cellular composition as well as cell wall structure. Even cells of different ages of the same organisms show different cell wall chemistry, thus the type of biomass is responsible for differences in metal remediation capacity. These factors also affect the nature and number of metal binding sites. In different organisms, metal biosorption differs with the chemical nature of outer surfaces. Micro-organisms also show selectivity in metal sorption depending upon chemical nature of their outer surface. In *Penicillium*, the selectivity is in the following order: $Fe^{2+} > Cu^{2+}$, $Zn^{2+}$, $Ni^{2+} > Cd^{2+} > Pb^{2+} > VO_2^{2+}$ while in *R. arrhizus* it is $VO_2^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+} > Cu^{2+}$.

At a given equilibrium concentration, the biomass adsorbs more metal ions at low cell densities than at high densities. At lower biomass concentration, increase in specific metal uptake is due to the increase in metal to biosorbent ratio. The amount of metal adsorbed by the biomass increases with concentration of metals, but high percent removal will be achieved with low initial metal concentration. Thus, at a given concentration of biomass, the amount of metal uptake increases, but percent uptake decreases with increase in the initial metal concentration.

**Presence of cations and anions**

In metal sorption, the metal binding functional groups such as $COO^-$, $CO^-$, $OH^-$ and $SH^-$ are non-specific for binding cations. Different metal ions may compete with each other for the binding sites. To understand this competition, the Pearson's classification of metals based on the chemical co-ordination characteristics of the elements, provides useful information. Significant ionic competition

occurs between metals of the same class and between the soft and borderline metals. A high metal uptake has been observed with increased ionic radii and ionic charge of metals. *Rhizopus arrhizus* shows metal uptake in the increasing order of $Sr^{2+} < Mn^{2+} < Zn^{2+} < Cd^{2+} < Pb^{2+}$, which correlates with the covalent index of metal ions. Uptake of the desired metal decreases with increasing concentrations of other cations present in the solution.

In waste, a variety of anions such as sulphate, carbonate, nitrites, chloride, phosphate etc. are normally present along with metals. These anions form complexes with metal cations and reduce the metal binding to the cell-surfaces. Inhibition of copper uptake by *R. arrhizus* and cobalt by marine algae has been observed in presence of EDTA, $SO_4^{2-}$, $Cl^-$ and $SO_4^{2-}$, $PO_4^{2-}$, $CO_3^{2-}$ and $NO_3^-$ ions. Benjamin and Leckie (1981) have proposed three types of interactions due to the presence of anions

(a) Metal - anion complexes formed are non-adsorbing or weakly adsorbing that result in reduction in metal binding.

(b) Biosorbent - anion interactions either enhance or reduce metal binding, and

(c) Metal - anion complexes strongly bind to free metals and thus enhance metal uptake.

The presence of multimetal ions in the waste leads to the modification of the biosorption equilibrium as compared to a single metal system. The total metal absorbed in multimetal system is normally higher than the metal absorbed in individual tests. But the total capacity of adsorption is always lower than the sum of the individual adsorption capacities of metals taking part in the test.

**Role of pH**

pH of the system influences the binding sites on biomass as well as solubility of metals and so at pH lower than 2.0, there is hardly any metal uptake. At low pH, proton concentration is so high that metal ions compete with $H^+$ ions. At highly acidic pH, due to

repulsive force, wall ligands restrain the access of metal ions. As pH increases, more and more negatively charged ligands are exposed and show increased attraction of positively charge metal ions. However, at high pH, solubility of metal decreases that reduces the availability of free metal ions for binding. Metal anions such as $CrO_4^{2-}$, $AuCl^{3-}$, $Ag(CN)^{2-}$ show higher uptake in the acidic pH.

**Pre-treatment**

Physico-chemical treatment of biomass affects the metal uptake due to various phenomena. Treatment with acetone or boiling water works as cleanser, while heat and detergent washing expose additional metal binding sites. Enzymes destroy unwanted components and increase sorption ability. On the other hand, treatment with acids, acetone and methanol modify cell surface and shows a mixed influence on metal binding. Pretreatments are varied with the type of biomass and its source.

## CHROMIUM - AS A POLLUTANT

Chromium was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Louis-Nicholas Vauquelin. According to US Environmental Protection Agency (USEPA) < 0.1 ppm concentration of chromium in drinking water is permissible on the basis of health considerations. Cr (VI) is present as either dichromate in acidic environments or as chromate in alkaline environments (Thakur *et al.*, 2007).

Chromium is a major source of aquatic pollution in India and the main areas for their concentration are Tamil Nadu, Uttar Pradesh, West Bengal and nearly some chromite mines of Orissa.

Chromium (atomic number 24, atomic mass 51.99) has an outer electronic configuration of $3d^5$, $4S^1$ and belongs to VI B group or chromium group on the periodic table. In soil the ionic form of chromium that is absorbed by plants are $Cr^{+3}$ and $Cr^{+6}$. Cr(III) is absorbed more rapidly than Cr(VI). The different oxidation states of chromium are given in Table 7.4.

**Table 7.4: Different oxidation states of chromium and its compound (Thakur et al., 2007)**

| Sl. No. | Chromium | |
|---|---|---|
| | Oxidation state | Compounds |
| 1 | 0 | $Cr(CO)_6$ |
| 2 | + 1 (Unstable) | - |
| 3 | + 2 (Chromus) | $Cr(CH_3COO)_4$, CrO, $CrSO_4$ |
| 4 | + 3 (Chromic) (Stable) | $CrCl_3$, $Cr_2O_3$, $Cr_2(SO_4)_3$ |
| 5 | + 4 (Unstable) | $CrO_2$ |
| 6 | + 5 (Unstable) | $CrF_5$ |
| 7 | + 6 (Stable) | $K_2Cr_2O_7$, $K_2Cr_2O_4$, $CrO_3$ |

Note: $Cr^{+3}$ is maximum stable, because it is not oxidizing or reducing agent

**Uptake and toxicity of chromium**

Chromium is a toxic, non–essential elements to plants, hence they do not posses specific mechanisms for its uptake. Therefore, the uptake of this heavy metal in plants is through carriers used for the uptake of essential metals for plant metabolism. The pathway of Cr (VI) transport is an active mechanism involving carriers of essential anions such as sulphate (Cervantes, 2001). Fe, S and P are also known to compete with chromium for carrier binding (Wallace, 1976). Chromium directly enters to the food chain through plant metabolism and cause harmful effects on living organisms. Metals and their "free radicals" are highly reactive attacking other cellular structures. The ability of metals to disrupt the function of essential biological molecules, such as protein, enzyme and DNA is major cause of their toxicity.

**Conventional treatment methods for chromium containing effluents**

Some of the conventional treatments methods are: precipitation, ion-exchange method, electrochemical cells, reverse osmosis and biological methods.

Chemical approaches are available for metal remediation, but are often expensive to apply and lack of the specificity required to treat target metals against a background of competing ions. In addition, such approaches are not applicable to a cost-effective remediation of large-scale subsurface contamination *in situ* (Table-7.5).

**Table 7.5: Different technologies for the removal of heavy metals from the industrial wastewater depends on various factors**

| Technology | Conc. Depen-dence | pH | Suspended solids | Effluent Conc. (mg/L) | Regeneration | Sludge generation |
|---|---|---|---|---|---|---|
| Biosorption | Yes | Yes | Yes | <1 | Yes | No |
| Hydroxide Precipitation | No | No | Yes | 2-5 | No | Yes |
| Sulfide Precipitation | No | No | Yes | <1 | No | Yes |
| Ion Exchange | Yes | Some | No | <1 | Yes | Yes |
| Evaporation | Yes | Yes | Yes | 1-5 | --- | No |
| Reverse Osmosis | No | Some | No | 1-5 | No | No |
| Adsorption | Yes | Some | Yes | 1-5 | Yes | No |

**Biological methods**

Microbial research and need for new methods of water treatment have led to great deal of expansion in the filed of biological methods of industrial effluent clean-up. Microbes require heavy metals as human require certain metals in their diet. There are four commonly known pathways by which microbes accumulate heavy metals like:

(a) Binding to the cell surface

(b) Intracellular accumulation

(c) Extra-cellular precipitation

(d) Volatilization.

Living biological systems are well-suited for the treatment of water. The mining industry has used engineered living systems in the remediation of lagoons contaminated with heavy metal. Algae with excellent metal adsorption are deliberately grown in the lagoons. As discussed earlier, the term "biosorption" is used to describe the accumulation of metal ions from effluents/solution by material of biological origin (microbial or plant). Biosorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents.

Biosorbents are prepared from the naturally abundant and/or waste biomass of algae, fungi or bacteria. A variety of uptake

mechanisms is involved including adsorption and ion exchange. It has been widely suggested that this behavior could be utilized in wastewater treatment but, to date, there has been little commercial success in this field.

**Advantages of Biosorption**

- Non-living cells are less sensitive to metal ion concentration (toxicity effects).
- Can be operated at ambient conditions of pH and temperature.
- Low operating cost.
- Produce stable sludge.
- Volume of sludge can be minimized.
- Supply of nutrients is not required.
- Dead biomass can also be procured from industrial sources as a waste product from the fermentation processes.

The need for economical, effective and safe methods for removing heavy metals from wastewater has resulted in the search for unconventional materials that may be useful in reducing the levels or accumulation of heavy metals in the environment. The newly discovered metal sequestering properties of certain types of microbial biomass of fungi, bacteria and algae offers considerable promise (Volesky, 1995) and offer an alternative to the existing methods for metal detoxification and their recovery. The most successful biotechnological processes utilize biosorption and bioprecipitation, in addition to other processes such as binding by specific macromolecules. Previous studies on metal biosorption have been restricted to simple solutions of only one metal. Biomass, which is available in large quantities as a waste product, their potential utilization as a metal biosorbent, is of interest. As bio-commercial exploitation of the technique is realized so far, much wider application is possible and needs to be seriously considered. The feasibility and efficiency of a biosorption process depends not

only on the properties of the biosorbents but also on the composition of the wastewater.

## SOME OF THE CHROMIUM BIOREMEDIATION PROCESS USING NATIVE MICROORGANISMS

As discussed earlier for the biological remediation of chromium from the contaminated sites has been well practiced by several authors. From the toxicological point of view hexavalent chromium is more toxic than trivalent chromium for biological system. Many genera of facultative anaerobes are capable of reducing Cr(VI) to Cr(III) under appropriate conditions as in Table 7.6 and are wide spread in nature. Although chromium reducing bacteria are widespread, high cell densities are generally required for significant Cr(VI) reduction from the contaminated sites. Growth of microbial cell depends on several factors and also influences the reduction capability. The factors are:

**Electron Donor-** Chromium reducing bacteria may utilize a variety of organic compounds as electron donors for chromium reduction (Table 7.6). However, the majority of the known electron donors are natural aliphatic compounds, mainly low-molecular weight carbohydrates, amino acids and fatty acids.

**Table 7.6: Microbial populations that transform Cr(VI) to Cr(III)**

| Organism | Substrate(S) | Redox condition |
|---|---|---|
| 1 | 2 | 3 |
| Achromobacter eurydice | Acetate, glucose | Anaerobic |
| Aeromonas dechromatica | Galactose, mannose, melibiose, sucrose, fructose, lactose, cellobiose, arabinose, mannitol dulcitol, sorbitol, glycerol | Anaerobic |
| Agrobacterium radiobacter | Glucose, fructose, maltose. lactose, mannitol, glycerol | Aerobic |
| Bacillus cereus | Acetate, glucose | Anaerobic |
| Bacillus sp. | Glucose | Aerobic |
| Bacillus subtilis | Acetate, glucose | Anaerobic |

| 1 | 2 | 3 |
|---|---|---|
| Desulfovibrio vulgaris | Hydrogen | Anaerobic |
| Enterobacter cloacae | Acetate, glycerol, glucose | Anaerobic |
| Escherichia coli | Acetate | Anaerobic |
| Escherichia coli ATCC 33456 | Glucose, acetate, propionate | Aerobic,an aerobic |
| Micrococcus roseus | Acetate, glucose | Anaerobic |
| Pseudomonas aeruginosa | Acetate, glucose | Anaerobic |
| Pseudomonas dechromaticans | Peptone, glucose | Anaerobic |
| Pseudomonas chromatophila | Ribose, fructose, fumarate, lactate, acetate, succinate, butyrate, glycerol, ethylene glycol | Anaerobic |
| Pseudomonas ambigua G-1 | Nutrient broth | Aerobic |
| Pseudomonas fluorescens LB 300 | Glucose | Aerobic |

**Cr(VI) Concentration** – The rate of chromium(VI) reduction depends on the concentration of Cr(VI). The time required for complete reduction increased progressively as the initial concentration of Cr(VI) increased in culture (Wang *et al.*, 1989; Shen and Wang, 1994; Wang and Xiao, 1995). In case of *E. cloacae* culture (Komori *et al.*, 1989), where the trend of reduction was opposite as higher reduction rates at lower concentrations.

**Dissolved Oxygen** – Bacterial reduction of Cr(VI) may occur both aerobically and anaerobically (Table 7.6). Under aerobic conditions, organisms may reduce Cr(VI) through the action of a soluble reductase (SR), using NADH or endogeneous electron reserves as an electron donor. Organism may also reduce Cr(VI) under anaerobic conditions via the mediation of either a soluble reductase, a membrane-bound reductase, or both, with the possible involvement of cytochrome *b, c* and *d* (Fig. 7.5).

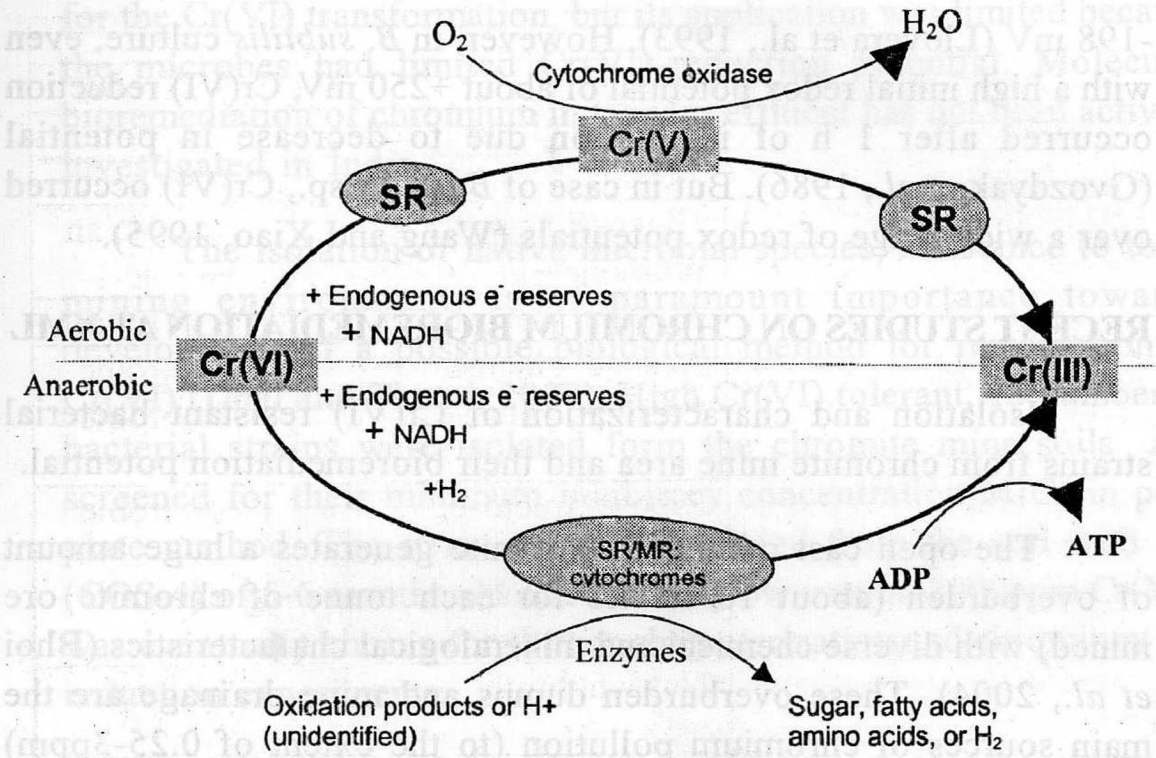



**Fig. 7.5: Mechanisms of Cr(VI) reduction in bacteria. SR-soluble reductase; MR-membrane-bound reductase**

**Other Electron Acceptors**– Inhibition of sulphate and nitrate on Cr(VI) reduction has not been reported for aerobic culture. Sulphate up to 1mM and nitrate at 200 mM had no effect on chromate reduction either with whole cells or cell-free supernatant fluid of *Pseudomonas putida* (Ishibashi *et al.*, 1990). In case of *Bacillus* sp. (Wang and Xiao, 1995), sulphate at 10mM and nitrate at 16mM did not affect on Cr(VI) reduction by whole cells and cell free extract.

**pH, Temperature and Redox Potential** - Experimental conditions like pH and temperature are great influence on Cr(VI) reduction which generally coincide with the optimal growth conditions of cells. It has been reported that for *E. coli*, the optimum pH and temperature for maximum Cr(VI) reduction at pH 7 and about 36°C temperature (Shen and Wang, 1994). In case of *Bacillus* sp., the optimal condition for Cr(VI) reduction were pH 7 and 30°C temperature (Wang and Xiao, 1995). The best range of redox potential for Cr(VI) reduction has not yet been conclusively established. Using washed cells of *A. radiobacter* pre-grown under different carbon and energy sources, the rate of Cr(VI) reduction was shown to be greater in cell suspensions at -240mV than that at

-198 mV (Llovera et al., 1993). However, in *B. subtilis* culture, even with a high initial redox potential of about +250 mV, Cr(VI) reduction occurred after 1 h of incubation due to decrease in potential (Gvozdyak *et al.*, 1986). But in case of *Bacillus* sp., Cr(VI) occurred over a wide range of redox potentials (Wang and Xiao, 1995).

## RECENT STUDIES ON CHROMIUM BIOREMEDIATION AT NML

Isolation and characterization of Cr(VI) resistant bacterial strains from chromite mine area and their bioremediation potential.

The open cast mining of chromite generates a huge amount of overburden (about 10 tonnes for each tonne of chromite ore mined) with diverse chemical and mineralogical characteristics (Bhoi *et al.*, 2004). These overburden dumps and mine drainage are the main sources of chromium pollution (to the extent of 0.25-3ppm) (Envis News, 2006), into the inland fresh water and farmyard lands in and around the mining sites. Chand *et al.* (1994) used coconut jute and baggase for Cr(VI) removal. Tripathy et al. (1994) have used *Pseudomonas florescence* for Cr(VI) detoxification. Mise and Sudhakar (1995) used activated carbon prepared from a biological agent for Cr(VI) adsorption. Singh *et al.* (1994) have used formalin-treated acacia bark for Cr(VI) removal. Brady *et al.* (1994) used nonviable bio-sorbent for removal of trivalent and hexavalent forms of chromium. In another experiment, Brady et al. have used various algal cultures for Cr(VI) bioaccumulation. Blake *et al.* (2003) used *Pseudomonas* sp. for Cr(VI) removal. Akhtar and Mohan (1995) have used *A. niger* for Cr(VI) removal from electroplating effluent. Shen and Wang (1994) have used *E. coli* for Cr(VI) removal. Pethkar and Paknikar (1994) observed that, as compared to other metals, biosorption of chromium by *A. niger* is the lowest.

In India, the biosorption-biotransformation of Cr(VI) in an activated sludge process has been tried by Gopalan and Veeramani (1994). They isolated many bacterial strains for this purpose and compared the biotransformation potential of those isolates. Philip *et al.* (1998, 1999) tried the Cr(VI)-reduction potential of *B. cosgulance*, a bacterial isolate from chromium-contaminated soils. They tried to understand the mechanism of Cr(VI) transformation in an aerobic environment. They developed an immobilization microbial reactor

for the Cr(VI) transformation, but its application was limited because the microbes had limited Cr(VI)-reduction potential. Molecular bioremediation of chromium in tannery effluent has not been actively investigated in India.

The isolation of native microbial species, resistance to toxic mining environment, is of paramount importance towards development of a possible biological method for remediation of Cr(VI) (Dhal and Thatoi, 2007). High Cr(VI) tolerant, 13 number of bacterial strains were isolated form the chromite mine soils and screened for their minimum inhibitory concentration(MIC) in pour plate method. The strain (CSB-4), isolated from the soil with ore (COS-4) of 5-6 months old and able to grow even in 2000ppm Cr(VI), was eventually chosen for further characterization and chromium(VI) reduction experiments.

Morphological and biochemical characterization and 16SrRNA gene sequencing were carried out for the identification of bacterium. The bacterium is rod shaped with a diameter of 2-4 ìm, gram positive, motile and strictly aerobic. The bacterium formed elliptical spores with dimensions in the range 1.8-3.0´0.8-5.0 ìm. The colonies were convex and white in colour. The strain could utilize D-glucose, L-arabinose, D-fructose and sucrose but not D-mannitol and D-xylose and citrate. The characteristic features of these bacteria are: to reduce nitrates, showing positive to catalase, oxidase tests as well as hydrolysis of starch (Table 7.7 and Fig. 7.6).

**Table 7.7: Morphological, physiological and biochemical characteristics of bacterial strain**

| Morphological and Physiological tests | Result | Biochemical tests | Result |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| Gram's reaction | +ve Rod | Indole | - |
| Cell diameter | > 1.0 µm | MR | + |
| Spore round | - | VP | + |
| Sporangium swollen | - | Citrate utilization | + |

| 1 | 2 | 3 | 4 |
|---|---|---|---|
| Para sporal crystal | - | Catalase | + |
| 6.8pH in Nutrient broth | + | Oxidase | + |
| 5.7pH in NB | + | Nitrate reduction | + |
| Growth at 5°C | - | pH in VP broth, < 6<br>> 7 | -<br>+ |
| Growth at 40°C | + | Acid from<br>D-Glucose<br>L-Arabinose<br>D-Xylose<br>D-Mannitol | <br>+<br>+<br>+<br>+ |
| Anaerobic growth | - | Gas from glucose | + |

**+ positive, - negative**

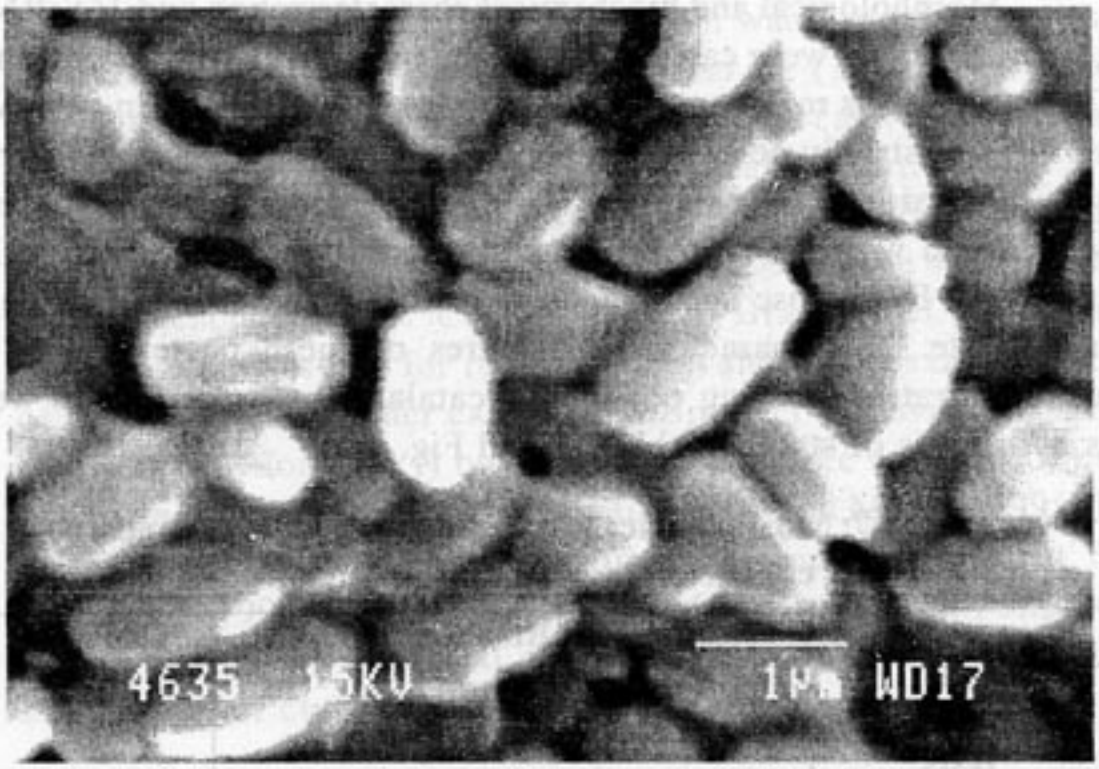



**Fig. 7.6: Scanning Electron Microscopic (SEM) of *Bacillus* sp.**

## BIOSORPTION OF CR(III) FROM TANNERY EFFLUENTS USING ASPERGILLUS NIGER

Cr(III) is toxic at higher concentration with its permissible limits of 0.05 and 0.1 ppm concentration in potable and tannery effluents respectively (Buljan, 1996; Stupar *et al.*, 1999; Dominik *et*

*al.*, 2007; Srivastav and Thakur, 2006). The interaction of metallic ions with microbe cell surface depends not only on the nature of biosorbent used but also on the solution chemistry of the metal to be removed (Valix and Loon, 2003; Kapoor and Viraraghavan, 1998; Yan and Viraraghavan, 2000). The solubility diagram (Calfa and Torem, 2008) of chromium in water at 25°C is given in Fig. 7.7. It is widely known that the most stable valence state of chromium, in aqueous media, is the trivalent species. Chromium exists in its trivalent form predominantly at lower pH. As the pH rises, Cr(III) precipitates as $Cr(OH)_3$.

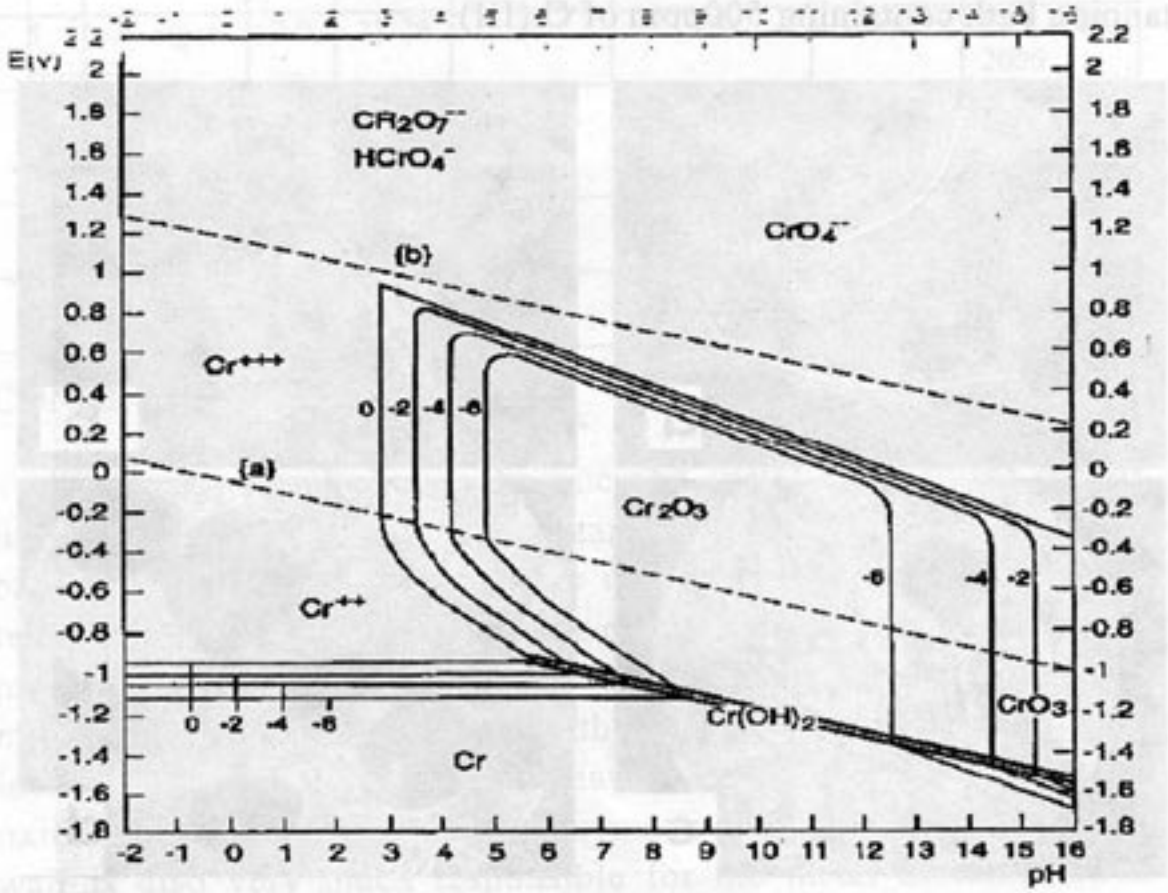



**Fig. 7.7: Pourbaix diagram of chromium in water at 25°C [Concentration=1M]**

It was reported that the biosorption of Cr(III) was rather specific and was not affected by the presence of other cations or anions in solution. The kinetics of Cr(III) uptake was rapid, in the pH range of 4 to 6.

Pure culture of *Aspergillus niger* (MTCC-281 obtained from IMTECH, Chandigarh) was cultivated in Czapek Dox broth at 35°C

and 2.5 pH in an orbital shaker. The biomass was adapted over a wide range of Cr(III) concentration (100-1000ppm) at 35°C and 2.5 pH. The fully grown biomass was sonicated [*Model-* SONICSÔ, Vibracell] to release electro-statically bound Cr(III) on cell surfaces and centrifuged at 10,000rpm for 10 min. to separate the biomass from the solution. The biomass settled after centrifugation was used in biosorption experiments.

Fungal species was used in different forms in the biosorption studies viz., live, autoclaved and alkali-treated. The treated fungal biomass has been used for the biosorption experiments from a model tanning bath containing 500ppm of Cr(III).

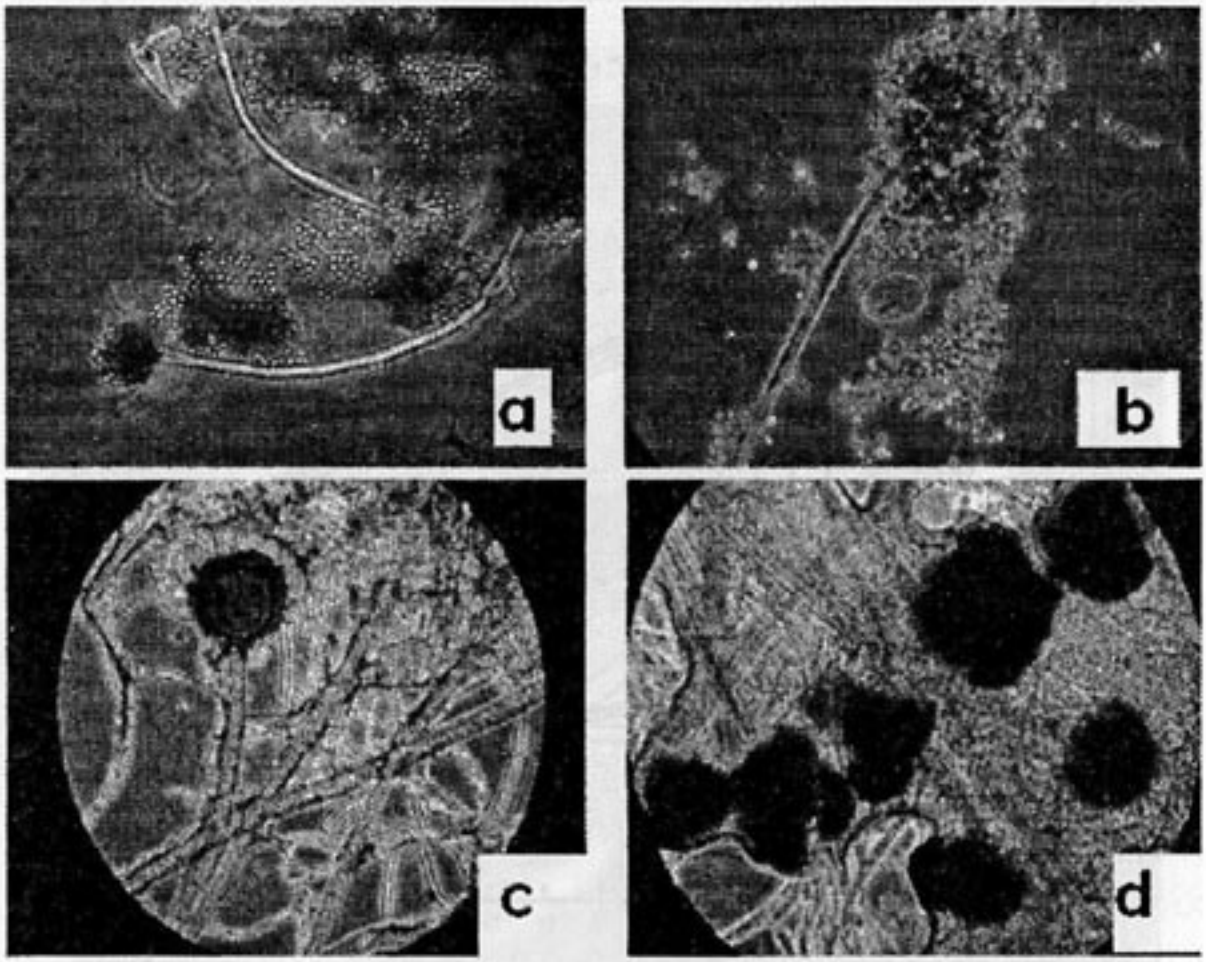



**Fig. 7.8(a-d): Microscopic observation of fungal adaptation to 1000ppm Cr (III) at pH=2.5 and 35°C (1000X) (a – A. niger adapted in 24h, b – adapted in 48h, c – adapted in 72h and d – adapted in 96h)**

**Table 7.8: Biosorption of Cr(III) using *Aspergillus* species**

| S. No | Fungual species | Experimental condition | | | Biosorption capacity ($mg\ g^{-1}$) | Biomass Type | Reference |
|---|---|---|---|---|---|---|---|
| | | pH | Temp (°C) | Initial Metal Ion (mg/L) | | | |
| 1 | *A.niger* | 3.5 | 30 | 50 | 6.6 | Fungal pellets | Dursun *et al.*, 2003 |
| 2 | *A. niger* | 2.0 | 30 | 150 | 100.3 | Live | Goyal *et al.*, 2003 |
| 3 | *A. niger* | 2.0 | 30 | 250 | 101 | Live | Goyal *et al.*, 2003 |
| 4 | *A. oryzae* | 5.0 | 30 | 240 | 83.7 | Live | Sepehr *et al.*, 2005 |
| 5 | *A. niger* | 2.0 | 22-28 | 50 | 13 | Dead | Park *et al.*, 2005 |
| 6 | *A. niger* | 6.0 | 30 | 50 | 8.9 | Fungal pellets | Srivastava *et al.*, 2006 |
| 7 | *Aspergillus* sp. | 4.5 | 25 | 6mM | 1.36 | Live | Zafar *et al.*, 2007 |
| 8 | *A. niger* | 2.5 | 35 | 500 | 45.5 | NaOH treated | Dhal *et al.*, 2009 |

## CONCLUSIONS

As described above, the microbe–metal interactions results in the bioremediation of metal contaminants. The different genera of bacterial and fungal species were involved in these processes for remediation of toxic metals. In this chapter, the main focus is on the principle and mechanisms of bioremediation by the metal resistant microbial species. The chemo-lithotropic bacterial species were involved for the metal bioremediation by transforming the metal oxidation states because of the enzymatic action. The microbial cell wall is also very much responsible for the metal bioremediation which adsorbes/binds the metal from the contaminated solutions through complex formation and ion exchange. In general four major mechanisms viz. (i) biosorption/ chemisorption, (ii) Intracellular accumulation, (iii) Extracellular precipitation/mineralization and (iv) transformation/reduction of metal ion (valence state) are responsible for the bioremediation processes. At the time of metal-microbe interaction, the environmental parameters such as pH, temperature, redox potential and metal concentration etc. are also important for metal bioremediation process.

A typical algal cell wall consists of a fibrillar skeleton and an amorphous embedding matrix. The main acidic groups responsible for metal uptake are the carboxyl group of uronic acid (guluronic, mannuronic, and glucuronic) as well as sulfonate groups. Hydroxyl groups are present in all polysaccharides, but they become negatively charged only at pH > 10. In case of gram positive bacteria, peptidoglycan layer is a linear polymer of alternating glucosamine and muramic acid with peptide side chains. These side chains bear some carboxyl group at the terminal amino acid and additional functional groups on certain intermediate amino acids like asparagines, cysteine or aspartic acids. In teichoic and teichuronic acid of peptidoglycan, phosphodiesters and carboxyl groups are contributing to the negative charge of the biomass and enable ion exchange. But, in gram negative bacteria, the outer membrane which contains lipopolysaccharides and phospholipids creates a negative surface charge conductive for binding of metals. Certain bacteria produce $SO_4^{2-}$ or $S^{2-}$ or enzymes that liberate $HPO_4^{2-}$. These ligands can form micro-precipitates with metal cations. Similarly to algae and bacteria, the cell wall is the main site of metal interaction in fungi. The inner microfibrillar layer of the fungal cell wall usually consists of chitin, cellulose or in rare cases; non-cellulosic α-glucan can take part in metal interaction. In some cases, phosphorylated polysaccharides and carboxyl groups are responsible for the negative charge in the fungal wall, whereas the amino groups of the chitosan creates a positive charge for interaction with the metals.

Reduction of hexavalent chromium from a synthetic solution using chromite mine soil bacteria (*Bacillus* sp.) and biosorption of trivalent chromium from a model tanning bath using *A. niger* - a fungal species are also described. These results reveal the potential use of the microbial species for the bioremediation of metal polluted sites. The main advantage of biological reduction of Cr(VI) occurs near the neutral pH and over a moderate temperature range implies that no costly chemical reagents and extensive energy inputs are required. On the other hand, metals and their derivatives can interact with the fungi in various ways depending on the metal species, organism, and environment, while fungal metabolic activities can also influence speciation and mobility. The biomass immobilizes the metals species by the sorption onto cell components or expolymers,

which results in the bioremediation of chromium from the effluent.

## REFERENCES

Akhtar, N., Mohan, P.M. (1995): Bioremediation of toxic metal ions from polluted lake waters and industrial effluents by fungal biosorbent, Curr. Sci. **69**(12), 1028-1030.

Avery, S.V., Tobin, J.M. (1993): Mechanism of adsorption of hard and soft metal ions to *Saccharomyces cerevisiae* and influence of hard and soft anions, Appl. Environ. Microbiol. **59**, 2851-2856.

Bae, W., Chen, W., Mulchandani, A., Mehra, R.K. (2000): Enhanced bioaccumulation of heavy metals by bacterial cells displaying synthetic phytochelatins, Biotechnol Bioeng. **70**, 518–524.

Basu, M., Bhattacharya, S., Paul, A.K. (1997): Isolation and characterization of chromium-resistant bacteria from tannery effluents, Bull. Environ. Contami. Toxicol. **58**, 535–542.

Benjamin, M.M., Leckie, J.O. (1981): Multiple-site adsorption of Cd, Cu, Zn, and Pb on amorphous iron oxyhydroxide, J. Colloid. Inter. Sci. **78**, 209–221.

Beveridge, T.J., Murray, R.G.E. (1980): Sites of metal deposition in the cell wall of *Bacillus subtilis*, J. Bacteriol. **141**, 876–887.

Beveridge, T.J. (1981): Ultrastructure, chemistry and function of the bacterial wall. Int. Rev. Cytol. **72**, 229–317.

Beveridge, T.J. (1989): Interactions of metal ions with components of bacterial cell walls and their biomineralization. *In: Metal-Microbe Interactions* (Poole, R.K., Gadd, G.M., eds.), pp. 65–83. *Spec. Publ. Soc. Gen. Microbiol.* 26.

Blake R.C.H., Choate, D.M., Bardjan, S., Revis, N., Barton, L.L., Zocco, T.C. (2003): Chemical transformation of toxic metals by a pseudomonas strain from a toxic waste site, Environ. Toxicol. Chem. **12**(80), 1365.

Brady, D., Stoll, A., Duncan, J. R. (1994): Biosorption of heavy metal cations by non-viable yeast biomass, Environ. Technol. **15,** 429–438.

Brady, D., Stoll, A.D, Starke, L., Duncan, J.R. (1994) Bioaccumulation of metal cations by Saccharomyces cerevisiae, Appl. Microbiol. Biotechnol. **41**, 149–154.

Brady, D., Duncan, J.R. (1994): Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*, Biotechnol. Bioeng. **44**, 297-302.

Brierley, C.L. (1990): Bioremediation of metal-contaminated surface and groundwater, Geomicrobiology, **8**, 201– 223.

Buljan, J. (1996) Pollution limits for discharge of tannery effluents into water bodies and sewers., World Leather, 9, 65-68.

Calfa, B.A. and Torem, M.L. (2008): On the fundamentals of Cr(III) removal from liquid streams by a bacterial strain., Min. Engg, **21**, 48-54.

Carney, T. (1993): *Contaminated Land*, p. 4, Blackie, London.

Cervantes, C., Gracia, J.C., Devars, S., Corona, F.G., Tavera, H.L., Torres-guzaman carlos, J. (2001): Interactions of chromium with microorganisms and plants, FEMS Microbial Rev. **25**, 335-47.

Chand, S., Agrawal, V.K., Kumar, P. (1994): Removal of hexavalent chromium from waste water by adsorption, Indian J. Environ. Heal. **36**(3), 151.

Collins, Y.E., Stotzky, G. (1992): Heavy metals alter the electrokinetic properties of bacteria, yeasts and clay minerals, Appl. Environ. Microbiol. **58**, 1592–1600.

Dhal, B., Thatoi, H.N. (2007): Isolation and characterization of bacterial strains occurring in Baula chromite mine soils of Orissa: potential for biotechnological applications, Asian J. Microbiol. Biotechnol. Environ. Sci. **9**(4), 359-364.

Dhal, B., Abhilash, Mehta, K.D. Pandey, B.D. (2009): Biological remediation of trivalent chromium from effluents, In: *Proc. 13th Int. Conf. on Non-Ferrous Minerals & Metals*, 10-11th July, Bhubaneswar, Eds: C.R. Mishra & S. Majumdar, Tech – 4/1 – 4/9.

Dominik, J., Vignati, D.A.L., Koukal, B., Pereira de Abreu, M.H., Kottelat, R., Szalinska, E., Bas, B. and Bobrowski, A. (2007): Speciation and environmental fate of chromium in rivers contaminated with tannery effluents, Eng. Life Sci. **7(2)**, 155–169.

Dursun, Y.A., Uslu, G., Cuci, Y., Aksu, Z. (2003): Bioaccumulation of copper (II), lead (II), chromium (VI) by growing *Aspergillus niger*, Proc. Biochem. **38**, 1647-1651.

Dursun, A.Y., Uslu, G., Tepe, O., Cuci, Y. and Ekiz, H.I. (2003): A comparative investigation on the bioaccumulation of heavy metal ions by growing *Rhizopus arrhizus* and *Aspergillus niger.*, J. Biochem. Eng., **15**, 87-92.

ENVIS Newsletter (2006): State of Environment: Chromite Mining & Related issues, May - July 06, **5**(1):1-6. *http://www.cesorissa.org*

Ewan, K.B., Pamphlett, R. (1996): Increased inorganic mercury in spinal motor neurons following chelating agents, Neurotoxicology, **17**, 343– 349.

Ferris, F.G., Beveridge, T.J. (1986): Site specificity of metallic ion binding in *Escherichia coli* K-12 lipopolysaccharide, Can. J. Microbiol. **32**, 52–55.

Fourest, E., Canal, C., Roux, J.C. (1994): Improvement of heavy metal biosorption by mycelial dead biomasses (*Rhizopus arrhizus, Mucor miehei and Penicillium chrysogenum*): pH control and cationic activation, FEMS Microbiol Rev. **14**, 325– 332.

Gadd, G.M. (1988): Accumulation of metals by microorganisms and algae. In: *Biotechnology – A Comprehensive Treatise*, Vol. 6b (Rehm, H.-J., Reed, G., eds.), pp. 401–433. VCH Verlagsgesellschaft, Weinheim, Germany.

Gopalan, R., Veeramani, H. (1994): Studies on microbial chromate reduction by *Pseudomonas* sp. in aerobic continuous suspended growth cultures, Biotechnol. Bioeng. **43**, 471–472.

Goyal, N., Jain, C.S., Banerjee, C.U. (2003): Comparative studies on the microbial adsorption of heavy metals, Adv. Environ. Res. **7**, 311-319.

Gvozdyak, P.I., Mogilevich, N.F., Rylskii, A.F., Grishchenko, N.I. (1986): Reduction of hexavalent chromium by collection strains of bacteria, Mikrobiologiya **55**, 962-965.

Hartmeier, W., Berends, A., (1995): Biosorption of heavy metals by *Bacillus amyloliquefaciens*: contribution of cell walls, Med. Fac. Landbouww. Univ. Gent., **60**(4b), 2585– 2588.

Hoyle, B.D., Beveridge, T.J. (1984): Metal binding by the peptidoglycan sacculus of *Escherichia coli K-12*, Can. J. Microbiol. **30**, 204–211.

Huang, C., Huang, C., Morehart, A.L. (1990): The removal of copper from dilute aqueous solutions by *Saccharomyces cerevisiae*, Water. Res. **24**, 433– 439.

Ishibashi, Y., Cervants, C., Silver, S. (1990): Chromium reduction in *Pseudomonas putida*, Appl. Environ. Microbiol. **56**, 2268-2270.

Jiang, W., Saxena, A., Song, B., Ward, B.B., Beveridge, T.J., Myneni, S.C.B. (2004): Elucidation of functional groups on Gram-positive and Gram-negative bacterial surfaces using infrared spectroscopy, Langmuir **20**, 11433– 11442.

Kapoor, A. and Viraraghavan, T. (1998) Biosorption of heavy metals on *Aspergillus niger*: Effect of pretreatment, Biores. Technol., **63**, 109-113.

Komori, K., Rivas, A., Toda, K., Ohtake, H. (1989): Factors affecting chromate reduction in *Enterobacter cloacae* strain HOI, App. Microbiol. Biotechnol. **31**(4), 567-570.

Krauter, P., Martinelli, R., Williams, K., Martins, S. (1996): Removal of Cr(VI) from ground water by *Saccharomyces cerevisiae*, Biodegradation **7**, 277– 286.

Langley, S., Beveridge, T.J. (1999): Effect of O-side-chainlipopolysaccharide chemistry on metal binding, Appl. Environ. Microbiol. **65**, 489–498.

Llovera, S., Bonet, R., Simon-Pujol, M., Congregado, E. (1993) : Chromate reduction by resting cells of *Agrobacterium radiobacter* EPS-916, Appl. Environ. Microbiol. **59,** 3516-3518.

Luef, E., Prey, T., Kubicek, C.P. (1991): Biosorption of zinc by fungal mycelial wastes. Appl Microbiol Biotechnol. **34**, 688–692.

Lynch, J.M. (1988): The terrestrial environment. *In: Microorganisms in Action: Concepts and Applications in Microbial Ecology* (Lynch, J.M., Hobbie, J.E., eds.), pp. 103–131. Blackwell, Oxford, UK.

Macaskie, L.E., Dean, A.C.R. (1989): Microbial metabolism, desolubilisation and deposition of heavy metals: metal uptake by immobilized cells and application to the detoxification of liquid wastes, Adv. Biotechnol. Process. **12**, 159–172.

Malik, A. (2000): Studies on bio-desulfurization of coal. Ph.D. Thesis, Indian Institute of Technology, Delhi, India.

Malik, A., Dastidar, M.G., Roychoudhury, P.K. (2001): Biodesulfurization of coal: effect of pulse feeding and leachate recycle, Enzyme Microb Technol. **28**, 49–56.

Mise, S.R., Sudhakar, P. (1995): Adsorption studies of Cr(VI) from synthetic aqueous solution by activated carbon derived from Ziziphus zuzuba seed shells, JIPHE **1**, 61.

Mohl, W., Motschi, H., Schweiger, A. (1988): Magnetic resonance studies of Cu(II) adsorbed on the surface of the bacterium *Klebsiella pneumonia*, Langmuir **4**, 580–583.

Moore, J.W. (1990): Inorganic contaminants of surface water residuals and monitoring priorities. New York: Springer-Verlag, p. 178– 210.

Mueller, J.G., Cerniglia, C.E., Pritchard, P.H. (1996): Bioremediation of Environments Contaminated by Polycyclic Aromatic Hydrocarbons, *In Bioremediation: Principles and Applications*, pp. 125–194, Cambridge University Press, Cambridge.

Park, D., Yun, S.Y., Jo, H.J., Park, M.J. (2005): Mechanism of hexavalent chromium removal by dead fungal biomass of *Aspergillus niger*, Wat. Res. **39**, 533-540.

Pethkar, A.V., Paknikar, K.M. (1994): Screening of soil fungi for biosorption of toxic and precious metals from solution. In Abstracts: *National Conf. Fungal Biotechnol.*, Bhopal, Hune, pp. 24–26.

Philip, L., Iyengar, L., Venkobachar, C. (1998): Cr (VI) reduction by *Bacillus coagulans* isolated from contaminated soils, J. Environ. Eng. ASCE, **124**(12), 1165–1170.

Philip, L., Iyengar, L., Venkobachar, C. (1999): Immobilized microbial reactor for the biotransformation of hexavalent chromium, Int. J. Environ. Poll. **11**(2), 202–210.

Rai, L.C., Gaur, J.P., Kumar, H.D. (1981): Phycology and heavy metal pollution. Biol. Rev. **56**, 99 -151.

Ruhling, A., Tyler, G. (1973): Heavy metal pollution and decomposition of spruce needle litter, *Oikos* **24(3)**, 402-416

Senthilkumar, S., Bharathi, S., Nithyanandhi, D., Subburam, V. (2000): Biosorption of toxic heavy metals from aqueous solutions, Biores. Technol. **75**, 163– 165.

Sepehr, N.M., Nasseri, S., Assadi, M.M., Yaghmaian, K. (2005): Chromium bioremoval from tannery industries effluent by *Aspergillus oryzae,* Iran. J. Environ. Heal. Sci. Eng. **2(4)**, 273-279.

Shen, H., Wang, Y.T. (1994): Biological reduction of chromium by *E. coli*, J. Environ. Eng. **120,** 560–568.

Silver, S., Misra, T.K. (1988): Plasmid-mediated heavy metal resistance, Ann. Rev. Microbiol. **42**, 717–743.

Singh, D.K., Saksena, D.N., Tiwari, D.P. (1994): Removal of Cr(VI) from aqueous solution, Indian J. Environ. Heal. **36(4)**, 272.

Srivastava, S., Thakur, I.S. (2006): Isolation and process parameter optimization of *Aspergillus* sp. for removal of chromium from tannery effluent, Biores.Technol. **97(10)**, 1167-1173.

Stainer, R.Y., Doudoroff, M., Adelberg. Edward A., (1986): The Microbial World, 2nd Edition, Englewood Cliffs, NJ, Prentice Hall Press.

Stupar, J., Vrtovec, M., Kocijancic, A. and Gantar, A. (1999) Chromium status of tannery workers in relation to metabolic disorders., J. Appl. Toxicol., **19**,437–446.

Sugawara, H., Yoda, A., Kitamoto, K., Yamasaki, M. (1997): Isolation and characterization of nickel-accumulating yeasts, Appl. Microbiol. Biotechnol. **48**, 373–378.

Thakur, R., Sharma, G.D., Dwivedi, B.S., Khatik, S.K. (2007): Chromium: As a pollutant, J. Ind. Poll. Contr. **23**(2), 209-215.

Tripathy, A.K., Khrae, S., Ganguli, A. (1994): Isolation and characterization of pseudomonas strains for detoxifying Cr in tannery effluents, *In Proc. Symp. Environ. Biotechnology*, Nagpur, March 28–29.

Tyler, G. (1974): Heavy metal pollution and soil enzymatic activity, Plant Soil. **41**, 303–311.

Valix, M. and Loon, L.O. (2003): Adaptive tolerance behaviour of fungi in heavy metals, Min. Eng., **16**, 193-198.

Valls, M., Atrian, S., de Lorenzo, V. and Fernandez, L.A. (2000): Engineering a mouse metallothionein on the cell surface of Ralstonia eutropha CH34 for immobilization of heavy metals in soil. Nat. Biotechnol. **18**, 661-665.

Veglio, F., Beolchini, F., Gasbarro, A. (1997) Biosorption of toxic heavy metals: an equilibrium study using free cells of *Arthrobacter* sp. Proc. Biochem. **32**, 99– 105.

Volesky, B., Holan, Z.R. (1995): Biosorption of heavy metals, Biotechnol. Prog. **11**, 235– 50.

Volesky, B. (1994): Advances in biosorption of metals: selection of biomass types, FEMS Microbiol. Rev. **14**, 291– 302.

Wallace, A., Soufi, S.M., Cha, J.W., Romney, E.M. (1976): Some effects of chromium toxicity in bush bean plants grown in soil, Plant Soil **44**, 471-3.

Wang, P.C., Mori, T., Komori, K., Sasatsu, M., Toda, K., Ohtake, H. (1989) Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions, Appl. Environ. Microbiol. **55**, 1665-1669.

Wang, Y.T., Xiao, C. (1995): Factors affecting hexavalent chromium reduction in pure cultures of bacteria, Water. Res. **29**, 2467-2474.

Yan, G. and Viraraghavan, T. (2000): Effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii*, Wat. S.A., **26(1)**, 119-123.

Zafar, S., Aqil, F., Ahmad, I. (2007): Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil, Biores. Technol. **98**, 2557-2561.