APPLIED MICROBIOLOGY

Microbial interactions with inorganic pollutants: acid mine drainage, microbial accumulation of heavy metals and radionuclides

S. R. Dave

Department of Microbiology
School of Sciences
Gujarat University
Ahmedabad 380 009.
Email: shaileshrdave@yahoo.co.in

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Introduction

Inorganic and organic contaminants of environment, in general, and public water supplies in particular, have been a matter of concern in relation to public health for nearly a century. Contaminants of health concern in the range of 1 mg/L or less are referred as trace contaminants. Data available in the literature shows that lead was the first trace contaminant to be brought under scrutiny due to the prevalent use of lead pipes. Zinc and copper were questioned in 1923 and 1926, respectively due to the use of galvanized pipes and increased use of copper salts for algae control. During this period, the presence of iodide and fluorides were also reported to have harmful effects on health. Inorganic chemical constituents of concern include nutrients, non-metallic constituents, metals, metalloids and gases. The chemical constituents of wastewater and polluted land are typically classified as inorganic and organic. It was in 1962, U. S. public health drinking water standards were advanced and limits were set on the levels of trace contaminants of public health significance other than copper and zinc. The outbreak of 'Itai-Itai' occurred among farmers who drank cadmium-contaminated water from Jintsu river in Japan. Biomagnification of mercury through natural food chain to humans takes place largely through fish and evidence of certain organic and inorganic compounds as carcinogens has refocused the attention of public health organizations on trace contaminants.

The sources of inorganic non-metallic and metallic constituents in water are from the surrounding land, ground water, mining wastes, effluents from domestic and industrial water softeners, industrial effluents and others. Trace quantities of many metals such as cadmium, chromium, copper, iron, lead, manganese, mercury, nickel and zinc are important constituents of most waters. Many of these metal are essential to living cells. All living organisms require varying amounts of iron, chromium, copper, zinc and cobalt for growth and the absence of these could even limit growth. Although macro- and micro-amounts of these metals are required for optimum growth, the same metals are highly lethal when present in elevated concentrations. The concentrations of various inorganic constituents can greatly influence the utility of the waters. The major non-metallic inorganic constituents of concern are nitrogen, phosphorus, chlorides, sulphur, alkalinity, hydrogen ion and others. The hydrogen ion is very important, as concentration of most chemical constituents is dependent on hydrogen ion concentration of the solution. The existence of most biological life prefers pH range of 6 to 9. Alkalinity results from the presence of the hydroxides (OH^-), carbonates (CO_3^{2-}) and bicarbonates (HCO_3^-) of calcium, magnesium, sodium, potassium and ammonia. Presence of borates, silicates and phosphates can also contribute to alkalinity. The alkalinity of water has very little significance in public health.

Nitrogen, phosphorus, iron and sulphur are essential for the growth of microorganisms, plants and animals, and hence, these are known as nutrients or biostimulants. Nitrogen and sulphur are required in the synthesis of proteins. The most common and important forms of nitrogen in the aquatic or terrestrial environments are ammonia gas (NH_3), ammonium ion (NH_4^+), Nitrogen gas (N_2), nitrite ion (NO_2^-) and nitrate ion (NO_3^-). Most sources of nitrogen in environment are of biological origin.

The principal sources of nitrogen compounds are:

(i) The nitrogenous compounds of plant and animal origin,
(ii) Sodium nitrate, and
(iii) Atmospheric nitrogen

Phosphorus is responsible for noxious algal blooms in stagnant surface water. And much attention is focussed on controlling its entry in surface water from domestic and industrial discharge as well as natural runoff. The usual forms of phosphorous in water are orthophosphate, polyphosphate and organic phosphate. The orthophosphates: PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^{2-}$ and H_3PO_4 are available for biological metabolism without further breakdown. Polyphosphates undergo hydrolysis in aqueous solutions and revert to the orthophosphate forms. Organically bound phosphorus is of minor importance in domestic wastes, but it can be important constituent of industrial wastes and wastewater.

Sulphur is released by degradation of protein. The sulphate ion occurs naturally in most water supplies, it gets reduced biologically under anaerobic conditions to sulphide, which in turn combines with hydrogen to form hydrogen sulphide (H_2S), which causes concern due to colour and pungent odour. The sources of metals in wastewater are mainly discharges from residential dwellings, ground water infiltration, mining drains, and commercial and industrial discharges. Some sources of metals or metalloids and their discharge limits are shown in Table 1. When composted sludge is applied in the field, there is a threat of arsenic, cadmium, copper, lead, mercury, molybdenum, nickel, selenium and zinc pollution in soil.

Table 1: Typical discharge limits for toxic constituents found in secondary effluent

Metal	***Symbol***	**Average daily discharge limit (μg/L)**	**Concentration threshold of inhibitory effect on heterotrophic organisms (mg/L)**
Arsenic	As	20	0.05
Cadmium	Cd	1.1	1.0
Chromium	Cr	11	10[a], 1[b]
Copper	Cu	4.9	1.0
Lead	Pb	5.6	0.1
Mercury	Hg	2.1	0.1
Nickel	Ni	7.1	1.0
Selenium	Se	5.0	
Silver	Ag	2.3	
Zinc	Zn	58	1.0

[a] Total chromium
[b] Hexavalent chromium

Interaction with Inorganic Pollutants

Microorganisms play a significant role in the conversions of elements and other inorganic compounds in the environment. In the biosphere the conversion and cycling of nitrogen is the next most important process after the transformation of carbon. According to Robinson and Robbins (1970), the major nitrogenous compound released to the atmosphere is ammonia and most of the ammonia released is mainly by the activities of heterotrophic microorganisms on land and in the oceans.

Biologically evolved ammonia is the major source of nitrogen gas emitted to the atmosphere. Estimated annual global formation of NH_3 and NO_2 due to biological activity is about $4.9X10^9$ and $1.5X10^8$ tons/year, respectively. In comparison to it, all other sources contribute only $4.3X10^8$ tons/year. Natural organic substrates are attacked by bacteria and fungi, and the nitrogen present in such substrates is ultimately released as ammonium. Ammonium is oxidised by nitrifiers to nitrate and downward transport of nitrate contaminates ground water, which is then carried to wells and surface waters used for drinking purpose. The uncontrolled use of synthetic fertilisers, rapid growth of large urban regions, development of industrial centres, large feedlots and poultry houses release nitrogenous pollutants in copious amounts. Microbial activity converts these compounds to nitrate, nitrite or ammonium. If the nitrate content is more than 22 ppm, it is responsible for methaemoglobinemia in infants and livestock. Human infants sometime receive excessive amount of nitrate in the food, which is linked with microbial production of nitrates in soil, which is assimilated through the roots and accumulated in plants. Beets, spinach, celery and lettuce are prominent nitrate accumulators. Nitrite is inhibitory to the plant growth, whereby the affected plants are stunted, and become chlorotic and sometimes even die. A high nitrite level in water-logged soils is sometimes due to the indigenous nitrate-reducing bacteria.

The nitrogen oxides are important group of air pollutants. Many denitrifying bacteria and fungi are reported to generate N_2O and NO. The produced NO gas is oxidised in the atmosphere to N_2O. Many species of *Achromobacter, Bacillus, Chromobacterium, Micrococcus, Pseudomonas, Serratia* and *Aspergillus flavus, Penicillium atrovenetum, Fusarium solani* and others are reported to reduce nitrate to gaseous products mainly N_2O.

Sulphur Compounds

Hydrogen sulphide (H_2S) is formed by many sulphate-reducing microorganisms, and it is harmful to human beings, animals, higher plants, microorganisms and even non-living materials. Sulphate reducers are ubiquitous in mud, swamp and poorly drained soils, where they will propagate using sulphate as terminal electron acceptor and thus produce hydrogen sulphide. *Desulfovibrio desulfuricans* is known to produce H_2S. *Clostridium nitrificans* is also responsible for the reduction of sulphate. On the other hand, many heterotrophic and autotrophic bacteria and fungi oxidise sulphide. Among them *Acidithiobacillus, Thiobacillus, Leptospirillum, Baggiatoa* and *Thiothrix* are most important. Some of them oxidise sulphides, thiosulfate, tetrathionates to sulphate, and ferrous to ferric. Many of these tolerate or require highly acidic conditions and produce pH<1. Some of the sulphur conversions are shown in Table 2.

Many organic compounds are also attacked by bacteria, fungi and actinomycetes, and sulfur from such compounds is converted to H_2S. The production of H_2S has also been reported from decay of algae, and it is a common transformation in ocean and lake bottom sediments.

Hydrogen sulphide is highly toxic even at less than 0.1 ppm. Hydrogen sulphide in water has detrimental effect on hatched fish and growth and survival of fish eggs. It is offensive and contributes to the foul odour that affects upto one to two Km surrounding area.

Microorganisms are also responsible for the production of sulfur dioxide. Some wine yeast are responsible for synthesis of SO_2 from sulfate but are unable to reduce further. *Microsporum gypseum* has also been reported to excrete sulfite, when grown in media containing cystine.

Table 2: Microbial conversion of sulphur

Substrate	Organisms	Product
S^0, $S_2O_3^{3,}$ S_4O_6	*Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans, Thiobacillus thioparus*	H_2SO_4
S^0	Thiobacillus denitrificans	SO_4
H_2S	*Thiobacillus* spp.	S
	Baggiatoa spp.	
	Thiothrix spp.	
	Photosynthetic sulphur bacteria	
Metal sulphide	*Acidithiobacillus ferrooxidans*	$MSO_4+H_2SO_4$
SO_4	*Desulfovibrio* spp.	H_2S

Mercury

Environmental pollution with mercury has gained widespread recognition only after 1950, when 116 people were poisoned irreversibly and 43 died during 1953-1960 due to consumption of mercury contaminated fish from Minamata Bay area of Japan, which received nearby vinyl chloride factory waste. Similar episode had also occurred in Niigata, where fish were contaminated with mercury discharged from a vinyl chloride factory. Microorganisms contribute to mercury poisoning because they transform mercury to methylmercury, which is extremely toxic to human than inorganic mercury cations. Direct evidence for microbial involvement in mercury transformation comes from the work of Kimura and Miller (1964). *Clostridium cochlearium* has been reported to produce methylmercury from $HgCl_2$, HgO and $Hg(NO_3)_2$.

Arsenic Compounds

Arsenic is a strong poison for animals, humans and higher plants. Arsenite is more toxic than arsenate. The volatile trimethylarsine is also toxic to human. Arsenic as little 0.2 ppm in drinking water exerts its toxic effect. Microbial transformation of arsenic first became evident, when human poisoning was reported in rooms containing wallpapers coloured with arsenic-containing pigments. The arsenic pigments are not toxic but fungi grown on the wallpaper liberated the volatile trimethylarsine, which was responsible for the toxic effect.

Spores of *Fusarium, Aspergillus, Paecilomyces, and Penicillium* are capable of generating trimethylarsine from arsenic containing compounds. Apart from fungi *Methanobacterium, Desulfovibrio, Micrococcus* and others are also responsible for generating volatile arsenic compounds. Species of *Micrococcus, Yeast, Chlorella* and *Pseudomonas* interact with arsenate and arsenite and reduce or oxidise them.

Acid Mine Drainage

Minerals and processing of minerals involve many complex operations. Some of them are responsible for the production of solid and aqueous wastes. Acidic water generated from

sulphidic and coal mines cause a major environmental pollution problem. Active as well as abandoned mines are responsible for acid generation, termed as Acid Mine Drainage (AMD) or Acid Rock Drainage (ARD), which leads to contamination of rivers, streams, lakes, underground water as well as sea coasts. ARD is a worldwide problem. It is generally accepted that these diluted streams of sulphuric acid contaminated with metals, have to be treated. Mining process generates huge amount of overburden and waste rock. The waste rock, which includes non-mineralised or low–grade mineralised rocks, are removed from above or adjacent to the ore. This fragmented waste is generally placed in piles close to the mine. Due to drilling, blasting the explosives to fragment the rock and other activities of mining site expose the minerals to water, air and microorganisms. The outcome of the chemical and microbial activity is the production of sulphuric acid, which reacts with metal bearing rock and generates metal containing acid drainage from active as well as abandoned mines.

When sulphide minerals such as pyrite, sphalerite, chalcopyrite, galena, chalcocite and lignite are exposed to air and water, hydrogen ions are produced as shown below:

$$4FeS_2 + 15O_2 + 2H_2O \longrightarrow 4Fe^{3+} + 8SO_4^{2-} + 4H^+$$

When pyrite is chemically oxidized, in the presence of air and water, slightly acidic environment is created, which is conducive for the propagation of iron and sulphur oxidizing bacteria. These ubiquitous and indigenous chemolithotrophic bacteria are associated with mineral sulphides such as arsenopyrite, pyrite, chalcopyrite, sphalerite, galena, millerite, orpiment and antimonite; all these serve as energy sources for the microorganisms. The abundance of iron and sulphur in natural sulphide minerals makes it easier for the iron and sulphur oxidizers to colonize them. These organisms colonize the exposed mineral surface and derive their energy from such sulphidic and/or iron substrate. Among iron and sulphur oxidizers, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* play very vital role in ARD generation. *At. ferrooxidans* derives energy from the oxidation of ferrous, pyrite, sulphur, thiosulfate, tetrathionate and other inorganic sulphur and iron containing compounds. *At. ferrooxidans* enhances the chemical rate of pyrite oxidation by 5,00,000 to 10,00,000 folds. *L. ferrooxidans* is capable of oxidizing only ferrous iron. Almost all sulphide mineral and lignite deposits have pyrite (FeS_2) in less or more quantity, thus biocatalyzed oxidation of pyrite is the single most important reaction that contributes to ARD. Acid mine drainage from sulphidic and lignite mines are shown in Plate 1. The photograph also shows the oxidized iron in the form of ferric with brown coloration on the wall of mines as well as in collected waters.

Biooxidation of pyrite generates ferric iron, which is a very strong oxidizer. Ferric iron produced from pyrite biooxidation enhances further pyrite oxidation. As a result, ferrous iron, sulphate and hydrogen ions are produce as shown below:

$$FeS_2 + 14Fe^{3+} + 8H_2O \longrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

The ferrous iron is further oxidized by *At. ferrooxidans* and *L. ferrooxidans*.

$$4Fe^{2+} + O_2 + 4H^+ \longrightarrow 4Fe^{3+} + 2H_2O$$

Once again this ferric will attack pyrite and this cyclic process continues.

During this process, ferric iron also reacts with sulphate and soluble potassium and forms jarosite by following reaction:

$$K^+ + 3Fe^{3+} + 2SO_4^{2-} + 6H_2O \longrightarrow KFe_3(SO_4)_2(OH)_6 + 6H^+$$

$$K + 3Fe^{3+} + 2HSO_4^- + 6H_2O \longrightarrow KFe_3(SO_4)_2(OH)_6 + 6H^+$$

(A)

(B)

Plate 1: Acid Mine Drainage at (A) Sulphidic mine site and (B) Lignite mine site having mine drainage water pH below 3.0m and high amount of dissolved ferric, sulphate, copper and other base metals

The jarosite production is induced due to the presence of NH_4^+ and Ag. As can be evident from these equations, jarosite generation is an acid-producing reaction. When this solution seeps from the sulphide rich environment with pH>2.5, the soluble ferric iron undergoes hydrolysis and generates still more acid.

The ferric hydrolysis is directly proportional to pH, so during lime or limestone treatment of ARD, this acid generation from iron hydrolysis should also be considered.

The generated ferric ion and sulphuric acid enhances the oxidation and solubilization of metals from the ore, rock and soil. Ultimately water seeping out from rock, in many instances, is heavily loaded with toxic metals and hydrogen ions.

Many abandoned and active mines showed the pH of ARD water as low as < 2 and metal ion concentration more than few hundred milligrams per litre.

At. thiooxidans also interacts with metal sulphides as well as reduced sulphur compounds and produces sulphate and hydrogen ion pollution as depicted in the equation:

$$MS + 2SO_2 + 2H^+ \longrightarrow M^{2+} + SO_4^{2+} + H_2O$$

Where M stands for Cu, Zn, Ni etc.

$$2S^0 + 2H_2O + 3O_2 \longrightarrow 4H^+ + 2SO_4^{2-}$$

Mining environment also contains carbonate in the form of calcite, dolomite and other acid consuming materials, which react with generated acid in the drainage water and neutralise the acidity. The extent of acid producing and acid consuming reactions decide whether the mine drainage is acidic or alkaline. Both chemical and biological reactions of pyrite oxidation are exothermic and are responsible for increase in the temperature to as high as 60° to 70°C in the centre of dump or heap. The increased temperature enhances the growth of moderate to extreme thermophiles. Depending upon the temperature of the ecosystem, mesophilic *At. ferrooxidans, At. thiooxidans, Leptospirillum* will be succeeded by moderate thermophiles and then by extremely thermophilic *Sulfolobus, Sulfobacillus, Acidinus* and *Metallosphaera*. Thus, depending on the pH and temperature, the microbial interactions with environment will continue by one or the other group of microbes. ARD is of worldwide serious problem, which is very difficult to control. Once it is activated, it is always better to prevent by starting mining activities. So sulphidic materials that are acidic need special disposal techniques. Generation of ARD and metallic pollutants in mine drainage could be stopped or minimised by the addition of surfactants and/or slow releasing biocides, which inhibit the growth of *Acidithiobacillus, Leptospirillum* and the thermophilic bacterial species. The applied chemical inhibitors get, however, diluted after some time and/or adsorbed to rock surfaces and gradually become ineffective. Thus, frequent addition of these is required. Particularly in case of application of such chemicals at depth in piles of sulphide bearing rocks that are colonized by bacteria, it is almost impossible. Therefore, if ARD is once initiated, it is virtually uncontrollable. Thus, to prevent the initiation of ARD, the acid generating sulphide minerals should not be allowed the exposure of air and water. In the absence of air and water, both chemical and biological activities responsible for ARD generation get inhibited. The best time of implementation of ARD prevention is when mining activity is first started. The mining activity such as blasting and crushing of sulphidic rocks makes them more vulnerable to chemical and biological oxidation due to increased sulphide–surface exposure to ARD-conducive conditions. The major management steps, which help in the prevention or control of ARD, include:

1. Mixing the acid producing minerals with acid consuming rocks.
2. Capping the waste piles with clay and earth or vegetation to promote evapo-traspiration and prevention of erosion.
3. Piling acid producing rocks on impermeable pads and collecting the acid mine drainage at specific place.

4. Encapsulating acid producing rock with low permeability clay, which minimise acid and water contact.
5. Storing of tailings and finely ground sulphidic materials in line storage and maintaining a water layer over the material, which minimises air contact. Such treatments prevent both chemical and biological oxidation and provide economical management options.

Prediction of ARD Generation

Two types of rocks are normally present at mining sites. One is acid generating and the other is acid consuming. Thus, it is very essential to do the chemical and biological characterization of the rocks to predict acid generating or consuming potential and accordingly they should be stock piled. The above characteristics can be quantified by carbon sulphur method, biological acid producing potential (BAPP) test, humidity cell test and large column leach test. However, none of these methods is approved by Environmental Protection Agency. In this situation, often it is necessary to perform field assessment on stored waste materials to evaluate the potential for ARD, which will assess the effectiveness of the control measures taken to prevent ARD. Microorganisms play a significant role in ARD generation, and thus various biological tests are also used to predict ARD generation potential. The conventional procedure is to enumerate *At. ferrooxidans, L. ferrooxidans* and moderately and extremely thermophilic iron oxidisers by plate or MPN method. The indirect assessment can be done by nitrogen, protein and ATP analysis. Now a days, group specific molecular probes are available for the study of microbial population in acid mine drainage. The study of microbial diversity in mine waste heaps and mineral leaching environments provide useful data in terms of utilisation of sulphate, nitrate, ferric iron and manganese. The respirometry is also quite useful as indirect measurement method for bacterial activity in solid mine wastes.

Ferroplasma acidamanus is believed to be one of the major culprits for the formation of ARD, as it is isolated from ARD having pH as low as 0.5. In normal conditions, if the ARD is not prevented or controlled, the mine drainage water reaches to a pH as low as 2 or below. Such an acidic water when passes through the minerals it solubilizes sulphates, carbonates and metal ions present in the rock. Thus, ARD is always having the amount of such pollutants above the permissible limits, which are responsible for serious damage to surrounding aquatic and terrestrial ecosystems.

Method for Prevention or Control of ARD

Several conventional methods for treatment of acid mine waters are available depending upon the volume of the wastewater, the type and concentration of contaminants present. The commonly used method is the chemical neutralization of waste followed by precipitation of metals. But such active treatments require the installation of a plant with agitated reactors, precipitators, clarifiers and thickeners as well as costly reagents. Moreover, such plants require high maintenance and disposal of resulting metal leaded sludge is once again a problem. The alternative of such high cost procedure is the passive treatment with the application of anoxic alkali producing and sulphate-reducing organisms combined with wetland arrangement based on biological and physico-chemical processes such as oxidation, reduction, adsorption, absorption and precipitation. This type of system is slower than physical or chemical method but it is long lasting and generating minimum waste and they are ecofriendly. To activate the microbial system, it is necessary to add the fertilizers having ammonia and phosphate as nutrients to enhance the growth of microorganisms, which also

neutralizes the acidity as well as precipitates the metals as sulphides. Due to these recent developments, the generation of sludge is 6 to 10 times lower and the toxic metals are removed to a 1-100 ppb level. And this process gives recovery of valuable metals like copper, zinc, cobalt and others. Moreover metal oxides are easily soluble compared to metal sulphides, and thus the metal ions removed by chemical methods lead to underground pollution, when such precipitates are disposed as land filling material.

Bioremediation of Metals and Radionuclides

There has been a long history of association between metals and human development. The increased use of metals during the industrial revolution of the nineteenth century and thereafter heavy metals have become essential to modern society because of the range of metal products used. Metals and metal products are needed for urbanisation and industrialisation. We cannot think of these developments without metals. The rapid urbanization, industrialisation and mining developments throughout the world have become a serious concern to the environment due to pollution in general and metallic pollutants in particular.

Water is primary requirement for all human activities, ranging from drinking to agricultural production and industrial development to all forms of large-scale operations. Ever since man appeared on the face of the earth, he has exploited and modified the water supplies to his advantage in many ways. Unfortunately man has caused large perturbance in the ecosystems, which can permanently disturb the balanced state of the natural cycles, which we know as pollution. Pollutants have certain intrinsic properties, which determine the likely effect that they will have, if they are emitted or discharged into the environment. These properties are grouped into two types: effect generating properties and pathway determining properties. Pollution of water bodies and soil has made sustainable management of water resources a very complex task throughout the world. The current trends suggest that the situation is likely to become worse in the coming decades. Water is a primary requirement for all human activities. As the total global population increases, there is an increase in water requirement. The amount of fresh water is limited, and therefore, man has to depend on just 0.62% water found in fresh water lakes, rivers and groundwater supplies. Under such circumstances, it becomes important to identify the major water and soil pollutants, which are natural organic wastes, synthetic organic compounds, plant nutrients, metals, inorganic chemicals, sediments, radioactive substances, thermal discharge and pathogens. Out of these contaminants, pollution due to metals has been a major cause of concern since a long time, owing to its non-degradability.

Heavy metals occur in different environments, to a varying extent. In fact, the ability of water body to support aquatic life, as well as its suitability for drinking purpose and other uses depends on its trace elements. Metals such as Co, Cr, Cu, Fe, I, Mn, Mo and Zn, when present in trace amounts are essential for the physiological functions of plants and animals, whose deficiency causes disease under normal living conditions. When the same metals enter lakes, streams, rivers, oceans and other water bodies at higher concentration, they get dissolved or lie suspended in water or get deposited on the bed. This results in pollution of water whereby the quality of the water deteriorates that affects aquatic ecosystems. Metals can also seep down and affect the ground water deposits. Metallic pollutants have many sources. The most important of them is due to direct or indirect human activities, such as the city sewage, industrial waste discharge, agricultural, burning fuels and run-off mine that drains into rivers, and natural phenomenon like acid rain, acid mine drainage, volcanic

eruption and corrosion of water pipes. Water pollution by heavy metals as a result of human activities is causing serious ecological problems in many parts of the world, as metals are mobilized and carried into food web and at every level of food chain, concentration of the metals increase and pass on to the next higher level. This phenomenon is known as biomagnification. This situation is aggravated by the lack of natural elimination processes for metals. As a result, metals shift from one compartment within the aquatic environment to another, including the biota, often with detrimental effects.

Metallic pollutants cause direct toxicity both to eukaryotic and prokaryotic life forms. Heavy metals are known to have hazardous effects on human beings (Table 3). 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 lights up a candle to give light and at the same time to create disasters, which makes life dark. So we must work together to lessen metallic pollutants from the environment.

Table 3: Toxic effects of some heavy metals in effluents on human and their maximum permissible limits

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

*The threshold values given are for release of effluents in sewage or wastewater.

Conventional Remediation Methods

Much of the environmental awareness has been directed towards the preservation of water quality and the restoration of contaminated surface and ground waters as well as metal

contaminated land. The legislation enacted long ago to protect surface and ground waters has been revitalized through reauthorization of laws, establishment of stricter standards, broadening of definitions of regulated waste and increased enforcement of existing regulation. Achieving regulatory standards now in effect has necessitated revamping existing water treatment processes and developing and implementing new processes. Presently two approaches are generally available to prevent the metal pollution or reduce it to very low level:

1. Use of selected recovery and/or removal process.
2. The substitution of metallic compounds in the manufactured products.

When the later option is not feasible, it is essential to take all in-house precautions in the process to reduce the generation of metal bearing wastewater. Widely used conventional processes namely, chemical precipitation, ion exchange, membrane separation, solvent extraction and adsorption are used to tackle the problem of metallic pollutants. Among these methods, the most common is chemical precipitation. However, this method may be cost effective but it requires a relatively large amount of space for the clarifier, it produces a typical wet bulky sludge and generally requires final filters for polishing, if small residual levels of metal are required. Moreover, the chemical methods require large inputs of certain chemicals that cause secondary pollutions. Other available processes mentioned above are relatively expensive, which involve either elaborate or costly equipments or high operational costs and energy requirements. The ultimate disposal of the contaminants may also be a problem after these treatments. Moreover, methods and technology available or which are used in developed countries for the mitigation of such metallic pollutants, cannot be adopted in our country due to economic constrains. Microbial interactions with metallic pollutants offer many options of exploiting the microbes for metal remediation from the environment without generating any secondary pollution. Thus, there is an urgent need for development of environment friendly and economically viable bioremediation technologies.

Bioremediation Technologies

As seen in previous section, various physical and chemical processes are available for metal remediation but all of them suffer from significant drawbacks including incomplete metal removal. Moreover, such processes may be ineffective or extremely expensive when initial metal concentrations are in the range of 10 to 100 mg/L. Thus, new technologies are required that can reduce metal concentration to environmentally acceptable levels at affordable costs. Metal remediation was observed as early as 1940's by Ruchloft who observed that activated sludge efficiently removed Plutonium^{239} from contaminated wastewater. Goodman and Roberts (1971) in UK used mosses as indicators of aerial metal levels. Later, microbes such as *Ascophyllum nodusum*, strains of *Bacillus, Pseudomonas, Streptomyces*, fungi imperfectii and *Ascomycetes* have been used for bio-monitoring of heavy metals. Several reports of remediation of metals from aqueous state by bacteria, fungi, yeast and algae have been documented in the literature (Table 4).

Microbial and phytoremediation provides a promising technology for economical removal and recovery of metals. Agriculture wastes such as tree bark, peanut skin, onion skin, melon seed, waste tea leaves, rice bran etc. have been used for sorption of heavy metal ions. According to David E. Salt, more than 300 species of plants are known to accumulate metals, such as cadmium, copper, manganese, nickel, selenium or zinc in high levels from contaminated water or soil. The wild mustard *Thlaspi goesingense*, a plant that grows in the Austrian Alps, can accumulate 10,000 parts per million of nickel in their tissue, while normal

plants can accumulate 10 to 100 ppm of nickel. Harvesting of precious metals like gold could be possible by cultivating carrot, red beet, onion and radish. Carrot roots and radish are reported to accumulate as high as 48.3 and 113 mg of gold per kg of dry weight, respectively. This bioremediation process is called more specifically a 'phytoremediation'. The use of such rare plants has increasingly been examined as a potential, practical and more cost effective technology than soil replacement, solidification or washing strategies of polluted soil. Categories of phytoremediation include phytoextraction, phyto-volatilization and rhizofiltration. But the metal hyper accumulating plants found in nature would not be used for phytoremediation because they are small and have lower growth rates.

Table 4: Maximal metal accumulation by bacteria, fungi and yeast

Metals	Microorganism	Maximal concentration in biomass (% dry wt.)	Remarks
Cadmium	*Saccharomyces cerevisiae*	3.12	
Copper	*Rhizopus arrhizus*	1.71	
	Penicillium notatum	8.00*	Incubation time : 30 d
Zinc	*Penicillium notatum*	2.30*	
	P. spinulosum	0.13	
Uranium	*Aspergillus niger*	21.50	
Thorium	*Rhizopus arrhizus*	18.50	
Lead	*Penicillium* spp.	0.61	
Silver	*Rhizopus arrhizus*	5.40	Expected maximum uptake capacity calculated from isotherm
Chromium		3.10	
Copper		1.60	
Cadmium		3.00	
Mercury		5.80	
Lead		10.40	
Uranium		19.50	
Zinc		2.10	

The potential of certain types of microbial biomass live or dead to concentrate or remove metals has been well established (Table 5). Biosorption processes using *Pseudomonas mendocina, Pseudomonas putida, Saccharomyces cerevisiae, Aspergillus niger*, *Penicillium* sp., *Chlorella* sp., *Scenedsmus* sp. and others have been applied efficiently for the removal of chromium, copper, nickel, lead and zinc from electroplating, battery manufacturing, tannery, cooling tower and other industrial effluents. Leach solution from active and abandoned mine sites containing metallurgical and chemical wastes were successfully treated by immobilised microbial biomass. Polyacrylamide immobilised yeast biomass showed 90, 55 and 45% copper, cadmium and zinc removal, respectively from electroplating waste while, algal biomass gave 95 and 43% lead and zinc removal respectively from zinc mine effluent. Mercury is also an important metal and considerable volumes of mercury containing effluent are being discharged into public sewer systems. Mercury uptake and biosorption by laboratory grown isolates and natural consortia of sewage sludge have been documented.

Available information also focuses attention on microbial enzyme based volatilisation of mercury by *Pseudomonas putida*, *Acidithiobacillus thiooxidans* (formerly *Thiobacillus thiooxidans*), *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*), *Beijerinckia mobilis* and *Azotobacter vinelandii*.

Table 5: Type of waste biomass studied for metal sorption

	Biomass used	Metals removed
1.	Plants	Copper, zinc
2.	Seaweed	Copper, zinc, nickel
3.	Sewage sludge	Copper, zinc, nickel, silver, mercury
4.	Fermentation wastes	Copper, zinc, nickel, silver, mercury, uranium, cadmium
5.	Laboratory grown specific biomass	Copper, zinc, nickel, silver, mercury, cadmium, gold, lead

Biosorption is an ideal process for the treatment of high volume low concentration complex waste. Metal bioremediation is a hybrid phenomenon and an interdisciplinary approach, which seems essential for developing the technology to a successful process application stage. Application of waste microbial biomass from fermentation industry and regeneration of biosorbent improves the process economy.

Biosorption enjoys a lion's share in metal bioremediation technology. The term "biosorption" has been used to encompass metal uptake by whole biomass either living or dead via physicochemical mechanism such as adsorption, ion exchange, precipitation, particulate entrapment as well as metabolism dependent uptake. In the following section, various mechanisms and technological aspects are discussed.

Bioremediation of Radionuclides

Pollution of environment by radionuclides takes place as a result of increasing industrial activity and wastes that arise due to experimental explosions of nuclear weapons and waste of reprocessing plants of nuclear fuels and use of isotopes for medical purposes. Radionuclides have less concentration as a pollutant but have a long half-life. As a result the impact of radionuclide pollutants is growing with time. Most radionuclides exist as cations and have good sorption ability. There are several reports on the biotechnological techniques for the removal or detoxification of uranium, caesium, iodine as well as radioactive cobalt, thorium, americium, antimony and technetium. Immobilized cells of *Citrobacter* sp. is able to generate heavy metal resistance due to acid type phosphatase which, in presence of organic phosphate, releases HPO_4^{2-} that precipitates uranium and lanthanum as their phosphates, since they are highly insoluble. Plutonium is also reported to be removed as $Pu(HOP_4)_2$ as the insoluble product. Microorganisms are also reported to remove ^{241}Am (Americium), $^{238\text{-}239}Pu$ (Plutonium) and ^{237}Np (Neptunium). Apart from *Citrobacter*, species of *Pseudomonas, Arthrobacter, Rhodotorula, Streptomyces* and *Aspergillus* are also reported for considerable sorption of technetium from the waste. *Acidithiobacillus ferrooxidans* and *A. thiooxidans* are known to reduce the radioactivity of technitium-99 to more than 70%. The live cells exhibit 5-fold higher sorption than dead cells indicating metabolic dependent activity. The uptake of uranium occurs in 3 steps: 1. nitrogen complexes with uranyl ions and results in adsorption.

In the 2nd step, the precipitation will take place. And at the end, the hydrolysed uranium is deposited in polymer network. In case of thorium, the uptake takes place in external cell walls and ultimately gets precipitated. The chitin-amide nitrogen and free radicals associated with chitin play significant role in uranium and thorium removal. *Rhizopus arrhizus* removed maximum uranium and plutonium at pH 6-7, where as the optimum pH for ^{241}Am, ^{144}Ce, ^{147}Pm, $^{152+152}$Eu was pH 2. This biomass is proved to be promising sorbant for the treatment of radioactive effluent from nuclear industries.

Mechanisms

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. Major mechanisms of biosorption are shown in Figure 1. The physico-chemical reactions between microorganisms and metals can be divided into six distinct processes, which are explained below:

1. 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 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.

2. 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.

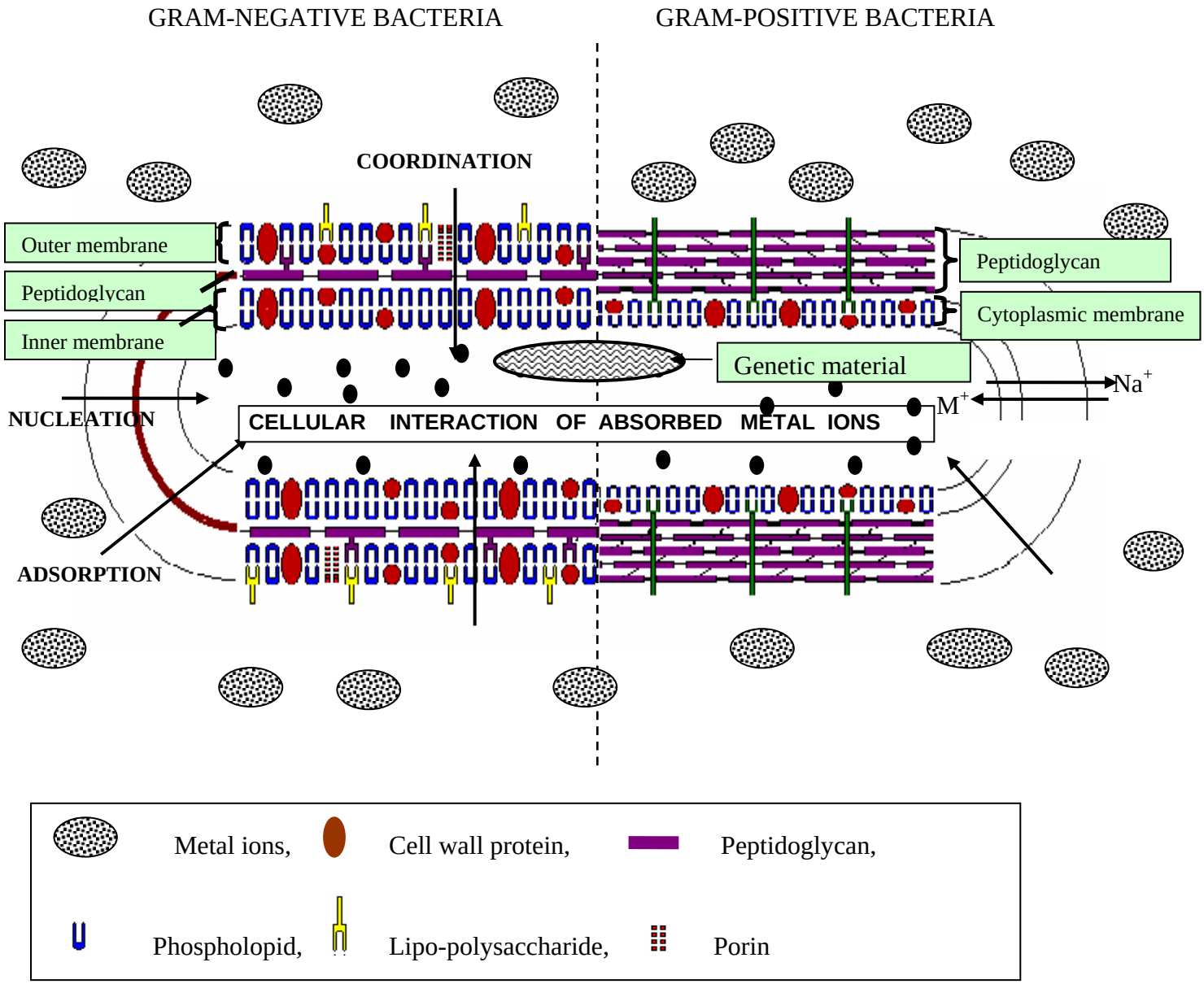

Figure 1: Schematic representation of Gram positive and Gram negative bacteria showing metal remediation sites

3. 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.

4. 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 with divalent metal and forms $MHPO_4$ precipitate at cell surface. This mechanism is very significant in the removal of metal or radionuclide from effluents having phosphate containing organic substrates.

5. 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 is changing 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 great 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 polyfunctional metal binding sites for metals. Ionic charges and covalent binding have been reported in *Chlorella, Ulothrix, Chlamydomonas, Spirulina* and *Sargassum*.

6. 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

Factors intrinsically related to the biosorbents, biosorbets 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:

Biomass

Microorganisms exhibit a very high diversity, and thus they differ in their cell wall structure and composition. Growth of microorganisms is associated with changes 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. Microorganisms 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 was due to the increase in metal to biosorbent ratio. The amount of metal adsorbed by the biomass increased 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 was observed with increased ionic radii and ionic charge of metals. *Rhizopus arrhizus* showed metal uptake in the increasing order of $Sr^{2+} < Mn^{2+} < Zn^{2+} < Cd^{2+} < Pb^{2+}$, which correlated with the covalent index of metal ions. Uptake of the desired metal decreased 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 the presence of EDTA, SO_4^{2-}, Cl^- and SO_4^{2-}, PO_4^{2-}, CO_3^{2-} and NO^{3-} ions.

Benjamin and Leckie have proposed three types of interactions due to the presence of anions

1. Metal - anion complexes formed are non-adsorbing or weakly adsorbing that results in reduction in metal binding.

2. Biosorbent - anion interactions either enhance or reduce metal binding, and
3. 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.

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 show a mixed influence on metal binding. Pretreatments are varied with the type of biomass and its source.

Equilibrium of Biosorption

In the beginning, metal biosorption rate is very high as compared to the rate of desorption. As sorption process progresses, metal sorption slows down as available free binding sites decrease and corresponding desorption rate increases until the rate of both sorption and desorption become equal. At this point the biomass binding sites is saturated with metal ions and no net uptake of metal occurs. This state gives equilibrium and it follows an adsorption isotherm. Adsorption isotherms show the solute concentration in the adsorbed state as a function of its concentration in the solution at constant temperature. The isotherm indicates the relative affinity and adsorption capacity of biosorbent for metal ions.

On the basis of laboratory scale data with the application of mathematical models it is possible to optimize the reactor configurations and prediction of the effects of particle size, metal and biomass concentration on the process efficiency.

Non-living biomass in metal remediation

Non-living biomass is also used efficiently for the removal of various metals from waste. The application of non-living biomass in this technology has the following merits:

1. It is not subjected to toxicity limitations due to metal concentration.
2. Costly nutrients for growth are not needed. Moreover, this reduces the disposal problem of spent media.
3. Biomass from fermentation industry can be available at a cheap rate, which is waste for fermentation industry.
4. No physiological constrain like live biomass in the system.
5. The process is very rapid.
6. Due to non-living nature of the biosorbent operating conditions such as pH, temperature, metal concentration evenly fluctuates. It does not disturb the process.
7. Aseptic maintenance is not required.
8. Metal desorption can be done with stronger reagents as compared to live biomass. Thus recovery is faster and more concentrated.

There can be a few disadvantages due to the use of non-living biomass as compared to the use of living biomass:

1. Process will be saturated early and thus, frequent desorption is required.
2. Chances of process improvement are limited.
3. Biological transformation of metal valancy state is not possible. Thus limited use in the reduction in metal toxicity. E.g. Dead biomass cannot convert As^{3+} to As^{5+}, which is possible with living biomass.

Metal Recovery from Biomass

One of the important industrial applications of biosorption is to recover back the metal ions from the biosorbent, simultaneously regenerating the biosorbents for reuse. Effective and viable biosorption technology also requires highly efficient and economical method of desorption, which should not damage the biomass so the regenerated biomass can be reused in subsequent cycles of sorption for metal removal. Some metal ions show marked pH dependence for binding to biomass. Such metal can be desorbed easily from the biosorbent altering the pH, whereas metal ions with little influence of pH on their binding can be stripped by the addition of specific ligands with high affinity for the metal ions. Dilute HCl, H_2SO_4 and HNO_3 have been successfully used for desorption of metals from the biomass. Use of concentrated acid gives faster and enhanced desorption but it may cause permanent damage to the biomass surface structure resulting in a great reduction in metal sorption in the next cycle. A controlled strength of EDTA, thiosulphate, citric acid, acetate, lactic acid, sodium, carbonate and bicarbonate can also be used for metal elution without much disturbing the biosorption ability of biosorbent for the further cycle.

In spite of the advantages mentioned above, the use of free microbial biomass in metal remediation system may present some unique problems. The foremost problem with free biomass is the physical state of the native biomass. They are very small in size in the range of micrometer and highly fragile, so very difficult to separate from the processed effluent and often responsible for pressure drops across a fixed-bed column during down-flow operation. Microbial cells have low mechanical strength and low rigidity thus they collapse when used in packed or fluidized bed reactors with large volume of solution. Free cells are suitable only for discontinuous reactors. Thus, the biomass needs to be modified by various immobilization techniques to generate biomass in the form of particles of desired size, mechanical strength and rigidity with retaining native properties of the biomass.

Immobilization of Cells

In recent years immobilization technology of whole cells has improved considerably. Biomass is immobilized with polyacrylamide, silica, agar, agarose, calcium alginate, K-carrageenan, diatomaceous earth, ceramics, glass beads, polyvinyl foams, polyurethane and epoxy resin. Immobilization techniques can be classified as physical or chemical depending on the type of binding. The use of glutraldehyde for the immobilization of non-viable fungal biomass for copper, nickel and cadmium removal has been reported. The influence of resting time on metal biosorption in column was investigated and it was found that resting time improved the sorption efficiency and rate.

When immobilized biomass is employed for metal sorption, a number of factors can be taken into consideration, so that the number of cycles a biosorbent can undergo will increase without losing metal remediation efficiency. These appear critical for the overall economics of the process. The most significant factors influencing biosorption technology are contact time, flow speed, diffusion rate, column height, number of sorption-desorption cycles, metal sorption kinetics and disposal of used biomass. Some of these factors are taken care of by selecting proper immobilization material in the desired concentration, use of counter current flow, decreasing the size of the immobilized biomass beads and increasing the porosity of the beads. In order to analyse the kinetics of immobilised biomass, the following major assumptions are required for the development of a working model:

1. Immobilised biomass particle are spherical in geometry with uniform size.
2. Admix components and biomass in a uniform layer.
3. The admix do not accumulate the solute.
4. Local biosorption equilibrium exists in the pores of the biosorbent particles.
5. Accumulation of solute is negligible in the liquid, inside the pores of the particles.

Patents and Commercial Applications

Microbial biomass has the advantages such as competitive performance, selectivity for heavy metals, cheap, regenerative nature, minimum sludge generation, possible metal recovery and cheaper process equipments as compared to conventional ones. Some excellent products based on immobilized biomass such as: AMT-BIOCLAIM^{TM}, AlgaSorb^{TM}, Bio-Fix and BIOMAT$^{®}$ have been developed, patented and commercialized for detoxification of metal loaded wastewater or effluents (Table 6). Algasorb^{TM} contains algal biomass immobilised in a silica gel matrix. These have been successfully used to remove Ag, Al, Au, Cu, Co, Cr, Hg, Ni, Pb, Pd, Pt, U and Zn from contaminated effluents and process streams using column reactors. The regenerated biomass after desorption showed about 90% of the original metal uptake efficiency. AMT - BIOCLAIM^{TM} process was used in the form of fixed bed reactors containing 20Kg. of granular biosorbents and 80-90Kg. biosorbent was used in fluidised pulsed bed system. These granulated biosorbents was reported for removing Cd, Cr, Cu, Hg, Ni, Pb, U and Zn individually or in a mixed form. In certain cases, the processes resulted in removal efficiency of more than 99% with the outcoming effluents having as low metal concentration as 10-50 ppb. The application of Sulphate Reducing Bacteria (SRB) was also studied on a large commercial scale using 1800 m^3 concrete reactor with a waste treatment capacity of 7000 m^3 per day. The SRB reactors have been shown to remove 90% of the metal and 20% of the sulphate from coal mine drainage waters.

Table 6: Patented products for metal remediation

Patent product	Micro-organism used
AMT-BIOCLAIM ™	*Bacillus*
AlgaSorb ™	Fresh water alga
Bio-Fix	Yeast, alga, plants and bacteria
BIOMAT®	Cyanobacteria and purple autotrophic bacteria

Bio-Fix™ processes were developed at US Bureau of Mines, USA using thermally killed biomass of algae, yeast, bacteria and sphagnum peat moss. The biomass was immobilised using polysulfome – dimethylformamide mixture. These immobilised biosorbents were successfully used for at least 120 cycles without reduction in the sorption efficiency. This immobilised biomass gained enough strength and rigidity, so that they can be suitable for the replication in stirred tank reactors, fixed bed and fluidised bed columns.

Reactors for the Treatment

Conventional engineering systems used for wastewater treatment can be used for bioremediation process with minor modifications. Most metal bioremediation processes use non-viable immobilized biomass, thus either a batch or a column reactor can be used for the process. Column configuration offers greater metal-sorption capacity and higher efficiency, whenever immobilized biomass is incorporated into wastewater treatment technology.
Commonly used bioreactors in metal bioremediation technology are:

1. Stirred-tank reactors,
2. Packed-bed reactors,
3. Fluidized-bed reactors,
4. Dispersed-bed (air-lift) reactors.

The diagram of the above reactors is shown in Figure 2, 3, 4 and 5.

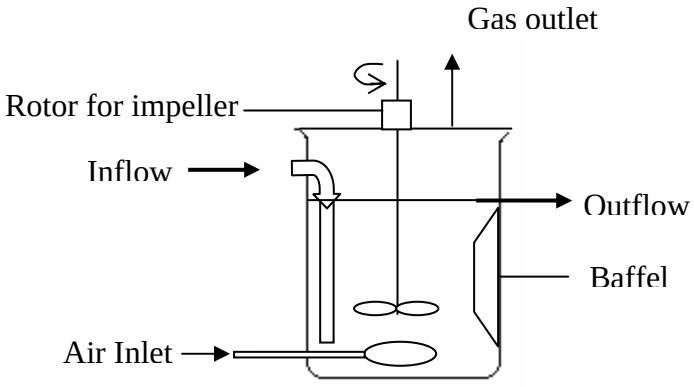

Figure 2: Stirred Tank reactor

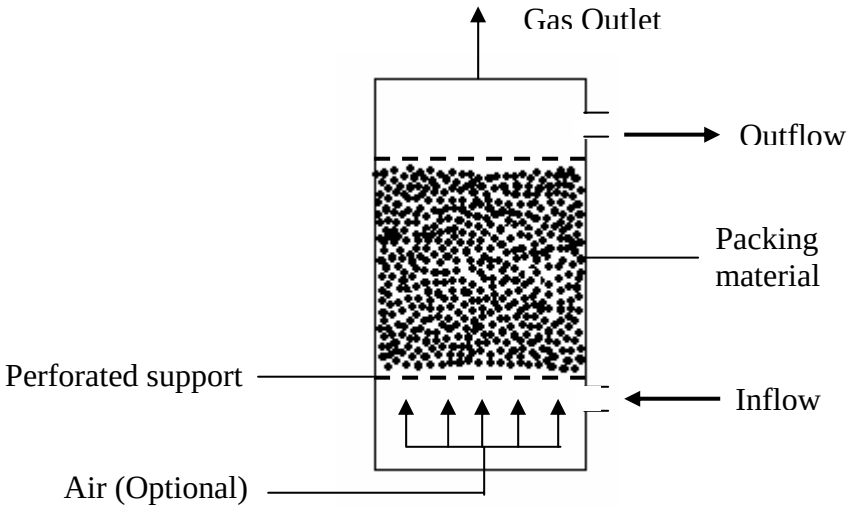

Figure 3: **Packed Bed Reactor**

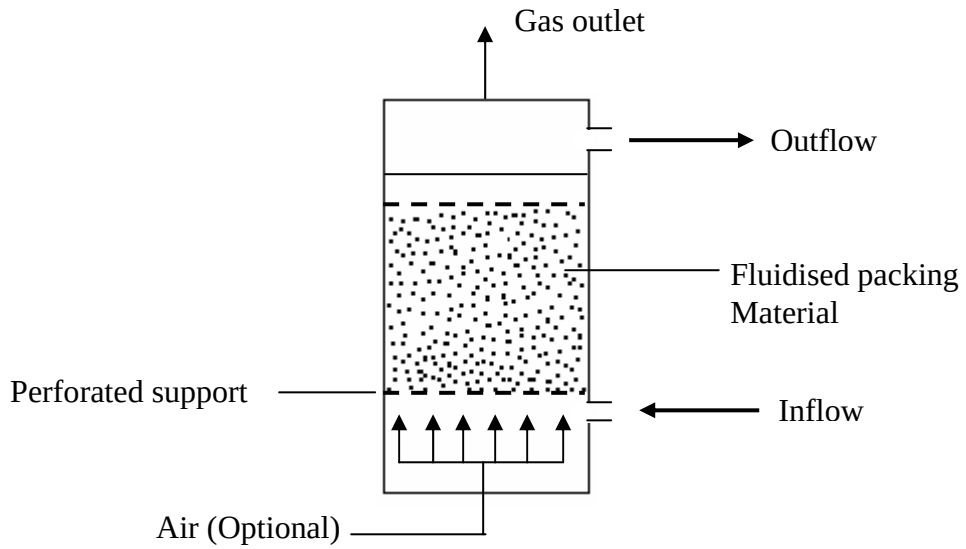

Figure 4: Fluidised Bed Reactor

Considerable experience has been gained in the use of above type of reactors for metal remediation and pollution control in the industries using ion exchange resins and activated carbon. The most common of such reactors are the upflow or downflow packed bed reactors and the continuous fluidized bed reactors. Pilot scale data for both types of the reactors for biosorption from industrial effluents are available. Application of the above two types of reactors are found to be of economically acceptable choice.

Criteria for selection of biomass for bioremediation depend on availability and cost of biomass, uptake rate of metal, single or multiple metal sorption ability of the biomass, ability to withstand desorption cycles and influence of environmental factor on metal bioremediation. The future application of this technology can be extended for public health point of view, for the removal of metallic pollutants from food, food products, herbal-based medicinal product preparation and juices of carrot, grapes and oranges.

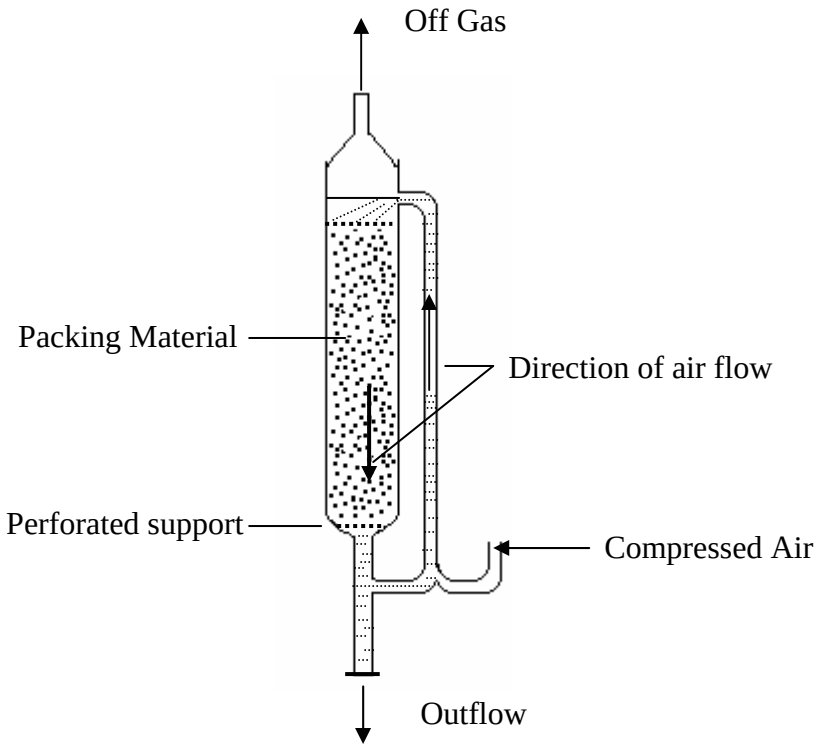

Figure 5: Dispersed bed (Air Lift) Reactor

If we look into the challenges in the development of metal bioremediation processes, much work has been done at international level. India is lagging behind in the development of such bioremediation processes, in spite of enormous industrial development, which has led to the generation of huge volumes of metal containing wastes, which cannot be treated by conventional methods.

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