Lectures in
Microbial Bioremediation Of Water And Sewage Water
For Diploma Students

Part 2
Bioremediation

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Chapter 2
Bioremediation

Bioremediation is defined as the process by which microorganisms are stimulated to rapidly degrade hazardous organic pollutants to environmentally safe levels in soils, sediments, substances, materials and ground water. Recently, biological remediation process have also been devised to either precipitate effectively immobilize inorganic pollutants such as heavy metals.

- **BIO-AUGMENTATION:** is the process when microorganisms are imported to a contaminated site to enhance degradation.
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- **BIOSTIMULATION** involves the addition of fertilizers to increase the bioavailability within the medium.
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- **LAND FARMING** is a bioremediation treatment process that is performed in the upper soil zone or in biotreatment cells.

POTENTIAL STRATEGIESFOR BIOREMEDIATION

Microorganisms are mainly used in bioremediation to eliminate heavy metals (elements with densities above 5g/cm3) from the polluted environment. In addition to the natural occurrence of heavy metals, they are widely used in industry, agriculture, and military operations. These processes have led to the continuous accumulation of heavy metals in the environment, which raises threats to public health and ecosystems. The high concentrations of heavy metals in the environment were also attributed to several life-threatening diseases, including cancer and cardiovascular ailments. The elimination of heavy metals requires their concentration and containment as they cannot be degraded by any biological, physical, or chemical processes). Therefore, employing microorganisms in heavy metal elimination and environmental cleaning is an effective approach due to their varied ability of interacting with heavy metals. For instance, microorganisms can transform heavy metals from one oxidative state or organic complex to another. Mainly, microorganism-based remediation depends on the resistance of the utilized microbe to the heavy metal that is either activated independently or through metal stress. Microorganisms perform the remediation of heavy metals through three different processes (**Figure1**): **Biosorption**, **Bioaccumulation** and **Siderophore Formation.**

Bioaccumulation

Bioaccumulation takes place when the absorption rate of the contaminant is higher than the rate of losing it. Thus, the contaminant remains contained and accumulated inside the organism.

Bioaccumulation is a toxicokinetic process that affects the sensitivity of living organisms to chemicals. Organisms can normally resist concentrations of chemicals up to certain levels, beyond which these chemicals become toxic and endanger the organism. The sensitivity of organisms to chemicals is highly variable depending on the types of organisms and chemicals involved.

Bioaccumulation candidate organisms should have a tolerance ranging between one or more contaminants to higher levels. Furthermore, they may demonstrate superior biotransformational capabilities, changing the toxic chemical to a non-toxic form which enables the organism to reduce the toxicity of the contaminant while keeping it contained. Several different organisms are used for the study of bioaccumulation and as indicators for increased levels of pollutants, including plants.

It was shown that bacteria produce metal-binding proteins such as metallothioneins (MTs) in order to increase the metal binding capacity as a response to increased metal exposure (Figure1B) .

Siderophore Formation

Siderophores are selective and specific iron chelating agents secreted by living organisms such as bacteria, yeasts, fungi (Figure1C) and plants. Siderophores have a relatively low molecular weight and an extremely high binding affinity to trivalent metal ions (Fe3C), which is poorly soluble and predominantly found in oxygenated environments (Neilands, 1995; Chuetal., 2010). There are three different types of siderophores; namely, hydroxamate siderophores, catecholates (phenolates) siderophores and carboxylate siderophores. The hydroxamate siderophores are the most common group of siderophores. Itconsists of C(DO) N-(OH)R, where R is an amino acid and its derivative which are mainly produced by bacteria and fungi. The catecholate siderophores binds with iron through the formation of the hexadentate octahedral complex. Several well-known bacteria such as E. coli and Salmonella typhimurium produce these type of siderophores (Searleetal., 2015). The carboxylate siderophores bind to iron ions through the carboxyl and hydroxyl groups, and is produced by rhizosphere bacteria suchas Rhizobium as well as several other types of bacteria.

It was shown that trivalent forms of metals (other than iron) having similar chemistry can stimulate bacteria to produce siderophores, for instance, Al, Ga, and Cr. Thus, the positive effects of siderophores on remediation such as reducing bioavailability and metal toxicity is not limited to iron but can be extended to several other toxic heavy metals. Stimulation of siderophore synthesis by heavy metals in the presence of high iron concentrations in *Pseudomonas aeruginosa* and *Alcaligenes eutrophus* bacteria was reported in an early study.

Biosurfactants Production

Surfactants or surface-active agents are substances that alter the prevailing conditions of surfaces through adsorption leading to lower surface tension between liquids or

between a liquid and a solid. Thus, they can generally classified into(1) high molecular weight polymers binding tightly to solid surfaces and(2)low molecular weight molecules efficiently lowering the surface and interfacial tensions.

Biosurfactants are surfactants produced or secreted by living organisms such as microbes(**Figure1D**). Although biosurfactants have been commonly used for organic pollutants remediation, several studies have also reported that biosurfactants are able to complex and remediate heavy metals such as Cd, Pb, and Zn.

Rhamnolipids are amajor class of biosurfactants that are produced by *P. aeruginosa* and several other organisms. They are glycolipids with rhamnose moiety comprising of a glycosyl head group and a 3- (hydroxyalkanoyloxy) alkanoic acid (HAA) fatty acid as the tail Rhamnolipids have several potential applications in industry and as additives for environmental remediation. The ability to capture heavy metal ions through electrostatic or complexation techniques has been attributed to anionic biosurfactants. These complexations lead to an increase in the apparent solubility of metals. Therefore, the bioavailability of metals is affected through their reduction by common metabolic by-products, which leads to the formation of less soluble metal salts including phosphate and sulfide precipitates.

There are several other biosurfactants produced by a wide range of bacterial and yeast species such as exocellular polymeric surfactants in the form of polysaccharides, proteins, lipopolysaccharides, lipoproteins or complex mixtures. For instance, several species of *Acinetobacter* demonstrated robust production of high molecular weight emulsifiers.

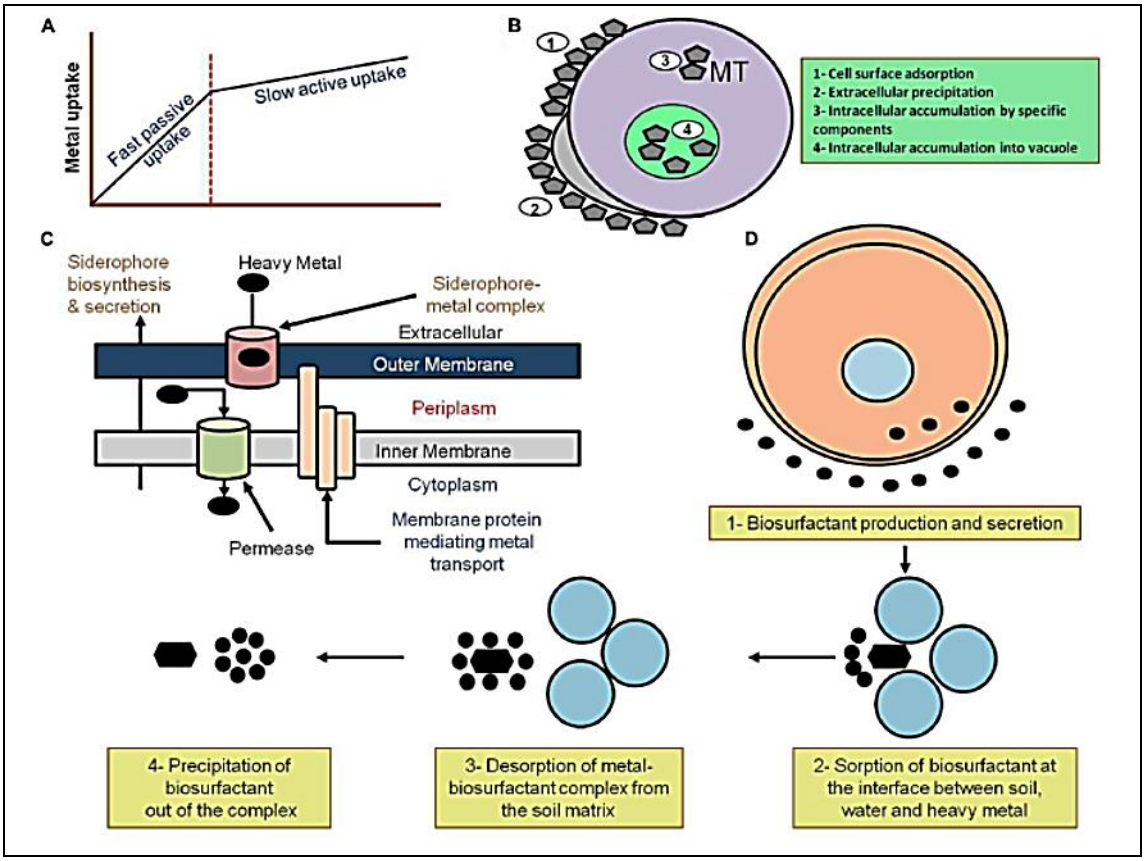


FIGURE 1| Mechanism of microbial remediation. (A) Passive and active heavy metal uptake by biological materials. The uptake of heavy metals canbe either passive (fast) through adsorption onto the cell surface or any extracellular components such as polysaccharides, or alternatively active(slow) through sequestration of the heavy metals via interaction with metallothioneins (MT) into the cell. Adapted from Scragg (2005). (B) Mechanisms of heavy metal biosorption by bacterial cells. Bacterial biosorption of heavy metals through(1) cell surface adsorption, (2) extracellular precipitation, (3) intracellular accumulation through special components, such as metallothioneins (MT) or, (4) intracellular accumulation into vacuoles. Adapted from Banik etal. (2014). (C) Heavy metal remediation via siderophoreformation. Bacterial heavy metal remediation takes place through formation of the siderophore aided by membrane protein-mediated metal transport and the formation of siderophore-metal complexes. Adapted from Banik etal. (2014). (D) Mechanism of bacterial heavy metal remediation through biosurfactant production. The precipitation of heavy metals takes place through sorption and desorption at the soil–water-heavy metal matrix leading to heavy metal precipitation. Adapted from Banik etal. (2014).

Rhizoremediation: the combinatorial effects of bio/phytoremediation

Rhizoremediation is the combination of two approaches, i.e., phytoremediation and bioaugmentation, for cleaning contaminated substrates. Rhizoremediation refers to the exploitation of microbes present in the rhizosphere of plants utilized for phytoremediation purposes. Heavy metal resistant rhizospheric and endophytic bacteria are ecologically friendly and cost effective toward the reclamation of heavy metal polluted soil.

The exploitation of metal resistant siderophore-producing bacteria (SPB) present near the rhizosphere provide nutrients (particularly iron) to the plants that could reduce the deleterious effects of metal contamination. The siderophore not only solubilizes iron from minerals or organic compounds, it can also form stable complexes with environmental concern metals such as Al, Cd, Cu, Ga, In, Pb, Zn, and radionuclides. Bacteria, mainly plant growth promoting rhizobacteria (PGPR), and fungi, mainly arbuscular mycorrhizal fungi (AMF), are used as pure cultures or co-cultures for bioaugmentation. PGPR such as Agrobacterium ,Alcaligenes, Arthrobacter, Azospirillum, Bacillus, Burkholderia, Serretia, Pseudomonas, and Rhizobium are generally used for metal extraction with plants. The siderophore synthesized by P. fluorescens improves Fe uptake in tomato, carnation, oats, vine, and maize.

The siderophore –producing and arsenate-reducing Pseudomonas sp. Bacterial strain plays a key role in the ability to convert arsenate to arsenite as well as promote plant growth and increase in the biomass of the fern Pteris vittata (Lampisetal., 2015). This suggests that the presence of rhizobacteria in soil can improve the efficiency of arsenic phytoextraction in hyperaccumulator plant species as well

Bioremediation Of Sewage

Sewage is a very complex mixture of wastes, usually dominated by fecal materials but also containing toxic chemicals that have been dumped into the disposal system by industries and home owners. Many advanced sewage-treatment technologies utilize microbial processes to both oxidize the organic matter associated with fecal wastes and to decrease the concentrations of soluble compounds or ions of metals, pesticides, and other toxic chemicals. The latter effect, decreasing the aqueous concentrations of toxic chemicals, is accomplished by a combination of chemical adsorption as well as microbial biodegradation of complex chemicals into their simpler, inorganic constituents. Microbial processes are relied upon in many sewage treatment systems including activated sludges, aerated lagoons, anaerobic digestion, trickling filters, waste stabilization ponds, composting, and disposal on land.

Bioremediation Of Acidification

In some situations, artificial wetlands can be engineered to treat acidic waters associated with coal mining or other sources of acidity. Coal mining disturbs soil and fractures rocks exposing large quantities of pyritic sulfur to atmospheric oxygen. Under such conditions, certain species of bacteria oxidize the sulfide of the mineral pyrites to sulfate, generating large quantities of acidity in the process known as acid mine drainage.

The resulting acidity is often treated by adding large quantities of acid-neutralizing chemicals such as lime or limestone. However, it has also been recently demonstrated that natural, acid-consuming, ecological processes operate in wetlands. These processes can be taken advantage of in constructed wetlands to decrease much of the initial acidity of acid mine drainages, and thereby reduce the costs of conventional treatments with acid-neutralizing chemicals.

The microbial processes that consume acidity are various, but they include:

(1) the chemical reduction of sulfate to sulfide at the oxygen-poor interface between the sediment and the water column and around plant roots,
(2) the reduction of ferric iron to ferrous in the same anoxic microhabitats, as well as
(3) the primary productivity of phytoplankton, which also consumes some acidity.

A less intensive type of bioremediation can be used to mitigate some of the deleterious ecological effects associated with the acidification of surface waters, such as lakes and ponds. In almost any fresh waters, fertilization with phosphate will greatly increase the primary productivity of algae and vascular plants. In acidic waters, this process can be taken advantage of to reduce the acidity somewhat, but the most important ecological benefit occurs through enhancement of the habitat of certain aquatic animals. Ducks and muskrat, for example, can breed very successfully in fertilized acidic lakes, because their habitat is improved through the vigorous growth of vegetation and of aquatic insects and crustaceans. However, the productive but still acidic habitat remains toxic to fish. In this case, manipulation of the ecosystem by fertilization mitigates some but not all of the negative effects of acidification.

Bioremediation Of Metal Pollution

Metals are common pollutants of water and land because they are emitted by many industrial, agricultural, and domestic sources. In some situations, organisms or ecological processes can be successfully utilized to concentrate metals that are dispersed in the environment, especially in water. The metals can then be removed from the system by harvesting the organisms. **For example**, metal polluted waste waters can be treated by encouraging the vigorous growth of certain types of algae, fungi, or vascular plants, usually by fertilizing the water within some sort of constructed lagoon. This bioremediation system works because the growing plants and microorganisms absorb metals from the water (acting as so called biosorbents), and thereby reduce their concentrations to a more tolerable range.

The plants can then be harvested to remove the metals from the bioremediation system. In some cases, the plant biomass may even be processed to yield metal products of economic value.

Bioremediation Of Spilled Hydrocarbons

Accidental spills of petroleum or other hydrocarbons on land and water are regrettable but frequent occurrences. Such spills can range in size from a few gallons that may be spilled during refueling to enormous spillages of millions of tons as occurred to both the sea and land during the Gulf War of 1991. Once spilled, petroleum and its various refined products can be persistent environmental contaminants. However, these organic chemicals can also be metabolized by certain microorganisms, whose processes transform the toxins into simpler compounds, ultimately to carbon dioxide, water, and other inorganic chemicals.

Numerous attempts have been made to increase the rates by which microorganisms break down spilled hydrocarbons. In some cases, specially prepared concentrates of bacteria that are highly efficient at metabolizing hydrocarbons have been "seeded" into spill areas in an attempt to increase the rate of degradation of the spill residues. Although this technique has sometimes been effective, it commonly is not. This is because the indigenous microbial communities of soils and aquatic sediments contain many species of bacteria and fungi that are capable of utilizing hydrocarbons as a metabolic substrate. After a spill, the occurrence of large concentrations of hydrocarbons in soil or sediment stimulates rapid growth of those microorganisms. Consequently, seeding of microorganisms that are metabolically specific to hydrocarbons does not always make much of a difference to the overall rate of degradation.

More important, however, is the fact that the environmental conditions under which spill residues occur are almost always highly sub-optimal for their degradation by microorganisms. Most commonly, the rate of microbial breakdown of spilled

hydrocarbons is limited by the availability of oxygen or of certain nutrients such as nitrate and phosphate. Therefore, the microbial breakdown of spilled hydrocarbons on land can be greatly enhanced by occasionally tilling the soil to keep conditions aerated and by fertilizing with nitrogen and phosphorus while keeping conditions moist but not wet. Thus, bioremediation systems for dealing with soils contaminated by spilled gasoline or petroleum can be based on simple tillage and fertilization.
Similarly, petroleum refineries may utilize a bioremediation process called land farming, in which oily wastes are spread onto land, which is then tilled and fertilized until microbes reduce the residue concentrations to an acceptable level.

After some petroleum spills, more innovative approaches may prove to be useful. For example, it is difficult to fertilize aquatic habitats, because the nutrients simply wash away and are therefore not effective for very long. In the case of the Exxon Valdez spill in Alaska in 1989, research demonstrated that nutrients could be applied to soiled beaches as an oleophilic (that is, oilseeking), nitrogen and phosphorus-containing fertilizer. Because of its oleophilic nature, the fertilizer adhered to the petroleum residues and was able to significantly enhance the rate of oil degradation by the naturally occurring community of microorganisms. This treatment was applied to about 73 mi (118 km) of oiled beach and proved to be successful in speeding up the process of degradation of the residues by increasing the rate of oxidation by about 50%. No attempts were made in this case to "seed" the microbial community with species specifically adapted to metabolizing hydrocarbons. It was believed that hydrocarbon-specific microbes were naturally present in the beach sediment and that their activity and that of species with broader substrate tolerances only had to be enhanced by making the ecological conditions more favorable, that is, by fertilizing.

BIOSORPTION

Many industrial activities result in heavy metal dispersion in the environment worldwide. Heavy metals are persistent contaminants, which get into contact with living organisms and humans creating serious environmental disorders. Metals are commonly removed from wastewaters by means of physical-chemical processes, but often microbes are also enrolled to control metal fate. When microorganisms are used as biosorbents for metal entrapment, a process called "biosorption" occurs. Biosorption efficiency is significantly influenced by many parameters such as environmental factors, the sorbing material and the metal species to be removed, and highly depends on whether microbial cultures are alive or dead. Moreover, the presence of biofilm agglomerates is of major importance for metal uptake onto extracellular polymeric substances. In this chapter, the effect of the above mentioned variables on biosorption performance was reviewed. Among the environmental factors, pH rules metal mobility and speciation. Temperature has a lower influence with an optimal value ranging between 20 and 35 °C.

The co-presence of more metals usually decreases the biosorption efficiency of each single metal. Biosorption efficiency can be enhanced by using living microorganisms due to the interaction with active functional groups and the occurrence of transport phenomena into the cells. The existing mathematical modeling approaches used for heavy metal biosorption were overviewed. Several isotherms, obtained in batch conditions, are available for modeling biosorption equilibria and kinetics. In continuous systems, most of the models are used to predict the breakthrough curves. However, the modeling of complex continuous-flow reactors requires further research efforts for better incorporating the effect of the operating parameters and hydrodynamics.

Introduction

Wastewaters from mineral processing and industrial activities are often characterized by high metal concentrations. Heavy metals are toxic and non-biodegradable compounds that can result in serious health disorders for human beings if over-discharged in the hydrosphere (Zhuang et al. 2009). Several physical-chemical processes, such as adsorption, coagulation, flocculation, ion exchange, membrane separation or precipitation can be used to treat heavy-metal containing wastewaters (Fu and Wang 2011). However, the use of microbes to rule metal mobility in the environment has recently gained increasing attention by the scientific community.

Among the bioremediation technologies used for metal immobilization and sequestration, biosorption has shown promising removal efficiencies with several heavy metals, e.g. Cd, Cu, Ni, Pb and Zn (Tsezos 2001; Pardo et al. 2003; Wang et al. 2006; Lakzian et al. 2008). Biosorption is a complex combination of processes aimed at the entrapment of a substance onto the surface of a living/dead organism or extracellular polymeric substances (EPS).

Many mechanisms contribute to the overall process, such as adsorption, precipitation and intracellular accumulation of metal compounds, with each mechanism significantly depending on

(i) the biosorbent used,
(ii) the substance to be sorbed,
(iii) pH and temperature,
(iv) presence of competing metals and ions and
(v) the possible presence of metabolic activity (Gadd 2009).

Many biological materials are suitable for maintaining biosorption due to high efficiency, cost effectiveness and particular affinity with metals, metalloids and other pollutants (Gadd 1990; Bailey et al. 1999). The potential of archaea, bacteria, fungi, algae, yeasts and agricultural wastes as biosorbents has been largely studied and reviewed (Fourest and Roux 1992; Wang and Chen 2009). Moreover, since most microorganisms live in the form of biofilms, the different nature of the cell agglomerates and the presence/composition of EPS further contribute to biosorption (Flemming 1995; Comte et al. 2008).

Besides metal removal, biosorption has also been used in metal recovery applications (Simmons and Singleton 1996; Mata et al. 2009). The high market price of some precious metals, e.g. gold, silver, platinum and palladium, implies the use of cheap technologies in order to maintain the operating costs low and reduce the amount of chemicals that other technologies require (Das 2010). Although the potential of biosorption in this direction appears to be enormous, the control of the operating parameters for the development of a selective metal biorecovery strategy in multi-metal systems needs further investigation.

There is a growing interest of the scientific community in biosorption, as reported by the increasing number of scientific publications in Fig. 2.1. In the first part of this chapter, the biological sorption mechanisms occurring onto microbial biomass (living and dead) and EPS are described. More specifically, the mechanisms involved in biosorption have been classified depending on the microbial metabolism and the location of occurrence. Furthermore, the existing relations between biosorption mechanisms have been individuated. The second part of the chapter focuses on the classification of the most used mathematical models for heavy metal biosorption on microbial biomass. Mathematical models are classified in two main categories: models for (*i*) batch and (*ii*) continuous systems.

The first category is further subdivided in sorption equilibrium models and kinetics batch models and summarizes the main modeling approaches introduced to reproduce the behavior of batch systems in terms of maximum sorption capacity and kinetics. Continuous models are aimed at reproducing the dynamic behavior of column bioreactors. Finally, a short analysis of further research directions and biosorption future perspectives are given.

The application of biosorption at the industrial scale has not been yet exploited, mainly due to the complexity of the mechanisms involved for both metal removal and recovery. Therefore, a mathematical model appears as a support tool to gain essential information for the identification of the key factors affecting biosorption efficiency and stability.

At the current state, mathematical tools have been developed to simulate biosorption by using isotherm models. **Langmuir and Freundlich isotherms** are the best known models but their application has been mainly addressed to simple batch systems (Liu and Liu 2008). The modeling of a complex system, such as a continuous-flow bioreactor, requires the use of more powerful mathematical tools. Thus, dynamic models, capable of simulating the biosorption mechanisms and the interaction between substances and biofilm agglomerates, are more appropriate.

The discovery and further development of biosorption phenomena provide a basis for a whole new technology aimed at the removal of various pollutants or the recovery of valuable resources from aqueous systems. Today, biosorption is one of the main components of environmental and bioresource technology.

Microbes have been widely used in the process of environmental clean-up and are known as bioremediators. Their ability to absorb metal ions from aqueous solutions either as living or dead biomass as well as derived products has been exploited.

Physico-chemical mechanisms of removal, which may be encompassed by the general term 'biosorption", include adsorption, ion exchange and entrapment which are features of living and dead biomass as well as derived products. In living cells, biosorption can be directly and indirectly influenced by metabolism.

Pioneering research on biosorption of heavy metals at **McGill University** in Montreal has led to identification of a **number of microbial biomass** types which are extremely effective in concentrating metals. **Some of the biomass** types come as a waste by-product of large-scale industrial fermentations (the mold *Rhizopus* or the bacterium *Bacillus subtilis*). **Other metal-binding** biomass types, certain abundant seaweeds (particularly brown algae e.g. *Sargassum, Ecklonia*), can be readily collected from the oceans. These biomass types, serving as a basis for metal biosorption processes, can accumulate in excess of 25% of their dry weight in deposited heavy metals: Pb, Cd, U, Cu, Zn, even Cr and others. Research on biosorption is revealing that it is sometimes a complex phenomenon where the metallic species could be deposited in the solid biosorbent through different sorption processes of ion exchange, complexation, chelation, microprecipitation, etc.

Definitions:

Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions.

Biosorption is the binding and concentration of heavy metals from aqueous solutions even very dilute ones) by certain types of inactive, dead, microbial biomass.

Biosorption: the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells.

Biosorption: the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake .

Biosorption: the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells. Metal sequestering by different parts of the cell can occur via various processes: complexation, chelation, coordination, ion exchange, precipitation, reduction.

Biosorption is the property of certain biomolecules (or types of biomass) to bind and concentrate selected ions or other molecules from aqueous solutions. As opposed to a much more complex phenomenon of bioaccumulation based on active metabolic transport, biosorption by dead biomass (or by some molecules and/or their active groups) is passive and occurs primarily due to the 'affinity' between the biosorbent and adsorbate.

Biosorption is a process in whichsolids of natural origin are employed for binding heavy metals. It is a promising alternative method for treating industrial effluents, mainly because of low cost and high metal binding capacity.

Biosorption is a rapid phenomenon of passive metal sequestration by non-growing biomass.

Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria which was found responsible for this phenomenon. **Opposite to biosorption** is metabolically driven active **bioaccumulation** by living cells. That is an altogether different phenomenon requiring a different approach for its exploration.

Biosorption is possible by both living and non-living biomass. **Bioaccumulation** is a growth dependent process and **Biosorption** involves mechanism like ion exchange, chelation and complexation .

Metal binding appears to be at least a **two-step process**, Where 1-**stoichometric** interaction between the metal and the reactive chemical groups in the cell wall, 2- an **inorganioc deposition** of increased amount of metal.

Thus:

Bioaccumulation is an active metabolic process driven by energy from a living organism and requires respiration It occurs by absorbing contaminants which are transferred onto and within the cellular surface.

Biosorption is a metabolically passive process, and the amount of contaminants a sorbent can remove is dependent on kinetic equilibrium and the composition of the sorbents cellular surface.

1. reversible process
2. occurs at a faster rate
3. produce higher concentrations

Heavy metals are chemical elements with a specific gravity that is at least 5times the specific gravity of water. **Examples**: **Arsenic 5.7, Cadmium 8.65, Iron 7.9, Lead 11.34 Mercury 13.546.**

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Toxic metals are Cr, Cu, Mn, Ni, Sn and Zn. once dispersed in there biosphere, these metals cannot be recovered or degraded. Hence, environmental effects of metal pollution are said to be permanent.

Symptoms of Heavy metal Toxicity:

mental confusion, headaches , short term memory loss, pain in the muscles and joints, gastrointestinal upsets, food intolerances, vision problems, chronic fatigue or allergies.

Heavy metal, present in different types of industrial effluents, is responsible for environmental pollution. Traditionally, metal removal was made by chemical precipitation.

Three countries, the United States, Germany and Russia,with only 8% of the world's

population consume about 75% of the world's most widely used metals.

Table 2. Comparison of the features of biosorption and bioaccumulation.

Features	Biosorption	Bioaccumulation
Cost-effectiveness	High, as biosorbents used are mainly waste biomass released from industrial, agricultural and other sources. Cost involves mainly transportation and other simple processing charges.	Low, as the living-cell maintenance is cost prone.
pH	Metal uptake is strongly influenced by pH; however, process can be operated under wide range of pH conditions.	In addition to uptake, the living cells themselves are affected under extreme pH conditions.
Temperature	No influence.	Severely affected.
Maintenance	Easy, as biomass is inactive.	External metabolic energy is needed in maintenance of culture.
Selectivity	Poor, but can be improved by modification/processing of biomass.	Better than biosorption.
Versatility	Good, as the binding sites can accommodate a variety of ions.	Not very flexible, as the process is prone to high metal/salt conditions.
Uptake capacity	Very high, as biomasses are reported to accommodate an amount of toxicant nearly as high as their dry weight.	Low, as living cells are sensitive to high toxicant concentration.
Uptake rate	Usually rapid.	Usually slower than biosorption, as intracellular accumulation is time-consuming.
Regenerability and reusability	High with possible reuse over a number of cycles.	Low, as toxicants are intracellularly accumulated.
Toxicant recovery	Possible, but with proper selection of eluant.	Not possible.

The complex structure of microorganisms implies that there are ways for the metal to up taken up by the microbial cell.

According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

1.Metabolism dependent
2.Non -metabolism dependent.

Physical adsorption:

Physical adsorption takes place with the help of van der Waals' forces. In 1998, Kuyucak and **Volesky** hypothesized that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomasses of algae, fungi and yeasts takes place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells.

Some of the key features of biosorption compared to conventional processes include:
1. competitive performance
2. heavy metal selectivity
3. cost-effectiveness
4. regenerative
5. no sludge generation.

Biosorption is particularly economical and competitive for environmental applications in detoxifying effluents from, for example:

1. metal plating and metal finishing operations
2. mining and ore processing operations

3. metal processing
4. battery and accumulator manufacturing operations
5. thermal power generation (coal-fired plants in particular)
6. nuclear power generation.

Biosorbents can be classified into:
a. Inactive organisms (mainly) include algae, fungi and bacteria
b. Their derivatives which are termed as **biopolymers**.

What are typical biosorbents ?

1. Some of the biomass types come as a waste by-product of large-scale industrial fermentations (the mold *Rhizopus*, the bacterium *Bacillus subtilis* and waste activated
2. sludge).
3. Other metal-binding biomass types, certain abundant seaweeds (particularly brown algae e.g. *Sargassum*, *Ecklonia*), can be readily collected from the oceans.
4. Biopolymers are normally extracted from inactive organisms and processed before use (e.g. *Ca-Alginate*)
5. These biosorbents can accumulate in excess of 25% of their dry weight in deposited metals: Pb, Ag, Au, U, Cu.

Types of native biomass that have been used for preparing biosorbents

Category	Examples
Bacteria	Gram-positive bacteria (*Bacillus* sp., *Cornynebacterium* sp., etc) Gram-negative bacteria (*Escherichia* sp., *Pseudomonas* sp., etc), Cyanobacteria (*Anabaena* sp., *Synechocystis* sp., etc.)
Fungi	Molds (*Aspergillus* sp., *Rhizopus* sp., etc), Mushrooms (*Agaricus* sp., *Trichaptum* sp., etc.) and Yeast (*Saccharomyces* sp., *Candida* sp., etc.
Algae	Micro-algae (*Clorella* sp., *Chlamydomonas* sp., etc.) Macro-algae (green seaweed (*Enteromopha* sp., *Codium* sp., etc.), brown seaweed (*Sargassum* sp., *Ecklonia* sp., etc.) and red seaweed (*Geildium* sp., *Porphyra* sp., etc.))
Industrial wastes	Fermentation wastes, food/beverage wastes, activated sludges, anaerobic sludges, anaerobic sludges, etc.
Agricultural wastes	Fruit/vegetable wastes, rice straws, wheat bran, soybean hulls, etc.
Natural residues	Plant residues, sawdust, tree barks, weeds,
Others	Chitosan-driven materials, cellulose-driven materials, etc.

Parameters Affecting Biosorption

For each biosorbent, many factors can influence metal uptake onto biomass at different rates determining the overall biosorption performance (Park et al. 2010). The influence of some important parameters affecting biosorption is described in the following sections and reported in Table 2.1.

1. pH

pH highly influences metal biosorption and is probably the most important parameter controlling biosorption extent. Indeed, pH plays a crucial role in defining

the chemical properties of metals, the availability of biomass functional groups and the competition among metallic ions for adsorption sites (Friis and Myers-Keith 1986; Galun et al. 1987; Comte et al. 2008).

Generally, low pH keeps metals in solution, represses microbial activity and increases the competition between cations for binding sites resulting in lower biosorption efficiencies (Gadd and White 1985). However, biosorption of anionic metal species (e.g. $CrO4^{2-}$ and $SeO4^{2-)}$ is often enhanced under acidic conditions. As pH rises, the biosorptive removal efficiency of cationic metals increases albeit may induce metal precipitation at a pH above 7.0 (Park et al. 2010). In some cases, biosorption is pH independent. For instance, Ag+, Hg2+ and AuCl4 − form strong covalent complexes with the biosorbent resulting in a negligible effect of pH (Gadd 2009).

2. Temperature

In contrast with the effect of pH, influence of temperature on biosorption is observed in the range 20–35 °C (Aksu et al. 1992). A better uptake can however be achieved by increasing the operating temperatures up to 50 °C (Tsezos and Volesky 1981), although high temperatures have shown contrasting effects on biosorption system behavior. For instance, a high temperature determines an increase in surface activity and kinetic energy of the adsorbate, although the biosorbent structural integrity can be irreversibly damaged (Park et al. 2010). Moreover, the effect of temperature on the biosorption efficiency depends on whether living or dead cells are used. A higher temperature normally results in a higher number of cells and an enhanced biosorption efficiency. However, the impact of other parameters (i.e. pH) has to be simultaneously evaluated (Congeevaram et al. 2007).

3. Biosorbent Dosage and Size

The concentration of the biomass used as sorbent is also an important parameter to take into account for evaluating biosorption performance. A high biomass content increases the overall biosorption efficiency but also causes interference between the binding sites with a consequently lower specific uptake (Gadd et al. 1988; Park et al. 2010). With regard to the size of the biosorbent, small particle sizes are desirable in batch assays in order to increase the surface area and enhance the contact between metals and sorbent. On the other hand, in continuous-flow applications the use of small bio-particles results in channeling and clogging of the systems affecting biosorption efficiency (Park et al. 2010).

4. Metal Ions Coexistence and Metal Speciation

The removal of ionic metal species can be affected by the coexistence of further metal ions or other anions. Many studies are contradictory and not all biosorption mechanisms are well understood. This is because the presence of more metals only plays an indirect role on the biosorption of a particular species as the influence of pH or other parameters is often more relevant. Generally, the concomitant presence of several metals affects the biosorption efficiency of single metals, whilst no influence is observed on the total metal binding capacity (Akthar et al. 1996).

For instance, uranium uptake onto *Rhizophus arrhizus* cells is particularly affected by the presence of Fe2+ and Zn2+ (Tsezos and Volesky 1982). Similarly,

inhibition of cobalt uptake has been observed in the presence of uranium, lead, mercury and copper whereas no effect on uranium uptake has been revealed in the presence of the same metals (Sakaguchi and Nakajima 1991).

The solubility, mobility and bioavailability of metals also change depending on **metal speciation**. Anions like chloride or carbonate can influence metal speciation promoting the formation of complexes and affecting the extent of biosorption. Furthermore, the **concomitant** presence of other ions hinders biosorption yields. Phosphate may affect biosorption since PO_4^{3-} has been reported to compete with some metals (such as As(V)) for binding sites or form insoluble metal precipitates (Darland and Inskeep 1997; Gadd 2009; Papirio et al. 2014).

Biosorption Mechanisms

Besides the factors described above, biosorption mechanisms strongly depend on the metabolism of microbial cells and the location of metal removal (Ahalya et al. 2003; Abbas et al. 2014). Biosorption can be (i) metabolism dependent or (ii) non-metabolism dependent according to the activity of biomass. Depending on the location of the metal removal, biosorption can occur via the mechanisms proposed by Ahalya et al. (2003):

(a) Extra cellular accumulation/precipitation;
(b) Cell surface sorption/precipitation;
(c) Intracellular accumulation.

Mechanism of Biosorption Activity

Biosorption can be performed by many mechanisms occurring under different operating and environmental conditions. Due to the complexity of the biological materials used as biosorbents and the wide range of parameters affecting biosorption, the specific mechanisms involved are usually hard to determine (Gadd 2009).

However, biosorption can occur through a single mechanism or a combination of several processes such as adsorption, chelation, complexation, electrostatic interaction, ion exchange and microprecipitation (Veglio and Beolchini 1997; Volesky 2001; Wang and Chen 2006; Vijayaraghavan and Yun 2008).

Thus the mechanism of biosorption is complicated and not fully understood, the following have been used to describe the mechanism of biosorption activity;

1. Complexation
2. Chelation
3. Coordination
4. Ion Exchange
5. Microprecipitation
6. Reduction

I. COMPLEXATION

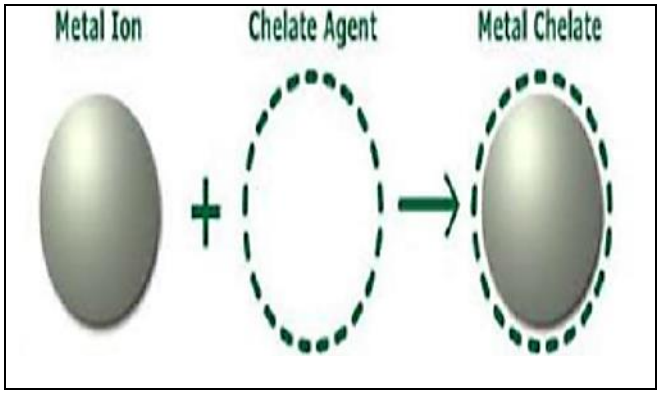
- May be electrostatic or covalent
- Complex formation of metal ions with organic molecules involves ligand centers in the organic species i.e. the presence of an atom or atoms having lone pair electrons to donate.

- Hardsoft acid-base classification used to explain this.
- Hard acids metals (Na, K, Ca, Mg) essential nutrients for microbial growth.

2. CHELATION

Complex formation with multidentate ligands is termed **chelation**; complexes are chelates. It is the formation of metal ion complex in which the metal ion is associated with a charged or uncharged electron donor referred to as ligand. Furthermore, the coordination number refers to the number of ligand atoms surrounding the central atom, where most metal cations engage in coordinations of 2, 4, 6, and 8, with 4 and 6 being the most common. In the case of polymers these values may be lower due to steric effects. A proton complex has a coordination number of one, as opposed to the higher coordination numbers found in metal complexes. **Although** this terminology is typically employed for aqueous **complexation** with small ligands, the terms are often applied in the literature when dealing with more complex molecules, thus this outline is intended to serve as a basis for its usage.

A chelate is a cyclic complex formed between a metal and a compound that contains two or more ligands (binding site).



- Organic molecules containing more than one functional group with donor electron pairs can simultaneously donate these to a metal atom. This can result in the formation of a ring structure involving the metal atom a process termed 'CHELATION'
- Chelated compounds (eg EDTA) are more stable than complexes involving monodentate ligands.
- Stability tends to increase with the number of chelating sites available on the ligand.

The terms **inner-sphere** and **outer-sphere** complex are used to distinguish between binding which is, respectively, largely covalent in character or chiefly electrostatic in nature.

In the first case, the interacting ligand is immediately adjacent to the metal cation.

In the second case, ions of opposite charge are attracted and approach each other within a critical distance and effectively form what is termed an ion pair. In outer-sphere complexes, the metal ion or the ligand or both generally retain

their coordinated water when the complex is formed. In other words, the metal ion and the ligand are most often separated by one or more water molecules.

3. CO-ORDINATION

Any combination of cations with molecules or anions containing free electron pairs (bases) is termed coordination, also known as complex formation. Coordination or complex formation, in turn, may be either electrostatic (i.e. Coulombic) or covalent in character. The heavy metal cation that is bound is often designated as the central atom, and is distinguished from the anions or molecules with which it forms a coordination compound, the ligand(s). When the ligand is composed of several atoms, the atom responsible for the basic or nucleophilic nature of the ligand is termed the ligand atom. A base containing more than one ligand atom, a multidentate complex, may occupy more than one coordination position in the complex.

- Metal atoms have preferences for specific donor atoms ("hard/hard" /"soft/soft") and the stereochemical arrangements that play an important role in the binding with the available ligands on the microbial cell.
- Limited information of surface complexation models, based on the theory of surface coordination chemistry, is available to describe metal biosorption; however the preferences of the metal species should be considered to explain observed metal biosorption capacities and to elucidate biosorption mechanisms

4. ION EXCHANGE

The gram-positive bacteria, principally members of the genus Bacillus, have enhanced capacity for metal binding because of a significant negative charge density. This is due to the structure of the cell wall with teichoic and teichuronic acids attached to the peptidoglycan network. The phosphodiesters of teichoic acid and the carboxylate groups of the teichuronic acid thus contribute ion-exchange capacity to the cell wall.

1. Cation exchange

- In biopolymers the most likely cation binding ionisable groups to be involved are: carboxyl, organic phosphate and organic sulphate.
- Carboxylic acids are widely distributed in biopolymers being most commonly found as side-chain constituents of proteins, the uronic, neuraminic and muramic acids, and related substituted monosaccharides of polysaccharides.
- Phosphodiester links impart negative charge to the nucleic acid backbone while both diester and monoester groupings are found most commonly in bacterial polysaccharides and related macromolecules.
- Lipoprotein and lipopolysaccharides are also likely to contain phosphodiester as part of the lipid moiety.

2. Anion exchange.

- On biopolymers can take place on a variety of organic nitrogen- based groupings.

- In proteins, amino (Lysol side chain and N-terminal) imidazole (histidyl) and guanidine (arginyl) groupings are common centres of positive charge.
- Centres of positive charge in nucleic acids will occur with protonation of amino groups on purine or pyrimidine rings or with protonation of heterocyclic nitrogen atoms.

5. PRECIPITATION

- The term precipitation in most cases refers to the formation of insoluble inorganic metal precipitates. However, in the case of metal biosorption by microbial cells, organic metal
- precipitates may also be formed. This may be more easily understood when metals are bound to Extracellular Polymeric Substances (EPS) excreted by some prokaryotic (bacteria, archaea) and eukaryotic (algae, fungi) microorganisms.
- The precipitates may be formed and remain in contact with or inside the microbial cells or may be independent of the solid phase of the microbial cell. In the later case, the presence of the solid phase-microbial cell or biofilm also plays a favourable role in the phenomenon of precipitation.

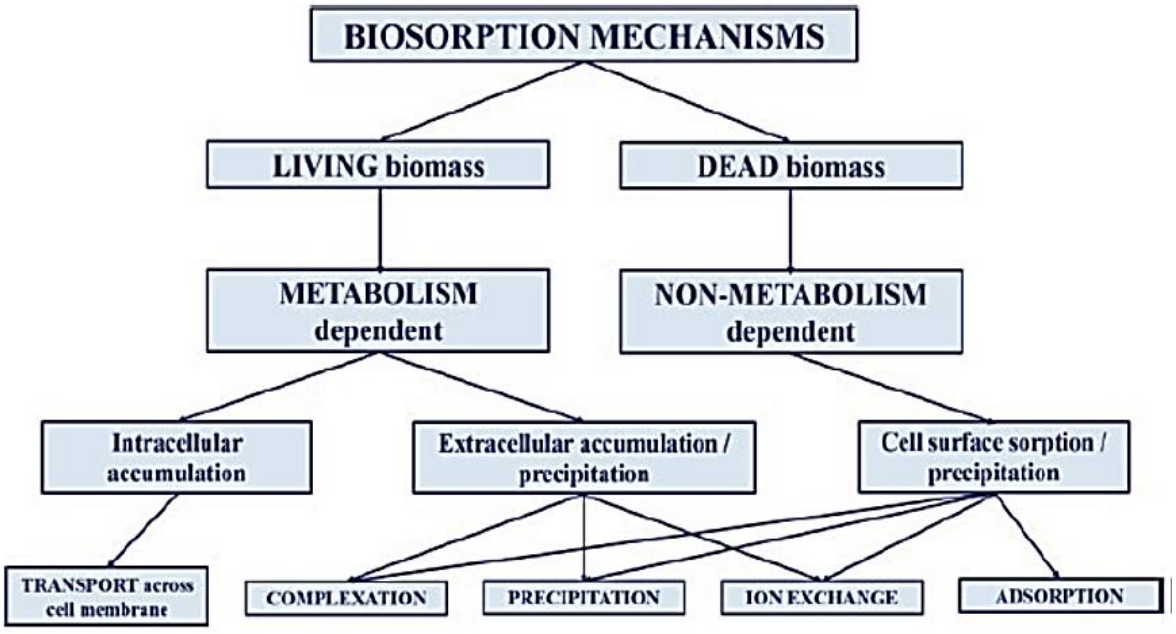
6. REDUCTION

- The biosorption mechanism is a two-step process: initiation of the uptake at discrete points by chemical bonding, then reduction of the metal ions .
- Also soft metals like gold and palladium are first bound on sites on and within the cell wall and these sites act as nucleation points for the reduction of metals and growth of crystals and elemental gold and palladium have been obtained.

The key factors controlling and characterizing these mechanisms are;

1. The type of biological ligands available for metal sequestering;
2. The status of the biomass, i.e. living /non-living;
3. The chemical, stereochemical and coordination characteristics of the targeted metals and metal species;
4. The characteristics of the metal solution such as pH and the presence of competing co-ions.

The following Figures report a flow diagram and a schematic illustration, respectively, of the biosorption mechanisms depending on the presence/absence of metabolism and the location of the metals sorbed.



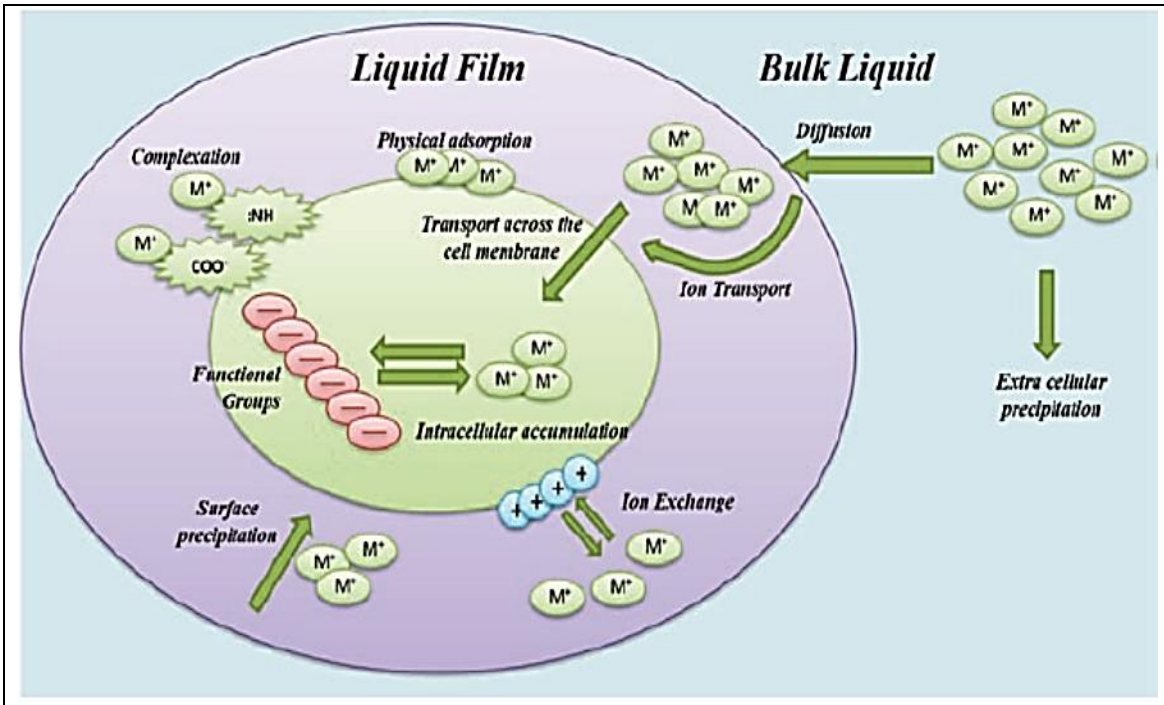
Biosorption mechanisms correlation according to metabolism and metal removal location. Note the complexity of the process and the high number of the concomitant mechanisms contributing to the extent of biosorption.

Despite the different classification, all biosorption mechanisms are strictly related to each other and can occur with both metabolic and non-metabolic microbial activity.

Non-metabolism dependent mechanisms are mainly rapid and reversible physical-chemical interactions between metals and the functional groups on the cell surface (Kuyucak and Volesky 1988). On the contrary, intracellular uptake phenomena (**bioaccumulation**) are ruled by cellular metabolism and occur at lower rates (Goyal et al. 2003). Metal precipitation can be either affected by microbial metabolism, when performed with compounds produced by microorganisms (Sag and Kutsal 2001), or not when a simple chemical interaction between metals and cell surface takes place (Scott and Palmer 1990).

The mode and characteristics of **metal binding** to biomass also depend on the presence of other substances and the condition of the biomass itself. Besides the cellular wall, metal binding can also occur onto extracellular polysaccharides (McLean et al. 1992) and is different when related to living or dead biomass (Das et al. 2008).

In the next subsections, the interactions between metals and microorganisms will be deepened. In particular, the attention will be focused on the differences between metal sorption onto cell walls and EPS. Since biosorption primarily occurs on the microbial surface, it is of major importance to know whether microbes are present as single cells or complex agglomerates, e.g. flocs, granules or biofilms, held together by EPS. Moreover, some important differences between metal removal occurring onto living and dead biomass will be outlined.



Schematic illustration of the main mechanisms involved in biosorption. Metabolism-dependent mechanisms include the transport across the cell membrane and intracellular accumulation by reacting with active functional groups. In contrast, ion exchange, physical adsorption and complexation also occur in the absence of cellular metabolism

Metal: Cell Wall Interaction

Bacterial cell wall is fundamental for cell integrity and is characterized by the presence of ***N*-acetylmuramic** acid (peptidoglycan) and **poly-*N*-acetylglucosamine** right out of the cytoplasmic membrane (Rogers et al. 1980). 10 to 20% and 90% of the cell wall of Gram-negative and Gram-positive bacteria, respectively, is made by **peptidoglycan** (Kolenbrander and Ensign 1968; Vijayaraghavan and Yun 2008). The remaining part of the outer membrane consists of **phospholipids** and **lipopolysaccharides** (Sheu and Freese 1973). The membranes are made of **anionic** functional groups which are the main elements determining the capability of cell walls to bind metals (Beveridge
and Murray 1976; Sherbert 1978). **Hard metals** preferentially bind with oxygen-containing ligands, whereas **soft metals** bind with nitrogen- or sulfur-containing ligands (Avery and Tobin 1993).

As an example, **carboxylic groups** observed on the *Streptomyces pilosus* cell walls clearly affect **Cu and Pb removal** as reported by Golab et al. (1995). Similarly, **Cd reduction and Pb uptake** can be efficiently performed by dried *Sargassum* species characterized by a high amount of carboxylic groups (Fourest and Volesky 1996). Besides COOH groups, the presence of Cu on the microbial cell surface is due to the interaction with **phosphoryl groups** (Mullen et al. 1989). **Amine groups** also contribute to metal biosorption, especially the binding of Cr ions by using *Pseudomonas aeruginosa* (Kang et al. 2007).

The chemical modification of **lipids**, **carboxylic** and **amino** functional groups affects Pb, Cu and Cd biosorption (Mashitah et al. 1999). After the **esterification** of

carboxyl and **methylation** of amine groups, a lower metal biosorption is observed (Kapoor and Viraraghavan 1997).

Different binding groups are involved in Cd biosorption depending on pH (Boyanov et al. 2003). Cd is mainly adsorbed onto **phosphoryl sites** at a pH of 3.4, whereas Cd interaction with carboxyl groups prevails at a pH ranging from 5.0 to 7.8. The biosorption efficiency also depends on the microbial culture and metal used. For instance, *B. subtilis* and *B. licheniformis* cell walls bind approximately 30 times more Cu2+ than *E. coli* (Beveridge and Fyfe 1985). Regarding the different metals used, *Staphylococcus aureus* cell walls have a higher affinity with Ce3+ than Cu2+.

In **conclusion**, the role of the cell wall functional groups, the particular microbial species and the metallic contaminant used are fundamental for metal binding. A careful selection of the most appropriate biosorbent is thus essential for achieving a proper process efficiency.

Metal Interaction with Living and Dead Biomass

The extent of metal removal is substantially different when biosorption is performed with living or dead biomass. Metal uptake onto dead cells is mainly carried out through the interaction between the cell wall and contaminant (Veglio and Beolchini 1997). In contrast, biosorption with living cells is a more complex combination of processes.

Biosorption can occur through the interaction with active amine and sulfhydryl functional groups, denatured in dead biomass, onto the cell wall and/or through different transport phenomena for intracellular accumulation (Gadd 2001, 2009). Furthermore, the biosorption performance can be **positively or negatively affected** by **metabolic activities** (e.g. respiration, nutrient uptake and metabolite release) which specifically modify the cell surrounding environment.

The predominant biosorption mechanism and efficiency also depend on the operating conditions, metal type and concentration as well as the microbial culture used as reported in Table 2.2.

Living cells perform Cu uptake better than dead cells mainly through intracellular accumulation (Golab et al. 1995). **Cu accumulation** has been observed in the cytoplasmic fraction of algae (Nakajima et al. 1979) and yeasts (Naiki and Yamagata 1976). **Strontium** biosorption is more effective with living biomass as covalent bonds are formed instead of weaker ionic bonds with denatured biomass (Avery and Tobin 1992).

The use of living cells, however, results in lower metal sorption efficiencies in non-buffered systems under acidic conditions. For instance, a higher Cr6+ biosorption by dead cells of two Bacillus sphaericus strains (OT4b31 and IV(4)10) has been reported at a stable pH of 4.0 (Srinath et al. 2002; Velسsquez and Dussan 2009), mainly owing to the good interaction between metal and biomass under acidic conditions. Cr6+ uptake is indeed driven by acid adsorption for the higher concentration of H+ involved in the anion exchange (Sharma and Forster 1993; Kratochvil et al. 1998). The metabolic activity of cells usually leads to an increase of pH that decreases the biosorption efficiency.

Besides Cr6+, the enhancement of metal uptake when using dead cells of *Myxococcus xanthus* and *Saccharomyces cerevisiae* has been reported for other metals such as La3+, Co2+, Mn2+, Pb2+, Co2+, Ag+, Zn2+, Cd2+, Cr3+ and Ba2+ (Omar et al. 1997).

With regard to **metal recovery**, the use of living and dead cells results in a different heavy metal desorption efficiency. It is generally reported that the metals intracellularly entrapped cannot be extracted from biomass, unless microbial cells are disrupted (Wong et al. 1993; Costley and Wallis 2001). Therefore, a lower metal recovery has been observed with living biomass, as intracellular accumulation is the main mechanism for metal biosorption.

For instance, the **desorption efficiency** of Cu2+ and Zn2+ from dead cells of *Pseudomonas putida* CZ1 was 95.3 (±2.6)% and 83.8 (±4.3)%, respectively. Conversely, desorption only reached 72.5 (±1.8)% and 45.6 (±1.2)% for Cu2+ and Zn2+, respectively, by using living cells (Chen et al. 2005). Similarly, a higher desorption efficiency was also observed for Cd2+ by using dead (91.2%) than living (70.2%) *Bacillus cereus* RC-1 cells (Huang et al. 2013).

Interaction Between Metals and Extracellular Polymeric Substances

The presence of EPS results in increasing biosorption yields, especially in systems involving bacterial colonies forming biofilms. EPS are biopolymers produced by cell activities such as active bacterial secretion, shedding of cell surface and cell lysis materials. Moreover, EPS can derive from organics adsorption from the environment (Wingender et al. 1999). The composition of EPS is made of organic substances with a higher amount of carbohydrates and proteins and a smaller fraction of humic, uronic and nucleic acids (Sponza 2002). EPS have an abundance of negatively charged functional groups that make them a potent biosorbent to be used for metal sorption (Ledin 2000; Flemming and Wingender 2003; Wang et al. 2014).

EPS can be divided into two main groups: bound and soluble. The bound EPS mainly consist of organic matter produced by microbes and attached to the microbial aggregates (Nielsen et al. 1997). Soluble EPS are composed by hydrolyzed products from attached organic matter, organic molecules released by cell lysis and soluble polymers produced by microbes (Comte et al. 2006). Besides different characteristics, the two groups have different metal biosorption efficiency: Cu2+, Pb2+, Ni2+ and Cd2+ removal is higher with soluble EPS than bound EPS (Comte et al. 2006). A better protonic ion exchange of soluble EPS results in more dissociated sites for metal entrapment and thus a higher biosorption efficiency. In contrast, a higher biosorbed Pb(II) and Cu(II) percentage for attached compared to suspended biomass can be obtained at various metal concentrations (Black et al. 2014). This mainly occurs when a higher EPS content is in the attached than suspended biomass. Similarly, a higher biosorption efficiency for Cu(II) can be observed in the bound (subdivided in loosely and tightly bound EPS) than soluble EPS for both wild-type and mutant type strains (Hou et al. 2013).

The interaction of EPS with heavy metals also depends on pH. Besides modifying the chemical properties of metals, the pH affects the activity of functional groups in biopolymers and the competition of heavy metals for the biosorbent sites

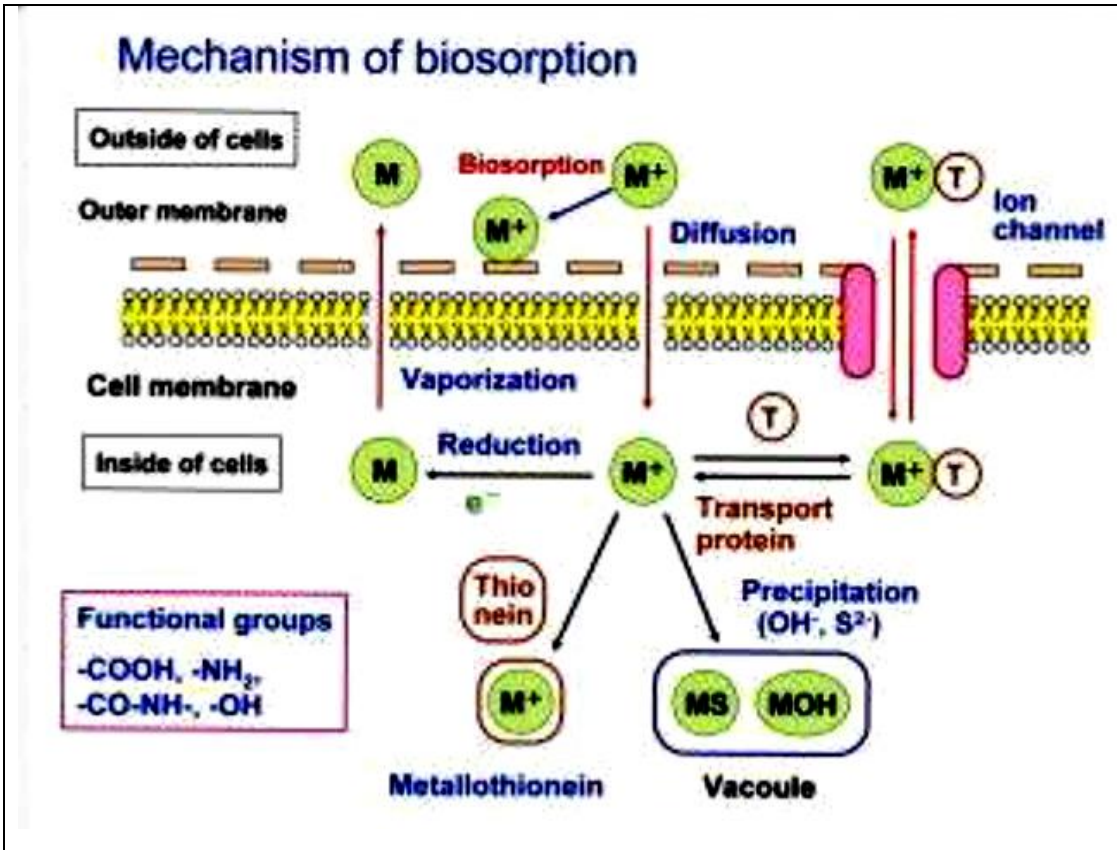
(Salehizadeh and Shojaosadati 2003). An increase of Pb and Hg uptake can be achieved at acidic pH by EPS of *Azotobacter chroococcum XU1* (Rasulov et al. 2013). On the contrary, a lower metal biosorption at alkaline pH is mainly attributed to metal precipitation as hydroxide (Salehizadeh and Shojaosadati 2003). Feed metal concentration and the coexistence of other metals also affect biosorption onto EPS (Rasulov et al. 2013). Pb and Hg biosorption increases at increasing metal concentrations although the saturation of the binding sites occurs more quickly (Lakzian et al. 2008). But, the simultaneous supplementation of Ni and Zn highly affects the adsorption of Pb and Hg onto EPS of *Ensifer meliloti* MS-125 (Lakzian et al. 2008). Depending on the environmental conditions the bacterial strains are isolated from, biosorption of a particular metal is preferred. For instance, the EPS of *Rhizobium etli*, isolated from a manganese rich environment, show a preferential uptake of Mn2+ compared to Zn2+, Pb2+, Co2+, Ni2+ and Cu2+ (Foster et al. 2000).

Metal biosorption by EPS can occur through a set of processes such as complexation, ion exchange and surface precipitation (Li and Yu 2014). Metal removal by ion attraction significantly depends on pH (Pardo et al. 2003). Alkaline conditions generally favor ion exchange due to the lower presence of protons competing with metals for the binding sites (Lopez et al. 2000). However, high pH leads to the transformation of soluble metals into hydroxylated monomeric and polymeric species and then into crystalline oxides that precipitate, resulting in lower amounts of metal sorbed (Kushwaha et al. 2012). Regarding metal complexation, metal binding occurs due to the deprotonated form of the reactive sites (Morlay 2000). Metal-EPS complexation can be related to the concepts of hard and soft acids and bases, assuming that inner and outer-sphere complexes are those produced during the metal binding with EPS (Avery and Tobin 1993). Complexes and bond typology can be different according to the metals and the functional groups involved. Nitrogen in the amino-sugar and oxygen in the hydroxyl and carboxyl groups mainly bind metals with strong covalent characteristics (e.g. Pb2+, Cu2+ and Zn2+) forming inner-sphere complexes (Ha et al. 2010; Fang et al. 2011). On the contrary, metals such as Cd2+ and Ni2+ form weak covalent bonds with EPS showing low adsorption affinity (Joshi and Juwarkar 2009).

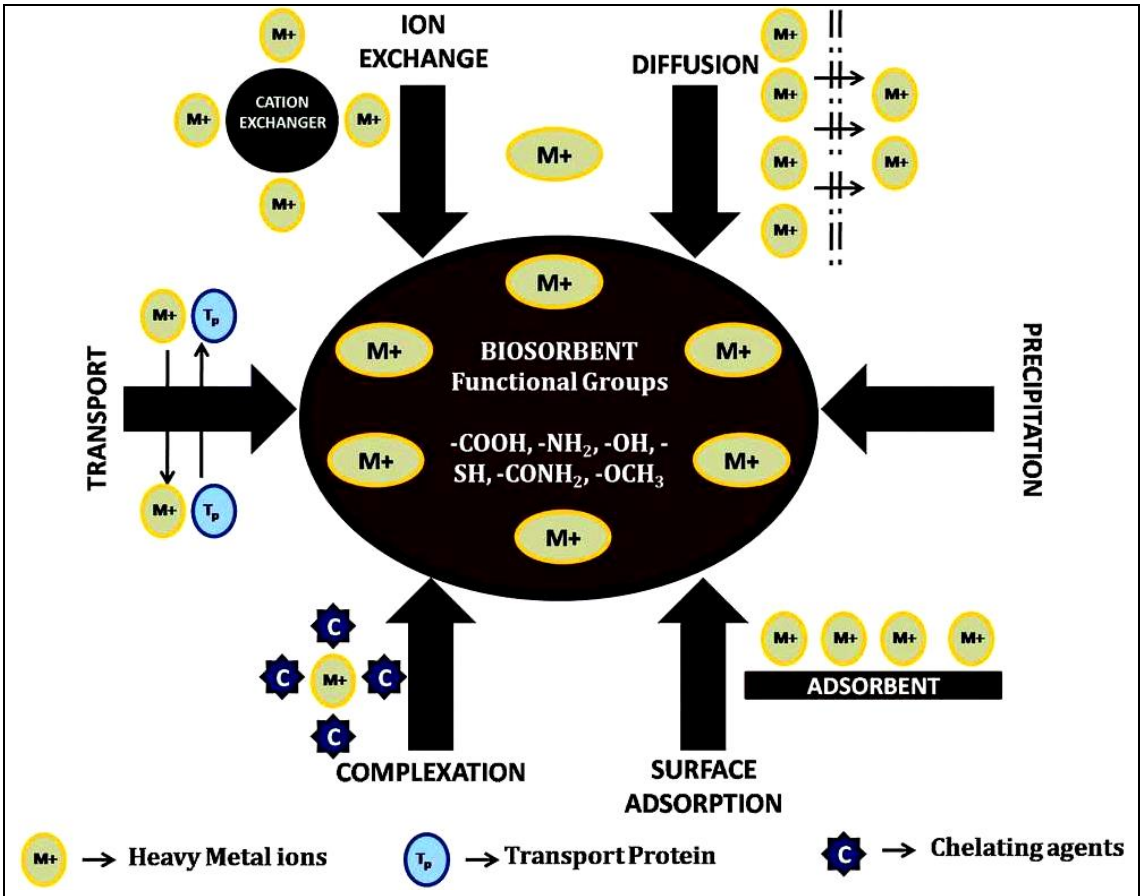
EPS composition and related functional groups significantly depend on various parameters, such as cell cultivation time, presence of organic substrates (e.g. volatile fatty acids), salt concentration (e.g. NaCl and CaCl2), and C/N ratio (Sheng et al. 2006). A different EPS composition results in a variable binding capacity of EPS towards metals (d'Abzac et al. 2013). The metal species to be biosorbed also influences the EPS composition in terms of proteins/carbohydrates ratio (Sheng et al. 2005). Therefore, all the operating conditions adopted in a particular study case affect the chemical nature of EPS and the extent of heavy metal biosorption.

Desorption of metals previously entrapped onto EPS can decrease biosorption performance. A study on Hg2+ and Sb5+ adsorption/desorption has shown that pH, temperature and the coexistence of chelating agents and competitive cations affect the process efficiency (Zhang et al. 2013). A pH shock highly affects Hg desorption under acidic conditions, whereas no effect has been observed in alkaline systems. In the same way, Hg desorption can occur in the presence of complexing agents (e.g. ethylenediaminetetraacetic acid) or cations competing for the binding sites (e.g. Ca2+). Unlike pH, a temperature range from 10 to 30 °C does not affect Hg and Sb entrapment, indicating insignificant effects of temperature on biosorption (Zhang et al. 2013).

Metal biosorption by EPS cannot be considered as a reversible process as metals can form stable complexes with EPS. This results in a less efficient metal biorecovery. But, on the other hand, the irreversibility of heavy metal biosorption generally leads to a lower metal release in the environment (Malik 2004). A hysteresis trend in the sorption/desorption of Cd and Pb from EPS has been observed after a significant variation of pH (from 11.8 to 2.1) resulting in about 30% of metals irreversibly sorbed onto EPS (Guibaud et al. 2008). Despite the possibility of stable metal-EPS complexes formation, metal desorption occurs and has to be taken into account besides the influence of several operational parameters.



Mechanism of biosorption



Metal sequestering by different parts of the cell.

BIOSORPTION MECHANISM IN FUNGI

Fungal cell walls are made mostly of polysaccharides, which constitute typically about 80% of the dry weight, to which a wide array of different proteins, often heavily glycosylated, are anchored in various ways. These proteins are present in lower proportions, 3–20%, and lipids, pigments and inorganic salts are present in much smaller amounts Fibrillar Chitin, cellulose, β –glucan Matricial α-Glucan, chitosans, polyuranides, glucoproteins, lipids, inorganic salts and pigments

The bulk material of the cell wall is usually in the form of β[1→3]-glucan. This forms a very stable hydrogen-bonded triple helix in solution, and probably *in vivo* . The packing of these triple helix structures appears to be controlled by the size and frequency of very short (1→6) side chains, sometimes consisting of only a single glucose monomer.

the outermost layer of the cell wall consists of diverse proteins bearing polysaccharide side chains composed of mannose. The usual explanation is that these are attached through their mannan side chains via α[1→3] linkage with the β[1→6] glucan. However, this is only a model. Real life appears to be very much more complex, involving a wide variety of different interactions between glycoproteins and bulk cell-

wall materials. Wall composition frequently varies markedly between species of fungi. This has been shown by electron microscopy, which revealed that cell walls of mycelial fungi contain mostly chitin, which is a polymer of n-acetylglucosamine and may constitute 25–30% of the dry weight of the cell, and the cell wall of yeast contains about 29% glucan, 31% mannan, 13% protein, 8.5% lipid and 3% ash. The protein is present in the form of complexes: glucan protein, mannan protein I and glucomannan protein II. α -Glucan is absent in yeast. Various metal-binding groups, viz. amine, imidazole, phosphate, sulphate, sulphhydryl and hydroxyl, are present in the polymers [73]. However, their availability depends on the fungal species used as biosorbent.

Advantages of using fungal biosorbents

Firstly, fungus shows excellent metal-binding capacity because of the variety of functional groups present due to a high percentage of cell-wall material.

Secondly, compared with some biosorbents such as plant products or algal biomass, fungus is easy to cultivate at large scale as it has a short multiplication cycle. Moreover, it can be easily grown using unsophisticated fermentation techniques and inexpensive growth media. Apart from these, the yield of biomass is also quite high.

Thirdly, fungal biomass is easily available as industrial waste products e.g. *Aspergillus niger* (waste from citric acid production) and *Saccharomyces cerevisiae* (brewery industry waste). A variety of fungal biomass types arise from many industrial fermentations and the food, brewing and distilling industries, and these also receive continuing study. This provides an economic advantage to the fungal biosorbents, compared with
other types of microbial biomass. Fourthly, a major portion of fungal biosorbents are non-pathogenic; thus they are generally regarded as safe and are easily accepted by the public when applied practically.

The metal uptake by fungal biomass takes place by two basic processes.

The **first is by** living organisms, where the metal uptake is dependent on the metabolic activity.

The **second process** involves metal uptake by dead and living cells as a result of the chemical functional groups of the cell and, in particular, the cell wall. It should be noted that the metal uptake by the second process may also be involved during the metabolism-dependent metal uptake of growing cells (Gadd, 1986).

The cell wall of the fungi is the first to come into contact with metal ions in solution, where the metals can be deposited on the surface or within the cell wall structure before interacting with the cytoplasmic material or the other cellular parts. In extreme cases, for the living cells, intracellular uptake may take place due to the increased permeability as a result of cell-wall rupture and subsequent exposure of the metal-binding sites (Gadd, 1990). The metal uptake by the cell wall has been broadly based on two mechanisms: uptake directed by functional groups like phosphate, carboxyl, amine and phosphate diester species of these compounds. The second uptake mechanism results from physicochemical inorganic interactions directed by adsorption phenomena. The removal mechanisms for radionuclides result from the

combination of the above two processes, while for other heavy metals, the first process seems to play an important role.

Tobin ***et al.*** (1984) reported that the metal uptake was **independent of the ionic charge or electrostatic strength** and was influenced by the **ionic radius**. The **carboxylate** and/or **phosphate** ligands were proposed to be actively involved along with the **hydroxy** and **amide functional groups**, which would form relatively weak bonds with metal ions. Treen-Sears (1986) also reported that uptake of metals depended on the **ratio of phosphate to carboxyl residues**.

It was also indicated that ion exchange may be the principal mechanism for metal sequestering during the rapid initial uptake phase. Fungal cell walls contain chitin and chitosan. Chitin and chitosan have been shown to sequester metal ions. Chitin and chitosan contents of the fungal cell wall can change during growth of mycelia and this can account for the variations in the metal-uptake capacity with the cell age.

Uranium biosorption

Uranium has been shown to be deposited throughout the cell wall of non-living ***R. arrhizus*** cells exposed to uranium solutions. IR spectroscopy revealed the sequestered uranium to be associated with the nitrogen of the chitin monomer N-acetyl-Dglucosamine (NAGI). The pure chitin had a very low uranium uptake (6 mg/g). Based on experimental results, Tsezos and Volesky (1982~) proposed a three-step process to describe the biosorption of uranium by non-living cells of ***R. arrhizus.*** The first process involves the formation of a complex between the dissolved uranium ionic species and the chitin network present in the cell wall. The complex forms between the uranium and the amine-nitrogen of the chitin crystallates. The second process involves the adsorption of additional uranium by the chitin network close to that complexed by the chitin nitrogen. Thus, the complexed uranium could be acting as nucleation sites for further deposition of uranium. The third process is the hydrolysis of the uraniumchitin complex formed by the first process and the precipitation of the hydrolysis product in the cell wall. Tsezos and Volesky (1982b) observed a different removal mechanism for biosorption of thorium.

The first process involves the formation of a coordinated complex between thorium and nitrogen of the cell-wall chitin. Pure chitin exhibited a capacity of 8 mg/g. The biosorptive uptake capacity of thorium by ***R. arrhizus*** was found to be greater than 170 mgig, thus suggesting other processes to be involved apart from the complexation by chitin.

The second process involved the adsorption of hydrolyzed thorium ions by the outer layers of the ***R. arrhizus*** cell wall. Tsezos (1983) and Tsezos and Volesky (1982a)
suggested that uranium forms a complex with the chitin nitrogen and a free radical (hydroxyl group) was suggested to participate in the uranyl ion coordination to nitrogen. The presence of CU*+ and Fe2’ reduced the uptake of uranium, possibly due to the competition for the nitrogen sites of the chitin crystalline network. This resulted in a reduced number of nucleation sites created as a result of the first mechanism.

The interactions of metals with proteins is well known (Spiro, 1981) and can also be involved in biosorption of metals. Muraleedharan and Venkobachar (1990) showed that proteins in ***Ganodemza lucidurn,*** a macro-fungus, do not play any substantial role by themselves in copper (II) uptake. It was also indicated that chitin did not play a significant role in copper uptake by G. ***lucidum.*** EPR spectra of G. ***lucidurn,*** the chitin fraction of G. ***lucidum*** and the fraction devoid of chitin, indicated the presence of a free radical, which seemed to be present in a very stable cell-wall matrix. Tsezos & Mattar (1986) and Muzzarelli ***et al.*** (1979) indicated the presence of this radical to be associated with chitin nitrogen, in contrast to the observations of Muraleedharan and Venkobachar (1990).

The recent data suggest the structural polysaccharides of G. ***lucidum*** to be the main site of interaction. The biosorption site is believed to be oxygen dominated and the majority of the metal taken up was exchanged with calcium and hydrogen present on the cell wall .

Siegel (1990) suggested that biosorption of UO, ion by various purified cell wall polymers is in the following order: chitin > cellulose phosphate > carboxymethyl cellulose > cellulose; and the difference between the highest and lowest is only 20%. To date, the biosorption mechanism of metal ions by fungal biomass has been studied largely in relation to chitin, its deacetylated derivative, chitosan, and cellulose. The fungal cell walls also contain glycans, proteins, lipids, polyuronides and melanin. The role played by these cell-wall fractions and structural polysaccharides is not fully understood, and needs to be studied in greater detail.

MECHANISMS OF BIOSORPTION OF DIFFERENT HEAVY METALS BY BROWN MARINE MACROALGAE

A few species of marine macroalgae, commonly known as brown algae, exhibit high metal binding capacities. These were higher than those of other types of biomass and other sorbents

Cell wall

Typical algal cell walls of Phaeophyta, Rhodophyta, and many Chlorophyta are comprised of a fibrillar skeleton and an amorphous embedding matrix. The most common fibrillar skeleton material is cellulose (Fig. 1). It can be replaced by xylan in the Chlorophyta and Rhodophyta in addition to mannan in the Chlorophyta. The Phaeophyta algal embedding matrix is predominately alginic acid or alginate (the salt of alginic acid; Fig. 7) with a smaller amount of sulfated polysaccharide (fucoidan; Fig. 6) whereas the Rhodophyta contains a number of sulfated galactans (e.g. agar, carregeenan, porphyran, etc.). Both the Phaeophyta and Rhodophyta divisions contain the largest amount of amorphous embedding matrix polysaccharides. This characteristic, combined with their well-known ability to bind metals, makes them potentially excellent heavy metal biosorbents.

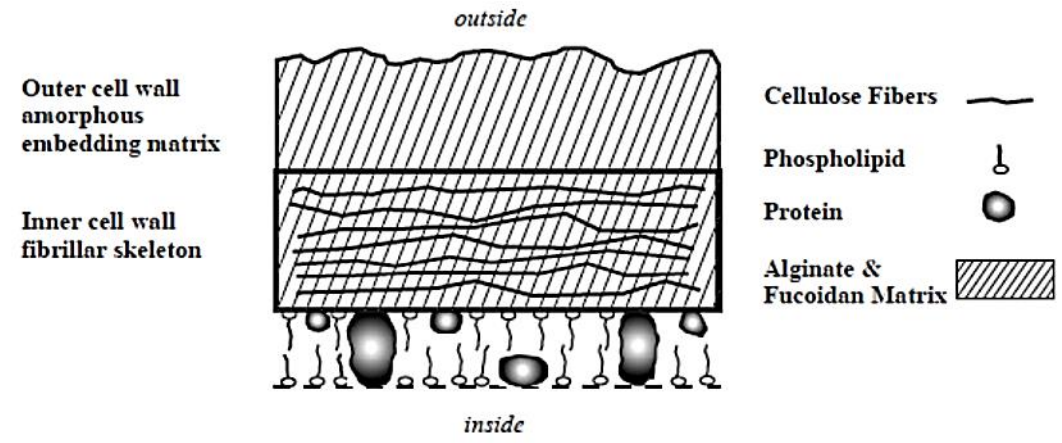


Fig. 1. Cell wall structure in the brown algae. After Schiewer and Volesky

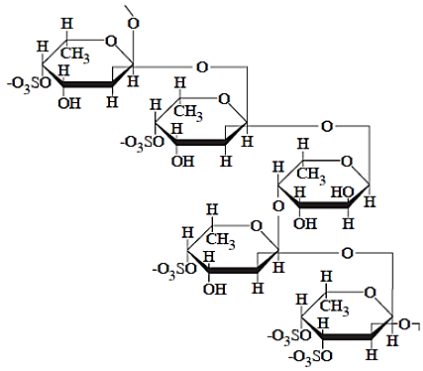
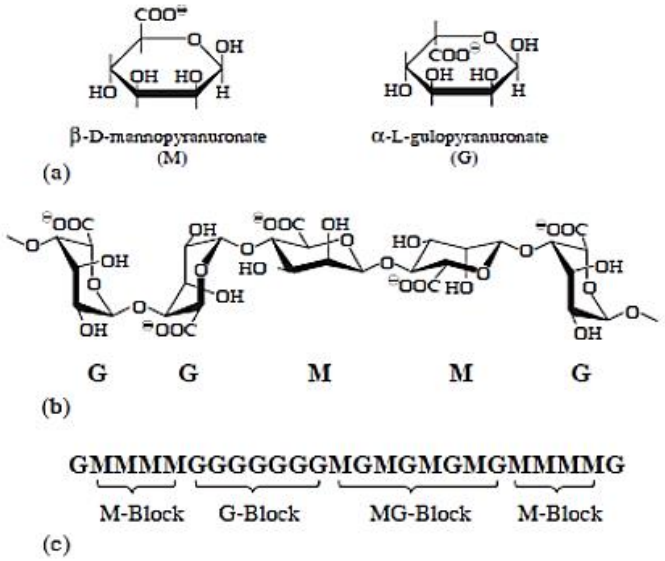
As mentioned above, the cell wall of algae is composed of at least two different layers. The innermost growth in length, they can display a predominantly longitudinal orientation. The mass fraction of cellulose may be 2–20% of the dry weight. For example, the cellulose content of *Ascophyllum* and *Laminaria* (Fig. 2) were determined to be 7% and 20%, respectively.

Extracellular polysaccharides

Fucoidan has been found to occur in several members of the family Laminariaceae Fucoidan is a branched polysaccharide sulfate ester with l-fucose 4-sulfate building blocks as the major component

Alginic acid occurs in all brown algae. It may be present in both the cell wall matrix and in the mucilage or intercellular material (Figs. 1,7) and can constitute between 10% and 40% of the dry weight (untreated) of the algae. Its abundance is dependent on the depth at which the algae are grown and it also displays seasonal variations. The latter may reflect changes associated with growth stages. The alginic acid content in *Sargassum longifolium* was found to be 17%.

Alginic acid or alginate, the salt of alginic acid, is the common name given to a family of linear polysaccharides containing 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues arranged in a non-regular, blockwise order along the chain

	
The structure of fucoidan, a branched polysaccharide sulfate ester with l-fucose building blocks as the major component with predominantly a β(1-2)-linkages.	Alginate structural data: (a) alginate monomers (M vs. G); (b) the alginate polymer; (c) chain sequences of the alginate polymer.

Biomass of brown marine macroalgae is a renewable biological resource, which is available in large quantities and can form a good base for the development of biosorbent material. Brown algae contain high concentrations of **alginic acid** and **sulfated polysaccharides**. It has been postulated that the function of these polysaccharides, which are absent in terrestrial plants, is to **enable marine algae to selectively absorb metallic ions** in a saline medium through ion exchange.

There are several chemical groups in biomass that can attract and sequester the metals: acetamido, amino, amido, sulfhydryl, sulfate, and carboxyl. Metal sequestration during biosorption follows complex mechanisms that include mainly ionic interactions and formation of complexes between metal cations and ligands contained in the structure of the cell wall biopolymers, as well as precipitation on the cell wall matrix.

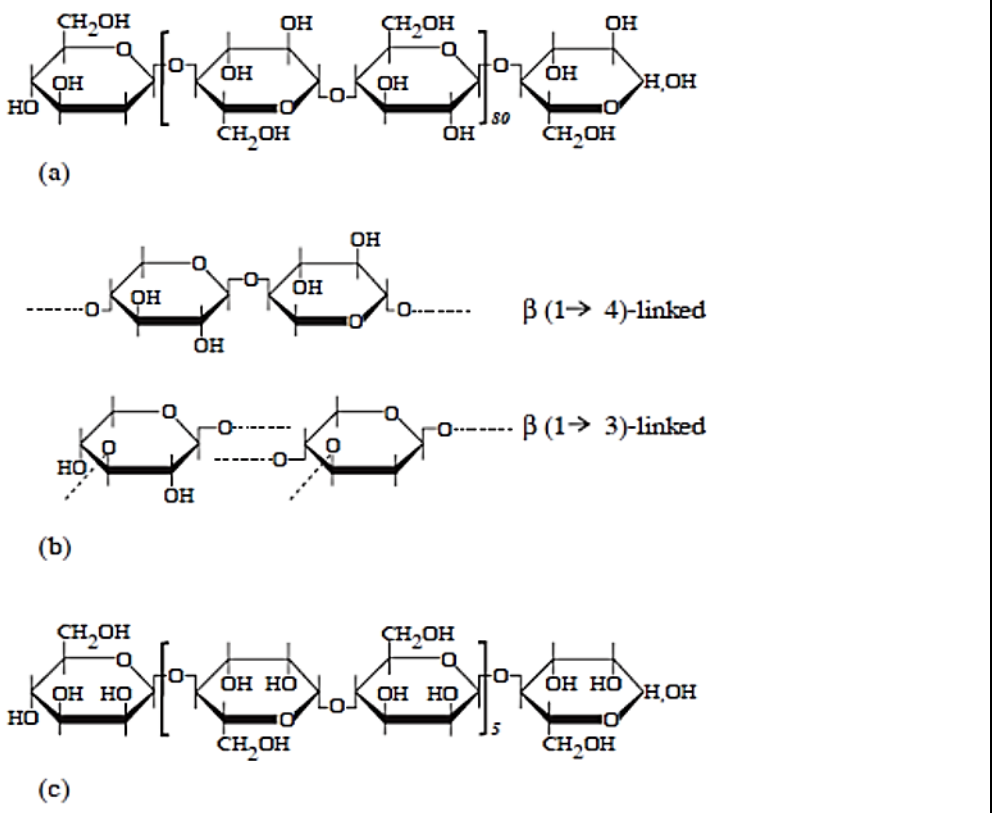


Fig. 5. The fibrillar molecules of algal cell walls. (a) Algal cellulose, aβ (1—4) -linked unbranched glucan, of the brown algae; (b) structural units present in xylan from the red algae, both β(1—3) and β(1—4)-linked forms have been isolated; (c) mannan, a β (1—4)-linked d-mannose from the red algae. After Percival and McDowell.

Mechanisms of biosorption

Key functional groups

The **carboxylic groups** are generally the most abundant acidic functional group in the brown algae. They constitute the highest percentage of titratable sites (typically greater than 70%) in dried brown algal biomass. The adsorption capacity of the algae is directly related to the presence of these sites on the alginate polymer, which itself comprises a significant component (up to 40% of the dry weight, of the dried seaweed biomass. The role of carboxylic groups in the adsorption process has been clearly demonstrated by a reduction in cadmium and lead uptake by dried *Sargassum* biomass following partial or complete esterification of the carboxylic sites.

The second most abundant acidic functional group in brown algae is the sulfonic acid of fucoidan. Sulfonic acid groups typically play a secondary role, except when metal binding takes place at low pH. Hydroxyl groups are also present in all polysaccharides but they are less abundant and only become negatively charged at pH>10, thereby, also playing a secondary role in metal binding at low pH.

There are several examples of metal biosorption mechanisms.

From the different techniques used and the known chemical composition of the algal cell wall, it was observed that biosorption of the metallic cations to the algal cell wall component was a surface process.

The main chemical groups involved in the metallic cation biosorption were apparently carboxyl, amino, sulfhydryl, and sulfonate. These groups were part of the algal cell wall structural polymers, namely, polysaccharides (alginic acid, sulfated polysaccharides), proteins, and peptidoglycans.

Ion-exchange process

It should be pointed out that the term **ion-exchange** does not explicitly identify the binding mechanism, rather it is used here as an umbrella term to describe the experimental observations. The precise binding mechanism(s) may range from physical (i.e. electrostatic or London–van der Waals forces) to chemical binding (i.e. ionic and covalent).

The term **sorption** would refer to binding of a metal cation to a free site as opposed to one that was previously occupied by another cation. It is distinct from adsorption that, strictly speaking, defines binding in terms of a physical rather than chemical surface phenomenon. In the case of biosorption of heavy metals by brown algal biomass, the mechanisms can be viewed, in principle, as being extracellular, or occurring discretely at the cell wall. Intracellular sorption would normally imply bioaccumulation by a viable organism.

It is reported that cobalt biosorption by nonliving biomass of the brown marine macroalgae *Ascophyllum nodosum* is predominantly an **ion-exchange process**. They suggested that the carboxyl groups of the cell wall alginates play an important role in cobalt binding. The contribution of other functional groups, such as the strongly acidic sulfate groups ($R\text{-}OSO_3^-$) present in the cell wall polymers (fucoidan, carrageenans) was estimated at 10% of the overall metal-binding sites of these seaweeds. It is also indicated that lead uptake occurred mainly through binding to carboxyl groups as well as nitrogen-containing groups.

Untreated biomass generally contains light metal ions such as K+, Na+, Ca2+, and Mg2+. These are originally bound to the acid functional groups of the alga and were acquired from seawater.

Treated biomass generally implies one of two chemical alterations. The first is protonation of the biomass with a strong acid such as HCl whereby the proton displaces the light metal ions from the binding sites (i.e. carboxylic, sulfonic, and others). In the second, the biomass is reacted with an aqueous solution of a given ion at high concentration so that the majority of sites are occupied by, for example, calcium or potassium.

In cases where the non-treated marine alga *Sargassum* was reacted with a (heavy) metal-bearing solution, a pH increase and the release of light metal ions was observed. This also was explained in terms of ion-exchange, whereby the observed release of light metals balanced the uptake of protons and heavy metals. When the heavy metal concentration was increased, little pH increase was observed and this was attributed to the fact that the maximum binding capacity of the biomass had been reached and all exchangeable sites were occupied by the heavy metal.

In related metal uptake experiments with treated biomass (protonated), the pH of *Sargassum* suspensions decreased. This was observed as either a continual but initially steep drop in pH in a free-drift system, or by the addition of base in a pH-stat system. Again, this was interpreted as ion-exchange between protons and the heavy metal ions at the binding sites

The main cadmium cation sequestration mechanism by the algal biomass was apparently **chelation**, while the main nickel cation sequestration mechanism was ion exchange. Lead cations exhibit a higher affinity to the algal biomass, and their binding mechanism included a combination **of ion exchange, chelation, and reduction reactions,** accompanied by metallic lead precipitation on the cell wall matrix.

During the ion-exchange process, calcium, magnesium, and also hydrogen cations in the algal cell wall matrix were replaced by the heavy metals investigated herein.

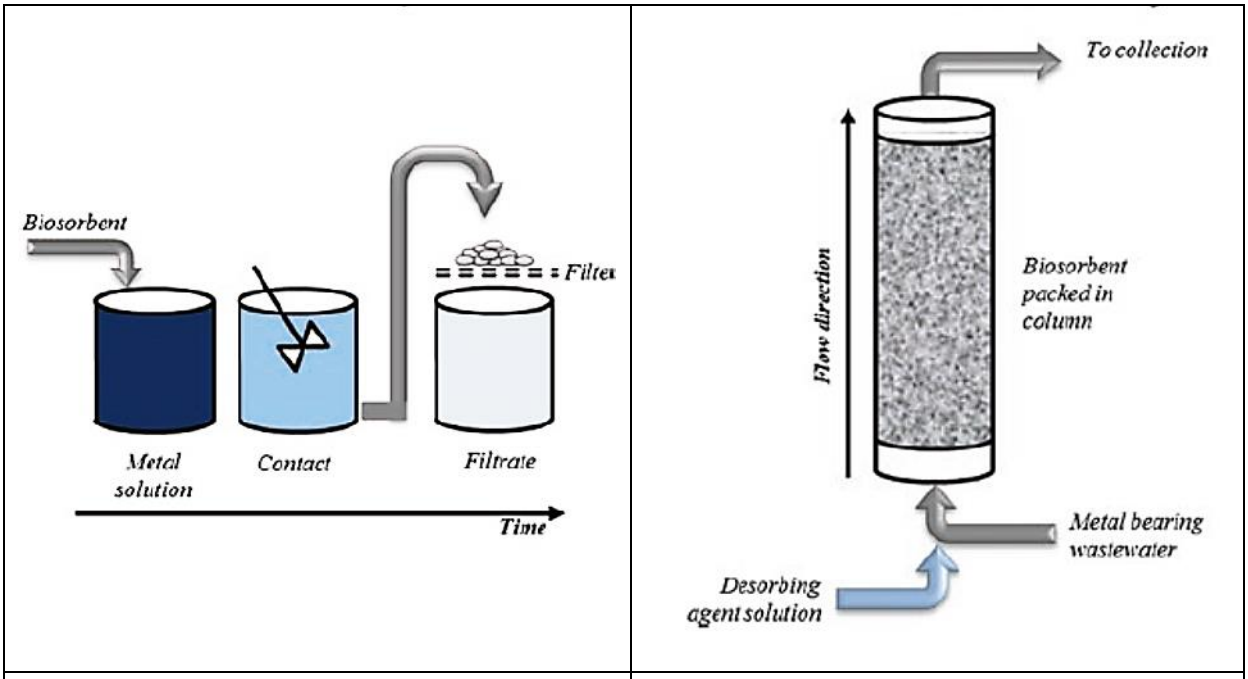
Carboxyl groups were the dominant species in the heavy metal biosorption by the *Sargassum* biomass, especially in the case of nickel. Groups containing nitrogen and sulfur, such as amino/amido and sulfonate/thiol, were also involved in the adsorption of the heavy metals tested, especially lead and cadmium.

Lead cations exhibit higher affinity to the algal biomass, and their binding mechanism included a combination of ion exchange, chelation, and reduction reactions, accompanied by metallic lead precipitation on the cell wall matrix. During the ion exchange process, calcium, magnesium, hydrogen cations, and probably other cations (sodium and potassium) in the algal cell wall matrix were replaced by the tested heavy metals.

Modeling Heavy Metal Biosorption in Batch and Continuous Systems

Parallel to the extensive experimental activity carried out during the last decades on heavy metal biosorption, several mathematical models, most in the form of empirical correlations, have been developed to elucidate and represent the heavy metal adsorption on biomass binding sites (Volesky 2003). Mathematical modeling represents a useful tool to describe the complex mechanisms characterizing the biosorbent and the solute interactions and assists in the optimization and design of biosorption processes.

Figure 2.4 shows the biosorption models classified into two main categories based on the mode of operation (batch and continuous) used to conduct the process:



A. Batch System | **B. Continuous System**

Fig. 2.4 Schematic illustration of batch (**a**) and continuous (**b**) experimental biosorption systems. Under batch conditions, a biosorbent is mixed with a metal-containing solution within a simple reactor operated till the thermodynamic equilibrium. In a continuous-flow system (i.e. a packed bed configuration), a synthetic wastewater is continuously fed to a biosorbent column with an upward or downward stream. The biosorbent regeneration for the sorption/desorption cycle is performed by switching the wastewater flow to an appropriate desorbing agent flow

2.3.1 Modeling of Batch Systems

Batch experiments have been mostly devoted to the collection of fundamental information, such as biosorption efficiency or rate, which plays a crucial role in designing biosorption systems (Aksu 2005). In this case, the experimental protocol is quite simple: a suitable mass of biosorbent is immersed in a solution containing single or multiple heavy metals until a thermodynamic equilibrium is reached between the metal concentrations on the solid sorbent and in the liquid phase. At this point, the biosorbent is separated from the liquid phase to be regenerated or disposed (Fig. 2.4a).

2.3.1.1 Modeling Sorption Equilibrium

The concept of biosorption isotherm is of major importance for the evaluation of the performance of any given sorption system. Any isotherm is related to the solute uptake and is obtained by plotting the solute uptake versus the equilibrium solute concentration in the liquid phase. In general, the solute uptake increases at increasing solute concentration until saturation. The batch equilibrium isotherm curves have usually been modeled by using empirical correlations or mechanistic equations (Park et al. 2010). The empirical models are not able to reproduce the mechanisms of solute uptake, but have widely been recognized as efficient tools for providing a suitable description of the

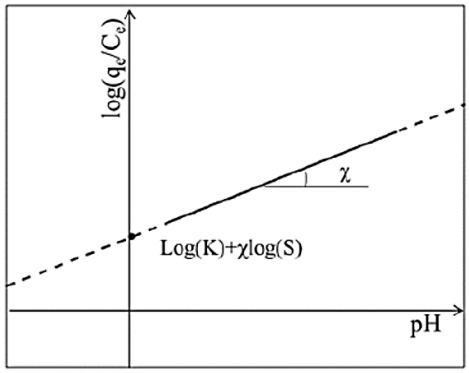
experimental behavior (Volesky 2003). These models can be classified based on the number of parameters involved (n-parameter models) and components included. In the last case, the models are obtained by extending the single component isotherm models to multi-metal systems and taking into account the interferences and competition phenomena for adsorption sites.

Such models can be only related to the individual isotherm parameters or can include some correction factors (Aksu 2005).

Table 2.3 summarizes the main features, including the equations and the relative degree of freedom, of the most used isotherm models. Among them, the Langmuir and Freundlich isotherms have widely and successfully been applied, as proved by the extensive application present in literature (Liu et al. 2001; Tokunaga and Hakuta 2002; Aksu 2002; Ozdemir et al. 2003; Wang et al. 2006; Luo et al. 2006; Parvathi and Nagendran 2007; Lakzian et al. 2008). The isotherm parameter values can be assessed by a linearization of the equation (two-parameter models), a trial and error procedure for higher order models or by using a non-linear optimization (for all the isotherms).

Parallel to the adoption of empirical equations, more complex mechanistic models have been suggested to effectively elucidate the sorption mechanisms. These models are based on specific hypotheses concerning the reactions between functional groups and heavy metals, and require a biomass characterization. Among them, the surface complexation model (SCM) and the ideal adsorbed solution theory (IAST) have successfully been applied to investigate the metal adsorption process (Daughney and Fein 1998; Fowle and Fein 1999; Volesky 2003; Vijayaraghavan and Yun 2008). The SCM was conceived as reported by Kurbatov et al. (1951) with the aim of describing the function of protons in metal adsorption at a macroscopic level. The model consists of mass law equations to describe reactions at individual surface sites and assume protons as the dominant potentially determining ions. The parameters involved in the resulting equations, including the equilibrium constants of surface reactions and the concentrations of functional groups, are discriminating factors in characterizing the efficiency of a sorbate/sorbent system. Such values are usually obtained by adopting Kurbatov plots for titration data, which consist in plotting pH on the x axis versus log(qe/Ce), with qe and Ce being the sorbed and free metal concentration at equilibrium, respectively, on the y axis (Fig. 2.5). A linear relationship is thus obtained. The slope χ corresponds to the number of moles of protons released per mole of metal complexed. The intersect with the y axis provides log(K), where K represents the equilibrium constant. Obviously K and χ are specific for each sorbent/sorbate system (Guibaud et al. 2008). The SCM has mainly been used to study metal adsorption on EPS as confirmed by the huge number of scientific works published on this specific topic (Pagnanelli et al. 2000; Guibaud et al. 2005, 2006, 2008; Comte et al. 2008; Wei et al. 2011).

Fig. 2.5 Schematic illustration of the linear trend (Kurbatov plot) obtained by plotting log(qe/Ce) on the y axis versus pH on the x axis. The slope of the linear trend is χ, whereas the intersect with the y axis is log(K)+χlog(S), with *S* being the concentration of complexation sites not associated with the metal (Guibaud et al. 2008)



2.3.1.2 Modeling Batch Adsorption Kinetics

Kinetic studies are aimed at describing the behavior of the sorption system on time (Volesky 2001) and have commonly been applied to study the contribution of the main rate controlling steps invariably involved in the sorption process. **Abbas et al.(2014) described the biosorption as a series of several consecutive elementary steps which reproduce:**

1. the diffusion of heavy metal ions from the liquid bulk to the liquid film surrounding the sorbent particles;
2. the ion transport from the boundary liquid film to the surface of the sorbent particles;
3. the transfer of heavy metals from the surface to the internal active binding sites; and
4. the interactions between the active binding sites and metals (Fig. 2.3).

Generally, the sorption reactions, as well as the external diffusion, are inherently very fast and thus do not constitute the rate-limiting steps of biosorption. In most cases, the intraparticle diffusion is recognized as the rate-limiting step and can be modeled by using the equation introduced by Weber and Morris (1963). As shown in Table 2.4, this equation results in a linear correlation between the variables, which includes the origin when the intraparticle diffusion represents the limiting step (Aksu 2005).

Various models have been proposed to quantify the kinetic behavior of sorption systems (Table 2.4). Some of them are related to the intra or extra particle mass transfer (Gerente et al. 2007), others are based on pseudo-first **(i.e. Lagergren)** and second-order kinetic equations, which introduce a direct proportionality between the process rate and the number of adsorption sites in first or second power (Michalak et al. 2013). **The Lagergren first order** and the **pseudo second order** equations, reported in Table 2.4, are both expressed as a function of the sorption capacity of the solid phase. However, the Lagergren equation has been found to fit better the initial 20–30 min of the sorption process. This is probably due to the trivial evaluation of the

real equilibrium sorption capacity, which is generally performed by using an extrapolation. **Contrary to the Lagergren model**, pseudo-second order kinetics are able to predict the sorption behavior over the entire time range and does not require to preliminary know the amount of solute sorbed at equilibrium when expressed in the linear form.

In addition, the second order rate constant *kII* can be expressed as a function of temperature by using the following Arrhenius type equation (Aksu 2001):

$$k_{II} = k_0 \exp\left(-\frac{E}{RT}\right)$$

where k0 is the temperature independent factor, E is the activation energy of sorption, R is the gas constant and T is the solution temperature. By plotting ln(kII) versus 1/T, a linear relationship is obtained whose slope corresponds to -E/R (Fig. 2.6). The activation energy usually assumes negative values as reported in many studies (Aksu 2001; Calero et al. 2009; Horsfall Jr. and Spiff 2005; Mobasherpour et al. 2014), confirming the exothermic nature of the adsorption phenomena and providing information about the rate controlling step of adsorption (Horsfall Jr. and Spiff 2005). Nevertheless, both first and second order kinetic equations require a presetting of the reaction order, which strictly depends on the reaction mechanism. Liu and Shen (2008) introduced a general rate law equation for biosorption which does not need a presetting a priori of the reaction order unless the sorption mechanisms are known. This equation states that biosorption kinetics follows the universal rate law for a chemical reaction and is written in terms of the adsorption sites available on the biosorbent surface (Liu and Liu 2008).

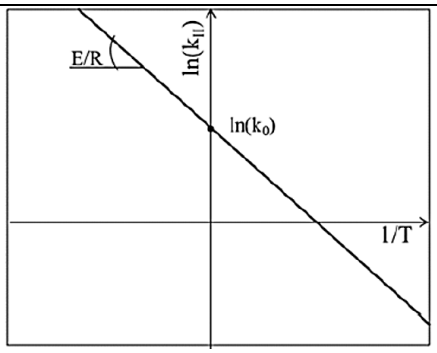


Fig. 2.6 Schematic illustration of the linear trend obtained by plotting Ln (kII) on the y axis versus 1/T on the x axis. The intersect with the y axis corresponds to ln(k0) and the slope to E/R:

2.3.2 Modeling of Continuous Systems

The continuous mode of operation has generally been used to test the technical feasibility of biosorption for real applications (Vijayaraghavan and Yun 2008). Mostly continuous stirred tank reactors, fluidized bed, moving bed and packed bed columns have been used (Kumar et al. 2016). Packed bed columns have been recognized as one of the most convenient configurations due to the higher sorbing capacity, the high operational yield and the technical feasibility (Vijayaraghavan and Yun 2008). A packed bed column usually consists of a cylindrical reactor filled with

sorbent, passed through by a metal-containing wastewater by gravity or pressure (Fig. 2.4b). The concentration of the solute in the outlet is found to increase over time as the biosorbent becomes saturated. The region of the bed where the adsorption takes place is named mass transfer zone or adsorption zone and moves forward until approaching the end of the bed (Le Cloirec and Andrès 2005).

The breakthrough curve, typically S-shaped, is obtained by plotting the normalized effluent concentration Ceff/Cin versus time and represents a valuable tool for evaluating the biosorbent efficiency. Indeed, the amount of solute removed at saturation can be easily evaluated by calculating the area above the breakthrough curve, whose slope provides information about the column service time. The main features of the breakthrough curve are the breakthrough and saturation/exhaustion points which theoretically correspond to an abrupt rise (inflection point) in the effluent concentration plot and the complete column saturation, respectively (Fig. 2.7). The time elapsed until attaining the breakthrough point directly affects the service time of the column. In laboratory experiments, packed bed columns are usually operated until the saturation point is attained. Conversely, in industrial applications, the column is usually regenerated when the effluent metal concentration exceeds a breakthrough/service point, which is prefixed depending on the metal toxicity. When the breakthrough point is attained, the effluent concentration can slowly rise to the saturation point (flattened breakthrough curve). However, it is preferable to have a steep slope which corresponds to a shorter mass transfer zone (Vijayaraghavan and Yun 2008). The shape of the breakthrough curve is affected by many parameters, such as flow rate, inlet metal concentration, pH, bed height and bed particle size (Kumar et al. 2016).

Most of the models for continuous sorption systems have been developed to predict the breakthrough curves. Some examples are reported in Table 2.5. The Adams-Bohart model is usually applied to the initial part of the breakthrough curve and is obtained by combining two kinetic equations, the first describing the solute transfer from the liquid phase, the second governing the sorption accumulation on the biosorbent. A similar equation has been obtained by Wolborska (1999), who also takes the solute axial diffusion into account. The two equations are transformed into the same expression in the case

$$k = \frac{\beta_a}{N_0}$$

The Thomas model has been used in the linear form to quantify the maximum adsorption capacity of the adsorbent bed.

In the Clark model, the breakthrough curve is obtained by adopting the Freundlich equation. The model introduced by Yoon and Nelson (1984) is much simpler as it does not require specific information about the adsorbate/adsorbent system.

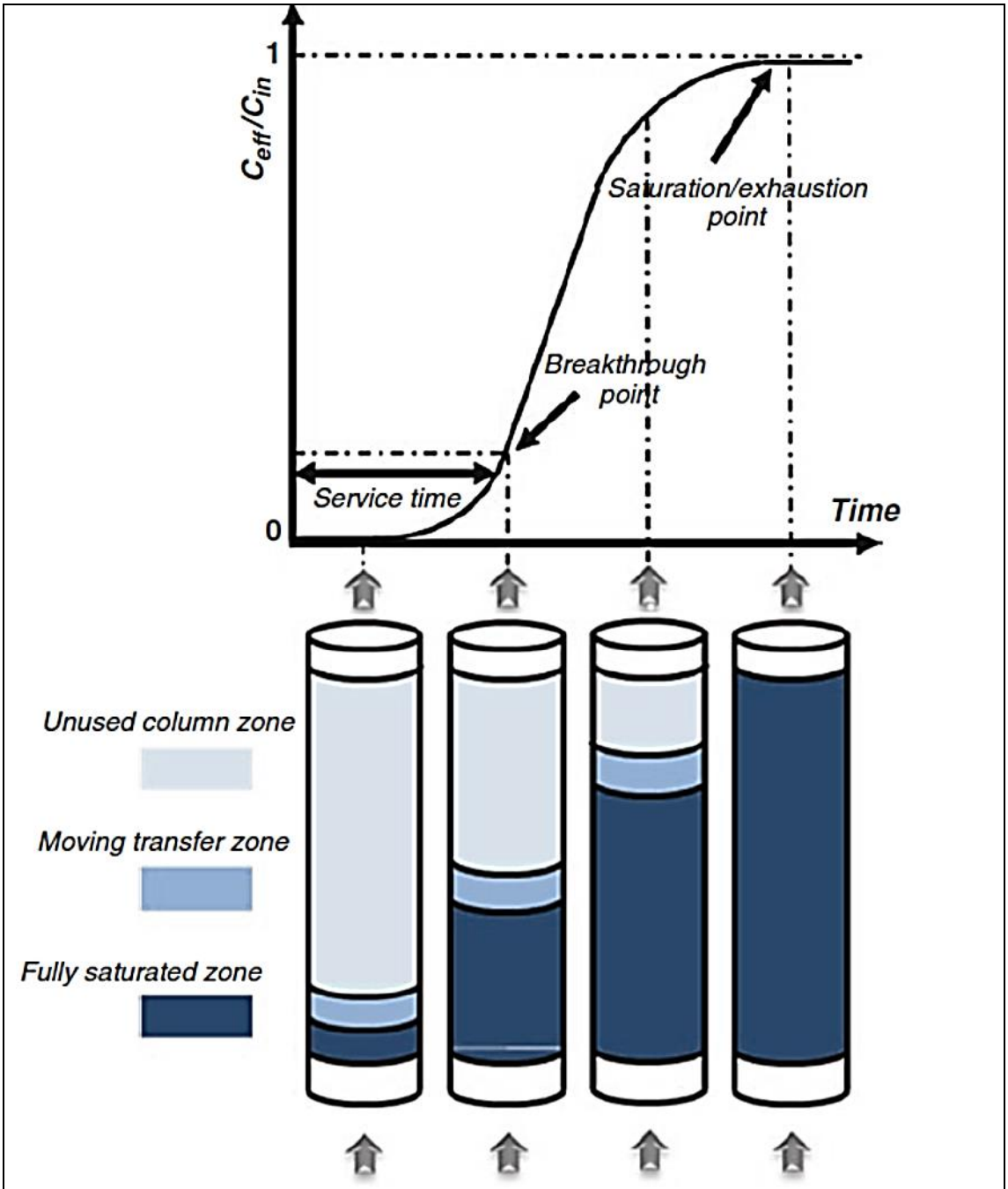


Fig. 2.7 Schematic illustration of the evolution of the mass transfer zone and the corresponding theoretical time-based breakthrough curve for metal sorption in a packed bed column. Ceff and Cin are the metal effluent and influent concentration, respectively (Adapted from Gupta et al. 2016)

Potential for applied remediation

Biosorption can be used to eliminate heavy metals from industrial effluents or to recover precious metals from processing solutions. The fully ‘‘loaded’’ biosorbent may concentrate heavy metals a thousand fold from their concentration in the liquid phase. This loading of the biomass may be reversed in order to ‘‘desorb’’ the metals and several studies have shown [41,67,68] elution of the biomass by acid aqueous solutions to be highly effective. The elution process does not significantly reduce the binding capacity of the biomass and several cycles may be employed. For example, Yang [86] used a Sargassum fluitans loaded fix-bed column to study uranium biosorption. He used a 0.1N HCl solution to elute the bound uranium and recovered 99.5% of the metal. Furthermore, the column was maintained continuously for 1 month over which time five biosorption–desorption cycles were carried out. The biosorption capacity of the substrate decreased by approximately 7% after the first cycle and was about 20% less than the fresh biomass on the fifth cycle. The observed drop in biosorption capacity between cycles was attributed to leaching of alginate. The overall metal concentration factor, defined as the ratio of the elution concentration to the influent concentration for a given biosorption cycle, was determined to be approximately 25. With a high concentration factor, it should be possible to reduce the volume of waste that is produced by applying an iterative metal sorption–desorption process such that only a small volume of solid waste is generated. According to this scenario, the biosorbent is regenerated and a highly concentrated metal solution is obtained. This concentrate may then be treated by either co-precipitation, flocculation or electro-winning. A toxic sludge would be generated by co-precipitation whereas the solid metal, a more desirable end-product, would be recovered from the concentrate by electro-winning.

A simplified schematic representation of the proposed ‘‘zero discharge’’ technology is shown in Fig. 11 where multiple ‘‘sorption’’ and ‘‘desorption’’ cycles are carried out.

The application of biosorption is particularly well suited as a refining technique where wastewater heavy metal concentrations range from 1 to 100 ppm. These levels can be lowered to drinking water standards with the existing biosoption technology. The main advantages of the biosorption process over traditional techniques are the high effluent quality it generates, its terms of operation under a broad range of service conditions and its cost-effectiveness. The bottom line is the inexpensive nature of brown algal biosorbents.

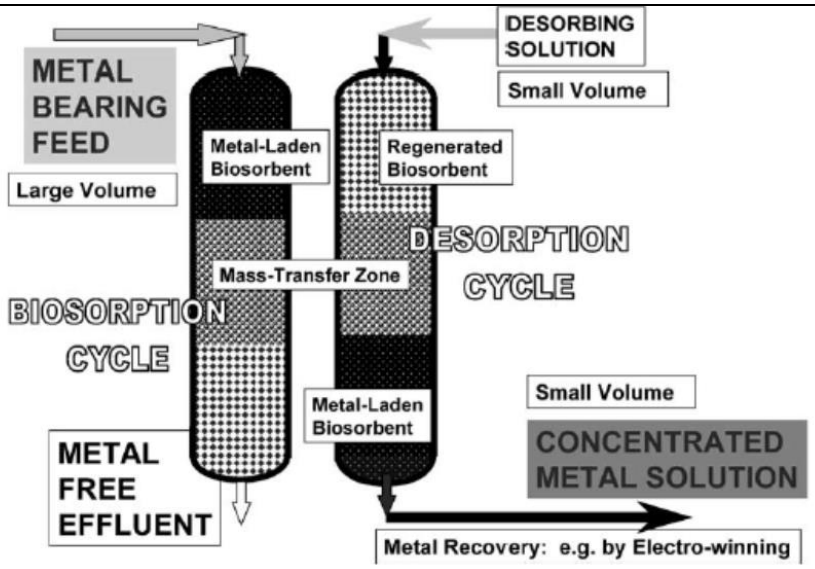


Fig. 11. Schematic diagram of possible biosorption implementation using packed bed columns for biosorption and desorption.

Desorption methods for regeneration of biosorbenets and recovery of metals or dyes

One of the important industrial applications of biosorption is recovery of loaded pollutants (especially valuable metals) from the biosorbent and simultaneous regeneration of the biosorbent for reuse. In fact, the usefulness of a specific biomass as a biosorbent depends not only on its biosorptive capacity, but also on the ease of its regeneration and reuse.

Category	Detailed methods
Physical	microwaving, heating, etc
Nondestructive Chemical	Acids (HCl, H2SO4, HNO3, H3PO4, acetic acid, etc); Alkalis (NaOH, NH4OH, etc); Organic solvents (methanol, ethanol, acetone, etc) Others (CaCl2, KSCN, Na2CO3, KHCO3, EDTA, etc)
Destructive	Incineration, dissolution into strong acids or alkalis, etc.

Biosorption Advantage

a. Low cost,
b. High efficiency,
c. Minimization of chemical or biological sludges,
d. The regenerate ability biosorbents, and
e. The possibility of metal recovery following adsorption.

Commercial Application

a. A potent alternative technique for treating industrial wastewaters containing metals and/or dyes.
b. It may be used for the purification and recovery of rare proteins, steroids, pharmaceuticals, and drugs that are valued in thousands of dollars per gram.
c. Many biosorption processes are under development or have been developed and patented for commercial applications. Pilot installations and a few commercial scale units have been constructed in the USA and Canada.

Conclusion

Over the last years, biosorption has received considerable attention from academic researchers, becoming one of the most promising and cost-effective alternative technologies for heavy metal removal and recovery from industrial wastewaters.

However, despite the high number of scientific studies on biosorption, several technical and scientific aspects still need to be clarified for the commercialization and the spread of this technology at industrial scale. Based on these considerations, future research may be focused on the characterization and identification of new materials to be used as biosorbents with higher cost-effectiveness and biosorption efficiency, enhancement of selective metal biorecovery through biosorption in multi-metal systems and development of analytical tools based on deterministic mathematical models able to describe multi-sorbate systems.