MICROBIAL BIOSORPTION OF HEAVY METALS

By

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DECLARATION

I, Bony Cyriac (2018-11-097) hereby declare that the seminar report titled 'Microbial biosorption of heavy metals' has been completed by me independently after going through the references cited here and I haven't copied from any of the fellow students or previous seminar reports.

Vellanikkara | Bony Cyriac
Date: 25/ 01/2020 | (2018-11-097)

CERTIFICATE

This is to certify that seminar report titled ‘Microbial biosorption of heavy metals’ for the course MICRO. 591 has been solely prepared by Bony Cyriac (2018 - 11 - 097) under my guidance, and he has not copied from any seminar reports of seniors, juniors or fellow students.

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1. Introduction

Industrialization to a larger degree is responsible for the contamination of environment especially water where lakes and rivers are overwhelmed with a large number of toxic substances. Heavy metals are reaching hazardous levels when compared with the other toxic substances. Heavy metals are a unique group of naturally occurring compounds. Their continuous release leads to overconsumption and accumulation. As a result, people around the globe are exposed to adverse consequences of these heavy metals.

As these metals are not biodegradable, they tend to accumulate in the living organisms and lead to various diseases and disorders which ultimately threaten human life. They can cause ill health, even when present in the range of parts per billion (ppb). Biosorption has emerged as an attractive option over conventional methods for the removal of heavy metal ions discharged from various industries and other sources which ultimately reach and pollute fresh water bodies.

1.1 Heavy metals

"Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 5 gcm^{-3} (Nies, 1999). According to United States Environmental Protection Agency, most common heavy metals, in order of abundance are Lead, Chromium, Arsenic, Zinc, Cadmium, Copper and Mercury (USEPA, 1996).

1.2 Sources of heavy metals

There are two main sources of introduction of heavy metals in the environment

(i) Natural sources which includes volcanic emissions, forest fires, deep-sea vents, and geysers.
(ii) Anthropogenic sources which includes mining and smelting sites, industries, vehicular emissions, sewage and sludge pollution and modern agricultural practices.

Industrial effluents are known to contain heavy metals which originate from metal plating, mining activities, smelting, battery manufacture, tanneries, petroleum refining, paint manufacture, pesticides, pigment manufacture, and printing and photographic industries.

1.3 Impacts of heavy metal contamination

Heavy metals contamination is becoming a serious issue of concern around the world as it has gained momentum due to the increase in the use and processing of heavy metals during various activities to meet the needs of the rapidly growing population. Soil, water and air are the major environmental compartments which are affected by heavy metals pollution. There are various ways through which heavy metals present risks to humans, animals, plants and ecosystems as a whole. Such ways include direct ingestion, absorption by plants, food chains, consumption of contaminated water and alteration of soil pH, porosity, colour and its natural chemistry which in turn impact on the soil quality.

Heavy metals can affect plants directly by reducing the plant biomass, seed germination, root elongation, water status, mineral uptake, photosynthesis, respiration and protein content. Indirectly heavy metals affects plants by causing cell death, altering enzyme activity, causing DNA damage and even chromosomal aberrations. In humans, it can lead to a numerous diseases of which major symptoms are head ache, fatigue, respiratory illness, nausea, gastroenteritis, skin irritations and can even lead to cancer.

2. Methods for removal of heavy metals

Since last many decades, various physical and chemical methods were employed to remove metals from environment. The list is given below.

> **i. Physical methods**: Ion exchange, membrane technology, reverse osmosis, and evaporation recovery, filtration.
>
> **ii. Chemicals methods**: Chemical precipitation, electrochemical treatment, oxidation/reduction.

Chemical precipitation and electro chemical treatment are ineffective, especially when metal ion concentration in aqueous solution is as low as 1–100 mgL^{-1}. These methods also produce a large amount of sludge, which is difficult to be treated. Ion exchange, membrane technologies and the activated carbon adsorption process are extremely expensive, especially when treating a large amount of water and wastewater containing heavy metal in low concentration, so they cannot be used at large scale.

Much has been discussed about all physico-chemical methods and different aspects in recent years, hence it can be concluded that these conventional methods have significant disadvantages, which include incomplete metal removal, high capital costs, high reagents and/or energy requirements, and generation of toxic sludge or other waste products that require disposal. These disadvantages, together with the need for more economical and effective methods for the recovery of metals from wastewaters, have resulted in the development of alternative separation technologies (Zhao *et al*., 2015).

3. Biosorption

Biosorption can be defined as the ability of biological materials to accumulate heavy metals from waste water through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). It is a complex process that depends on different factors like cell physiology, physicochemical factors such as pH, temperature, contact time, ionic strength, and metal concentration, chemistry of the metal ions, cell wall composition of microorganisms. Biosorption of different heavy metals e.g. cadmium, silver, lead, nickel etc. by using microorganisms like fungi, algae or bacteria was studied by different scientists.

3.1. Significance

In recent years, research attention has focused on biological methods, which have emerged as an effective alternative to conventional abiotic methods of heavy metal remediation. Of the different biological methods, bioaccumulation and biosorption have been demonstrated to possess good potential to replace conventional methods for the removal of metals. Some confusion has prevailed in the literature regarding the use of the terms 'bioaccumulation' and 'biosorption', based on the state of the biomass.

Therefore, bioaccumulation is used to describe the process involving living cells, whereas the biosorption mechanisms involve the use of dead biomass. To be precise, bioaccumulation can be defined as the uptake of toxicants by living cells. In general, the use of living organisms may not be an option for the continuous treatment of highly toxic organic/inorganic contaminants. The toxicant can transport into the cell and accumulate intracellularly, across the cell membrane and through the cell metabolic cycle. Beyond this point, an organism's metabolism may be interrupted, resulting in death of the organism. This scenario can be avoided in the case of dead biomass, which is flexible to environmental conditions and toxicant concentrations, and thus the use of dead biomass seems to be a preferred alternative for the majority of metal-removal studies reported.

3.2. Advantages of microbial biosorption

The advantages of using dead cells can be summarized as given (Michalak *et al*., 2013).

- Absence of toxicity limitations

- Absence of requirements for growth media and nutrients in the feed solution
- Easy absorbance and recovery of biosorbed metals
- Easy regeneration and reuse of biomass
- Possibility of easy immobilization of dead cells
- Avoidance of sudden death of the biomass population
- Easy mathematical modelling of metal uptake reactors.

4. Microbes as biosorbents

Various microorganisms like bacteria, fungi, yeasts, algae and cyanobacteria have proved to be potential metal biosorbents

4.1 Bacteria

Among microorganisms, bacteria constitute the most abundant, versatile, most diverse creature on this planet earth. They are basically classified on the basis of their morphology as rod, cocci or spirillum. A bacterium has relatively simple morphology consisting of cell wall, cell membrane, capsule, slime layer and internal structures mitochondria, Golgi apparatus, ribosomes, endoplasmic reticulum. Slime layer contains functional groups like carboxyl, amino, phosphate or sulfate for metals chelation. Cell wall in general, is responsible for surface binding sites and binding strength for different metal ions depending on different binding mechanisms. Various bacterial species e.g. *Bacillus*, *Pseudomonas*, *Escherichia* exhibit biosorption property because of their small size and ability to grow in different environmental conditions.

Gram staining divides bacteria in two broad categories; Gram positive and Gram negative. Gram negative mostly constitute pathogens although pathogens are also reported in Gram positive. Gram positive bacteria are comprised of thick peptidoglycan layer connected by amino acid bridges, also known to contain polyalcohols and teichoic acids. Overall, Gram positive bacterial cell wall comprised of 90% peptidoglycan. Some teichoic acids are linked to lipids of lipid bilayer forming lipoteichoic acid. These lipoteichoic acids are linked to lipids of cytoplasmic membrane. They constitute linkage of peptidoglycan to cytoplasmic membrane. This results in cross linking of peptidoglycan forming a grid like structure. These teichoic acids are responsible for negative charge on cell wall due to presence of phosphodiester bonds between teichoic acid monomers.

On the other hand, Gram negative bacterial cell wall contains an additional outer membrane composed of phospholipids and lipopolysaccharides. Gram negative cell wall contains 10–20% peptidoglycan. The negative charge on the Gram negative bacteria is due to lipopolysaccharides, teichoic acids, and teichuronic acids. Extracellular polysaccharides also exhibit the property of metal binding. They are not present in all Gram negative bacteria. Moreover, those species that contain them, they can be easily removed by chemical washing or mechanical disruption.

4.1.1. Biosorption by bacteria

Bacterial cell wall encountering the metal ion is the first component of biosorption. The metal ions get attached to the functional groups (amine, carboxyl, hydroxyl, phosphate, sulfate, and amine) present on the cell wall. The general metal uptake process involves binding of metal ions to reactive groups present on bacterial cell wall followed by internalization of metal ions inside cell. More metal is uptaken by gram positive bacteria due to presence of glycoproteins. Less metal uptake by gram negative bacteria is observed due to phospholipids and LPS.

Table 1. Bacterial species in used metal biosorption

Metal	Biosorbent
Copper	*Thiobacillus ferrooxidans*
Cadmium	*Pseudomonas* sp.
Lead	*Corynebacterium glutamicum*
Nickel	*Pseudomonas aeruginosa*
Zinc	*Escherichia coli*

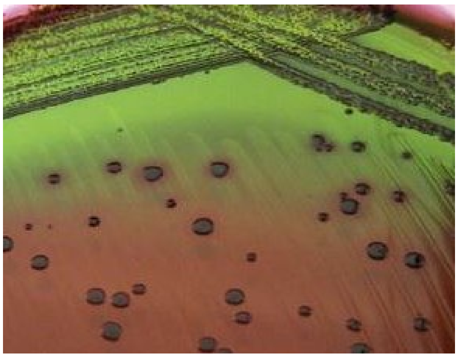


Plate 1. *Escherichia coli*

Plate 2. *Bacillus* sp.

4.1.2 Biosorption of heavy metal polluted soil using bacteria isolated from soil

Oyewole *et al*. (2019), conducted a study based on the biosorption of copper and chromium polluted soil using bacteria isolated from soil. Bacterial species isolated were *Pseudomonas*, *Bacillus*, *Micrococcus*, *Escherichia*, *Streptococcus*, *Enterobacter* and *Staphylococcus*. The isolated bacteria were screened for potential to biosorb copper and chromium. For the bacterial isolates, the highest biosorption rates of chromium (89.67%) and copper (90.89%) by *Pseudomonas aeruginosa* were observed at 20 ppm on day 21 and 15 ppm on day 14, respectively (Fig 1 &2). The results of this study revealed the ability of *Pseudomonas aeruginosa* to biosorb copper and chromium from the environment.

Table 2. Screening of bacterial isolates for biosorption of chromium and copper

Bacterial isolates	**Chromium (%)**	**Copper (%)**
Bacillus lentus	38.03	36.33
Escherichia coli	4.47	8.63
Micrococcus roseus	36.56	20.33
Enterobacter aerogenes	22.09	14.87
Pseudomonas aeruginosa	**45.59**	**42.69**
Staphylococcus aureus	19.94	17.28
Streptococcus species	13.87	4.86
Control	0.41	0.65

Figure 1. Biosorption of chromium by *Pseudomonas aeruginosa*

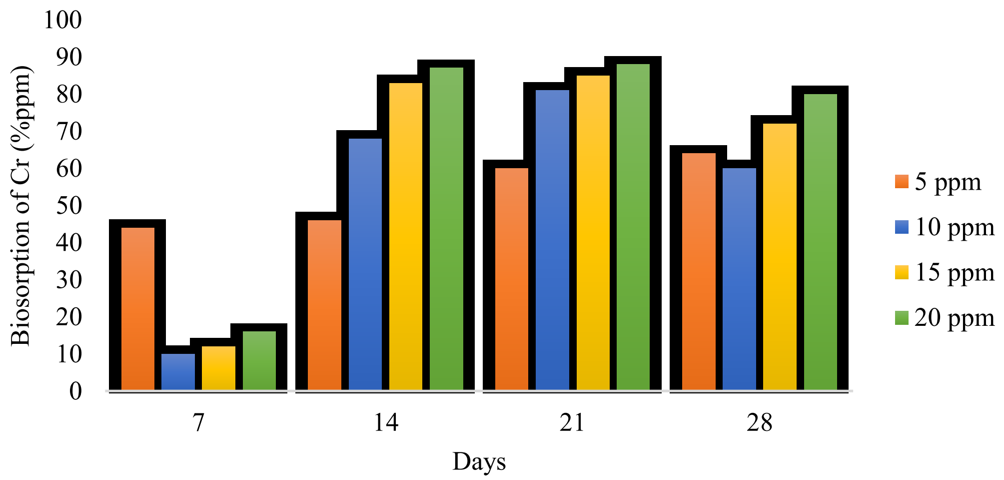
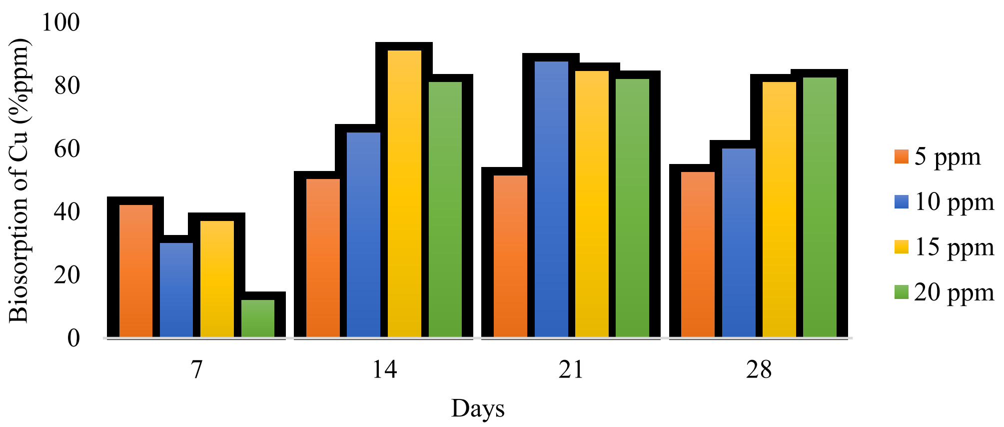


Figure 2. Biosorption of copper by *Pseudomonas aeruginosa*



4.2 Fungi

Fungi are eukaryotic living organism which includes yeasts, mushrooms, molds, etc. The cell wall structure of fungi offers good metal binding properties. Fungi in living and dead both forms can be used as biosorbent material. Metal uptake by fungi involves two processes (i) active uptake or bioaccumulation or intracellular uptake, it is dependent on cell metabolism and (ii) biosorption or passive uptake which involves binding of metal ions to surface of cell wall and it is independent of cell metabolism. The energy independent metal uptake mechanism can be affected by temperature, metabolic inhibitors, etc. Metal uptake by fungi was reported both active and passive. Active uptake occurred only with living cells. In this case, the interaction of metal ions with cell surface functional groups may involves ion exchange, complexation or just physical adsorption.

4.2.1. Biosorption by fungi

Fungal cell wall exhibit excellent metal biding properties due to its components. The cell wall of fungus is composed mainly of chitins, mannans, glucans, in addition to lipids, polysaccharides, pigments e.g. melanin. Fungal cell wall is reported to be made up of 90% polysaccharides. The functional groups which are involved in metal binding includes carboxyl, phosphate, uranic acids, proteins, nitrogen containing ligands, chitin or chitosan.

Biosorption ability of fungal cells can be manipulated by physical of chemical treatments including autoclaving, heat processes or dimethyl sulfoxide, laundry detergent, orthophosphoric acid, formaldehyde, gluteraldehyde, NaOH, respectively. Macrofungi also called as mushrooms, grow wild in all types of environments ranging from forests to polluted soils and water bodies. They uptake the metals in their fruiting bodies, mycelia and sporocarps.

Table 3. Fungal species used in metal biosorption

Metal	Fungi
Arsenic	*Pencillium chrysogenum*
Cadmium	*Aspergillus cristatus*
Chromium	*Penicillium canescens*
Copper	*Fomes fasciatus*
Lead	*Rhizopus nigricans*
Mercury	*Aspergillus flavus*
Nickel	*Aspergillus niger*

4.2.2 Yeasts

Yeasts are famous organisms while studying biosorption. *Saccharomyces cerevisiae* is well known yeast which is considered a model system to study biosorption. They are easy to grow, non-pathogenic and give high biomass yield using simple growth medium. The availability of complete genome information makes its genetic engineering an easy job. They are also considered ideal experimental organism in molecular biology experimentation.

The property of biosorption by yeast cells is affected by various factors including properties of metal ions (valency, radius), cell age of *S. cerevisiae* cells, conditions of culture (composition of growth medium, carbon source), Biosorption conditions (initial concentration of metals and biomass, availability of metal ions, temperature, pH, other ions in growth medium). Moreover, the large size of yeast makes them promising candidates for metal bioremediation. *Saccharomyces cerevisiae* is a widely studied yeast strain. Its different forms are already studied for its biosorption properties including immobilized versus fess cell, living versus dead cells, engineered versus non engineered cells, cultural versus waste cells etc.

4.2.2.1 Biosorption by yeast

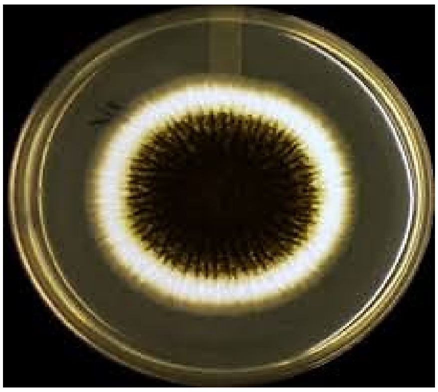
The free form of yeast cells is not considered good candidates for biosorption. Free cells face the problem of separation of solid liquid phase. This problem seems to be less effective in flocculating cell. Pretreatment of yeast cells can result in increased surface to volume ration for binding of metal with the metal binding sites. It is reported that pH above 5 optimizes the metal biosorption in yeast cells. In yeasts, higher concentration of heavy metals can be accumulated by bioaccumulation process than biosorption. However, general biosorption is responsible for the major uptake of heavy metals for many filamentous fungi.

4.2.3 Biosorption of heavy metal polluted soil using fungi isolated from soil

Oyewole *et al*. (2019), conducted a study based on the biosorption of cadmium and nickel polluted soil using fungi isolated from soil. Fungi isolated were *Aspergillus niger*, *Penicillium notatum* and *Aspergillus flavus*. The isolated fungi were screened for potential to biosorb cadmium and nickel. The fungi isolates, *P. notatum* showed highest biosorption rate for cadmium at 10 ppm with 77.67%. *Aspergillus niger* showed highest biosorption rate for nickel with 81.07% after 28 days of incubation (Fig. 3 & 4). The results of this study revealed the ability of *A. niger* and *P. notatum* to biosorb cadmium and nickel from the environment.

Table 4. Screening of fungal isolates for biosorption of cadmium and nickel

Fungal isolates	Cadmium (%)	Nickel (%)
Aspergillus niger	19.18	**43.69**
Aspergillus flavus	17.00	42.69
Penicillium notatum	**38.81**	20.00
Control	1.84	1.65



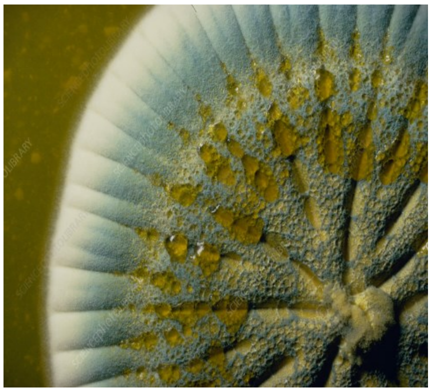


Plate 3. ***Aspergillus niger***

Plate 4. ***Pencillium notatum***

Figure 3. Biosorption of nickel by *Aspergillus niger*

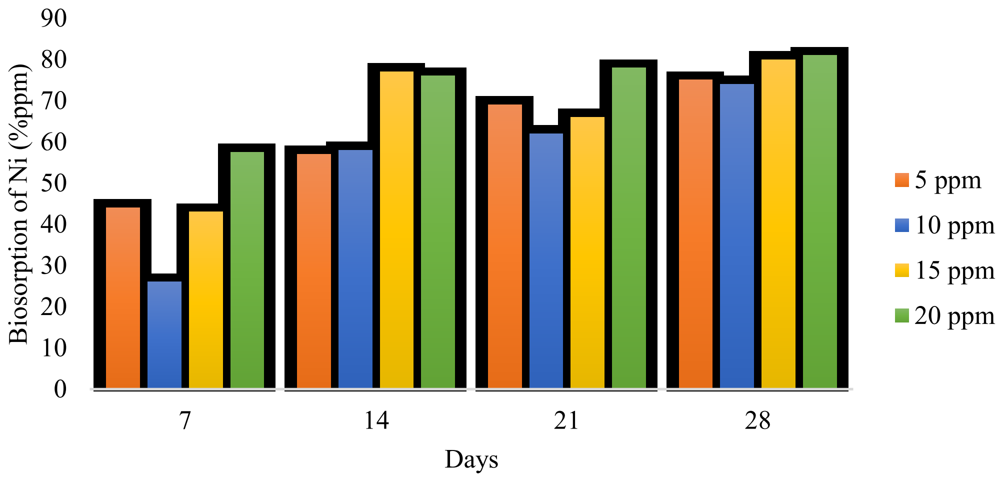
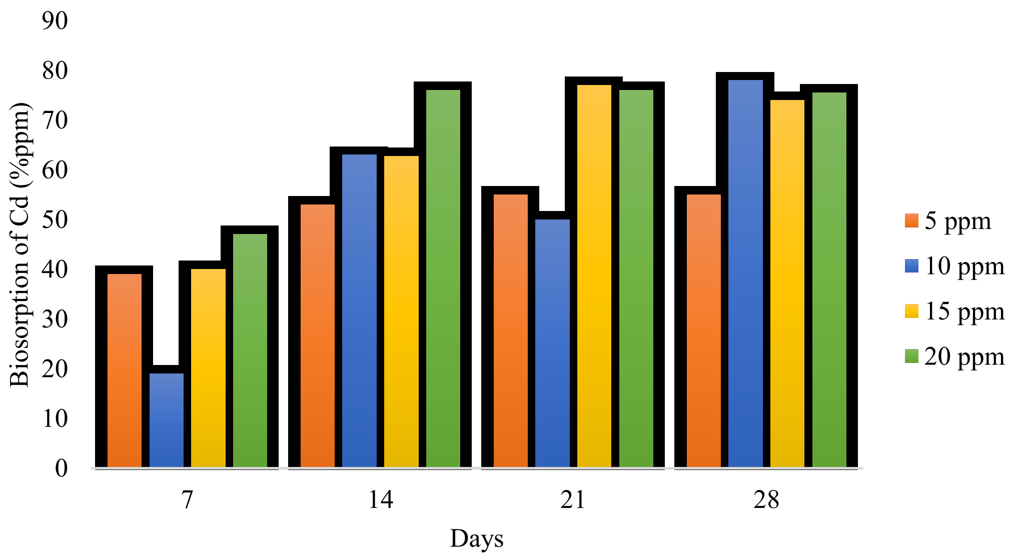


Figure 4. Biosorption of cadmium by *Pencillium notatum*



4.3 Algae

Algae are aquatic plants that lack true roots and stems. It can range from micro algae to macro algae. They are autotrophic. They can grow in big biomass even when less nutrition is provided. They are considered good biosorbent material because of their big size, high sorption capacity and no production of toxic substances. Mostly they are classified as microalgae (fresh water or green algae), macro algae (marine or brown algae) and red algae. Among these three classes, brown alga is reported to have higher metal uptake capacity.

The following features are responsible for binding of heavy metal ions to algae surface; algae species, ionic charge of metal and chemical composition of metal ion solution. Metal ion binding sites on algal surface includes sulfhydryl, hydroxyl, phosphate, sulfate, imidazole, amine, carboxyl groups. The metal uptake mechanism of algae is similar to that of bacteria that is bonding of metal ions with the surface followed by internalization. Two mechanisms in algal biosorption is involved: (1) ion exchange method where ions present on algal surface Ca, Mg, Na, K they are displaced by metal ions, (2) complexation between functional groups and metal ions.

4.3.1. Biosorption by algae

Algal cell wall is made up of polysaccharides (alginic acid, chitin, xylan, mannan) which provides functional groups (sulfate, hydroxyl, phosphate, imidazole, amino, amine) known to act as metal binding sites. As far as metal binding mechanism is concerned, ionic charge and covalent bonding are hypothesized. Carboxyl and sulfate groups are involved in ionic bonding whereas amino and carboxyl groups are involved in covalent bonding between metal ion and functional group. In response to metal ions, phytochelatins are produced inside the algal body.

Table 5. Algal species used in metal biosorption

Metal	**Algae**
Cadmium	*Sargassum* sp.
Chromium	*Sargassum tenerrimum*
Cobalt	*Spirogyra hyaline*
Copper	*Sargassum* sp.
Lead	*Calotropis procera*

Mercury	*Spirogyra hyaline*
Nickel	*Ascophyllum nodosum*

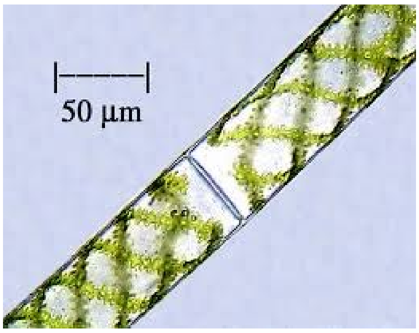


Plate 5. *Spirogyra hyaline*

Plate 6. *Sargassum* sp.

4.4 Cyanobacteria

Cyanobacteria are cosmopolitan microorganisms which play an important role in many ecosystems. The heavy metal Biosorption properties of cyanobacteria is same as that of algae.

4.4.1 Removal of heavy metals by biosorption using cyanobacteria isolated from fresh water pond

The study was aimed at assessing the biosorption of heavy metals (cadmium, mercury and lead) using the living/dead cells of *Oscillatoria limosa* (Sivakami *et al*., 2015). Results indicate that the order of uptake of Cd, Hg and Pb was found to be the order of 82, 78 and 72% respectively (Fig. 5, 6 and 7.). The study also indicated that the metal uptake appeared to be a concentration independent phenomenon where an increase in metal concentration resulted in an increased uptake of metal.

Figure 5. Cadmium uptake by *Oscillatoria limosa*

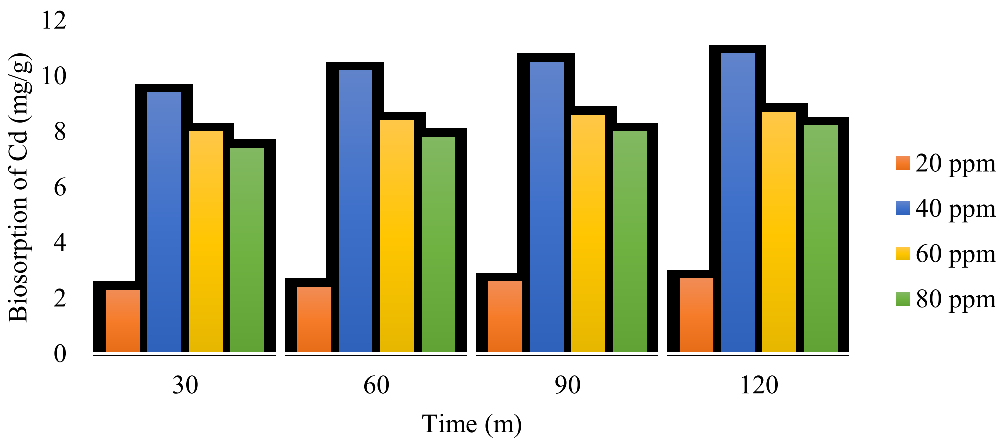


Figure 6. Mercury uptake by *Oscillatoria limosa*

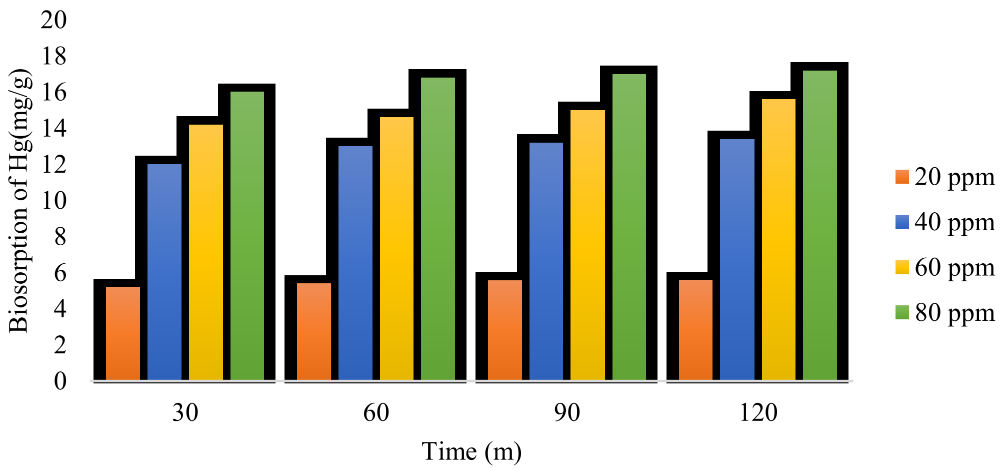
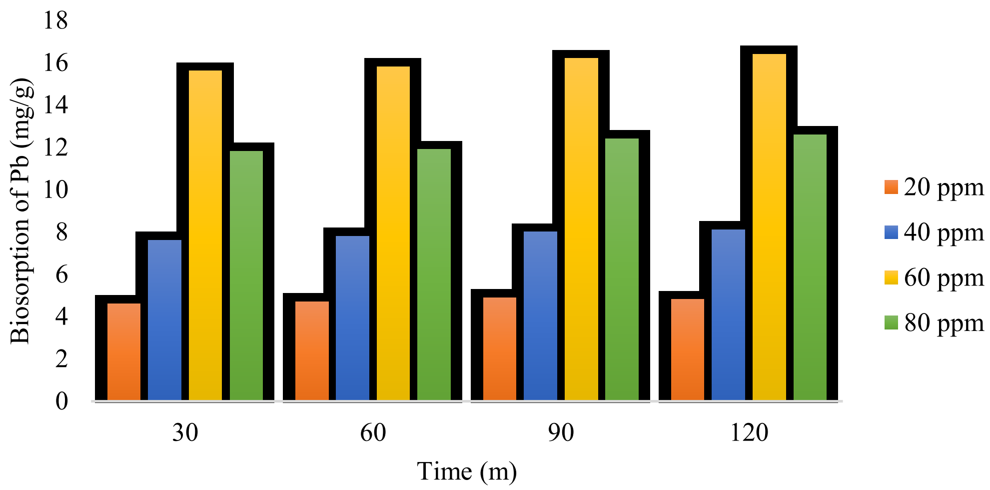


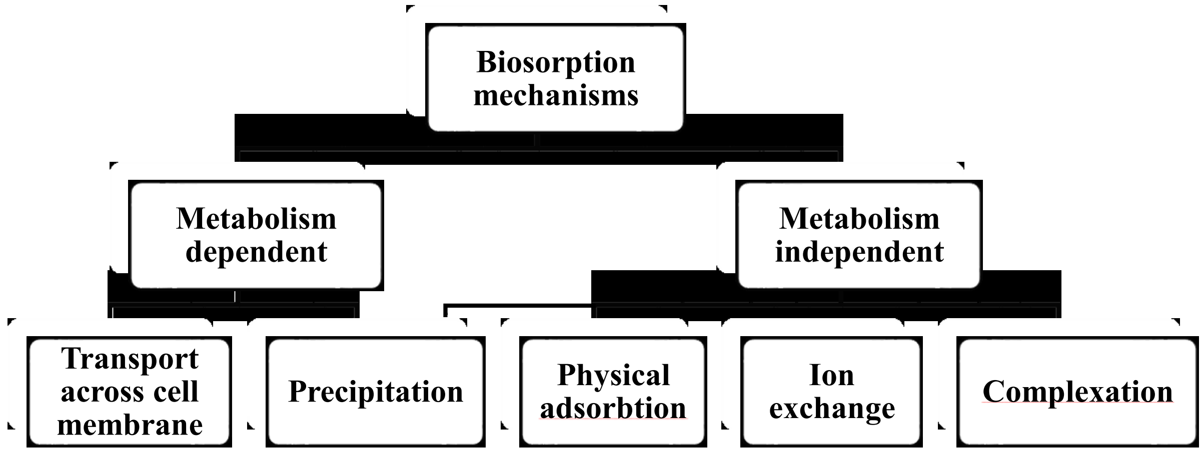
Figure 7. Lead uptake by *Oscillatoria limosa*



5. Mechanisms of microbial biosorption

The process of heavy metal ion binding to bacterial cell wall (peptidoglycan) can be metabolism dependent or independent.

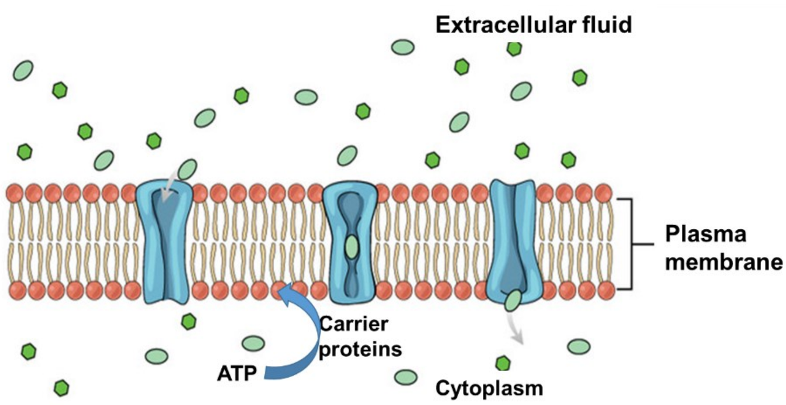
Figure 8. Classification of microbial biosorption mechanisms



5.1. Transport across cell membrane

Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically important ions such as potassium, magnesium and sodium. The metal transport systems may become confused by the presence of heavy metal ions of the same charge and ionic radius associated with essential ions. This kind of mechanism is not associated with metabolic activity. Basically biosorption by living organisms comprises of two steps. First, a metabolism independent binding where the metals are bound to the cell walls and second, metabolism dependent intracellular uptake, whereby metal ions are transported across the cell membrane. (Gadd, 1988).

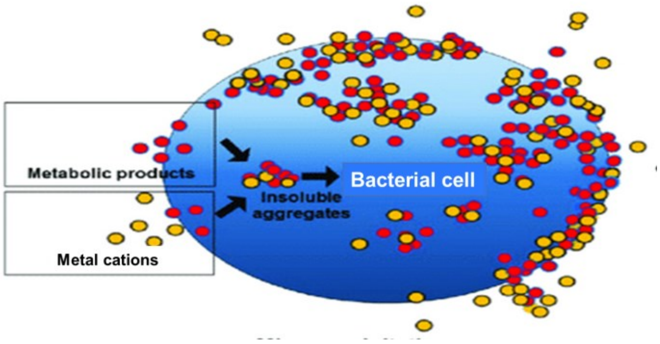
Figure 9. Transport of heavy metals across cell membrane



5.2. Precipitation

Precipitation may be either dependent on the cellular metabolism or independent of it. In the former case, the metal removal from solution is often associated with active defense system of the microorganisms. They react in the presence of a toxic metal producing compounds, which favour the precipitation process. In the case of precipitation not dependent on the cellular metabolism, it may be a consequence of the chemical interaction between the metal and the cell surface. The various biosorption mechanisms mentioned above can take place simultaneously.

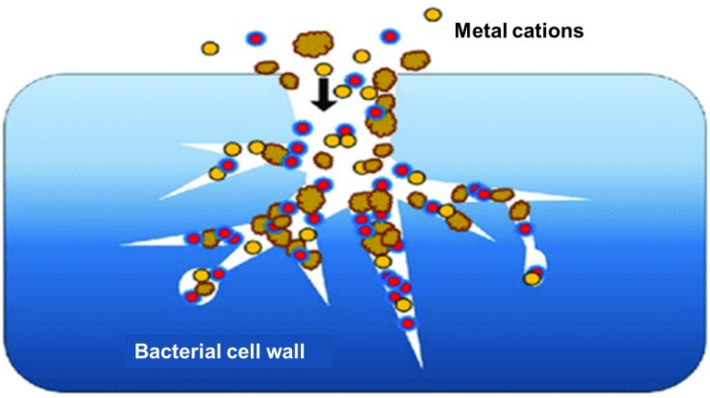
Figure 10. Precipitation



5.3. Physical adsorbtion

Physical adsorption takes place with the help of van der Waals' forces. Kuyucak and Volesky (1988), 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. Electrostatic interactions have been demonstrated to be responsible for copper biosorption by bacterium *Zoogloea ramigera* and alga *Chiarella vulgaris* (Aksu, 1992), for chromium biosorption by fungi *Ganoderma lucidum* and *Aspergillus niger*.

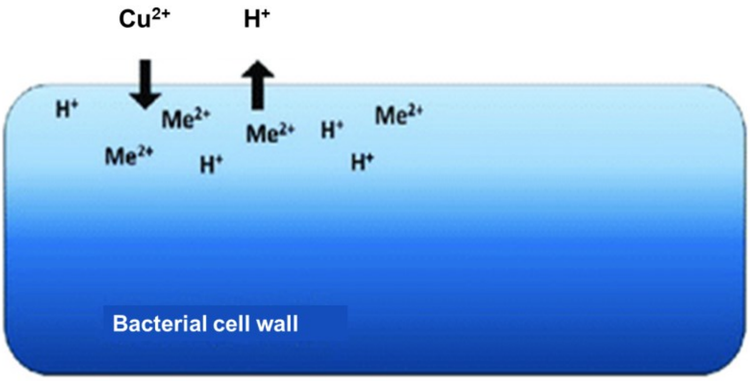
Figure 11. Physical adsorbtion



5.4. Ion exchange

Cell walls of microorganisms contain polysaccharides and bivalent metal ions exchange with the counter ions of the polysaccharides. For example, the alginates of marine algae occur as salts of K^+, Na^+, Ca^{2+},and Mg^{2+}.These ions can exchange with counter ions such as Co^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}resulting in the biosorptive uptake of heavy metals (Kuyucak and Volesky 1988). The biosorption of copper by fungi *Ganoderma lucidium* and *Aspergillus niger* was also uptaken by ion exchange mechanism.

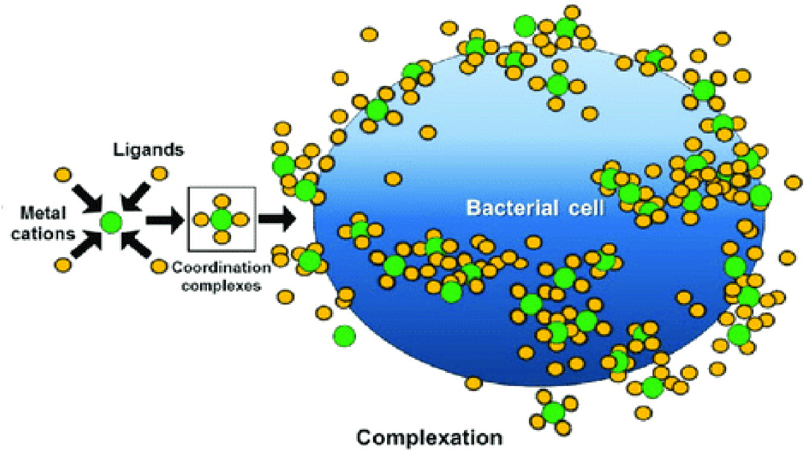
Figure 12. Ion exchange



5.5. Complexation

The metal removal from solution may also take place by complex formation on the cell surface after the interaction between the metal and the active groups. Aksu (1992), hypothesized that biosorption of copper by C. *vulgaris* and Z. *ramigera* takes place through both adsorption and formation of coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides. Complexation was found to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by *Pseudomonas syringae.* Microorganisms may also produce organic acids (e.g., citric, oxalic, gluonic, fumaric, lactic and malic acids), which may chelate toxic metals resulting in the formation of metallo-organic molecules. These organic acids help in the solubilisation of metal compounds and their leaching from their surfaces. Metals may be biosorbed or complexed by carboxyl groups found in microbial polysaccharides and other polymers.

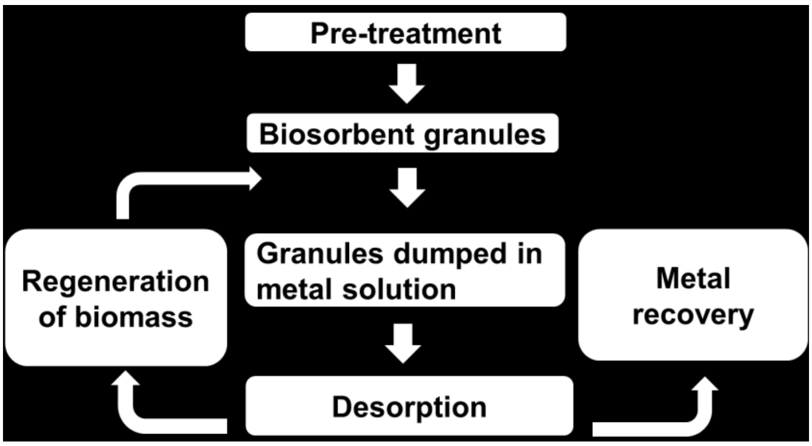
Figure 13. Complexation



6. Process of microbial biosorption

A successful biosorption process requires the preparation of a good biosorbent. The process starts with the selection of the most promising type of biomass. Pretreatment and immobilization are done to increase the efficiency of the metal uptake. Biosorption experimental studies are carried out by batch and column processes, and, finally, the adsorbed metal is removed by a desorption process and the biosorbent can be reused for further treatments.

Figure 14. Flowchart showing process of microbial biosorption

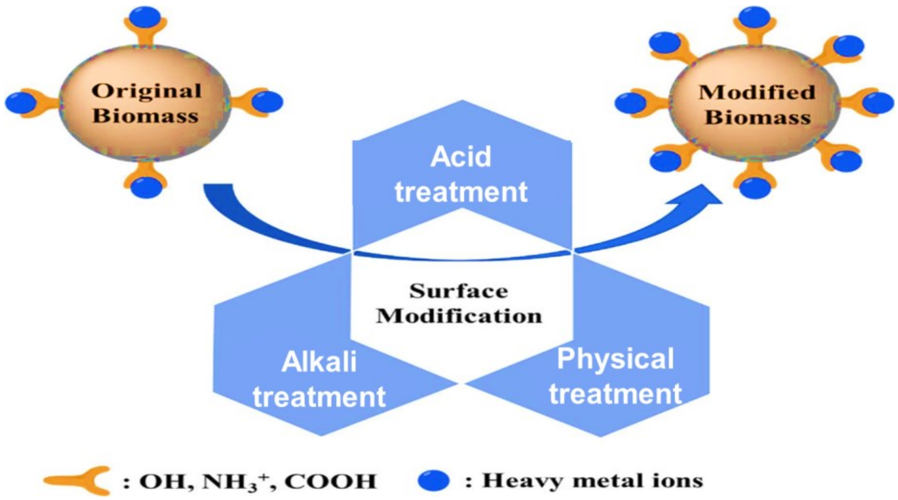


6.1 Pre-treatment of biosorbents

Biosorbents are prepared initially by pretreating the raw biomass with different chemicals that cause cell wall modification of the biomaterial by creating derivatives with altered metal-binding abilities and affinities, which in turn affect the biosorption potential. It has been suggested that the pretreatment modifies the surface characteristics/groups either by removing or masking the groups or by exposing more metal-binding sites.

Common chemical pretreatments include acid, alkali, ethanol and acetone treatments of the biomass. The success of a chemical pretreatment strongly depends on the cellular components of the biomass itself. Alkali treatment of fungal biomass has increased the metal-uptake capacity significantly, whereas acid treatment of biomass almost has no influence on metal biosorption. *Aspergillus niger* mycelium was modified by introducing additional carboxy, ethaldiamino groups, which increased metal biosorption. The biosorption of cadmium and lead ions from synthetic aqueous solutions using yeast biomass was investigated. The baker's yeast cells were treated with caustic, ethanol and heat, and the highest metal uptake values for Ca^{2+} and Pb^{2+} were obtained by ethanol-treated yeast cells.

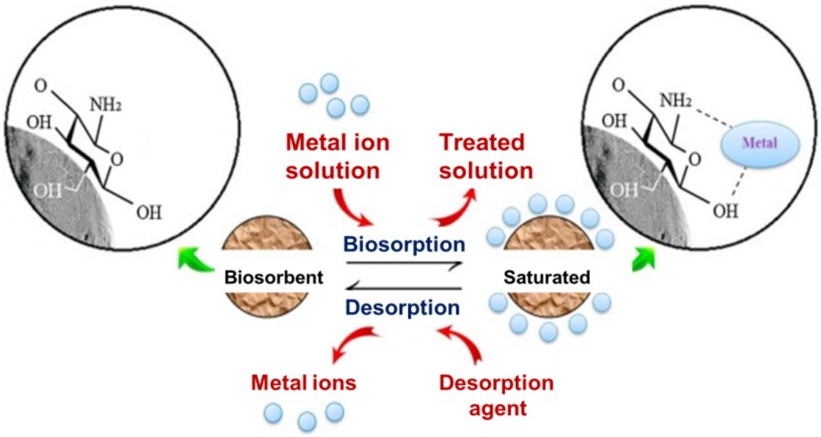
Figure 15. Pre-treatment process



6.2 Desorption and regeneration of biomass

The biggest achievement of a biosorption process is to concentrate the solute, i.e. sorption followed by desorption. Desorption is of utmost importance when the biomass reparation/generation is costly, as it is possible to decrease the process cost and also the dependency of the process on a continuous supply of biosorbent. A successful desorption process requires the proper selection of eluents, which strongly depends on the type of biosorbent and the mechanism of biosorption. Also, the eluent must be (1) non-damaging to the biomass, (2) not costly, (3) environmentally friendly and (4) effective. Several investigators have conducted exhaustive screening experiments to identify appropriate eluants for this process. Even though some chemical agents perform well in desorption, they may be detrimental to the biosorbent. Dilute mineral acids (HCl, H_2SO_4, and HNO3) have been used for the removal of metals from fungal biomass, and also organic acids (citric, acetic, and lactic) and complexing agents (EDTA, thiosulphate etc.) can be used for metal elution without affecting the biosorbent.

Figure 16. Desorbtion process



7. Factors affecting microbial biosorption

The key factors affecting biosorption efficiency include initial concentration of metal ions, temperature, solution pH, contact time, ionic strength and biosorbent concentration.

7.1. Effect of initial solute concentration

The initial solute concentration seems to have impact on biosorption, with a higher concentration resulting in a highsolute uptake. This is because at lower initial solute concentrations, the ratio of the initial moles of solute to the available surface area is low; subsequently, the fractional sorption becomes independent of the initial concentration. However, at higher concentrations, the sites available for sorption become fewer compared to the moles of solute present and; hence, the removal of solute is strongly dependent upon the initial solute concentration. It is always necessary to identify the maximum saturation potential of a biosorbent, for which experiments should be conducted at the highest possible initial solute concentration.

7.2 Effect of temperature

Temperature seems to affect biosorption only to a lesser extent within the range from 20 to 35 °C. Higher temperatures usually enhance sorption due to the increased surface activity and kinetic energy of the solute; however, physical damage to the biosorbent can be expected at higher temperatures. Due to the exothermic nature of some adsorption processes, an increase in temperature has been found to reduce the biosorption capacity of the biomass. High temperatures above 45°C may results in damage to proteins which in turn affects metal uptake process. It is always desirable to conduct/evaluate biosorption at room temperature, as this condition is easy to replicate.

7.3 Effect of pH

Solution pH usually plays a major role in biosorption, and seems to affect the solution chemistry of metals/ dyes and the activity of the functional groups of the biomass. For metals, the pH strongly influences the speciation and biosorption availability of the metal ions. At higher solution pH, the solubility of metal complexes decreases sufficiently allowing precipitation, which may complicate the sorption process. The activity of binding sites can also be altered by adjustment of the pH. For instance, during the biosorption of metal ions by bacterial biomass, pH 3 to 6 has been found favorable for biosorption, due to the negatively charged carboxyl groups (pKa=3–5), which are responsible for the binding metal cations via ion exchange mechanism. In the case of the biosorption of dyes, different dye classes require different pH ranges

7.4 Effect of contact time

The time required to attain maximum biosorption depends on the type of biosorbent, metal ion, and their combination. The rate of biosorption is rapid initially (within an hour) with almost 90% of the metal binding because all the active sites are vacant and available for metal ion biosorption. But with increase in time the rate of biosorption decreases due to increase in percentage saturation by metal ions remaining in the solution. Most of the Cd and Zn ions are biosorbed onto *Aspergillus niger* biomass within the first 6 h and there is no further biosorption after 24 h. *Bacillus cereus* and *Pseudomonas aeruginosa* biosorb Zn ions with an equilibrium contact time of 30 min.

7.5 Effect of ionic strength

Another important parameter in biosorption is the ionic strength, which influences the adsorption of solute to the biomass surface. The effect of ionic strength may be ascribed to the competition between ions, changes in the metal activity, or in the properties of the electrical double layer. When two phases, e.g. biomass surface and solute in aqueous solution are in contact, they are bound to be surrounded by an electrical double layer owing to electrostatic interaction. Thus, adsorption decreases with increase in ionic strength. Some inorganic ions, such as chloride, may form complexes with some metal ions and therefore, affect the sorption process.

7.6 Effect of biosorbent concentration

The dosage of a biosorbent strongly influences the extent of biosorption. In many instances, lower biosorbent dosages yield higher uptakes and lower percentage removal efficiencies. An increase in the biomass concentration generally increases the amount of solute biosorbed, due to the increased surface area of the biosorbent, which in turn increases the number of binding sites. Conversely, the quantity of biosorbed solute per unit weight of biosorbent decrease with increasing biosorbent dosage, which may be due to the complex interaction of several factors. An important factor at high sorbent dosages is that the available solute is insufficient to completely cover the available exchangeable sites on the biosorbent, usually resulting in low solute uptake. Also, as suggested by Gadd (1988), the interference between binding sites due to increased biosorbent dosages cannot be overruled, as this will result in a low specific uptake.

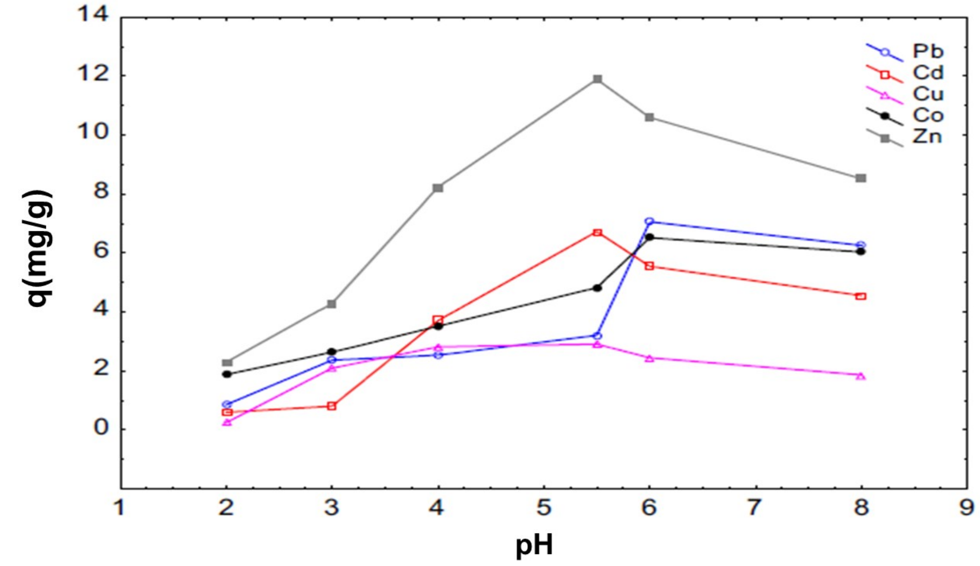
8. Effect of various factors on biosorption of heavy metals by *Saccharomyces cerevisiae*

Farhan and Khadom (2015), evaluated the performance of the yeast *Saccharomyces cerevisiae* to remove heavy metals from aqueous solutions. The effect of pH, temperature, initial concentration, contact time, and biosorbent dosage on biosorption capacity was studied and reported as below.

8.1 Effect of pH

Metal uptake is a rapid process at pH values (5.0–6.0), and the order of accumulated metal ions is Pb>Zn>Cr>Co.>Cd>Cu.

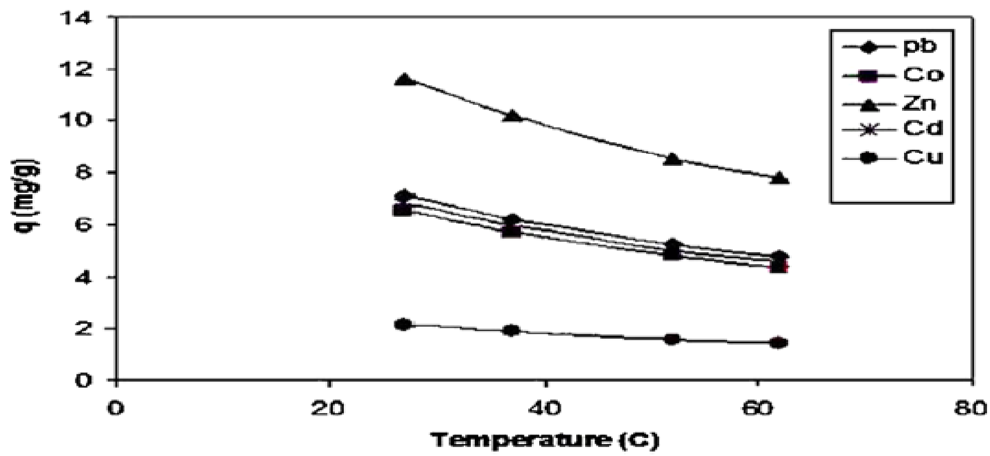
Figure 17. Effect of pH on heavy metal biosorption by *S. cerevisiae*



8.2 Effect of temperature

Temperature has an influence on the biosorption of metal ions, but to a limited extent under a certain range of temperature. The increase of temperature indicating a decrease of sorption capacity and the maximum equilibrium uptake occurred at 27°C as shown in Fig. It is important to mention that the biosorption process is usually not operated at high temperature because it will increase the operational cost. Since adsorption reactions are normally exothermic, biosorption capacities increase with decrease in temperature. The decrease in biosorption capacity between 27 and 62°C may be due to the damage of active sites in the yeast. Many other researchers have also observed the same results.

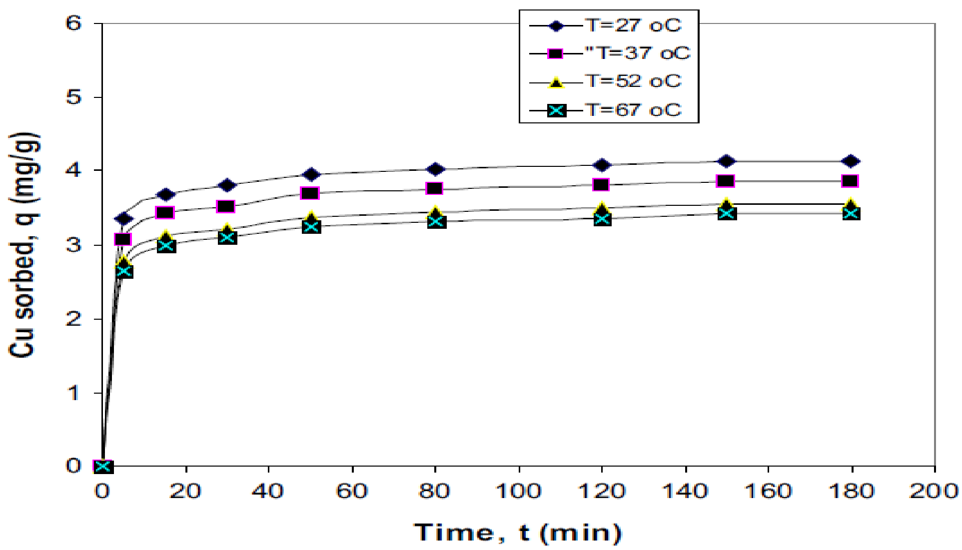
Figure 18. Effect of temperature on heavy metal biosorption by *S. cerevisiae*



8.3 Effect of time

It is seen that the biosorption capacity increases with an increase in time at constant temperature. The amount of metal ion sorbed per unit mass of sorbent increases sharply up to 5, and 30 min and increases thereafter, slowly reaching equilibrium. The short contact time of biosorbent with metal solution for biosorption suggests that adsorption onto the biosorbent surface is the main mechanism of uptake.

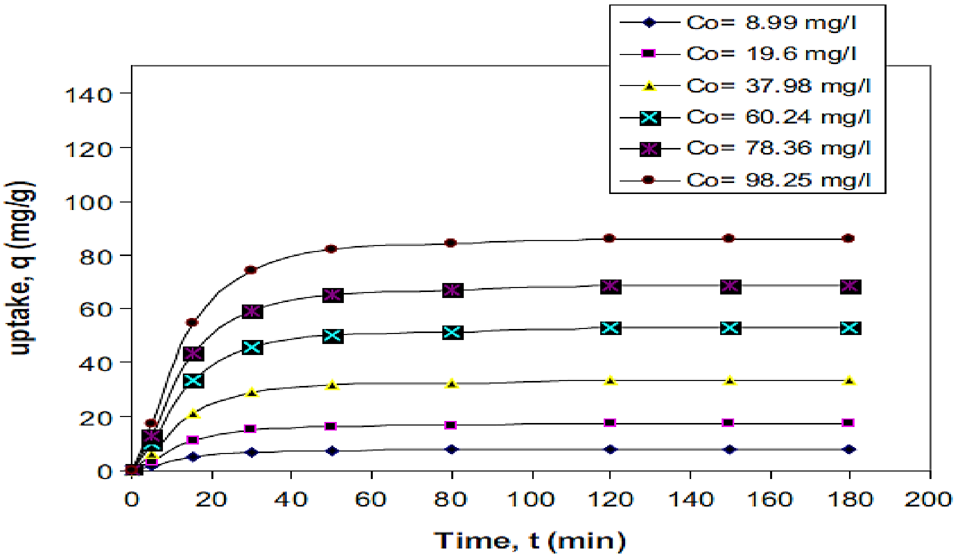
Figure 19. Effect of temperature on biosorption of copper with time



8.4 Effect of initial concentration

Biosorption has been observed to increase as initial concentration increases; this may be attributed to the active binding sites available for available sorbate ions. Figure shows that biosorption is very fast for all metal ions in the first 5 min, while for the remaining time period, the metal concentrations in the liquid continued to diminish and reach an equilibrium concentration value. The faster first phase of metal biosorption may be attributed to the surface adsorption due to the action of ion exchange with the participation of some functional groups, while the second lower phase may represent diffusion of metal ions into the cell.

Figure 20. Effect of initial concentration on biosorption of cobalt



9. Conclusion

Microbial biosorption is a useful alternative to conventional systems for the removal of toxic heavy metals from industrial effluents. It is a “promising-age old technology” which is under-exploited. However, further studies are required for commercialization of microorganisms for large scale application.

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11. Discussion – Questions & Answers

1. Is microbial biosorption commercially exploited.? If so can you name some products.?

Microbes used as biosorbents is still in the infancy stage and microbial biosorption is not commercially exploited. Some products under reseach trials are Sorb-Ex, Alga-Sorb, Bio-Claim etc.

2. In the conclusion you have mentioned biosorption as a "promising-age old technology" which is under-exploited. Can you please clarify.?

The technology as such was discovered in 1980s, but nothing is new in it. And higher ability of microbes to remove heavy metals compared to other adsorbants and heavy metal remediation process is well proved in laboratory conditions. Since microbial biosorption is a complex procedure involving various factors, laboratory scale study is not sufficient for commercial use. Field trials are being taken up but, data are still not available. So, there should be more researches for taking up these technologies.

3. Weather both dead and living microorganisms can be used for biosorbtion.?

Bioaccumulation is used to describe the process involving living cells, whereas the biosorption mechanism involves the use of dead biomass. To be precise, bioaccumulation can be defined as the uptake of toxicants by living cells. In general, the use of living organisms may not be an option for the continuous treatment of highly toxic organic/inorganic contaminants. The toxicant can transport into the cell and accumulate intracellularly, across the cell membrane and through the cell metabolic cycle. Beyond this point, an organism's metabolism may be interrupted, resulting in death of the organism. This scenario can be avoided in the case of dead biomass, which is flexible to environmental conditions and toxicant concentrations, and thus the use of dead biomass seems to be a preferred alternative.

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF HORTICULTURE, VELLANIKKARA

Department of Agricultural Microbiology

MICRO 591: Masters Seminar

Name: Bony Cyriac	Venue : Seminar hall
Admission No.: 2018-11-097	Date : 12-12-2019
Major advisor: Dr. K. Surendra Gopal	Time : 11.30 am

Microbial biosorption of heavy metals

Abstract

Human and industrial activities produce and discharge wastes containing heavy metals into the water resources making them toxic, which threatens human health and ecosystem. Conventional methods for the removal of metal ions such as chemical precipitation and membrane filtration are extremely expensive, inefficient at low concentrations of metal and generate large quantities of sludge and other toxic products that require careful disposal (Zhao *et al*., 2015). These disadvantages, together with the need for more economical and effective methods for the recovery of metals from waste water, have resulted in the development of alternative separation technologies. Biosorption is one of the alternatives for conventional methods.

Biosorption can be defined as the ability of biological materials to accumulate heavy metals from waste water through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). Microbial biosorption includes the use of bacteria, fungi, algae and cyanobacteria for biosorption of heavy metals. Biosorption has become popular due to low cost, high efficiency for dilute effluents, no additional nutrient requirements and minimum quantity of chemical and/or biological sludge to be disposed-off (Michalak *et al*., 2013). For bacterial isolates, the highest biosorption rates for chromium (89.67%) and copper (90.89%) were by *Pseudomonas aeruginosa* at 20 ppm on day 21 and 15 ppm on day 14 respectively. The fungal isolates, such as *Pencillium notatum* showed the highest biosorption rate for cadmium (77.67%) at 10 ppm and *Aspergillus niger* showed the highest biosorption rate for nickel (81.07%) at 20 ppm after 28 days of incubation (Oyewole *et al*., 2019).

The mechanism of microbial biosorption is a complex process which involves the binding of sorbate onto the biosorbent by transport across cell membrane, precipitation,

physical adsorption, ion exchange and complexation. Process of microbial biosorption also involves pre-treatment, preparation of biosorbent granules, sorption of heavy metals and de-sorption. The key factors affecting biosorption efficiency include initial concentration of metal ions, temperature, solution pH, contact time, ionic strength and biosorbent concentration. Studies on the effect of various factors on biosorption of heavy metals by *Saccharomyces cerevisiae* showed that the heavy metal uptake is a rapid process at pH 5.0-6.0. The maximum equilibrium uptake occurred at 27°C and biosorption capacity increased with an increase in time at constant temperature. An increase in biosorption has also been observed with an increase in the initial concentration of heavy metals (Farhan and Khadom, 2015).

Microbial biosorption is a useful alternative to conventional systems for the removal of toxic heavy metals from industrial effluents. It is a "promising-age old technology" which is under-exploited. However, further studies are required for commercialization of microorganisms for large scale application.

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