Management of Heavy Metal Pollution by using Bacterial Biomass

Yogeshwari Yadav[1], Ragini Gothalwal[1], R.K.Tenguriya[2]

[1]Department of Biotechnology Barkatullah university, Bhopal M.P.-462026, India
[2] Division of Microbiology, Department of Botany, Gov., M.V.M.Bhopal, M.P.-462008, India

Abstract

Municipal solid waste containing a wide range of organic and inorganic compounds which causes contamination to the environment. Heavy metals are major components of inorganic compounds and cannot degrade. Due to their toxic nature, it's necessary to remove from environment. Conventionally metal removal by classis techniques involve expensive methodologies and not get to successful optimum results. Currently the biological methods such as Biosorption have receiving attention to remove heavy metals from environment. The technique also has significant role in recovery of metals of economic importance from various solution phases, as it is economic and producing less quantity of toxic products using biological material e.g. bacteria. Various other biomaterials are also used to bind pollutants e.g. fungi, algae and wastes of agricultural and industrial products. The biosorbents of bacterial origin has proven their good performance with low cost and feasibility. They have multivariate functional units for biosorption, which is dependent on various physicochemical parameters. In this review the problems associated with bacterial biosorption are analyzed, and suitable remedies are discussed. The ways of heavy metals removal with significance of methods, procedural ways of biosorption studies and the role of bacteria as biosorbent are also focused.

Keywords - *Biosorption, Bacteria, Pollution, Heavy metal, Municipal Solid Waste (MSW*)

I. INTRODUCTION

With rapid industrialization all over the world, pollution is drastically increasing. Increasing urbanization, industrialization and population explosion in a developing country have created enormous problems of environmental pollution in terms of generating the variable quantity and quality of solid and liquid wastes [13]. This waste contains all the substances that enter in human metabolism, such as food, beverages, pharmaceuticals, a huge variety of household chemicals and the substances discharged from trade and industry to the sewer system. [79].Shrivastav *et al.,*[84] reported 3.4 to 4.0 billion tons of municipal and industrial waste and around 300 million tons of hazardous waste are annually produced worldwide. In the coming years this figure will be increasing rapidly which in turn will increase the global demand for solutions that convert waste into energy or electricity.[80,19].Urban India produces 188,500 tons per day (68.8 million tons/year) of municipal waste per captia waste generation rate of 500 grams/person/day. The waste amount will increase rapidly due to industrialization in the country by the year 2020.[1,74].Indian cities are generating eight times more municipal solid waste (MSW) than they did in 1947 because of changing life styles and population explosion .The rate of increase of MSW generated per capita is estimated at 1 to 1.33% annually [57].MSW generation rate in small towns are lower than those of metro cities which varies between 200-300 gms/capita for small towns, 300-400 gms/capita for medium cities and 400-600 gms/capita for larger cities as per the Planning Commission report and the per capita generation rate of MSW in India ranges from 0.2 to 0.5 kg/ day [69].It was also estimated that the total MSW generated by 217 million people living in urban areas was 23.86 million ton/year in 1991, and more than 39 million ton in 2001[60] and per capita waste generation rate is changing from decades to decades (0.2 kg/capita in 1981 and 0.47 kg/capita) will be in 2035 due to change of economic growth[27].

According to the Central Pollution Control Board [23] India generated 1, 43,449 tons per day (TPD) of municipal solid waste during 2014-15, with an average waste generation of 0.11 kg/capita/day (GIZ, 2015). According to the report of the Task Force on Waste to Energy (WtE), 2014, of the Planning Commission, the 7935 urban centers of India generate 1,70,000 TPD i.e. 62 million tons of MSW annually. The Central Pollution Control Board (CPCB) had conducted a survey of solid waste management in 299 cities and has given the data (Table-1) of waste generation for different cities.

Table-1: Status of MSW generation, collection, treatment and disposal in class-I cities [22, 23]

S.N.	Name of the State	No. of cities	Municipal population	Municipal solid waste	Per capita generated

				(t/day)	(kg/day)
1	Andhra Pradesh	32	10,845,907	3943	0.364
2	Assam	4	878,310	197	0.223
3	Bihar	17	5,278,361	1479	0.280
4	Gujarat	21	8,443,972	3805	0.451
5	Haryana	12	2,254,353	623	0.276
6	Himachal	1	82,054	35	0.427
7	Karnataka	21	8,283,448	3518	0.376
8	Kerala	146	3107358	1220	0.393
9	Madhya Pradesh	23	7225833	2286	0.316
10	Maharashtra	27	22727186	8589	0.378
11	Manipur	1	198535	40	0.201
12	Meghalaya	1	223366	35	0.157
13	Mizoram	1	155240	46	0.297
14	Orissa	7	1766021	646	0.366
15	Punjab	10	3209903	1001	0.312
16	Rajasthan	14	4979301	1768	0.355
17	Tamil Naidu	25	10745773	5021	0.467
18	Tripura	1	157358	33	0.210
19	Uttar Pradesh	41	14480479	5515	0.381
20	West Bengal	23	13943445	4475	0.321
21	Chandigarh	1	504094	200	0.397
22	Delhi	1	8419084	4000	0.475
23	Pondicherry	1	203065	60	0.295
		299	128113865	48134	0.376

Table-2: Per Capita Quantity of Municipal Solid Waste in Indian Cities [57]

S.N.	Population	Waste Generation Rate Kg/capita/day
1	Cities with a population < 0.1 million (8 cities)	0.17-0.54
2	Cities with a population of 0.1–0.5 million (11 cities)	0.22-0.59
3	Cities with a population of 1–2 million (16 cities)	0.19-0.53
4	Cities with a population > 2 million (13 cities)	0.22-0.62

Table-2 suggests the per capita quantity of municipal solid waste in Indian cities. It also suggests that

average municipal solid waste production varies from 0.21 to 0.50 Kg per capita per day in India. The urban population of India is approx. 341 million in 2010. MSW quantities are expected to increase from 34 million tons in 2000 to 83.8 million tons in 2015 and 221 million tons in 2030.It is also reported that per capita per day production will increase to 1.032 kg, and urban population to 586 million in 2030.The higher the household income and standard of living, the higher will be the amount of MSW generated [27].

II. CHARACTERISTICS OF MUNICIPAL SOLID WASTE

The agricultural use of municipal waste has been a common practice of waste disposal nowadays [40]. As compared to the western countries, MSW differs greatly with regard to the composition and hazardous nature, in India [24].Many categories of MSW are found such as domestic waste, commercial waste, institutional waste, industrial waste, construction and demolition waste, and sanitation waste. Since it also contains various types of plant nutrients, organic matter toxicants and considerable amount of toxic metals and other microbial pollutant such as *Campylobacter sp., Enterohemorrhagic, Escherichia coli (*e.g., *E. coli O157:H7), enterotoxigenic Staphylococcus aureus, enterotoxigenic Bacillus cereus, Listeria monocytogenes, Salmonella sp., Shigella sp., Yersinia enterocolitica, protozoa Cryptosporidium sp., Cyclospora cayetanensis, Giardia sp., Entamoeba histolytica, helminths such as Ascaris sp.,* and viruses, in particular, *adenoviruses, enteroviruses, noroviruses*, and *rotaviruses.*[24,40 and 89]. The physical and chemical characteristic of solid waste varies depending on population size and consumption pattern. According to Joshi *et al.,* [52] MSW in India approximately has 40-60% composite waste, 30-40% inert waste and 10-30% recyclable waste. Analysis carried out by NEERI reveals that, in total Indian waste consists of Nitrogen content(0.64±0.8)%, Phosphorous(0.67 ±0.15)% and C/N ratio(26±5)%. The report of the Earth Engineering Centre, 2012 stated that the calorific value of the waste taken largely from 7 large metropolises varied between 6.8-9.8 MJ/Kg (1620-2340 Kcal/kg.).Waste from smaller cities has low calorific value less than 800Kcal/kg. It is known that a calorific value of over 2800 Kcal/kg is required for feasible incineration. [84].Approximately more than 450 cities in India generate more than 17 million cubic meters of raw waste per day [52]. Generally this waste material contains a wide range of inorganic and organic compounds cause contamination, these include heavy metals, combustible and putriscible substances, hazardous wastes, explosives and petroleum products. Major component of inorganic contaminants are heavy metals and pose a different problem than organic contaminant [14, 53, 56].

III. HEAVY METALS AND THEIR TOXICITIES

Heavy metals are considered one of the most common and hazardous pollutants having a specific density of more than 5 g/cm3 .Metals, like copper, iron, manganese, zinc are essential for life processes whereas others, like cadmium, nickel and mercury have no physiological function but often results in harmful disorders at a higher concentration. [66]

Heavy metal contamination is defined as the increased levels of toxic metals in the environment which can be subtle, silent, stalking killers. Many anthropogenic activities such as soldering, mining, refining of ores, combustion of fossil fuels, fertilizers and pesticides, metallurgy and municipal solid wastes are result in metal contamination. Metals ions are reported as priority pollutants, due to their mobility in natural ecosystems and due to their toxicity. The problem associated with metal ions pollution is that they are not biodegradable and are highly persistent in the environment. Thus they can be accumulated in living tissues, causing various diseases and disorders. [95].Some of the most important studied causes of heavy metal poisoning are Itai-Itai disease caused due to polluting Jinzugawa river by cadmium of the Mitsui Mining & Smelting Company, Japan and Minimata disease caused by mercury toxicity from contaminated fish in Japan.[12].The potential health hazards of some metal ions as given by the Table:3

Due to their toxic nature, the management of heavy metals is of special concern; therefore it is necessity to remove metal ions from environment. In this respect, many physicochemical methods have been developed. Some of these methods are illustrated in Fig: 1.

Each of these methods has its advantages and disadvantages so that they are inadequate to deal with various environmental problem; the advantages and disadvantages of the conventional methods for metal removal are listed in Fig: 2

Table-3: Health Hazards of some Heavy metal

Metal	Source	Effect on Humans	References
Arsenic	Atmospheric deposition, mining, pesticides, rock sedimentation	Brain damage, cardiovascular and respiratory disorder, conjunctivitis, dermatitis, skin cancer	[66]
Chromium	Dyeing, electroplating, Paints production, steel fabrication, tanning, textile	Bronchopneumonia, chronic bronchitis, diarrhea, emphysema, headache, irritation of the skin, itching of respiratory tract, liver diseases, lung cancer, Nausea.	[45]
Copper	Copper polishing, mining, paint, plating, printing operations	Abdominal pain, anemia, diarrhea, headache, liver and kidney damage, metabolic disorders, nausea, vomiting	[44]
Lead	Coal combustion, electroplating, manufacturing of batteries, mining, paint, pigments	Anorexia, chronic nephropathy, damage to neurons, high blood pressure, hyperactivity, insomnia, Risk factor for Alzheimer's disease.	[16]
Nickel	Electroplating, nonferrous metal, paints, porcelain enameling	Cardiovascular diseases, chest pain, dermatitis, dizziness, dry cough and shortness of breath, headache, kidney diseases, lung and nasal cancer, nausea	[68]

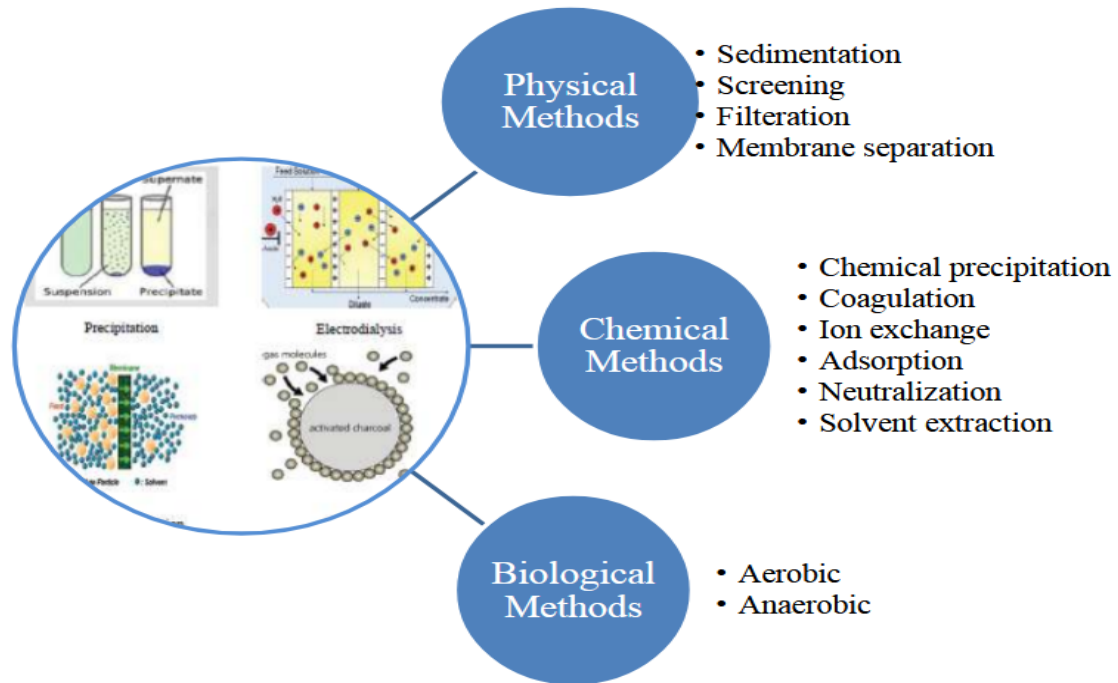**Fig: 1 Conventional Technology for Heavy Metal Removal**

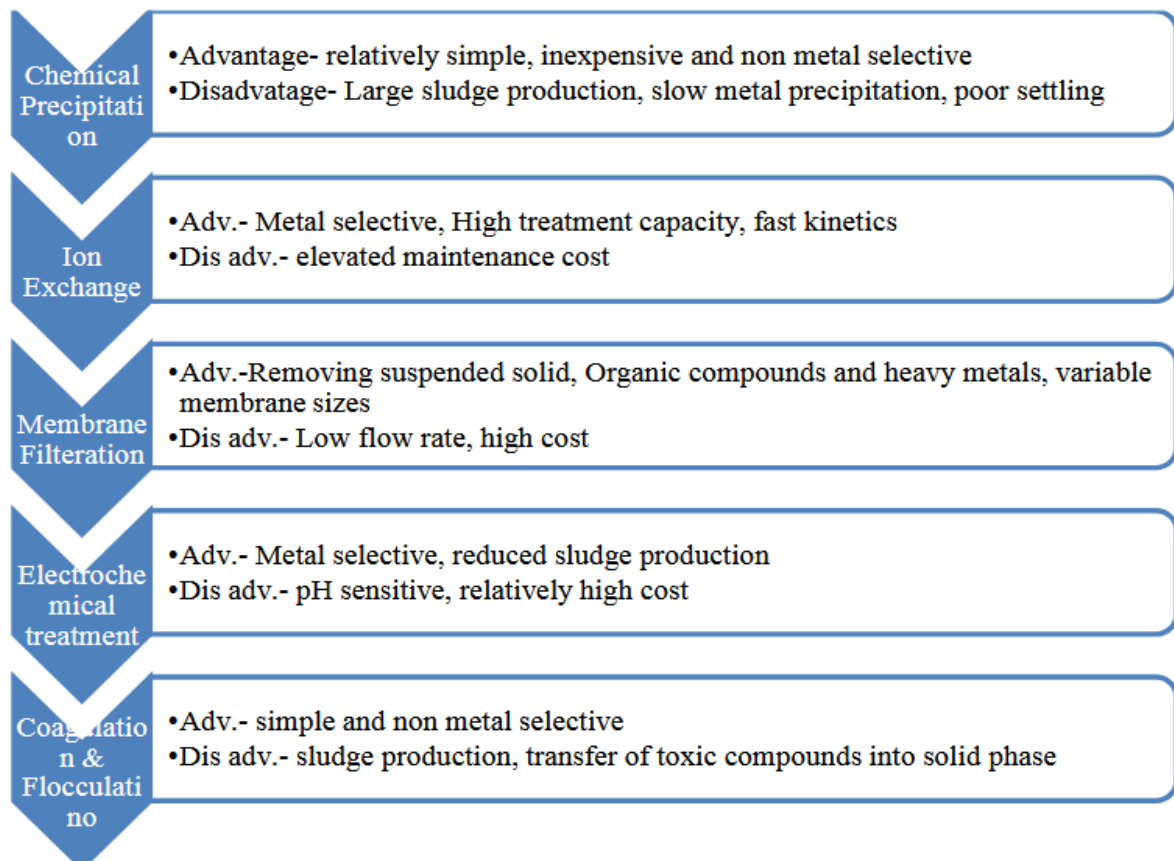**Fig: 1 Conventional Technology for Heavy Metal Removal**

Chemical Precipitation	• Advantage- relatively simple, inexpensive and non metal selective • Disadvatage- Large sludge production, slow metal precipitation, poor settling
Ion Exchange	• Adv.- Metal selective, High treatment capacity, fast kinetics • Dis adv.- elevated maintenance cost
Membrane Filteration	• Adv.-Removing suspended solid, Organic compounds and heavy metals, variable membrane sizes • Dis adv.- Low flow rate, high cost
Electrochemical treatment	• Adv.- Metal selective, reduced sludge production • Dis adv.- pH sensitive, relatively high cost
Coagulation & Flocculatino	• Adv.- simple and non metal selective • Dis adv.- sludge production, transfer of toxic compounds into solid phase

Fig: 2 Advantages and Disadvantages of Conventional   methods of Metal Removal

Therefore, the search for efficient, eco-friendly and cost effective techniques for the removal of heavy metal has been initiated. Biological methods such as Biosorption is an efficient, economical and ecofriendly method which uses inexpensive biomaterials to sequester environmental pollutants from aqueous solution by a wide range of physicochemical mechanisms, including ion exchange,chelation,complexation,physical adsorption and surface micro precipitation[30].

IV. BIOSORBENTS MATERIAL

Various waste materials and microorganism have been reported as a biosorbents for the removal of metal ions. Any biological material which exhibits its affinity and concentrates the heavy metals even in a dilute solution is called as biosorbent material. A number of microbial biosorbents like bacteria, fungi, yeast and cyanobacteria [4, 93] have

been studied for the removal of toxic metals from waste streams. Studies using biosorbents have shown that both living and dead microbial cells are able to uptake metal ions and offer potential inexpensive alternative to conventional absorbents. However, living cells are subject to toxic effect of the heavy metals, resulting in cell death.Morever, living cells often require the addition of nutrients and hence increase the BOD and COD in the waste water. For these reasons the use of non living biomaterials or dead cells as metal binding compounds has been gaining advantage because toxic ions do not affect them. And dead cells require less care and maintenance and are cheaper and dead biomass could be easily regenerated and reused [17].Table: 4 show some examples of types of native biomass that have been used for preparing microbial biosorbents.

Table: 4   Types of Microbial Biomass [4, 84, 93]

Category	Examples
Bacteria	Gram +ve Bacteria (Bacillus sp.,Cornebacterium sp., etc. Gram – ve Bacteria (E.coli, Pseudomonas sp., etc. Cyanobacteria(Anabaena sp.,Synechocystis sp.,etc.
Fungi	Molds (Aspergillus sp., Rhizopus sp., etc. Mushrooms (Agaricus sp., Trichoderma sp., etc. Yeast(Saccharomyces sp.,Candida sp., etc.
Algae	Micro-algae (Chlorella sp., Chamydomonas sp., etc. Macro-algae(green seaweed-Enteromorpha sp.,etc);(brown seaweed-Sargassum sp.,etc);(Red seaweed-Geildium sp.,Porphyra sp.,etc).
Industrial waste	Fermentation wastes, food/beverages wastes, activated sludges,anaerobic sludge etc.
Agricultural wastes	Fruits/vegetable wastes, rice straws, wheat bran etc.

Table: 5 Metal Biosorption by Various Bacterial Sp.

SN	Metal	Organism	References
1.	Chromium(iv)	*Aeromonas caviae ,Bacillus coagulans,Bacillus licheniformis,Bacillus megaterium ,Bacillus thuringiensis,Chryseomonas luteola, Pseudomonas sp ,Staphylococcus xylosus ,Zoogloea ramigera ,Giobacillus thermodenitrificans*	[84.82]
2.	Copper	*Bacillus subtilis IAM 1026,Bacillus thurengiensis OSM29,Enterobacter sp.J1,Micrococcus luteus IAM 1056 ,Pseudomonas aeruginosa PU2 Pseudomonas putida ,Giobacillus sp., ThioBacillus ferrooxidans,Zoogloea ramigera ,Arthrobacter sp.*	[67,75]
3.	Cadmium	*Aeromonas caviae ,Bacillus circulans ,Enterobacter sp., Pseudomonas putida ,Pseudomonas sp.,Staphylococcus xylosus, Streptomyces pimprina,Streptomyces rimosus ,Giobacillus thermodenitrificans*	[2526]
4.	Lead	*Bacillus sp. (ATS-1),Corynebacterium glutamicum, Enterobacter sp. J1, Giobacillus thermodenitrificans, ,Pseudomonas aeruginosa PU21 ,Pseudomonas putida, Streptomyces rimosus, Streptoverticillium cinnamoneum*	[25.26,67]
5.	Mercury	*Bacillus* sp	[12]
6.	Zinc	*Aphanothece halophytica, Pseudomonas putida ,Streptomyces rimosus , Streptoverticillium cinnamoneum ,ThioBacillus ferrooxidans*	[59,67]
7.	Nickel	*Bacillus thuringiensis, Streptomyces rimosus*	[68,97]
8.	Iron	*Streptomyces rimosus ,Giobacillus thermodenitrificans*	[5.25]
9.	Silver	*Pseudomonas sp,Lactobacillus sp ,Giobacillus thermodenitrificans*	[91,59]
10.	Gold	*Acinetobacter calcoaceticus, Erwinia herbicola, Pseudomonas aeruginosa and P. maltophilia, Streptomyces erythraeus Spirulina platensis*	[93]
11.	Uranium	*Arthrobacter nicotianae IAM, Bacillus licheniformis IAM 111054 ,Bacillus megaterium IAM 1166, Bacillus subtilis IAM 1026, Corynebacterium equi IAM 1038 , Micrococcus luteus IAM 1056, Nocardia erythropolis IAM 1399,Zoogloea ramigera IAM 12136*	[62]

V. BIOSORBENTS FROM BACTERIAL BIOMASS

Bacteria are the most abundant and versatile of microorganisms and constitute a significant fraction of the entire living terrestrial biomass of ~1018 g [63]. Bacteria were
used as biosorbents because of their ability to grow under controlled conditions, their small size, their

VI. BACTERIAL BIOSORBENTS-CELL WALL STRUCTURE AND BIOCHEMISTRY

The cell wall of bacterial biomass acts as the primary component for interacting with pollutants like metal ions where they are sorbed on the surface or within the cell wall [32].Functional groups which is present on the cell wall of bacterial biomass plays important roles in the biosorption process. [32]. A variety of functional groups located on the bacterial cell wall are known to be included in metal biosorption. These include carboxyl, amine, and hydroxyl, phosphate, and sulfhydryl groups [90]. The

ubiquity and their resilience to a wide range of environmental situation [43].

Table: 5 shown the name of several bacterial species which may be used for metal biosorption. Gutnick *et al.,* [43] suggested that among all the bacterial sp.*Bacillus* has been identified as having high potential of metal sequestration and has been used in commercial biosorbents preparation.
cell walls of Gram positive bacteria is thick due to presence of thick peptidoglycan layer (20-80 nm) containing teichoic acid and lipoteichoic acid in them [6] as shown in Fig: 3.Peptidoglycan accounts for 50 % or more of the dry weight of the wall of some gram positive species.Teichoic acid appears to extend to the peptidoglycan surfaces and as they are negatively charged, helpful to give the gram positive cell wall a negative charge. The phosphoryl groups of secondary polymers and the carboxyl groups of the peptide chains provide negatively charged sites in the gram-positive cell wall, helps in cation sequestering mechanism [70].
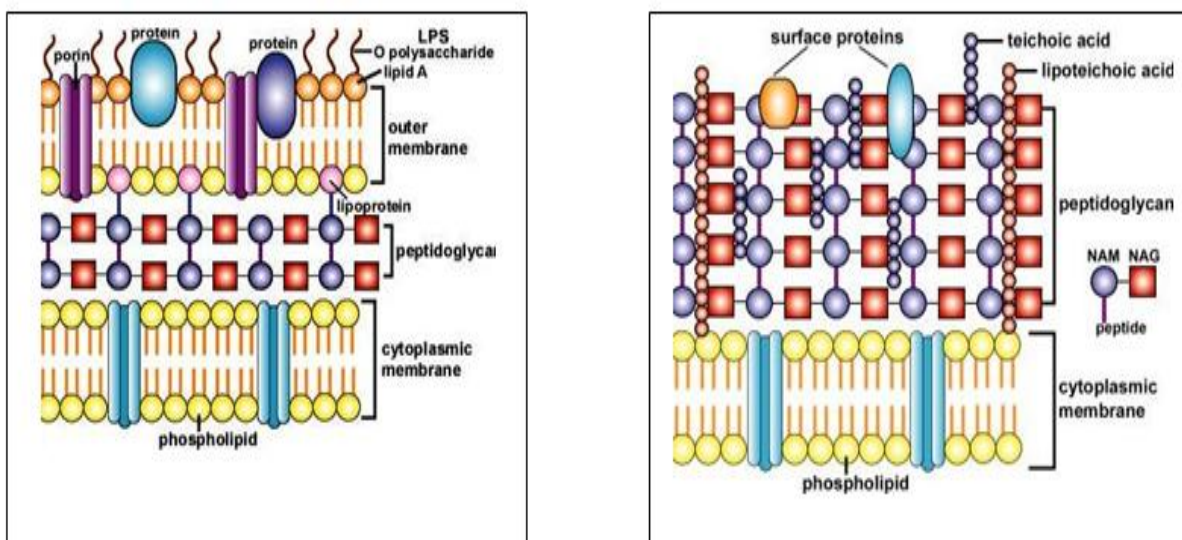

Fig:3 Structure of Gram -ve and Gram +ve bacterial cell wall[65]

Gram-negative bacteria are thinner than gram positive containing monolayer peptidoglycan (10-15thick), Lipopolysaccharide (LPS), phospholipids and surface protein [5].The phosphate groups within LPS and phospholipids have been demonstrated to the primary sites for metal interaction [70].

VII. MECHANISM OF BACTERIAL BIOSORPTION

The mechanism of metal biosorption by bacterial biomass occurs through lipid peroxidation,

complexation, coordination, physical adsorption, chelation, ion exchange, precipitation and/or a combination of these processes[30] as shown in Fig:4.Micro-precipitation is a common phenomenon in metal binding by bacteria but complexation by extracellular substances or by N and O ligands in the cell wall, as well as electrostatic attraction to charged groups in the cell wall may also occur [5].The metabolism independent process of metal binding to the cell walls of bacterial biomass and external surfaces is the main mechanism present in the case of non-living biomass and involves an adsorption

process, such as ionic and physicochemical adsorption[25].The functional group present on the surface of bacterial cell wall as they are negatively charged and abundantly available, carboxyl groups actively participate in the binding of metal cations.[31].

Exopolysaccharide (EPS) substances have also been shown to bind metal ions selectively with high metal accumulating potentials. These polymers have anionic potentials and hence bind metal cations and sometimes form capsules or loose aggregates around cells. [39,85].

Cell walls of gram-negative bacteria are thinner than the gram-positive bacteria and are also not heavily cross-linked. They have an outer membrane which is composed of an outer layer of lipopolysaccharide (LPS), phospholipids and Proteins [40].

Gourdon *et al.,* [26] compared the Cd^{2+} biosorption capacities of gram-negative and gram-positive bacteria.Glycoproteins present on the outer side of gram-positive bacteria cell walls have been suggested to have more potential binding sites for Cd^{2+} than the phospholipids and LPS and hence are responsible for the observed difference in capacity.Carboxyl group modification caused a marked reduction in metal uptake by *Bacillus sp.* In *B. subtilis*, teichoic acid and in *Bacilllus licheniformis*, teichoic acid and teichouronic acid were found to be the prime sites for metal binding.

In E.coli outer membrane, the phosphoryl groups of the LPS and phospholipids have been found to be the most probable binding sites for metal cations. [6].

In *Streptomyces longwoodensis*, phosphate residues were suggested to be the primary constituents responsible for uranium binding.

Joo *et al.,* [50]. Explained their finding by the fact that Gram-positive bacteria normally showing lower levels of surface complexation due to the heavily cross-linked peptidoglycan layer, while in Gram negative bacteria, most of their lipo-polysaccharide (LPS), phospholipids, and proteins are exposed on the cell surface and is responsible for efficient metal binding capacity.

The biosorption properties of dead sulfate reducing bacteria (SRB) such as *Desulfovibrio fructosivorans* for the removal of Cu (II) and Fe (III) from aqueous solutions were studied by Quan *et al.,* [72]. He studied that, the maximum specific metal biosorption was 93.25 mg·g-1 at pH 4.5 for Cu (II) and 88.29 mg·g-1 at pH 3.5 for Fe (III).The ability of *Streptomyces lunalinharesii* to sorb copper and zinc from aqueous solutions was investigated by Venu *et al.,* [92] through batch experiments and maximum capacities for Cu (II) and Zn (II) biosorption were found to be 11.53 and 13.64 mg/g, respectively.
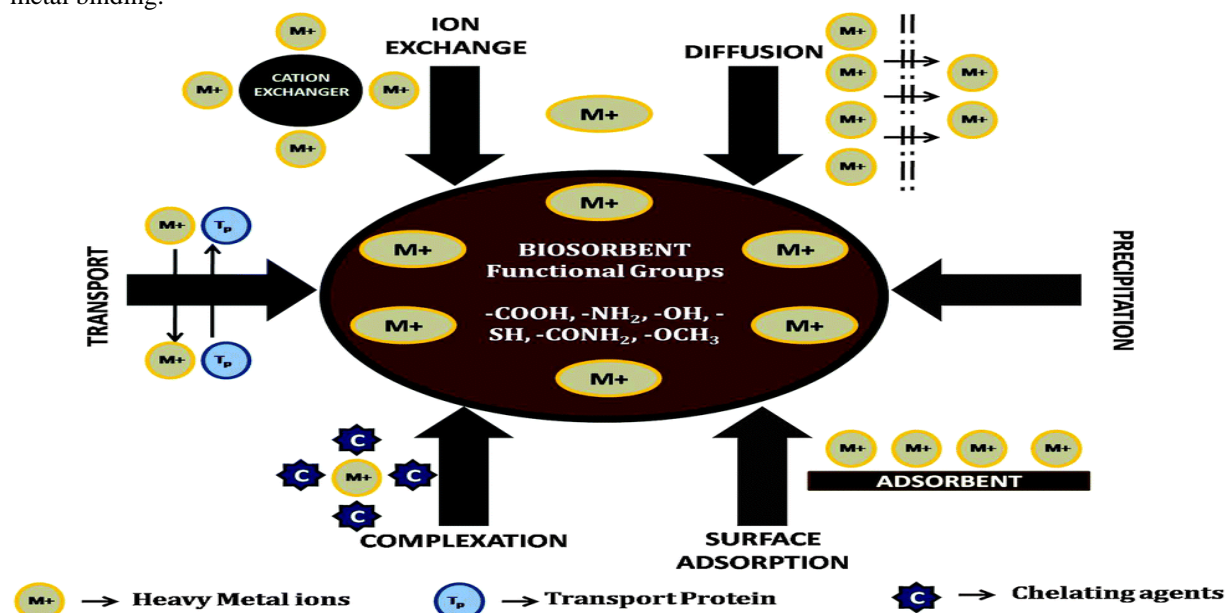

Fig: 4 Mechanism of Bacterial Biosorption [97]

High abilities of gold biosorption by gram negative bacterial strains viz. *Acinetobacter calcoaceticus*, *Erwinia herbicola, Pseudomonas aeruginosa* and *P. maltophilia* from a solution containing hydrogen tetra chloroaurate have been

reported by Tsuruta [88].Magneto tactic bacteria (MTB) have been investigated by Huiping *et al., [48]* as biosorbent for the adsorption of Au (III) from aqueous solution.

Bacterial strains viz. BP 7126 (a *Pseudomonas sp*. strain isolated from a silver mine and in sediments of the river in Austria), ER 121 (a slime producing alkali genes eutrophus strain, isolated from soil contaminated by a non-ferrous industrial plant in Belgium) and AS 302: *Pseudomonas mendocinu* strain, isolated from a copper mine in Likasi South in Zaire showed the potentiality of silver biosorption [86]. Silver biosorption by *Lactobacillus sp* was reported by [31].Also, the biosorption of Pt (IV) ions from aqueous solutions using the bacterial sp. *Desulfovibrio desulfuricans, Desulfovibrio fructosivorans were* investigated and the maximum biosorption capacity obtained was 95.2 mg/g.

The sorption of four heavy metals cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb), on living and nonliving *Bacillus subtilis* B 38 was investigated by Wang *et al.,*[95]using batch experiments. The nonliving biomass generally showed greater or similar adsorptive capacities as compared with the living biomass, the bacterium had a stronger affinity to the cationic heavy metals than to the anionic one, and the equilibrium sorption amounts were 210.6, 332.3, and 420.9 mg/g for Cd (II), Hg (II), and Pb (II).

Several bacterial biosorbents showed potential for the recovery of precious metals such as gold, silver and platinum group metals (Ru, Rh, Pd, Os, Ir, and Pt) from wastewater. Due to high market prices, recovery of precious metals from effluents is interesting. [29] Evaluated the biosorption capacities of palladium and platinum using three different species of *Desulfovivbrio: Desulfovibrio desulfuricans, Desulfovibrio fructosivorans* and *Desulfovibrio vulgaris*. They reported that the most promising Pd and Pt biosorption results were obtained using *D. desulfuricans* with a maximum uptake of 128.2 mg/g and 62.5 mg/g for Pd and Pt accumulation respectively, at pH 2. Won *et al.,* [97] applied their developed biosorbent, PEI-modified *E. coli* biomass, to ICP wastewater containing Pt ions and successfully recovered metallic form of platinum with a recovery efficiency of over 98.7% by a combined method of biosorption and incineration.

VIII. CHARACTERIZATION OF BACTERIAL SURFACE

Characterization of bacterial biomass and the biosorption mechanisms can be elucidated using different methods, including potentiometric titrations,Fourier transform infrared spectroscopy, X-ray diffraction, X-ray photoelectron spectroscopy, Scanning electron microscopy; Atomic force microscopy, Transmission electron microscopy and Energy dispersive X-ray microanalysis [71,91].

The nature of the binding sites and their involvement during biosorption can be approximately evaluated using FT-IR, which is helpful to understand the surface-bonding mechanism. Jin *et al* [51] analyzed the FT-IR spectra of Pd^{2+} exposed *Alcaligenes* sp.BAPB.1 several band formations such as O–H, C–O, N–H, N–H, C–N, N–H, and P–O stretching allowed to predict the possible involvement of hydroxyl, amino, amide, carbonyl, and phosphate groups in the biosorption of Pd^{2+}. Similar type of studied was identified by Oves *et al.,* [67]. He used FT-IR spectra to confirm the presence of carboxyl, amine and phosphonate groups of *B.thuringiensis* which may be involved in the biosorption of Cd^{2+}, $Cr3^+,Pd^{2+},Cu^{2+}$ and Ni^{2+} .

To analyze the morphology of the cell surface before and after biosorption, SEM micrographs are often used. With the aid of SEM photographs, Vijayaraghavan *et al.,* [81] used SEM photographs to show the pattern of *C. glutamicum* immobilization within a polysulfone matrix.

EDAX can provide information regarding the chemical and elemental characteristics of a biomass. Tunali *et al.,* [87] analyzed both Pb (II) and Cu (II) loaded *Bacillus sp.* using EDAX, and confirmed the involvement of an ion exchange mechanism during their biosorption. In order to elucidate the chemical nature of bacterial cell bound lanthanum, Kazy *et al.,* [61] employed XRD analysis, and confirmed the involvement of cellular carboxyl and phosphate groups in the binding of lanthanum by *Pseudomonas sp.*

AFM is an ideal tool for determining changes in surface morphology. For example, AFM was used to investigate the cell surface morphology of raw and polyallyamine hydrochloride (PAH)-modified *E. coli* biomass. The surface morphology of biomass was prone to change when it was modified by cross-linking reaction with polymer. This superficial change was attributed to the addition of polymer to the biomass surface. Pan *et al.,* [71] showed AFM images of *Bacillus cereus* cells under the exposure to different amounts of Pb^{2+} ions solutions, and the biomass shape has been changed from a rod-like structure to a spindle-like structure after Pd^{2+} biosorption. They mentioned that these morphological changes of the sample can be attributed to the interactions between heavy metal and the surface of *B. cereus* biomass.

Mechanism of silver biosorption was reported by Tsezos *et al.,*[86] on three bacterial strains namely *Arthrobaccer sp*, *Alkaligenes eutrophus* and *Pseudomonas mendocina*.The inference was drawn as a result of TEM microscopic analysis and EDAX analysis. A further in depth study was done by Kasthuri *et al.,* who used FTIR and TEM analysis to prove that silver precipitated as nanoparticles as a result of interaction of Ag^+ with carbonyl groups. Mullen *et al.,* (1998) revealed the

presence of Ag^{2+} as discrete particles at or near the cell wall of both gram-positive and gram-negative bacteria and the presence of silver were confirmed by energy dispersive X-ray analysis (EDAX).

IX. FACTORS AFFECTING METAL IONS BIOSORPTION

A. Solution pH:

The solution pH is one of the most important variables which affect the speciation of metals in solution through hydrolysis, complexation and redox reactions during metal recovery [36].This factor is capable of influencing not only the binding site dissociation state, but also the solution chemistry of the target metal in terms of hydrolysis, complexation by organic and or/ inorganic ligands and redox potentials .In the biosorption phenomenon, the pH value affects two aspects: metal ion solubility and biosorbent total charge, since protons can be adsorbed or released. The acidity of the medium affects the competition ability of hydrogen ions with metal ions to active sites on the biosorption surface[36].

The different functional groups such as carboxyl, sulfhydryl, hydroxyl, and amino groups are present on the surface of biosorbents structure.Deprotonation of these functional groups occurs on increasing pH and these behave as negatively charged moieties which start attracting the positively charged metal ions. On the other side as the pH is lowered, the overall surface charges will become positive, which will inhibit the approach of positively charged metal cations.

Magnetotactic bacteria (MTB) as biosorbents for the adsorption of Au (III) ions from aqueous solution have been investigated [48].The optimum pH condition was noted 1–5.5 for Au (III). The effect of pH on the binding capacity of Ag+ to *Lactobacillus sp.* strain A09 was studied by Lin *et al.,* [62] it was found that binding rate constants were pH dependent with maximum rate constant and minimum half life period occurred near pH 5.0.

B. Temperature:

Temperature effects are confined to metabolism-dependent metal accumulation. At a low temperature (0-5°C) little or no metal is sequestered through metabolic process by viable biomass. Temperature increase to 40°C caused only a slight binding increase, whereas temperatures of 60°C or more caused a change in the texture of the sorbent and a loss in the sorption capacity due to the material deterioration.[48]. According to Aksu *et al.,* [1] temperature seems not to influence the biosorption performances in the range of 20–35 °C. In case of magnetotactic bacteria (MTB), Huiping *et al.,* [48] reported that an increase of temperature in the interval of 15-20 °C dealt with an increase in the adsorption

yield of Au (II) and Cu (II) and began to decrease slowly with increasing temperature from 25 to 35 °C. As a whole, both metal ions were effectively adsorbed by MTB in the range of 10–35 °.

C. Ionic strength:

Ionic strength influences the adsorption of solute to the biomass surface ([10].Ionic strength is the competition between ions or the changes in the metal activity. When two phases, e.g.biomass surface and solute in aqueous solution in contact, they are bound to be surrounded by an electrical double layer owing to the electrostatic interaction. Thus, adsorption decreases with increases in ionic strength [35].

D. Biosorbent dosage:

The biosorbents dosages strongly influences the extent of biosorption.Lower biosorbents dosages yield higher uptakes due to high surface area and availability of more sorption sites.[91]Conversely, with increase in biosorbent dosage, the decrease in adsorption capacity may be observed due to unsaturated binding sites and reduction in the surface area.

E. Initial metal concentration:

The initial solute concentrations have an impact on biosorption.Higher solute concentration(initial)resulting in a high solute uptake(final),due to the elevated surface area of the biosorbent which in turns increases the number of binding sites([67]However at higher concentration, the sites available for sorption become fewer compared to the moles of solute and hence, the removal of solute is strongly dependent upon the initial solute concentration[91].Oves *et al.,* [67]demonstrated that rate of biosorption decreased with an increases in metal ion concentration of copper. The maximum biosorption of copper was recovered at a low initial metal ion concentration for example it was 91.8% at 25 mg/l while it was 82.7% at 150 mg/l.

F. Desorption and recovery:

Desorption is very much necessary when the biomass preparation/ generation is costly. It is possible to decrease the process cost and also the dependency of the process on a continuous supply of the biosorbent through desorption. A successful desorption process requires the proper selection of elutants, which strongly depends on the type of biosorbent and the mechanism of biosorption.Elutant must be i) non damaging to the biomass ii) less costly, iii) eco-friendly and iv) effective.[35].

Dilute inorganic acids (e.g. hydrochloric acid, nitric acid, sulphuric acid, acetic acid, EDTA, Nitrilotriacetic acid are commonly used agents for desorption of heavy metals on biomass. Metals are

eluted with high yield, but using a higher concentration of acid or a longer period of time can damage the biomass, thereby worsening the accumulation of biomass in successive sorption [29,37].For Cu desorption from live yeast *Saccharomyces cerevisiae* can be used hydrochloric acid (1 M) or a mixture of acids (acetic, lactic, nitric). In both cases, copper is almost completely removed from the biomass, but there is always a subsequent reduction in binding capacity of cells.

Very good and frequently used elution agents are various organic chelating agents. Using ethylenediaminetetraacetic acid (EDTA) can very effectively remove lead and zinc ions from the dead biomass *Streptoverticillium cinnamoneum,* but the recovery is reduced the subsequent sorption of about 20–30% Quite successful, this reagent can also be used for the metals desorption from the activated sludge, where only intracellular bound metals are not removed. When this reagent was used for the desorption of copper from live yeast *Saccharomyces cerevisiae,* only about 60% of bound copper was released (Junghans and Straube 1991). The good chelating agents are further nitrilotriacetic acid (NTA) and less common diethylenetriaminopenta acetic acid (DTPA) NTA is suitable for desorption of cadmium, zinc and copper, but for other metals is not so suitable agent. NTA recovered 70–95% of bound cadmium from immobilized bacteria *Zoogloea ramigera* depending on the amount of accumulated [37, 44, 45, and 94]

X. CONCLUSION AND FUTURE PERSPECTIVES

Removal of heavy metal ions from contaminated sites is a great challenge. Heavy metal remediation by using physicochemical techniques is expensive and often not suitable for voluminous effluents containing complexing organic matter as well as low metal ion concentration. Biotechnological approaches may be an alternative for such traditional methods.Biosorption can be effectively used for the removal of heavy metal ions from dilute complex solutions with high efficiency and in a rapid manner.Thus, Biosorption can be an ideal method for the treatment of high volume low concentration complex waste waters.

However most of the reports on biosorption have been restricted to the laboratory scale. And there is a need to extrapolate this technology to commercial levels. In addition, the mechanisms involved in biosorption are still not fully understood thereby limiting applications of the biosorption process. The Development of biosorption process for management of heavy metal pollution needs to be investigated further in terms of modeling, understanding more clearly biosorption mechanisms, developing methods for regeneration and immobilization of biosorbents and treating contaminated sites at large scale levels.

CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest.

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