

Microbes as Heavy Metal Removers in Industrial Waste

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Abstract: *Environmental pollution is the greatest threat posed to the humanity and the biosphere as a whole. Among these water pollution is of great significance. Water pollution causes undesirable changes in the physico-chemical and biological properties of water, which may be harmful to the activities of man and domestic species. Now water pollution is a global problem due to rapid increase in industrialization. The effluent from industries contain heavy metals like Cu, Pb, Zn, Hg, Cd, Mn etc. all of which are toxic to animals. The heavy metals discharged from industries into lakes and ponds reach the human through the food chain. The special characteristics of heavy metal chemicals is their strong attraction to biological system and slow elimination from it. Metal toxicity means the uptake of metals by the cell. The time required to develop tolerance mechanisms are influenced by biotic and abiotic factors. Many microorganisms acquire a mechanism that enables them to repair metal toxicity damages.*

Keywords: Pollution, heavy metal, toxicity, microbial cell, Biosorption

1. Introduction

The microorganisms resistant to heavy metals by exclusion of metal from body cell membrane or by active transport of metal from the cell. These organisms can accommodate the heavy metals temporarily inside the cell, therefore they are not available to produce the toxic effects. Some metals at higher concentrations while others at very low concentrations are toxic. Toxicity index of 1 ppm metals is $Hg > Cu > Cd > Pb$. All chemicals in the industrial waste are toxic to animals and may cause death or sublethal pathology of the liver, kidney, heart, reproductive systems, respiratory systems of nervous system in both invertebrate and vertebrate aquatic animals and their consumption cause changes in humans. Lead poisoning damages central nervous system and may cause death in children. Cadmium affects respiratory system and also cause a number of heat diseases and is a potential carcinogen. Cadmium is the major pollutant metal which more mobile in soil components due to low affinity of cell component towards it. The biosorption frequency employed for a range of process by which the biomass removing metals and other toxic substances from the solution. The cell wall of bacteria have several metal binding components, which contribute to biosorption process. These components include polysaccharides, glycoproteins, lipopolysaccharides associated with proteins.

Metallothionein protein can complex metals is controlled by metallothionein gene and its regulator. An important consideration for practical utilization of microorganisms for accumulation, separation or recovery of metals is the amount of microorganisms of metals that can be accumulated by microbial cell vary from few microorganisms per gram of cells to several percentage of the dry cell weight. The ability to accumulate a particular metal varies among microbial species influences by properties of both metals and microorganisms. It

is also influenced by properties of both metals and microorganisms. It is also influenced by parameters like temperature, pH and concentrations of other metal ions. The metal accumulation is observed in association with extracellular products at or within the cell membrane and intracellularly. Knowledge of chemical and physiological reactions that occur during metal uptake enables specification and control of process parameters to increase the rate, quantity and specificity of metal accumulation. By knowing those inherent properties it is possible to enhance the ability of microorganisms to accumulate metals from the environment. There are several processes which lead to metal accumulation in organisms. Biosorption involve complex reactions between the metal species and the charged cellular components. Subsequent to absorption precipitation or crystallization of metals also take place.

The insoluble metal species can be physically entrapped in the microbiologically produced extracellular polymers. The metabolically mediated metal uptake involves the specialized transport systems, enzymes and energy expenditure. Accumulation also involves undersigned non-metabolic mediated processes. The metabolically mediated accumulation is usually intracellular under control of plasmid linked gene. The non-metabolically mediated accumulation result from biosorption and precipitation of metal extracellularly or within cell wall matrix and intracellularly. The metal and the metal containing molecules undergoes transformation reactions that are mediated by microorganisms to prevent its toxicity. These mechanisms include metal oxidation-reduction, methylation, alkylation, metal complexation, dealkylation etc. Taxonomically diverse group of heterotrophic bacteria utilize metallic cation as the terminal-electron acceptor under anaerobic conditions. During this metals is reduced to a lower valency state, several metabolic elements process multiple valency states which can be potentially utilized by microbes.

Oxidation/reduction enhances metal mobility and stimulates movements of metal from the cell. Zn binding protein in metal resistant strain of *Alcaligenes enterophus* CH34 (Ramacle and Verchaval, 1992)

2. Mechanisms of Metal Removal

- 1) Adsorption to cell surface :Microorganisms can bound metals as a result as a result of interaction between metal ions and the negatively charged microbial surface .
- 2) Complexation:Microorganisms can produce organic acids like citric acid which may chelate toxic metals result in the formation of metallorganic molecules.
- 3) Precipitaion: By producing ammonia,H₂S or organic bases eg. Cadmium to CdS Which prepared as electron dense granules at the cell surface.
- 4) Volatilisation : a) $Hg^{2+} \rightarrow Hg^0$ (volatile species)
 -----> $Hg+CH_3$
 b) Hg^{2+} -----↑ ↓
- 5) $Hg(CH_3)_2$ **Dimethyl mercury**
- 6) Intracellular accumulation of metals by specific system transport.

Active process take place in two phases:

Metal	Organism	Metal uptake (g metal/ gcell dry weight)	Reference
Ag	<i>Pseudomonas maltophilia</i>	0.32	Charley and Bull (1979)
	<i>Thiobacillus ferroxidans</i>	0.18	Charley and Bull (1979)
	<i>Thiobacillus thioxidans</i>	0.25	Pooley(1982)
Co	<i>Proteus vulgaris</i>	0.08	Neyland <i>et al</i> (1952)
	<i>Zoogloea sp.</i>	0.25	Friedman and Dugan (1968)
Cu	<i>Zoogloea sp.</i>	0.34	Friedman and Dugan (1968) Norberg and Persson (1984)
	<i>Zoogloea ramigera</i>	0.17	Norberg and Rydin (1984)
Ni	<i>Zoogloea sp.</i>	0.13	Friedman and Dugan (1968)
Pb	<i>Citrobacter sp.</i>	0.35	Aickin <i>et al.</i> (1979)
U	<i>Rhizopus arrhizus</i>	0.18	Tsezos and Volesky (1982)
	<i>Sach.cerevisiae</i>	0.15	Strandberg <i>et al</i> (1981)
	<i>Pseudomonas aeruginosa</i>	0.15	Strandberg <i>et al</i> (1981)
Th	<i>Pencillin chrysogemum</i>	0.08	Jilek <i>et al</i> (1975)
	<i>Rhizopus arrhizuz</i>	0.17	Tsezos and Volesky (1982)

3. Materials and Methods

Isolation of Organism

The effluent waste water samples were collected from different industrial areas which are expected to contain heavy metals. The pH of the samples were noted.The serial dilutions of the sample were done using saline and incubated into nutrient agar medium and incubated for overnight at 37° C.The pure colonies developed after incubation was noticed. A single colony from the nutrient agar surface was taken and then incubated into nutrient broth medium and incubated for overnight in an incubator at37° C. The isolated organism may be resistant to heavy metals because of its growth in the effluent waste water containing heavy metals.

- 1.Rapid cation binding to negatively charged group of the cell wall
- 2.Subsequent metabolism dependant intracellular uptake

Physico chemical properties of organic metal differ from those of pure metal.The adsorption of metal ions by solid surface is an important mechanism that control trace metal concentration in the aquatic system(Hem,1976).Adsorption is the most common mechanism of metal removal that take place at mineral solute interface (Dudly et al ,1988.,Zachara et al ,1991).Brierley et al (1989) patented processed involving immobilized bacteria, fungi and algae to remove heavy metals. Ramamurthy and Kushner (1975) revealed the experiment on the binding of mercury and other metal ions by microbes. Beveridge and Murray (1976) and Shumate et al (1976) carried out biological removal of metal ions from effluents. Brown and Lester (1976) studied metal removal from activated sludges and role of bacterial extracellular polymers in waste removal.Strandberg et al (1981)proposed the microbial cell as biosorbent of heavy metals. Moore and Ramamoorthy (1984)studied heavy metals in natural water and Barness et al (1991) proposed a new process of microbial removal of heavy metals.

Nutrient Agar Medium

Ingredients

Peptone – 5 g NaCl - 5 g Lab lemco-3g Agar- 15 g pH -7.2

Metal assay

The amount of metals in the given sample was detected by using AAS (Atomic Absorption Spectrophotometer).

Identification of Bacterial Strains

The pure strain of bacteria obtained was identified based on the morphological, staining and Biochemical properties according to the *Bergey's Manual of Systematic Bacteriology*. The first step in the identification of an unknown bacterial organism is to analyze the morphological characters.

I. Morphological characters

- a) **Shape:** The shape of an organism was checked from good gram stained slide
- b) **Size:** The size of the organism was determined by an ocular micrometer on a good gram stained slide.
- c) **Motility:** Motility is checked by hanging drop examination from broth culture
- d) **Cellular arrangement:** Hanging drop examination from broth cultures were done. Noted whether occurred singly in pairs or in Chains
- e) Staining reactions
 - 1) **Gram staining:** Divided the bacteria into Gram positive or Gram negative
 - 2) **Endospore staining:** Only a small group of bacteria found to be capable of producing endospore. Placed the slide containing bacterial culture over a beaker of boiling water. A large droplet had condensed on the underside of the slide, allowed it to stand with a 5 % aqueous solution of malachite green and washed with tap water. Treated with 0.5 % safranin or 0.05 % basic fuschin for 30 seconds ,washed ,dried and examined for endospore formation.

II. Determination of Resistance to heavy metals

The metal stocks of heavy metals like zinc and cadmium was prepared from ZnCl₂ and CdSO₄ at 500 ppm.Prepared different ppm solutions from the stock as 5 ppm, 10 ppm, 25 ppm etc. All samples wrer made upto 50 ml ,using the nutrient broth. Inoculated the given organism isolated from effluent. Incubated overnight.

The growth of the organism was determined by the turbidity in the media.The organism showed different growth characteristic in different metal concentrations. The turbidity of each sample was measured by using UV spectrophotometer. The turbidity values of each sample were used to get growth curve by plotted it against time in hours.

III. Metal uptake studies in Bacillus species

Microorganisms accumulate heavy metals through various uptake mechanisms. The growth characteristics of the organism under such treatment is given in table -4.The amount of metal accumulated by the microorganisms Bacillus sp. Was determined by assaying the metal present in the medium with inoculums at 0 hour and after 96 hours. The difference was calculated to estimate the amount of metal up taken by Bacillus sp.(Table-3)

Determination of heavy metal Resistance

Different concentrations of heavy metals viz.25, 50, 100, 150, 200, 250, 300 ppm metal level were used. The metals selected are Zn and Cd, which are supported to be present in the effluent water from the industrial area. The organism showed optimum growth at 50 ppm of Zn.

Table 1: Initial metal concentration in the effluent samples in ppm

Samples	Zn	Mn	Cu
1	0.184	0.455	0.053
2	0.080	0.724	0.038
3	0.165	0.533	0.000

Table 2: Metal concentration in culture media augmented with effluents after microbial growth for 96 hour (ppm)

Samples	Zn	Mn	Cu
1	0.076	0.044	0.026
2	0.110	0.176	0.015
3	0.082	0.034	0.000

Table 3: Amount of metal assimilated(in mg) by Bacillus sp. After 96 h of growth

Samples	Zn	Mn	Cu
1	0.108	0.411	0.027
2	0.070	0.548	0.023
3	0.083	0.499	0.000

Table 4: Absorbance at 660nm showing growth characteristics of Bacillis sp

Samples	Absorbance at 0 hour	Absorbance at 96 hour
Control	0.0262	2.1210
1	0.0262	1.8029
2	0.0262	2.2700
3	0.0262	2.1977

Table 5: Percentage of heavy metal uptaken by Bacillus sp. After 96 h of incubation

Samples	Zn	Mn	Cu
1	58.69	90.32	50.94
2	38.69	75.70	60.52
3	50.30	93.62	0.00

4. Results and Discussion

Modern industrialization has resulted in the pollution of water bodies in and around industrial belts. The water samples collected from various locations showed varying concentration of metal ions, when subjected to assay in an ASS. The assay indicated the presence of Zn, Cu, Mn at different concentration . The value of concentration of heavy metal are given in Table-1.The microorganisms including bacteria have the ability to accumulate metals from the surrounding environment. Many bacteria show resistance to heavy metals due to their ability to toxify them. Microorganisms can be used to treat aqueous stream containing heavy metals for removal, concentration and recovery of toxic and valuble heavy metals.

Barnes et al (1991) proposes a new process of microbial removal of heavy metals. Accumulation of metals by microbial cell occur by both metabolic and non-metabolic processes. Metabolic dependant mechanism includes metal precipitation as sulphide, hydroxides etc. and intracellular compartmentalization. In this experiment the isolated the

heavy metal resistant bacteria from the effluents of industrial area. The organism isolated was found to uptake heavy metals. The morphological characteristics and biochemical properties were studied and found that the organism belongs to the genus *Bacillus*. The resistance of the organism towards the heavy metals was confirmed by the growth of microorganism in the sample containing heavy metals. After 24 hours of incubation at 37 °C the bacteria showed optimum growth at 50 ppm of zinc. The organism showed increases growth with increase in metal concentration upto 50 ppm after which the growth declined gradually. Apart from growing the bacteria, *Bacillus* in known concentration of heavy metals added into culture media, using the effluent containing heavy metals at different dilutions for augmenting the medium.

The effluent contained Zn, Mn and Cu in different combinations. After incubating bacterium in a medium supplemented with effluent found considerable decrease in the amount of heavy metals in the medium. The metal content in media after incubation with *Bacillus* sp. For 96 hours is given in Table-2. It can be concluded that substantial amount of heavy metals were accumulated by *Bacillus* sp. The percentage of metal accumulated by this organism after proper incubation period is given in Table-5.

Determination of heavy metal resistance

Different concentrations of heavy metals viz. 25, 50, 100, 150, 200, 250, 300 ppm levels were used. The metal selected are Zn, Cd which are supposed to be present in the industrial area. The organism showed optimum growth at 50 ppm of Zn.

5. Summary and Conclusion

In this study an attempt was made to screen bacteria capable of absorbing heavy metals like Cu, Zn and Mn that present in the industrially polluted water. Isolated organisms were later identified by studying morphological and biochemical properties. The organism was found to be *Bacillus* sp. And was resistant to Zn upto concentration of 50 ppm. From the analysis on metal uptake by *Bacillus* sp. Subjected to treatment with effluent containing heavy metal, it was observed that substantial amount of metal accumulation does take place. Further there was no considerable decrease in the rate of organism under various heavy metal treatments of lower concentrations.

As microorganisms like *Bacillus* are basic in food chain and any accumulation of metals by these organisms pose considerable threat to organism higher up in the food chain. Thus it may lead to biomagnifications and associated consequences. If these organisms were used for the recovery of heavy metal from low level metal containing surface run off waters it will be highly beneficial. This approach of heavy metal recovery with appropriate scaling up designs will be a valuable tool for future, in pollution abatement measures. It will get several advantages over conventional processes like precipitation, ion-exchange resins etc. In addition to that it will be cost effective also.

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