



REMOVAL OF HEAVY METALS FROM INDUSTRIAL WASTE WATER USING BACTERIAL BIOMASS

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ABSTRACT:

Industrial development results in the generation of industrial effluents, and if untreated results in water, sediment and soil pollution. Industrial wastes and emission contain toxic and hazardous substances, most of which are detrimental to human health. The key pollutants include heavy metals, chemical wastes and oil spills etc. Heavy metal resistant bacteria have significant role in bioremediation of heavy metals in wastewater. The objective of this work is to study the role of bacteria in removing the heavy metals present in the industrial effluent. Five effluent samples out of nine were selected for this study due to high content of heavy metals. The heavy metals Pb and Cu were removed by *E.coli*. The average Pb reduction was 45% and Cu reduction was recorded as 62%. The heavy metals Cd, Ni and Co were removed by *P.aeruginosa*. The average Cd reduction was 56%, average Ni reduction was 34% and average Co reduction was recorded as 53%. The heavy metals Cd and Cu were removed by *E.aerogens*. The average Cd reduction was 44% and average Cu reduction was recorded as 34%.

KEYWORDS:

Bacterial biomass, Heavy Metal, Industrial waste water

INTRODUCTION

Rapid industrial development have led to the recognition and increasing perceptible of interrelationship between pollution, public health and environment. Industrial development results in the generation of industrial effluents, and if untreated results in water, sediment and soil pollution. (1) Industrial wastes and emission contain toxic and hazardous substances, most of which are detrimental to human health (2)

Heavy metals from industrial processes are of special concern because they produce water or chronic poisoning in aquatic animals (3). While some heavy metals are purely toxic with no cellular role (4), other metals are essential for life at low concentration but become toxic at high concentrations (5), high concentration of all heavy metals inhibits activity of sensitive enzymes (6).

Heavy metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA. Toxicity of these

heavy metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions. Also, toxicity can occur as a result of alterations in the conformational structure of the nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance. Heavy metals are not biodegradable and tend to be accumulated in organisms and cause numerous diseases and disorders (7).

Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption (8) for heavy metal removal from waste streams are not cost effective (9) and hence biological approach has been considered as an alternative remediation for heavy metal contamination.

To survive under metal stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation

and complexation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state (10). The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

1. Metabolism dependent; and
2. Non metabolism dependent.

According to the location where the metal removed from solution is found, biosorption can be classified as:

1. Extra cellular accumulation/ precipitation;
2. Cell surface sorption/ precipitation; and
3. Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defence system of the microorganism, which reacts in the presence of toxic metal. During non metabolism dependent biosorption, metal uptake is by physicochemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells metabolism.

Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., nonmetabolism dependent is relatively rapid and can be reversible (11). In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (12). Further, it may be dependent on the cell's' metabolism if, in the presence of toxic metals, the microorganism produces compounds that favour the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface.

MATERIALS AND METHOD

Sample Collection

Industrial waste water samples were collected from a nearby industrial area in Akola in sterile plastic bottles and were brought to the lab aseptically. The samples were stored at 4°C for further use

Estimation of heavy metals:

Estimation of heavy metal present in collected effluent will be carried out as per American Public Health Research.

Isolation and screening of Gram negative bacteria:

The screening of metal tolerant Gram negative bacteria was carried out by broth method or plate diffusion method. Isolated organism was identified on the basis of their morphological, biochemicals and cultural characteristics by adopting standard methods.

Biomass cultivation:

Isolated metal tolerant gram negative bacteria were cultivated in the form of biomass on shaker for incubation with suitable medium. The high yield of biomass production we evaluated in the presence of different sugar sources (like glucose, sucrose, maltose etc).The carbohydrate source with appropriate concentration providing optimum biomass production was carried ahead for further study.

Biosorption experiment:

The treatment of the effluent with the bacterial biomass was done in Erlenmeyer flasks containing 150 mL of each samples and 15.0 ± 1.0 mg of cells. The flasks were kept, under constant agitation, at $30-35 \text{ }^\circ\text{C} \pm 2^\circ\text{C}$ for 48 hours. After 48 hours, cells were separated from the medium and residual metal concentrations were determined by Atomic Absorption Spectrophotometer (AAS).

RESULTS AND DISCUSSION

Isolation of heavy metal resistant bacteria

Numerous studies have revealed a number of bacterial species which are capable of removing metals from aqueous environment. In the present study three bacteria were isolated: *E.coli*, *P.aeruginosa* , *E.aerogens* from

waste water by serial dilution and pour plating method using Nutrient agar supplemented with different heavy metal salts. The biochemical characteristics of the isolated bacteria are given in Table 1.

Table 1: Biochemical characterization of isolated bacteria

Characters	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>E. aerogens</i>
Gram reactions	Gram negative	Gram negative	Gram negative
Colour on CLED	NA	Greenish yellow	Yellow
Cetrimide agar	Grey	NA	NA
Indole	-ve	+ve	+ve
Methyl red	-ve	-ve	+ve
Voges-Proskers	-ve	+ve	-ve
Citrate	+ve	+ve	-ve
Catalase	+ve	+ve	+ve
Urease	NA	+ve	NA
Coagulase	NA	NA	NA
Oxidase	+ve	+ve	NA
Desulfurase	NA	+ve	-ve
Colour on Endo Agar	Colour less to pink	Pink mucoid	Red with metallic sheen

Determination of Heavy Metals Concentration in Industrial Effluent

The concentration of Heavy metals in the effluent samples was determined by AAS. And the metals Cd, Cu, Ni, Pb and Co were determined in the effluent samples. The concentration of Cadmium (Cd) ranged from 0.48 to 1.62mg/l, concentration of Nickel (Ni) ranged from 0.14 to 0.31mg/l, concentration of Lead (Pb) ranged from 0.59 to 1.75 mg/l, concentration of copper (Cu) ranged from 0.21 to 1.35 mg/l, and concentration of cobalt ranged from 0.08 to 0.91mg/l. Five samples A,C,E,G and H out of nine, were selected for this study due to high content of heavy metals (Table 2).

Table 2: Heavy metal content in the Effluent sample

S.No	Sample	Cd mg/l	Ni mg/l	Pb mg/l	Cu mg/l	Co mg/l
1.	A	0.85	0.31	1.43	0.21	0.60
2.	B	ND	0.01	0.09	ND	0.02
3.	C	1.54	0.14	1.75	1.35	0.91
4.	D	0.09	ND	ND	0.06	0.05
5.	E	0.48	0.22	0.59	0.42	0.24
6.	F	0.04	ND	0.02	0.04	0.01
7.	G	1.02	ND	1.15	ND	0.08
8.	H	1.62	0.18	1.32	0.36	0.32
9.	I	ND	0.15	ND	0.12	0.06

Treatment of metal by bacterial biomass (*E.coli*)

The reduction in heavy metal concentration in the effluent sample by *E.coli* is indicated in Table 3. The heavy metals Pb and Cu were removed by *E.coli*. The average Pb reduction was 45% and Cu reduction was recorded as 62%.

Treatment of metal by *P. aeruginosa*

The reduction in heavy metal concentration in the effluent sample by *P.aeruginosa* is indicated in Table 4. The heavy metals Cd, Ni and Co were removed by

P.aeruginosa, the average Cd reduction was 56%, average Ni reduction was 34% and average Co reduction was recorded as 53%.

Treatment of metal by *E. aerogens*

The reduction in heavy metal concentration in the effluent sample by *E.aerogens* is indicated in Table 5. The heavy metals Cd and Cu were removed by *E.aerogens* The average Cd reduction was 44% and average Cu reduction was recorded as 34%.

Table 3: Treatment of metal by bacterial biomass (*E.coli*)

Sample	Pb (before treatment) mg/l	Pb (after treatment) mg/l	Cu (before treatment) mg/l	Cu (after treatment) mg/l
A	1.43	0.79	0.21	0.08
C	1.75	0.88	1.35	0.48
E	0.59	0.33	0.42	0.17
G	1.15	0.69	ND	ND
H	1.32	0.73	0.36	0.14

Table 4: Treatment of metal by bacterial biomass (*P. aeruginosa*)

Sample	Cd (before treatment) mg/l	Cd (after treatment) mg/l	Ni(before treatment) mg/l	Ni(after treatment) mg/l	Co(before treatment) mg/l	Co(after treatment) mg/l
A	0.85	0.36	0.31	0.22	0.60	0.29
C	1.54	0.62	0.14	0.09	0.91	0.39
E	0.48	0.22	0.22	0.15	0.24	0.11
G	1.02	0.44	ND	ND	0.08	0.05
H	1.62	0.78	0.18	0.12	0.32	0.15

Table 5: Treatment of metal by *E.aerogens*

Sample	Cd(before treatment) mg/l	Cd(after treatment) mg/l	Cu(before treatment) mg/l	Cu (after treatment) mg/l
A	0.85	0.51	0.21	0.14
C	1.54	0.82	1.35	0.93
E	0.48	0.26	0.42	0.27
G	1.02	0.60	ND	ND
H	1.62	0.86	0.36	0.25

CONCLUSION

From the present study it could be concluded that bacteria play a very important role in the removal of heavy metals from waste water. In the present study five effluent samples out of nine were selected to study the removal of heavy metals by bacteria. After treatment it was found that *P.aeruginosa* and *E.coli* were able to remove Cd from the effluent samples with an average reduction of 56% and 44% respectively. Removal of Ni was recorded by *P.aeruginosa* with an average reduction recorded of 34%. Pb was removed by *E.coli* with an average reduction of 45%. Cu was removed by both *E.coli* and *E.aerogenes* with an average reduction recorded of 62% and 34% respectively. Co was removed by *P.aeruginosa* and the average reduction recorded was 53.

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