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Biosorption of Heavy Metals Using *Pseudomonas* Species

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Abstract

The quality of life on Earth is linked inextricably to the overall quality of the environment. In early times, we believed that we had an unlimited abundance of land and resources today. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects. A better approach than these traditional methods is to completely destroy the pollutants if possible or at least to transform them to innocuous substances Biosorption can be defined as the uptake of organic and inorganic metal species both soluble and insoluble; by physico-chemical mechanism such as adsorption. In living cells, metabolic activity may influence Biosorption because of changes in pH; organic and inorganic nutrients and metabolites in the cellular microenvironment. It is the binding and concentration of heavy metals from aqueous solutions (even very dilute ones) by certain types of inactive, dead, microbial biomass. Biosorption is a passive metabolism independent physio-chemical interaction between heavy metals ions and microbial surface. It is a non-directed, physio-chemical interaction that may occur between metal/radionuclide species and microbial cells. The microorganisms respond to these heavy metals by several processes; including transport across the cell membrane. In the present investigation, the ability of isolated bacterial strains i.e., *pseudomonas* species towards sorption of Nickel, Chromium, Zinc and Magnesium was evaluated by characterizing the biosorption of these metals.

Keywords: Biosorption, *Pseudomonas*, Heavymetals, bacteria, physiochemical interaction.

Contents

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1. Introduction

The speedy development and increasing sophistication of various industries in the past century has remarkably increased the amount and complexity of toxic waste effluents, which may be bioremediated by appropriate plants and microbes, either natural occurring or tailor-made for the specific purpose. The bioremediation and natural attenuation area has both basic research and field application foci for the environmental biotechnology. The basic research foci are co-metabolism, bio-treatability, biotransformation kinetics, and modeling of biogeochemical processes. The field application foci are co-metabolic techniques, biogeochemical assessment techniques, and modeling of attenuation and environmental fate (1).Modern industry is, to a large degree, responsible for

contamination of the environment. Lakes, rivers and oceans are being overwhelmed with many toxic contaminants. Among toxic substances reaching hazardous levels are heavy metals (2).

Biosorption is a process with some unique characteristics. It can effectively sequester dissolved metals from very dilute complex solutions with high efficiency. This makes biosorption an ideal candidate for the treatment of high volume low concentration complex waste-waters (3).Bioremediation uses biological agents, mainly microorganisms, yeast, fungi or bacteria to clean up contaminated soil and water (4). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (5). Metals are extensively used in several industries, including mining, metallurgical, electronic, electroplating and metal finishing. The presence of metal ions in final industrial effluents is extremely undesirable, as they are toxic to both lower and higher organisms. Under certain environmental conditions, metals may accumulate to toxic levels and cause ecological damage (6). Of the important metals, Mercury, lead, cadmium, Arsenic and Chromium (VI) are regarded as toxic; whereas, others, such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing levels in the environment are of serious concerns (7).

The Biosorption process involves a solid phase adsorbent (sorbent, biosorbent, biological material) and a liquid phase (solvent, normal water) containing a dissolved species tobe sorbed (sorbate or metal ions). Due to the higher affinity of the sorbent for the sorbate species, the latter is attracted and removed by different mechanisms. The process continues till equilibrium is established between the amounts of solid bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the substrate determines its distribution between the solid liquid phases. The major advantage of biosorption over conventional treatment methods include cost, higher efficiency, minimization of chemical and biological sludge, regeneration of biosorbent and possibility of metal recovery (8).
The *Pseudomonas* species is an effective bacterium for the reclamation of oil/metal contaminated soils (9) by producing surfactants and tolerate to certain heavy metals. A number of mine tailing reclamation studies have emphasized a strong association between the establishment of a stable plant community and abundance and composition of microorganisms (10). There is an urgent need to study the occurrence of indigenous microorganisms in heavy metal polluted sites. It may provide new insight into bacterial diversity under unfavorable conditions, new isolates and probably new genetic information on heavy metal resistance, which could be exploited in revegetation in future (11). The objective of this work was to isolate bacteria from the contaminated soil and evaluate them for use in biosorption of heavy metals. To study the bacterial biosorption of heavy metals like chromium, zinc, nickel, lead, ferrous from the industrial waste as well as their tolerance level.

2. Materials and Method

Isolation of Bacteria

Serial dilution: A small measured volume or weight of sample was mixed with a large volume of sterile water called dilution blank. Dilutions were made in multiplies of 10. A single dilution was calculated as follows:
Dilution = Volume of the sample/ Total volume × Dilution factor
The dilution blanks were labeled as 10^{-1} to 10^{-10} and it was filled with 9 ml of sterile distilled water or saline water. Initial dilution prepared by adding 1 ml of sample into dilution blank. Then the contents were mixed thoroughly, from these diluents 1 ml of sample was transferred to the 10^{-2} dilution. The same procedure was repeated upto 10^{-10}dilution (12).

Simple Staining

The smear was prepared from the given sample and heat fixed the slide. Crystal violet stain was added 1-2 drops. It was washed with tap water for 3 minutes to remove excess stain. Then the smear was air dried and then observed under microscope.

Gram Staining

The smear was prepared from the given sample and heat fixed. The smear was flooded with crystal violet for 3 minutes. The smear was washed with running water and the gram's iodine was added and kept for 1 minute and washed with running tap water. Then the decolorizing agent (ethyl alcohol) was added drop wise to remove excess crystal violet stain on the smear. The smear again washed with running water then the saffranin was added and kept for 3 minutes. The smear was air dried and observer under oil immersion at 100X magnification. The slide was washed with tap water and stained with saffranin for 30 seconds then the smear was decolorized with alcohol. The slide was examined under oil immersion objective.

Endospore staining

The smear was prepared from the given sample and heat fixed. The smear was flooded with Malachite Green stain and allowed to stand for 5 minutes. The saffranin solution was added and kept for 30 seconds. The slide was examined under 100X magnification.

Viability and Motility of Bacterial cell

The smear was prepared in the cavity slide and a cover slip was placed over it. Then the slide was examined under 100X magnification.

Isolation of pure strain

1 ml of 0.1 M concentration of developed biomass was taken as a sample for serial dilution. The serial dilution was done up to 10^{-10} dilution. From this sample 10^{-3}, 10^{-4}, 10^{-5}, 10^{-7}, 10^{-9}, 10^{-10} dilutions were taken and plated. From those plates the dominant colonies were identified and isolated. Then the dominant colonies were counted by using colony counter and sub-cultured. From this subculture the cell count was done using haemocytometer. Again the sub-culturing process was done, and then the straining and biochemical tests were done.

Biochemical Tests

Indole Production Test: The sterile tubes were taken and it is filled with 5 ml of sterile peptone broth. The culture sample was inoculated into the broth and the tubes were kept into the incubator at 37˚C for 24 hrs. After incubation the Kovac's reagent was added.

Hydrogen Sulphide Test: The sterile tubes were taken and it is filled with 5 ml of sterile peptone broth. The culture sample was inoculated into the broth and the tubes were kept into the incubator at 37˚C for 24 hrs.After incubation period the results were observed.

Methyl Red Test

MR-VP broth was prepared and sterilized. The sterile tubes were taken and the broth was poured. Test organisms were inoculated and the tubes were kept into the incubator for 24 hrs at 37˚ C. After 24 hrs. the methyl red indicator was added to the test tubes.

Voges-Proskaur Test: 5 ml of sterile MR-VP broth was taken in sterile tubes and the tube organisms were inoculated. Then the tubes were incubated at 37˚C for 24 hours. After incubation the barrit's reagent was added.

Citrate Utilization test: Simmons's citrate agar media was prepared and sterilized. 5 ml of medium was poured into the sterile tubes and the slants were prepared and the test organisms were streaked on the agar surface on the slant and incubated at 37˚C for 24 hours.

Heavy Metal Tolerance Assay

To explore the tolerance of the isolates to the heavy metals optimal culture conditions were used with varying initial heavy metal concentrations. To each freshly prepared growth medium, Chromium was amended as Cr (VI) using Potassium dichromate salt (Cr (VI) concentrations ranging from 10 – 100 mg/ 100 ml). Nickel was amended as Ni (II) using Nickel sulphate salt (Ni (II) concentrations ranging from 10 – 100 mg/ 100 ml). Zinc was amended as Zn (II) using Zinc sulphate (Zn (II) concentrations ranging from 10 – 100 mg/ 100 ml). Magnesium was amended as Mg (IV) using Magnesium sulphate (Mg (IV) concentrations ranging from 10 – 100 mg/ 100 ml). After 24 hours incubation, the biomass was measured. The extent of tolerance was compared and the normalized biomass was calculated, i.e., biomass at each heavy metal concentration per biomass using a control.

3. Results and Discussion

Identification of pure strains: The isolated and distinct colonies o Pseudomonas species on the selective media were sub-cultured and obtained in the form of pure culture and identified on the basis of their morphology and biochemical characteristics.

Biochemical characteristics: The biochemical characteristics of the isolated bacterial strain were shown in table 1 and figures 1, 2 and 3.

Antibiotic sensitivity: The antibiotic activity of the *pseudomonas* to the various antibiotics were shown in table 2 and figure 4.

Metal tolerance concentration Assay

Metal tolerance concentration to the various heavy metals were shown in the table 3 and figures 5, 6, 7 and 8.

Table 1: Morphological / Physiological/ Biochemical characteristics.

Morphological/Physiological/ Biochemical characteristics.	**Isolated Bacterial Strain**
Gram Stain	Negative
Cell Shape	Rod
Growth at temperature	37˚C
Voges-proskaur test	positive
H_2S production	Negative
Indole production test	Negative
Citrate utilization	Positive
Methyl Red test	Negative

Table 2: Antibiotic sensitivity assay

S.No	Bacteria	Resistant to	Ring diameter (cm)
1	*Pseudomonas*	Ampicillin	0.9
		Kanamycin	1.5
		Tetracyclin	2.3
		Vanamycin	1.7

Table 3: Metal tolerance concentration of nickel

Concentration (mg/100 ml)	Nickel OD at 560 nm	Chromium OD at 560 nm	Zinc OD at 560 nm	Magnesium OD at 560 nm
Without organism	1.256	1.425	1.159	2.501
100	0.013	0.662	0.381	1.963
125	0.007	0.550	0.296	1.487
150	0.001	0.371	0.277	1.252

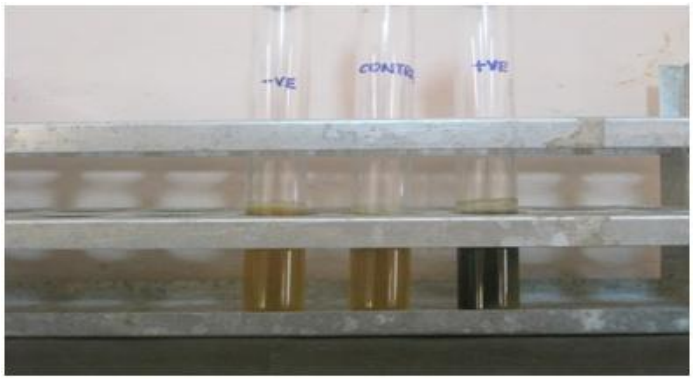

Figure 1: Hydrogen Sulphide Test

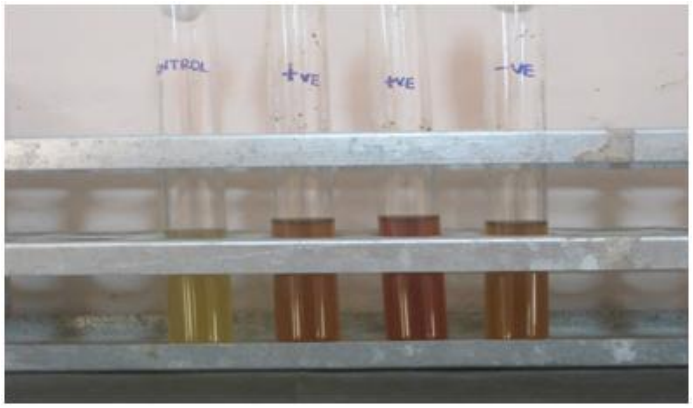

Figure 2: Methyl Red Test

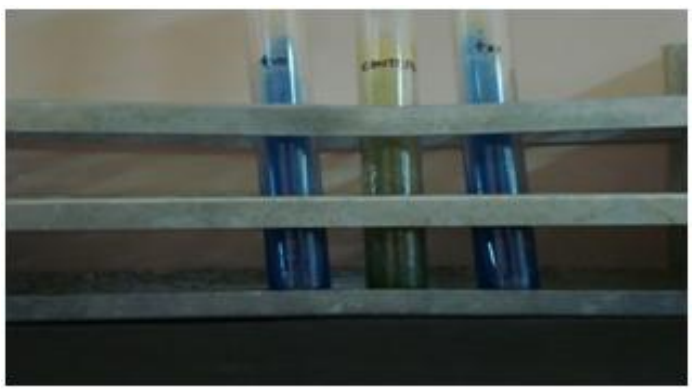

Figure 3: Citrate Utilization test

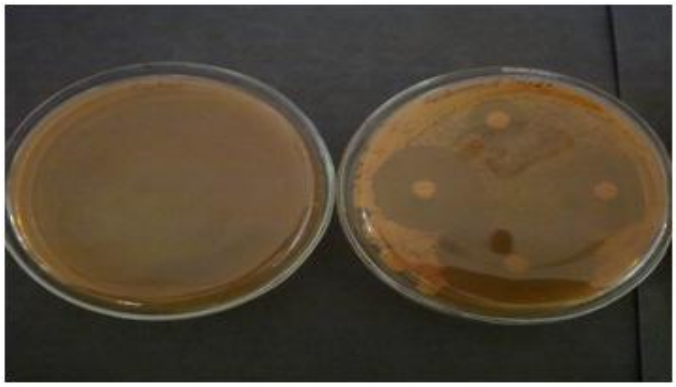

Figure 4: Antibiotic Resistance

Figure 5: Metal Tolerance Concentration of Nickel

Figure 6: Metal Tolerance Concentration of Chromium

Figure 7: Metal Tolerance Concentration of Zinc

Figure 8: Metal Tolerance Concentration of Magnesium

4. Conclusion

The microbes play a vital role in the remediation of heavy metals and other pollutants.Biosorption of three heavy metals namely Nickel, Chromium, Zinc and Magnesium were conducted using individual cultures of Pseudomonas species. The results obtained from the study revealed that the above-said biosorption method can be very efficient for the treatment of soils contaminated with toxic heavy metals. Consequently, the *Pseudomonas* sp. which was isolated form soil was more potent bioremediation agent. The antibiotic susceptibility of these metal sorption bacteria indicate that those metal tolerant and metal utilizing bacteria highly sensitive atomost of the various antibiotics.

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