



RESEARCH ARTICLE

HEAVY METAL BIOSORPTION POTENTIAL OF *PSEUDOMONAS SP.* ISOLATED FROM INDUSTRIAL WASTEWATER OF HARIDWAR DISTRICT, INDIA.

Namitajoshi, Deep gupta and Anubala.

1. Department of Environment science, KanyaGurukulcampus ,Haridwar India.
2. Department of civil engineering, COER.

Manuscript Info

Manuscript History

Received: 23 August 2017
Final Accepted: 25 September 2017
Published: October 2017

Abstract

The present study was done to evaluate the heavy metal biosorption potential of two bacterial strains isolated from industrial wastewater of Haridwar industrial area. The wastewater samples were collected from four different sites., i.e., HI, BI, OD and DI. The heavy metal concentrations were determined after digestion of wastewater samples. The heavy metal resistant bacteria were isolated and screened for their Biosorption potential. The minimum inhibitory concentration (MIC) of Pb, Cr, Ni and Zn was determined by agar diffusion method in 25, 50, 100, 200 & 400 ppm concentrations. The isolates were identified and characterized molecularly with 16S rRNA gene sequence analysis and were revealed 99% similarities of BIN and BOD with *Pseudomonas geniculata* and *Pseudomonas hibiscicola* respectively. Biosorption experiments indicate that, BIN and BOD strain could bioadsorb metals in the order Pb>Cr>Zn>Ni and Ni>Cr>Pb>Zn.

Copy Right, IJAR, 2017,. All rights reserved.

Introduction:-

The quality of life on earth is linked inextricable to the overall quality of the environment. The development or new inventions in different fields like astronomical, medical, agricultural, corporate, engineering etc are serving human living too easy in present days. Human activities are intimately tied to the environment and bio-friendly behavior of human beings often has harmful consequences on environment. With the rapid development of industries heavy metal pollution has become one of the most serious environmental problems. Heavy metals affect many biological processes such as respiration, photosynthesis, reproduction and metabolism which cause a partial or total damage to living organisms^{1, 2}. Both natural and anthropogenic activities are responsible in accumulation of wide ranges of toxic heavy metals in environment and thus global concern. Contaminants vary in their tendency to end up in water held in the soil or in the underlying ground water (by leaching through the soil), volatilize (evaporate) into the air and binding tightly to the soil³. The fates and behavior of heavy metals are controlled by process of sinks and processes of remobilization addressing as entering compartment⁴. The process of sinks consist of adsorption and co-precipitation, precipitation and incorporation in biological activity and the process of remobilization comprise elevated salt concentration, changes in redox reaction, lowering of pH, increasing use of organic complexing agents and biochemical process. The search for new and innovative technology for the remediation of heavy metals pollution has attracted the attention on the biosorption potential of certain micro-organism. The use of microbial biomass like bacteria, fungi, algae and yeast as a Biosorbent is a potential alternative over chemical methods for removal of metals from soil and industrial wastewater by the method known as biosorption. Biosorption is a passive removal of metals using non-living biomass as adsorbent material which is metabolism-independent, in contrast to

bioaccumulation i.e., metabolism dependent process⁵. It is a property of certain types of inactive, non-living microbial biomass to bind and concentrate heavy metals from very dilute aqueous solution. It is particularly the cell wall structure of certain bacteria, algae and fungi, which was found responsible for the biosorption⁶. The main mechanism of biosorption include mechanism of absorption, ion-exchange, surface complexation and precipitation⁷. Bacteria and fungi used across various industries (e.g., Nutritional, Pharmaceutical) become post production waste that may be valuable, free of cost Biosorbent. Potent metal biosorbents among bacteria include genera *Bacillus*, *Pseudomonas*, *Streptomyces*, *Micrococcus* and *Escherichia coli*. In the present study two *Pseudomonas sp.* were isolated from industrial wastewater sample and further evaluated for biosorption capacity of Lead, Chromium, Nickel and Zinc in laboratory experiment

Material and Methods:-

Sampling:-

Industrial wastewater samples were collected from four different sites i.e., Haridwar industrial area (HI), Bahadrapur Industrial area (BI), Open drainage of SIDCUL (OD) and direct effluent (DI). The samples were collected in sterile bottles and stored at 4°C in the refrigerator to prevent volume change due to evaporation.

Heavy metal analysis of wastewater samples:-

Samples were digested by taking 20 ml of sample in 100 ml glass beaker. 10 ml of nitric acid and 5 ml of perchloric acid were added to sample and evaporated on hot plate for 2 hrs by slow boiling. After evaporation to near 10 – 20 ml, the samples were dissolved with 10 ml nitric acid, filtered and diluted to 50 ml with distilled water. Total metal concentration (Cr, Ni, Co, Cu, Fe, Cd, Pb and Zn) of digested samples were analysed by using AAS⁸.

Enumeration and Identification of heavy metal resistant bacteria species:-

Wastewater samples were serially diluted upto 10^{-5} and were spread on Nutrient agar plates enriched with 25 ppm of heavy metal (Pb, Zn, Cr & Ni) individually incubated at 37°C for 24 hr⁹. After incubation, colony forming unit of each plate was calculated and according to morphology, bacterial isolates were purified further by streaking on nutrient agar plate. Bacterial isolates were maintained on agar slants. The minimum inhibitory concentration (MIC) of isolates at which no growth occurred was determined by agar diffusion method¹⁰. The concentration range was 25 ppm to 400 ppm. According to MIC, the two bacterial isolates were identified by Gram's staining and some biochemical test such as Indole test, Methyl red (MR) and Voges-Proskauer etc.

The molecular characterization of selected isolates was done by 16S sequence analysis. The bacterial genomic DNA was extracted using standard protocol¹¹. The strains were amplified by PCR using two bacterial 16S primers, 5'-AGAGTTGATCMTGGCTCA-3' (27F) for forward sequencing and 5'-CGGTTACCTTGTTACGACTT-3' (1492R) for reverse sequencing. 16S rRNA sequences were deposited to Genbank database to get the accession number and most similar sequence alignment using www.ncbi.nlm.gov/BLAST¹² was identified. The nucleotide sequences were aligned with MUSCLE and phylogenetic tree was constructed with the help of MEGA 5.2 software.

Biosorbent preparation:-

All the strains were inoculated separately into 100 mL nutrient broth in 500 mL conical flasks and incubated on a shaker at 150 rpm for 24 h at 28°C. The cells were grown to late exponential phase, harvested by centrifugation (REMI, India) at 10,000 rpm for 30 min at 4°C and washed three times with deionized water. Cell suspensions for assay of biosorption potential of live bacteria were prepared by resuspending the cell pellet in deionized water. Biomass concentration in cell suspensions were determined by drying an aliquot in a preweighed aluminum foil container to a constant weight at 80°C¹³

Biosorption experiment:-

Dried and powdered living biomass (10.0 mg \pm 0.1) of four isolates was inoculated separately into 100 mL of metal solution containing 25 to 400 ppm of Ni, Cr, Zn and Pb. Metal-free and biosorbent-free solutions were prepared as controls. The flasks (250 mL) were kept on rotatory shaker for 24 h at 30°C and 150 rpm. After 24 h, cells were harvested from the medium and content of supernatant were analyzed after proper digestion and dilution by AAS. The optimum pH and temperature were maintained for the growth of microorganisms in batch culture¹⁴

Biosorption capacity i.e. amount of metal ion (mg) bioadsorbed/g of dried biomass was calculated using the following equation:

$$Q = ((C_i - C_f)V) / m$$

Whereas Q = mg of metal ions uptake per gram biomass (mg/g), C_i is initial concentration of metallic ions (mg/L); m is dried mass of biosorbent in the reaction mixture (g) and V is volume of reaction mixture (mL).

Results and Discussion:-

Heavy metal concentration:-

Heavy metal content of wastewater sample was analysed by AAS. Wastewater samples were highly contaminated with Chromium and Lead metal. The concentration of chromium in DI sample was found 41.16 ± 0.4 ppm. Cobalt and copper were not detectable in BI and OD sample as it showed low level of contamination by industries.

Screening and Minimum Inhibitory concentration of Bacteria:-

After serial dilution of soil sample, bacterial isolates were selected based on colony morphology and color. These isolates were further purified by streaking separately on nutrient agar plates. Isolated bacteria were further screened for resistance against heavy metals. Among ten isolates only two bacterial strains were selected for Biosorption and minimum inhibitory concentration were determined. These isolates were named as BIN and BOD. The minimum inhibitory concentration (MIC) of bacterial isolates has been depicted in Table 2. MIC range of isolates against various metal concentrations was in the range of 25 mg/g to 400 mg/g. MIC was found species specific and metal dependent.

Identification:-

The selected two bacterial isolates were positive to some biochemical tests (Table 3). Based on these biochemical tests and Bergey's manual of bacteriology¹⁵, isolates were identified as *Pseudomonas sp.*

Biosorption experiment:-

The biosorption potential and percentage removal of heavy metal by *Pseudomonas geniculata* (BIN) and *Pseudomonas hibiscicola* (BOD) were shown in table 3 & 4. Percentage removal was also represented in figure 32 & 33. The order of biosorption potential of BIN and BOD was $Pb > Cr > Zn > Ni$ and $Cr > Pb > Ni > Zn$ respectively. The maximum Biosorption by BIN was observed in Pb i.e., 13.03 ± 0.47 mg/g, 29.7 ± 0.33 mg/g and 56.96 ± 0.18 mg/g at initial concentration 25, 50, and 100 ppm respectively whereas minimum biosorption potential was observed in Nickel metal i.e., 9.43 ± 0.31 mg/g, 19.5 ± 0.20 mg/g and 32.1 ± 0.03 at initial concentration 25, 50 and 100 ppm respectively. The maximum percentage removal was observed 59.9% at 50 ppm initial concentration of Pb metal and the minimum percentage removal was observed 32.1% at 100 ppm of Ni. For BOD, the maximum biosorption potential of *Pseudomonas hibiscicola* showed for Chromium i.e., 12.3 ± 0.66 mg/g & 30.2 ± 0.08 mg/g at 25 & 50 ppm; Lead was 60.8 ± 0.47 mg/g at 100 ppm and Zinc was 100.4 ± 0.52 mg/g & 121.03 ± 0.54 mg/g at 200 & 400 ppm. The percentage removal of Pb, Zn, Cr and Ni by *Pseudomonas hibiscicola* represented in figure 26. The percentage removal by *Pseudomonas hibiscicola* of lead was 46.6%, 55.2% & 60.8%; Zinc was 37.33%, 47.33% 58.9%, 50.2% & 30.2%; Chromium was 49.33%, 60.46% & 54.1% and 53.4%, 50.8%, 33.43% 36.58% and 35.87% at 25, 50, 100, 200 & 400 ppm respectively.

Table 1:-Metal concentration in wastewater samples (In ppm)

Metals	HI	BI	OD	DI
Cr (ppm)	16.2 ± 0.3	14.5 ± 0.19	9.85 ± 0.06	41.16 ± 0.4
Pb (ppm)	26.8 ± 0.14	20 ± 0.18	17.8 ± 0.3	26.8 ± 0.7
Ni (ppm)	6.35 ± 0.17	2.03 ± 0.16	0.17 ± 0.02	8.4 ± 0.1
Zn (ppm)	4.16 ± 0.02	3.79 ± 0.2	1.8 ± 0.01	14.25 ± 0.08
Co	1.5 ± 0.69	2.83 ± 0.02	ND	3.10 ± 0.003
Cu	3.5 ± 0.06	2.9 ± 0.008	ND	3.3 ± 0.16
Cd	0.155 ± 0.006	ND	0.01 ± 0	1.5 ± 0.03
Fe	1.5 ± 0.2	2.38 ± 0.09	0.9 ± 0	2.8 ± 0.02

Table 2:- Minimum inhibition concentration of four isolates („+++“ is 75% inhibition, „++“ is 50% inhibition, „+“ is 25 % inhibition, „-“, is no inhibition).

Metals	Initial concentration	BIN	BOD
Pb	25	+++	+
	50	+++	+
	100	+++	+
	200	-	-
	400	-	-
Zn	25	+++	+
	50	++	+
	100	-	+
	200	-	+
	400	-	+
Cr	25	++	++
	50	++	+
	100	+	-
	200	-	-
	400	-	-
Ni	25	++	+
	50	+	+
	100	+	+
	200	-	+
	400	-	+

Table 3:- Identification of isolates

Test	B(IN)	B(OD)
Indole	-	-
Methy-Red	-	-
Voges-proskauer	+	+
Citrate Utilisation	-	-
Gas production from glucose	-	-
Starch hydrolysis	-	-
Nitrate Reduction	-	-
Catalase test	+	+
Oxidase	+	-
Urea hydrolysis	+	+

Table 4:-Biosorption potential and percentage removal of heavy metal (Pb, Zn, Cr& Ni) by *Pseudomonas geniculata* (BIN)

Initial concentration	Biosorption potential (mg/g)	% Removal	Biosorption potential (mg/g)	% Removal	Biosorption potential (mg/g)	% Removal	Biosorption potential (mg/g)	% removal
	Pb	Pb	Zn	Zn	Cr	Cr	Ni	Ni
25 ppm	13.03 ± 0.47	52.13333	13.6 ± 0.5	54.4	11.1 ± 0.51	44.53333	9.43 ± 0.31	37.73333
50 ppm	29.7 ± 0.33	59.53333	28.4 ± 0.81	56.93333	25.9 ± 0.41	51.86667	19.5 ± 0.20	39
100 ppm	56.96 ± 0.18	56.96667	-	0	36.83 ± 1.68	36.83333	32.1 ± 0.06	32.16667
200 ppm	-	0	-	0	-	0	-	0
400 ppm	-	0	-	0	-	0	-	0

Table 5:-Biosorption potential and percentage removal of heavy metal (Pb, Zn, Cr& Ni) by *Pseudomonas hibiscicola* (BOD)

Initial concentration	Biosorption potential (mg/g)	% Removal	Biosorption potential (mg/g)	% Removal	Biosorption potential (mg/g)	% Removal	Biosorption potential (mg/g)	% removal
	Pb	Pb	Zn	Zn	Cr	Cr	Ni	Ni
25 ppm	11.66 ± 0.33	46.6667	9.33 ± 0.33	37.3333	12.3 ± 0.66	49.3333	13.3 ± 0.86	53.4666
50 ppm	27.6 ± 0.11	55.2	23.66 ± 0.17	47.3333	30.2 ± 0.08	60.4666	25.4 ± 0.15	50.8
100 ppm	60.8 ± 0.47	60.8333	58.9 ± 0.52	58.9333	54.1 ± 1.0	54.1	33.4 ± 1.20	33.4333
200 ppm	-	0	100.4 ± 0.52	50.2	-	0	73.16 ± 1.49	36.5833
400 ppm	-	0	121.03 ± 0.54	30.2583	-	0	143.5 ± 1.32	35.875

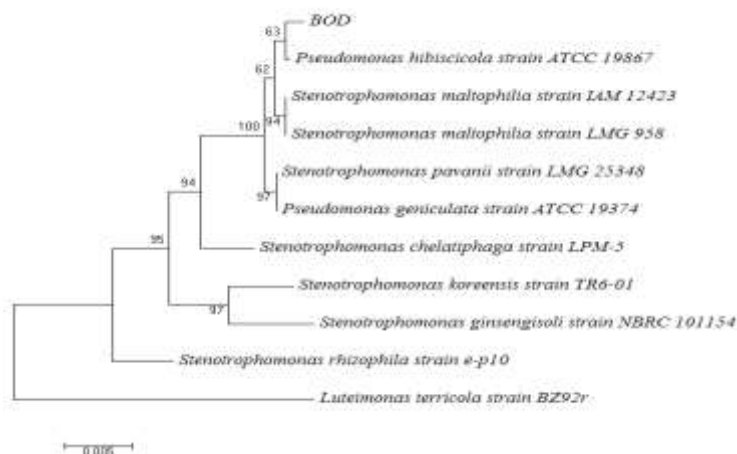
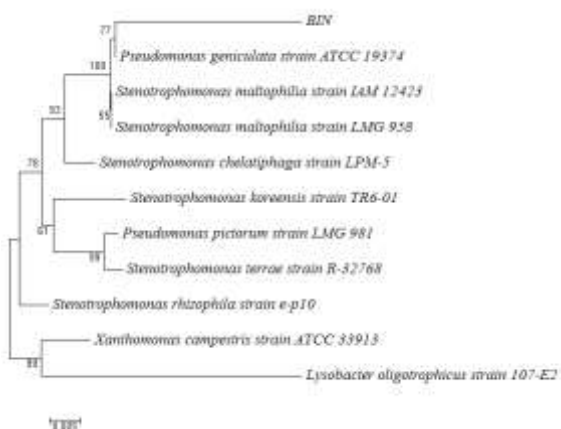
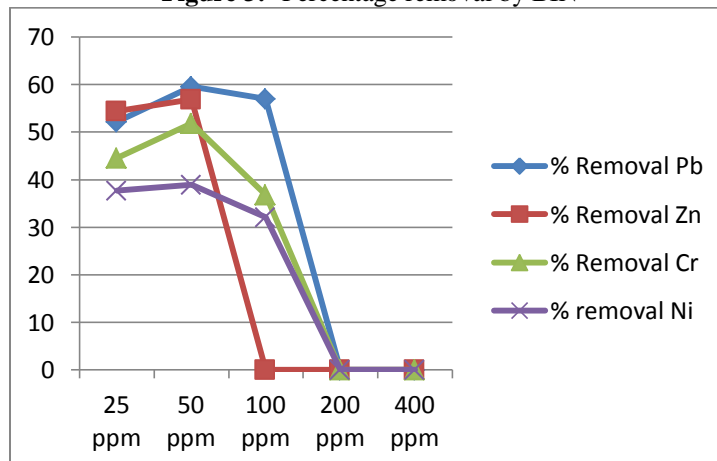
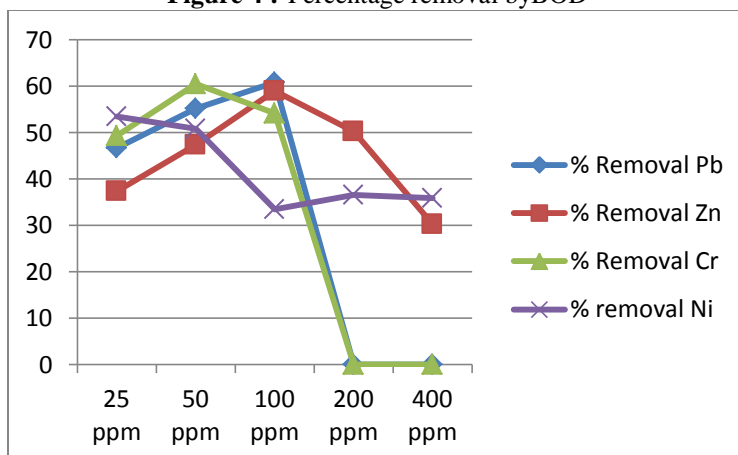
Figure 1:- Phylogenetic tree of BIN**Figure 2 :-** Phylogenetic tree of BOD

Figure 3:- Percentage removal by BIN**Figure 4 :-Percentage removal byBOD****References:-**

1. Mulligan, C. N., Yong, R. N. and Gibbs, B. F. (2001). Remediation technologies for metal-contaminated soil and groundwater: an evaluation. *Engin. Geol*, 60: 193-207.
2. Anderson, D. (2003). Introduction to heavy metal monitoring, European Environment Agency (EPA), environment assessment report no. 10, Europe's environmental :- the 3rd assessment, published on web by Epa.
3. Shayley H., McBride M, and Harrison E. (2009). Sources and impacts of contamination in soils. Cornell waste management Institute. 1-6.
4. Fostner, U. and Wittmann, GTW (1983). Metal pollution in the aquatic environment Germany: Springer- Verlag Berlin Heidelberg.
5. - Hussien, H., Farag, S. and Moawad, H. (2003). Isolation and characterization of *Pseudomonas* resistant to heavy metals contaminants. *Arab Journal of Biotechnology*. 7: 13-22.
6. Volesky, B., 1990. Biosorption of Heavy metals. *Biotechnology Progress*. 11, 235-250.
7. Ananya, N., Ramachandra, T. V., KANAmadi, R. D., (2003). Biosorption of Heavy metals. *Res. Journal of Chem. Env*. 7, 4:71-70. ISSN 0972-0626.
8. APHA
9. Aneja, K. R. (2010). In: Experiments in microbiology, plant pathology and biotechnology (4th edition). New age international (pvt) ltd., New Delhi.
10. 16. Hansson, P.J.; Edwards, N.T. and Andrews, J.A. (2003). Effect of different tree species on soil properties. *Journal of Applied Ecology*. 23: 657-666.
11. 17. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual (2nd edn), Cold Spring Harbour, New York.

12. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739.
13. Puranik, P. R. and Paknikar, K. M. (1999): Biosorption of lead, cadmium and zinc by *Citrobacter* strain MCM B-181: characterization studies. *Biotechnol. Prog.*, 15: 228-237.
14. Cybulski Z, Dzurla E, Kaczorek E, Olszanowski A, (2003). The influence of emulsifiers on hydrocarbon biodegradation by pseudomonadacea and Bacillacea strains. *Spill Science and technology bulletin* 8:503-507
15. John G H; Noel R K; Peter H A S; James T S and Stanley T W.(1994) *Bergey's manual of Determinative Bacteriology* (9th edition). Lippincott Williams & Wilkins, New York.