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Research Article

BIOSORPTION OF HEAVY METALS BY ACCLIMATED MICROBIAL SPECIES, *ACINETOBACTER BAUMANNII*

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ABSTRACT

Objective: The biosorption of the heavy metal species was achieved using the microbial biomass of *Acinetobacter baumannii* isolated from soil and sludge and further used as bioremediating agent in situ.

Material and method: The isolated *Acinetobacter baumannii* was allowed to grow in synthetic media amended with heavy metal solution. The waste samples, both solid and liquid were collected and chemical parameters was checked viz. pH, temperature, BOD, COD, TDS, chloride and calcium. The heavy metals Cd, Cr, Cu, Mn, Pb, and Zn were determined in liquid waste and industrial wastewater, while metals viz. Cr, Co, Mn, Ni and Zn were measured in the leachate form.

Result and discussion: The bioremediation of waste was carried out by the biosorption process in a batch process by using the micro-organism, *Acinetobacter baumannii.*

Conclusion: It was found that *Acinetobacter baumannii* reduced Ni by 56% and Cr by 68%., which leads to the conclusion that microbes can tolerate against the heavy metals due to several resistance and catabolic potentials.

Key Words: Heavy metals, biological oxygen demand, chemical oxygen demand, biosorption, *Acinetobacter baumannii.*

INTRODUCTION

With the rapid development of several industries (mining, surface finishing, energy and fuel producing, fertilizer, pesticide, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leather, photography, electric appliance manufacturing, metal surface treating) and aerospace and atomic energy installations, waste containing metals are directly or indirectly being discharged into the environment causing serious environmental pollution and even threatening human life (Volesky B 1990) Beside the fact that these metals kill microorganisms during biological treatment with a consequent delay of the process of purification, their presence in the environment is known to be detrimental to both flora and fauna as it enters into the food chain. This leads to their bioaccumulation causing more harm to the higher trophic level organisms.The physico-chemical methods, such as chemical oxidation or reduction, evaporative recovery, filtration, ion exchange, electrochemical treatment, chemical precipitation, and membrane technologies are being widely used to remove heavy metal ions from the industrial effluents. All biological systems contain redox elements that function in physiological regulation and maintenance of homeostasis (F. N. MBAOJI et al. 2008) .These processes may be ineffective or expensive, especially when the heavy metal ions are in solutions containing in the order of 1-100 mg dissolved heavy metal ions/L (Volesky B 1990). Henceforth, it is of extreme importance to find an eco-friendly option to clean up the contaminated environment and consequently, preserve the health of the deteriorating organisms. In this regard, several approaches have however, been attempted out of which bioremediation is a highly acceptable and inexpensive technology that involves the usage appropriate microbes and plants, either naturally occurring or man-made to tackle heavy metal issues. Volesky and Holan (1995) reported that different types of biomass were capable of efficiently accumulating heavy metal ions (Oves et al. 2013). Among the various bioremediation options, many scientists spread over different countries have used live or dead culture of bacteria (Gawali et al. 2014), fungi (Dhankhar et al 2011), yeast (Ruta et al. 2010) and algae (Poole et al. 1989) to biosorb heavy metals. But bacterial population has emerged out to be the most important biosorbent used for metal removal among several other microbial populations. There are many mechanisms involved in the biosorption process out of which, some are not fully understood. Biosorption mechanism may be classified according to dependence on the cell's metabolism which is called metabolism dependent or according to the location where the metal removed from solution is found which is called Non -metabolism dependent/ metabolism independent like extra cellular accumulation/ precipitation, Cell surface sorption/ precipitation and Intracellular accumulation (Davis TA et al 2003) .The biosorption of a specific metal by a certain bioabsorbent depends on various factors such as the availability and accessibility of sites and affinity between the site and metals in the bioabsorbent.

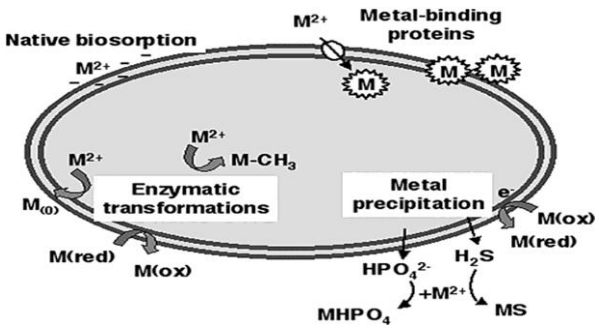

Fig:1

How bacteria cope with toxic concentrations of heavy ions. The scheme summarizes the various means by which bacteria react to the presence of metals (M_{2^+}) in the medium, with reference to the cellular compartment that harbours the response. These mechanisms include the intra- or extracellular binding (and thus immobilisation) of the metal with a cognate protein (frequently a metallothionein) or a matching anion, the biotransformation of the toxic ion into a less noxious or more volatile form, and the dissimilatory reduction of the metal. (Valls et al. 2002)In the life system of living organisms, metals have an integral role. Fluoride contamination is one of the major health problems all around the world(singh et al . 2016). Generally, the effluents released from various industries cause the increase in fluoride level in ground water which tends to be toxic to both aquatic and terrestrial life But the pollution from man-made resources can easily create elevated metal conditions leading to disastrous effects towards environment. Several metals such as Na, Mg, Na, K, Ca, and S serve as microelements and are used to build the plant body and Fe, and Co, Cr, Cu, Mn, Ni and Zn serve as micronutrients and are used as components of various enzymes and process. While many other metals (Ag, Al, Cd, Au and Pb) have no biological role and they are non-essential. The purpose of the present work was to investigate the ability of *Acinetobacter baumannii* (MTCC No.-11451) to accumulate the heavy metals and to use as bioremediation agent in situ.

MATERIAL AND METHOD

Sample Collection

Solid and liquid waste samples were collected by the Standard method APHA, 1995 from the industries and landfills of Doon valley, Uttarakhand,India viz. Srinagar, Chamba, Devprayag, Rudraprayag, Pauri, Rishikesh, Haridwar, Haldwani, Dehradun, Nanital, Lakshar, Uttarkashi, Mussorie and Kotdwar. Samples were collected in the pre sterilized covered glass bottles to protect from contamination and were stored at 4°C for further analysis.

Measurement of Physico-Chemical Parameter of the waste samples

The physico-chemical parameters were measured in all samples for screening of the samples. BOD and COD were measured by LaMonte Dissolved Oxygen test Kit Code 5856, according to the procedure mentioned in the manual provided with the kit. The pH, temperature, chloride, TDS and total hardness of samples was measured by digital pH meter, thermometer, ion selective electrode (ISE), Volhard's method and titration with EDTA respectively (Kumar et al. 2010)

Measurement of Metal Concentration in Solid waste

As metal concentration can't be measured directly in the solid waste, leach ate from solid waste was prepared to determine the heavy metal concentration according to the Standard method (Srivastava et al. 2005, Ferrari et al. 1999).The 10% solid waste was prepared as leach ate by the following procedure:

- 100 g of solid waste was added to 1000ml of distilled water.
- The above suspension was kept on a rotary shaker at 180 rpm at30±1°C for 24 hr for continuous shaking.
- The suspension was first filtered by glass wool followed by Whatman filter paper 42.
- It was further centrifuged at 3000 rpm for 15 min to remove the fine suspended particles.
- The test dilutions (2%, 5% and 10%) with double distilled was made to use the final supernatant.
- Due to high heavy metal concentration, bioremediation process was carried out with four samples viz. DD, HR, MR, and RK.

Measurement of Metal Concentration in Liquid waste

The estimation of heavy metals in the supernatant, obtained from the filtration of liquid sample by Whatman filter paper 42 was done by inductive coupled plasma mass spectrometry (ICP-MS).

Microbial strains

Acinetobacter baumannii was isolated from the soil and sludge by pour plating method and serial dilution. Strains were maintained in agar slants containing nutrient broth and then, further characterized on the basis of bio-chemical procedures and morphologically. In order to maintain the active metabolic activity, microorganisms were transferred weekly to the fresh medium.

Culture medium and Heavy metal exposure

Culture was allowed to grow in synthetic medium with different heavy metal concentration. The concentration of metals Cd, Cr, Co, Cu, Mg, Mn, Ni, Pb and Zn were 25 g/l, 30 g/l, 100 g/l, 150 g/l and 200 g/l. Beef extract (3.0 g), peptone (10.0 g), sodium chloride (5.0 g) and disodium phosphate (1.0 g) were dissolved in one liter of distilled water to make the nutrient broth with pH range 7.3 - 7.5. The medium was autoclaved at 121°C for 20 min. The cells were cultured in the nutrient broth and further cultures were maintained in agar slants (nutrient broth with 25 g/l agar). The cells were inoculated in nutrient broth and kept under agitation in rotary shaker, at 85 rpm 30-35°C ± 2°C for 48 hrs. Further the cells to be used for biosorption were separated by biosorption.

Biosorption Experiments

In all the experiments, cells were obtained in the Erlenmeyer flasks at the same growth stage from single cultivation. The flasks contained 130 ml of each sample with 13.0±1 mg of cells and were used to conduct experiments for heavy metals biosorption. Cells and waste were maintained in contact for 48 hrs under constant agitation, at 30-35°C ± 2°C to obtain equilibrium. After 48 hours, cells were separated from the medium and residual metal concentration was monitored by ICP-MS. The optimum pH and temperature maintained form the growth of microorganisms in the batch culture (Cybulski Z et al. 2003, Hietala K.A. and Roane T.M. 2009). The pH and temperature were recorded daily.

Statistical Analysis

The data obtained were not distorted and had a normal distribution before statistical analysis. All statistical analysis was performed with Statistical analysis System Programs Statistical Analysis Systems. 2001, Snedecor GW et al. 1982). One way analysis of variance (ANOVA) at p=0.05 was used to analyze the data statistically.

RESULTS AND DISCUSSION

Biochemical Tests on Acinetobacter baumannii

The isolates of *Acinetobacter baumannii* species were taken, which appear as coccobacilli on Gram strain. *Acinetobacter baumannii* strains grew well on usual culture mediums and produced colonies by 2-3mm diameter at 18-24 hours. They produced a pale yellow to white grayish pigment on the solid medium. *Acinetobacter baumannii* strains presented a large metabolic activity. They had the capacity to produce acid from glucose and xylose. The production of acid from urea test had variable reactions. All strains were positive to Simmons citrate. The negative reactions: the acid production from sucrose, esculin hydrolysis, H_2S on TSI, nitrate reduction and methyl red. Table 1 represents the results of the tests performed on *Acinetobacter baumannii*.

Physico-Chemical Parameter of the waste samples

The physical and chemical parameters, which were analyzed in the present study, are viz. temperature, pH, BOD, COD, TDS, fluoride and chloride listed in table 2. It is apparent from table 2 that water contains heavy metals, which makes it unsuitable to be discharged into the environment. The temperature ranges from 18 to 340° C and pH ranges from 3.1 to 9.6. The BOD is measured to know the degree of pollution which is 8.5 to 53 mg/l in the waste. The COD ranged from 98 to 780mg/l and total dissolved solids found in the range of 105 to 850mg/l. The calcium ions ranged from 45mg/l to 280mg/l. Maximum permissible limit of chloride is 1000 mg/L with desirable limit of chloride is being 250 mg/L as per Indian standards, but here chloride is found to be in more quantity than the permissible limits.

Table 1. Biochemical test of acclimated microorganism.

S No.	Test, substrate	Acinetobacter baumannii
1	Morphology	coccobacilli
2	Motility	non-motile
3	Fermentative or oxidative	O
4	Catalase	+
5	Oxidase	-
6	Growth on MacConkey agar	+
	Acid from:	+
7	Glucose	+
8	Xylose	-
9	Sucrose	-
10	Esculin hydrolysis	-
11	TSI acid: Slant	-
12	H_2S: on TSI	-
13	Simmons citrate	+
14	Urea	V
15	Nitrate reduction	-
16	Methyl red	-

Key reactions: O= oxidative; + = positive reaction; - = negative reactions; V= variable reactions.

Table 2. . Physico-chemical analyses of waste sample.

S No.	Sites(Codes)	Sample	pH	Temp (°C)	BOD (mgl^{-1})	COD (mgl^{-1})	TDS (mgl^{-1})	Ca (mgl^{-1})	Cl^- (mgl^{-1})
1	**Srinagar**	**Liquid**	6.2	26	29	375	152	182	233
	(SN)	**Solid***	6.1	30	32	783	149	162	252
2	**Chamba**	**Liquid**	8.5	24	21	556	182	49	321
	(CB)	**Solid**	6.9	30.1	56	354	153	181	240
3	**Devprayag**	**Liquid**	4.6	20.4	13.4	118	423	123	136
	(DG)	**Solid**	3.5	34	18.9	171	154	184	335
4	**Rudraprayag**	**Liquid**	7.1	23.5	9.5	149	346	118	326
	(RG)	**Solid**	4.1	29.9	18	181	159	131	198
5	**Pauri**	**Liquid**	7.2	28	24	134	161	124	450
	(PG)	**Solid**	6.9	31	13.2	194	306	138	120
6	**Rishikesh**	**Liquid**	7.4	24	18.4	271	427	142	234
	(RK)	**Solid**	7.6	32	16	435	146	230	424
7	**Haridwar**	**Liquid**	6.9	26	13.5	489	492	115	358
	(HR)	**Solid**	7.5	32	24	681	581	162	120
8	**Nanital**	**Liquid**	7.7	20	17	232	146	98	240
	(NL)	**Solid**	4	22	33	322	154	121	234
19	**Kotdwar**	**Liquid**	5.6	26	14.5	100	439	135	210
	(KD)	**Solid**	8.7	34	27.3	171	442	205	285
10	**Mussorie**	**Liquid**	6.3	18.5	21.4	181	689	242	235
	(MR)	**Solid**	10	27	33.4	240	337	301	315
11	**Uttarkashi**	**Liquid**	7.1	22	12.5	125	152	60	126
	(UK)	**Solid**	6.2	28	23.6	162	112	46	175
12	**Lakshar**	**Liquid**	5.2	26	18.5	169	216	81	234
	(LS)	**Solid**	3.6	34	36	491	110	100	124
13	**Dehradun**	**Liquid**	7.2	25	13.5	661	861	283	550
	(DD)	**Solid**	5.6	31	18.7	658	363	126	512
14	**Haldwani**	**Liquid**	6.6	26.2	15.2	248	134	168	421
	(HD)	**Solid**	6.2	30	19.9	556	159	155	150

Metal Analysis of Sample

The metal ion analysis of waste sample was done by ICP-MS and heavy metals Cd, Cr, Cu, Mn, Pb, and Zn were determined in liquid waste and industrial wastewater, while metals viz. Cr, Co, Mn, Ni and Zn were measured in the leach ate form, which was made from solid waste sample. Desirable limit of cadmium, chromium, lead and zinc is as follows: $0.01mgl^{-1}$, $0.05mgl^{-1}$, $0.05mgl^{-1}$ and $5.0mgl^{-1}$, respectively. The concentration the metals is listed in Table 3.

Biosorption of metals by Acinetobacter baumannii

Acinetobacter baumannii reduced the nickel and chromium concentration 56% and 68.94 % respectively, at pH 4.3 and temperature 35°C. The pH increases from 4.3 to 5.2 and temperature increases from 35°C to 53°C. The results are listed in Table 4.

Table 3: Metal screening of the sample (mgl^{-1})

S No.	Sites(Codes)	Sample	Cd	Cr	Co	Cu	Mn	Ni	Pb	Zn
1	**Srinagar**	**Liquid**	0.003	0.09	ND	0.45	11.65	ND	0.32	7.23
	(SN)	**Solid***	ND	ND	0.15	ND	ND	0.86	ND	ND
2	**Chamba**	**Liquid**	0.05	0.13	ND	0.89	6.59	ND	0.15	6.98
	(CB)	**Solid**	ND	ND	0.08	ND	3.71	0.47	ND	ND
3	**Devprayag**	**Liquid**	0.018	ND	ND	0.79	8.99	ND	0.06	4.86
	(DG)	**Solid**	ND	0.09	0.31	ND	4.62	3.41	ND	3.45
4	**Rudraprayag**	**Liquid**	0.015	ND	ND	1.92	9.92	ND	0.03	4.62
	(RG)	**Solid**	ND	0.4	0.45	ND	ND	6.65	ND	ND
5	**Pauri**	**Liquid**	0.01	0.07	ND	1.23	16.1	ND	0.89	4.87
	(PG)	**Solid**	ND	0.16	0.23	ND	13.41	0.89	ND	ND
6	**Rishikesh**	**Liquid**	0.027	1.56	ND	1.92	14.98	ND	0.92	8.98
	(RK)	**Solid**	ND	1.01	0.42	ND	13.01	2.78	ND	8.98
7	**Haridwar**	**Liquid**	0.55	8.78	ND	3.99	21.09	ND	0.96	8.92
	(HR)	**Solid**	ND	2.59	0.55	ND	11.58	5.34	ND	8.13
8	**Nanital**	**Liquid**	4.8	ND	ND	0.15	5.9	ND	0.03	2.9
	(NL)	**Solid**	9.8	ND	0.089	ND	11.2	1.33	ND	ND
9	**Kotdwar**	**Liquid**	12.7	0.8	ND	3.92	13.46	ND	0.52	6.43
	(KD)	**Solid**	13.1	0.5	0.43	ND	13.01	6.1	ND	7.45
10	**Mussorie**	**Liquid**	0.28	1.8	ND	6.23	36.21	ND	0.43	9.12
	(MR)	**Solid**	ND	0.6	0.905	ND	14.22	8.1	ND	8.98
11	**Uttarkashi**	**Liquid**	7.63	ND	ND	0.09	ND	ND	0.06	4.12
	(UK)	**Solid**	ND	ND	0.02	ND	1.32	0.06	ND	ND
12	**Lakshar**	**Liquid**	0.009	0.029	ND	0.09	3.09	ND	0.05	5.45
	(LS)	**Solid**	ND	0.02	0.076	ND	ND	0.81	ND	ND
13	**Dehradun**	**Liquid**	0.47	1.9	ND	5.01	45.9	ND	0.66	11.9
	(DD)	**Solid**	ND	3.34	0.65	ND	31.26	5.9	ND	8.19
14	**Haldwani**	**Liquid**	ND	0.87	ND	0.38	ND	ND	0.22	4.81
	(HD)	**Solid**	5.45	0.56	0.087	ND	5.34	3.01	ND	2.55

*The parameters are in leach ate form. ND= Not Detected

Table 4: Biosorption of metals by *Acinetobacter baumannii*

S. No	Site	Sample	pH	Temp	Ni (Before)	Ni (After)	Cr (Before)	Cr (After)
1	DD	**Liquid**	4.3	35	ND	ND	1.85	0.59
		Solid*	4.5	37	5.5	1.93	3.34	1.06
2	HR	**Liquid**	4.7	41	ND	ND	8.56	2.73
		Solid	4.9	43	5.3	1.57	2.09	0.66
3	MR	**Liquid**	5.1	46	ND	ND	1.05	0.33
		Solid	5	52	7.6	2.58	0.56	0.17
4	RK	**Liquid**	5.5	53	ND	ND	1.45	0.46
		Solid	5.2	51	2.6	0.96	0.96	0.3

CONCLUSION

The increasing usage of the various heavy metals has led to a rapid increase in environmental pollution by several folds. The main purpose of metal remediation is the decrease of metal mobility and toxicity within the waste sample or removing the toxic metals from waste. Microbial biomass is one of the low-cost and efficient biosorbents, which perform several reactions to accomplish this goal. The process of biosorption has many attractive features including removal of metals ion over relatively broad range of pH and temperature [18]. In the present study, *Acinetobacter baumannii* was used for biosorption and the four samples out of fourteen were screened for biosorption study. After treatment, *Acinetobacter baumannii* reduced Ni by 56% and Cr by 68%. Henceforth, it may be concluded that microbes can tolerate against the heavy metals due to several resistance and catabolic potentials.

RFERENCES

1. Cybulski Z, Dzuirla E, Kaczorek , Olszanowski A, (2003). *The influence of emulsifiers on hydrocarbon biodegradation by*
2. *Pseudomonadacea and Bacillacea strains.* Spill Science and Technology Bulletin 8: 503 – 507.
3. Davis TA, Volesky B, Mucci A (2003) *A review of the biochemistry of heavy metal biosorption by brown algae.* Water Res 37: 4311-4330.
4. Dhankhar R., Hooda A. Fungal biosorption: *an alternative to meet the challenges of heavy metal pollution in aqueous solutions.* Environ. Technol. 2011;32:467–491.
5. Ferrari, B., Radetski, C.M., Veber, A.M., Ferard, J.F., (1999). *Ecotoxicological assessment of solid waste: a combined liquid and solid phase testing approach using battery of bioassay and biomarkers*. Env Toxic Chem 18, 1195-1202.
6. F. N. MBAOJI* , A. C. EZIKE, C. S. NWORU, C. A. ONYETO, I. A. NWABUNIKE, I. C. OKOLI, P. A. AKAH, *International Journal of Pharmacy and Pharmaceutical Sciences,Volume 8 Issue7,2016*,p 47-51
7. Gawali Ashruta A, Nanoty VD, Bhalekar UK (2014) *Biosorption of Heavy Metals from Aqueous Solution using Bacterial EPS,* Int. J. of Life Sciences, 2(4): 373-377
8. .Hietala K.A. and Roane T.M. (2009). *Microbial Remediation of Metals in Soils In Advances in* Applied Bioremediation, Soil Biology 17, Springer-Verlag Berlin Heidelberg. Pp. 201-220
9. Kumar, Ashok, Bisht, B.S., Talwar, Amitabh and Chandel, Deepika (2010b*). Physico-Chemical and Microbial Analysis of Ground Water from Different Regions of Doon Valley*, Inter. Jou Appl. Envtl Sci. (IJAES) 5(3); 433-440
10. Marc Valls, Víctor de Lorenzo (2002) *Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy*

metalpollution: http://dx.doi.org/10.1111/j.1574-6976.2002.tb00618.x 327-338

11. Mohammad Oves, Mohammad Saghir Khan, and Almas Zaidi, *Biosorption of heavy metals by Bacillus thuringiensis strain OSM29 originating from industrial effluent contaminated north Indian soil,* Saudi J Biol Sci. 2013 Apr; 20(2): 121–129.. doi: 10.1016/j.sjbs.2012.11.006
12. Mustapha MU, Halimoon N (2015) *Microorganisms and Biosorption of Heavy Metals in the Environment: A Review Paper.* J Microb Biochem Technol 7: 253-256. doi:10.4172/1948-5948.1000219
13. Neethu CS, Mujeeb Rahiman KM, Saramma AV, Mohamed Hatha AA (2015) *Heavy-metal resistance in Gram-negative bacteria isolated from Kongsfjord, Arctic*. Can J Microbiol 61: 429-435
14. Poole R.K., Gadd G.M. IRL Press; Oxford: 1989. *Metals: Microbe Interactions*. pp. 137.
15. Richa, Kumar Dinesh, Gupta, S.K, (2005). Bioremediation of Municipal sludge by vermitechnology and toxicity assessment by Allium cepa, Elsevier Journal of Bioremediation Technology; doi: 10.1016/j.biortech.2005.01.029
16. Ruta L., Paraschivescu C., Matache M., Avramescu S., Farcasanu I.C. *Removing heavy metals from synthetic effluents using "kamikaze" Saccharomyces cerevisiae cells.* Appl. Microbiol. Biotechnol. 2010; 85:763–771.
17. Snedecor GW, Cochran WG. (1982). Statistical Methods. 7th ed. The low State University Press. Ames, IA
18. Statistical Analysis Systems. (2001). SAS® User's Guide: Statistics, Version 5 Edition Cary, NC.
19. Tej Pratap Singh,C.B.Majumder.Fluoride Removal From Sewage Water Using Sweet Lemon Peel as Biosorbents, *International Journal of Pharmacy and Pharmaceutical Sciences,Volume 8 Issue7,2016*,p 85-92
20. .Volesky B, Biosorption and biosorbents, in *Biosorption of heavy metals,* edited by B Volesky (CRC Press, Boca Raton Florida) 1990a, 3-5.
21. B, *Removal and recovery of heavy metals by biosorption. In: Biosorption of heavy metals.* Boston, USA, CRC press, 1990b, p. 7-4