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Title Key: Chromate uptake in single and multiple …

Chromate uptake in single and multiple metal artificial effluents using a screened bacterial consortium and antibiotic tolerance associated with it

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Abstract:
The consortium showed 66.9 and 30.3 % chromate uptake respectively in AEI and AEII respectively, prepared in distilled water and 42.16 and 35.56 % uptake respectively in phosphate buffer. A very high efflux has been observed for both the concentrations of dichromate in case of AE II effluent along with a decrease in plate counts in presence of multiple metal effluents. Out of the cultures and antibiotics tested , 10 of the 12 cultures showed resistance to Erythromycin and Ampicillin, 6 to Chloramhenicol and 4 to Tetracycline at 10 µg/ml.
Key words: Consortium, Chromate , metals, antibiotics.

Introduction :

Heavy metals have a wide range of uses right from leather and tanning ,wood preservation , metallurgical uses to oil refining and chemical syntheses. The environment has a mechanism of tackling pollution, however when the quantum of pollution is large enough or crosses its threshold limits, the natural flora is unable to do must justice. In such cases laboratory bred cultures are introduced into the ecosystem to counter such pollutants. Since the natural mineralization of metals is a slow process, pollution by heavy metals constitutes one of the most important environmental problems of industrial societies.(Hernandez *et al*.,1998).

Material and methods:

Collection of samples: Five different effluent and soil samples from disposal sites of electroplating industries were collected. Three of these were from metal plating industries in and around the industrial township of Ludhiana and one each from Waluj (Aurangabad, India) and Kalwa (Thane, India).(Bhattacharya,2017)

A bacterial consortium of 12 organisms was raised from enrichments using above mentioned samples and selective microbiological techniques.(Bhattacharya and Lomte, (2009;2011).

Antibiotic susceptibility tests:

Antibiotic susceptibility with respect to the isolates of the consortium was demonstrated on Muller-Hinton medium (Bauer *et al.*,1966) by disc diffusion method.(Verma *et al*.,2004). The following

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antibiotics mentioned below were used for preliminary studies at 10µg/ml. Penicillin, Erythromycin, Streptomycin, Ciprofloxacin, Rifamycin, Ampicillin, Tetracycline and Chloramphenicol (Faisal and Hasnain, 2004; Bopp *et al*.,1983) were also used at 50µg/ml. The antibiotic disks were placed on freshly prepared lawns of the isolates followed by measurements of zones of inhibition.

Chromate uptake in single metal and multiple metal artificial effluents:

Metal uptake in a defined medium has the advantage of providing nutrients for growth which therefore also generates a proportional amount of biomass. This is not possible in a non nutrient medium such as a buffer or electroplating effluent. It has been reported earlier that total amount of metal biosorption in a multiple metal system is lower than that observed in a single metal system. (Hussein *et al*.,2004).The extent of chromate remediation and viable counts that make it possible was monitored with respect to time in an artificially prepared effluent containing either a single metal ion (only chromate) or different heavy metals.

Plate counts reduced drastically in effluents containing no nutrients. Therefore artificial effluent (AE-I) was prepared in sterile distilled water (pH 7.0) supplemented with 0.1% yeast extract. It was incorporated either with sterile 10µg/ml dichromate or several heavy metals (AE-II) at 10µg/ml such as nickel chloride, mercuric chloride, zinc sulphate, silver sulphate, magnesium sulphate, copper chloride, cadmium chloride, lead sulphate, manganese sulphate along with 10 µg/ml potassium dichromate respectively. The effluents were inoculated with 10% of the consortium.(A_{600} =0.85). 10 µl of 10^{-4} dilution of both the effluents were spread on nutrient agar plates and incubated at room temperature for 24 h. for recording the viable cell counts/ml. The same was also monitored at 24h. interval. Initial Cr(VI) was recorded from metal(s) containing uninoculated sets of both the effluents. Residual dichromate was estimated over a 120 h. period using diphenyl carbazide method**.** Phosphate buffer pH 6.8 was prepared and sterilised. It was then incorporated with presterilised 0.1% yeast extract along with 50 µg/ml dichromate only or different heavy metal salts (AE-II) at 10µg/ml along with 50 µg/ml dichromate . Consortium (A_{600} =1.25) was inoculated at 10% of the final volume.CFU/ml, and residual dichromate was estimated after 24h.and thereafter for a period of 5 days.

Results and discussion:

The following cultures were used for formulating the consortium for use in chromate detoxification as well as the present study.

Table 1 :Identification of isolates selected for chromate bioremediation.

Sr.no	Tentative culture designation	Identification of the cultures based on biochemical tests.
1	12*	*Bacillus brevis*

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2	17b	*Bacillus subtilis*
3	10bW1	*Alkaligenes sp.*
4	6b	*Listeria sp.*
5	17Sy	*Caryophanon sp.*
6	8s	*Cellulomonas sp.*
7	PCD2	*Curtobacterium pusillum*
8	Pcont	*Corynebacterium xerosis*
9	10bw	*Sporolactobacillus inulinus*
10	S1Cr	*Curtobacterium citreum*
11	17sw	*Bacillus laterosporus*
12	10by	*Micrococcus luteus*

Antibiotic susceptibility tests:

An implication of heavy metal tolerance in the environment is that it may contribute to the maintenance of antibiotic resistance genes by increasing the selective pressure of the environment. The isolates constituting the consortium showed metal resistance but did not show multiple drug resistance. Out of 8 different antibiotics (µg/ml) selected, resistance to Chloramphenicol and Ampicillin was noted in some of the isolates at concentrations tested. The isolates of the chromate detoxifying consortium were otherwise largely sensitive to most of the other antibiotics at concentrations tested. However they show resistance at lower concentrations of the drugs used.(10 µg/ml). Faisal and Hasnain (2004), also observed similar resistance in several strains of *Brevibacterium* sp with respect to Streptomycin, Ampicillin, Tetracycline, Kanamycin and Chloramphenicol.

Table 2: Antibiotic susceptibility/tolerance in isolates of the consortium at 10µg/ml.

Sr.no	**Culture designation**	**Diameter of zones of inhibition (mm)**

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		P	E	Cp	S	A	T	Cl	R
1	*B. brevis*	20	10	32	30	R	R	R	14
2	*C. tenue*	28	R	36	34	R	R	R	14
3	*M. luteus*	29	R	29	38	24	R	17	27
4	*C.pusillum*	R	R	19	18	R	R	32	R
5	*Alcaligenes sp.*	32	R	28	22	R	12	R	12
6	*B. subtilis*	16	R	19	17	R	12	R	15
7	*Cellulomonas sp*	15	R	26	23	R	13	R	13
8	*S. inulinus*	15	R	22	25	R	13	15	R
9	*Listeria sp.*	14	R	16	17	R	R	R	12
10	*B .laterosporus*	12	R	22	19	R	14	16	R
11	*Curtobacterium citreum*	R	R	30	32	R	15	14	15
12	*Corynebacterium xerosis*	13	10	34	27	R	R	13	R

Table 2a: Antibiotic susceptibility/tolerance in isolates of the consortium at 50µg/ml.

Sr.no	Culture designation	Diameter of zones of inhibition (mm)							
		P	E	Cp	S	A	T	Cl	R
1	*B. brevis*	13	10	30	28	6	7	8	11
2	*C. tenue*	20	6	34	30	9	9	R	13
3	*M. luteus*	21	9	27	38	20	9	15	25
4	*C.pusillum*	9	6	16	16	6	9	28	9
5	*Alcaligenes sp.*	28	7	24	20	9	10	R	10
6	*B. subtilis*	11	6	16	15	R	10	R	12
7	*Cellulomonas sp*	13	8	23	21	R	11	8	11
8	*S. inulinus*	13	9	20	23	R	11	13	9
9	*Listeria sp.*	12	9	14	15	7	9	8	11
10	*B .laterosporus*	11	8	20	18	R	13	14	9
11	*Curtobacterium citreum*	9	6	29	28	R	12	12	10
12	*Corynebacterium xerosis*	12	10	32	25	R	7	11	8

Penicillin(P),Erythromycin(E),Streptomycin(S),Ciprofloxacin(Cp),Rifamycin(R), Ampicillin(A),Tetracycline(T),Chloramphenicol(Cl), R-resistance.

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Chromate uptake in single metal and multiple metal artificial effluents:

It may be observed from table 3 and 3a that a drastic reduction in CFU occurred with respect to both artificial effluents, AEI and AEII after 24h. of inoculation. Chromate uptake at 10µg/ml (Table 3) was monitored over a period of 120 h. 66.9 and 30.3 % uptake occurred in AEI and AEII respectively which was prepared in distilled water. Chromate uptake at 50µg/ml (Table 25a) was also monitored in phosphate buffer over a period of 120 h. 42.16 and 35.56 % uptake occurred in AEI and AEII respectively. A very high efflux has been observed for both the concentrations of dichromate in case of AE II effluent.

Table 3: Chromate uptake and plate counts in single and multiple metal artificial effluents in distilled water.

Time elapsed	Artificial effluent-I			Artificial effluent-II		
	CFU/ml	Residual Cr(VI) µg/ml	% cumulative uptake	CFU/ml	Residual Cr(VI) µg/ml	% cumulative uptake
0	$2x10^8$	7.46	0	$1.3x10^9$	7.46	0
24	$9x10^5$	6.59	11.67	200	5.03	32.58
48	$7x10^6$	6.54	12.34	17	3.47	53.49
72	$1.23x10^6$	5.38	27.89	22	3.47	53.49
96	$1.2\ x10^4$	5.03	32.58	13	4.68	37.27
120	$3x10^4$	2.47	66.9	2	5.20	30.3

Table 3a: Chromate uptake and plate counts in single and multiple metal artificial effluents prepared in phosphate buffer pH.6.8.

Time elapsed (h).	Artificial effluent-I			Artificial effluent-II		
	CFU/ml	Residual Cr(VI) µg/ml	%cumulative Uptake	CFU/ml	Residual Cr(VI) µg/ml	% cumulative uptake
0	$2.9x10^{10}$	55.56	0	$3.3x10^9$	59.12	0
24	$1.4\ x10^7$	52.01	6.39	65	38	35.73
48	$1.7x10^7$	43.9	20.99	12	45.15	23.63
72	$1.23x10^5$	22.12	60.19	5	32	45.88
96	$1.2\ x10^4$	30	46.01	7	40.12	32.14
120	$3x10^4$	32.14	42.16	2	38.10	35.56

It was observed by Burd *et al*.(1998) that when metals were added to a non growing bacterial suspension in distilled water, the metal did not decrease the colony counts (CFU) in the suspension after 5 days of incubation at 25°C . This explains the fact that metal toxicity will be higher in a nutritional medium than in distilled water or buffers, as a nutritional medium will support growth and toxicity will be more pronounced on growing or proliferating cells.

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A similar study by Gopalan and Veermani (2004), was carried out with respect to reduction of hexavalent chromium in three bench-scale CSTR (continuosly stirred tank reactors) using *Pseudomonas* sp. capable of giving 83 to 87% chromate reduction in 72 h batch assays with 60 mg Cr(VI)/l in a synthetic medium.

The extent of chromate uptake in single and multiple metal effluent was investigated over a period of 5 days. There was a 50 % decrease in CFU after 5 days of inoculation of the consortium in distilled water. CFU increased from 10^5 to 10^6 on the second day which was due the presence of yeast extract, other wise the reduction in viable counts was very severe in the absence of nutrients.

The decrease in CFU was sharper in multiple metal effluents because of the combined toxicity of several heavy metals. It was reported by Utigikar *et al*.(2000) that single metal effluent shows a higher uptake as compared with metal biosorption in a multiple metal system. Cr(VI) has been shown to be abiotically reduced in presence of Mn(II) to Cr(III) (Perez-Benito and Arias, 2001).Although 50% uptake was noted in multiple metal effluents , it could not be correlated with the corresponding viable counts which were too low for such reductions to occur. Thus the reduction was largely abiotic due to manganese in the effluent.

Reduction in CFU was also observed in phosphate buffer containing either Cr(VI) or other metal salts along with chromium. Chromate reduction was higher in phosphate buffer containing only chromium. This may be because of a physiological pH provided by the buffer that allowed the cells to survive. Release of accumulated Cr(VI) was observed in multiple metal effluent solutions prepared in phosphate buffer as well as in distilled water on account of cell lysis or even due to efflux. Anions (e.g. chromate) also play a negative role in the adsorption phenomenon.(Ajmal *et al*.,1993). Thus some efflux was also noted in the effluent containing only chromate in both distilled water as well as phosphate buffer. It was indicated by Alvarez *et al*.(1999), that decreased chromate uptake by resistant cells may be either by an efflux system, by a blockage in chromate uptake, or by both processes.

Conclusion :

Many have speculated and have even showed that a correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be located closely together.(Spain, 2003). Calomiris *et al*. (1984), noted a positive correlation between tolerance to high levels of Cu^{2+}, Pb^{2+}, and Zn^{2+} and multiple antibiotic resistance among bacteria from distribution waters but not detected in bacteria from raw waters. Similarly toxicity shown by multiple metals is more than single metal, however some abiotic detoxification also may be noted because of metal – metal interactions.

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