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Bacterial chromate uptake by chemoautotrophic and myxotrophic free and embedded cells in enrichments obtained from rock samples

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ABSTRACT

Rocks, mineral ores, sediments or soil are often associated with inorganic matter such as metals. Thus inherent organisms are exposed to the toxicity of many of these metals. Chromate uptake was observed in enrichment cultures. Since organisms do not exist in natural habitat as pure culture, therefore the study was conducted in native consortia. Almost 30% of the chemoautotrophic enrichments did not show any chromate uptake. The chromate uptake was 10 % or less in 23% of the samples. The highest uptake was 34% at 50 µg/ml (phosphate buffer pH 6.8) in the enrichment obtained from haematite. Only 6% of the enrichments showed uptake less than 1% or no uptake at all at 100µg/ml of chromate. The uptake at 100µg/ml in the enrichment obtained from haematite was further reduced by about 50% when compared with 50 µg/ml chromate uptake. The uptake in myxotrophic free living cells in enrichments ranged from 17 to 96%. The highest uptake in myxotrophic biofilms on glass coupons was found to be 56% at 50µg/ml chromate in phosphate buffer pH 6.8.

Key words: Chemoautotrophic, myxotrophic, bacteria, biofilms, chromate.

INTRODUCTION

Heavy metals have a great ecological significance due to their toxicity and accumulative behavior [1]. The designation autotroph means "self feeding." These are able to grow on a medium that is free of a carbon source . Lithotrophic means "rock eating," and also a term that reflects on the ability of these bacteria to grow in apparently unfavourable environments.

Groups of microorganisms that stay together because of exopolymeric materials produced by them have been found to colonise most substrates in surface waters called as biofilms. Autotrophic biofilms are currently the subject of a large number of studies focusing on the regulations of the organic carbon content in shallow environments [2]. Chromium is a toxic metal of widespread industrial use and exists in several oxidation states. [3] of which hexavalent and trivalent chromium occur under highly oxidizing and reducing conditions. Many bacterial strains isolated from natural sources have been found to possess unique properties which make them useful for commercial processes and environmental cleanups [4]. Hexavalent chromium has greater solubility and hence higher toxicity compared to trivalent chromate that is insoluble and far less toxic. Chromate uptake by chemoautotrophic as well as

myxotrophic bacteria associated with indigenous rocks found in Marathwada region, has been investigated in this study.

Methodology followed for chromate bioremediation

Biofilms are a way of life for microorganisms. They grow together and enrich the community using conjugative gene transfers. They also carry out many activities that may not be possible in isolation. Biofilms are characterized by their spatial structure and the heterogeneous distribution of the microorganisms that work together in a fused and lively way. Chemoautotrophic bacteria were enriched from different rock samples, sediments and mineral ore samples. Chromate reduction was studied in these consortia. Chromate uptake in mineral salts medium at different chromate concentration in free living cells as well as in biofilms was studied. The consortia was further grown myxotrophically in biofilms on glass coupons and chromate uptake was noted once again.

MATERIALS AND METHODS

First enrichment of chemoautotrophs

The chemoautotrophs were enriched in a modified mineral salts medium [5] without any carbon source. In all 18 different rock/ore/clay/sediments were powdered using a mortar and pestle and inoculated at 4% concentration in the above mentioned growth media. The flasks were kept at room temperature (40^{o}C) under static conditions for up to 3 months with intermittent addition of 5ml of media every 10 days to supplement for the water loss due to evaporation. Presence of organisms in the enrichment was confirmed by Gram's staining procedure periodically. All the results indicated are averages of samplings done in triplicate.

Chemoautotrophic uptake of chromate

In order to detect chromate uptake by whole cells, 5 ml of the enrichment was transferred aseptically in eppendorf tubes and spun at 10,000 rpm in a preset microcentrifuge. (Bioera). The resultant pellet was washed with sterile saline and transferred to phosphate buffer pH6.8 containing 50 or 100 µg/ml of dichromate and incubated at 30^{o}C for 24-72h along with appropriate controls. Hexavalent chromate was recorded using diphenyl carbazide reagent.[6].

Second enrichment of myxotrophs along with chromate uptake by non growing cells

Myxotrophs were enriched in Glucose mineral salts medium supplemented with 1% yeast extract and incubated at room temperature for 48 to 72 h. The myxotrophic enrichment (5ml) was spun at 10,000 rpm for 10 min. The consortium was washed in sterile water. Myxotrophic chromate uptake at 100µg/ml was recorded in the cell pellet obtained after centrifugation in phosphate buffer (pH 6.8; room temperature) containing 100µg/ml after for 3 days. Since nutrient medium was not provided the cells may be considered as viable but not growing.

Chromate uptake in myxotrophic biofilms

Five ml of the above mentioned enrichment was centrifuged and the pellet after adequate rinsing was transferred to sterile Glucose minimal medium [7] along with presterilised hung glass coupons so as to allow the development of biofilms on the glass coupons. The incubation and biofilm development was allowed for 10 days at 40 ^{o}C.

Chromate uptake with 2 glass coupons containing biofilms was set in parallel sets in phosphate buffer (pH 6.8) containing 50µg/ml and held at 30^{o}C for 24h. Initial chromate in uninnoculated controls were also recorded along with final uptake after 24 h. as before.

RESULTS AND DISCUSSION

Grams staining of the first enrichment was done and recorded in table 1.Since the medium was devoid of any carbon source , the organisms that were enriched were therefore chemoautotrophic in nature. Typical Gram-ve rods were observed in mineral salts medium which may be again indicative of the presence of chemoautotrophs.

Table 1: Grams staining of the enrichment culture after three months of enrichment

Sr. No	Rock /sediment/mineral ore	Grams's character and morphology of chemoautotrophically grown culture.
1	Red oxide	Gram –ve rods and Gram +ve cocci
2	Green sand stone	Gram –ve rods
3	Chalcopyrite	Gram + rods, Gram –ve rods
4	Bauxite	Gram –ve rods
5	Limonite	Gram –ve rods and Gram +ve cocci
6	Friable glauconitic sandstone	Gram –ve rods , Gram +ve cocci
7	Dulomite	Gram –ve rods , Gram +ve cocci
8	China clay	Gram –ve rods, sheathed cells
9	Marble	Gram –ve rods , sheathed cells
10	Laterite	Gram –ve rods , sheathed cells
11	Red white sand stone	Gram –ve rods, Gram +ve cocci, sheathed cells
12	Magnetite	Gram –ve rods, Gram +ve cocci, sheathed cells
13	Quartz muscovite schist	Gram –ve rods , Gram +ve cocci,
14	Green stone	Gram –ve rods , Gram +ve cocci,
15	Yellow sand stone	Gram –ve rods , Gram +ve cocci,
16	Chromite	Gram –ve rods , Gram +ve cocci,
17	Haematite	Gram –ve rods , sheathed cells
18	Fine grained sandstone	Gram –ve rods , Gram +ve cocci,

Table 2: Chemoautotrophic chromate uptake by cell pellet obtained from first enrichment at 50µg/ml in phosphate buffer, pH 6.8

Sr. No for samples used	Rock /sediment/mineral ore	% chromate uptake
1	Red oxide	0.21
2	Green sand stone	7.87
3	Chalcopyrite	1.27
4	Bauxite	14.05
5	Limonite	16.74
6	Friable glauconitic sandstone	0
7	Dulomite	28.71
8	China clay	5.71
9	Marble	0
10	Laterite	0
11	Red white sand stone	0
12	Magnetite	0
13	Quartz muscovite schist	0
14	Green stone	24.12
15	Yellow sand stone	20.17
16	Chromite	12.67
17	Haematite	33.98
18	Fine grained sandstone	0

Table 3: Chemoautotrophic chromate uptake at 100µg/ml in phosphate buffer using consortia

Sr. No for samples used	Rock /sediment/mineral ore	% chromate uptake
1	Red oxide	0.10
2	Green sand stone	3.45
3	Chalcopyrite	0.54
4	Bauxite	6.58
5	Limonite	8.66
6	Friable glauconitic sandstone	0.1
7	Dulomite	15
8	China clay	2.2
9	Marble	0
10	Laterite	0
11	Red white sand stone	0
12	Magnetite	0.1
13	Quartz muscovite schist	0
14	Green stone	12.12
15	Yellow sand stone	10.34
16	Chromite	6.78
17	Haematite	17
18	Fine grained sandstone	0.12

Microbial transformations for different metallic minerals have been reported by Chiong et al. [8] and Lebedeva and Lyalikova [9]. The transformations include redox conversions of inorganic forms, inorganic to organic form and vice versa [10]. Some of these transformations permits the bacteria to augment their tolerance towards heavy metals. The highest chromate uptake was by the enriched consortia obtained from haematite. Almost 30% of the enrichments did not show any chromate uptake. The chromate uptake was 10 % or less in 23% of the samples. The highest uptake was 34% in the enrichment obtained from haematite. Abiotic controls were appropriately set as presence of variable amounts of iron and manganese present in the samples used can also carry out chromate reduction [11].

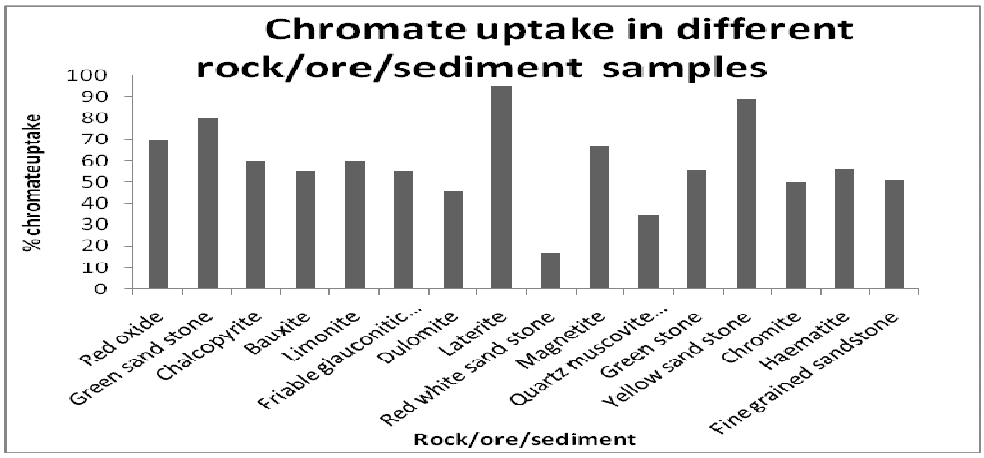
Only 6% of the enrichments showed uptake less than 1% or no uptake at all . The uptake at 100µg/ml in the enrichment obtained from haematite was further reduced by about 50% when compared with 50 µg/ml chromate uptake. Chen and Hao [12], have reported that microbial remediation of high concentrations of Cr(VI) in the environment may be limited by its toxicity. Specific Cr (VI) reduction, unit weight of Cr reduced/unit weight of biomass was greater at higher concentration of chromate [13].

Table 4: Myxotrophic chromate uptake by free living cells at 100µg/ml in phosphate buffer using consortia

Sr. No used	Rock /sediment/mineral ore	% chromate uptake
1	Red oxide	70.12
2	Green sand stone	80.11
3	Chalcopyrite	60.24
4	Bauxite	55.34
5	Limonite	60.12
6	Friable glauconitic sandstone	55.19
7	Dulomite	46
8	Laterite	95.12
9	Red white sand stone	17.08
10	Magnetite	66.78
11	Quartz muscovite schist	34.6
12	Green stone	55.67
13	Yellow sand stone	89
14	Chromite	50.12
15	Haematite	56.33
16	Fine grained sandstone	51.11

The enrichments that carried out some chromate uptake were selected for myxotrophic uptake. The myxotrophic chromate uptake was higher compared to the chemoautotrophic chromate. Since the myxotrophs were provided with glucose and yeast extract, energy output for the organisms was certainly more than in absence of the sugar. It has been reported by Fulladosa et al.[14] that the difference in uptake depending on the presence of different carbon sources could be as a result of differences in bacterial metabolism.

Fig1: Chromate uptake at 100µg/ml in different enrichments obtained from rocks/ores/minerals



The uptake ranged from 17 to 96%. Abiotic uptake if any was deducted from biological uptake and reported. Enrichments from Laterite followed by yellow sand stone and green sand stone were the highest chromate reducers.(Table 4 and Fig 1).

Table 5: Myxotrophic chromate uptake at 50µg/ml in phosphate buffer using biofilms entrapped on glass coupons

Sr. No	Type of sample	% chromate uptake by biofilm on the first glass coupon	% chromate uptake by biofilm on the second glass coupon
1	Red oxide	40.75	68.52
2	Green sand stone	55.56	25.93
3	Chalcopyrite	38.89	50
4	Bauxite	35.19	35.19
5	Limonite	35.19	16.69
6	Friable glauconitic sandstone	38.89	31.49
7	Dulomite	27.78	75.92
8	Laterite	81.48	24.08
9	Red white sand stone	7.41	31.49
10	Magnetite	37.04	25.96
11	Quartz muscovite schist	11.12	22.23
12	Green stone	24.08	20.38
13	Yellow sand stone	53.71	51.86
14	Chromite	20.38	20.4
15	Haematite	27.78	27.8
16	Fine grained sandstone	18.52	19.62

Biofilms are formed by heterogeneous clusters of cells and voids connected by channels [15]. Biofilms on glass coupons showed a comparatively lower colonization and hence a lower uptake compared to free living cells. The uptake was also not identical for 2 glass coupons used and showed considerable variation on account of unequal colonization as in case of the biofilm developed from laterite, Dulomite and green sand stone. However, as the films have been developed on inert surfaces can be reused , the process may be of interest. The highest uptake stood at 56 % as in case of the enrichment obtained from green sand stone. Extracellular polymers of bacteria are composed of polysaccharides, proteins, RNA, DNA and lipids. The material has charged functional groups and has both adsorptive and adhesive properties. They display a high affinity toward certain metal ions thereby causing transport of metal in the environment [16].Whole cell myxotrophic uptake is better and can go up to even 95% at 50µg/ml as in case of the consortium obtained from laterite.

CONCLUSION

Chemoautotrophs on account of their slower growth rate and poor energy output in absence of any added carbon source are therefore not able to produce enough biomass. Added to this was the toxicity of chromate that resulted in the killing of cells and thus a lower uptake has been recorded. Myxotrophs consortia with an average colony count of 10 6 to10 9 / ml were able to carry out chromate reduction more efficiently.

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