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REDUCTION OF CARCINOGENIC HEAVY METALS IN INDUSTRIAL EFFLUENTBY MICROBIAL STRAIN

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ABSTRACT

Bioreduction of carcinogenic heavy metal like Cr-VI to Cr-III is possible by using Microbes like Bacillus Megaterium and Burkholderia isolated from the sludge of electroplating industries effluent contaminated site, Satpur, Maharashtra. Consortium cultures were isolated in aerobic condition and characterized using 16S rRNA partial sequence analysis. Immobilized microbes cell and extracellular enzyme of resultant CRS were found highly effective for bio-reduction of toxic Cr-VI present in effluent of electroplating industry. by at optimized conditions. Bioreduction of Cr-VI is the most promising, eco-friendly and cost effective technology for 100% detoxification of heavy metals in industrial pollutants.

KEYWORDS: CRS, Bioreduction, Microbes, Bacillus Megaterium, Burkholderia Species, Effluent

INTRODUCTION

The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment.

Due to the rapid industrialization and development of industries, wastewaters contaminated with heavy metals are directly or in directly discharged into the environment, especially in developing countries. Due to their elemental and non-biodegradable nature, heavy metals always and regardless of their chemical form, create serious risk, when released into the environment.

Chromium is majorly released into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc. This metal is of major concern because of its larger usages in developing countries and its non degradability nature. (Shankar Congeevaram et al.,2007) Hexavalent chromium is highly soluble in water, highly mobile, mutagenic and carcinogenic to human. Heavy metals, Cr exist in nature in two stable oxidation states Cr-III andCr-(VI) is found highly toxic because of its high mobility and strong oxidizing property.).Cr-(VI) is classified as Class-A carcinogen by US(EPA,HPA2007, COSTA, M 2003) According to the World Health Organization (WHO) the allowable (Thacker, U.et al., 2006, Viti, C et al., 2003, Pal, A et al., 2004) in drinking water is 0.05 mg L^{-1}.There are several strategies have developed to resist chromate mainly through chromate reduction and chromate efflux. The main role of these strategies is to depress chromate toxicity to cells. Hence, chromate-reducing bacteria are able to reduce highly soluble chromate Cr(VI) to thermo dynamically stable and less toxic trivalent chromium Cr(III), (Cheung and Gu, 2007; He et al. 2011).

Inside the cells Cr(VI) is partially reduced to highly unstable Cr(V),Cr(IV) radical, (Sunitha, 2013) which leads to the formation of reactive oxygen species (ROS) and ultimately converted to Cr(III).The process produced reactive oxygen

species(ROS) can damage the DNA(Kokenge Meli 2012).Chromium(III) is rather benign, less mobile, forms water insoluble compounds in aqueous solution and easily absorbed in soils and waters, whereas Cr(VI), which is the toxic form of chromium, is readily adsorbed (Zahoor A et al., 2009). The conventional treatment methods used for this purpose include chemical precipitation, lime coagulation, ion exchange, chemical oxidation, electro dialysis, ultrafiltration and solvent extraction(Ozturk, S.; 2009).However, chemical processes are inefficient, energy intensive and prohibitively expensive and having problem of solid waste management (Natarajan S. et al.,1988).Bioremediation offers an alternative, ecofriendly, economical and effective procedure. A number of mechanisms by which microorganisms tolerate and remove heavy metals have been proposed. Microorganisms modulate metal toxicity by maintaining a low intracellular concentration of toxic metals via extracellular complexation and precipitation(B. Prasenjit et al., 2005) .

As per the present study the immobilized CRScell as well as its extracellular were found potentially efficient to reduce 100% of chromium –(VI) in industrial effluent(Camargo F.A.O et al., 2004).

MATERIALS AND METHODS

Microorganisms Isolation and Characterization

The effluent was collected from a disposal site of electroplating industry MIDC area Nashik, India at weekly intervals for five weeks, pooled together and stored at 4 °C for analysis.

The collected effluent was analyzed for following physicochemical parameters Dissolve oxygen, Biological oxygen demand (BOD) , chemical oxygen demand (COD),CaCo3 alkanility, total hardness, Cadmium, Chromium, Zinc, Choride , total Sulphate, total phosphate, total nitrate, colour, turbidity, pH(Ray,S.,Ray,M.2009).

Table 1: Physiochemical Parameters of Effluent

Sr. No.	Parameters	Results
1	Colour	Yellow
2	pH	7.5
3	DO	22.5 mg/lit
4	BOD	10.5 mg/lit
5	COD	28mg/lit
6	CaCo3	N.D
7	Total hardness	121 mg/lit
8	Cd	N.D
9	Cr	820 mg/lit
10	Zn	885 mg/lit
11	Cl^-	810 mg/lit
12	Sulphate	625 mg/lit
13	Sulphite	N.D
14	Total phosphate	0.01 mg/lit
15	Nitrate	< 1 mg/lit

To isolate chromium resistant bacteria 1 gm. Sample of effluent contaminated sludge from electroplating industry was mixed in 50 mL of the sterile distilled water (R.S.Baiandetal., 2001). Diluted sample of this solution was spread on agar-agar nutrient plates. The growth of two different type of bacterial colonieswereobservedafter24hofincubationatroom temp they were sub culture on nutrient agar plate. Isolated colonies were inoculated and spread on separate agar-agar nutrient plates to get isolated CRS. Fresh inoculums from overnight culture of Selected strains were characterized morphologically, biochemically, and physiologically by 16 S rRNA sequencingas Bacillus Megaterium and. Burkholderia sp.

Table 2: Biochemical Characters Table

S. No	Biological Characters	Bacillus sp	Burkholderia sp.
1	Indol test	+ve	-ve
2	Methyl Red test	-ve	-ve
3	Voges-Proskauer test	-ve	-ve
4	Citrate test	+ve	+ve
5	Mannitol fermentation	+ve	-ve
6	Starch Hydrolysis	+ve	-ve
7	(EMB)	-ve	+ve
8	Urease test	+ve	-ve
9	Catalase	+ve	+ve
10	oxidase	-ve	+ve
11	Nitrate reductase	+ve	+ve
12	Mc ConckyTest	-ve	-ve

Table 3: Morphological Characters

S. No	Morphological Characters	Bacillus sp.	Burkholderia sp
1	Colony Shape	Circular	circular
2	Colony color	white	yellow
3	Colony elevation	Convex	convex
4	Colony margin	Rod	rods
5	Gram character	+ve	-ve

Optimization of Growth Media

Fresh overnight inoculums of both the CRS were supplemented on various type of nutrient media prepared by using different type of Carbon sources and Nitrogen Sources to get various combinations and allowed to grow for 48 hr. Growth of both the CRS were occur on various type of nutrient agar plate growth media for particular species has been optimized by colony count method.

Table 4: Carbon and Nitrogen Sources Taken

S. No	Source of Protein	Source of Glucose			
1	Peptone –(P)	Glucose- (G)	Beef Extract-(B)	NaCl - (NC)	Agar agar powder (AA)
2	Peptone-(P)	Sucrose-(Su)			
3	Peptone-(P)	Starch –(S)			
4	NH_4Cl –(N)	Glucose-(G)			
5	NH_4Cl –(N)	Sucrose-(Su)			
6	NH_4Cl –(N)	Starch-(S)			
7	Urea –(U)	Glucose –(S)			
8	Urea –(U)	Sucrose-(Su)			
9	Urea –(U)	Starch-(S)			

Table 5: Various Combinations of Carbon and Nitrogen Source for Optimization of Growth Media

S No	Combinations for Bacillus spe.	CFU for Bacillus spe	Combinations for Burkholderia spe.	CFU for Burkholderia spe
1	P-G-B-NC-AA	$10.8x10^7$	P-G-B-NC-AA	$47x10^6$
2	P-Su-B-NC-AA	$3X10^7$	P-Su-B-NC-AA	$16x10^6$
3	P-S-B-NC-AA	$11.5x10^7$	P-S-B-NC-AA	$13x10^6$
4	N-G-B-NC-AA	00	N-G-B-NC-AA	$7x10^6$
5	N-Su-B-NC-AA	00	N-Su-B-NC-AA	$22x10^6$
6	N-S -B-NC-AA	00	N-S-B-NC-AA	$2x10^6$
7	U-G-B-NC-AA	00	U-G-B-NC-AA	$9x10^6$
8	U-Su -B-NC-AA	00	U-Su-B-NC-AA	$2x10^6$
9	U-S-B-NC-AA	00	U-S -B-NC-AA	$7x10^6$

Immobilization of CRS Cell and Enzyme

Chromate reducing strain were allowed to grow in nutrient broth for 48hr. Turbid broth samples containing mass of microbes were centrifuged for 2 hr. at 500 rpm. to get pallets of microbes cell then separated from supernant containing extracellular enzyme (Chandraraj Krishnan et al.,2009)secreted by microbes. Enzyme was purified by ammonium sulphate precipitation method. For immobilization of both CRS cell and enzyme, sodium alginate was dissolved in flask containing100 ml of water and then stirred uniformly to give uniform solutions of sodium alginate.

The pallet of CRS cell as well as purified enzyme of Burkholderia sp. and Bacillus species were mixed in separate solutions of sodium alginate and mixtures were drop in the independent conical flasks containing solution of calcium chloride to get beads containing microbes. Beads were store in 1% CaCl2.in separate flasks.

Effluent of plating industry, containing Cr-(VI) was diluted with distilled water to get 100ppm concentration of Cr(VI).

50 ml of stock solution was taken in four 250ml conical flask. 50gm.of beads containing microbescell, and enzymes of were put in each solution. The solutions were kept at room temperature and periodic metal uptakes were analyzed for Cr-VI by UV-Visible spectrophotometer at λmax=540nm.Condition for pH for effective uptake of toxic Cr-VI was optimized by adjusting pH from highly acidic range to basic range by drop wise addition of1N NaOH (Todd R. Sandrin et al., 2003). Condition for concentration for effective uptake of toxic Cr-VI was optimized by examining effluent with various concentrations as 25mg/l, 50mg/l, 75mg/l and 100mg /l.(M. Vidali 2001).

RESULTS AND DISCUSSIONS

Results of reduction of toxic Cr-VI were studied for both CRS by using immobilized microbes cell and Enzyme with respective period, pH of solution, Concentration of solution, and e-donors on Effluent.(Adel M et al.,2002)

Effect of Time

Diluted effluent containing 100 mg Cr-VI was analyzed after every24 hr. for reduction of toxic Cr-VI with respect to time by both the encapsulated microbes cells and encapsulated enzymes ofthe CRS Bacillus megaterium by using UV-Visible spectrophotometer at λmax=540 .Both encapsulated microbes cells and encapsulated enzymes ofthe CRS were highly effective to reduced 100% toxic Cr-VI from effluent within 144 hr. Bioreduction by both Encapsulated Microbes cells Encapsulated Microbes and enzyme of CRS-Burkholderia sp. were found efficient to reduse CrVI from effluent.

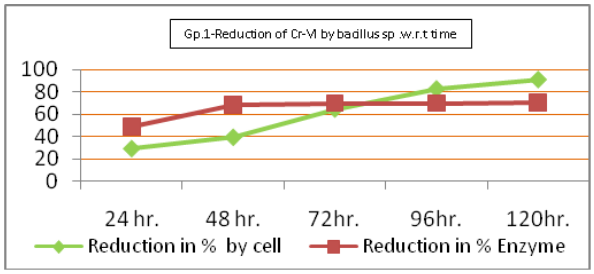

Figure 1: Reduction of Cr-VI by Bacillus sp w. r. t Time

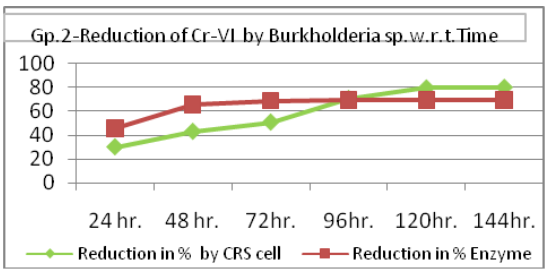

Figure 2: Reduction of Cr-VI by Burkholderia w. r. t Time

Effect of pH

Bioreduction of Cr-VI was highly affected by pH of the solution At high pH Cr-VI is in at precepited form and not avilable for bioreduction.pH of solution varied from acidicto basic range by dropwise addition of NaOHsolution.It was

observed rate of bioreduction was high at acidic pH than basic pH. And rate of bioreduction was more in acidic pH. It was maximum at pH-5.

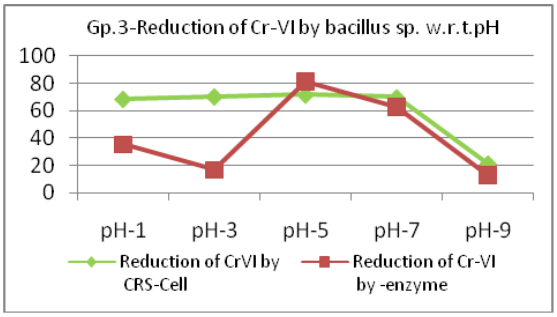

Figure 3: Reduction of Cr-VI by Bacillus sp .w. r. t pH

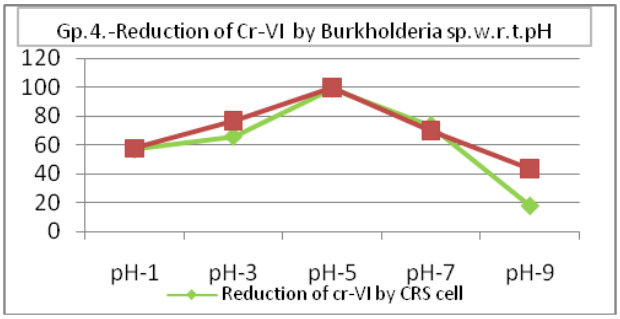

Figure 4: Reduction of Cr-VI by Burkholderia .w. r. t pH

Effect of Concentration

Stock solution of effluent consisting 100mg of toxic Cr-VI per lit was dilluted by distilled water for various oncentrations, that are 25%, 50%, 75%and100% analysis has been done after 48 hr for both ,encapsulaed CRS cell and enzyme of by using UV-Visible spectrophotometer at λmax=540 for Bacillus megaterium and Burkholderia sp. During analysisit was observed rate of bioreduction was high for 25% dilution.

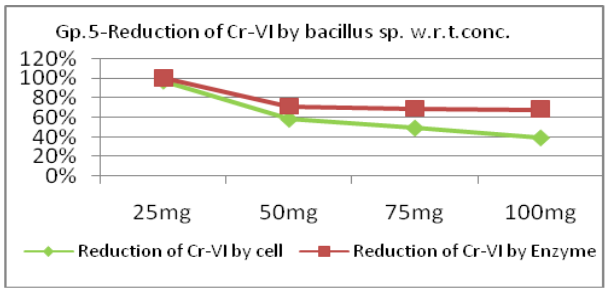

Figure 5: Reduction of Cr-VI by Bacillus. sp. w. r. t Concentration

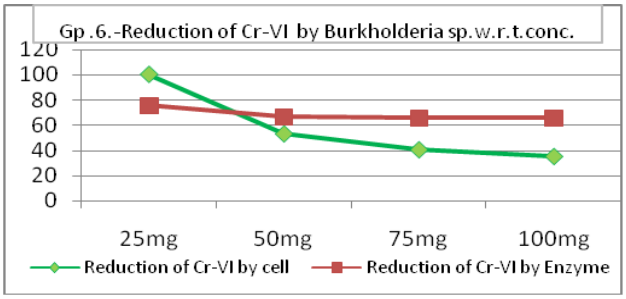

Figure 6: Reduction of Cr-VI by Bacillus sp. w. r. t Concentration

Effect of Electron Donor

250ml conical flask of effluent consisting 100mg of Cr-VI and CRS were supplimented with various e- donors like glucose, molasses, glycerol, succinate and starch by addition of 1% by volume to the flask.Flask was kept undisturbed for 48 hr.Uptake of Cr-VI was observedby using UV- Visible spectrophotometer at λmax=540 for both encapsulated CRS cells and enzymes ofthe Bacillus megaterium and Burkholderia sp. It was observed rate of bioreduction was increased with the addition of electron donor.

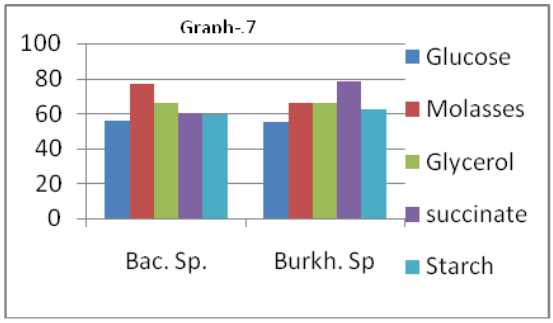

Figure 7: Effect of E- Donor on Bioreduction of Toxic Crvi by Bac. Sp, Burkh. Sp. by microbes Cell .

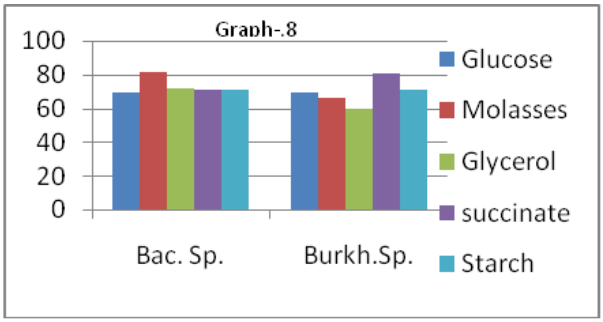

Figure 8: Effect of E- Donor on Bioreduction of Toxic Crvi by Bac. Sp, Burkh. Sp. Bymicrobes Enzyme

CONCLUSIONS

Conventional technologies to clean up heavy metals ions from the contaminated waste have been utilized but these technologies are not cost effective, having major problem of solid waste disposal and alternating to these more expensive technologies are the bioremediation methods which are eco -friendly, inexpensive and safe by which toxic Cr-VI could convert into its non toxic trivalent form and could reduce100% Cr-VI with in a week even in high acidic condition. Industrial effluent was treated with two types of isolated chromium resistant strain by encapsulated cells and encapsulated enzyme method at normal temperature condition. It is observed that bioreduction of toxic Cr-VI is more speedily by encapsulated enzyme method than encapsulated cells method at pH-5 .It is also observed that 100 % bioreduction is possible by encapsulated CRS cells method with in 144 hr by CRS **Bacillus megaterium** Where as by **Burkholderia species** can reduce-80%of Cr-VI. CRS enzyme method can reduced Cr-VI till 70 %by **Bacillus megaterium** as well as by **Burkholderia species** in 96 hrs. at pH-5. Uptake of Cr-VI can be enhance along with dilution. Rate of reduion of Cr-VI also can increased by 35-40% with addition of electron donor.

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