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Bioremediation of Toxic Cr- VI from Effluent of Electroplating Industries by Immobilized Microbes

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ABSTRACT

Microbes like CRS *StaphylococcusSeLB4* and *Bacillus megaterium* isolated from the sludge of Electroplating industry Satpur Nashik, Maharashtra are found highly effective for bioreduction of carcinogenic Cr(VI) present in effluent of electroplating Industry. In present study investigation and optimization of conditions for effective bioremediation of Chromium-VI from electroplating industrial effluent has been done. Bioremediation is the most promising, eco-friendly and cost effective technology widely used now a days for detoxification of toxic industrial pollutants and soil.

Key words: Encapsulated microbes cell, encapsulated microbes enzyme, CRS, *Bacillus Megaterium*, *Staphylococcus*

1. INTRODUCTION

Hexavalent chromium is a very dangerous carcinogen, oxidizing agent and mutagen listed as class A human carcinogen by the US-EPA It is released into the environment from many industrial processes including electroplating, leather tanning, dye and pigment manufacturing, wood treatment, textile dyeing steel and alloy industries Inside the cells Cr (VI) is partially reduced to highly unstable Cr(V) radical, which leads to the formation of reactive oxygen species (ROS) (Kokenge Meli, 2009). Wastewaters from these industries possess several toxic effects to life forms and the environment. Accumulation of these toxic metals in human has several adverse actions such as growth and developmental abnormalities, carcinogenesis, mental retardation . Various plants and biomasses have received attraction because of their heavy metal tolerance capacities which is potentially efficient and eco friendly

strategy for uptake of heavy metals. Toxic heavy metals can be degrade by the enzymes of plants (Malode S.N. et al., 2013). *Marinobactor Bryozoorun AY-17* can be used for degradation of organic compounds like azo dye (Shartate R.S. et al., 2013). Aquatic plants like water hyacinth found very effective for bioaccumulation of heavy metal (Ruchi Dubey et al., 2013). 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 and soluble (Zahoor and Rehman, 2009, H. Shen et al., 1994). Subsequently, bioreduction of Cr(VI) to Cr(III) is an effective way of combating Cr(VI) pollution and is the most promising practice with proved expediency in bioremediation (Sarangi and Krishnan, 2008). Bioreduction of Cr(VI) has been shown by several bacterial species like *Bravibacterium species* (Mohammad Faisal et al.,2000), *Pseudomonas Putina* (Deepali, 2011), *Achromobacter sp.* (Wani et al. 2007) and others (Viti et al. 2003; Pal and Paul, 2004; Thacker et al. 2006; Sultan and Hasnain, 2007; Sarangi and Krishnan, 2008). etc According to the World Health Organization (WHO) the allowable concentration of Cr(VI) in drinking water is 0.05 mg L^{-1} . Thus, it is essential to reduce Cr (VI) concentrations from water/wastewater to acceptable levels.

The conventional treatment methods used for this purpose include chemical precipitation, lime coagulation, ion exchange, chemical oxidation, electro dialysis, ultra filtration and solvent extraction (Sultan, S. and Hasnain, S. 2007). However, chemical processes are inefficient, energy intensive and prohibitively expensive (Camargo, F. A. O. et al. 2004) Bioremediation offers an alternative, eco friendly, economical and effective procedure which can be successfully used. (Natarajan S et al., 1988). Heavy metal waste management by bacteria is getting increased attention due to its efficient, affordable, and environmentally friendly advantages (Ozturk et al. 2009). Bioreduction of Cr (VI) has been demonstrated in several bacterial species. Effluent of electroplating industries is having pH range 4-8. Bacillus species reduces Cr(VI) in acidic pH (Pradnya Raut and R.S. Saler. 2013). Extracellular enzyme secreted by CRS can be used for effective reduction of Cr(VI) (Pradnya Ingle and R.S. Saler. 2014). In acidic media most of the bacterial species are unable to sustain. In present study bioremediation of toxic Cr(VI) has been demonstrated by chromate reducing microorganism in acidic condition.

2. MATERIALS AND METHODS

2.1. Microorganisms isolation and Characterization

The effluent samples containing Cr(VI) were collected from a disposal sites of varies electroplating industry MIDC area Nasik, 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, namely Dissolve oxygen, Biological oxygen demand (BOD), chemical oxygen demand (COD), CaCo3 alkalinity, total hardness, Cadmium, Chromium, Zinc, Chloride , total Sulphate, total phosphate, total nitrate, color, turbidity, pH (Gupta et al., 2009)To isolate the chromium resistant bacteria, 1 gm. Sample of effluent contaminated sludge of electroplating industry was mixed in 50 mL of the sterile distilled water(Ray S. et al., 2009). Diluted sample of this solution was spread on agar-agar nutrient plates. The growth of two bacterial colonies were observed after 24 hours of incubation at room temp. They were subculture on nutrient agar plate. Isolated colonies were inoculated and spread on separate agar-agar nutrient plates to get isolated colonies. Fresh inoculums from overnight culture of Selected strains were characterized morphologically, biochemically, and physiologically by 16 S rRNA sequencing as *StaphylococcusSeLB4, Bacillus Megaterium*.

2.2. Optimization of Growth Media

Fresh overnight inoculums of both the CRS were supplemented on various types of nutrient media prepared by using different type of Carbon sources and Nitrogen Sources (Table 1) to get various combinations and allowed to grow for 48 hours. Growth of both the CRS was occurring on various types of nutrient agar plate's growth media for particular species has been optimized by colony count method (Table 2). The strains were allowed to grow in nutrient broth for 48 hr. to get enough mass of strains. Broth containing cultures were centrifuged at 200rpm for 30 min to get pallet of strains.

2.3. Encapsulation of CRS

2.3.1. Encapsulation of CRS-Cell

Turbid broth samples containing mass of microbes were centrifuged for 2 hr. at 200 rpm. To get pallets of microbes. 2.5 gm. of sodium alginate was dissolved in each of two flask containing 100 ml of water and then stirred uniformly to give uniform solutions of sodium alginate. Both the flasks were labeled as F-1 and F-2. The pallet of *StaphylococcusSeLB4,* was mixed in solution labeled as F-1 and the pallet of *Bacillus megaterium* was mixed in solution labeled as F-2 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 two different flask.

Table 1
Various Carbon and Nitrogen sources

S.N	Source of Nitrogen	Source of Glucose			
1	Peptone –(P)	Glucose- (G)			
2	Peptone-(P)	Sucrose-(Su)			
3	Peptone-(P)	Starch –(S)			
4	NH_4Cl –(N)	Glucose-(G)			
5	NH_4Cl –(N)	Sucrose-(Su)	Beef Extract-(B)	NaCl - (NC)	Agar agar powder A.A
6	NH_4Cl –(N)	Starch-(S)			
7	Urea –(U)	Glucose –(S)			
8	Urea –(U)	Sucrose-(Su)			
9	Urea –(U)	Starch-(S)			

Table 2
Various combinations of carbon and nitrogen source for optimization of growth media

S N	Combinations for *Bacillus sp*	CFU	Combinations for *Staphylococcus sp*	CFU
1	P-G-B-NC-AA	$10.8x10^7$	P-G-B-NC-AA	$22x10^6$
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	$4x10^6$
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	9x106
8	U-Su -B-NC-AA	00	U-Su-B-NC-AA	2x106
9	U-S-B-NC-AA	00	U-S -B-NC-AA	$7x10^6$

2.3.2. Encapsulation of CRS- Enzyme

During growth microorganism secrets extracellular enzymes in the broth. After separation of microbes from broth, enzyme concentration and purification has been done by ammonium sulphate precipitization method. Precipitated enzyme dissolved is suitable buffer and enzyme encapsulation has been done by sodium alginate and CaCl2 and bead were store in the flask labeled asF-3 andF-4 containing 1% $CaCl_2$.

2.4. Preparation of stock solution

Effluent was collected from selected electroplating industries and diluted to get concentration of Cr(VI) 100 mg/lit.50 ml of stock solution was taken in two separate 250ml conical flasks. Every solution was enriched by 50gm. of beads. The solutions were incubated at room temperature and periodic uptakes of Cr (VI) were analyzed after every 24 hours by UV-Visible spectrophotometer.

2.5. Optimization of Conditions

For effective bioreduction of Cr (VI), pH condition was optimized by analyzing Cr(VI) reduction from effluent samples of various pH range. Variation of pH from highly acidic range to basic range has been made by drop wise addition of 1N NaOH and 1N H_2SO_4. Optimization of Initial Cr(VI) concentration present in effluent has been done by studying

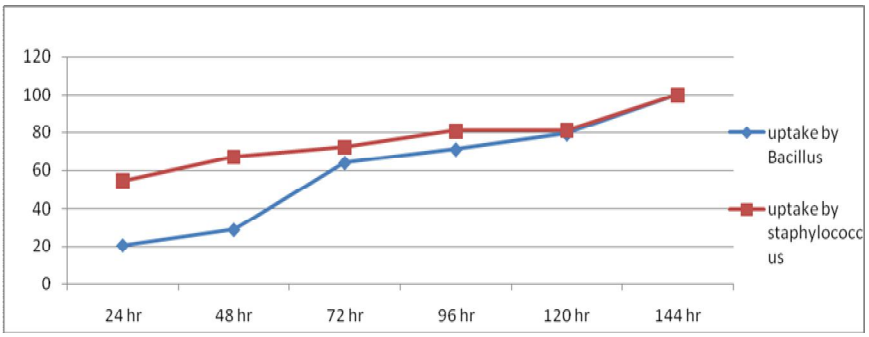

Figure 1

Bioreduction of toxic Cr(VI) *by B. megaterium and st aphylococcusSeLB4* (w.r.t.time) With Microbes – cell(% Reduction Vs Time.)

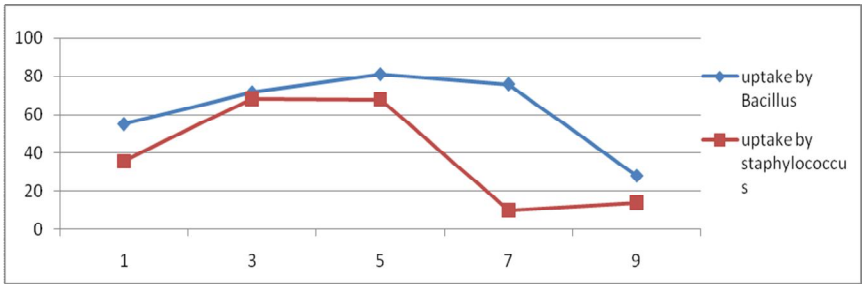

Figure 2

Bioreduction of Cr(VI) by *B.megaterium and StaphylococcusSeLB4* (w.r.t.pH) With Microbes – cell (% Reduction Vs pH)

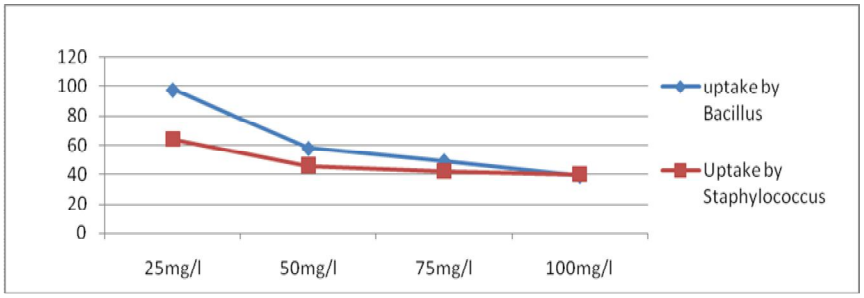

Figure 3

Bioreduction of toxic Cr(VI) *by B. Megaterium and StaphylococcusSeLB4* (w.r.t.conc.) With Microbes –cell (% Reduction Vs Conc.)

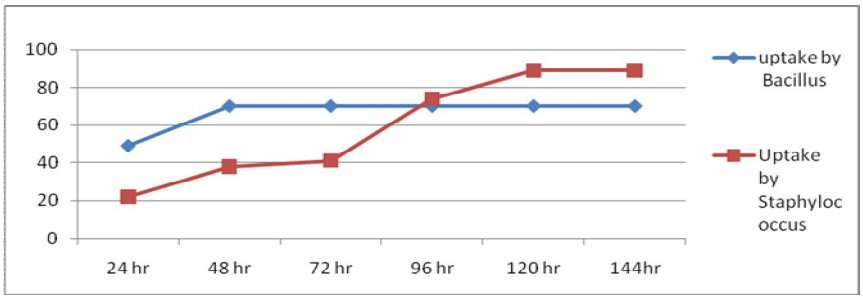

Figure 4

Bioreduction of toxic Cr(VI) *byB. Megaterium and StaphylococcusSeLB4* (w.r.t.time) with enzyme beads (% Reduction Vs Time)

reduction of Cr(VI) from various concentrated effluent samples such as 25 ppm , 50 ppm, 75ppm and 100 ppm. Concentrations of Cr(VI) from effluent samples were varied by diluting it by distilled water.

3. RESULT AND DISCUSSION

3.1. Results by Microbes cell Encapsulation Method

Both the isolated, encapsulated cells ofChromium reduction srtain -*StaphylococcusSeLB4* and *Bacillus megaterium* were able to reduse Cr(VI) from solution. Reduction of Cr(VI) was analysed with respect to time, pH and concentration.

3.1.1. Effect of Time

20 gm encapsulated microbes cells were added in the flask containingCr(VI)and allowed to bioreduction of Cr(VI) .Bioreduction of Cr(VI) was observed after every 24 hr.by UV.Visible spectrophotometer at λmax=540nm. Both the specie of CRS- *StaphylococcusSeLB4* and *Bacillus megaterium* reduced 100% toxic Cr(VI) from effluent within 144 hours as shown in Figure 1.

3.1.2. Effect of pH

pH of effluent was increases from acidic range to basic range by drop wise addition of 1 N NaOH .CRS were allowed to biodegrade Cr(VI) at different pH for 96 hr. It was observed that at pH 5, bioreductions of Cr VI was maximum by both the microbes species as shown in Figure 2.

3.1.3. Effect of concentration of effluent

Effluent was diluted to achieve various concentration of solution like 25mg/l, 50mg/l, 75mg/l and 100mg/l Uptake of Cr(VI) was observed for all concentrations. It was observed that uptake of Cr(VI) was increased with dilution by encapsulated microbes cell of both the organism as shown is Figure 3.

3.2. Results by Microbes Enzyme Encapsulation Method

Encapsulated enzymes of Both the isolated, Chromium reduction srtain -*StaphylococcusSeLB4* and *Bacillus megaterium* were able to redusc Cr(VI) from solution. Bioreduction of Cr(VI) by enzymes of CRS was analysed with respect to time , pH and concentration.

3.2.1. Effect of Time

Bioreduction of Cr(VI) was investigated by appling enzyme encapsulated bead on solution containing Cr(VI).Both the specie of CRS- *StaphylococcusSeLB4* and *Bacillus megaterium* allowed to biodegrade Cr(VI). Uptake of Cr(VI) was observed after every 24 hr. Bioreduction of Cr(VI) was 70% from effluent with in 96 hr. after that concentration remain constant as shown in Figure 4.

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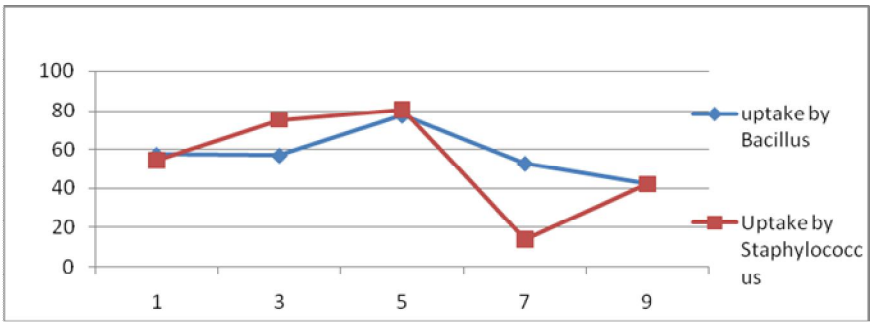

Figure 5

Bioreduction of toxic Cr(VI) *byB. megaterium and StaphylococcusSeLB4* (w.r.t.pH) with enzyme beads(% Reduction Vs pH)

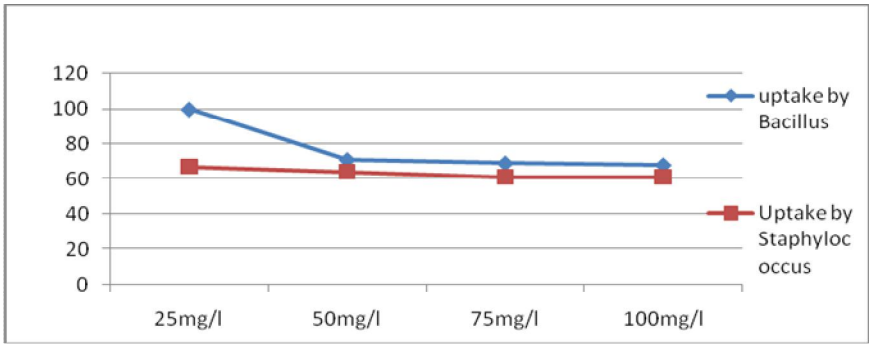

Figure 6

Bioreduction of toxic Cr(VI) byB. megaterium Staphylococcus *SeLB4* (w.r.t.conc.) with enzyme beads(% Reduction Vs Conc.)

3.2.2. Effect of pH

pH of effluent was increases from acidic range to basic range by drop wise addition of 1 N NaOH. CRS were allowed to biodegrade Cr (VI) at different pH for 96 hours. It was observed that at pH 7, bioreduction of Cr (VI) were maximum by both the microorganism as shown in Figure 5.

3.2.3. Effect of concentration of effluent

With dilution rate of Cr(VI) rediuction decreases initially and then become constant. For both the microbes as shown in Figure 6.

4. CONCLUSION

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 reduce hundred percent Cr-VI within a week even in high acidic condition Industrial effluent was treated with in capsulated chromium resistant bacterial isolate strains at normal temperature condition. This resulted in the reduction of 100% diluted effluent by using microbes cell within one week. The process of Cr(VI) reduction is fast by enzyme but up to 70 % within 96 hours at optimized pH-5 and at 25% dilution.

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