

Bioremediation of toxic Cr- VI from effluent of electroplating industries by immobilized microbes

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ABSTRACT

Microbes like CRS *Staphylococcus*SeLB4 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.

Keywords: Chromium; carcinogen; Bioremediation

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 (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 degraded by the enzymes of plants (Malode S.N. et al., 2013). *Marinobacter 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; Zahoor and Rehman, 2009; H. Shen et al., 1994; 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).

The 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). 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. 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). The growth of two bacterial colonies were observed after 24 hours of incubation at room temp. They were subculture on nutrient agar plate.

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)	Beef Extract-(B)	NaCl - (NC)	Agar agar powder A.A
2	Peptone-(P)	Sucrose-(Su)			
3	Peptone-(P)	Starch -(S)			
4	NH ₄ Cl -(N)	Glucose-(G)			
5	NH ₄ Cl -(N)	Sucrose-(Su)			
6	NH ₄ Cl -(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 <i>Bacillus sp</i>	CFU	Combinations for <i>Staphylococcus sp</i>	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	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

2.3.2. Encapsulation of CRS- Enzyme

During growth microorganism secretes extracellular enzymes in the broth. After separation of microbes from broth, enzyme concentration and purification has been done by ammonium sulphate precipitation method. Precipitated enzyme dissolved in suitable buffer and enzyme encapsulation has been done by sodium alginate and CaCl₂ and beads were stored in the flask labeled as F-3 and F-4 containing 1% CaCl₂.

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.

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 H₂SO₄. Optimization of Initial Cr(VI) concentration present in effluent has been done by studying 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 of Chromium reduction strains -*Staphylococcus SeLB4* and *Bacillus megaterium* were able to reduce 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 containing Cr(VI) and allowed to bioreduce Cr(VI). Bioreduction of Cr(VI) was observed after every 24 hr. by UV-Visible spectrophotometer at $\lambda_{max}=540\text{nm}$. Both the species of CRS- *Staphylococcus SeLB4* 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 increased 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 concentrations 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 organisms as shown in Figure 3.

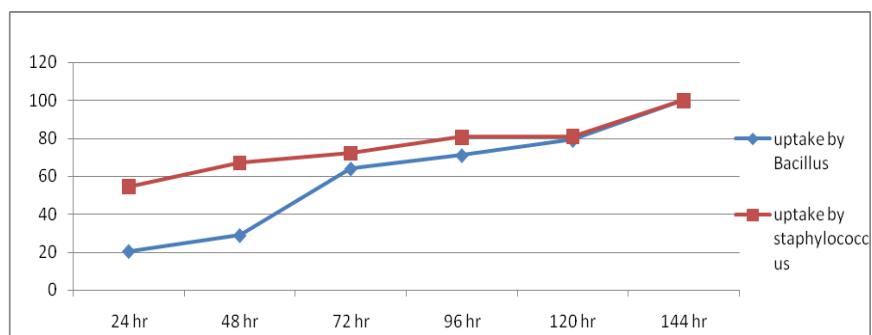


Figure 1

Bioreduction of toxic Cr(VI) by *B. megaterium* and *staphylococcusSeLB4* (w.r.t.time) With Microbes – cell(% Reduction Vs Time.)

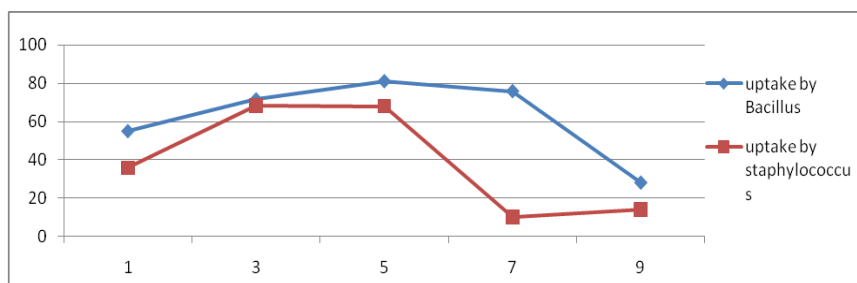


Figure 2

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

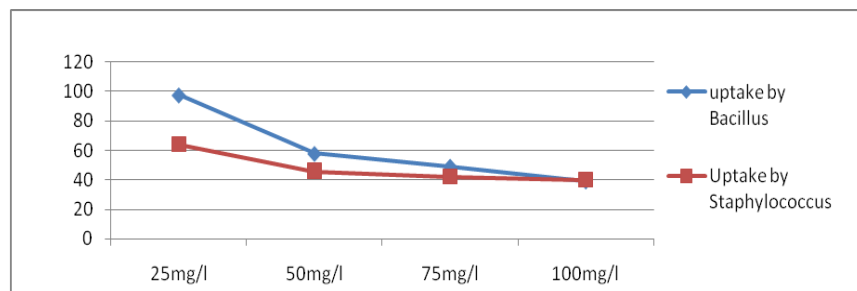


Figure 3

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

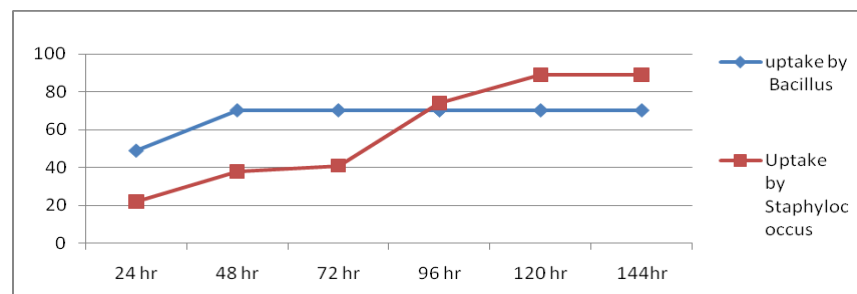


Figure 4

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

3.2. Results by Microbes Enzyme Encapsulation Method

Encapsulated enzymes of Both the isolated, Chromium reduction strain -*StaphylococcusSeLB4* and *Bacillus megaterium* were able to reduce 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 applying enzyme encapsulated bead on solution containing Cr(VI). Both the species of CRS- *Staphylococcus* *SeLB4* 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 within 96 hr. after that concentration remain constant as shown in Figure 4.

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.

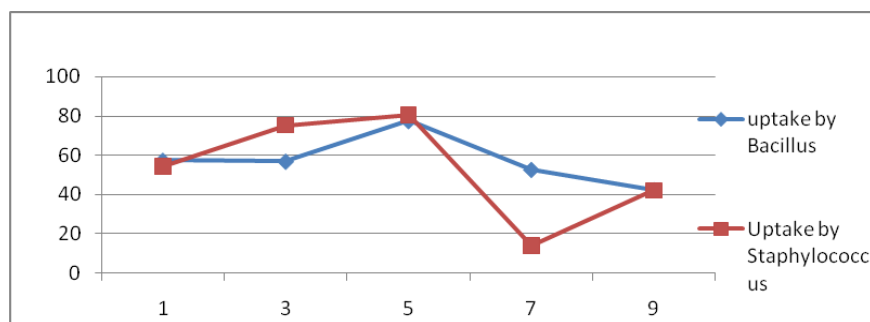


Figure 5

Bioreduction of toxic Cr(VI) by *B. megaterium* and *Staphylococcus* *SeLB4* (w.r.t. pH) with enzyme beads (% Reduction Vs pH)

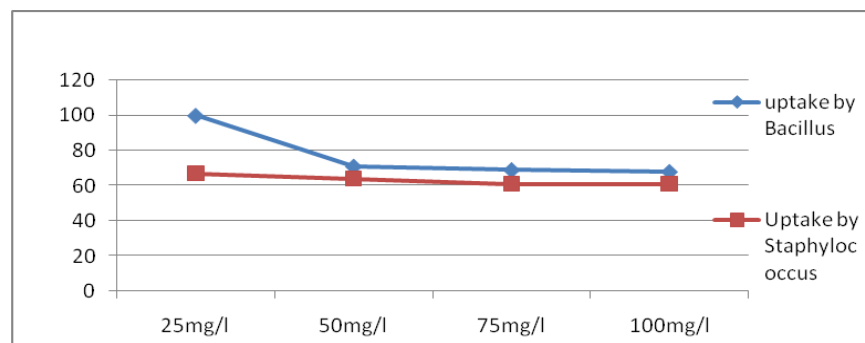


Figure 6

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

3.2.3. Effect of concentration of effluent

With dilution rate of Cr(VI) reduction 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 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. 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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Conflict of interest

The authors declare that they have no conflict of interest.

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Data and materials availability

All data associated with this study are present in the paper.

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