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Application of Cr (VI) Reducing Bacterial Cells and Enzymes for Its Bioremediation

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Abstract

Bioremediation of Cr(VI) pollutant, present in the effluent of electroplating and tannery industries majorly is the most cost-effective eco-friendly, and promising solution to reduce the toxic effect posed on the ecosystem by chromium (VI). Several acidophilic bacterial species have shown great potential to adapt and reduce class-A carcinogen Cr(VI) in the effluent to its nontoxic form Cr(III**).** Immobilized extracellular enzymes secreted by microbes during the development of the mechanism and the microbial cells both were used to accomplish the biological reduction of Cr(VI). All the bacterial species were isolated from the effluent sample and characterized as *Bacillus megaterium C31171* (CRS-W), *Staphylococcus* species *SeLB4* (CRS-Y1), and *Burkholderia species* (CRS-Y2) using 16S rDNA sequencing. Microbes-cells and extracellular enzymes secreted by microbes were separated. The growth of microbial species was monitored for the parameters like growth media and temperature.

Keywords: Bioremediation, Chromate reducing bacterial strains (CRS), Immobilized extracellular enzymes, Chromium (VI), Effluent

1 Introduction

In this era of the industrial revolution, environmental pollution due to the discharge of untreated effluent is one of the enormous problems of the world [1]. The effluent released from electroplating, tannery, wood preservation, textiles, leather, metallurgical, pigments, and dyes industries have led to contamination of primary water bodies and agriculture and can pose a serious risk to the environment [2]. Hence, great emphasis has been given to heavy metal reduction by the affluent as they are highly toxic even at very low concentrations. Chromium in VI oxidation state is classified as a class-A carcinogen [3-4] and so the priority pollutant according to the USEPA and can cause genetic mutation, lung cancer, allergies, skin irritation, respiratory tract disorder, chromosomal damage and tympanic membrane perforation [5-6], etc. Cr(VI) is a highly mobile and soluble ion having the ability to enter the human body by three different routes that are through the gastrointestinal route, through the air, and penetration through the epidermis. The biotoxicity of Cr(VI) is mainly due to its powerful oxidizing property [7] and its ability to cross the biological membranes, [8]. Various treatment technologies have been developed for the removal of toxic heavy metal cations in effluents are found expensive and have the issues like formation of side products and solid waste management. However biological methods like bioremediation are come up as economical and eco-friendly solutions for the reduction of toxic metal ions contaminants [9-10]. In present studies both immobilized extra-cellular enzymes and cells of different microbes were used for the bioremediation of Cr(VI) [11]. Purification and encapsulation of microbe cells and extracellular enzymes have been done using sodium alginate [12] [13]. Cr(VI) bioreduction analysis has been done with the optimization of parameters like the effect of time, pH, initial concentration of Cr (VI), and electron donor [14-15].

2 Materials and Methods

2.1 Collection of samples

Effluent samples were collected from the discharge sites of five different electroplating industries of Ambad and Satpur MIDC, Nashik, Maharashtra India. Samples were stored in plastic sealed bottles at 4^0 C.

2.2 Estimation of toxic metal contaminant Cr(VI)

Cr (VI) present in the effluent samples was confirmed and estimated with the help of atomic absorption spectroscopy and UV-Visible spectroscopy.

2.3 Physico-Chemical analysis of effluent

All the effluent samples were analyzed to study the physicochemical parameters like DO, BOD, COD, Total Hardness, Presence of Sulphate, Sulphite, Nitrate, Cr, Zn, and Cl using standard analytical techniques (APHA) [16].

2.4 Isolation and characterization of Cr (VI) reducing bacteria

Cr(VI) reducing bacterial strains were isolated from the sludge, and collected from the effluent discharge site. 1 gm. A sample of sludge was mixed in 10 ml of sterile distilled water[17]. A diluted sample of this solution was spread on nutrient plates and incubated at 37°C for 24 hours. After the incubation period, the plates were observed for growth on the media. Colonies obtained were picked and purified by many rounds of re-streaking. Out of various microbes samples 03 microbes species, BS-2, BS-4, and BS-7 were found effective for uptake of Cr(VI) from stock solution (100 mg Cr(VI) /liter)

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and they were labeled as per their colors. Fresh inoculums from an overnight culture of selected strains were characterized morphologically, biochemically, and physiologically by 16S rRNA sequencing as *Staphylococcus sp. CRS-Y1, Bacillus megaterium CRS-W and Burkholderia sp. CRS-Y2.* Morphological characterization tests were applied to all the chromate-reducing bacterial strains (CRS).

Table 1: Physico-chemical analysis of effluent

Sr. No.	Parameters	Results
1	pH	7.5
2	DO	22.5 mg/lit
3	BOD	10.5 mg/lit
4	COD	28mg/lit
5	Total hardness	121 mg/lit
6	Cd	Not. Detected
7	Cr	820 mg/lit
8	Zn	885 mg/lit
9	Sulphate	625 mg/lit
10	Sulphite	Not. Detected
11	Total phosphate	0.01 mg/lit
12	Nitrate	< 1 mg/lit

Table 2: Morphological characterization of Chromate reducing bacteria

Sr. No.	*Morphological Characters*	*Staphylococcus*	*Bacillus megaterium*	*Burkholderia*
1	Colony Shape	Circular	Circular	Circular
2	Colony color	Pale yellow	White	Yellow
3	Colony elevation	convex	Convex	Convex
4	Colony margin	Cocci	Rod	Rods
5	Gram character	+ve	+ve	-ve

2.5 Separation and purification of microbial cells and extracellular enzymes

The growth of Microbes cells and enzymes developed the turbidity in the broth solution at the end of incubation. Microbial biomass and enzyme secreted in the medium were separated by centrifuging the broth for half an hour at 2000 rpm to get a pallet of Microbial cells and solution containing enzyme secreted by microbes. To separate and purify the extracellular enzymes secreted, saturation and precipitation of the aqueous solution of an enzyme have been done with ammonium sulfate salt [20].

2.6 Encapsulation of microbial cells and microbial enzymes

Encapsulation of microbe cells and Microbe enzymes has been done using Sodium alginate (2.5 %) in two sets of three flasks (for three types of CRS) containing 100 ml of water each to give uniform solutions of sodium alginate. The purified enzymes of bacterial species and bacterial cells were mixed in sodium alginate solutions taken in the different sets of conical flasks and labeled. Encapsulated beads of microbial enzymes and cells were obtained by dropping the sodium alginate solution with CRs enzymes and CRS cells in the independent conical flask containing $CaCl_2$ solution [18]. Encapsulated beads were stored in a 1% $CaCl_2$ solution.

7. Reduction of hexavalent chromium by bacteria

To determine the capabilities of Cr(VI) reducing bacterial isolates, the diphenylcarbazide method was used [19]. 200mg of 1-5, diphenylcarbazide was dissolved in 100ml of 90% alcohol, and 10% sulphuric acid solution was prepared by adding 40ml of concentrated acid into the 360 ml of distilled water and mixed in the 1-5, diphenylcarbazide solution with continuous stirring, the solution was stored in the refrigerator. Initially, 50 ml of a stock solution containing Cr(VI) was taken in the beaker, and the addition of 1 mL of diphenylcarbazide solution (prepared by dissolving 0.25 g diphenylcarbazide in 100 mL acetone) and 1 drop of H_3PO_4 been done. The mixture was allowed to develop the red-violet color at room temperature for 10 minutes.1-5, diphenylcarbazide formed a red-violet complex, particularly with hexavalent chromium. Absorbance was checked in the UV-Visible spectrophotometer at 540 nm as shown in the figure. Furthermore, two sets of three beakers were filled with 50 ml of stock solutions; the first set was supplemented with 30 gm of three different types of encapsulated microbe **cells** while the second set was supplemented with 30 gm of three different types of encapsulated microbe enzymes. The solutions were kept at room temperature and periodic metal uptakes of Cr(VI) were analyzed by double beam UV-Visible spectrophotometer (Make-Jasco corporation Japan.) at λ_{max} = 540 nm.

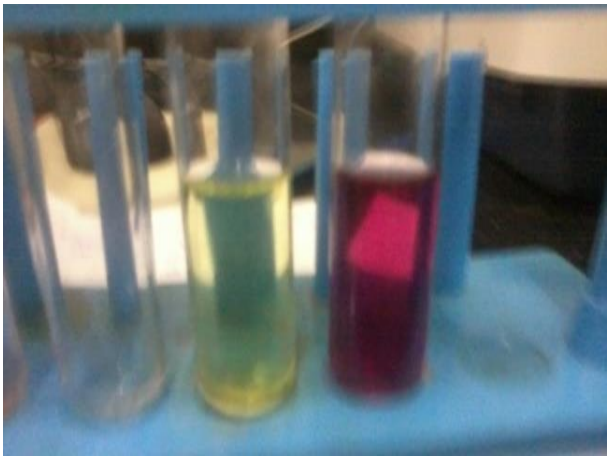

Fig. 1: 1, 5 Diphenyl carbazied complex form by Cr (VI)

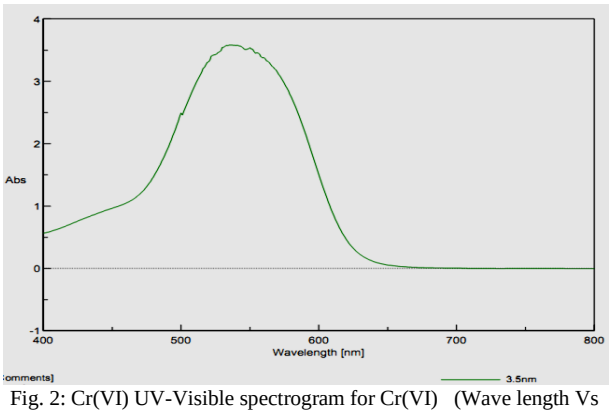

Fig. 2: Cr(VI) UV-Visible spectrogram for Cr(VI) (Wave length Vs Absorption)

2.8 Optimization of Parameters

Effective bioremediation of Cr(VI) has been achieved by optimizing the various bioremediation parameters. The reduction of Cr(VI) was analyzed for time. Time optimization has been done by monitoring the uptake of Cr-VI after every

interval of 24 hours. Suitable pH was optimized at various pH ranges varying pH from acidic to the basic range [21]. 0.1N NaOH and 0.1N H2SO4 solutions were used to vary the pH of effluent samples. Identification of the suitable electron donor for the bioremediation of Cr(VI), has been done by enriching the Cr(VI) containing effluent with various electron donors. Optimization of the initial Cr(VI) concentration present in effluent has been done by studying the reduction of Cr(VI) from effluent samples with various concentrations such as 25 ppm, 50 ppm, 75 ppm, and 100 ppm. Concentrations of Cr(VI) from effluent samples were varied by diluting it with distilled water.

3 Results and Discussions

All the parameters affecting the reduction of chromium (VI) like contact time, pH of the solution, initial concentration of chromium (VI), and e- donors were optimized using sodium alginate beads with biomass cells and sodium alginate beads with biomass enzyme. Solutions were incubated at room temperature and the reduction of chromium (VI) has been analyzed after every 24 hours by UV-Visible spectrophotometer at λmax=540nm by using 1, 5 diphenylcarbazide.

3.1 Time optimization time for Cr (VI) reduction from effluent using Microbe cells and Microbe enzymes

It was observed that as the contact time increases the rate of absorption of chromium (VI) increases.100% Cr(VI) was reduced within 144 hours by all three microbes.

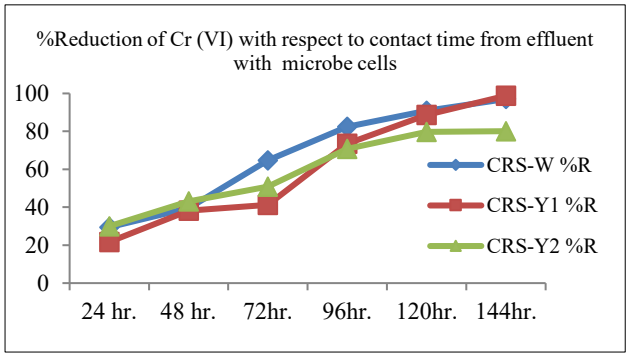

Fig. 3 Reduction of Cr(VI) in effluent w.r.t. time using microbe cells (Time in hours Vs % Reduction of Cr(VI))

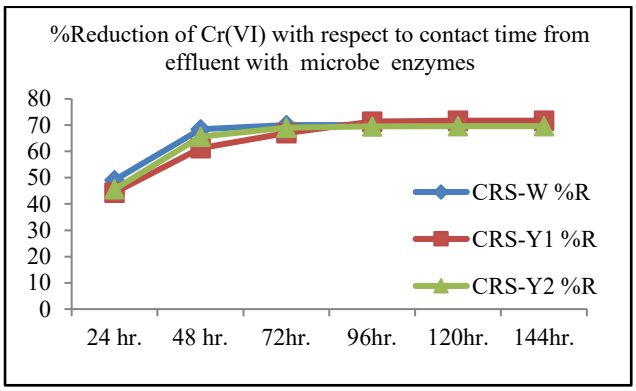

Fig. 4: Reduction of Cr(VI) in effluent w.r.t. time using Microbe enzymes (Time in hours Vs % Reduction of Cr(VI))

3.2 Optimization of pH for effective reduction of Cr(VI) in effluent using Microbe cells and Microbe enzymes

Diluted NaOH was used to vary the pH of effluent stock solution and Chromate Reducing bacterial cells and enzymes were allowed to bioremediate the Cr(VI) at different pHs for a certain time which was 96 hours. The reduction of Cr(VI) in the effluent was analyzed by UV-Visible spectrophotometer at λ max=540nm using 1,5 diphenylcarbazide. Results suggested that the rate of Cr(VI) reduction was maximum at 5 pH.

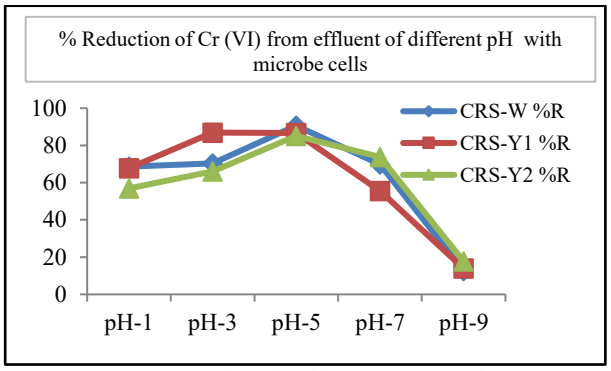

Fig. 5 Optimization of pH for effective reduction of Cr (VI) in effluent using microbe cells (pH Vs % Reduction of Cr (VI))

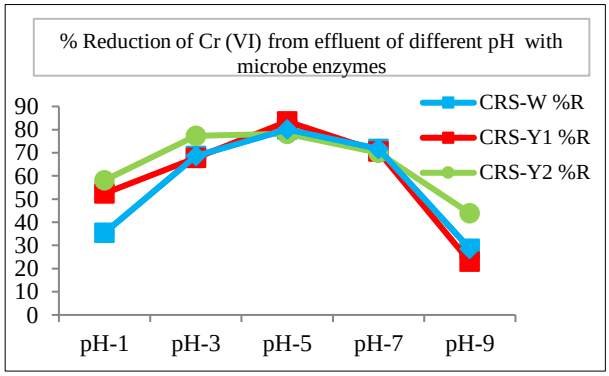

Fig. 6: Optimization of pH for effective reduction of Cr(VI) in effluent using microbe enzymes (pH Vs %Reduction of Cr(VI))

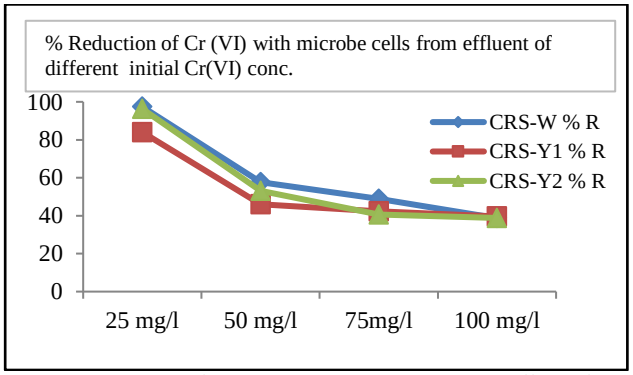

Fig. 7: Optimization of initial Cr(VI) concentration in the effluent for its effective reduction using microbe cells (Initial Conc. Vs % Reduction of Cr(VI)

3.3 Effect of initial Cr(VI) concentration on bioreduction

The effect of the initial concentration of Cr(VI) on its reduction was examined from different concentrated solutions such as 25 mg/l, 50 mg/l, 75 mg/l, and 100mg/l at neutral pH after the contact time of 48 hours by UV-Visible spectrophotometer at λ max=540nm using 1,5 diphenylcarbazide. Results suggested that the chromium (VI) reduction rate was maximum at a lower initial Cr(VI) concentration in the effluent. Both microbe cells, as well as

microbe enzymes, were found efficient to reduce Cr(VI) in effluent up to 100% at a lower initial concentration.

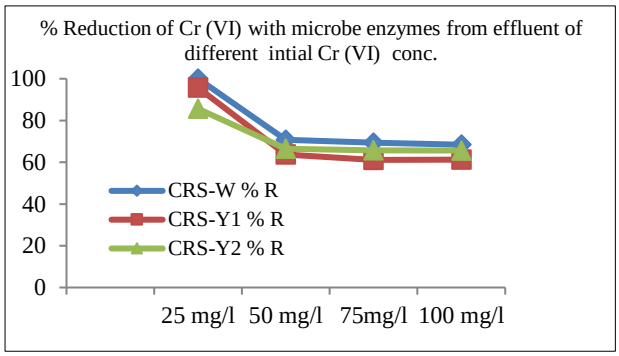

Fig. 8: Optimization of initial Cr (VI) concentration in the effluent for its effective reduction using microbe enzymes (Initial Conc. Vs % Reduction of Cr (VI))

3.4 Effect of electron donor enrichment on bioreduction

Electron donors such as glucose, succinate, glycerol, molasses, and starch were used to enrich the affluent. Chromium (VI) bioremediation results were investigated by using microbe cells and microbe enzymes of all three bacterial strains after the incubation period of 48 hours. CRS-W and CRS-Y1 reduced chromium (VI) effectively from effluent in presence of succinate while CRS-Y2 reduced Cr(VI) effectively in presence of molasses. The rate of Cr(VI) bioreduction was faster using microbe enzymes as compared to Microbe cells.

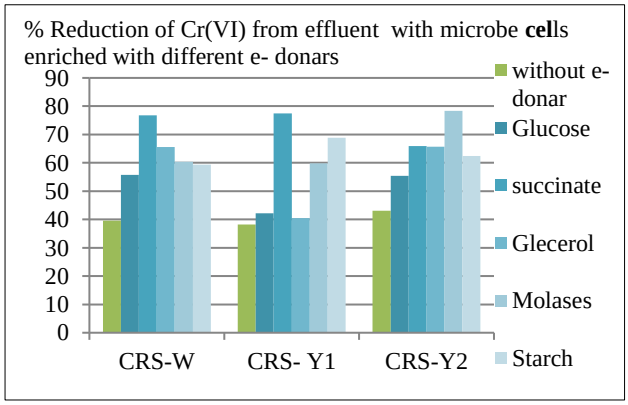

Fig. 9: Effect of e-donor enrichment on Cr(VI) reduction by using microbe cells

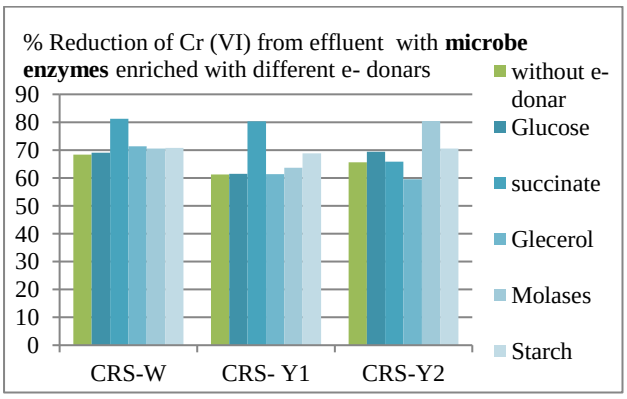

Fig. 10: Effect of e- donor enrichment on Cr(VI) reduction by using microbe enzymes respectively

3.5 Reduction of chromium (VI) from effluent under optimized conditions by using microbe cells and microbe enzymes

Under the optimized conditions of pH, initial concentration, and electron donor, bioreduction of Cr(VI) has been investigated which demonstrated the accomplishment of 100% reduction of chromium (VI) within 48 hrs by Microbe cells and by using microbe enzymes it has been accomplished with is 36 hrs.

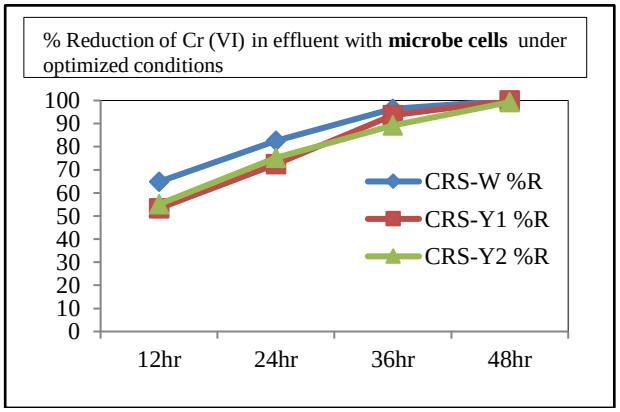

Fig. 11: Bio Reduction of Cr (VI) in effluent with microbe cells under optimized conditions

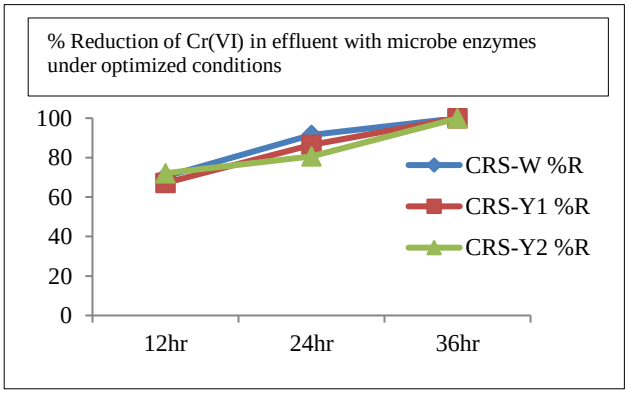

Fig. 12: Bio Reduction of Cr(VI) in effluent with microbe enzymes under optimized conditions

3.6 Reduction Mechanism of Cr(VI) to Cr(III)

Bio reduction of toxic Cr(VI) to Cr (III) in effluent has been accomplished by the extracellular enzymes. Extracellular enzymes secreted by microbes are efficient to converted Cr-VI to Cr-III and forming a complex with EPS (Extracellular polymeric Substance) released in the supernatant by bacteria. The presence of Cr(III) has been confirmed by atomic absorption spectroscopy.

4 Conclusions

1. All the isolated chromate-reducing bacterial strains were found highly efficient for the bioremediation of chromium (VI) from effluent.
2. Bioremediation of chromium (VI) has been increased by 30 % with the addition of suitable electron donors in the effluent samples.
3. Bioremediation of toxic Cr(VI), under the optimized conditions of pH, initial concentration of Cr(VI), and the suitable e- donor has been achieved 100% from effluent

within 48 hours by CRS-W, CRS-Y1, and CRS-Y2 using microbial cells whereas by using microbial enzymes bioremediation time could be reduced up to 36 hours.

4. Microbial enzymes were found more effective in the bioremediation of Cr(VI) as compared to Microbe cells.

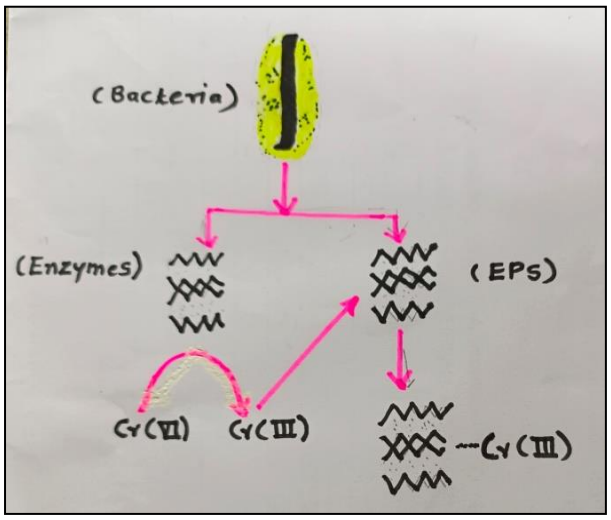

Fig. 13: Extracellular reduction of Cr (VI) to Cr (III)

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Ethical issue

The author is aware of and complies with, best practices in publication ethics specifically about authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. The author adheres to publication requirements that the submitted work is original and has not been published elsewhere in any language.

Author's contribution

The authors of this study have a complete contribution to data collection, data analysis, and manuscript writing.

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