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Treatment to the toxic metal contaminant from effluent with special reference of Cr (VI) using bioremediation techniques

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ABSTRACT

Toxic metal pollutant from industrial effluent can be reduced in to its benign form using most promising green technique; Bioremediation. Industrial effluent contaminated with heavy metals is directly or indirectly discharged into the environment; large quantities of biomaterial from environment have shown efficient removal of toxic metal pollutant during various metabolisms in microorganism. In present study bioremediation of toxic Cr (VI) that is Cr(VI) has been demonstrated by acidophilic chromate reducing microorganism isolated from the sludge of electroplating industry.

KEYWORDS; Bioremediation, Microbes, Chromium(VI), Staphylococcus Species., Bacillus Species. Burkholderia species, CRS-W, CRS-Y1,CRS-Y2.

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INTRODUCTION

Chromium in six oxidation state is highly toxic carcinogen; mutagen and oxidizing agent listed as class A human carcinogen by the US-EPA[3, 12]. It is released into the eco system from various processes of electroplating, leather tanning, textile dyeing, dye and pigment manufacturing, wood treatment, and the steel and alloy industries.

Introduction of toxic metals in human cells has several adverse actions such as growth and developmental abnormalities, carcinogenesis, mental retardation. Inside the cells Cr (VI) undergoes intercellular reduction to generate Cr^{+3} ultimately through the formation of short lived species Cr^{+5},$Cr^{+4.}$ This process produces reactive oxygen species (ROS) can damage the DNA[7]. Cr^{+3} is nonhazardous, less mobile, produces water insoluble compounds in aqueous solution and easily absorbed in soils and waters. According to the World Health Organization (WHO) the allowable Cr (VI) quantity in drinking water is 0.05 mg /L[10]. Thus, it is essential to reduce Cr(VI) concentrations from water/wastewater to acceptable levels[9]. Bioremediation can provide an eco friendly, effective and economical solution for removal of toxic metal contaminant[8].

MATERIAL AND METHODS

The effluent samples containing Cr (VI) were collected from a disposal sites of various electroplating industry MIDC, Nashik, India at weekly intervals for five weeks and stored at 4 °C for analysis. The collected effluent was analyzed for following physico-chemical parameters dissolve oxygen, Biological oxygen demand (BOD), Chemical oxygen demand (COD),$CaCO_3$ alkalinity, total hardness, cadmium, chromium, zinc chloride , total sulphate, total phosphate, total nitrate, color, pH.

Table1: 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	$CaCO_3$	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

1) Microorganisms isolation and Characterization

To isolate the Cr (VI) reducing bacteria, 1 gm. Sample of sludge contaminated with effluent from electroplating industry was mixed in 50 ml of the sterile distilled water[3]. Diluted sample of this solution was spread on agar-agar nutrient plates The growth of nine different type of bacterial colonies were observed after 24 hr. 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 of individual species. Fresh inoculums of all the obtained microbial colonies were further inoculated in to50ml of Lauria Bartini broth in 100 ml conical flask. LB broth samples containing microbes were centrifuged for 30 min at 2000 rpm to get pellets of microbes. Out of 09 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) and they were labeled as per their colors. Fresh inoculums from overnight culture of selected strains were characterized morphologically, biochemically and physiologically by 16 S rRNA sequencing as *Bacillus Sp.c31171* ***(CRS-W)***. *Staphylococcus sp.****(CRS-Y1)*** *and* *Burkholderia sp.****(CRS-Y2)***

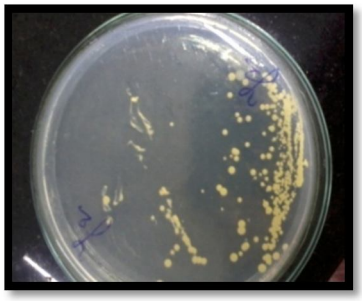


Fig.1 Bacillus Sp.c31171

Fig.2 Staphylococcus sp.

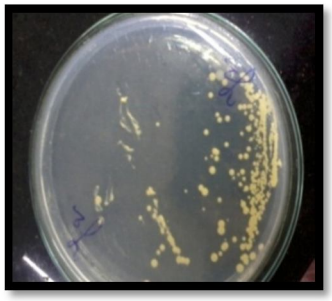


Fig.3 Burkholderia sp.

Table 2:Morphological Characterization

Sr. No.	Morphological Characters	CRS-W	CRS-Y1	CRS-Y2
1	Colony Shape	Circular	circular	circular
2	Colony color	white	Pale yellow	yellow
3	Colony elevation	Convex	convex	convex
4	Colony margin	Rod	cocci	rods
5	Gram character	+ve	+ve	-ve

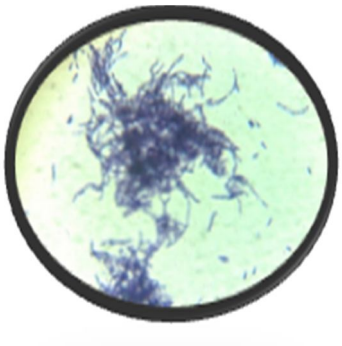


Fig.4 Bacillus sp.

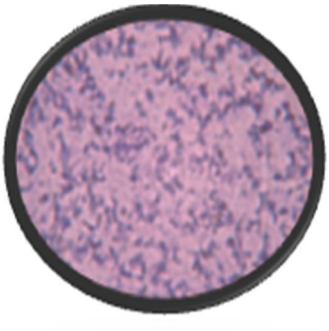


Fig.5 Staphylococcus sp.

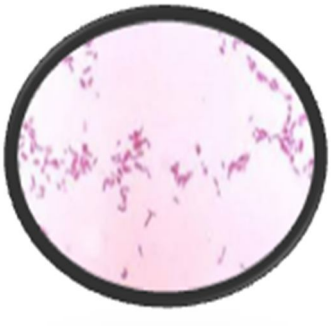


Fig.6 Burkholderia sp

Table 3: Biochemical characterization

Sr. no.	Biological characters	CRS-W	CRS-Y1	CRS-Y2
1	Indol test	+ve	-ve	-ve
2	Methyl Red test	-ve	-ve	-ve
3	Voges-Proskauer test	-ve	-ve	-ve
4	Citrate test	+ve	-ve	+ve
5	Mannitol fermentation	+ve	-ve	-ve
6	Starch Hydrolysis	+ve	+ve	-ve
7	(EMB)	-ve	-ve	-ve
8	Urease test	+ve	-ve	-ve
9	Catalase	+ve	+ve	+ve

2) Separation and purification of microbial cell and extracellular enzymes

Nutrient broth solutions were prepared in three 100 ml conical flasks (50ml each). Three flasks were inoculated with single colony of three different isolated CRS strain and incubated at 35^0C for 48 hours. Turbid broth containing microbes were centrifuged for half an hour at 2000rpm to get pallet of Microbial cell. These cells were separated from supernant containing extracellular enzyme secreted by microbes.

Aqueous solution of an enzyme was saturated with ammonium sulfate salt and was kept in the refrigerator overnight. The precipitate obtained was sediment in refrigerator centrifuged at 10000 rpm to get pallet of precipitate. Furthermore precipitate was dissolved in buffer solution. Using the salting out ammonium precipitate method, solubility of protein was altered. The hydrophobic amino acid molecules from aqueous solution are attracted by the salt ion. If solution is saturated and solubility of protein decreases, as high ionic strength was available in the solution, protein was completely precipitated out.

3) Encapsulation of microbial cell and microbial enzyme

Sodium alginate (2.5 gm) was dissolved in two set of three flasks containing100 ml of water in each and then stirred uniformly to give uniform solutions of sodium alginate. The purified enzyme pallet of *Bacillus sp, StaphylococcusY1 and Burkholderia s.* were mixed in solutions taken in the

conical flasks of Set - 1 labeled as MCF-1, MCF-2 MCF-3 respectively. Similarly wet mass of microbial cell (6% w/v) suspended in another set of flask labeled MEF-1, MEF-2 and MEF-3 respectively. Microbial cell encapsulated beads and microbial enzyme encapsulated beads ofall three species were obtained by dropping the individual's solution in the independent conical flask containing CaCl2 solution. Encapsulated beads were store in 1% CaCl2 solution.

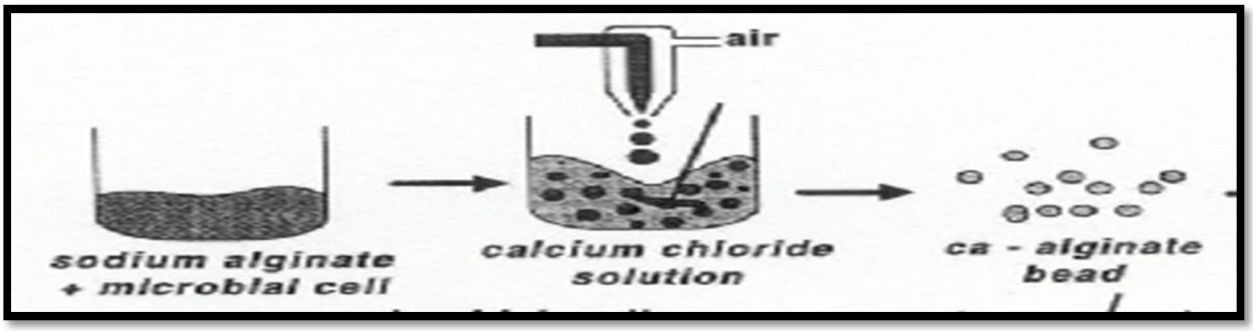


Fig.7 Encapsulation of Microbial cell

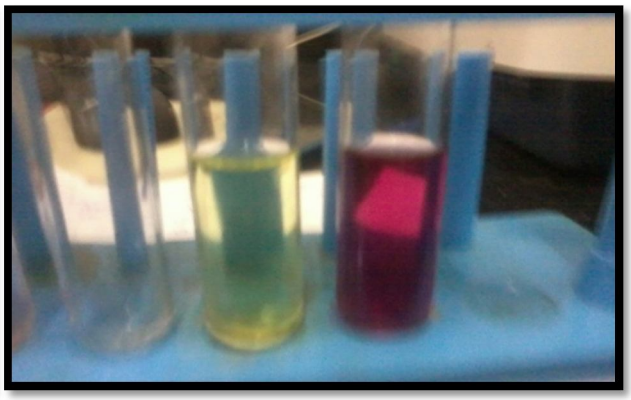
Fig: 8Encapsulated Microbial cell

4) **Preparation of stock solution and application of chromium reducing strains.**

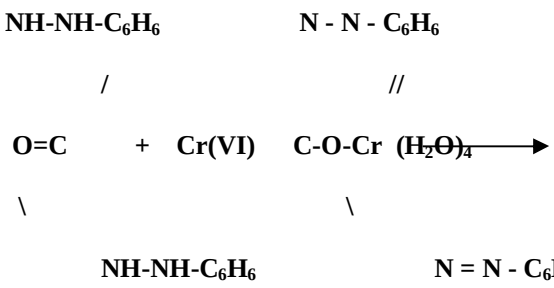
Effluent was collected from selected electroplating industry and diluted to get Cr (VI) conc. 100 mg/lit. 50 ml of stock solution was taken in six 250ml conical flask. Out of six, three flasks were supplemented with 30gm. of beads encapsulated with microbial cell and remaining three are supplemented with 30gm. of beads encapsulated with microbial enzymes of three different microbes respectively. The solutions were kept at room temperature and periodic metal uptakes were analyzed for Cr(VI) by UV-Visible spectrophotometer using 1, 5 diphenyl carbazied at λ max=540 nm.

1-5, diphenylcarbazide formed complex particularly with hexavalent chromium to form a red-violet colored product as shown in figure whose absorbance is checked in the spectrophotometer at 540 nm.

Cr (VI) Solution with Microbs cell



Red-violet coloredcomplex



Diphenyl carbazide

Diphenyl carbazide complex with Cr (VI) (Violet Red)

RESULTS AND DISCUSSIONS

1) Optimization of contact time for Cr (VI) reduction from effluent by usingmicrobial cell and microbial enzyme:

Time required for effective reduction of Cr (VI) was optimized by investigating Cr (VI) reduction using microbial cell as well as microbial enzyme from effluent. Results of reduction were examined after every 24 hours by UV-Visible spectrophotometer at λ max=540nm by using 1, 5 diphenyl carbazide.

Percent reduction of Cr (VI) has been calculated by eequation-2

As = (Absorption shown by Stock sol) =3.8

C ini = Initial Concentration of Cr (VI) in solution (100mg/lit),

D= concentration of Cr (VI) left in the solution,

A= absorption Cr (VI) of sample soil,

E= Concentration of Cr (VI) Reduced,

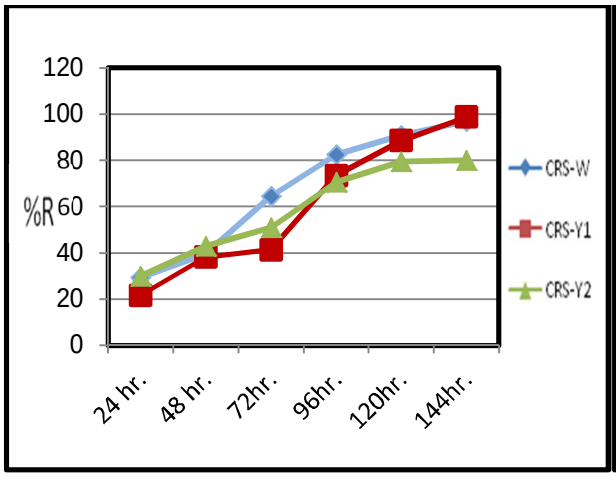
R= % reduction of Cr(VI)

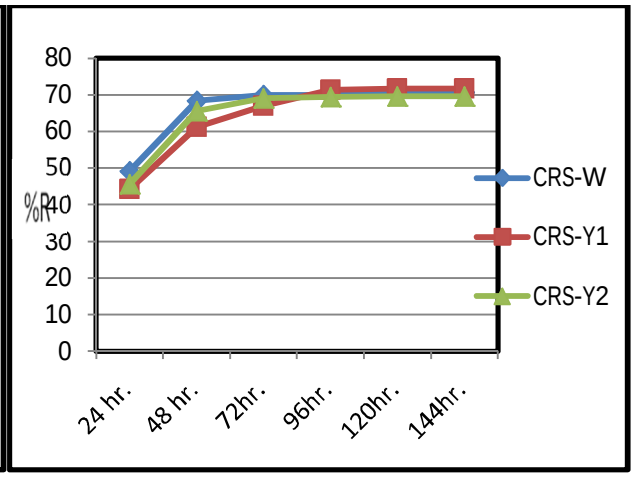
D= [A XC/ As] ---------- (Eq.1)

E = C initial-D

R = EX100/C initial ---------- (Eq.2)

It was observed that by using microbial cell, with the contact time, absorption of Cr (VI) increases as shown graph1 and using microbial enzyme it was observed that with time, reduction of Cr (VI) from effluent increased till 70 % rapidly with in 80 hr. and then became constant as shown in graph 2. It was also observed that furthermore reduction extends to 100% by addition of further 10 gm of enzyme beads within next 12 hours.

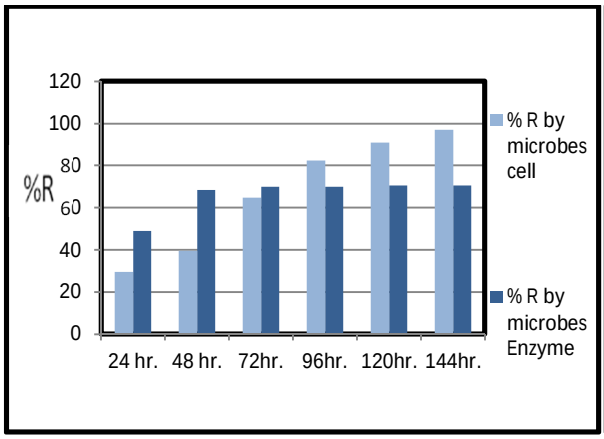


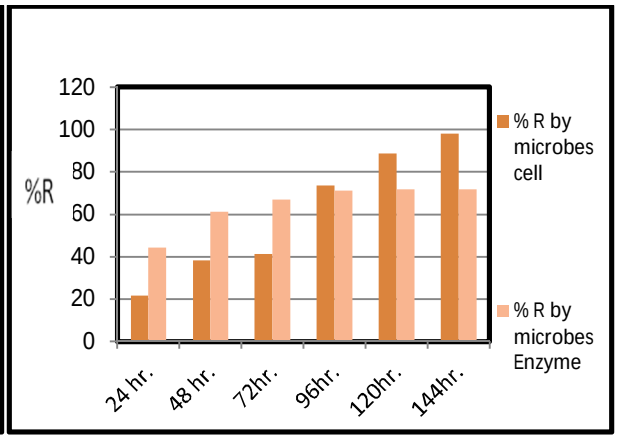


Graph-1: Effect of contact time on Cr (VI) reduction effluent using Microbial cell

Graph-2: Effect of contact time on Cr (VI) reduction from effluent using Microbial enzyme

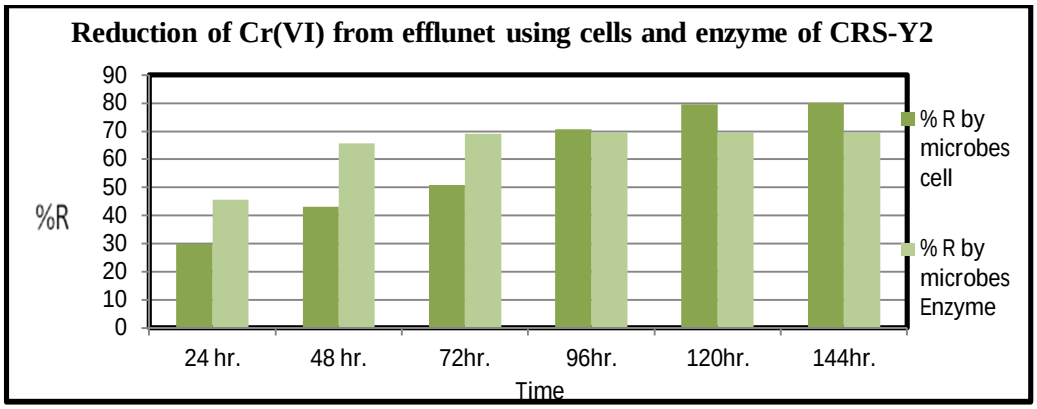
Results of Cr (VI) reduction from effluent using microbial enzyme was also compared with results obtained using microbial cell, results obtained are shown in graph No 3 and 4





Graph-3: Reduction of Cr (VI) from effluent by microbes and microbe enzyme by Bacillus sp. *(CRS-W)*

Graph-4: Reduction of Cr (VI) from effluent by Microbial cell cell and microbe enzyme by staphylococcus sp.(CRS-Y1)

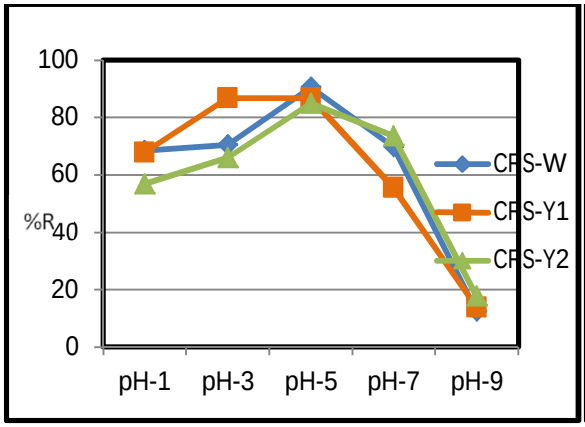


Graph-5: Reduction of Cr (VI) from effluent by Microbial cell and Microbial enzyme by (CRS-Y2)

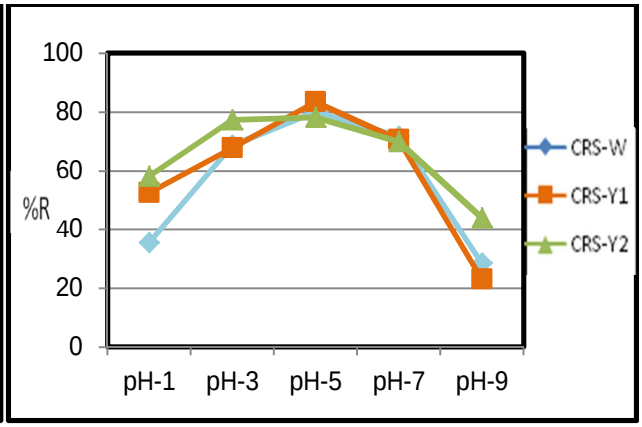
2) Optimization of pH for reduction of Cr (VI) from effluent of Cr (VI) by using microbial cell and microbial enzymes

Optimum pH for effective reduction of Cr (VI) has been observed from its effluent samples having various pH values such as pH-1, pH-3, pH-5, pH-7 and pH-9 and keeping concentration of samples constant that is 100mg / lit. pH of samples were varied by addition of 0.1 N HCl and 0.1 N NaOH. Reduction of Cr (VI) was analyzed by using both, microbial cells and microbial enzymes for all three different species after 120 hours.

Maximum reduction of Cr (VI) by CRS-W, CRS-Y1 and CRSY2 was obtained at pH-5. as shown is graph.



Graph-6: Effect of pH on Cr (VI) reduction using Microbial cell using Microbial enzymes



Graph- 7: Effect of pH on Cr (VI) reduction

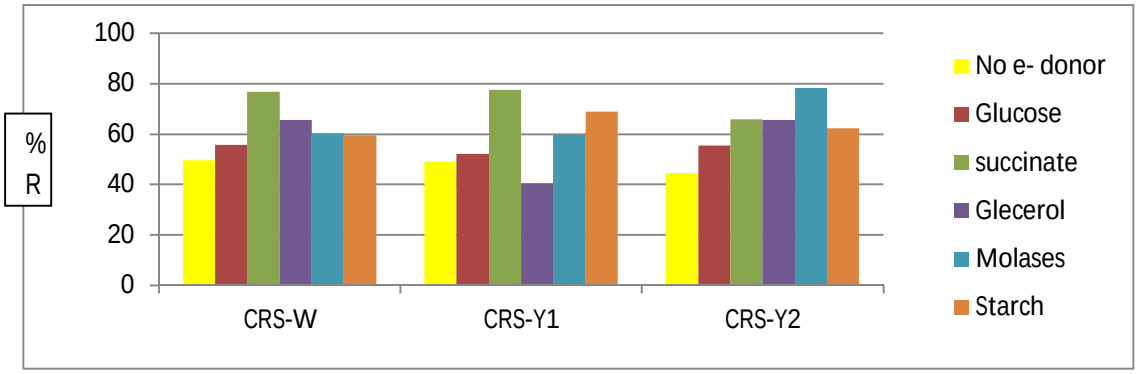
3) Effect of e^- donor on reduction of Cr (VI) from effluent by microbial cell and microbial enzymes

Effects of various electron donors were observed by enriching effluent samples with electron donors like glucose, molasses, glycerol, succinate and starch at 1% level solutions.

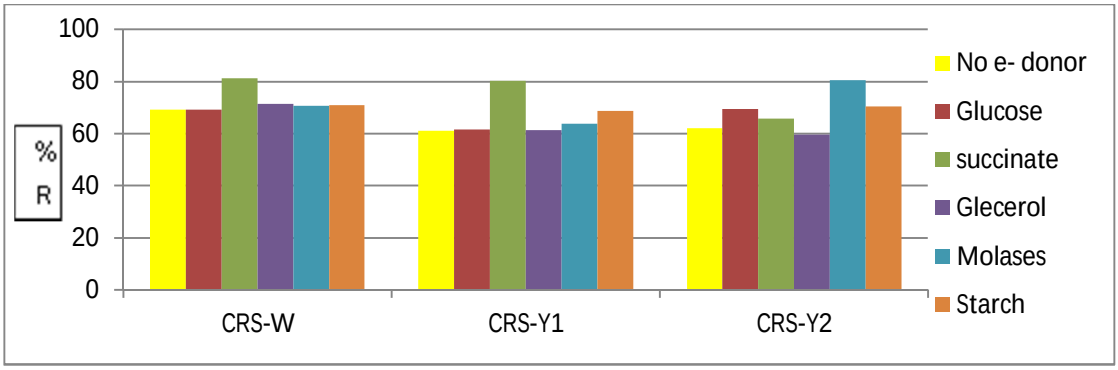
Cr (VI) reduction results were investigated by using both microbial cell as well as microbial enzyme of all three organisms after the incubation period of 48 hours.

The resultantCr (VI) reduction values clearly suggested that rate of Cr (VI) reduction was increased in presence of e^- donor as compared to Cr (VI) reduction in absence of them by all the microbe's species as shown in graph.

Both microbial cell as well as microbial enzymes of CRS-W and CRS-Y1 reduced Cr (VI) effectively in presence of e^- donor succinate while that of CRS-Y2 reduced Cr (VI) effectively in presence of e^- donor molassesas shown in graph 8 and 9.



Graph 8. Effect of e- donor on Cr (VI) reduction usingmicrobial cell

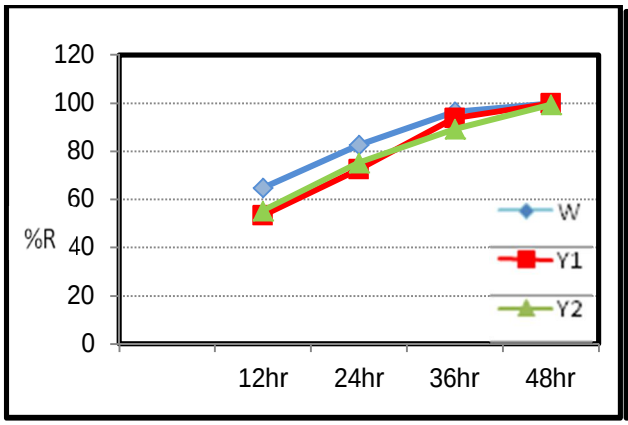


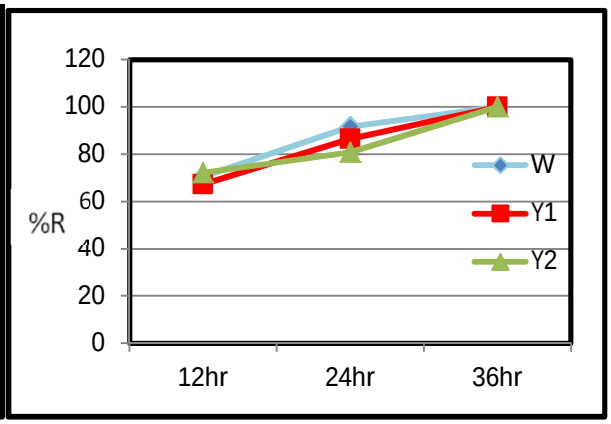
Graph 9.Effect of e- donor on Cr (VI) reduction usingmicrobial enzyme

4) Results of reduction of Cr (VI) from effluent with optimized condition by using microbial cell and microbial enzyme

Investigation of Cr (VI) reduction from effluent at optimized condition has been done after every 12 hours. It was observed that under the optimized conditions of pH and electron donor, toxic chromium was reduced up to 100% from the effluent within 48 hours of contact time usingCRS-W and CRS-Y1.Using microbial cell of CRS-Y2 Cr (VI) can be reduced up to 99% as shown in graph 10.

While using microbial enzyme toxic Cr (VI) from effluent reduced within 36 hours up to 100%as shown in graph.11





Graph. 10: Effect of Optimized condition on Cr (VI) reduction from effluent by microbial cell

Graph.11: Effect of optimized conditions on Cr(VI)reduction from effluent by microbial enzyme

CONCLUSIONS

- Conventional technologies to clean up heavy metals ions from the contaminated waste are not cost effective, having major problem of solid waste disposal. Alternating to these more expensive technologies bioremediation methods could be the most promising, eco -friendly, inexpensive and safe by which toxic Cr (VI) could convert into its non toxic trivalent form.
- Results obtained were indicated all the isolated microbes are efficient to reduce toxic Cr (VI) from effluent under the optimized conditions of parameter like pH and e- donor.
- Maximum reduction of Cr (VI) from effluent was obtained at optimum pH-5 for CRS-W, CRS-Y1 and CSR-Y2.
- Reduction of Cr (VI) was increases nearly by 30 % with the addition of suitable electron donor in effluent samples.
- Under the optimized conditions of pH (pH-5 For CRS-W,Y1and Y2), and suitable e- donor, Cr (VI) was reduced with rate of 2.08 % per hr. from effluent by using microbial cell and by using microbial enzyme reduction rate of Cr (VI) was 2.77% per hr.
- It can be concluded that Cr (VI) reduction rate was faster by using microbial enzyme than microbial cell.
- Thus method of microbe's application depends on availability of time as complete cleanup of toxic Cr (VI) is possible by microbial cell but time required is more as comparative to microbial enzymes.
- If the concentration of Cr (VI) in effluent is less and volume of effluent is more, use of micro bialenzymes are recommended and if the concentration is appreciable use of microbes-cells are preferable as they are capable to remove Cr (VI) completely from the effluent.

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