Bioremediation of Chromium (VI) In Electroplating Industrial Effluent

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Abstract: Heavy metal Cr in +6 oxidation state, introduced from various kinds industrial effluent is extremely toxic, carcinogenic and mutagenic in nature. Its direct discharge can contaminate the soils, ground water, sediments and surface waters and creates serious risk to environment which requires immediate attention for getting eco friendly and economical solution for effluent treatment containing Cr(VI). Large quantities of biomaterials have shown efficient uptake of heavy metals as a result of various metabolism for the removal of metals. Chromate reducing bacteria (CRS),Burkholderia sp (CRS-Y2) and Kocuria turfanensis (CRS-R), Microbes-cell and extracellular enzyme secreted by microbes isolated from discharge site of effluent, can be used to achieve bioremediation of toxic chromium(VI).

Key words: CRS, Microbes, Chromium(VI), extracellular enzyme Burkholderia species, Kokuria species.

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1 INTRODUCTION:

Chromium is having various uses in the industries because of its hardness, oxidizing property, corrosion resistivity. The rapid industrialization and extensive industrial uses of chromium leads to generation of large volumes of effluent containing chromium that is discharged into the environment.

Because of the elemental and non-biodegradable nature of Cr(VI), it is always retain its chemical form and create serious risk, when released into the environment [8]. Chromium is introduced in ecosystem through electroplating, tanneries, leather industries, metallurgy, chrome ore processing industries, wood preservation industries, Mine tailing and effluents from non-ferrous metals industries are the major sources of Cr-VI in the environment[3].

After entering into hydrosphere, chromium becomes a part of this ecosystem and distributed by various physicochemical interactions. [4].

Human health effects of Cr (VI) also include lung cancer, respiratory irritation, dermatitis, kidney and liver damage, and damage to various proteins and nucleic acids, [5].

In Toxicological overview (2007), U.S. Environmental Protection Agency classified Cr-VI as a Class –A Carcinogen. [2].

The biotoxicity of chromate is mainly because of its powerful oxidizing nature and its ability to cross biological membranes. Cr-VI can penetrate through the membrane because of its mobile and soluble nature.

Microbes-cell and extracellular enzyme secreted by Chromate reducing bacteria (CRS) is found effective for the bioreduction of toxic Cr(VI) in to less toxic Cr(III). Cr (III) is 100 times less toxic and 1000 times less mutagenic than Cr (VI) [6].

Encapsulated of microbes-cell and extracellular enzyme has been done using sodium alginate. Sodium alginate beads of microbes-cell and extracellular enzyme of all isolated CRS are applied initially on synthetic solution of Cr-VI and then on effluent containing Cr-VI.

For the effective bioreducion of Cr(VI), Optimization of factors like pH, initial concentration of Cr (VI) in effluent and e- donor has been done. Results of Cr-VI degradation are analyzed periodically by UV. Visible spectrophotometer at λmax=540 nm. Total concentration of Cr-VI is analyzed by atomic absorption spectroscopy.

2. MATERIALS AND METHOD

2.1 Isolation and Characterization of CRS

The effluent is collected from a discharged 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 is analyzed for following physicochemical parameters[7]..

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

Chromate tolerating bacteria are isolated from effluent discharge site. To isolate chromium resistant bacteria 1 gm. sludge contaminated with effluent of electroplating industry. Diluted sample of this solution is spread on agar-agar nutrient plates. 9 different bacterial colonies are appeared on the plates. Fresh inoculate of every bacterial strain is inoculated in the 09 different conic flasks containing 100ml of stock solution of Cr-VI Solution is incubated at 37^0 C for 48 hours. The uptake of Cr-VI from solutions is examined by Double beam UV-Visible spectrophotometer (Make-Jasco Corporation ,Japan) by using 1,5 diphenyl carbazied at λ max=540 nm. Out of 09 microbes samples 02 microbes species, are found effective for Cr-VI uptake from Cr-VI solution and they are labeled as per their colors. Selected strains are characterized morphologically, biochemically, and physiologically by 16 S rRNA sequencing as Burkholderia sp. and Kokuria sp.

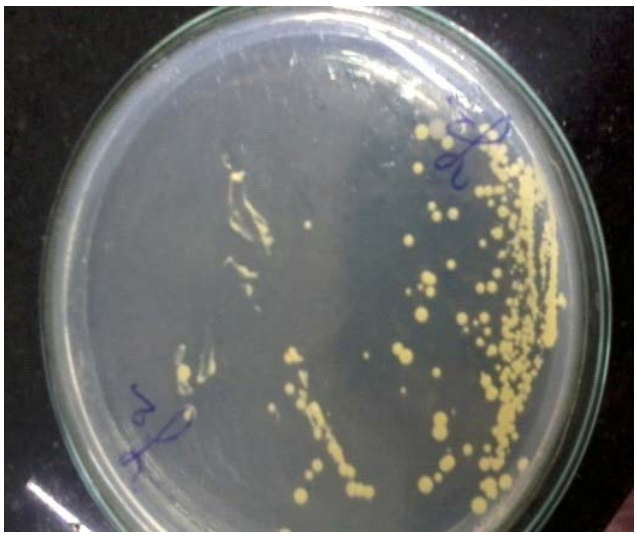

Figure1. Burkholderia species.

Figure 2. Kokuria species.

Table.2 Morphological characters

Sr.No.	Morphological characters	CRS-Y2	CRS-R
1	Colony Shape	circular	circular
2	Colony color	yellow	Redish
3	Colony elevation	convex	convex
4	Colony margin	rods	cocci
5	Gram character	-ve	+ve

Table.3 Biochemical characters

2.2 Optimization of growth media:

Fresh overnight inoculums of both the CRS is 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 are occur on various type of nutrient agar plate growth media for particular species has been optimized by colony count method.

2.3 Immobilization of CRS cell and enzyme:

CRS are allowed to grow in nutrient broth for 48hr. turbid broth samples obtained containing mass of microbes are centrifuged for 2 hr. at 500 rpm. to get pallets of microbes cell then separated from supernant containing extracellular enzyme secreted by microbes. Enzyme is purified by ammonium sulphate precipitation method. For immobilization of both CRS cell and enzyme, sodium alginate is dissolved in flask containing100 ml of water and then stirred uniformly to give uniform solutions of sodium alginate.

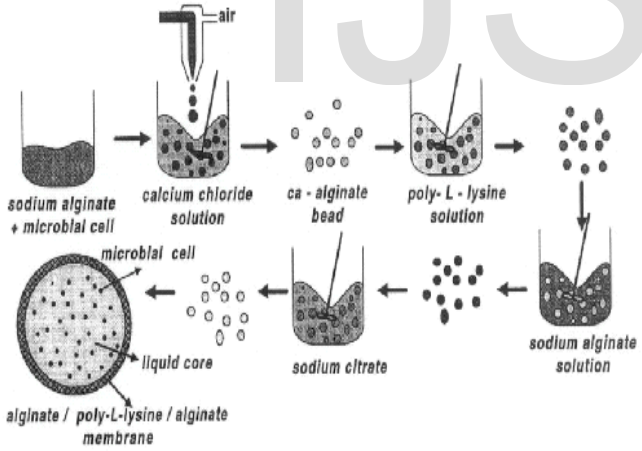

Figure 1. Encapsulation of Microbes and Enzyme

Sr. No.	Biological characters	CRS-Y2	CRS-R
1	Indol test	-ve	-ve
2	Methyl Red test	-ve	-ve
3	Voges-Proskauer test	-ve	-ve
4	Citrate test	+ve	±ve
5	Mc Concky Test	-ve	**-ve**
6	Mannitol fermentation	-ve	+ve
7	Starch Hydrolysis	-ve	-ve
8	Urease test	-ve	-ve
9	Catalase	+ve	+ve
10	oxidase	+ve	-ve

The pallet of CRS cell as well as purified enzyme of Burkholderia sp. and Kokuria sp. species are mixed in separate solutions of sodium alginate and mixtures are drop in the independent conical flasks containing solution of calcium chloride to get beads containing microbes. Beads are store in 1% CaCl2.in separate flasks. Effluent of plating industry, containing Cr-(VI) is diluted with distilled water to get 100ppm concentration of Cr(VI).

50 ml of stock solution is taken in four 250ml conical flask. 50gm. of beads containing microbes cell, and enzymes of are put in each solution. The solutions are kept at room temperature and periodic metal uptakes are analyzed for Cr-VI by UV-Visible spectrophotometer at λmax=540nm. Suitable pH for effective uptake of toxic Cr-VI is optimized by adjusting pH from highly acidic range to basic range by drop wise addition of 1N NaOH [10]. Suitable concentration for effective uptake of toxic Cr-VI is optimized by examining effluent with various concentrations as 25mg/l, 50mg/l, 75mg/l and 100mg /l. .[11]

Rate bioremediation is accelerated in presence of suitable e- donor .[1] which is optimized by examining reduction of Cr(VI) in presence of various e- donors.

3 Results And Discussion

Results of reduction of toxic Cr-VI are 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.

3.1 Effect of Time

Diluted effluent containing 100 mg Cr-VI is analyzed after every24 hr. for reduction of toxic Cr-VI with respect to time by both the microbes cells and enzymes of the CRS-Burkholderia sp.(Y2) and CRS kokuria sp. (R). Both microbes cells and enzymes of the CRS are highly effective to reduced toxic Cr-VI from effluent within 144 hr. Encapsulated Microbes and enzyme of CRS-Burkholderia sp.(Y2) are found efficient to reduce Cr(VI)from effluent.

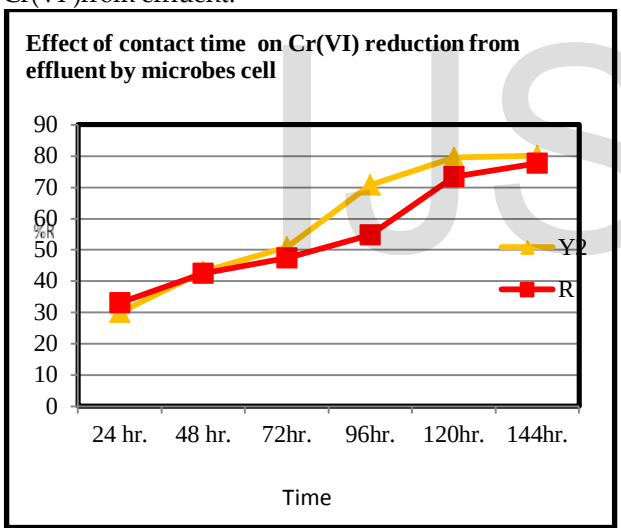

Figure 2. Reduction by CRS (Y2) and R **cell** w.r.t time

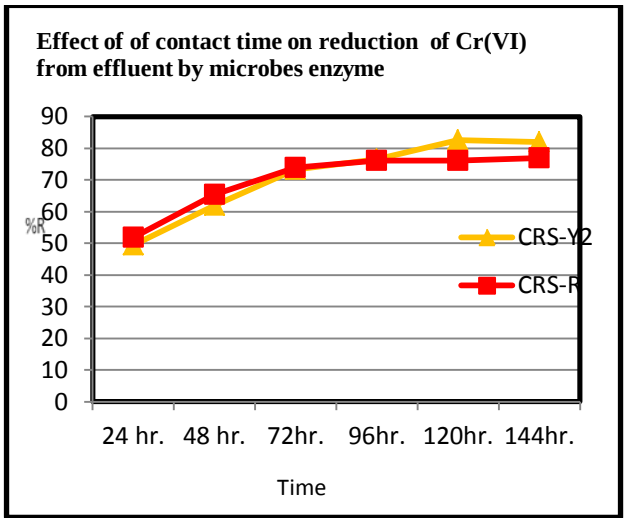

Figure 3. Reduction by CRS (Y2) and R **enzyme** w.r.t time.

3.2 Effect of pH

Bioreduction of Cr-VI is highly affected by pH of the solution At high pH Cr-VI is in at precipitate form and not available for bioreduction. pH of solution varied from acidic to basic range by drop wise addition of NaOH solution. It is observed rate of bioreduction is high at acidic pH than basic pH. And rate of bioreduction is more in acidic pH. It is maximum at pH-5.

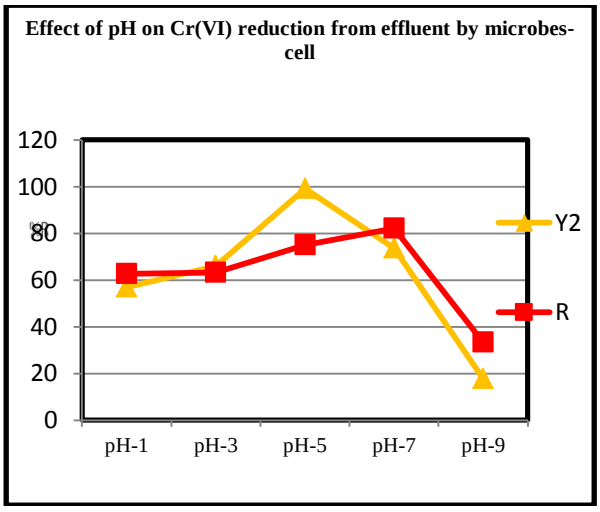

Figure 4. Reduction by CRS (Y2) and R **cell** w.r.t. pH

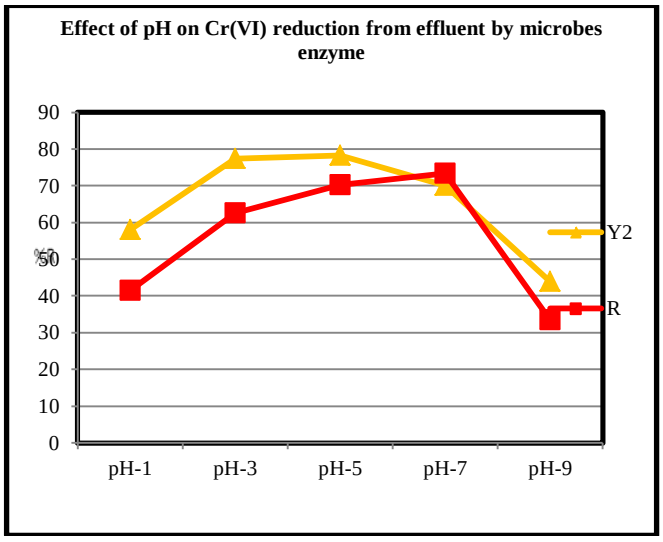

Figure 5. Reduction by CRS (Y2) and R **enzyme** w.r.t pH

3.3 Effect of concentration

Stock solution of effluent consisting 100mg of toxic Cr-VI per lit is diluted by distilled water to get different concentrations, that are 25%, 50%, 75%and100% analysis has been done after 48 hr for both ,encapsulated CRS cell and enzyme of by using UV-Visible spectrophotometer at λmax=540 for Burkholderia sp. and kokuria sp. . During analysis it is observed rate of bioreduction is high for 25% dilution.

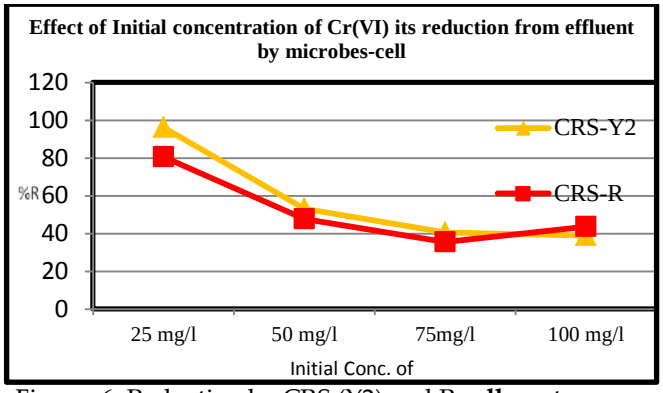

Figure 6. Reduction by CRS (Y2) and R **cell** w.r.t. conc.

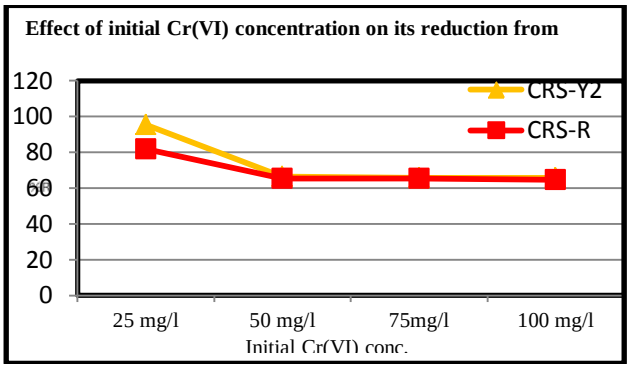

Figure 7.Reduction by CRS (Y2) and R **enzyme** w.r.t. conc.

3.4 Effect of electron donor:

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

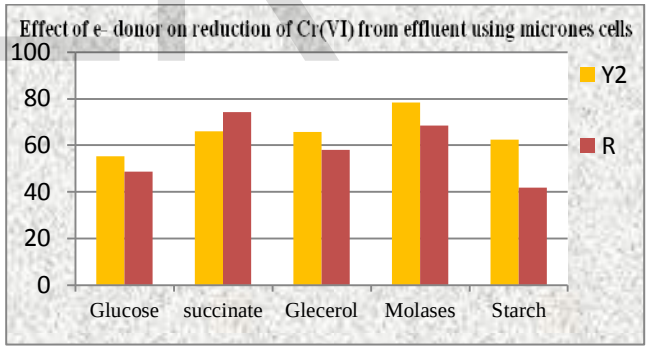

Figure 8. Reduction by CRS (Y2) and R **cell** w.r.t.e-donor

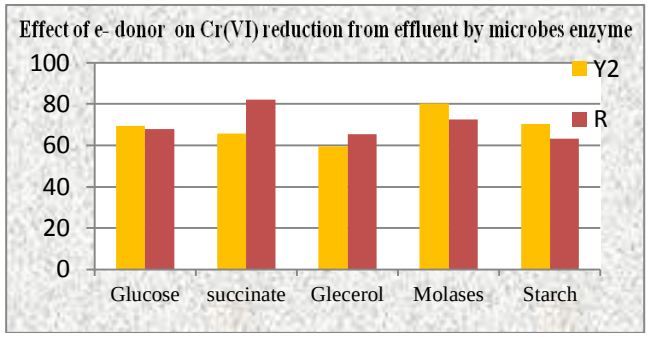

Figure 9 .Reduction by CRS (Y2) and R **enzyme** w.r.t.e-donor

3.5 Reduction of chromium (VI) from effluent with optimized condition

Reduction of chromium (VI) by both using microbes cell and enzyme is investigated by maintaining optimized condition of pH of effluent, initial concentration of chromium (VI) in the effluent and enrichment by suitable electron donor as shown in table.

Table.4 Reduction of chromium (VI) from effluent with optimized condition.

Sr. No.	CRS	pH	Initial concentration	Electron donor
1	CRS-Y2	5	25 mg/l	Molasses
2	CRS-R	7	25 mg/l	Succinate

It is observed that at optimized conditions of pH, initial concentration of Cr(VI) and electron donor as shown in table, toxic chromium is reduced more than 99% by CRS-Y2 and more than 87 % within 36 to 48 hr. by CRS-R as shown in figure.

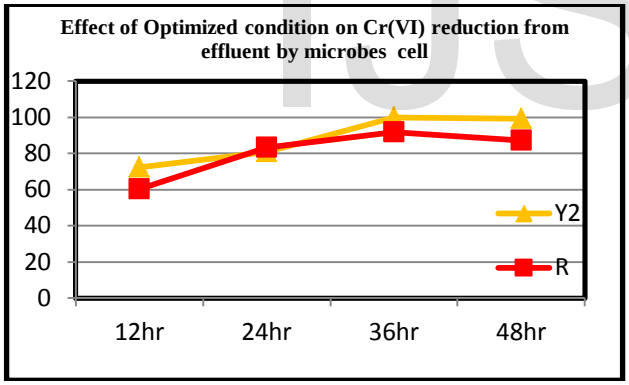

Figure 10. Reduction of Cr (VI) using microbes cell under optimized conditions oh pH, conc. and e- donor.

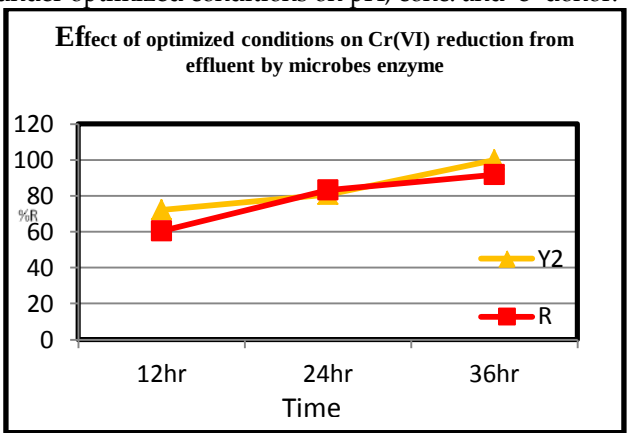

Figure 11. Reduction of Cr (VI) using microbes enzyme under optimized conditions oh pH, conc. and e- donor.

4 Conclusion

Conventional technologies to clean up heavy metals ions from the contaminated site have been utilized but these technologies are not cost effective, having major problem of solid waste disposal. Bioremediation is the most promising eco-friendly, inexpensive and safe alternative by which toxic Cr-VI could convert into its less toxic trivalent form and could reduce more than 85% Cr-VI within 2 days even in acidic condition. Industrial effluent is treated by two different methods using encapsulated cells of CRS and encapsulated enzyme of CRS at room temperature. It is observed that bioreduction of toxic Cr-VI is more speedily by encapsulated enzyme method than encapsulated cells method. It is also observed that 100 % bioreduction is possible by using encapsulated enzyme of **Burkholderia sp.** within 36 hrs. whereas **kokuria sp.** can reduce-90 % of Cr-VI. Encapsulated cells of **Burkholderia sp** can reduced Cr-VI till 100 % within 48 hrs. Whereas by cells of **kokuria sp.** can reduce 85% of Cr-VI within 48 hrs.. Uptake of Cr-VI can enhance along with dilution.

5 Acknowledgments

It is my privilege and honor to express my deepest gratitude to honorable Shefali madam and dignified head of IOE.

Dr. V. P. Wani, Bhujbal Knowledge City Nashik, India for inspiring guidance and useful intellectual suggestions.

6 References

1. Adel, M. Zayed and Norman Terry (2003). Chromium in the environment: factors affecting biological remediation. Plant and Soil; Vol. 249:139–156.
2. Assem, L. and Zhu, H. (2007). Chromium–toxicological overview. Health Protection Agency; Version 1:2-14.
3. Das Alok Prasad (2009). Bioreduction based bioremediation of hexavalent chromium Cr (VI) through potential indigenous microbes. M. Tech Thesis. National Institute of Technology, Rourkela, Orissa, India, Deem university.
4. Flora, De S. (2000). Threshold mechanisms and site specificity in chromium (VI) carcinogenesis. Carcinogenesis; Vol. 21(4):533-541.

5. Focarrdi Silvia, (2013). Microbial Reduction of Hexavalent chromium as a mechanism of detoxification. In: Biodegradation – Life of Science. (Rolando Chamy and Francisca Rosenkranz, Eds.). Intech: 321-346.
6. Morales-Barrera Liliana and Eliseo Cristiani-Urbina (2006). Biological reduction of hexavalent chromium: a potential. Water Environment Foundation; Vol.6: 4980-4984.
7. Patricia Warin (2012). Standard methods for examination of water and waste water. American Public Health Association (APHA); 22:3-18.
8. Rachna (2011). Bacterial Biosorbents for detoxification of heavy metals from aqueous solution. International Journal of Advances in Science and Technology; Vol. 2(6): 103-128.
9. Sarangi Abhipsa and Chandraraj Krishnan (2009). Enzymatic reduction of hexavalent chromium in bacteria. Envis Centre Newsletter; Vol. 7(2).
10. Todd, R. Sandrin and Raina, M. Maier (2003). Impact of metals on the biodegradation of organic pollutants. Environ Health Perspect; Vol.111 (8) : 1093–1101.
11. Vidali, M. (2001). Bioremediation. An overview Pure Appl. Chem.; Vol. 73(7): 1163–1172.