

Full Length Research Paper

Bioreduction of chromium (VI) to chromium (III) by a novel yeast strain *Rhodotorula mucilaginosa* (MTCC 9315)

Sabyasachi Chatterjee¹, Narayan Chandra Chatterjee² and Sikha Dutta^{2*}

¹Department of Biotechnology, University of Burdwan, Burdwan-713104, West Bengal, India.

²Department of Botany, University of Burdwan, Burdwan-713104, West Bengal, India.

Accepted 17 August, 2012

Introduction of heavy metal compounds like chromium, lead, arsenic and mercury into the environment generally induces morphological and physiological changes in the microbial communities hence exerting a selective pressure on the microorganisms. Generally, sites which are contaminated with heavy metals are the sources of metal resistant microorganisms. This paper describes the bioreduction of Cr⁶⁺ to Cr³⁺ by a novel strain *Rhodotorula mucilaginosa* (MTCC 9315). The yeast is very efficient in Cr⁶⁺ reduction. It is shown that the reduction of Cr⁶⁺ to Cr³⁺ species occurs extracellularly by analyzing the supernatant of the yeast extract peptone sucrose medium (YEPS) thus supporting the assumption about the existence of an extra-cellular pathway of Cr⁶⁺ reduction. Furthermore, it is demonstrated that *R. mucilaginosa* cells are salt tolerant. It is thus suggested that isolated yeast biomass can be used as sources of Cr⁶⁺ reducer, and the resin formulated in the present study may be used as biofilter to filter out Cr⁶⁺ ions. The yeast can also be a good supplier of Cr-chelators with potential pharmacological applications.

Key words: Chromium bioreduction, resin, industrial effluent, metal resistant microorganisms, yeast, biofilter.

INTRODUCTION

Chromium, one of the most common elements on earth, forms a wide range of stable compounds in trivalent Cr³⁺ and hexavalent Cr⁶⁺ states, being unstable in the forms of the intermediate valences Cr⁵⁺ and Cr⁴⁺. Cr⁶⁺ species are extremely toxic, whereas Cr³⁺ compounds are much safer; furthermore, they can be found in many kinds of food and are widely used as pharmaceuticals and dietary supplements (for example, chromium picolinate, chromium polynicotinate, chromium chloride) to prevent diabetes, lipoprotein abnormalities, and cardiovascular

diseases (Porter et al., 1999). While the molecular mechanisms of Cr³⁺ as a microelement are still unclear, experimental evidences have been obtained in support of the important role of this metal for the normal signal transduction pathway through the insulin-stimulated insulin receptor (Yamamoto et al., 2004).

Although Cr³⁺ supplements are widely used (for example, products containing chromium picolinate Cr(pyc)3 have annual sales of \$500 million), they possess some serious disadvantages which include: (1) poor solubility of chromium picolinate and chromium polynicotinate limits their low absorption up to 2 to 5%, and chelation of chromium chloride with large biomolecules restricts its consumption (Vincent, 2003); (2) the previous-mentioned salts and complexes have been shown to possess some deleterious effects, for example, they cause the damage of chromosomes in the Chinese hamster ovary cells (Stearns et al., 1995) and pathological ocular changes in rats (Amany et al., 2006). The synthesis of novel Cr³⁺ containing complexes, as

*Corresponding author. E-mail: sikha_bu_bot@yahoo.com. Tel: +91-9434672206.

Abbreviations: PDA, Potato dextrose agar; NA, nutrient agar; YEPD, yeast extract peptone dextrose; YEPS, yeast extract peptone sucrose; YEPM, yeast extract peptone maltose; EPS, exopolymeric substances.

well as microbial production of such compounds, is one of the possible ways of overcoming this problem. Taking into account significant kinetic inertness of Cr^{3+} salts in the formation of even thermodynamically stable complexes, it is promising to produce such complexes by using microbial cells capable of reducing Cr^{6+} (chromate, dichromate) to Cr^{3+} biocomplexes (Puzon et al., 2005; Ksheminska et al., 2008). Although, the genetic and biochemical aspects of this process have been insufficiently studied, it is known that chromate anions are transported into the cells through sulfate specific permease (s) coded, in the case of baker's yeast, by the genes SUL1 and SUL2 (Cherest et al., 1997) and can be reduced as a powerful oxidative agent to Cr^{3+} by cellular reducing systems which can include enzymatic and non-enzymatic pathways.

Glutathione and cysteine can be regarded as the most powerful non-enzymatic chromate reductants for microbial cells and ascorbate for higher organisms. The bacterial reduction of chromate is well established. It runs through the different enzymatic pathways which function under aerobic and anaerobic conditions with the use of hydrogenase, cytochrome c dependent electron transfer chains, nitrate reductase, flavin reductase, ferric reductase, some flavoproteins and nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) H-dependent reductases (Ksheminska et al., 2006). For eukaryotic microbial cells and primarily yeasts, the data on the chromate reducing systems are more ambiguous. It is generally unknown which system enzymatic or non-enzymatic, intracellular or extra-cellular one plays a leading role in the chromate detoxification process. Many yeasts like, *R. pilimanae* D-76, and *Pichia guilliermondii* ATCC 201911 have been reported for their reduction of Cr^{6+} to Cr^{3+} (Ksheminska et al., 2008). Many yeasts have been known for their reduction ability of Cr^{6+} to Cr^{3+} (Muneer et al., 2009).

Yeasts are ubiquitous unicellular microorganisms in natural environments or in industrial effluents and exposed to a variety of environments with respect to nutrient availability, temperature, pH, osmotic pressure, access to oxygen and water, etc., all of which induce stress responses (Hohmann and Mager, 1977); to which yeast cells have developed the mechanisms to adjust, with certain limits of environmental stresses. Elemental chromium (Cr) does not occur in nature, but is present in ores, primarily chromite (FeOCr_2O_3) (Hamilton and Wetterhahn, 1988).

Only two of the several oxidation states of chromium, Cr (III) and Cr (VI), are reviewed in this report based on their predominance and stability in the ambient environment and their toxicity in human beings and other animals. Chromium plays a key role in glucose and cholesterol metabolism and is thus an essential element to man and animals (Schroeder et al., 1962). Non-occupational exposure to the metal occurs through the ingestion of chromium-containing food and water, whereas occupational exposure occurs through inhalation

(Langard, 1982; Pedersen, 1982). Workers in the chromate industry have been exposed to estimated chromium levels of 10 to 50 $\mu\text{g}/\text{m}^3$ for Cr (III) and 5 to 1000 $\mu\text{g}/\text{m}^3$ for Cr(VI); however, improvements in the newer chrome-plating plants have reduced the Cr(VI) concentrations 10 to 40-fold (Stern, 1982).

Chromium (III) is poorly absorbed, regardless of the route of exposure, whereas chromium (VI) is more readily absorbed (Hamilton and Wetterhahn, 1988). Humans and animals localize chromium in the lung, liver, kidney, spleen, adrenals, plasma, bone marrow, and red blood cells (RBC) (Langard, 1982; ATSDR, 1989; Bragt and Van Dura, 1983; Hamilton and Wetterhahn, 1988). There is no evidence that chromium is biotransformed, but Cr (VI) does undergo enzymatic reduction, resulting in the formation of reactive intermediates and Cr (III) (Hamilton and Wetterhahn, 1988). The main routes for the excretion of chromium are through the kidneys/urine and the bile/feces (Guthrie and Langard, 1982).

Animal studies show that Cr (VI) is generally more toxic than Cr (III), but neither oxidation state is very toxic by the oral route. In long-term studies, rats were not adversely affected by ~1.9 g/kg/day of chromic oxide [Cr (III)] (diet), 2.4 mg/kg/day of Cr (III) as chromic chloride (drinking water), or 2.4 mg/kg/day of Cr (VI) as potassium dichromate (drinking water) (Ivankovic and Preussmann, 1975; MacKenzie et al., 1958). The respiratory and dermal toxicity of chromium are well-documented. Workers exposed to chromium have developed nasal irritation (at $<0.01 \text{ mg}/\text{m}^3$, acute exposure), nasal ulcers, perforation of the nasal septum (at $\sim 2 \mu\text{g}/\text{m}^3$, subchronic or chronic exposure) (Hamilton and Wetterhahn, 1988; ATSDR, 1989; Lindberg and Hedenstierna, 1983) and hypersensitivity reactions and "chrome holes" of the skin (Pedersen, 1982; Burrows, 1983; U.S Air Force, 1990). Among the general population, contact dermatitis has been associated with the use of bleaches and detergents (Love, 1983). Compounds of both Cr (VI) and Cr (III) have induced developmental effects in experimental animals that include neural tube defects, malformations, and fetal deaths (Iijima et al., 1983; Danielsson et al., 1982; Matsumoto et al., 1976). Considering the ecological benevolence of microorganisms for bioremediation of heavy metals, our interest has prompted us to use *R. mucilaginosa* isolated from industrial effluent of Chittaranjan locomotive, Chittaranjan, Burdwan, and West Bengal, India for reduction of Cr^{6+} to Cr^{3+} .

MATERIALS AND METHODS

Isolation and characterization

The organism was isolated from the effluent of Chittaranjan locomotive workshop effluent samples (50 ml) from Chittaranjan locomotive workshop, Burdwan; these were collected and analyzed for isolation of microorganisms. Sample (1 ml) was diluted upto 10^{-3} dilutions. 100 μl of each of different dilutions were plated separately

on potato dextrose agar (PDA) medium (pH 5.5) and nutrient agar (NA) medium (pH 7.0), incubated for 3 days at $30 \pm 1^\circ\text{C}$ for growth of fungal isolates and 2 days at 37°C for growth of bacterial isolates, respectively.

Developed colonies were randomly picked, isolated and cultured on fresh PDA and/or NA for growth of fungi and bacteria, respectively. The number of fungal colonies was more on PDA plates than the number of bacterial colonies on NA plates. Thus, the fungal isolates grown on PDA plates were selected for further experiments. Pure cultures of the isolates were obtained by streaking the colonies repeatedly on various media like PDA medium, yeast extract peptone dextrose medium (YEPM), yeast extract peptone sucrose medium (YEPS) and yeast extract peptone maltose (YEPM) medium. The isolated dominant colony was identified and characterized to the genus level on the basis of macroscopic characteristics (colony morphology, colour of the colony, appearance and shape of the colony) and on the basis of different microscopic characteristics (shape of the cells, diameter and texture of conidia, shape of the conidia).

Different biochemical tests were also performed and it has been confirmed that the isolate was yeast. The identification was also carried out to the genus and species level with 26S rDNA sequencing of D1/D2 domain of 629 nucleotide bases by using primers NL1 and NL4. Different restriction sites were pointed out using NEB cutter online software. Micrograph images were taken with a high resolution bright field microscope (Leica DMLB, Japan) and Scanning electron microscope (Nikon S530 Hitachi, Japan). Nucleotide sequencing was done at Institute of Microbial Technology, Chandigarh, India and deposited at IMTECH, MTCC, Chandigarh (MTCC number-9315) and at the National Center for Biotechnology Information (NCBI) (Accession number-GU074381).

A Varian (Spectra AA 55) flame atomic absorption spectrophotometer (FAAS, Australia) was used for measuring concentration of chromium species. All measurements were performed using integrated absorbance (peak area). Hollow cathode lamp for Cr was operated at 7.0 mA at wave length 357.9 nm and at a slit width of 0.2 nm. Air and acetylene flow rates were maintained at 3.5 and 1.5 L min^{-1} , respectively. Cr^{3+} and Cr^{6+} stock solutions were prepared from $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (Merck) and K_2CrO_4 (Merck), respectively. These solutions, 50 mg L^{-1} for Cr^{3+} and 5 mg L^{-1} for Cr^{6+} , were prepared in $0.5 \text{ mol L}^{-1} \text{ HNO}_3$ and double distilled water, respectively. These solutions were standardized against standard stock solutions of Cr^{3+} (1000 mg L^{-1}) supplied by Solutions plus inc. (Missouri, USA) which was tested against NIST SRM # 3108a using AAS.

Determination of optimum pH, temperature for yeast growth

Yeast cells were grown in media containing YEPS. 1 ml (10^8 cells/ml) of 24 h grown *R. mucilaginosa* cells was inoculated into every 25 ml ($25 \text{ ml} \times 5$) of YEPS medium and incubated at different temperature like 20, 25, 30, 35 and 40°C keeping pH 5.5 as constant and same experiment was repeated with different pH like 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 containing YEPS medium ($25 \text{ ml} \times 5$) keeping temperature 30°C constant for 24 h. All the experimental flasks were kept for random shaking at 120 rpm. After incubation, cells from every flask were centrifuged at 8000 rpm for 10 min, the pellet was collected, dried and dry biomass weighed.

Exopolymer extraction

For exopolymer extraction, 1 ml (10^8 cells/ml) of 24 h grown *R. mucilaginosa* cells was inoculated into YEPS medium (pH 5.5) and kept for incubation at 30°C for 72 h with constant shaking at

120 rpm. After incubation, cells were centrifuged at 8000 rpm for 10 min. Pellet was then treated with boiling water and 0.1 N HCl , again centrifuged at 8000 rpm for 10 min, pellet was discarded and in the supernatant double volume of 96% (v/v) chilled ethanol was added for 24 h at 4°C to precipitate exopolymer (Doughlas et al., 1988). Centrifugation process was repeated again for 10 min and supernatant was discarded. The ethanol precipitate was dialyzed for three days, freeze dried confirmed by Dubois et al. (1956) method and the rest of the sample was kept for further structural investigation.

Determination of salt stress tolerance of the yeast

It has been reported that the yeast cells having salt stress tolerance generally possess uronic acids or negatively charged molecules in the exopolymeric substances (EPS), and the number of uronic acids is directly proportional to the heavy metal chelation (Dae and Eui, 2003). So, trials were being made to determine the salt tolerance capacity of the yeast strain, for which 1 ml (10^8 cells/ml) of yeast cells were inoculated into YEPS medium supplemented with different concentrations of NaCl (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%) at 30°C and incubated for 72 h with shaking at 120 rpm. After incubation, cells were centrifuged at 8000 rpm for 10 min, the pellet was collected, dried and dry biomass was weighed. At the same time, yeast growth was measured spectrophotometrically at 540 nm (Abdel and Moghaz, 2010).

Column preparation and stability of the resin

Air dried resin (1.0 g) was immersed in deionised water and allowed to swell for 24 h. A glass column ($10 \times 1 \text{ cm}$) was packed with swollen beads to a bed volume of 2 ml. Before starting the experiment, the resin bed was thoroughly washed with $0.1 \text{ mol L}^{-1} \text{ HNO}_3$ followed by deionised water till the effluent was free from acid. Then, the resin bed was pre-conditioned by washing with buffer solutions of appropriate pH. Sorption and desorption characteristics for Cr^{3+} and Cr^{6+} in the column were studied at the optimum flow rate. Any adhering metal ions (not adsorbed) were completely washed out using solutions of appropriate pH. The sorbed Cr^{3+} and Cr^{6+} were completely eluted by 10 ml of 2 mol L^{-1} and 6 ml of $3 \text{ mol L}^{-1} \text{ HNO}_3$, respectively, at a flow rate of 5 ml min^{-1} . Concentration of each chromium species thus eluted was measured by FAAS. 250 mg of resin was shaken with 100 ml of acid or alkaline solutions of different ionic strengths (0.5 to $5 \text{ mol L}^{-1} \text{ HNO}_3$ and NaOH solutions were used for stability experiments) for 7 days then, filtered and washed with deionised water to remove acid or alkali and finally dried under vacuum. The metal ion exchange capacity was measured. The thermal stability of the resin was studied by thermogravimetry.

Chromium ion uptake as a function of pH

A batch technique was used, taking metal ion in excess to the resin. Capacities were determined in the pH range 2.0 to 10.0. To a glass stoppered centrifuge tube (diameter 2.0 cm) containing 30 mg of the dry resin in basic form, 10 ml of the desired pH solution was added. After equilibration of this mixture, 25 ml of 50 gm L^{-1} for Cr^{3+} and 5 gm L^{-1} for Cr^{6+} metal ion solutions were added. The mixture was shaken for 24 h. The pH of the equilibrating solution was adjusted by appropriate buffer solution. After 24 h, the solutions were filtered under suction and washed with deionised water to remove adhering metal ions. To confirm that the resin adsorbs chromium species, Fourier transform infrared (FTIR) spectra as well as scanning electron microscopy (SEM) images of the Cr^{3+} loaded



Figure 1. *Rhodotorula mucilaginosa* grown on yeast extract peptone sucrose agar.

resin were recorded (Das et al., 2010). The sorbed metal ion was eluted with suitable eluting agent. The concentration of the eluted metal ions was measured by flame atomic absorption spectroscopy (FAAS) using air-acetylene flame.

Bio-reduction of Cr^{6+} to Cr^{3+} by yeast species

The yeast species isolated was used to reduce Cr^{6+} to Cr^{3+} . The pH dependent separation of Cr species was performed using a resin which has been synthesized in the Department of Chemistry, Burdwan University, Burdwan (Figure 6). At first, 1 ml (10^8 cells/ml) of yeast cells was cultured in peptone sucrose medium at 30°C for 72 h (Li et al., 2008). Several standard potassium dichromate solutions of varying strengths (2, 4, 6, 8 $\mu\text{g ml}^{-1}$) were prepared by dilution method (50 ml of each). Then, 1 ml (10^8 cells/ml) of 24 h grown *R. mucilaginosa* was added to all solutions and kept for 72 h at 30°C . After that, 30 ml of culture medium was centrifuged at 8000 rpm for 10 min, and pH of the supernatant for every strength of potassium dichromate containing yeast culture was adjusted to 2.0 using appropriate buffer solution, and passed through the pre-conditioned resin column (Figure 6). Any Cr^{3+} produced in the solution will be retained by the resin in the column, and only Cr^{6+} will come out. Cr^{3+} was desorbed from the resin bed with 2.0 mol L^{-1} HNO_3 and measured by flame atomic absorption spectroscopy (FAAS). The percent bioreduction of Cr^{6+} to Cr^{3+} by the *R. mucilaginosa* was calculated.

RESULTS AND DISCUSSION

The yeast strain was identified as *R. mucilaginosa* by 26S rDNA partial gene sequencing (accession no. GU074381, NCBI) and with various restriction sites, (Figure 7) exhibited optimum growth on YEPS medium

containing 2% sucrose (Figures 1 and 8). An increase in concentration of sucrose (3 to 4 and then 5%) decreases the yeast biomass but otherwise increases the EPS production (Figure 9). YEPS, YEPD and YEPM media were proved to be better for growth of the yeast isolate than PDA medium. The optimum pH and temperature for the growth of the yeast were recorded to be 5.5 and 30°C , respectively (Figures 10 and 11). It was evident from the results that the yeast strain possesses stress tolerance property against the salinity stress (NaCl). NaCl stress at 1% concentration supplied with YEPS medium appeared to be the best for optimum biomass production (Figure 12) by the yeast cells, and after the foregoing said concentration, biomass production decreased. *R. mucilaginosa* cells were normally grown in YEPS broth (Figure 2) and with 0.5 mM of potassium dichromate containing YEPS broth (Figure 3) whereas the bursting out of intracellular materials forms a thin film in the presence of 8 mM of potassium dichromate on which the deformed yeast cells were observed under scanning electron microscope (Figure 4) studies. Budding *R. mucilaginosa* cells were observed under Leica microscope (Figure 5).

R. mucilaginosa was very effective to reduce Cr^{6+} toxicity by converting potentially toxic Cr^{6+} to relatively non-toxic Cr^{3+} species as shown in Table 1 and Figure 13. Thus, it may well be referred to as green method for reducing Cr^{6+} toxicity. The chemically synthesized resin acted as an important chelator for Cr^{3+} . The functionalities present in the solid polymeric matrix (resin) contain carboxylate anion; tertiary nitrogen (from amino

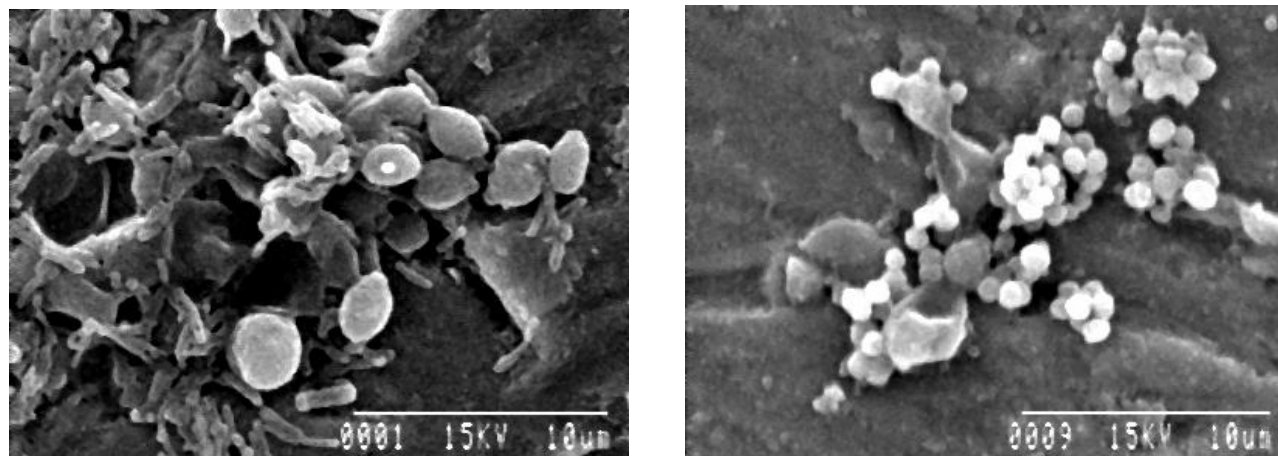


Figure 2. Scanning electron micrographic images of *Rhodotorula mucilaginosa* grown in yeast extract peptone sucrose broth.

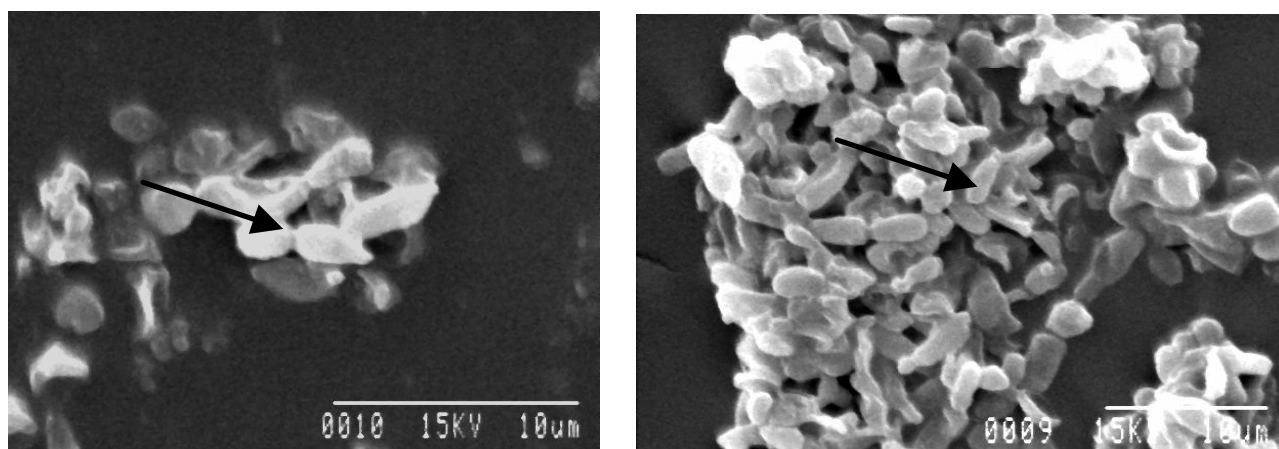


Figure 3. Scanning electron micrographic images of budding *Rhodotorula mucilaginosa* grown in yeast extract peptone sucrose broth with 0.5 mM of potassium dichromate.

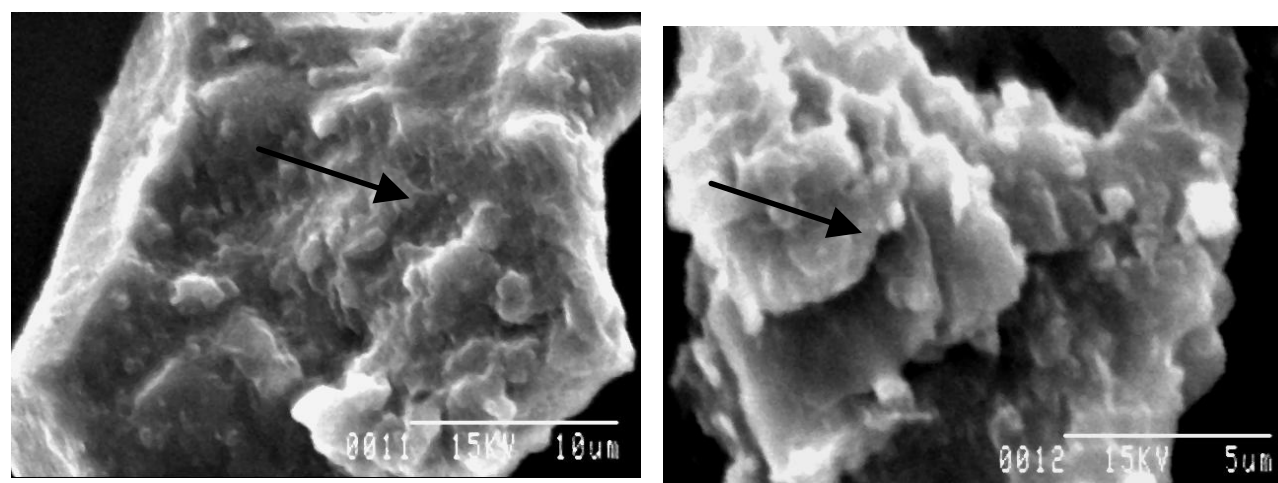


Figure 4. Scanning electron micrographic images of deformed *Rhodotorula mucilaginosa* grown in yeast extract peptone sucrose broth with 8 mM of potassium dichromate.

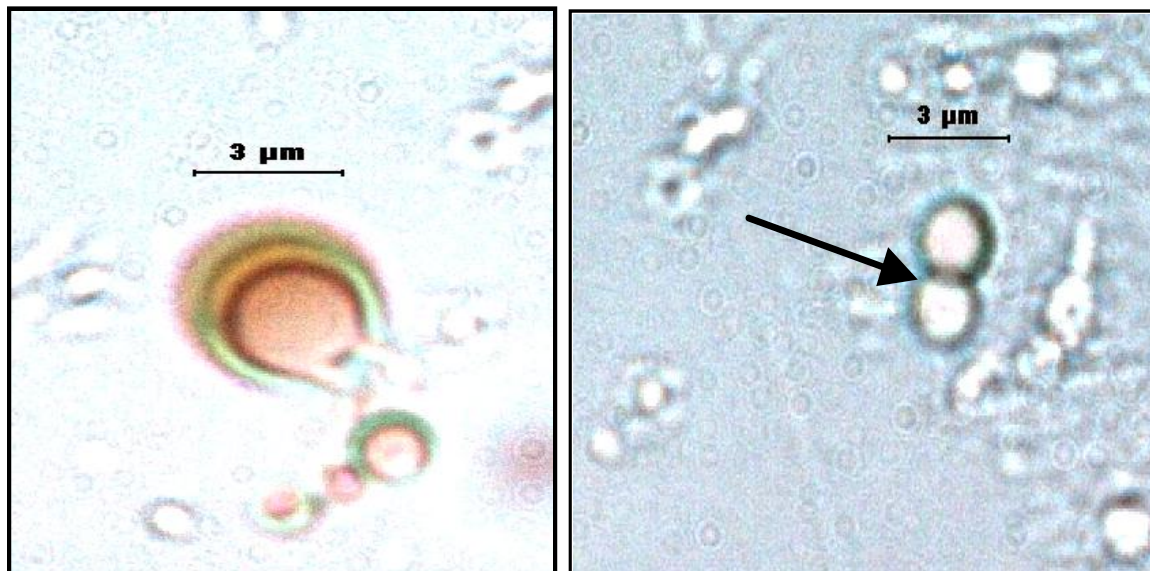


Figure 5. *Rhodotorula mucilaginosa* cell under Leica microscope (DMLB, Japan).

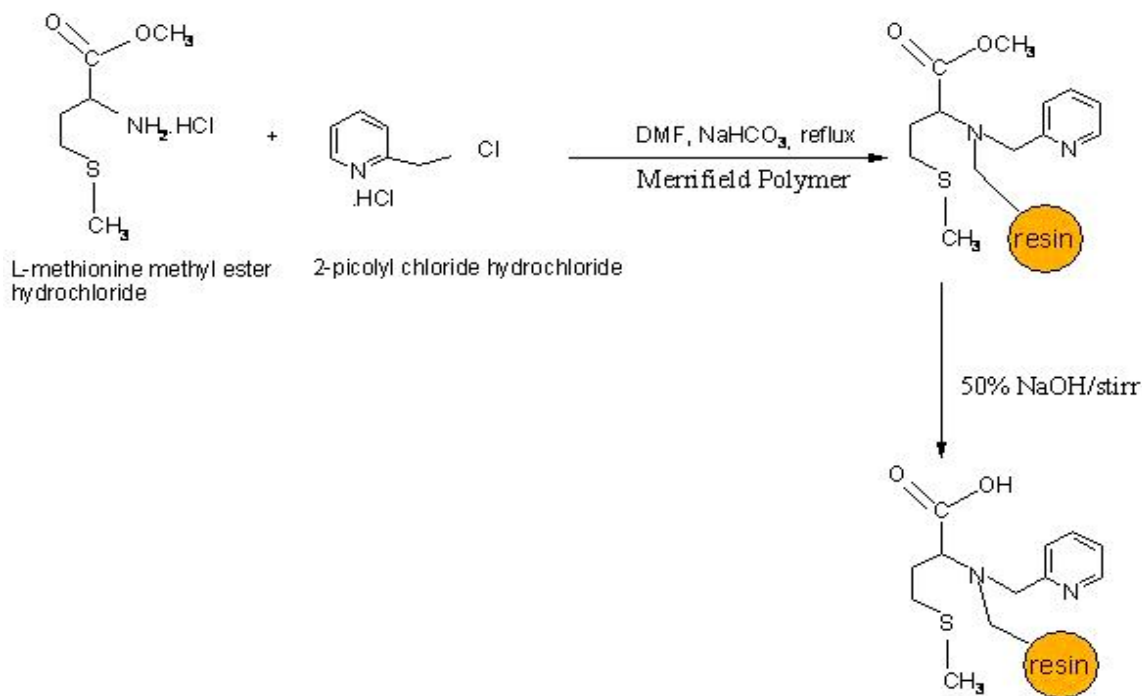


Figure 6. Structure of chemically synthesized resin.

acid part) and nitrogen from pyridine moiety may possibly be responsible for binding of Cr³⁺ at specific pH (Figure 6) (Das et al., 2010). It has been found that percent conversion of Cr⁶⁺ to Cr³⁺ was more in 2 μg ml⁻¹ potassium dichromate treated *R. mucilaginosa* cells (26.6%) and least in 8 μg ml⁻¹ potassium dichromate treated *R. mucilaginosa* cells (6.13%). Further experiments

suggests that as the concentration of Cr increased from 2 to 8 mM, the percent reduction of Cr⁶⁺ to Cr³⁺ also decreased as shown in Table 1 and Figure 13. This might be due to the toxicity imposed by the higher concentration of Cr⁶⁺ to the yeast cells and inhibited enzymatic reduction of Cr⁶⁺ to Cr³⁺. The pH of the metal solution also plays a crucial role in the passive microbial

Linear Sequence: 26S rDNA of D1D2

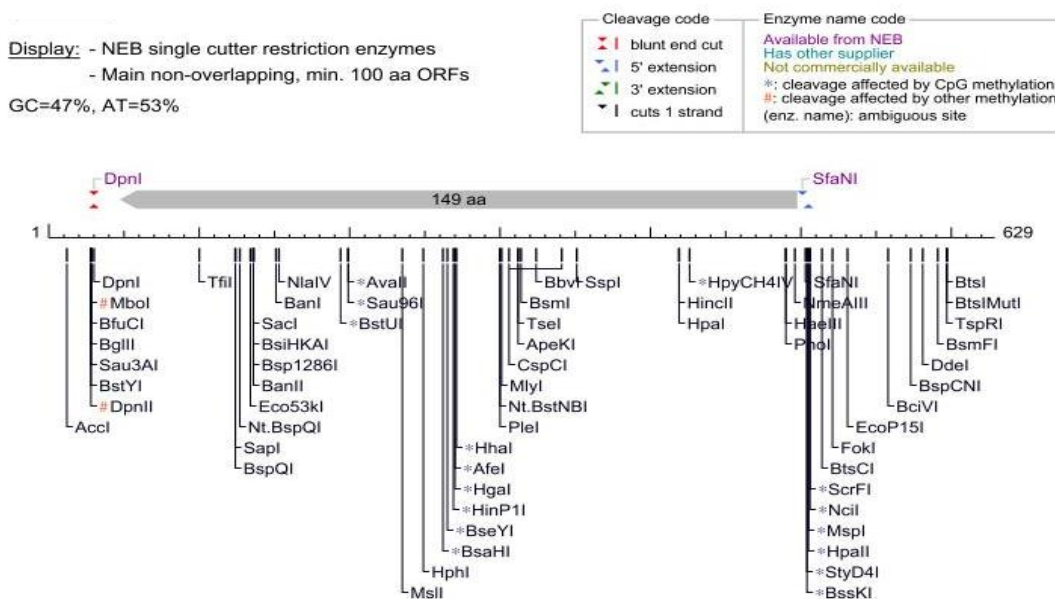


Figure 7. Different restriction sites of 629 sequences of D1/D2 Domain of 26S rDNA.

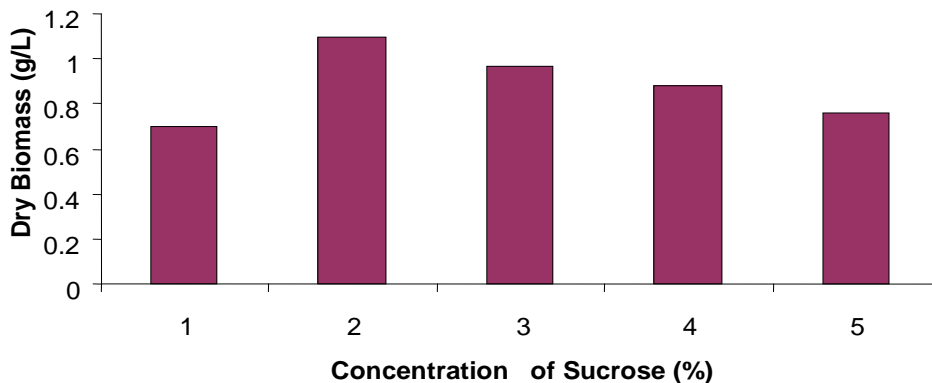


Figure 8. Effect of different concentration of sucrose on growth of *Rhodotorula mucilaginosa*.

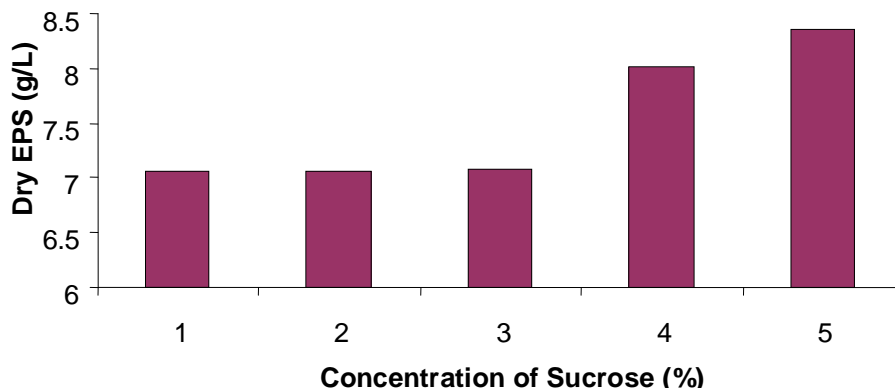


Figure 9. Different sucrose concentration (%) vs Dry EPS formation (g/L).

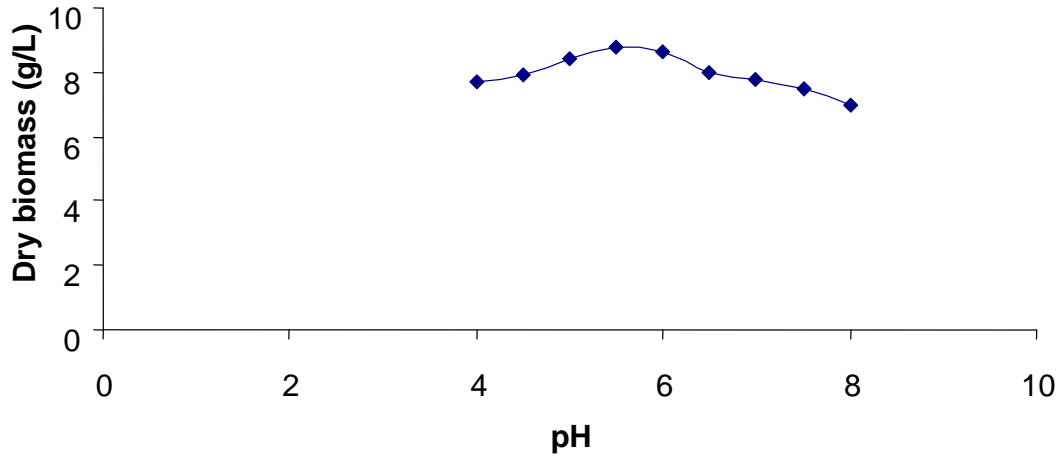


Figure 10. pH optimum for growth of *Rhodotorula mucilaginosa*.

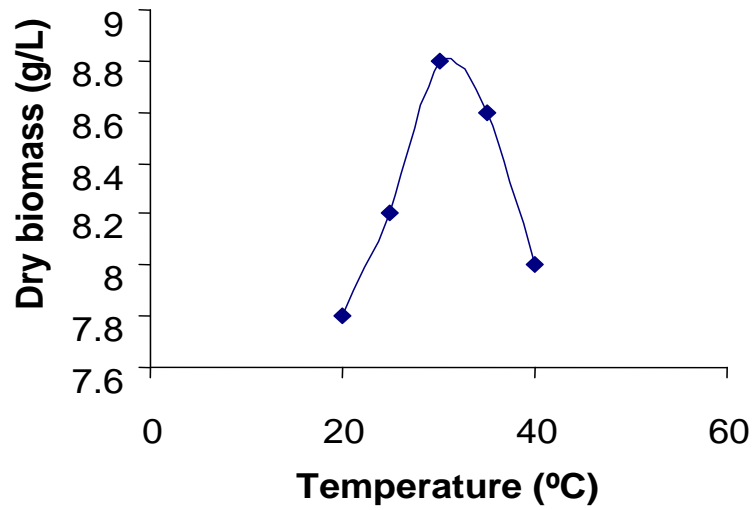


Figure 11. Temperature optimum for growth of *Rhodotorula mucilaginosa*.

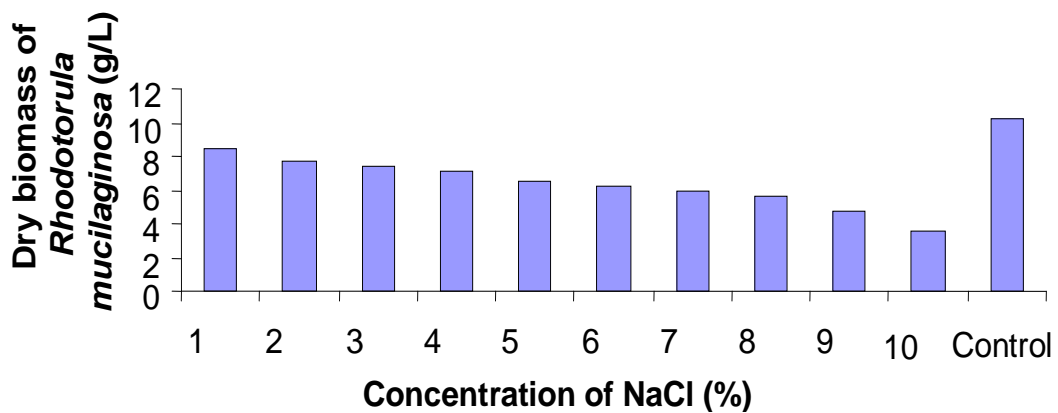
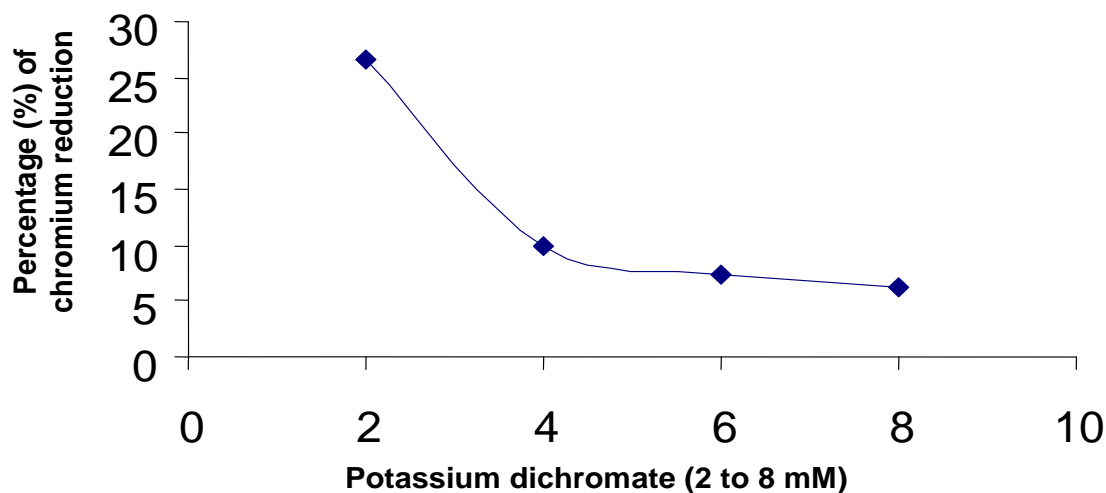


Figure 12. Effect of salt stress on growth of *Rhodotorula mucilaginosa*.

Table 1. The percentage reduction of Cr⁶⁺ to Cr³⁺ by *Rhodotorula mucilaginosa*.

Number of observation	Concentration of Cr ⁶⁺ (µg ml ⁻¹) originally present in the solution	Cr ⁶⁺ (mmol) present after treatment with yeast	Cr ³⁺ (mmol) formed	Percentage of Cr ⁶⁺ to Cr ³⁺ conversion
1	2.0	0.2182	0.058	26.6
2	4.0	0.14423	0.01278	9.90
3	6.0	0.4028	0.0299	7.43
4	8.0	0.5961	0.0365	6.13

**Figure 13.** Percentage of Chromium reduction from Cr⁶⁺ to Cr³⁺ by *Rhodotorula mucilaginosa*.

biosorption (Pardo et al., 2003). Affinity of anionic species towards the functional group present in cellular surface is affected by pH. At low pH values, cell wall functional groups are protonated and compete significantly with metal binding. As the pH increased further, the overall surface charges on the cells could become negative biosorption reduction. In general, these data indicate that the sorption capacity increased with increasing the initial metal ion concentrations corresponding to metal ions on the biomass surface. This sorption characteristic indicates that the surface saturation is dependent on the initial metal ion concentrations. At low concentrations, adsorption sites took the available metal more quickly (Fourest and Roux, 1992). However at higher concentrations, metals need more time to diffuse into the biomass surface by intra particular diffusion and greatly hydrolyzed ions will diffuse at a slower rate (Khambhaty et al., 2009; Bai and Abraham, 2001; Park et al., 2006; Tewari et al., 2005).

The present study demonstrated that this novel strain, *R. mucilaginosa* (MTCC 9315), effectively reduced Cr⁶⁺ to Cr³⁺ from industrial effluent and also exhibited salt stress tolerance. The same strain (*R. mucilaginosa*, MTCC 9315) is also capable of higher concentration of lead bioadsorption (Chatterjee et al., 2011). This investigation

is reporting for the first time a chemically synthesized resin for separation of Cr⁶⁺ and Cr³⁺. However, further studies with respect to metal-resin specificity, molecule responsible for conversion of chromium, chromium chelators applicability to various other types of metal-laden effluents and role of pigments if any in bioreduction of heavy metals will help in fine-tuning the bioremediation technology for large scale application.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the help of Prof. Pranab Roy, (Head Department of Biotechnology, the University of Burdwan) for the financial assistance and guidance. One of the authors Mr. Sabyasachi Chatterjee wishes to express gratitude to his wife Mrs. Pubali Chatterjee for her support throughout the work. The authors are thankful to the cooperation received from Dr. Subhrokanti Mukherjee (Department of Microbiology, The University of Burdwan), Dr. Debasish Das (Department of Chemistry, The University of Burdwan), Burdwan University Science Instrumentation Centre for providing SEM facility, Department of Environmental Science and Department of Chemistry, Burdwan University for Atomic

Absorption Spectrophotometric analysis.

REFERENCES

- Abdel NA, Moghaz EI (2010). Comparative study of salt tolerance in *Saccharomyces cerevisiae* and *Pichia pastoris* yeast strains. *Adv. Biores.* 1(1):170-177.
- Amany MA, Sawsan KH, Mosaad AWA (2006). Chromium-picolinate induced ocular changes: protective role of ascorbic acid. *Toxicology* 226:143-151.
- ATSDR (Agency for Toxic Substances and Disease Registry) (1989). Toxicological Profile for Chromium. Prepared by Syracuse Research Corporation under Contract 68-C8-0004. U.S. Public Health Service. ATSDR/TP-88/10.
- Bai SR, Abraham E (2001). Biosorption of Cr (VI) from aqueous solution by *Rhizopus nigricans*. *Bioresource Technol.* 79(1):73-81.
- Bragt PC, Van Dura EA (1983). Toxicokinetics of hexavalent chromium in the rat after intratracheal administration of chromates of different solubilities. *Ann. Occup. Hyg.* 27:315-322.
- Burrows D (1983). ed. Chromium, Metabolism and Toxicity. CRC Press, Inc., Boca Raton, FL. pp. 137-163.
- Chatterjee S, Gupta D, Roy P, Chatterjee NC, Saha P, Dutta S (2011). Study of a lead tolerant yeast strain BUSCY1 (MTCC 9315). *Afr. J. Micro-Biol. Res.* 5:5362-5372.
- Cherest H, Davidian JC, Thomas D, Benes V, Ansoerge W, Surdin-Kerjan Y (1997). Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. *Genetics* 145:627-635.
- Dae HC, Eui YK (2003). Characterization of Pb⁺² bioadsorption from aqueous solution by *Rhodotorula glutinis*. *Bioresour. Technol.* 25(5):271-277.
- Das S, Sahana A, Banerjee A, Lohar S, Karak D, Das D (2010). Pyridine appended l-methionine: A novel chelating resin for pH dependent Cr speciation with scanning electron microscopic evidence and monitoring of yeast mediated green bio-reduction of Cr(VI) to Cr(III) in environmental samples. *J. Hazard. Mater.* 185(2-3):1448-1457.
- Danielsson GRG, Hassoun E, Dencker L (1982). Embryotoxicity of chromium: Distribution in pregnant mice and effects on embryonic cells *in vitro*. *Arch. Toxicol.* 51:233-245.
- Douglas HB, Miriam EW, Michael S (1988). Variable expression of extracellular polysaccharide in the marine bacterium *Pseudomonas atlantica* is controlled by genome rearrangement. *Proc. Natl. Acad. Sci. USA.* 85:3923-3927.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith S (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350-356.
- Fourest E, Roux CJ (1992). Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. *Appl. Microbiol. Biotechnol.* 37(3):399-403.
- Guthrie BE, Langard S (1982). The nutritional role of chromium In Ed. Biological and Environmental Aspects of Chromium. Elsevier Biomedical Press, Amsterdam. pp. 117-148.
- Hamilton JW, Wetterhahn KE (1988). Chromium In: Handbook on Toxicity of Inorganic compounds, Seiler, H.G. and Sigel Eds., Marcel Dekker, New York. p. 239.
- Hohmann S, Mager PWH (1977). (Eds.) Yeast stress Responses. Topics in Current Genetics Vol 1.
- Iijima S, Matsumoto N, Lu CC (1983). Transfer of chromic chloride to embryonic mice and changes in the embryonic mouse neuroepithelium. *Toxicology* 26:257-265.
- Ivankovic S, Preussmann R (1975). Absence of toxic and carcinogenic effects after administrations of high doses of chromic oxide pigment in subacute and long-term feeding experiments in rats. *Fd. Cosmet. Toxicol.* 13:347-351.
- Khambhaty Y, Mody K, Basha S, Jha B (2009). Biosorption of Cr(VI) onto marine *Aspergillus niger*, experimental studies and pseudo-second order kinetic. *World J. Microbiol. Biotechnol.* 23:671-676.
- Ksheminska HP, Fedorovich D, Honchar T, Ivash M, Gonchar M (2008). Yeast tolerance to chromium depends on extra-cellular chromate reduction and Cr(III)-chelation. *Food Technol. Biotechnol.* 46:420-427.
- Ksheminska HP, Honchar TM, Gayda GZ, Gonchar MV (2006). Extra-cellular chromate-reducing activity of the yeast cultures. *Cent Eur. J. Biol.* 1:137-149.
- Langard S (1982). Absorption, transport and excretion of chromium in man and animals. In: Ed. Biological and Environmental Aspects of Chromium. Elsevier Biomedical Press, Amsterdam. pp. 149-169.
- Li JZ, Yuan LH, Hu DX (2008). Cadmium resistance in growing *Rhodotorula* sp. Y11. *Bioresource Technology* 99:1339-1344.
- Lindberg E, Hedenstierna G (1983). Chrome plating: symptoms, findings in the upper airways, and effects on lung function. *Arch. Environ. Health* 38:367-374.
- Love AHG (1983). Chromium - biological and analytical considerations. In: Burrows, D., Ed. Chromium: Metabolism and Toxicity. CRC Press, Inc., Boca Raton, FL, p. 1.
- MacKenzie RD, Byerrum RU, Decker CF, Hoppert CA, Langham RF (1958). Chronic toxicity studies II. Hexavalent and trivalent chromium administered in drinking water to rats. *Arch. Ind. Health* 18:232-234.
- Matsumoto N, Iijima S, Katsunuma H (1976). Placental transfer of chromic chloride and its teratogenic potential in embryonic mice. *J. Toxicol. Sci.* 2:1-13.
- Muneer B, Rehman A, Shakoori FR, Shakoori AR (2009). Evaluation of Consortia of Microorganisms for efficient removal of hexavalent Chromium from Industrial Wastewater. *Bull. Environ. Contam. Toxicol.* 82:597-600.
- Pardo R, HerGuedas M, Barrado E, Vega M (2003). Biosorption of cadmium, copper, lead and zinc by inactive biomass *Pseudomonas putida*. *Anal. Bioanal. Chem.* 37 (1): 26-32.
- Park D, Yun Y, Yim KH, Park JM (2006). Effect of Ni(II) on the Reduction of Cr(VI) by *Ecklonia* Biomass. *Bioresource Technology.* 97:1592-1598.
- Pedersen NB (1982). The effects of chromium on the skin. S. Langard, Ed. Biological and Environmental Aspects of Chromium. Elsevier Biomedical Press, Amsterdam. pp. 249-275.
- Porter S, Yuille J C, Lehman DR (1999). The nature of real, implanted, and fabricated memories for emotional childhood events: Implications for the recovered memory debate. *Law Hum. Behav.* 23:517-537.
- Puzon GJ, Roberts AG, Kramer DM, Xun L (2005). Formation of soluble organochromium(III) complexes after chromate reduction in the presence of cellular organics. *Environ. Sci. Technol.* (39)8:2811-2817.
- Schroeder HA, Balassa JJ, Tipton IH (1962). Abnormal trace metals in man: chromium. *J. Chron. Dis.* 15:941.
- Stearns DM, Wise JP Sr, Patierno SR, Wetterhahn KE (1995). Chromium (III) Picolinate produces chromosome damage in Chinese hamster ovary cells. *FASEB J.* 9:1643-1648.
- Stern RM (1982). Chromium compounds; production and occupational exposure. In: Langard, S., Ed. Biological and Environmental Aspects of Chromium. Elsevier, New York. pp. 7-47.
- Tewari N, Vasudevan P, Guha BK (2005). Study on biosorption of Cr(VI) *Mucor hiemalis*. *Biochem. Eng.* 23:185-192.
- U.S. Air Force (1990). Chromium. In: The Installation Restoration Program Toxicology Guide. Wright-Patterson Air Force Base, OH, pp. 72-81
- Vincent JB (2003). Chromium (III) complexes. *Drugs Future* 28:1079-1086.
- Yamamoto Y, Ueda E, Kojimaa Y, Hiromu Sakurai H (2004). The action mechanism of zinc (II) complexes with insulinomimetic activity in rat adipocytes. *Life Sci.* 75(6):7.