Removal of Cr (VI) by a halotolerant bacterium *Halomonas* sp. CSB 5 isolated from sāmbhar salt lake Rajastha (India)

P. Chandra and D. P. Singh✉

Department of Environmental Science Babasaheb Bhimrao Ambedkar (A Central) University, VidyaVihar, Lucknow (U.P.)-226005, India

Corresponding author: D. P. Singh. Department of Environmental Science Babasaheb Bhimrao Ambedkar (A Central) University, VidyaVihar, Lucknow (U.P.)-226005, India. Email: dpsingh_lko@yahoo.com

Abstract

A halotolerant chromate reducing bacterium CSB 5 isolated from the Sambhar Salt Lake (Rajasthan) was identified as *Halomonas* sp. CSB 5 by 16SrDNA sequence analysis. The isolate could tolerate up to 25% NaCl (w/v) and100 $\mu g\ mL^{-1}$ of Chromium hexavalent in the complex medium (CM). Removal of Cr (VI) at concentration of 20, 40 and 60 $\mu g\ mL^{-1}$ was found to be 98%, 90.2%, and 65.7% within a period of 60 hour.Effect of factors like pH and temperature on the total removal of Cr (VI) at 60$\mu g\ mL^{-1}$concentration showed maximum removal at pH 8.0 (77.9 %) and temperature 35°C (82.6%) in 60 hour. The maximum intracellular uptake and surface adsorption (3.22 ± 0.09 and 14.16 ± 0.49 $\mu g\ mg^{-1}$ dry wt.) in CMB 5 bacterium was observed at 75 $\mu g\ mL^{-1}$ of Cr (VI) concentration in 45 min.The results showed that contribution of surface binding was about 4-5 fold higher than the intracellular uptake. Bacterium showed concentration dependent increase in the chromate reductase activity with saturating rate at 60 $\mu g\ mL^{-1}$ concentration of chromium. The values of Km and Vmax with NADH of the bacterium was found to be 0.818 $\mu g\ mL^{-1}$ and 0.085 $\mu g\ mL^{-1}.min^{-1}.mg^{-1}$ protein, respectively. Fourier Transform Infrared Spectroscopy (FTIR) analysis of Cr (VI) binding on membrane surface showed changes in wavenumber between 3300-2800 cm^{-1}due to involvement of proteins and fatty acids in the binding of Cr (VI). The absorption peaks at wavenumbers 1654.9, 1580.3 cm^{-1}, 1248 and 1085.6 cm^{-1} shifted to lower frequencies due to interaction of Cr (VI) with functional groups like amides and phosphodiester. These results suggested that the isolated CSB 5 bacterium can be used as potential bioremediating agent for removal of Cr (VI) in salt loaded waste water.

Key words: Bioremediation, Chromium hexavalent, FTIR, Functional groups, *Halomonas* sp. CSB 5, Chromate reductase.

Introduction

Hexavalent chromium Cr (VI)is highly water soluble and is widely used in various industries including electroplating, leather, tanning, dye and pigment manufacturing etc. (1, 2). Leather processing industries (LPI) use chromium (chrome liquor or chrome powder) for tanning of rawleather and residual chromium is discharged in solid or liquid effluents. Soluble Cr (VI) is extremely toxic.If ingested in excess, it causes irritation in the stomach, which may ultimately lead to ulcers and damage to kidney and liver (3). Cr (VI) is mutagenic, carcinogenic, persistent in nature, and has toxic effects on biological systems due to its strong oxidizing nature (4). The Cr (III) form is less toxic and less bioavailable than Cr (VI), as it readily forms insoluble oxides and hydroxides above pH 5 (5,6).The removal of toxic metals by the Physico-chemical methods includes reverse osmosis, solvent extraction, lime coagulation, ion exchange, chemical precipitation, and membrane separation process, filtration, and incineration (7). All of these methods have many drawbacks, for instance, recurring expenses with elevated capital investments, incomplete metal removal, high energy requirement, generation of toxic sludge or other waste products that require safe disposal (8).

Recently, microorganisms have been reported as biological remediators and adsorbents to remove heavy metals from wastewater (9). Microorganisms can also cause chemical biotransformation of toxic metals which helps in detoxification of contaminated sites (10). Microbial reduction of Cr (VI) reduction was first reported in the late 1970s, when Romanenko and Koren'kov observed Cr (VI) reduction capability in *Pseudomonas* sp. grown under anaerobic conditions (11). Since then, several researchers have isolated new microorganisms that catalyze Cr (VI) detoxification under varying conditions (12, 13). Biosorption is the first stage in metal accumulation involving adsorption of metal onto the cell walls of microorganisms (14), followed by biotransformation of Cr (VI) to relatively nontoxic Cr (III) form by chromate reductase enzyme (12, 15, 16).The presence of high salinity (1–10% NaCl by w/v.) in the waste water stream hinders the biological treatment.Several groups have made significant contribution to biological treatment of saline wastewater. Kargi and Dincer treated waste waters rich in halogenated organics at different salt concentrations and showed COD removal decreased with increasing salt concentration (17). Reduction of Cr (VI) by *Bacillus* sp.PB2 in the presence of NaCl showed that removal efficiency was inversely proportional to NaCl concentration (18). Toxic materials present in saline conditions could impose an important challenge for treatment of industrial waste water as the soluble salts pose a serious problem for survival of microbes. The aim of present study was to isolate a halotolerant bacterium exhibiting both salt as well as Chromium (VI) tolerance, so that it can be exploited for bioremediation of Cr (VI) in saline waste water stream.

Materials and methods

Isolated bacterial strain and culture conditions

The bacterium used in this study was isolated from Sambhar Salt Lake, situated at 26° 52′ - 27° 2′ N, 74° 53 ′ - 75° 13′ E in the middle of a closed depression

in Aravalli schists in Rajasthan, India. Isolation of the bacterial culture was carried out by culture enrichment technique. Isolate was grown in CM media used for halophiles (19).The medium containing Yeast extract-10, KCl -2, FeCl$_3$ - 0.02, Casamino acid -7.5, tri-sodium citrate-3.0, MgSO$_4$ 7H$_2$O – 20, NaCl -100 (g L^{-1}) and supplemented with K$_2$Cr$_2$O$_7$ 20 µg mL^{-1} in the pH was maintained at 8± 0.02 by using HCl or NaOH and the flasks were incubated at room temperature for 24 h on an orbital shaker. After 24 h, the enriched bacterial strain was isolated by plating it on agar plate. The isolated strain was identified by biochemical and 16S rDNA analysis.

Characterization and identification by 16S rDNA gene Sequencing

After growth of the bacterial isolate on a solid medium, Catalase and oxidase activities were determined following Smibert and Krieg (20). The isolate was identified by 16S r DNA sequencing usingUniversal primers (forward primer; 27 F 5'- AGA GTTT GAT CMT GGC TCAG -3' and reverse primerwith modification; 1492 R 5'- TA CGG YTA CCT TGT TAC GAC TT-3').

Preparation of stock solution and reagent

Stock solution of Cr (VI) (1000 mgL^{-1}) was prepared by dissolving 2.829 g of K$_2$Cr$_2$O$_7$ in 1000 mL of Millipore Water and pH of the solution was adjusted to 6.5 using 0.1 M HCl or NaOH. For the preparation 1, 5-diphenylcarbazide solution (0.25 g) was added in 50 mL of acetone to minimize the deterioration and reagent was stored in sterilized brown colored bottle.

Growth measurement of culture at different NaCl Concentrations

The CM broth with different initial concentration of NaCl ranging from (0 %, 5%, 10%, 15%, 20% and 25% w/v) at pH 8.0 was inoculated with overnight grown bacterial culture to obtain an optical density of 0.05 and incubated at 35°C. Aliquots (3 mL) were withdrawn at regular time interval and were centrifuged at 8000 × g min^{-1} for 10 min at room temperature. Thebacterial growth was determined by measuring the absorbance of bacterial culture at 600 nm against distilled water as blank.

Measurement of growth andchromium removal

The CM broth with 10 % NaCl (w/v) and different initial concentration of chromium ranging from 20 to 100 µg mL^{-1} were inoculated with bacterial culture to obtainan optical density of 0.05 and incubated at 35°C under shaking condition (150 rpm). Aliquots (3 mL) were withdrawn at regular time interval and were centrifuged at 8000 × g min^{-1} for 10 min at room temperature. The supernatant was used to measure the residual chromium concentration at 540 nm (21). The growth was determined by measuring the absorbance of bacterial culture at 600 nm against blank. During the growth, viability and contamination of culture was checked by plating the culture on agar plates.

Effect of temperature

Overnight grown culture under shaking condition (Optical density = 0.05) inoculated in CM broth with 10 % NaCl (w/v), pH 8.0 and 60 µg mL^{-1}chromium concentration at different temperature 25, 30,35 and 40 °C.Aliquots (3 mL) were withdrawn at regular time interval and analyzed for growth as well as residual chromium concentration in the medium.

Effect of pH

The CM broth supplemented with 10% NaCl (w/v) and 60µg mL^{-1} concentration of Cr (VI) under different set for pH conditions (6-10), were inoculated with overnight grown bacterial culture to obtain an absorbance of 0.05. Aliquots (3 ml) were withdrawn at regular time interval to monitor the growth as well as residual level of chromium in the medium.

Cr (VI) uptake and adsorption by isolated strain

Mid exponential phase bacterial culture were harvested and after centrifugation in a (REMI Centrifuge Mumbai, India) at 8000 g for 10 min. and pellet dried in hot air oven at 40 °C overnight. In order to study the uptake and adsorption of Cr (VI) under different concentrations 15 -75 µg mL^{-1} using 1 mg mL^{-1}dry weight of cells (22). Samples were withdrawn at regular intervals of 15 min up to 60 min. The cell suspension was centrifuged and supernatant was discarded. Pellets were washed with (EDTA) 2 mM solution and centrifuged. The supernatant was accounted as EDTA washable metal fraction (adsorption). Intracellular Cr (VI) concentrations were determined in the EDTA washed pellets after digesting it with 10-15 mL HNO$_3$ and HClO$_4$ (ratio of 3:1) on hot plate at 60-80 °C until mixture became colorless. The solution was filtered through Whatman No. 42 filter paper before analysis. The accumulation and adsorption of chromium concentrations was expressed in terms of in µg mg^{-1} dry biomass after determination by Atomic absorption spectrophotometer (AA 240 FS: Fast Sequential AAS Varian, Netherland) at a wavelength 357.87 nm. The uptake rate was expressed as µg Cr (VI) mg-1 dry wt.

Preparation of cell-free extract

Cell free extracts (CFE) of CSB 5 bacterial strain was prepared by following the modified procedure described before (23). Chromium induced mid-exponential phase cells were harvested and washed twice with 10 ml of 50 mM potassium phosphate buffer (pH 7.0) and frozen in liquid nitrogen by dropping the1 gram cell pellet in liquid nitrogen for 6-8 min. To harvest the broken cells from the jar, a precooled spatula (dipped in liquid nitrogen) was used. The broken samples were added in 10 mL of phosphate buffer and centrifuged (10000 × g, 4°C, and 20 min.). The collected supernatant filtered through a 0.2 µm cellulose acetate membrane filter was used as a CFE. Total protein in the enzyme sample was estimated as described before (24), using bovine serum albumin (BSA) as standard.

NADH dependent chromate reductase assay

Activity of chromate reductase in crude cell extract was measured using Nicotinamide adenine dinucleotide (NADH) as an electron donor. The reaction mixture (1 ml) contained different concentration of Cr (VI) (15-90 µg mL^{-1}) in 800 µl of 50 mM potassium phosphate buffer (pH 7.0), and 50 µl of 0.1 mM NADH. An aliquot of 200 µl of CFE was added to initiate the reaction.

Table1. Morphological, physiological and biochemical characteristics of the isolate.

Morphological and Biochemical Test

Name of the strain	*Halomonas* sp.	Amylase	−
Cell shape	Rod shaped (laterally flagellated)	Inositol	−
		Fructose	+
	Yellow	Gelatin hydrolysis	−
Pigmentation	Motile	Indole production test	−
Motility	Negative	Pectinate	−
Gram nature	0-15	Citrate utilization	−
Optimum NaCl (% w/v) Conc. for growth	0-20	Methyl Red and Voges Proskauer test	−
Range of NaCl (%) conc.which support growth		Cellulose production test	−
Optimum pH for growth	7-8	Urease test	+
Optimum temperature		Maltose	−
Catalase	30-35	Sucrose	−
Acid form Carbohydrate	+	Lactose	−
	-	Mannitol	−

An assay mixture with given composition above except CFE or NADH was used as respective controls. Cr (VI) was quantified colorimetrically using DPC as the complexing reagent (25). One unit of chromate reductase activity of enzyme which reduced 1.0 μg Cr (VI) per minute. The concentration dependent chromium reductase activity was measured. The K_m and V_{max} values were derived from Lineweaver-Burk plot equation [$1/V = K_m/V_{max}.1/S + 1/V_{max}$].

Measurement of Cr (VI)

Chromium concentration in the supernatant was measuredwith time using hexavalent chromium specific colorimetric reagent 1, 5- diphenylcarbazide (DPC) 0.25% (w/v) as described elsewhere (21).The reaction mixture was set up in 10 mL volumetric flask as follows: 200 μL or 400 μL sample (supernatant) volume was made to 1 ml using glass distilled water, followed by addition of 330 μL of 6 M H_2SO_4 and 400 μL of DPC and final volume was made to 10 mL using Millipore water.The mixture of solution was shaken for 30 second to form a bright pink color, After 5 minutes the absorbance of the solution was read at 540 nm in UV-vis Spectrophotometer a model no. (1601 Shimadzu, Japan).

Measurement of FTIR spectra

Mid-exponential phase bacterial culture was collected by centrifugation at 7000 rpm for 10 minutes at 4°C, filter-sterilized Cr (VI) of different concentration (30 and 60 ppm) was added to the medium and incubated for 30 minutes. Control experiment consisted of cells without addition of Chromium. The aliquots of bacterial suspensions was withdrawn, centrifuged and washed three times with Millipore water and dried in hot air oven at 60°C for 8 hours. The dried bacterial cell samples and Potassium bromide (KBr) (1/100 ratio) were grinded pressed to form pellet by using a manual hydraulic press (150 lb).The IR spectra (400-4000 cm^{-1}) were obtained with resolution of 5-7 cm^{-1} with the 32 scan number for each spectrum, using (FTIR) (Thermo-Scientific Nicole 6700, USA).

Results

Isolation and characterization of bacterial strain

The bacterial strain CSB 5 was isolated from sambhar salt lake (saline water). The strain was a Gram-negative heterotrophic microorganism that formed sandy color, rod shape colonies with 1.5-2.0 mm in diameter (Table 1). Based on 16S rDNA gene sequence, the isolate CSB 5 was assigned to the genus *Halomonas*, which is included in the group Proteobacteria, subgroup Gamma, in the family of Halomonadaceae. The distance is measured in the phylogram along the horizontal branches. The phylogenetic tree provided evidence of 100% sequence homology of the test organism to *Halomonas* sp. CSB 5. A partial (1410 bp) 16S r DNA sequence was determined for the bacterial isolate CSB 5. The sequence was submitted in the Gen-Bank database with the accession number:KF 322103. The consensus sequence of isolate CSB 5 was compared with those deposited in Gen Bank using the BLAST program (26).The closest relative of the strain CSB-5 was *Halomonas* sp. (AB305208.1) and *Chromohalobacter salexigens* strain *(*HE662816.1).

Effect of NaCl and Cr (VI) on growth

The growth of isolate was measured in the presence of varying NaCl concentrations (0-25% w/v). In general the isolate exhibited NaCl dependent decline in the growth upto 25 % NaCl. However a sluggish growth continued upto 20 % NaCl concentration (Fig. 1A). These results revealed the halotolerant nature of the isolate. Maximum growth at all the concentrations of NaCl was observed after 72 hours of incubation. The growth of isolated bacterium at various concentrations of Cr (VI) (0-100 μg mL^{-1}) showed a concentration dependent decrease in the growth (Fig. 2b).A 50% growth inhibitory concentration of Cr (VI) was at 80 μg mL^{-1}.
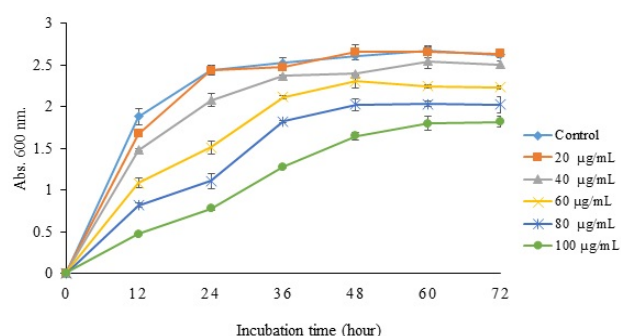
Removal of Cr (VI)

Bacterial removal of chromium at 20, 40 and 60 μg mL^{-1} concentration ofCr (VI) was found to be 98 %, 90.2%, 65% respectively, within a period of 60hour (at pH 8 and 35°C).The results showed that percent removal of chromium decreased with increase in Cr (VI) concen-

A
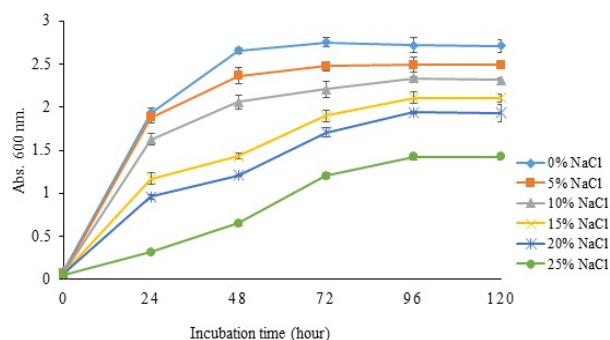
B

Figure 1. (A) Growth of bacterium CSB 5 at different NaCl (0-25%) concentrations at pH 8.0 **(B)** bacterial growth at different concentrations (0 -100 µg mL⁻¹) of Cr (VI). Results are expressed the mean ± SE of 3 replicates, significant at p ≤ 0.05.

tration, perhaps due to adverse effect of Cr (VI) on the microbial population. Results on time dependent measurement (Fig. 2) of residual Cr (VI) at initial concentrations of 20, 60, 80 and 100 µg mL⁻¹ exhibited that percent removal of Cr (VI) increased with increasing time of incubation.

Effect of pH and temperature on Cr (VI) reduction by bacterial strains

The Cr (VI) removal by CSB 5 was evaluated under four different temperatures, namely, 25, 30, 35 and 40°C (pH 8.0) for 60 hour. The Cr (VI) removal reached to optimum level (82.43, 82.60 %) at 30 and 35 °C, respectively (Fig.3A).An increase or decrease in temperature beyond the optimum temperature led to decline in Cr (VI) removal efficiency. Similarly, Chromium removal was studied under different pH (6.0, 7.0, 8.0, 9.0 and 10) conditions. Result showed the maximum removal
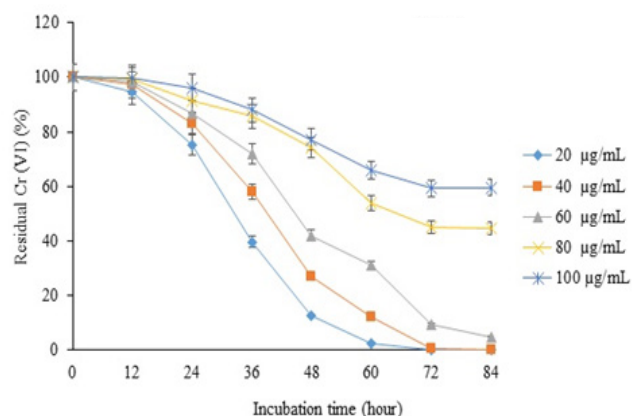
Figure 2. Percent residual of Cr (VI) (20-100 µg mL⁻¹) concentrations at different time interval. Results are expressed the mean ± SE of 3 replicates, significant at p ≤ 0.05.

of chromium between pH 7.0 to 8.0 (73.48 and 77.9 %), respectively. The metal removal efficiency declined at pH 6.0, 9.0 and 10.0 (Fig. 3B). The bacterial growth optima was found to be commensurating with pattern of pH or temperature dependent Cr (VI) removal. Apparently, there was a direct relationship between growth and chromium removal efficiency. These result indicated that the bacterium CSB5 requires optimum pH and temperature conditions for its growth as well as Cr (VI) removal.

Uptake and adsorption of Cr (VI) by dry Cells

The concentration dependent of intracellular uptake and surface adsorption of Cr (VI) in *Halomonas* sp.CSB 5 was studied in synthetic solutions of Cr (VI) containing different concentrations (15-75 µg mL⁻¹).The results showed (Fig. 5) highest uptake and adsorption rate of Cr (VI) after 45 min. of incubation. The uptake and adsorption of metal was increased with the increase in Cr (VI) concentration of the solution.The maximum uptake and adsorption (3.22 ± 0.09 and 14.16 ± 0.49 µg mg⁻¹ dry wt.)was observed at 75 µg mL⁻¹concentration of Cr (VI).
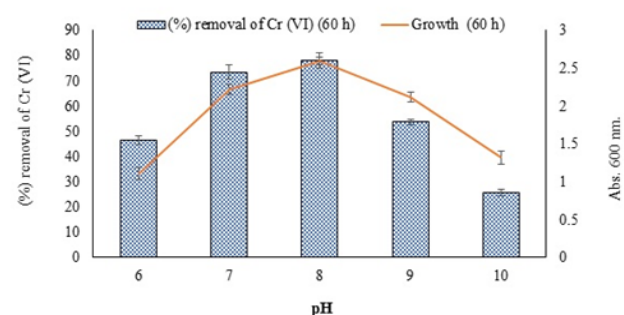
Chromate reductase activity

The crude cell free extract (CFE)was used to measure chromate reductase activity against different concentrations of chromate. The chromate reductase activity in CFE was about 2-3 fold higher in the cells pretreated with hexavalent chromium when compa-

A
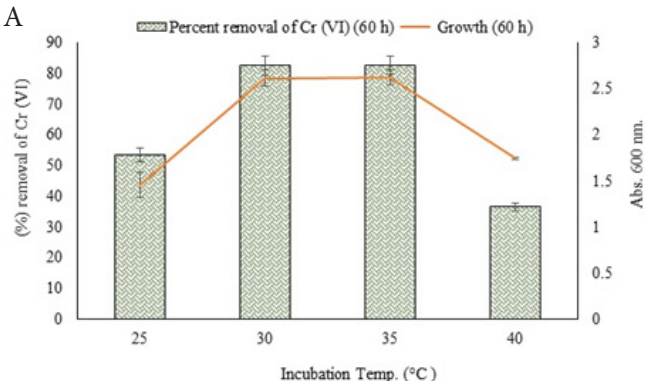
B

Figure 3. (A) Growth and percent removal of Cr (VI) by *Halomonas* sp. CSB 5 at different temperature (25-40 °C). The medium supplemented with 60 µg mL⁻¹Cr (VI) and 10% NaCl was incubated for 60 hour at pH 8.0. Results are expressed the mean ± SE of 3 replicates, significant at p ≤ 0.05. **(B)** Growth and percent removal of Cr (VI) by *Halomonas* sp. CSB 5 at different pH (6-10). The medium supplemented with 60 µgmL⁻¹ Cr (VI) and 10% NaCl was incubated for 60 hour at 35°C. Results are expressed the mean ± SE of 3 replicates, significant at p ≤ 0.05.
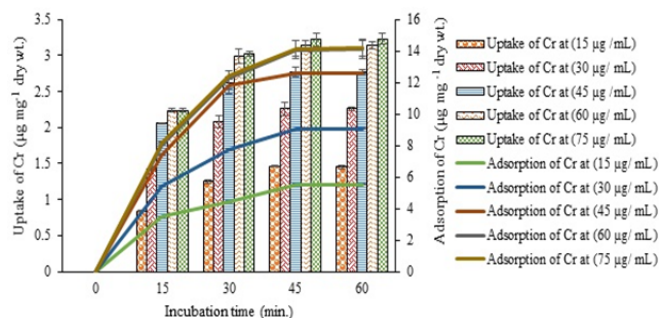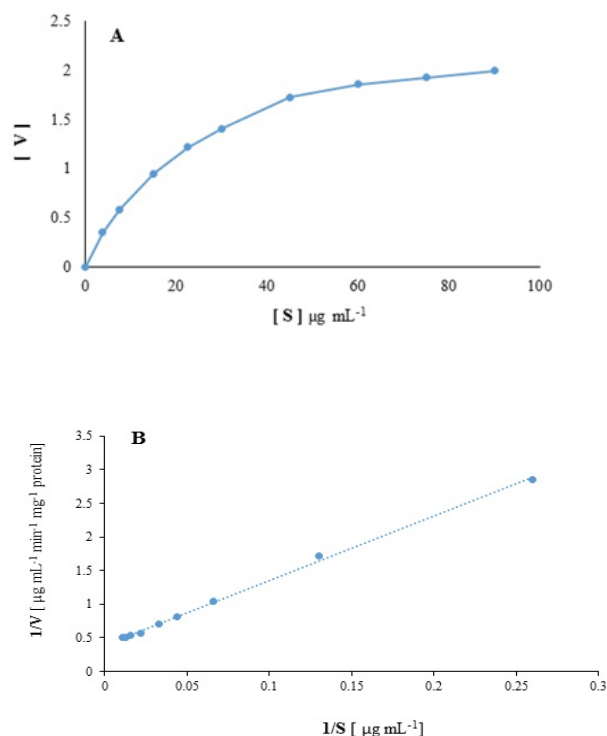
Figure 4. Uptake and adsorption of Chromium by dry biomass of *Halomonas* sp. CSB 5 exposed to different concentrations (15, 30, 45, 60 and 75 µg mL^{-1}) at pH 8.0. Results are expressed as mean ± SD of 3 replicates, significant at p ≤ 0.05.

Figure 5. The Figure 5A represents the Michaelis-Menten saturation kinetics for Chromate reductase activity (Rate of chromate reduction versus chromate concentration i.e., 0-100 ug mL^{-1}). The Figure 5B represents the Lineweaver- Burk plot of the chromate reductase activity of *Halomonas* sp. CSB 5.The values of **1/V** vs **1/S** were derived from Figure 5A.

red with that of without Cr (VI) treatment. The rate of NADH supported chromate activity reached to saturation level within 45 minutes of the incubation.Cr(VI) reduction by chromate reductase showed strong dependence on substrate concentration (15 to 45µg mL^{-1}). The rate of Cr(VI) reduction was the highest (62%) and at 60µg mL^{-1}concentration of Cr (VI). The initial velocity of chromate reductase activity linearly increased with chromate concentration, which followed Michaelis -Menten saturation kinetics with saturating rate at 60 µg mL^{-1} concentration of Cr (VI) (Fig. 5A). The values of Michaelis -Menten Constant (Km and Vmax) were calculated from the Lineweaver-Burk plot (Fig. 6B). The values of Km and Vmax in CFE in the presence of NADH were 0.818 µg mL^{-1} and 0.085 µg.min^{-1}.mg^{-1}protein, respectively.

IR spectral analysis

The IR spectra of the *Halomonas*sp.CSB 5 cells treated with 30 and 60 µg mL^{-1} concentration of Cr (VI) were obtained (Table. 2 and Fig. 6). The results showed changes in the peakat 3863.2 cm^{-1}in the cells (without Cr (VI) treatment). Which shifted to lower wavenumbers 3800.0 and 3729.1 cm^{-1} in the presence of 30 µg mL^{-1} chromium. This result suggested involvement of OH stretching in Cr (VI) binding (27). However, both the peaks disappeared at higher concentration of chromium (60 µg mL^{-1}). The wavenumber 3654.3 and 3465.6 cm^{-1} depicting slightly broad peaks in the untreated cells, indicated assigned to overlapping of hydroxyl and amines stretching on the bacterial surface (28), which completely disappeared due to chromium treatment. Further, emergence of new peaks at 3290.3, 3295.5 cm^{-1} mainly contributed by N-H stretching in the amide -A proteins suggested for involvement of membrane proteins in chromium binding (59). After treatment of chromium by 30 and 60 µg mL^{-1} concentration, new peaks appeared at 2959.2, 2926.7, 2856.2 and 2963.8, 2932.1 cm^{-1} which occurred due to adsorption of chromium by methyl groups of proteins and the fatty acid (membrane phospholipid) regions (29).The shifting of these bands indicated for the involvementof N-H and C-H of phospholipids and some amino acid side-chain vibrations in the adsorption process (30). The amide-I band at wavenumber 1654.9 cm^{-1}in untreated cells is shifted to 1651.5 cm^{-1} due to C=O stretching vibrational modes of the α-helical segment of protein at lower concentration

of chromium,but this band completely disappearedat higher concentrations of Cr (VI). The absorption peak at wavenumber 1580.3 cm^{-1}shifted to lower wavenumber 1540.7 and 1539.4 cm^{-1} due to adsorption of chromium. This result revealed an involvement of N-H stretching and C-N bending vibration in chromium binding. The wavenumbers 1454.5 and 1449.1 cm^{-1} appeared in the presence of chromium due to the asymmetric bending of –CH$_3$ attributed to methyl groups of proteins moiety (31). A characteristic peak of -COOH group often observed around 1403.8 cm^{-1}in untreated cells shifted to lower wavenumbers 1401.3 and 1403.3 cm^{-1}at 30 and 60 µg mL^{-1} chromium,indicated the symmetric stretching vibrations of −COO$^-$ functional groups of side chain amino acid, free fatty acids, or other derivatives (30). The absorption peak at wavenumber 1248.0 cm^{-1} shifted to lower frequencies 1239.3 and 1239.0 cm^{-1} due to adsorption of chromium by phosphodiester, and free phosphate (32). Emergence of a new absorption band at 1110.4 cm^{-1} was attributed to β (1-3) polysaccharide, indicating the role of polysaccharide in Cr (VI) binding (33). The involvement of absorption peak at wavenumber 1085.6 cm^{-1}, mainly contributed by phosphodiester group of nucleic acid, membrane phospholipids and amide- III, shifted to 1083.4 cm^{-1}at 30 µg mL^{-1} Cr (VI) concentration, suggesting major role of these functional groups in the surface binding of Cr (VI) (32).

Discussion

The halotolerant and Cr (VI) resistant strain *Halomonas* sp. CSB 5 isolated from Sambhar salt lake was identified by using 16S rDNA sequence analysis.Earlier the Cr (VI) resistant strain of *Halomonas* sp. MV-2007

Table 2. Assignment of functional groups associated with major vibration bands in mid-IR spectra of *Halomonas* sp.CSB 5.

Representing Range of Wavenumber in cm⁻¹	Control	30 ppm	60 ppm	Band Assignment
	Observed Wavenumber (cm⁻¹) at different concentration of Cr (VI)			
3800 -3500	3863.2	3800.00	0	O-H stretching of hydroxyl groups (27)
0	3654.3	0	0	O-H stretching vibrations (hydrogen bonding network may vary) (53)
3500 -3400	3465.6	0	0	O-H (asymmetric), N-H stretching (54)
3300 -3260	0	3290.3	3295.5	O-H (associated), N-H stretching (Amide- A) (54,59)
2960 -2950	0	2959.2	2963.8	CH_3 of lipids, C-H stretching (asym.) of the methyl groups from cellular proteins (53)
2930 -2920	0	2926.7	2932.1	CH_2 stretching (asym.) (30)
2862 -2843	0	2856.2	0	(C-H) of $> CH_2$ symmetric stretching (30)
2400 -2300	2371.0	2362.2	0	Asymmetric stretching band of CO_2 hydrates (50,59)
1670 -1630	1654.9	1651.5	0	Amide I band, C=O stretching of proteins in the form of α-helix (55)
1580 -1490	1580.3	1540.7	1539.4	Amide II band, N-H in plane bending , β sheet of amide (56,57)
1460 -1425	0	1454.5	1449.1	Asymmetric CH_3 bending of the methyl groups of proteins (31)
1440 -1405	1403.8	1401.3	1403.3	Symmetric stretching vibration of - COO^- free amino acids of side chain (30)
1270 -1230	1248.0	1239.3	1239.0	PO_2^- asymmetric stretching (32)
1109 -1112	0	0	1110.4	Polysaccharides (58)
1090 -990	1085.6	1083.4	0	Phosphodiester group of nucleic Acids and membrane phospholipids partially and amide-III (32)
630 - 530	571.3	619.3	620.1	Amide VI band, OCN deformation (27,59)

Wavenumber (cm⁻¹) and band assignment of Mid-IR spectra without chromium (Control) and Cr (VI) treated with 30 and 60 ppm.

isolated from Soap Lake, a chemical stratified alkaline lake located incentral Washington State, USA (34). *Halomonas* sp. TA-04 was isolated from stainless steel plant in the Taranto Gulf, Ionian Sea, Southern Italy (35). The wastewater from tannery and textile industries not only contain high concentrations of Cr (VI), but also show significant amount of soluble salts (36). Earlier removal of Cr (VI) was studied in the strain *Bacillus* sp. PB2 in the presence of NaCl, although the removal efficiency was found to be inversely proportional to NaCl concentration (18). In other reports, *Halomonas* sp. TA4 reduced the Cr (VI) in the presence of high concentration (16%, w/v) of NaCl (37).

In the present study, removal of Cr (VI) by *Halomonas* sp. CSB 5 showed concentration dependent increase in the presence of 10 % NaCl. However, higher concentration of Cr (VI) were found to have adverse effect on the survival of bacterium (38). The chromium removal in initial phases followed a slow rise, but reached to its maximum capacity within 45 minutes as observed by other workers (39).In a previous study, it was observed that the maximum removal (80.16%) of Cr (VI) by *Pseudomonas* sp.was observed at 10 mg L⁻¹ concentration within 72 hours. The removal of Cr (VI) by *Pseudomonas aeruginosa* and *Bacillus subtilis* at 25 mg L⁻¹of the Cr (VI) concentration had been reported to be about 78.6 and 33.5 %, respectively (8). However, few earlier studies have shown that an increase in the concentration of Cr (VI) lowered the growth, without affecting chromium removal efficiency ina *Brucella* sp.and *Halomonas* sp. TA-04 isolated from chromium-contaminated soil (21, 35).

Results on the growth response as well as Cr (VI) removal efficiency of bacterium CSB 5 over a wide range of temperature (20-40 °C) indicated an optimum temperature of 30-35° C for removal chromium. In previous

report also, the *Enterobacter cloacae* strain HO1exhibited optimum removal of Cr (VI) at 30°C (29). The Cr (VI) removal by *P. phragmitetus* evaluated under different temperatures showed optimum removal of Cr (VI) as well as optimum growth at 30°C (40). Growth and Cr (VI) removal study under different pH (6-10) conditions showed the highest growth as well as Cr (VI) removal at pH 8. In a previous report, the optimal pH for Cr (VI) removal by *Halomonas* sp. TA 4 pH was reported between pH 7.0-8.0 (37).

The present study showed that the contribution of surface adsorption process was about 4-5 fold higher in the removal of Cr (VI) as compared to intracellular uptake of Cr (VI) in the bacterial isolate. Comparatively, the present results showed significantly better performance by *Halomonas* sp. than that reported for *S. cerevisiae*(41).

A high rate of chromate reducing activity in the presence of NADH corroborated the earlier reports related to removal of Cr (VI) by *Halomonas* sp. TA-04 (42) and *E. coli* (43).The other studies on NADH dependent enzymatic reduction of Cr (VI) under aerobic conditions (44, 45, 46, 47, and 48) reported the involvement of soluble chromate reductase and NADH as external electron donor (49).

IR spectroscopic study on interaction of cell surface of *Halomonas* sp. CSB 5 with Cr (VI) revealed the nature of Cr (VI) binding. Shifting of the IR bands at 3863.2, 3465.6 cm⁻¹ with substantial decrease in wavenumber indicated the role of the hydroxyl and amine groups in chromium binding. The absorption peak at 2856.2 cm⁻¹,assigned to the –CH stretching in fatty acids, was also involved in the chromium binding.The peaks in the spectral region between 2400-2300 cm⁻¹ occurring due to an asymmetric stretching band of CO_2 hydrates, particularly near 2343 cm⁻¹, was influenced
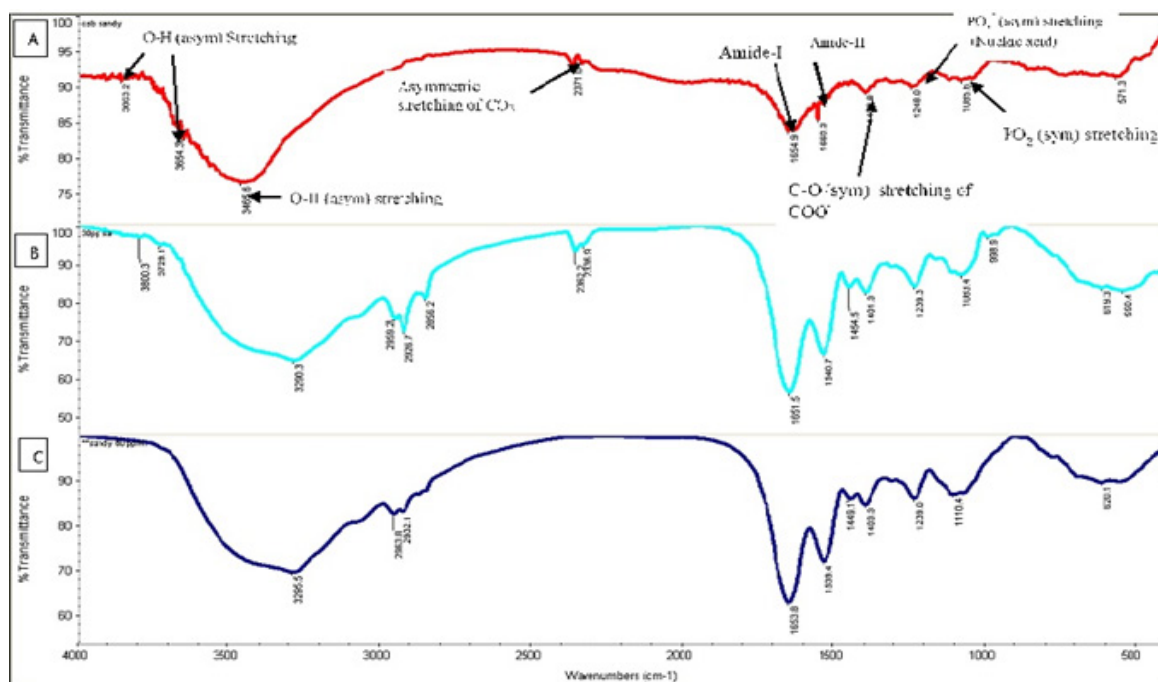
Figure 6. Comparative FTIR spectra of CSB 5 (A) Control (B) treated with 30 ppm (C) 60 ppm concentration of Cr (VI) [asym. (Asymmetric), sym. (Symmetric)]

by Cr (VI) binding as reported in *Candida dubliniensis*(50, 59). The spectral shift in this range of 2362.2, 2336.9 cm^{-1} observed in the Cr (VI) treated cells might be attributed to Cr (VI) induced the trans-gauche ratio in acyl chains (30, 51). Decline in the absorption peaks at 1654.9 and 1580.0 cm^{-1}in the presence of Cr (VI) was indicative of Cr (VI) interaction with amide groups.

The Cr induced shifting of the wavenumber to 1403.8 cm^{-1} with a decrease in intensity corresponding to O–H bending was assigned to binding of Cr (VI) with the carboxylate ions. A shift in the wavenumber at 1248.0 cm^{-1}suggested for possible role of PO_2^- and polysaccharide in the adsorption of Cr (VI) on to the cell surface (32).The FTIR spectra showed the presence of the electronegative functional groups (i.e., carboxyl, hydroxyl, phosphate and amino groups) on the Gram-negative bacterial surface (52), which are involved in binding of Cr (VI) (4).

From the present investigation, it was demonstrated that the isolated halotolerant bacterium *Halomonas* sp., showing cross resistance against Cr(VI) can be very efficient agent from mobilizing and removing the toxic chromium for the salt loaded waste water. They can also contribute to detoxification of Cr (VI) through chromate reductase activity.

Acknowledgment

References

1. Satarupa. D. and Paul. A.K., Reduction of hexavalent chromium by Immobilized Viable Cells of *Arthrobacter* sp. SUK 1201.*Bioremediation Journal.* 2014, **18**:1, 1-11.doi**:**10.1080/10889868.2013.8 34866

2. Cheung, K.H. and Gu, J.D. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: a review. *International Biodeterioration and Biodegradation.* 2007, **59** (1): 8-15. doi:10.1016/j.ibiod.2006.05.002

3. Babu, R.L., Vijayalakshmi, E., Naveen Kumar, M., Rajeshwari, H.P., Devaraju, K.S. and Chidananda S.S. Biosorption of Chromium (VI) and Lead (II): Role of *Spirulina platensis* in the Treatment of Industrial Effluent. *Bioremediation Journal*. 2013, **17** (4): 231-239. doi**:**10.1080/10889868.2013.827619

4. McLean, J. and Beveridge, T.J. Chromate reduction in Pseudomonad isolated from a site contaminated with chromate copper arsenate. *Applied and Environmental Microbiology*. 2001, **67:** 1076-1084.doi:10.1128/AEM.67.3.1076-1084.2001

5. Pellerin, C. and Booker, S.M. Reflections on hexavalent chromium: health hazards of an industrial heavy weight. *Environ Health Perspectives*. 2000, **108**: 402-407.

6. Rai, D., Sass, B.M. and Moore, D.A. Chromium (III) hydrolysis constants and solubility of chromium (III) hydroxide. *Inorganic Chemistry*. 1987, **26**: 345-349.**doi:**10.1021/ic00250a002

7. Donmez, G., Aksu, Z. Bioaccumulation of copper (II) and nickel (10) by the non-adapted and adapted growing *Candida* sp., *Journal of Water Research.* 2001, **35**:1425-1434. doi:10.1016/S0043-1354(00) 00394-8

8. Seker, A., Shahwan, T., Eroglu, A.E., Yilmaz, S. and Demirel. Z. Equilibrium Thermodynamic and kinetic studies for the biosorption of aqueous lead (II) Cadmium (II) and nickel (II) ions on S*pirulinaplatensis*. *Journal of Hazardous Materials.*2008,**154**:973-980.doi: 10.1016/j.jhazmat.2007.11.007

9. Elizabeth, K.M. and Anuradha, T.V.R. Biosorption of hexavalent chromium by non-pathogenic bacterial cell preparations. *Indian Journal of Microbiology.* 2000, **40:** 263-265.

10. Galvao, C.T., Mohn, W.W. and Victor. D.L. Exploring the microbial biodegradation and biotransformation gene pool. *Trends Biotechnology.* 2005,**23:**497-506.doi: 10.1016/j.tibtech.2005.08.002

11. Romanenko, V.I. and Korenkov, V.N. A pure culture of bacteria utilizing chromate and dichromate as hydrogen acceptors in growth under anaerobic conditions. *Mikrobiologiya.* 1977, **46**, 414- 417.

12. Ganguli, A. and Tripathi, A.K. Inducible periplasmic chromate

reducing activity in *Pseudomonas aeruginosa* from a leather tannery effluent. *Journal of Microbiology and Biotechnology,* 2001, **11:**355-361.

13. Ackerley, D.F., Gonzalez, C.F., Keyhan, M., Blake, R. and Matin, A. Mechanism of chromate reduction by the *Escherichia coli* protein, NfsA, and the role of different chromate reductases in minimizing oxidative stress during chromate reduction. *Environmental Microbiology*. 2004, **6** (8): 851-860. doi:10.1111/j.1462-2920.2004.00639.x

14. Margarita, E.R.C., Monica. A.P.S., Selma, G.F.L. Biosorption of chromium using factorial experimental design. *Process Biochemistry.* 2005, **40:** 779-788.doi:10.1016/j.procbio.2004.02.024

15. Camargo, F.A., Okeke, B.C., Bento, F.M. and Frankenberger, W.T. In vitro reduction of hexavalent chromium by a Cell Free Extract of *Bacillus* sp. ES 29 stimulated by Cu^{2+}. *Applied Microbiology and Biotechnology.* 2003, **62**: 569-573. doi:10.1007/s00253-003-1291-x

16. Pal, A. and Paul, A.K. Aerobic chromate reduction by chromium resistant bacteria isolated from serpentine soil. *Microbiological Research.* 2004, **159**:347-354. doi:10.1016/j.micres.2004.08.001

17. Kargi, F. and Dincer, A.R. Effect of salt concentration on biological treatment of saline wastewater by fed-batch operation. *Enzyme and Microbial Technology.*1996, **19:**529-537.doi: 10.1016/S0141-0229(96)00070-1

18. Okeke, B.C. Bioremoval of hexavalent chromium from water by a salt tolerant bacterium, *Exiguobacterium* sp. GS1. *Journal of Industrial Microbiology Biotechnology.*2008, **35** (12): 1571-1579. doi:10.1007/s10295-008-0399-5

19. Yun, L., Hua, X., Jingfang, L., Meixian, Z., Huarong, T. Purification and biological characterization of halocin C8, a novel peptide antibiotic from *Halobacterium* strain AS7092. *Extremophiles.* 2003, **7**:401-407.doi:10.1007/s00792-003-0335-6

20. Smibert, R.M. and Krieg, N.R. General characterization. In: Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R., Philips, G.B. (Eds.), *Manual of Methods for General Bacteriology. American Society for Microbiology.* 1981, 411- 442.

21. Urvashi, T., Rasesh, P., Yogesh, S. andDatta, M. Hexavalent chromium reduction by *Providencia* sp. *Process Biochemistry.* 2006, **41**: 1332–1337.doi:10.1016/j.procbio.2006.01.006

22. Asha, L.S., Murali, K.P., Nageswara, R.T. and Sharma, P.N. Uptake of Cr (VI) in the presence of sulphate by *Bacillus mycoides* in aerobic environment. *Indian journal of Biotechnology.* 2006, **5**: 506-509.

23. Richard, A. Smucker, and Robert, M.P. Liquid Nitrogen Cryo-Impacting: a New Concept for Cell Disruption. *Applied Microbiology.* 1975, **30** (3): 445-449.

24. Lowry, O.H., Rosebrough, N.J., Farr. A. L., and Randall, R.J. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*. 1951, **193:**265-275.

25. Urone, P.F. Stability of colorimetric reagent for chromium, s-diphenylcarbazide, in various solvents. *Analytical Chemistry*. 1955, **27**:1354−1355.**doi:**10.1021/ac60104a048

26. Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. Gapped BLAST and PSI-Blast: a new generation of protein database search programs. *Nucleic Acids Research*. 1997, **25**: 3389-3402. doi:10.1093/nar/25.17.3389

27. Jag Mohan. *Organic Spectroscopy Principles and Applications*. 2nd Edition, Narosa Publishing House. New Delhi, 2005.

28. Deng, S. and Ting, Y.P. Characterization of PEI-modified biomass and biosorption of Cu (II), Pb (II) and Ni (II).*Water Research*. 2005, **39:** 2167-2177. doi:10.1016/j.watres.2005.03.033

29. Wang, P.C., T. Mori, K. Komori, M. Sasatsu, K. Toda, and H. Ohtake. Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. *Applied and Environmental Microbiology.* 1989, **55**: 1665-1669. 0099-2240/89/071665-05$02.00/0

30. Dieter, N. FTIR and FT-Raman Spectroscopy in biomedical research. *Applied Spectroscopy Reviews*. 2011, **36** (2-3): 239-298. **doi:**10.1081/ASR-100106157

31. Fujioka, N., Morimoto, Y., Arai, T. and Kikuchi, M. Discrimination between normal and malignant human gastric tissues by Fourier transform infrared spectroscopy. *Cancer Detection and Prevention.*2004, **28**: 32-36. doi:10.1016/j.cdp.2003.11.004

32. Fukuyama, Y., Yoshida, S., Yanagisawa, S. and Shimizu, M. A study on the differences between oral squamous cell carcinomas and normal oral mucosa measured by Fourier transform infrared spectroscopy. *Biospectroscopy.*1999, **5:**117-126.doi:10.1002/(SICI)15206343(1999) 5:2<117::AID-BSPY5>3.0.CO; 2-K

33. Adriana, S., Susan, K. and Kathleen, M. Gough. A synchrotron FTIR microspectroscopy investigation of fungal hyphae grown under optimal and stressed conditions. *Analytical and Bioanalytical Chemistry.*2006, **387**:1779-1789. doi:10.1007/s00216-006-0850-2

34. Van Engelen, M.R., Peyton, B.M., Mormile, M. R. and Pinkart, H.C. Fe (III), Cr (VI), and Fe (III) mediated Cr (VI) reduction in alkaline media using a *Halomonas* isolate from Soap Lake, Washington. *Biodegradation.* 2008, **19**: 841- 850.doi:10.1007/s10532-008-9187-1

35. Silvia, F., Milva, P., Giacomo, L, Simone, G., Marcella, R., Pamela, D.B. and Silvano, E.F. Hexavalent chromium reduction by whole cells and cell free extract of the moderate halophilic bacterial strain *Halomonas* sp. TA-04. *International Biodeterioration and Biodegradation.* 2012, **66**: 63-70.doi:10.1016/j.ibiod.2011.11.003

36. Kiran, B., Kanshik, A. and Kanshik, C.P. Biosorption of Cr (VI) by native isolate of *Lyngbyaputealis* (HH-15) in the presence of salts. *Journal of Hazardous Materials*. 2007, **141**: 662- 667. doi:10.1016/j.jhazmat.2006.07.026

37. Shakoori, A.R., Makhdoom, M.and Haq, R.U. Hexavalent chromium reduction by a dichromate-resistant gram-positive bacterium isolated from effluents of tanneries. *Applied Microbiology and Biotechnology.* 2000, **53**:348-351.doi:10.1007/s002530050033

38. Deepali, B.D. Joshi and Jain, C.K. Biosorption of hexavalent chromium through bacterial strain. *National Journal of Life Science.*2007, **4** (1): 41-45.

39. Siegel, S., Keller, P., Galun, M., Lehr, H., Siegel, B. and Galun, E. Biosorption of lead and chromium by *Penicillium* preparations. *Water, Air and Soil Pollution*. 1986, **27**(1-2): 69-75. doi:10.1007/BF00464770

40. Yan Shi, L., Chai, Z. Y., Qingxiu, J., Runhua, C. and Yuehun, C. Identification and hexavalent chromium reduction characteristics of *Pannonibacter phragmitetus. Bioprocess and Biosystems Engineering.* 2012, **35**:843-850.doi:10.1007/s00449-011-0668-y

41. Krauter, P., Martinelli, R., K. Williams and Martins, S. Removal of Cr (VI) from Ground Water by *Saccharomyces Cerevisiae, Biodegradation Journal.*1996, doi:10.1007/BF00115741

42. Focardi, S., Pepi, M., Landi, G., Simone, G. S., Ruta, M, Biasio, P. D. and Focardi, S. E. Hexavalent chromium reduction by whole cells and cell free extract of the moderate halophilic bacterial strain *Halomonas*sp. TA-04. *International Biodeterioration& Biodegradation*. 2012, **66**: 63-70.doi: 10.1016/j.ibiod.2011.11.003

43. Shen, H., Wang, Y.T. Characterization of Enzymatic Reduction of Hexavalent Chromium by *E. coli* ATCC 33456. *Applied and Environmental Microbiology*. 1993, **59**: 3771-3777.0099-2240/93/113771-07$02.OO/0

44. Park, C.H., Keyhan, M., Wielinga, B., Fendorf, S., and Matin, A. Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase. *Appl. Environ. Micrbiol*. 2000, **66**: 1788-1795.doi:10.1128/AEM.66.5.1788-1795.2000

45. Bae, W., Lee, H., Choe, Y., Jahng, D., Lee, S., Kim, S., Lee, J. and Jeong, B. Purification and characterization of NADH dependent Cr (VI) reductase from *E. coli* ATCC 33456, *Journal of Microbiology*. 2005, **43**: 21-27.

46. Elangovan, R., Abhipsa, S., Rohit, B., Ligy, P. and Chandraraj, K. Reduction of Cr (VI) by a *Bacillus* sp. *Biotechnology Letters*. 2006, **28**: 247-252.doi:10.1007/s10529-005-5526-z

47. Desai, C., Jain, K. and Madamwar, D. (2008). Hexavalent chromate reductase activity in cytosolic fractions of *Pseudomonas* sp. G1DM21 isolated from Cr (VI) contaminated industrial landfill, *Process Biochemistry*. 2008, 43: 713-721. doi:10.1016/j.procbio.2008.02.015

48. Martorell, M.M., Fern´andez, P.M., Farina, J.I., and Figueroa, L.I.C. Chromium hexavalent reduction by cell-free extracts of *Pichia jadini* and *Pichia anomala* isolated from textile-dye factory effluents, *International Biodeterioration& Biodegradation*. 2012, **71**: 80-85.doi:10.1016/j.ibiod.2012.04.007

49. Cervantes, C., Campos, G.J. Reduction and efflux of chromate by bacteria. *MicrobiolMonor*. 2007, **6:** 407-419. doi:10.1007/7171_2006_087

50. Schultz. C.P., Eysel. H.H., Mantsch, H.H. and Jackson. M. Carbon Dioxide in Tissues, Cells, and Biological Fluids Detected by FTIR Spectroscopy. *The Journal of Physical Chemistry*. 1996, **100**, 6845-6848.**doi:**10.1021/jp953254t

51. Kilimann, K.V., Doster, W., Vogel, R.F., Hartmann, C., Gänzle, M.G., 2006. Protection by sucrose against heat-induced lethal and sublethal injury of *Lactococcus lactis* and FTIR study. *BiochimicaetBiophysicaActa*, 2006, **1764 (7)**: 1188-1197. doi: 10.1016/j.bbapap.2006.04.016

52. Zeynep, E.U., Alper, D.O., Evren, T., Sibel, A. and Turgay, T. Chromium (VI) Biosorption and Bioaccumulation by Live and Acid-Modified Biomass of a Novel *Morganella morganii* Isolate. *Separation Science and Technology*. 2014, **49**: 6, 907-914. **doi:**10.1 080/01496395.2013.866681

53. Wu, J.G., Xu, Y.Z., Sun, C.W., Soloway, R.D., Xu, D.F., Wu, Q.G., Sun, K.H., Weng, S.F. and Xu, G.X. Distinguishing malignant from normal oral tissues using FTIR fiber optic techniques. *Biopolymer (Biospectroscopy)*. 2001, **62**: 185-192.doi:10.1002/bip.1013

54. Schulz, H. and Baranska, M. Identification and qualification of valuable plant substances by IR and Raman spectroscopy. *Vibrational Spectroscopy*. 2007,**43**:13-25.doi: 10.1016/j.vibspec.2006.06.001

55. Paluszkiewicz, C. and Kwiatek, W.M. Analysis of human cancer prostate tissues using FTIR microscopy and SXIXE techniques. *Journal of Molecular Structure*. 2001, 565-566, 329-334.

56. Sethuraman, P. and Balasubramanian, N. Removal of Cr (VI) from aqueous solution using *Bacillus subtilis, Pseudomonas aeruginosa* and *Enterobacter cloacae. International Journal of Engineering Science and Technology*. 2010, **2**(6): 1811-1825.

57. Eckel, R., Huo, H., Guan. H.W., Hu, X., Che, X. and Huang, W.D. Characteristic infrared spectroscopic patterns in the protein bands of human breast cancer tissue. *Vibrational Spectroscopy*. 2001, **27:** 165-173. doi:10.1016/S0924-2031(01)00134-5

58. Ahalya, N., Ramachandra, T.V, Kanamadi, R.D., Biosorption of heavy metals. *Research Journal of Chemistry and Environment*. 2003, **7**:71-79.

59. Chandra. P., Barsainya, M. and Singh, D.P. A Fourier Transform Infrared (FTIR) spectroscopic study on cellular changes in the *Marinococcus luteu s*sslb 1 under different salinity regime. *International Journal of Pharma and Bio Sciences*. 2014,**5** (4): (B) 848 – 854.

60. Singh, V. K., Singh, M. P. Bioremediation of vegetable and agrowastes by *Pleurotus ostreatus*: a novel strategy to produce edible mushroom with enhanced yield and nutrition. *Cell. Mol. Biol*. 2014, **60 (5)**: 2-6. doi: 10.14715/cmb/2014.60.5.2

61. Vishnoi, N., Singh, D. P., Biotransformation of arsenic by bacterial strains mediated by oxido-reductase enzyme system. *Cell. Mol. Biol*. 2014, **60 (5)**: 7-14. doi: 10.14715/cmb/2014.60.5.3

62. Srivastava, A. K., Vishwakarma, S. K., Pandey, V. K., Singh, M. P., Direct red decolorization and ligninolytic enzymes production by improved strains of *Pleurotus* using basidiospore derived monokaryons. *Cell. Mol. Biol*. 2014, **60 (5)**: 15-21. doi: 10.14715/cmb/2014.60.5.4

63. Kumari, B., Rajput, S., Gaur, P., Singh S. N., Singh D. P., Biodegradation of pyrene and phenanthrene by bacterial consortium and evaluation of role of surfactant. *Cell. Mol. Biol*. 2014, **60 (5)**: 22-28. doi: 10.14715/cmb/2014.60.5.5

64. Pandey, V. K., Singh, M. P., Biodegradation of wheat straw by *Pleurotus ostreatus. Cell. Mol. Biol*. 2014, **60 (5)**: 29-34. doi: 10.14715/cmb/2014.60.5.6

65. Pathak, V. V., Singh, D. P., Kothari, R., Chopra, A. K., Phycoremediation of textile wastewater by unicellular microalga *Chlorella pyrenoidosa. Cell. Mol. Biol*. 2014, **60 (5)**: 35-40. doi: 10.14715/cmb/2014.60.5.7

66. Pandey, A. K., Vishwakarma, S. K., Srivastava, A. K., Pandey, V. K., Agrawal, S., Singh, M. P., Production of ligninolytic enzymes by white rot fungi on lignocellulosic wastes using novel pretreatments. *Cell. Mol. Biol*. 2014, **60 (5)**: 41-45. doi: 10.14715/cmb/2014.60.5.8

67. Ayaz E., Gothalwal, R., Effect of Environmental Factors on Bacterial Quorum Sensing. *Cell. Mol. Biol*. 2014, **60 (5)**: 46-50. doi: 10.14715/cmb/2014.60.5.9

68. Singh, M. K., Rai, P. K., Rai, A., Singh, S., Alterations in lipid and fatty acid composition of the cyanobacterium *Scytonema geitleri* bharadwaja under water stress. *Cell. Mol. Biol*. 2014, **60 (5)**: 51-58. doi: 10.14715/cmb/2014.60.5.10

69. Singh, M. P., Pandey, A. K., Vishwakarma, S. K., Srivastava, A. K., Pandey, V. K., Singh, V. K., Production of cellulolytic enzymes by *Pleurotus* species on lignocellulosic wastes using novel pretreatments. *Cell. Mol. Biol*. 2014, **60 (5)**: 59-63. doi: 10.14715/cmb/2014.60.5.11

70. Tewari, S., Arora, N. K., Talc based exopolysaccharides formulation enhancing growth and production of *Hellianthus annuus* under saline conditions. *Cell. Mol. Biol*. 2014, **60 (5)**: 73-81. doi: 10.14715/cmb/2014.60.5.13

71. Kumar, M., Singh, P., Tripathi, J., Srivastava, A., Tripathi, M. K., Ravi, A. K., Asthana, R. K., Identification and structure elucidation of antimicrobial compounds from *Lyngbya aestuarii* and *Aphanothece bullosa. Cell. Mol. Biol*. 2014, **60 (5)**: 82-89. doi: 10.14715/cmb/2014.60.5.14

72. Arun, N., Vidyalaxmi, Singh, D. P., Chromium (VI) induced oxidative stress in halotolerant alga *Dunaliella salina* and *D. tertiolecta* isolated from sambhar salt lake of Rajasthan (India). *Cell. Mol. Biol*. 2014, **60 (5)**: 90-96. doi: 10.14715/cmb/2014.60.5.15

73. Prakash, S., Singh, R., Lodhi, N., Histone demethylases and control of gene expression in plants. *Cell. Mol. Biol*. 2014, **60 (5)**: 97-105. doi: 10.14715/cmb/2014.60.5.16

74. Singh, A. K., Singh, M. P., Importance of algae as a potential source of biofuel. *Cell. Mol. Biol*. 2014, **60 (5)**: 106-109. doi: 10.14715/cmb/2014.60.5.17

75. Dixit, S., Singh, D. P., Role of free living, immobilized and nonviable biomass of *Nostoc muscorum* in removal of heavy metals: An impact of physiological state of biosorbent. *Cell. Mol. Biol*. 2014, **60 (5)**: 110-118. doi: 10.14715/cmb/2014.60.5.18