Estimation of Exopolysaccharides (EPS) Producing Ability of Cr (VI) Resistant Bacterial Strains from Tannery Effluent

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Abstract: Chromium is a known heavy metal and recognized as a carcinogen to the biological systems. Previously isolated Cr (VI) resistant *Exiguobacterium* UE1 and UE4 were used in this study. These strains were analysed for exopolysaccharides (EPS) production for the remediation of Cr (VI) contaminated soils. Both the strains could tolerate about 250µg/ml of Cr (VI) stress. Strain UE1 showed 100% Cr (VI) removal whereas UE4 reduced 99.2% at an initial concentration of K_2CrO_4 100µgml^{-1}. Optimum growth was observed at 37ºC and pH 7 for both strains. Strains exhibited significant EPS production under Cr (VI) stress and non-stress conditions. However, UE1 showed increased production of released as well as loosely bound EPS (0.36g/100ml and 0.152g/100ml respectively) under Cr (VI) supplemented condition. Thin Layer Chromatography (TLC) technique confirmed the presence of sugars in EPS samples after hydrolysis. Fourier Transforms Infrared Spectroscopy (FTIR) analysis showed the involvement of various functional groups such as hydroxyl group and aromatic compounds in the binding of Cr (VI) ions to the EPS. These findings suggest that strains UE1 and UE4 isolated from local tanneries of Pakistan can be used for remediation of Cr (VI) pollutes soils.

Keywords: Chromium reduction, exopolysaccharides, protein estimation, tanning industries, bioremediation, heavy metals, FTIR and TLC etc.

INTRODUCTION

Industrial and domestic effluents significantly pollute our environment because of increasing urban development. Industrial processes like electroplating and mining, tanning, metal processing etc. are the cheif cause of heavy metal pollution in waste water [1]. This has prompted noteworthy amounts of substantial metals being dumped into physical biological systems [2]. The metals of most concern are copper (Cu), chromium (Cr), manganese (Mn), zinc (Zn), lead (Pb), mercury (Hg), and cadmium (Cd) [3]. Anthropogenic activities likewise generate conditions in which the considerable metals are assimilated into new mixtures and may spread overall [4]. Chromium is the most commonly used heavy metal in various industries [5]. According to the US Environmental Protection Agency (EPA), the acceptable value of Cr is 0.05mgL^{-1} [6].

The oxidation state of chromium is changed under different environmental conditions. The high oxidation potential of Cr (VI) makes it more toxic and lethal to biological systems, whereas Cr (III) is relatively less toxic and insoluble [7, 8]. Cr (III) has the most stable valence state [9]. Cr (VI) is more lethal as it can enter in cells and have carcinogenic effects [10]. Breathing and retaining of Cr (VI) comprising materials can lead to puncture of the nasal septum, asthma [11], bronchitis, pneumonitis and liver and high rate of bronchogenic carcinoma [12]. Cr (VI) is responsible to cause severe abnormalities in human body so, there is a need to treat the Cr (VI) contaminated sites to reduce the toxic pollutants [13]. The conventional techniques used for Cr (VI) removal includes chemical precipitation, ion exchange, filtration, chemical oxidation and reduction, electrochemical treatment, reverse osmosis, evaporative recovery and solvent extraction [14]. These traditional technologies were often ineffective and expensive to be used for heavy metal reduction of polluted sites [15].

To survive in soil containing high concentrations of Cr (VI), microbes have established a variety of metal resistance mechanisms. Metal adsorption, extracellular precipitation, mineralization, enzymatic oxidation or reduction to a less toxic form, and efflux of heavy metals from the cell are few of the mechanisms described [16]. Cr (VI) removal by biosorption refers toward many types of non-active metal uptake by biomass which may even be dead [17]. This metabolism independent biosorptive metal uptake happens rapidly, efficiently and sometimes as a complex phenomenon [4].

Microbes synthesize and secrete extracellular polymeric substances plays an significant role in bacterial adaptation to different stress conditions [18, 19]. EPS is involved in the formation of symbiotic and pathogenic interactions with hosts and are proposed to be a major constituent of biofilm mature structures.

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They are also beneficial for protection of bacterial cell from desiccation [20], for continuing major cellular functions, crystallizing capability and waste degradation kinetics [21,22]. Furthermore, EPS are also involved in chelation of different heavy metals from polluted sites by binding ionic forms of heavy metals into polymeric substances [23]. EPS is an amalgam of different substances consisting of polysaccharides, proteins, various organic and inorganic compounds etc. [24].

Microorganisms for example bacteria, algae or fungi are being reported to grow in Cr (VI) stress environment by producing EPS, that are involved in Cr (VI) reduction [25, 26]. EPS are very beneficial for the survival of these microbes in stress conditions. So, EPS producing ability of microbes could be utilized for the cleaning of Cr (VI) contaminated areas [27]. Currently, microorganisms are utilized for remediation of Cr (VI) polluted soils, as they have potential to detoxify Cr (VI) to less toxic and insoluble Cr (III) as compared to expensive techniques [13].

The purpose of this research work was to analyze Cr (VI) reduction potential and EPS producing ability of previously isolated chromium resistant bacterial strain from tannery wastes. Quantification of protein and carbohydrate under stress and non-stress conditions was another aim of this study.

MATERIALS AND METHODS

Cr (IV) Resistant Bacterial Strains

Cr (VI) resistant bacterial strains UE1 (KC668296) and UE4 (KC668297) used were previously isolated by Batool *et al*. (2014) from tannery industries (Sialkot, Pakistan) [28]. These strains were taken from the Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan and were regrown on Luria Bertani (LB) agar supplemented with an initial concentration of 100µgml^{-1} of Cr (VI) stress.

Maximum Resistivity

The maximum resistivity of bacterial isolates was determined by inoculating strains systematically supplemented with higher concentrations of K_2CrO_4 on LB-agar plates. The bacterial strains showing resistance at a specific concentration were further inoculated on higher concentrations. The process was initiated with initial concentration of 100µgml^{-1} then preceded with 250µgml^{-1} and 500µgml^{-1} until maximum resistance level was achieved.

Metal and Antibiotic Resistance Profile

To estimate multiple metal resistance, five different metals were used and their (Minimum inhibitory concentration) MIC was determined. Salts of different heavy metals used were copper sulphate ($CuSO_4$), cobalt nitrate [$Co(NO_3)_2$], lead nitrate [$Pb(NO_3)$], nickel chloride ($NiCl_2$) and zinc chloride ($ZnCl_2$). MIC was estimated by using plate dilution method. Multiple antibiotic resistances (ampicillin, erythromycin, tetracycline, chloramphenicol and gentamycin) were determined by following the same method.

Estimation of Reduction Potential of Cr (VI)

Cr (VI) reduction potential was determined by Diphenylcarbazide method [22]. It was estimated calorimetrically by reaction with diphenylcarbazide in acid solution. Bacterial strains were regrown in LB-broth supplemented with Cr (VI) stress (100µgml^{-1}). After 24hrs, cultures were taken in sterile eppendroffs and centrifuged for 5min at 12,000rpm. Pellets were discarded and 50µl supernatant was taken. To the supernatant, orthophosphoric acid and diphenylcarbazide was added and incubated for 15minutes at room temperature. A purple-violet colored complex of unknown composition was produced. Optical density was observed at 540nm and Cr (VI) reduction potential was determined.

Extraction, Purification and Characterization of EPS

These strains were screened for EPS production by growing on E and P media [29, 30]. Different components of E medium were prepared and autoclaved separately. All components were mixed under aseptic conditions (supplemented with 100µgml^{-1} of Cr (VI) stress). Strains were inoculated in P and E media and incubated at 37ºC for 5 to 7 days [6]. Solvent extraction method was performed by centrifuging bacterial cultures and supernatant was further used [31]. Extracted EPS were filtered and refined by using centrifugation [32]. For protein estimation of exopolysaccharides, Bradford's method was used [33]. Reducing and non-reducing sugars were determined by phenol sulfuric acid assay [34].

Estimation of Released Exopolysaccharides (REPS) and Loosely Bound Exopolysaccharides (LEPS)

Exopolysaccharides (EPS) is made up of different layers i.e. the outer layer consists of released REPS

which solubilizes in the supernatant while inner layers consist of tightly bound EPS and remain attached to the cells. So, the tightly bound EPS can be removed from cells by different treatments.

Cr (VI) resistant strains were cultured in LB-broth supplemented with (100µgml^{-1}) and without Cr (VI) stress and incubated at 37ºC for 3days. Cultures were centrifuged at 5000g for 20min. Supernatant was separated for the quantification of REPS and sterilized, lyophilized, weighed and stored at -20ºC. LEPS was isolated by addition of milliQ water to pellets and vortexed. The samples were then placed on shaking water bath at 30ºC for 1hr. Then the process for REPS isolation was repeated for the isolation of LEPS [35].

Thin Layer Chromatography (TLC)

TLC was performed for the identification of different compounds present in extract. Extract and standards were spotted on the TLC plate. After spotting, TLC plate was air dried. Water/ethyl acetate/n-butanol in a 4:5:4 ratios was used as running solvent. TLC plate was positioned in the tank in such a way that the spots did not dip in the solvent. The plate was left in the tank for solvent to rise about one third of the plate, and then it was allowed to dry. Acid digestion was used for partial breaking of complex polymers into simple and then was run on TLC plate. Standard solutions (1mgml^{-1}) of carbohydrates were also run in same system as control. Double developed TLC was observed under UV illuminator for detection of any spot that is visible under UV only. The TLC plate was then stained by spraying it with H_2SO_4/methanol reagent. TLC plate after staining with reagent and baking for few minutes for color development was observed. The constituent displaying UV absorbance and fluorescence was marked and scanned.

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis

FTIR spectrum analysis was done to describe alteration in position of functional groups that existed along the surface of EPS produced by bacterial strains (UE1 and UE4) grown with (100µgml^{-1}) and without Cr (VI). The EPS fractions extracted from two strains were centrifuged and lyophilized. The lyophilized samples were weighed and 20mg was taken for FTIR analysis by KBr disc method with the range of 500-4000 cm^{-1} [23].

RESULTS

Cr (IV) Resistant Bacterial Strains

Bacterial strains UE1 and UE4 were streaked on high concentration of K_2CrO_4 in LB-agar plates and were further streaked on higher concentrations until sensitivity level was achieved.

Maximum Resistivity

Maximum resistance was determined for Cr (VI) resistant strains. Cultures were grown with increasing concentration of Cr (VI) (100µgml^{-1}, 250µgml^{-1} and 500µgml^{-1}) until maximum resistance level was attained. The maximum resistivity level of each bacterial isolate towards K_2CrO_4 was 250µgml^{-1}.

Metal Resistance and Antibiotic Resistance Profile

The ability of bacterial strains to tolerate heavy metals other than chromium such as copper ($CuSO_4$), cobalt [$Co(NO_3)_2$], lead ($PbCl_2$), zinc ($ZnCl_2$) and nickel ($NiCl_2$) was found which indicated the multiple metal resistance characteristics (Table **1**). Antibiotic susceptibility of the chromium resistant strains towards five different antibiotics was observed. UE1 and UE4 showed resistance against all of them.

Estimation of Reduction Potential of Cr (VI)

Cr (VI) reduction potential of bacterial strains was determined. Cr (VI) reduction ability of UE1 and UE4 was 100%, and 99.2% after incubation at with 100µgml^{-1} concentration of Cr (VI) respectively (Figure **1**).

EPS Extraction, Purification and Characterization

These strains were screened for EPS production by growing on E and P media. Strain UE1 exhibited weak

Table 1: Metal Resistance Profile of Chromium Resistant Bacterial Strains Isolated from Leather Tannery

Bacterial strains	Concentration (µgml^{-1})				
	$CuSO_4$	$NiCl_2$	$Pb(NO_3)_2$	$Co(NO_3)_2$	$ZnCl_2$
UE1	600	1400	750	250	300
UE4	600	1400	750	250	300

growth while UE4 showed rich growth on E and P medium after 24hrs incubation.

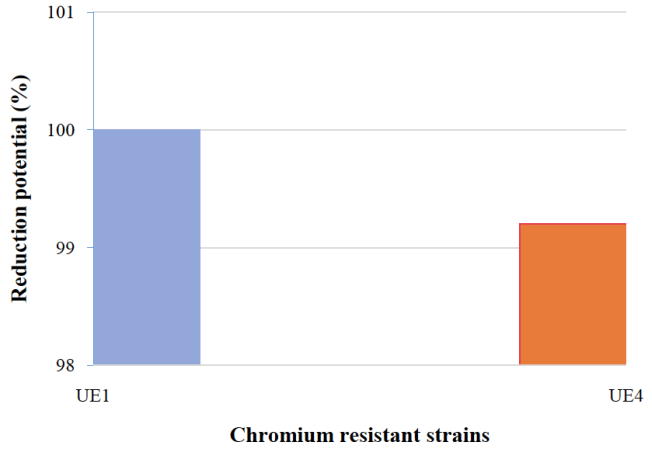

Figure 1: Determination of Cr (VI) reduction of bacterial strains.

Released and bound EPS were observed and quantified under stress and non-stress conditions for both strains. The effect of Cr (VI) on EPS production was also analyzed (Figure **2**). Protein estimation was done for EPS and its concentration was calculated by comparing optical densities of EPS with standard curve. Protein concentration was 3.366±0.072 μgml^{-1} and 4.382±0.062 μgml^{-1} for UE1 and UE4, however, the concentration became high under Cr (VI) stress conditions i.e. 7.861±0.038 μgml^{-1} and 13.891±0.020 μgml^{-1} respectively. Similar results were observed for carbohydrate content. Low concentration of carbohydrates was exhibited by UE1 and UE4 strains 4.293±0.021 μgml^{-1} and 9.064±0.072 μgml^{-1} and the value raised to 5.278±0.053 μgml^{-1} and 12.542±0.034 μgml^{-1} respectively under Cr (VI) stress conditions (Table **2**).

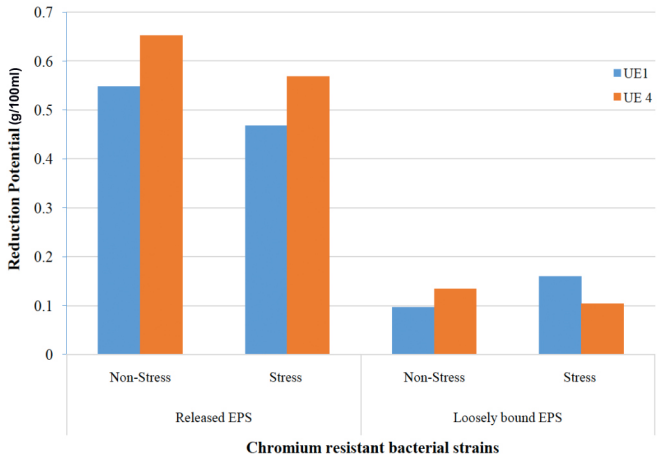

Figure 2: Quantification of released and bound EPS of chromium resistant bacterial strains under stress and non-stress condition.

Table 2: Protein and Carbohydrate Estimation of EPS Produced by Bacterial Strains under Stress and Non-Stress Conditions by Bradford's Assay and Phenol Sulfuric Acid Method Respectively

Samples	Protein concentrations (μgml^{-1})	Carbohydrate concentrations (μgml^{-1})
UE1	3.366±0.072	4.293±0.021
UE1{Cr(VI)}	7.861±0.038	5.278±0.053
UE4	4.382±0.062	9.064±0.072
UE4{Cr(VI)}	13.891±0.020	12.542±0.034

Thin Layer Chromatography (TLC)

Double developed TLC of EPS extracts was observed under UV illuminator after drying. Spots visible under UV were marked. Brownish black spots appeared after spraying and baking with reagent, showed retention time for all standards carbohydrates and light brown spots indicated the carbohydrates present in samples. Retention factor (0.52) for all standards, mixture of standards and sample was almost same (Figure **3**). Bands of carbohydrate standards were visible after staining while bands of all samples were visible only under UV illuminator and retention time for all samples was almost same.

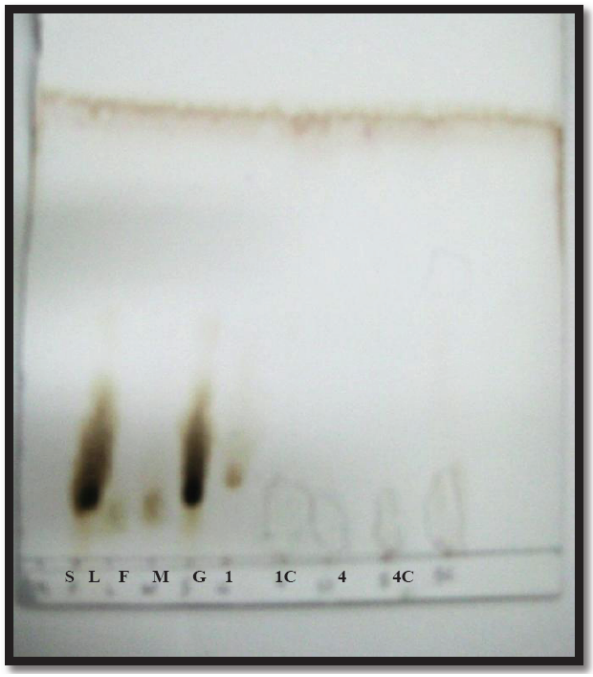

Figure 3: Double developed TLC plate after staining (S: sucrose, L: lactose, F: fructose, M: maltose, G: glucose, 1: EPS of sample UE1, 1C: EPS of sample UE1 with chromium, 4: EPS of sample UE4, 4C: EPS of sample UE4 with chromium).

Fourier Transforms Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was performed to analyze different functional groups involved in EPS formation. Different peaks obtained in FTIR spectra indicated the occurrence of a number of functional groups and hence a complex nature of exopolysaccharides. Peaks for UE1 and UE1-Cr at 722.96 cm^{-1} and 723.05cm^{-1} indicated presence of glycerol and polysaccharides (600cm^{-1} to 750cm^{-1}), 1376.79 $cm^{-1}$$cm^{-1}$, 1376.60$cm^{-1}$ showed methane group (1300cm^{-1} to 1400cm^{-1}), 1459.65cm^{-1}, 1459.33cm^{-1} specified aromatic compounds. Peak at 2967.91 cm^{-1} for strain UE1 with (100µgml^{-1}) and without Cr (VI) stress revealed presence of hydroxyl (-OH) group (2800 cm^{-1} to 3600 cm^{-1}) in exopolysaccharides. FTIR spectra for UE4 and UE4-Cr also represented a number of peaks. Some peaks in both conditions were similar but different functional groups under stress and normal conditions indicated role of binding of Cr (VI) to EPS composition. Peak at 722.90cm^{-1}, 723.02 cm^{-1} showed the presence of glycerol compounds, peaks at 1376.81 cm^{-1} and 1376.96 cm^{-1} stated functional group methane group and 1460.18cm^{-1}, 1460.14cm^{-1} specified aromatic compound. Peaks at 2960.20cm^{-1} and 2964.06cm^{-1} indicated hydroxyl group.

DISCUSSION

Rapid industrialization in developing countries is a basic reason for increased economic growth but ultimately leading to environmental pollution [36]. Chromium is widely used heavy metal in different industries [37]. Many scientists isolated Cr (VI) resistant bacteria and utilized their ability to reduce Cr (VI) for remediation purposes [38].

Extracellular polymeric substances are being defined as biological materials produced under stress environments that are characterized as ecofriendly and have distinct chemical properties [34]. This study involved the screening of already isolated chromium resistant bacteria from heavy metal polluted water and contaminated soil for exopolysaccharides production and further studied for their role in chromium removal.

Isolated bacterial strains UE1 and UE4 showed resistance till 250µgml^{-1} concentration. Isolates were gram-positive small rods. The Cr (VI) resistant bacterial strain isolated by Mistry *et al*. (2009) were also gram positive cocci with elongated edges. Different types of bacteria isolated from Cr (VI) polluted soils that belongs to genera such as *Serratia, Sphaerotilus, Pseudomonas, Brucella, Arthrobacter, Bacillus, Ochrobactrum, Microbacterium, Acinetobacter, Staphylococcus, Exiguobacterium, Stenotrophomonas Brevibacterium, Rhizobium,* etc. are capable for effective reduction of Cr (VI) to Cr(III) [39].

Both the strains were also checked for multiple metal resistance (nickel, copper, cobalt, lead, and zinc). The resistance against very high concentrations of nickel was also reported in *Alcaligenes eutrophus* CH34 that have altered themselves to their surroundings containing high degrees of heavy metals. Harmful metal exposure to bacteria could result in the development of multiple metal and antibiotic resistance. So, bacteria when exposed to stress conditions may develop resistance against it [40, 41]. Antibiotic resistance was observed against gentamycin, chloramphenicol, ampicillin, tetracycline and erythromycin.

Cr (VI) removal of microorganisms is their ability to reduce toxic forms of heavy metals to less toxic form and that is very essential for their survival in stress conditions. Hexavalent chromium removal of UE1 was 100% after 24hrs of incubation whereas UE4 was 99.2% (100µgml^{-1}). Similar results were described by Shakoori *et al*. (2000) showed high Cr (VI) reduction potential of bacteria isolated from tannery effluent [42]. So, they could be useful for the purification of Cr (VI) contaminated water.

Microorganisms produce exopolysaccharides (EPS), which play an important role in their survival and growth. UE1 revealed high level of released as well as loosely bound EPS as compared to UE4, suggesting that UE1 has high potential of EPS production under Cr (VI) stress conditions (100µgml^{-1}) [32]. Previously, it is also reported that under stress conditions, bacterial cells secrete EPS for their survival. Rate of EPS production is dependent on the amount of stress of heavy metal and bacterial strain present in that environment [43, 44, 45]. Bacterial cells produce EPS and may alter their characteristics to protect themselves from Cr (VI) stress [46, 47]. Both strains exhibited more carbohydrate content than protein content.

Thin layer chromatography (TLC) technique was performed to separate the carbohydrate compounds in EPS. TLC confirmed the presence of different sugar in EPS. Similar results were reported by Kachlany *et al*., (2001) [48]. FTIR technique confirms the presence of

various functional groups in EPS. It was observed that different peaks of functional groups were present under stress and non-stress conditions and presence of Cr(IV) affected the EPS composition. There was only a small difference in peaks of EPS samples when observed under stress and non-stress conditions. A peak at 4327.68cm^{-1} in EPS of UE1 under stress conditions indicated presence of saturated hydrocarbons. Peak at 723.05cm^{-1} for exopolysaccharides [42], peaks at 1376.60cm^{-1} and 1459.33cm^{-1} for aromatic compounds and 2967.91cm^{-1} for hydroxyl groups [43] was indication of exopolysaccharides. Iyer *et al*. (2005) also reported different types of peaks observed for EPS that are related to these findings and confirming the presence of various organic and in-organic compounds in EPS [26]. Functional groups that are involved in biosorption reported by Pradhan *et al*. (2007) and Volesky *et al*. (2007) are phosphoric amines, carboxyl, carbonyl and hydroxyl groups [49, 50]. Some of these charged ions were present in EPS of produced by both strains may interact in chelation of Cr (VI) and aid in survival of bacteria under chromate stress [51].

CONCLUSION

Isolated chromium resistant bacterial strain UE1 and UE4 exhibited increased Cr (VI) removal and EPS production that could be used for the remediation of chromium polluted soils. As EPS are involved in Cr (VI) uptake from surroundings and hence making it unavailable to other living organisms.

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ABBREVIATIONS

EPS = Exopolysaccharides

FTIR = Fourier Transforms Infrared Spectroscopy

TLC = Thin Layer Chromatography

EPA = Environmental Protection Agency

MIC = Minimum Inhibitory Concentration

REPS = Released Exopolysaccharides

LEPS = Loosely Bound Exopolysaccharides

REFERENCES

[1] Rajbanshi A. Study on heavy metal resistant bacteria in Guheswori sewage treatment plant. Our Nature 2009; 6(1): 52-57. https://doi.org/10.3126/on.v6i1.1655

[2] Sanyahumbi D, Duncan, JR. Zhao, M. Hille, R. Removal of lead from solution by the non-viable biomass of the water fern *Azolla filiculoides*. Biotechnol Lett 1998; 20(8): 745-747. https://doi.org/10.1023/A:1005386703592

[3] Namasivayam C, Ranganathan, K. Removal of lead (II) by adsorption onto" waste" iron (III)/chromium (III) hydroxide from aqueous solution and radiator manufacturing industry wastewater. Ind Eng Chem Res 1995; 34(3): 869-873. https://doi.org/10.1021/ie00042a019

[4] Young RV, Sessine, S. World of chemistry. Gale Group Farmington Hills; 2000.

[5] Cervantes C, Campos-García, J. Devars, S. Gutiérrez-Corona, F. Loza-Tavera, H. Torres-Guzmán, JC. Moreno-Sánchez, R. Interactions of chromium with microorganisms and plants. FEMS Microbiol Rev 2001; 25(3): 335-347. https://doi.org/10.1111/j.1574-6976.2001.tb00581.x

[6] Ucun H, Bayhan, YK. Kaya, Y. Cakici, A. Algur, OF. Biosorption of chromium (VI) from aqueous solution by cone biomass of *Pinus sylvestris*. Bioresour Technol 2002; 85(2): 155-158. https://doi.org/10.1016/S0960-8524(02)00086-X

[7] Chen JM, Hao, OJ. Microbial chromium (VI) reduction. Crit Rev Environ Sci Technol 1998; 28(3): 219-251. https://doi.org/10.1080/10643389891254214

[8] Goyal N, Jain, S. Banerjee, U. Comparative studies on the microbial adsorption of heavy metals. Adv Environ Res 2003; 7(2): 311-319. https://doi.org/10.1016/S1093-0191(02)00004-7

[9] Kotaś J, Stasicka, Z. Chromium occurrence in the environment and methods of its speciation. Environ Pollut 2000; 107(3): 263-283. https://doi.org/10.1016/S0269-7491(99)00168-2

[10] Costa M. Potential hazards of hexavalent chromate in our drinking water. Toxicol Appl Pharmacol 2003; 188(1): 1-5. https://doi.org/10.1016/S0041-008X(03)00011-5

[11] Fernández-Nieto M, Quirce, S. Carnés, J. Sastre, J. Occupational asthma due to chromium and nickel salts. Int Arch Occup Envi 2006; 79(6): 483-486. https://doi.org/10.1007/s00420-005-0078-z

[12] Srivastava S, Singh, A. Sharma, A. Studies on the uptake of lead and zinc by lignin obtained from black liquor–a paper industry waste material. Envi Technol 1994; 15(4): 353-361. https://doi.org/10.1080/09593339409385438

[13] Dhal B, Thatoi, H. Das, N. Pandey, B. Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: a review. J Hazard Mater 2013; 250: 272-291. https://doi.org/10.1016/j.jhazmat.2013.01.048

[14] Kurniawan TA, Chan, GY. Lo, WH. Babel, S. Physico–chemical treatment techniques for wastewater laden with heavy metals. Chem Eng J 2006; 118(1): 83-98. https://doi.org/10.1016/j.cej.2006.01.015

[15] Ozdemir G, Ceyhan, N. Ozturk, T. Akirmak, F. Cosar, T. Biosorption of chromium (VI), cadmium (II) and copper (II) by *Pantoea sp*. TEM18. Chem Eng J 2004; 102(3): 249-253. https://doi.org/10.1016/j.cej.2004.01.032

[16] Monteiro CM, Castro, PM. Malcata, FX. Metal uptake by microalgae: underlying mechanisms and practical applications. Biotechnol Prog 2012; 28(2): 299-311. https://doi.org/10.1002/btpr.1504

[17] Volesky B. Detoxification of metal-bearing effluents: biosorption for the next century. Hydrometallurg 2001; 59(2): 203-216. https://doi.org/10.1016/S0304-386X(00)00160-2

[18] Titus S, Gaonkar, S. Srivastava, R. Karande, A. Exopolymer production by a fouling marine bacterium *Pseudomonas alcaligenes*. Indian J Mar Sci 1995; 24(2): 45-48.

[19] Ramos A, Boels, IC. de Vos, WM. Santos, H. Relationship between glycolysis and exopolysaccharide biosynthesis in *Lactococcus lactis*. Appl Environ Microbiol 2001; 67(1): 33-41. https://doi.org/10.1128/AEM.67.1.33-41.2001

[20] Pal S, Manna, A. Paul, A. Production of poly (β-hydroxybutyric acid) and exopolysaccharide by *Azotobacter beijerinckii* WDN-01. World J Microbiol Biotechnol 1999; 15(1): 11-16. https://doi.org/10.1023/A:1008825009825

[21] Fusconi R, Godinho, M. Screening for exopolysaccharide-producing bacteria from sub-tropical polluted groundwater. Braz J Biol 2002; 62(2): 363-369. https://doi.org/10.1590/S1519-69842002000200020

[22] Rand M, Arnold, E. Michael, J. Standard methods for the examination of water and wastewater, a publication of American Public Health Association and Water Pollution. Control fed creation, Washington DC 1976; 476-478.

[23] Batool R, Yrjälä, K. Shaukat, K. Jamil, N. Hasnain, S. Production of EPS under Cr (VI) challenge in two indigenous bacteria isolated from a tannery effluent. J Basic Microbiol 2015; 55(9): 1064-1074. https://doi.org/10.1002/jobm.201400885

[24] Guo X, Wang, X. Liu, J. Composition analysis of fractions of extracellular polymeric substances from an activated sludge culture and identification of dominant forces affecting microbial aggregation. Sci Rep 2016; 6. https://doi.org/10.1038/srep28391

[25] Guibaud G, Comte, S. Bordas, F. Dupuy, S. Baudu, M. Comparison of the complexation potential of extracellular polymeric substances (EPS), extracted from activated sludges and produced by pure bacteria strains, for cadmium, lead and nickel. Chemosphere 2005; 59(5): 629-638. https://doi.org/10.1016/j.chemosphere.2004.10.028

[26] Iyer A, Mody, K. Jha, B. Accumulation of hexavalent chromium by an exopolysaccharide producing marine *Enterobacter cloaceae*. Marine Poll Bull 2004; 49(11): 974-977. https://doi.org/10.1016/j.marpolbul.2004.06.023

[27] KiliSc NK, D€onmez, G. Environmental conditions affecting exopolysaccharide production by *Pseudomonas aeruginosa, Micrococcus* sp. and *Ochrobactrum* sp. J Hazard Mater 2008; 154: 1019–1024. https://doi.org/10.1016/j.jhazmat.2007.11.008

[28] Batool R, Qurrat-ul-ain, K. Naeem, A. Comparative study of Cr (VI) removal by *Exiguobacterium* sp. in free and immobilized forms. Bioremed J 2014; 18(4): 317-327. https://doi.org/10.1080/10889868.2014.938722

[29] Kölbel-Boelke J, Tienken, B. Nehrkorn, A. Microbial communities in the saturated groundwater environment I: Methods of isolation and characterization of heterotrophic bacteria. Microb Ecol 1988; 16(1): 17-29. https://doi.org/10.1007/BF02097402

[30] Clark J, Munnecke, D. Jenneman, G. Insitu microbial enhancement of oil production. Dev Ind Microbiol 1981; 22: 695-701.

[31] Kim SJ, Murthy, HN. Hahn, EJ. Lee, HL. Paek, KY. Parameters affecting the extraction of ginsenosides from the adventitious roots of ginseng (Panax ginseng CA Meyer). Sep Purif Technol 2007; 56(3): 401-406. https://doi.org/10.1016/j.seppur.2007.06.014

[32] Muralidharan J, Jayachandran, S. Physicochemical analyses of the exopolysaccharides produced by a marine biofouling bacterium, *Vibrio alginolyticus*. Process Biochem 2003; 38(6): 841-847. https://doi.org/10.1016/S0032-9592(02)00021-3

[33] Spector T, Refinement of the Coomassie blue method of protein quantitation: A simple and linear spectrophotometric assay for≤ 0.5 to 50 µg of protein. Anal Biochem 1978; 86(1): 142-146. https://doi.org/10.1016/0003-2697(78)90327-5

[34] Dubois M. Colorimetric method for determination of sugars and related substances. Anal Chem 1956; 28(3): 350-356. https://doi.org/10.1021/ac60111a017

[35] Kim JU, Kim, Y. Han, KS. Oh, S. Whang, KY. Kim, JN. Kim, SH. Function of cell-bound and released exopolysaccharides produced by *Lactobacillus rhamnosus* ATCC 9595. J Microbiol Biotechnol 2006; 16(6): 939-945.

[36] Schell LM, Gallo, MV. Denham, M. Ravenscroft, J. Effects of pollution on human growth and development: an introduction. J Physiol Anthropol 2006; 25(1): 103-112. https://doi.org/10.2114/jpa2.25.103

[37] Sundar VJ, Ramesh, R. Rao, PS. Saravanan, P. Sridharnath, B. Muralidharan, C. Water management in leather industry. J Sci Ind Res 2001; 60(6): 443-450.

[38] Faisal M, Hasnain, S. Microbial conversion of Cr (VI) in to Cr (III) in industrial effluent. Afr J Biotechnol 2005; 3(11): 610-617.

[39] Malaviya, P, Singh, A. Bioremediation of chromium solutions and chromium containing wastewaters. Crit Rev Microbiol 2016; 42(4): 607-633.

[40] Ball MM, Carrero, P. Castro, D. Yarzábal, LA. Mercury resistance in bacterial strains isolated from tailing ponds in a gold mining area near El Callao (Bolívar State, Venezuela). Curr Microbiol 2007; 54(2): 149-154. https://doi.org/10.1007/s00284-006-0347-4

[41] Kumar U, Bandyopadhyay, M. Fixed bed column study for Cd (II) removal from wastewater using treated rice husk. J Hazard Mater 2006; 129(1): 253-259. https://doi.org/10.1016/j.jhazmat.2005.08.038

[42] Shakoori AR, Makhdoom, M. Haq, RU. Hexavalent chromium reduction by a dichromate-resistant gram-positive bacterium isolated from effluents of tanneries. Appl Microbiol Biotechnol 2000; 53(3): 348-351. https://doi.org/10.1007/s002530050033

[43] Mangaiyarkarasi MM, Vincent, S. Janarthanan, S. Rao, TS. Tata, B. Bioreduction of Cr (VI) by alkaliphilic *Bacillus subtilis* and interaction of the membrane groups. Saudi J Biol Sci 2011; 18(2): 157-167. https://doi.org/10.1016/j.sjbs.2010.12.003

[44] Kumar MA, Anandapandian, KTK. Parthiban, Z. Production and characterization of exopolysaccharides (EPS) from biofilm forming marine bacterium. Braz Arch Biol Technol 2011; 54(2): 259-265. https://doi.org/10.1590/S1516-89132011000200006

[45] Dogan NM, Kantar, C. Gulcan, S. Dodge, CJ. Yilmaz, BC. Mazmanci, MA. Chromium (VI) bioremoval by *Pseudomonas* bacteria: role of microbial exudates for natural attenuation and biotreatment of Cr (VI) contamination. Environ Sci Technol 2011; 45(6): 2278-2285. https://doi.org/10.1021/es102095t

[46] Brejerov E, Stratilov, ZHE. Sasinkov, V. Ebringerov, A. Effect of salt stress on the production and properties of extracellular polysaccharides produced by *Cryptococcus laurentii*. Z Naturforsch C 2005; 60: 444–450.

[47] Morel MA, Martha, CU. Olivera-Bravo, S. Callejas, C. Gill, PR. Castro-Sowinski, S. Cellular and biochemical response to Cr (VI) in *Stenotrophomonas* sp. FEMS Microbiol Lett 2009; 291(2): 162-168. https://doi.org/10.1111/j.1574-6968.2008.01444.x

[48] Kachlany SC, Levery, SB. Kim, JS. Reuhs, BL. Lion, LW. Ghiorse, WC. Structure and carbohydrate analysis of the exopolysaccharide capsule of *Pseudomonas putida* G7. Environ Microbiol 2001; 3(12): 774-784. https://doi.org/10.1046/j.1462-2920.2001.00248.x

[49] Pradhan S, Singh, S. Rai, LC. Characterization of various functional groups present in the capsule of Microcystis and study of their role in biosorption of Fe, Ni and Cr. Bioresource Technol 2007; 98: 595–601. https://doi.org/10.1016/j.biortech.2006.02.041

[50] Volesky B. Biosorption and me. Water Res 2007; 41: 4017–4029. https://doi.org/10.1016/j.watres.2007.05.062

[51] Omoike A, Chorover, J. Spectroscopic study of extracellular polymericsubstances from *Bacillus subtilis*: aqueous chemistry and adsorption effects. Biomacromolecules 2004; 5: 1219–1230. https://doi.org/10.1021/bm034461z

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