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Biosorption of Chromium by *Bacillus subtilis* Isolated from Ganga River

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ABSTRACT

Water pollution by heavy metals due to discharge of industrial and anthropogenic waste leads to serious environmental and health problems as most of these heavy metals are carcinogenic in nature. In the present study chromium biosorption capacity of live and dead biomass of bacterial strain HGB1 isolated from Ganga River in Haridwar, which was examined as *Bacillus subtilis*, following 16S rDNA sequence analysis, was examined for different physical parameters such as pH, time of incubation and temperature. Experimental results indicate that the *Bacillius subtilis* has maximum tolerance capacity up to 1000 mg.L^{-1} with highest metal uptake of 95.64%, 97.25% and 97.11% at pH 3, 60 minutes, 2.5 mg/mL biomass respectively in case of dead biomass. In case of living biomass, highest metal uptake was 81.64%, 96.79 % and 95.89% at pH 7, 72hr and 32°C respectively. The surface chemical functional groups of *Bacillus subtilis* identified by FTIR were amino, carboxyl, hydroxyl and carbonyl groups. The morphological changes were examined by SEM analysis.

INTRODUCTION

Metals naturally occur in earth crust and remain distributed in environment according to their properties and the environmental factors (Khlifi & Hamzachaffai 2010). Notably out of 92 naturally occurring elements, approximately 30 metals are toxic to humans (Sharma & Singh 2015), which basically comprise of heavy metals. In recent decades, heavy metals are widespread in environment and cause water, air and soil pollution (Simly & Sumithra 2017). Heavy metals refer to all those metals and metalloids with atomic density greater than 4 g/cm^3 or 5 times or more than water (Huton & Symon 1986, Battarbee et al. 1988, Nriagu et al. 1995, Hawkes 1997). Out of these heavy metals chromium contamination in aquatic system is of great concern as it is 7^{th} most abundant element in the earth crust (Mohanty & Kumar 2013). Chromium enters into various environmental grids, i.e. air, water and soil through various anthropogenic and industrial wastes (Mondal et al. 2017, Sharma & Singh 2018). In aqueous environment two forms of chromium exist, which are trivalent and tetravalent chromium. The trivalent chromium [Cr(III)] is considered as essential trace elements, which plays role in metabolism of mammals whereas tetravalent chromium [Cr(IV)] is considered as toxic and carcinogenic for mammals (Puentes-Cardenes et al. 2012). The toxic effects of chromium from environmental samples can be treated by the methodologies such as ion-exchange, electrochemical treatment, chemical reduction, etc. but all these techniques are highly expensive, labor intensive, and also generate secondary wastes, which are difficult to be managed, and also these methods are not applicable when the concentration of heavy metals are low (Rakhunde et al. 2012). In recent years a great attention was paid for the use of biological matters as biosorbent for the removal of heavy metals from the environment (Aravindhan et al. 2012). These biosorbents are readily available, cheap and possess high biosorption efficiency. Both live and dead biomass can be used as biosorbent (Saha & Orvig 2010, Samuel et al. 2015). In the present study, live and dead biomass of *Bacillus subtilis* was used as biosorbent for the biosorption of chromium from the aqueous solution. *Bacillus subtilis* is a Gram positive bacteria mainly composed of peptidoglycan and teichoic acid. Peptidoglycan is a polymer of acetyl glucosamine and acetyl muramic acid which displace carboxyl and hydroxyl functional groups. On the other hand, teichoic acid is composed of copyranosyl glycerol phosphate and it displace phosphate and hydroxyl functional groups. These functional groups play main role in forming the bonds between metals and biomass (Sivaprakash et al. 2009). The experimental data were analyzed using kinetics and adsorption isotherms, while Fourier Transform Infrared (FT-IR) spectroscopy was used for the identification of functional groups and SEM was used for the analysis of surface changes present on chromium unloaded and chromium loaded biomass.

MATERIALS AND METHODS

Bacterial isolation from Ganga samples: Bacterial isolates were isolated from Ganga river through serial dilution

method (Waksman & Fred 1922). The 1 mL of water sample was suspended in 9 mL of sterile distilled water blanks and diluted up to 10^{-3} dilution; 0.1 mL of suspension was spreaded on nutrient agar plates and incubated at 30±2°C.

Selection of chromium tolerant bacterial isolate: Ability of 18 bacterial isolates to tolerate chromium was determined by well diffusion method (Hemambika et al. 2011). Chromium salt solution of different concentrations, i.e. 20, 40, 60, 80 and 100 mg/L were prepared and 0.1 mL of these chromium solutions were added in 8mm well prepared nutrient agar plates that were spread with 24 hours old culture of bacterial isolates and then were incubated at 30±2°C for 24 hours and after that incubation zone of inhibition was measured.

Molecular identification of chromium resistant bacteria: The partial sequencing of 16S rDNA of chromium tolerant bacteria was carried out commercially by Eurofins Genomics India Private Limited. Later to accurately identify the strain by software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA 7.

Metal solution preparation: A stock solution of 1000 mg/L concentration was prepared by dissolving potassium dichromate ($K_2Cr_2O_7$) known quantity in double distilled water and stored in refrigerator (Hussein et al. 2004).

Biosorbent Preparation

(a) Preparation of live biomass: Selected and identified *Bacillus subtilis* strain was grown and maintained on the nutrient agar slants and a loopful pure culture was transferred in sterile nutrient broth and incubated at 30 ± 2°C in a shaker incubator for overnight and used as live biomass.

(b) Preparation of dead biomass: *Bacillus subtilis* culture was grown in nutrient broth at 30±2°C for 24 hours and harvested by centrifuging at 10000 rpm for 10 min at 4°C. Cell pellets, thus obtained, was washed twice with distilled water to make sure that no media remain attached to pellets and then they are dried for 6 hours at 80°C in hot air oven.

Biosorption experiments: The biosorption experiment were conducted during different physical parameters in 100 mL Erlenmeyer flasks containing 50 mL of 100 $mg.L^{-1}$ chromium salt solution; biosorption studies were performed using 0.5 to 2.5 $g.L^{-1}$ of died and living biomass (Aravindhan et al. 2011). The flasks with test solutions were agitated at 120 rpm using a shaker incubator at room temperature for different time intervals, i.e. 20, 40, 60, 80 and 100 minutes for dead biomass and 24, 48, 72, 96 and 120 hours for live biomass and different pH such as 3, 5, 7, 9 and 11 (Congeevaram et al. 2007). The batch biosorption experiments were also done at different temperatures for biosorption. The change in metal concentration after biosorption was determined by AAS technique. For isotherm study cell weight was kept constant, i.e. 1 mg/mL (Gelagutashvili 2013).

Kinetics studies: To study biosorption kinetics, varying biosorbent dose, i.e. 0.5, 1.0, 1.5, 2.0 and 2.5 g was used for the biosorption of 100 mg/L chromium in aqueous solution. Pseudo first order and Pseudo second order were used to determine the biosorption kinetics of *Bacillus subtilis* biosorption. First order rate expression was given by Lagergren (Namasivayam & Kanchana 1993).

$$\log(q_e - q) = \log q_e - \frac{K_1}{2.303} \quad \ldots(1)$$

Where, q_e is amount of chromium absorbed at equilibrium, q is amount of chromium absorbed while *t* is time in minutes in which Cr absorbed, k_t and q_e were first order rate constant and these are determined by slopes and intercepts of plots of log (q_e –q) versus t at different biomass dosages.

Second order kinetics was given by (Ho & McKay 1999).

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e t} \quad \ldots(2)$$

Where, k_2 and q_e were determined by the intercept of the slope of plot obtained by plotting *t/qt* versus time *t*.

Biosorption Characterization Through Surface Studies

Fourier transforms infrared (FT-IR) spectroscopy: FT-IR was used for the analysis of chemical nature of biosorbent surface before and after chromium loading. For FT-IR analysis chromium unloaded and loaded pellets of *Bacillus subtilis* were obtained and oven dried at 80°C for 2 hours, and 0.1 g of finely grounded biomass pellets were mixed with KBr for analysis (Rezaei 2013).

Scanning electron microscopy: For SEM analysis culture of *Bacillus subtilis* was centrifuged at 6000 rpm for 15 minutes, the supernatant was discarded and bacterial pellets were washed 3-4 times with 0.1M phosphate buffer (pH 7.2) after which pellets were fixed in 2.5% glutaraldehyde and dehydrated with 30-90% ethanol and finally dehydrated with 100% ethanol for 8-10 minutes. Chromium loaded and non-loaded samples were coated with gold 90 Å thick layer under vacuum to improve the quality of the image (Michalak et al. 2014).

RESULTS AND DISCUSSION

Total of 15 bacterial isolates were isolated from Ganga water samples. All these isolates were now screened for chro-

Table 1: Ability of bacterial isolates to tolerate chromium (average of triplicates).

Isolates	Chromium tolerance ability (± SE)					
	10 mg/L	20 mg/L	40 mg/L	60 mg/L	80 mg/L	100 mg/L
HGB1	-	-	-	-	-	-
HGB2	-	3.6±0.3	5.2±0.1	6.6±0.3	6.8±0.05	7.7±0.1
HGB3	-	-	-	-	-	4.4±0.05
HGB4	-	-	-	-	-	-
HGB5	-	4.3±0.3	5.6±0.2	6.8±0.02	8.6±0.3	10.8±0.02
HGB6	-	-	-	-	-	13.1±0.01
HGB7	-	-	-	-	6.5±0.01	7.9±0.01
HGB8	-	-	4.9±0.03	6.6±0.06	7.6±0.1	9.6±0.1
HGB9	-	-	6.3±0.33	6.9± 0.01	8.8±0.01	10.5± 0.5
HGB10	-	-	-	-	-	-
HGB11	-	-	-	5.1±0.05	8.3±0.66	10.3±0.66
HGB12	-	-	-	-	-	-
HGB13	-	3.3±0.3	4.6±0.33	5±0.01	6.4± 0.02	7.8±0.01
HGB14	-	-	-	-	-	-
HGB15	-	-	-	-	-	-

mium tolerance capacity through well diffusion method (Table 1). The growth of isolate, which is chromium resistant, will show no or very less zone of inhibition.

From the results (Table 1), it was found that HGB1 isolate shows no zone of inhibition up to 100 mg/L concentration of chromium, further on the basis of 16S rDNA sequencing HGB1 was found to be *Bacillus subtilis* (Fig. 1).

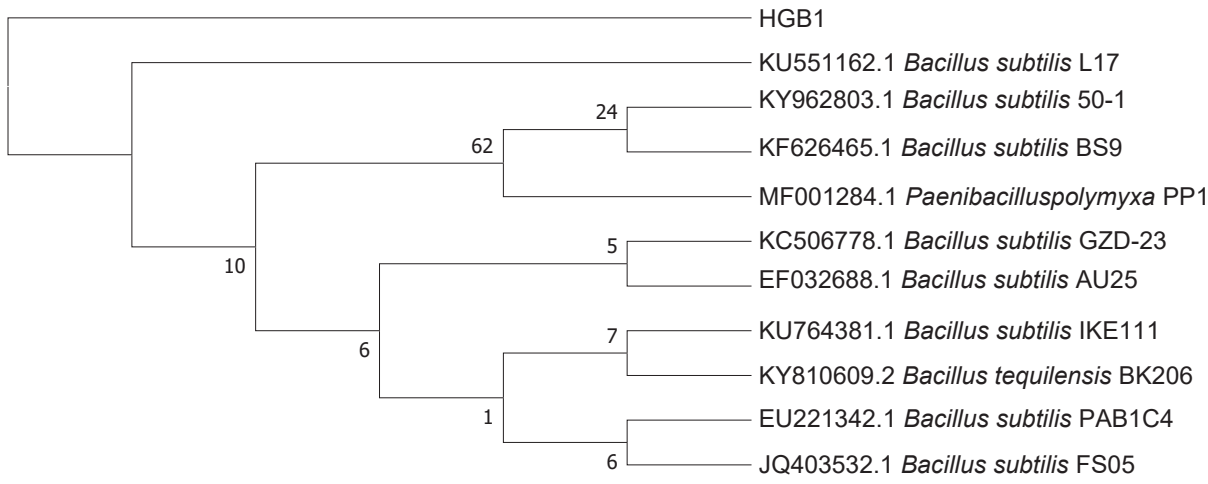


Fig. 1: Phylogenetic tree showing the relationship on the basis of 16S rDNA gene sequencing.

Table 2: Biosorption assay at different physical parameters.

Heavy metals	Microorganisms	Initial Concentration (mg/L)	Parameters	% of Removal		
			pH	**Dead**	**Live**	
Chromium (Cr)	*Bacillus subtilis*	100	3	81.89	2.06	
			5	92.65	19.89	
			7	83.09	91.34	
			9	74.78	22.40	
			11	34.99	3.68	
			Temp., °C	**Dead**	**Live**	
Chromium (Cr)	*Bacillus subtilis*	100	25	80.31	69.92	
			30	91.94	87.27	
			35	95.91	93.61	
			40	86.11	73.93	
			45	66.44	67.02	
			Time (min)	**Dead**	**Time (Hours)**	**Live**
Chromium (Cr)	*Bacillus subtilis*	100	20	79.72	24	61.48
			40	88.92	48	87.12
			60	97.25	72	94.02
			80	98.80	96	95.01
			100	98.95	120	95.97
			Biomass dose (mg/mL)	**Dead**	**Live**	
Chromium (Cr)	*Bacillus subtilis*	100	0.5	83.34	38.02	
			1.0	89.79	53.87	
			1.5	92.78	79.92	
			2.0	94.89	87.22	
			2.5	97.11	95.71	

Effect of pH change: The pH of the solution had a significant role in the removal of chromium; at particular pH the adsorption becomes maximum, below or above that pH the biosorption rate decreases (Zhou & Kiff 1991). In the present study the effect of pH on biosorption was determined by varying pH from 3 to 11, and it was found that in case of dead and live biomass at pH 5 and pH 7 biosorption was maximum, i.e. 92.65 % and 91.34 % respectively (Fig. 2). Aravindhan et al. (2011), Garcia et al. (2016) and Sukumar et al. (2014) also studied the effect of pH on biosorption of chromium by *Bacillus subtilis* and they also found that pH 2, 4.5 and 6 and are optimum for the biosorption (Table 2). Sethuraman & Balasubramanian (2010) studied the effect of pH on live biomass of *Bacillus subtilis* biosorption capacity and found that pH 7 was optimum. The possible reason for this might be that pH may affect the ionization state of the chemical functional groups such as carboxyl, amino and hydroxyl that are responsible for the binding of heavy metals (carry a positive charge). On low pH these functional groups become protonated and increase positive charge on the biosorbent and decrease the attachment of metal ions on biosorbent. On the other hand at high pH value, functional group becomes deprotonated and hence increase negative charge on the biosorbent, that is why more and more metal ions get attached to the surface of biosorbent (Comte et al. 2008 and Krishnani et al. 2008).

Effect of time of incubation: The incubation time was one of the important parameters that affect biosorption efficiency. In present study, the effect of time on 100 mg/L concentration of chromium was examined, and observed that 60 minutes and 72 hours was optimum for dead and live biomass and they show 95.91% and 93.61% removal of chromium respectively (Figs. 3 & 4). The similar study made by Murthy et al. (2012) showed 75.6% biosorption at

Fig. 2: Effect of pH on biosorption of chromium by dead and live biomass.

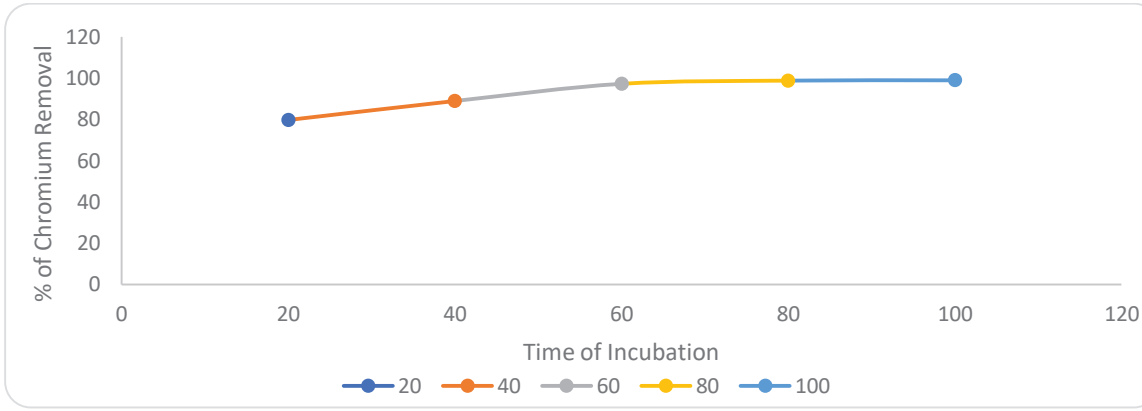


Fig. 3: Effect of time of incubation on the metal uptake capacity of dead biomass.

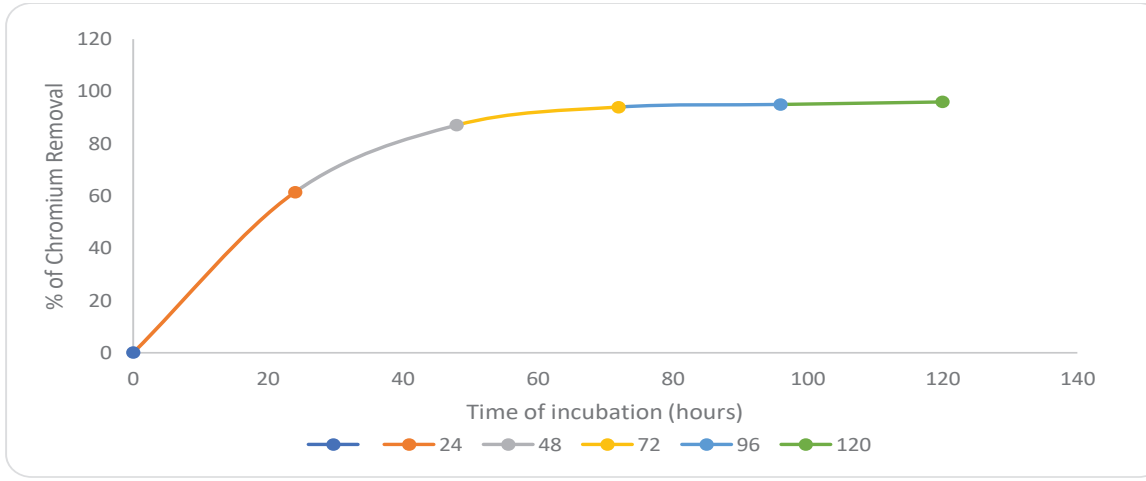


Fig. 4: Effect of time of incubation on the metal uptake capacity of live biomass.

72 hours to be optimum while studying biosorption of 200 mg/L lead by *Bacillus cereus*. Giri (2012) studied biosorption capability of living *Bacillus cereus* for 50 mg/L Pb and Cd and found 50.11% reduction in 48 hours.

Effects of temperature: To study the effects of temperature on the biosorption of 100 mg/L chromium by *Bacillus subtilis,* we carried our experiment on temperature ranging from 25°C to 45°C. It was found that the maximum biosorption was attained at 35°C, i.e. 95.91% and 93.61% for dead and live biomass respectively, which kept on slightly increasing with further increase in temperature (Fig. 5). These results indicate that the process of metal uptake was endothermic in nature. Similar results were also observed by Congeevaram et al. (2007) when they studied biosorption of Cr(VI) and Ni by *Micrococcus* sp. Aravindhan et al. (2012) studied the effect of temperature on the biosorption of Cr(III) using *Bacillus subtilis* biomass and found that at 30°C, biosorbent achieved maximum biosorption of Cr, i.e. 71.69%. Similar results were also obtained by Murthy et al. (2012), Ahmed & Kibret (2013), Kareem et al. (2014), Verma (2017) supporting the present study.

Effect of biomass dose: To study the effect of biomass dos-

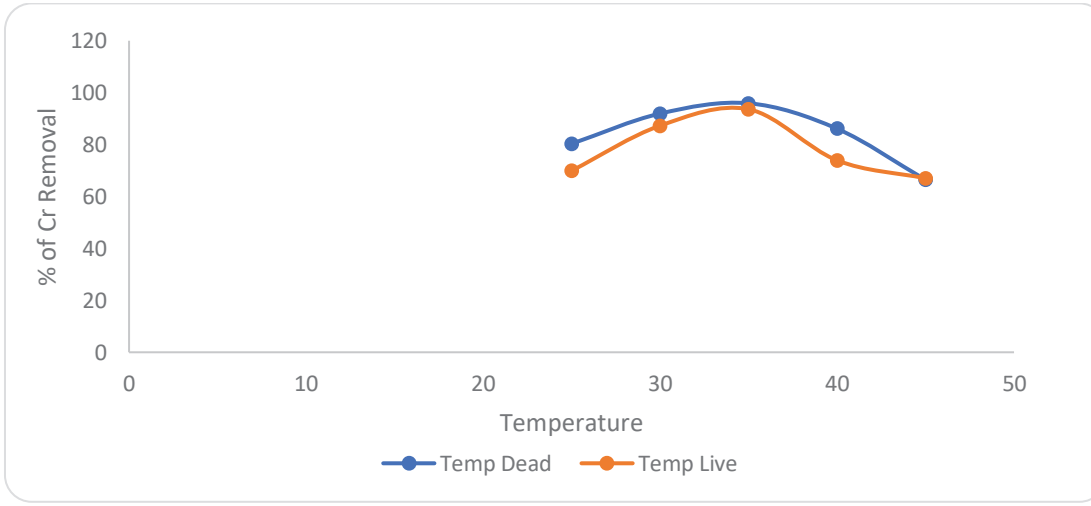


Fig. 5: Effect of temperature on metal uptake capacity of dead and live biomass.

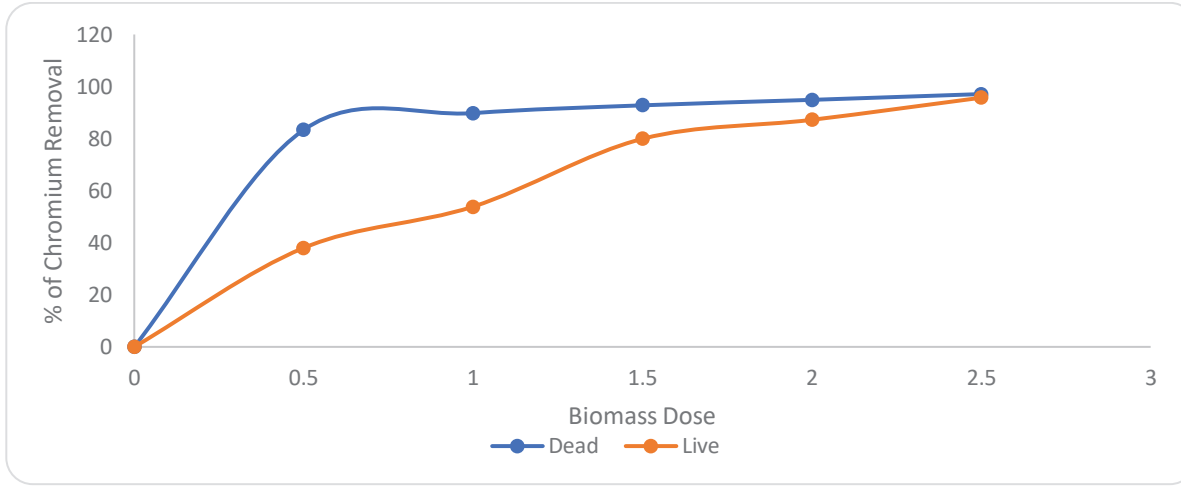


Fig. 6: Effect of biomass dose on metal uptake capacity of dead and live biomass.

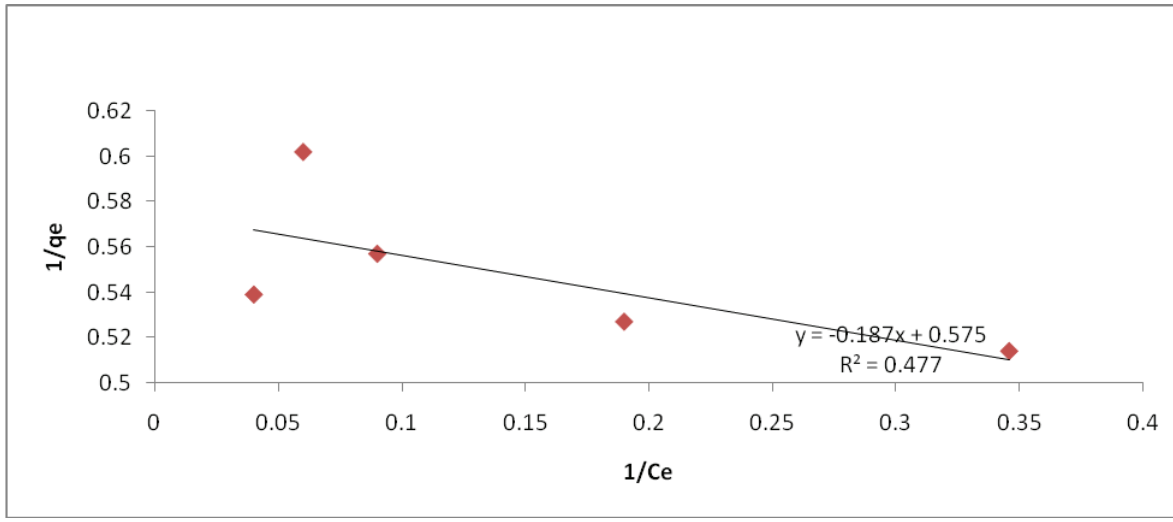


Fig. 7: Langmiur isotherm for chromium by *Bacillus subtilis* at pH 5, 60 min, 350°C temperature.

age on 100 mg/L heavy metals aqueous solution, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/mL of biomass dosage were considered, and it was found that the percentage uptake increased with increase in biomass (Fig. 6). During the study with *Bacillus subtilis*, % biosorption increases to 97.11% for dead biomass and 95.71% for live biomass. The same was observed by Aravindhan et al. (2011) and Bala-Kumaran et al. (2013).

Isotherm Analysis

Langmuir isotherm: The data obtained in the study of 100 mg/L aqueous solution of chromium showed the value of R^2 (Langmuir) as 0.004 (Fig. 7) and R^2 (Freundlich) as 0.909 (Fig.8) for *Bacillus subtilis* (Table 3). The value of 1/n further supports the Freundlich equation. Thus, equilibrium experimental results of chromium ions have been

Table 3: Parameters of isotherm models for chromium.

Biosorbent	Langmuir Isotherm			Freundlich Isotherms		
	q^o	B	R^2	r^2	K_f	n
Bacillus subtilis	2.89	1.019	0.477	0.921	2.15	0.4

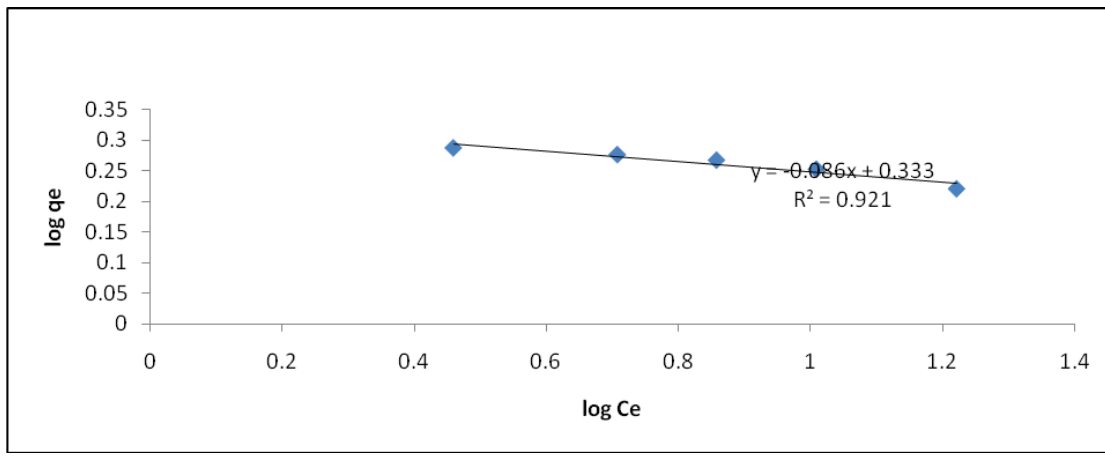


Fig. 8: Freundlich isotherm for chromium by *Bacillus subtilis* at pH 5, 60 min, 35°C temperature.

fitted in Langmuir and Freundlich models according to the Vijaykumar et al. (2012). If the value of the coefficient is less than 1, absorption is favorable. Similar pattern was also observed by Wierzba (2015).

Kinetic analysis: To calculate a suitable kinetic model, metal uptake data were plotted against time. The linear line shows the favorable biosorption process. In the present study, the results fitted in second-order kinetics (Table 4, Figs. 9 & 10).

FT-IR analysis: The spectra that were obtained during FT-IR analysis of chromium unloaded biomass (Fig. 11) show a broad and strong peak at 3447.82 cm^{-1} indicating the presence of hydroxyl groups. Another peak 2928 cm^{-1} lies in the range of 2930-2925 cm^{-1} which was considered for the presence of C-H group. The strong peak occurring at 1636 cm^{-1} indicates the presence of amino (N-H) group. Peaks at 1384.55 and 1056.80 cm^{-1} lie in the range of 1430-1280 cm^{-1} and 1.350-900 cm^{-1}, which represent the presence of

Table 4: Parameters of pseudo kinetics for chromium.

Biosorbent	Pseudo first order			Pseudo second order		
	K_1	q_e	R^2	K_2	q_e	R^2
Bacillus subtilis	0.0004	1.945	0.773	0.00041	2.75	0.999

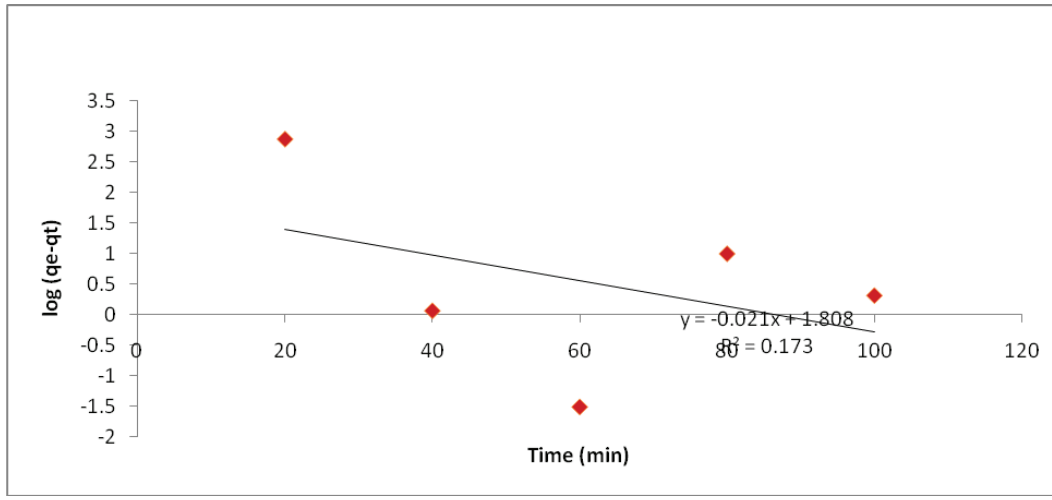


Fig. 9: First order kinetics for chromium by *Bacillus subtilis* at 60 min, pH 5, 35°C temperature.

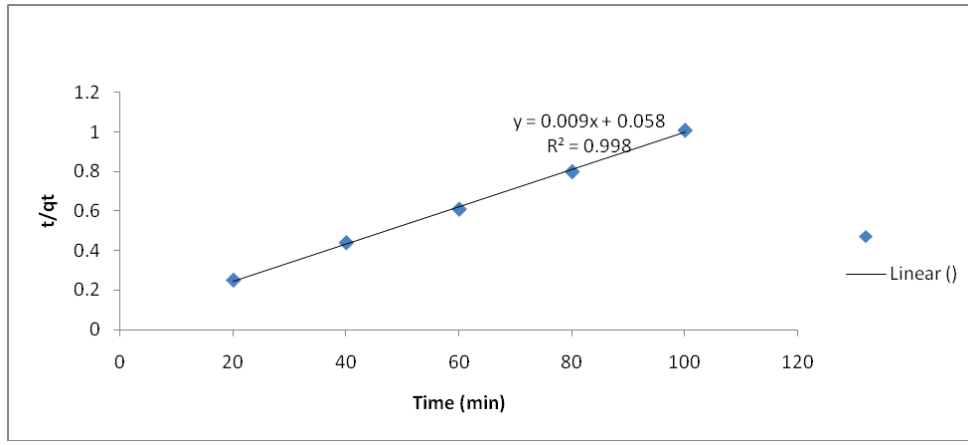


Fig. 10: Second order kinetics for chromium by *Bacillus subtilis* at 1 mg/mL biomass concentration, pH 5, 30°C.

O-H and C-N stretching respectively (Jaafar et al. 2015 and Yun et al. 2001). A peak at 680 cm^{-1} may be assigned to OH variation (Coates 2000). On the study of chromium loaded biomass (Fig. 12) peak of O-H group changes from 3469.68 cm^{-1} to 3447.82 cm^{-1}.

SEM analysis: Scanning electron microscopy (SEM) of *Bacillus subtilis* before and after biosorption of chromium clearly revealed the surface texture difference before and after biosorption (Fig. 13).

CONCLUSION

The present study has demonstrated the ability of *Bacillus subtilis,* isolated from Ganga river, as biosorbent for the removal of chromium ions from aqueous solution. Its biosorption ability was depend upon various factors, i.e. pH, temperature and time of incubation. Kinetics and equilibrium data also fitted with pseudo first and second order and Langmuir isotherm model; these models provide the information that the present process was favorable process-

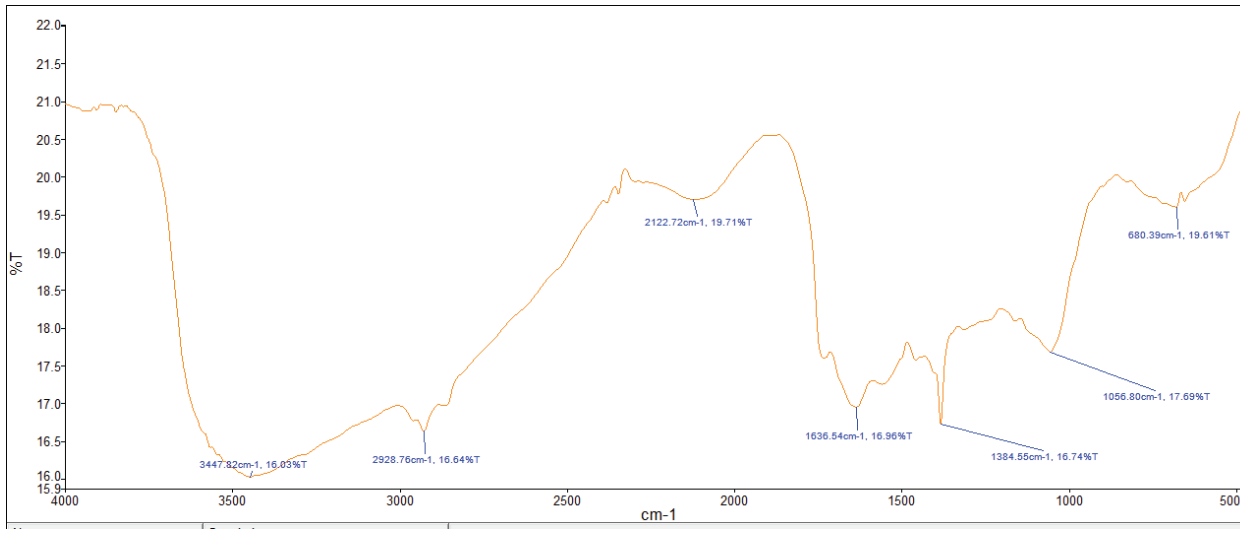


Fig. 11: FT-IR analysis of chromium unloaded *Bacillus subtilis*.

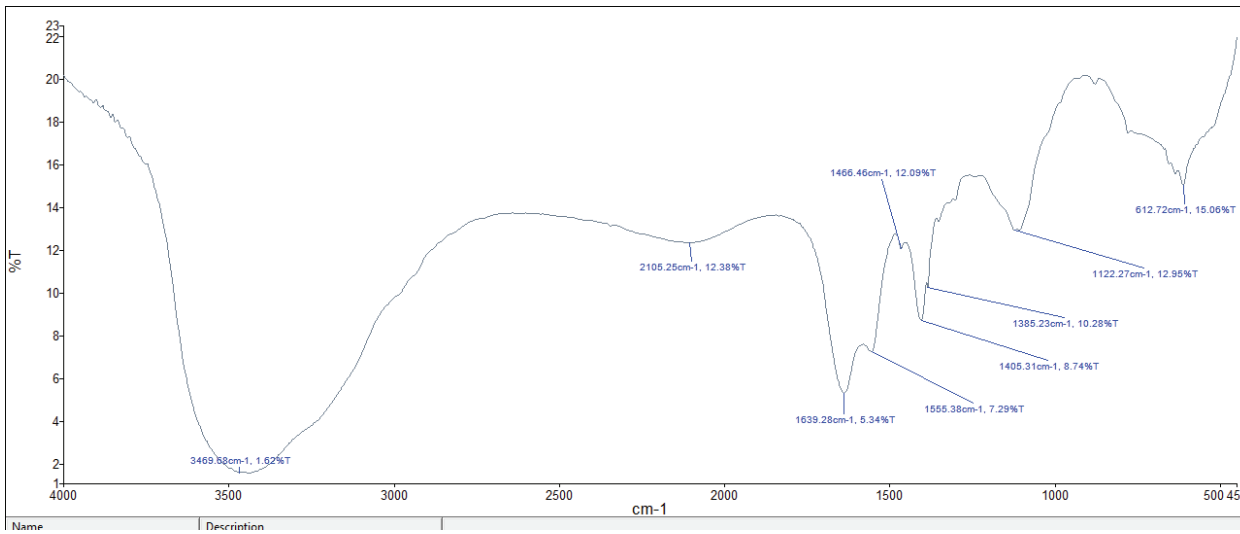
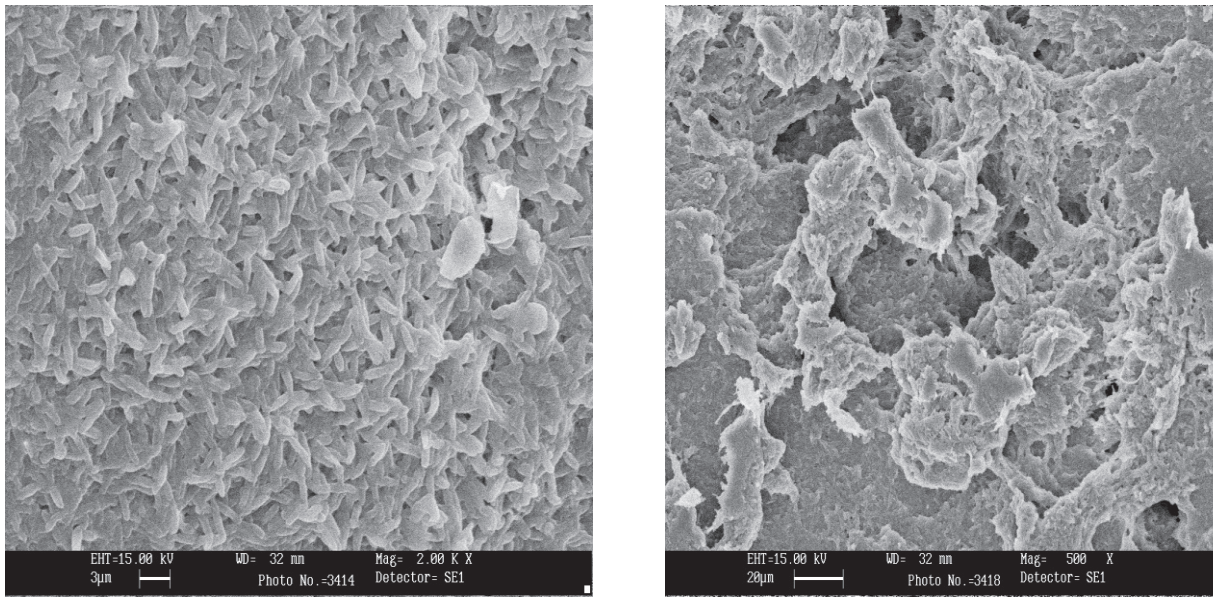


Fig. 12: FT-IR analysis of chromium loaded *Bacillus subtilis*.



(a) (b)

Fig. 13: Scanning electron microscopy (SEM) micrographs of *Bacillus subtilis* before (a) and after (b) of biosorption.

es. The proposed biosorption process can be successfully applied for the treatment of the waste that contains chromium ions.

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REFERENCES

Arvindhan, R., Fathima, A., Selvamurugan, M., Rao, J.R., and Balachandran, U.N. 2012. Adsorption, desorption and Kinetic study on Cr (III) removal from aqueous solution using *Bacillus subtilis* biomass. Clean Technol. Environ. Policy, 14: 727-735.

Battarbee, R.W., Anderson, N.J., Appleby, P.G., Flower, R.J., Fritz, S.C., Haworth, E.Y., Higgitti,S., Jones, V.J., Kreiser, A., Munro, M.A.R., Natkanshi, J., Oldfeild, F., Patrick, S.T., Richard-son, N.G., Rippey, B. and Stevenson, A.C. 1988. Lake Acidification in United Kingdom. ENSIS, London.

Coates, J. 2000. Interpretation of infrared spectra, a practical approach. In: Meyers, R.A. (Ed), Encyclopedia of Analytical Chemistry. John Wiley and Sons Ltd, Chichester, pp. 10815-10837.

Comte, S., Guibaud, G. and Baudu, M. 2008. Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values. J. Hazard Master, 151: 185-193.

Congeevaram, S., Dhanarani, S., Park, J., Dexillin, M. and Thamaraiselvi, K. 2007. Biosorption of chromium and nickel by heavy metals resistant fungal and bacterial isolates. Journal of Hazardous Material, 146: 270-277.

Garcia, R., Campas, J., Cruz, J. A., Calderon, M. E., Raynal, M. E. and Buitron, Y. G. 2016. Biosorption of Cd, Cr, Mn and Pb from aqueous solutions by *Bacillus* sp. strains isolated from industrial waste activate sludge. TIP Rev. Esp. Ciene. Quim. Biol., 19(1): 5-14.

Gelagutashvili, E. 2013. Comparative study on heavy metals biosorption by different types of bacteria. Open Journal of Metal, 3: 62-67.

Giri, A.K. 2012. Removal of arsenic (III) and chromium (IV) from the water using phytoremediation and bioremediation technique. PhD Thesis, Odisha, India.

Hawkes, J.S. 1997. Heavy metals. J. Chem. Educ., 74(11): 1374.

Hemambika, B., Johmcy, M.R. and Kannan, V.R. 2011. Biosorption of heavy metals by immobilized and dead fungal cells: A comparative assessment. Journal of Ecology and the Natural Environment, 3(5): 168-175.

Hussein, H., Ibrahim, S. F., Kandeel, K. and Hassan, M. 2004. Biosorption of heavy metals from waste water using *Pseudomonas* sp. Electronic Journal of Biotechnology, 7(1).

Hutton, M. and Symon, C. 1986. The quantities of cadmium, lead, arsenic entering the U.K. environment from human activities. Science of the Total Environment, 16: 1677-1700.

Jaafar, Sulami, A. A., Taee, A. A. and Aldoghachi, F. 2015. Biosorption of some heavy metals by metals by metal resistant *Bacillus thuringiensis* isolated from soil in Basra Government, Iraq. Journal of Biology, Agriculture and Healthcare, 5: 106-114.

Khilfi, R. and Hamza-chaffai, A. 2010. Head and neck cancer due to heavy metals exposure via tobacco smoking and professional exposure. A review. Toxicology and Applied Pharmacology, 248: 71-88.

Krishnani, K.K., Meng, X.G., Christodoulatos, C. and Boddu, V.M. 2008. Biosorption mechanism of nine different heavy metals onto biomatrix from rice husk. J. Hazard Mater., 153: 1222-1234.

Kumar, R., Bishnoi, N. R. and Garima, Bishnoi, K. 2008. Biosorption of chromium (VI) from aqueous solution and electroplating wastewater using fungal biomass. Chemical Engineering Journal, 135: 202-208.

Michalak, J., Wolowiee, P. and Chojnacka, K. 2014. Determination of

exposure to lead of subjects from South-Western Poland by human hair analysis. Environmental Monitoring and Assessment, 186: 1259-2267.

Mohanty, M. and Kumar, P.H. 2013. Effect of ionic and chelate assisted hexavalent chromium on mung bean seedlings (Vigna Radiata. Wilczek Ark- 351) during seedling growth. JSPB, 9(2): 232-241.

Mondal, N.K., Samanta, A., Dutta, S. and Chattoraj S. 2017. Optimization of Cr (VI) biosorption onto Aspergillus niger using 3- level Box Behnken design, equilibrium, kinetic, thermodynamic and regeneration studies. Journal of Genetic Engineering and Biotechnology, 24: 65-71.

Namasivayam, C. and Kanchana, N. 1993. Removal of congo red from aqueous solutions by waste banana pith. Pertanika, 1: 32-42.

Puentes-Cardenas, I.J., Pedroza- Rodriguez,A.M., Navarrete-lopez, M., Villegas-Garrido, T. L. and Cristiani-Urbina, E. 2012. Biosorption of trivalent chromium from aqueous solutions by *Pleurotus ostreatus* biomass. Environ. Eng. Manag. J., 11: 1741-52.

Rakhunde, R., Deshpande, L. and Juneja, H.D. 2012. Chemical speciation of chromium in water: A review. Crit. Rev. Environ. Sci. Technol., 42(7): 776-810.

Rezaei, H. 2013. Biosorption of chromium by using *Spirulina* sp. Arabian Journal of Chemistry, 9(6): 846-853.

Saha, B. and Orvig, C. 2010. Biosorbent of hexavalent chromium elimination from industrial and municipal effluents. Coord. Chem. Rev., 254: 2959-2972.

Sahin, Y. and Ozturk. 2005. Biosorption of Chromium (IV) ions from aqueous solution by the bacterium *Bacillus thuringiensis*. Process Biochem., 40: 1895-1901.

Samuel, M. S., Abigail, M. E.A. and Ramalingam, C. 2015. Biosorption of Cr (VI) by *Ceratocystis paradoxa* MSR2 using isotherm modeling, kinetic study and optimization of batch parameters using response surface methodology. PLOS one, 10(3).

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

Sharma, V. and Singh, P. 2015. Heavy metals pollution and its effect on environment and human health. International Journal of Recent Scientific Research, 6(10): 7752-7755.

Sharma, V. and Singh, P. 2018. Correlation study on physico-chemical parameters of river Ganga, during different seasons in Haridwar. International Journal of Research in Advent Technology, 6(9): 2261-2272.

Sivaprakash, A., Aravindhan, R., Rao, J. R. and Nair, B.U. 2009. Kinetics and equilibrium studies on the biosorption of hexavalent chromium from aqueous solutions using *Bacillus subtilis* biomass. Appl. Ecol. Environ. Res., 7: 45-57.

Smily, J.R.M.B. and Sumithra, P.A. 2017. Optimization of chromium biosorption by fungal adsorbent, *Trichoderma* sp. BSCR02 and its desorption studies. Hayati Journal of Biosciences, 24(2): 65-71.

Sukumar, C., Janaki, V., Kannan, S.K. and Shanti, K. 2014. Biosorption of chromium (VI) using *Bacillus subtilis* SS-1 isolated from soil samples of electroplating industry. Clean Technologies and Environmental Policy, 16(2): 405- 413.

Vijaykumar, G., Tamilarsan, R. and Dharmendira kumar, M. 2012. Adsorption, kinetic, equilibrium and thermodynamic studies on the removal of basic dye Rhodamine-B from aqueous solution by the use of natural adsorbent perlite. Journal of Material Environmental Science, 3(1): 157-170.

Waksman, S.A. and Fred, E.B. 1922. A tentative outline of the plate method for determining the number of microorganisms in the soil. Soil Sci., 14: 27-28.

Wierzba, S. 2015. Biosorption of lead (II), zinc (II) and nickel (II) from industrial wastewater by *Strenotrophomonas maltophilia* and *Bacillus subtilis*. Polish Journal of Chemical Technology, 17(1): 79-87.

Yun, Y.S., Park, J.M. and Volesky, B. 2001. Biosorption of trivalent chromium on the brown seaweeds biomass. Environ. Sci. Technol., 35: 4353-4358.

Zhou, J.L. and Kiff, R.J. 1991. The uptake of copper from aqueous solution by immobilized fungal biomass. J. Chem. Technol. Biotechnol., 52: 317- 340.