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Biosorption Studies on Nickel and Chromium by *Kocuria* sp. BRI 36 Biomass

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Abstract

Present study focuses on sorption mechanism of *Kocuria* sp. BRI 36 biomass for Ni^{2+} and Cr^{3+} removal from aqueous solution. Exposure of cells to the metals caused very minor changes in cell structure as revealed by Field Emission Scanning Electron Microscopy. Maximum Ni^{2+} removal of 98% and Cr^{3+} removal of 76 % was recorded at equilibrium conditions using 0.8 % biomass, 70 min contact time at 30°C with optimum pH of 4.0 and 5.0 for Ni^{2+} and Cr^{3+} respectively. The experimental data was found to be better suited in Langmuir isotherm model with correlation coefficient R^2 of 0.99 for Ni^{+2} and Cr^{3+}. The maximum adsorption capacity (Q_{max}) was found to be 10.41 mg/g for Ni^{2+} and 5.2 mg/g for Cr^{3+}. Separation factor values between 0 to 1.0 and surface coverage values approaching 1.0 supported Langmuir model of adsorption isotherm. Fourier transform infrared spectroscopy analysis confirmed involvement of hydroxyl, carbonyl, amide and carboxyl groups in metal binding. Metal removal above 70 %, moderate biomass requirement and possibility of its reuse make *Kocuria* sp. BRI 36 a novel system for Ni^{2+} and Cr^{3+} removal from aqueous solutions.

Keywords: Heavy metals, Biosorption, FTIR, FESEM

INTRODUCTION

Biological methods prove are eco- friendly, cost effective and sustainable alternatives to physic-chemical methods for removal of heavy metals from the environment. Biosorption or passive uptake and bioaccumulation or active uptake [1] are the two most common mechanisms reported. Application of bioaccumulation on a large scale demands availability of nutrients for growing biomass which in turn causes increase in biological oxygen demand (BOD) or chemical oxygen demand (COD) in the effluent [2]. On the contrary, passive mechanism of biosorption involves various functional groups like, carboxyl, amine, hydroxyl, phosphate, sulfhydryl etc. that are present on cell wall of microorganisms. Physical adsorption, chelation, ion exchange, inorganic precipitation and/or combination of these mechanisms had been suggested in biosorption of heavy metals [3,4].

Studies on adsorption isotherms help to decide the equilibrium relationships between adsorbent and adsorbate. Understanding the ratio between quantity adsorbed and the remaining in the solution at constant parameters (temperature, pH, contact time and metal concentration) at equilibrium assists in describing specific isotherm model that would facilitate designing definite conditions for bioremediation applications. Freundlich and Langmuir isotherms are the simplest known relationships describing the adsorption equation [5]. The paper discusses isotherm studies and FESEM observations. Comparative analysis of FTIR spectra revealed functional groups involved in metal binding. Previously we had reported the results of heavy metal removal by this Antarctic oceanic isolate [6,7,8]. Removal of zinc by *Kocuria carniphila* and cadmium by *Kocuria polaris* by biosorption had been published by Ebaa et al [9]. *Kocuria rosea* had been shown to have ability to remove mercury by biosorption [10]. Presumably, this is the first report on Ni^{2+} and Cr^{3+} adsorption studies in *Kocuria* sp.

MATERIAL AND METHODS

Organism:

The halotolerant *Kocuria* sp. BRI 36 was used in this work. The organism was grown in Mineral Salt Medium (MSM) at 25±2°C for 48 h with shaking at 120 rpm [11]**.** It was used at 10% inoculum concentration for all further experiments.

Chemicals and reagents

All chemicals used were of analytical grade. The media components were purchased from Hi Media Laboratories Pvt. Ltd. (Mumbai, India). The stock solutions of cadmium, nickel, lead and chromium at concentration of 1000 ppm were purchased from Sigma-Aldrich.

Field emission scanning electron microscopy (FESEM)

In order to study effect of adsorbed heavy metals on cell morphology we undertook FE-SEM by growing BRI 36 in MSM supplemented with Ni^{2+} and Cr^{3+} individually, each at 50 ppm. BRI 36 grown under similar conditions without metal

served as control. At the end of 48h of incubation, the samples were removed, centrifuged at 8000 rpm for 15 min. Cell pellet was used for smear preparation on glass slide as described by Ishii et al. [12]. The samples were then coated with chromium by using an sputter apparatus (Quarum) and observed under NOVA NANOSEM/450 (FEI) operating at 10kV.

Preparation of biomass

Kocuria sp. BRI 36 was maintained on MSM. Biomass was produced by growing the bacterial culture in MSM broth as mentioned above. Cells were harvested by centrifugation at 10000 rpm for 15 min. Cell pellet was washed three times with distilled water and dry biomass was prepared by oven drying at 60°C for 48 h as described by Oves et al [13]. Dry biomass of *Kocuria* sp. BRI 36 was then used as biosorbent.

Biosorption studies

Biosorption experiments were performed in 50 ml of Ni^{2+} and Cr^{3+} solutions of the desired concentrations individually in 250 ml stoppered conical flasks containing appropriate weight of adsorbent biomass. The flask contents were shaken using a mechanical shaker at 120 rpm for 30°C. Biomass was separated by centrifugation at 8000 rpm for 15 min and supernatant was analyzed for residual Cr^{3+} and Ni^{2+} concentration using atomic absorption spectrophotometer (Thermo Fisher Scientific, AA 203). All the experiments were performed in triplicates. Parallel experiments in the absence of biomass served as control.

The percent metal removed by the biomass is expressed as:

$$\%\text{Removal} = \frac{\mathbf{100\ (C_i - C_f)}}{\mathbf{C_i}}$$

C_i is the initial metal ion concentration (mg/l) and C_f is the equilibrium metal concentration (mg/l).

Influence of various parameters

Initially BRI 36 biomass of 0.1 g was exposed to Ni^{2+} and Cr^{3+} individually (each at 10 ppm concentration) for different time intervals (10 to 100 min) at 30°C. Samples were withdrawn at different time intervals and processed as described above. In order to determine the effect of pH on the adsorption of the metal ions, 10 ppm metal solutions were used with constant shaking at 30°C. The effect of biosorbent concentration was determined by equilibrating biomass (0.2 to 1.5 %) with metal concentration of 10 ppm individually for optimized time. The influence of initial metal ion concentration on biosorption was studied at equilibrium conditions by varying Ni^{2+} and Cr^{3+} concentration individually in the range of 10 to 250 ppm. The effect of temperature on biosorption process was examined by varying temperature in the range of 10 to 60°C at previously optimized parameters.

Langmuir isotherm

The total amount of metal adsorbed on cell surface was calculated as:

$$\text{Qe}=\frac{(C_i - C_f)\,V}{M}$$

Where,

Q_e = Metal ion uptake capacity (mg/g)

C_i = initial concentration of metal in solution (mg/l)

C_f = final concentration of metal in solution (mg/l)

M = Dry weight of biosorbent (g)

V = solution volume (l)

The difference between initial metal concentration and final metal concentration was considered as metal bound to the cell surface.

The biosorption equilibrium isotherm was determined by Langmuir model (Eq. 1) and Freundlich model (Eq. 2) [14]. Langmuir model considers that adsorption takes place at specific homogeneous sites within the adsorbent, and it has been used successfully for many adsorption processes of monolayer adsorption.

$$\mathrm{Q_e} = \frac{\mathrm{Q_{max} b c_f}}{1 + \mathrm{b c_f}} \quad (1)$$

Where,

Q_{max} is the maximum metal uptake under the given conditions, b is a constant associated to the affinity between the biosorbent and sorbate.

According to linerized Langmuir model

$$1/\mathrm{Q} = 1/\mathrm{Qmax}\ (1/\mathrm{b\ Cf} + 1)$$

Separation factor (SF)

The shape of the isotherm can be used to predict whether adsorption system is favorable or unfavorable in a batch adsorption system at different metal concentrations and time. Accordingly, the essential feature of Langmuir isotherm is expressed in terms of dimensionless constant called the separation factor (SF) [15],

$$S_f = 1/\ (1 + bC_i)$$

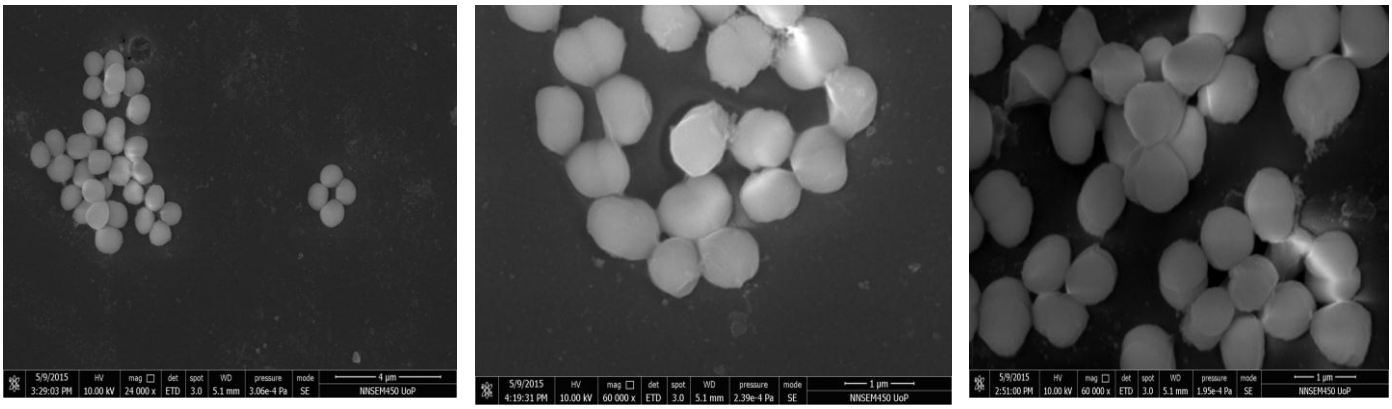
Surface coverage (Ø)

Surface coverage (Ø) is number of adsorption sites occupied divided by number of adsorption sites available. The adsorption behavior of the metal ions on the biomass was determined by the formula,

$$\mathrm{bC_i} = \text{Ø}/(1 - \text{Ø})$$

From above equation, surface coverage was calculated as:-

$$\text{Ø} = \mathrm{bC_i}/1 + \mathrm{bC_i}$$



(a) **(b)** **(c)**

a) Without metal stress, b) Ni^{2+} loaded cells and c) Cr^{3+} loaded cells

Figure 1. Field Emission Scanning Electron Microscopy (FESEM): Effect of heavy metals on surface morphology of *Kocuria* sp. BRI 36

Fourier transform infrared spectroscopy (FTIR)

In order to confirm interactions between heavy metals and functional groups on the cell surface, 1.0 mg each of metal exposed and metal free dry biomass was used for FTIR analysis (Brucker, tensor 37). The conditions used were 16 scans at a resolution of 4cm^{-1} measured between 400 and 4000 cm^{-1}.

RESULTS AND DISCUSSION

Field emission scanning electron microscopy (FESEM)

The present studies were carried out to observe the effect of metal adsorption on cellular morphology by using FESEM. Metal exposed cells exhibited minor changes in cell topology **(Figure 1).** The cell wall of bacteria acts as the first defense system against any external stress. In *Sinorhizobium* sp. morphological indications were observed by Jobby et al.[16] under Ni stress in the form of increase in cell size. Similarly, deformation and cell surface damage were observed in *M. amorphae* CCNWGS0123 in presence of copper [17]. Thus, absence of significant cell distortion in case of BRI 36 cells suggest possibility of reuse of biomass for Ni^{2+} and Cr^{3+} removal by adsorption.

Effect of contact time

For Ni^{2+} and Cr^{3+}, maximum metal removal of 47 % and 15 % was observed within the first 70 min **(Figure 2)** indicating suitability of BRI 36 biomass for bioremediation of heavy metals. Optimum contact time in the range of 60 to 80 min was earlier reported by many researchers [18,19].

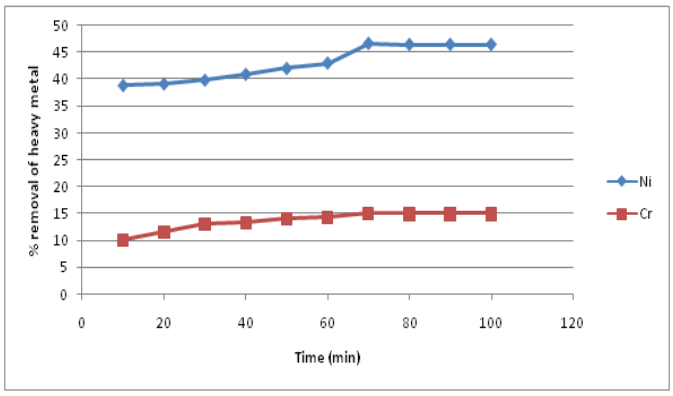


Figure 2. Effect of contact time (Reaction volume – 50 ml, metal concentration – 10 ppm, Biomass –0.2 %, at room temperature)

Effect of pH

As shown in **Figure 3** percent removal of 74 for Ni^{2+} and 42 for Cr^{3+} were observed at pH 4.0 and 5.0 respectively. At acidic pH levels, heavy metals tend to form free ionic species. Hence, more protons become available to saturate metal binding sites. This means that at extremely acidic pH of 2.0 or 3.0 the adsorbent surface might be positively charged, thus reducing the attraction between adsorbent and metal cations. While at pH more than 7.0 precipitation of metal hydroxides causes immobilization of metal ions, thus interfering with biosorption process. This reasoning backs our observations of highest metal removal at pH 4.0 and 5.0. Earlier many researchers had documented pH range of 4.0 to 6.0 for passive sorption mechanism [19,20].

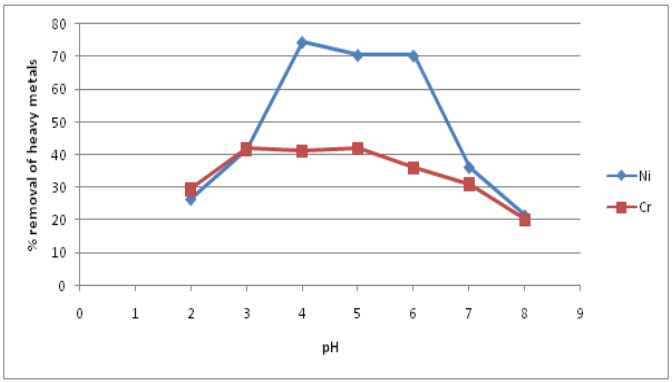


Figure 3. Effect of pH (Reaction volume – 50 ml, metal concentration – 10 ppm, contact time – 70 min, Biomass –0.2 % at room temperature)

Effect of biosorbent concentration

Our results depicted that metal adsorption increased with an increase in biosorbent upto 0.8% and then it leveled off **(Figure 4).** It may be attributed to non availability of adsorption sites caused by their overlapping due to excess biomass [21]. Maximum Ni^{2+} removal of 94 % and Cr^{3+} removal of 68 % were observed as shown in the figure**.** Metal removal in the range of 60 to 95 % had been published earlier by Pandey et al.[22] during their studies on bacterial biomass of *Calotropis procera*. Fourest and Roux (1992)[23] suggested absence of competing protons produced during metabolism in nonviable biomass might result into higher affinity of metals as compared to viable biomass. Thus requirement of moderate amount of biomass for Ni^{2+} and Cr^{3+} removal is another beneficial property of *Kocuria* sp BRI 36 biosorbent.

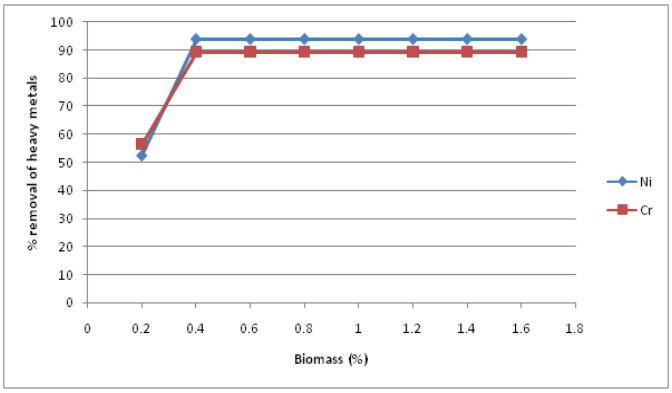


Figure 4. Effect of Biomass (Reaction volume – 50 ml, metal concentration – 10 ppm, pH for Ni^{2+} – 4.0 and for Cr^{3+}- 5.0, contact time – 70 min, biomass- 0.8% at room temperature

Effect of initial metal concentration

The adsorption equilibria of Ni^{2+} and Cr^{3+} were obtained at 76 and 47 ppm respectively **(Figure 5).** The greater metal uptake at initial concentration of each metal could probably due to a rapid metal adsorbing ability of bacterial biomass [23]. An unavailability of surface binding sites or surface saturation may be the probable explanation for decrease in metal exclusion at higher metal concentration.

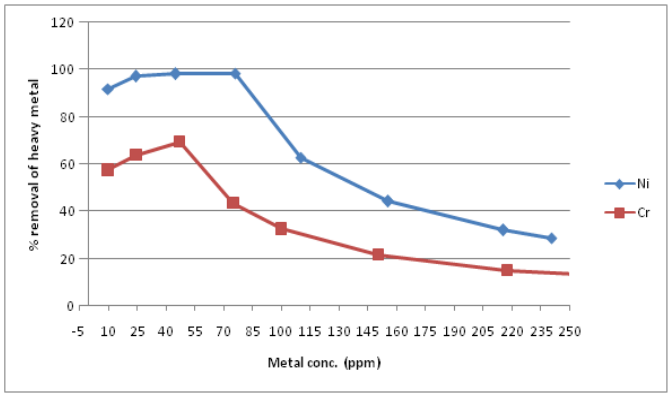


Figure 5. Effect of Initial metal concentration (Reaction volume – 50 ml, contact time – 70 min, Biomass –0.8%, pH – 4.0 and 5.0 for Ni and Cr respectively, at room temperature)

Effect of temperature

Adsorption of metal ions increased with increase in temperature upto 30°C for both the metals **(Figure 6)**. The decrease in biosorption capacity between 30 and 60° C may be due to the damage of active sites present on bacterial cell surface. Besides, exothermic nature of adsorption reactions may also cause decrease in sorption capacities with increase in temperature. Our observations agreed with previous reports [24,25,26]. Thus under optimized conditions of contact time, pH, biomass concentration, initial metal concentration and temperature maximum metal removal of 98.39% for Ni^{2+} and 76.91 % for Cr^{3+} was observed.

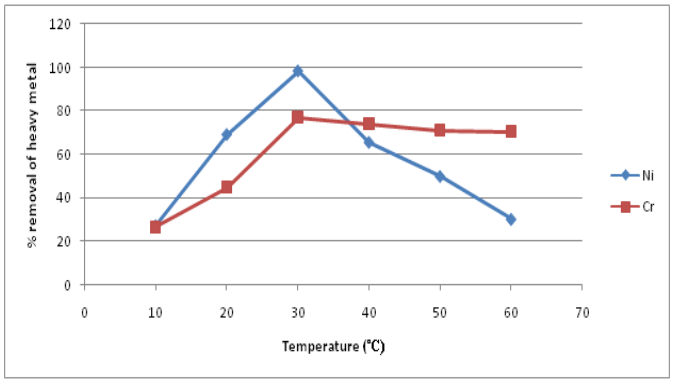


Figure 6. Effect of temperature Reaction volume – 50 ml, contact time – 70 min, Biomass –0.8%, pH – 4, Metal concentration – for Ni^{2+} 76.11 and for Cr^{3+} 47.26)

Adsorption isotherm

Suitability of specific model for an equilibrium data is essential for an industrial application. This study helps in comparative analysis of various biomaterials under different operational parameters [27]. In view of this, studies were undertaken to determine best fit isotherm model for the data obtained. Langmuir isotherm (Eq.1) explains monolayer

adsorption onto a surface with finite number of binding sites. The model is based on the assumption of identical adsorption energies with no transmigration of the adsorbate in the plane of surface. Correlation coefficient R^2 value of 0.99 for Ni^{+2} and Cr^{3+} indicates monolayer adsorption **(Table 1).** Thus our observations better fitted in Langmuir model than Freunlich adsorption isotherm.

Table 1. Langmuir isotherm constants for Ni^{2+} and Cr^{3+} adsorption by *Kocuria* sp. BRI 36

Metal ions	Langmuir isotherm		
	Q_{max}	K	R^2
Ni	10.41	0.0387	0.932
Cr	5.208	0.103	0.989

The maximum biosorption capacity (Q_{max}) of *Kocuria* sp. BRI 36 biomass was 10.41 mg/g for Ni^{2+} and 5.2 mg/g for Cr^{3+} (Table 1). Previously many researchers studied sorption capacities of various bisorbents [28-31]. The Q_{max} values were found to be in the range of 4.23 mg/g **(Figure 7)** to 8.92 mg/g **(Figure 8)**. This suggest the effectiveness of *Kocuria* sp. 36 biomass as a potential biosorbent for treatment of Ni^{2+} and Cr^{3+} contaminated water.

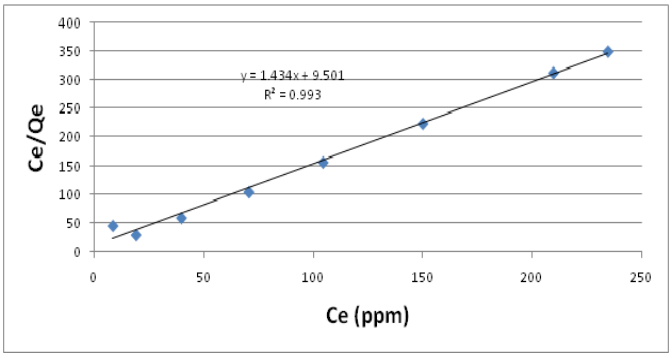


Figure 7. Langmuir adsorption isotherm for Ni^{2+} by *Kocuria* sp. BRI 36 biomass (Q_{max} = 10.41 mg/g, b = 0.038 L/mg).

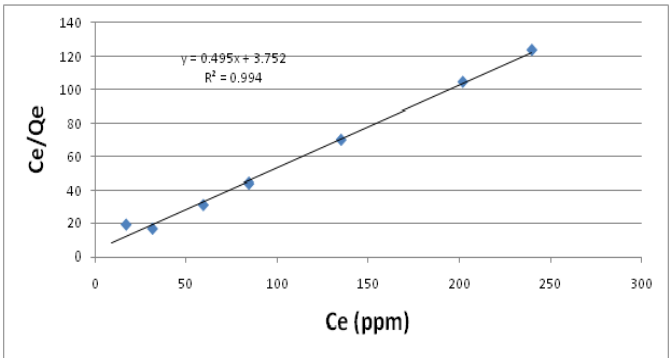


Figure 8. Langmuir adsorption isotherm for Cr3+ by *Kocuria* sp. BRI 36 biomass (Q_{max} = 5.2 mg/g, b = 0.1 L/mg)

The shape of Langmuir isotherm implies suitability of the biosorption system in a batch adsorption process. Separation factor (SF), a dimentionless constant helps to understand shape and nature of sorption mechanism. SF value between 0 and 1 indicates favorable isotherm. Accordingly SF values for nickel and chromium were calculated for BRI 36 biomass and plotted against initial metal concentration as shown in **Figure 9.** Biosorption increases with increase in metal concentration upto 76 ppm for Ni^{2+} and 47 ppm for Cr^{3+}. SF values of 0 to 1.0 for both Ni^{2+} and Cr^{3+} reconfirmed Langmuir isotherm as favorable model for adsorption of both metals onto BRI 36 biomass. Surface coverage value (Ø) for Ni^{2+} and Cr^{3+} helped to understand their adsorption pattern onto BRI 36. The value nearing 1.0 **(Figure 10)** for both the metals implied that BRI 36 biomass surface was fully covered with metal monolayer as metal concentration increases. However no significant change in surface coverage value at high metal concentration suggested that reaction rate becomes almost independent of the metal concentration [32].

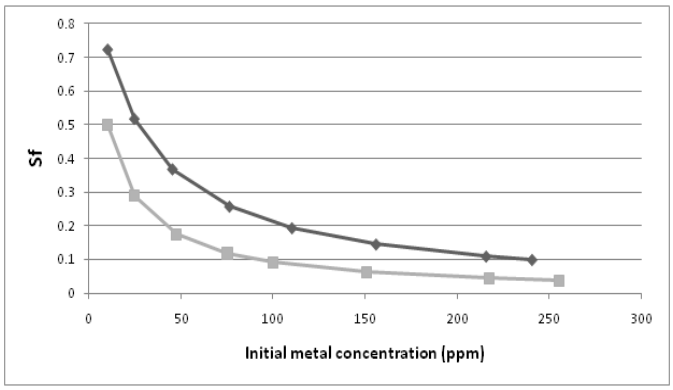


Figure 9. Separation factor profile for biosorption of Ni^{2+} (■) and Cr^{3+} (♦) as function of initial metal concentration

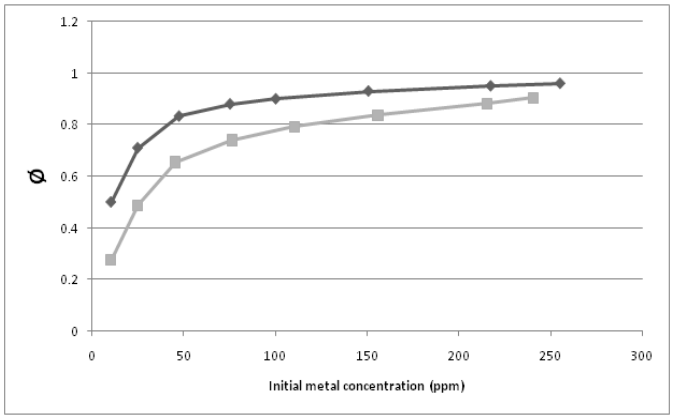


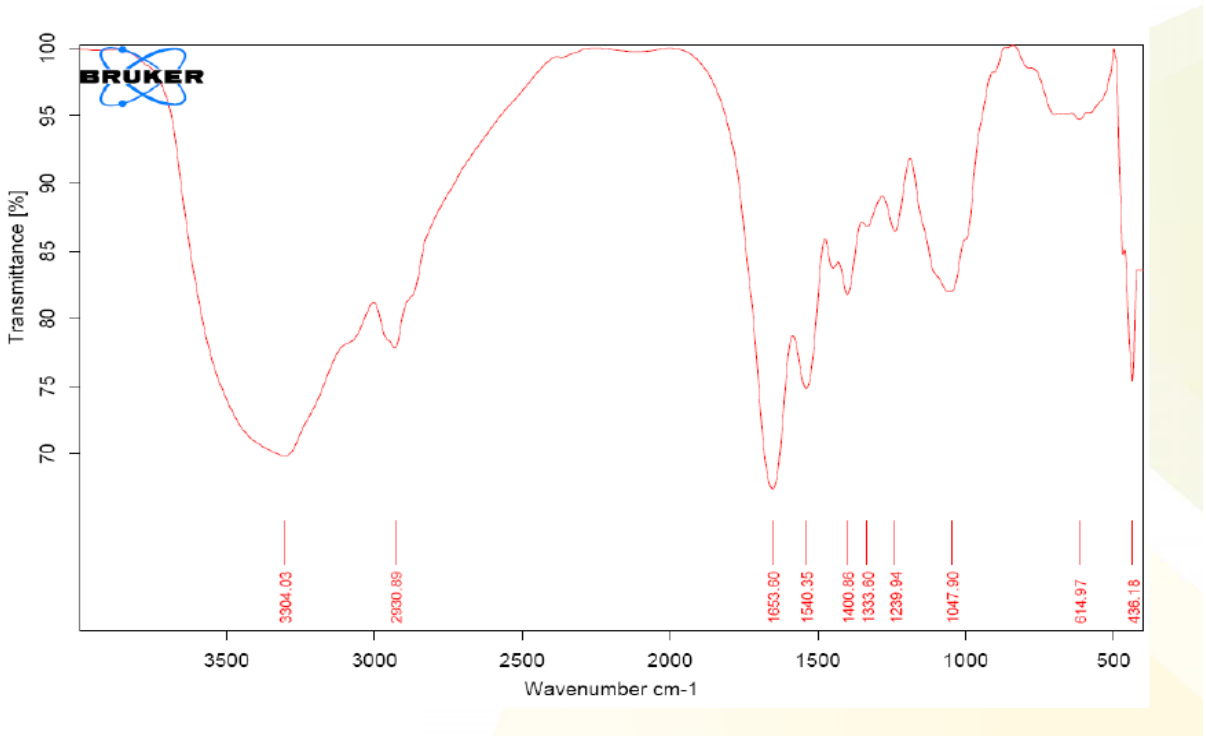
Figure 10. A graphs of surface coverage values against initial metal concentration of Ni^{2+} **(■)** and Cr^{3+} (♦) metal ions

Fourier Transform Infrared Spectroscopy (FTIR)

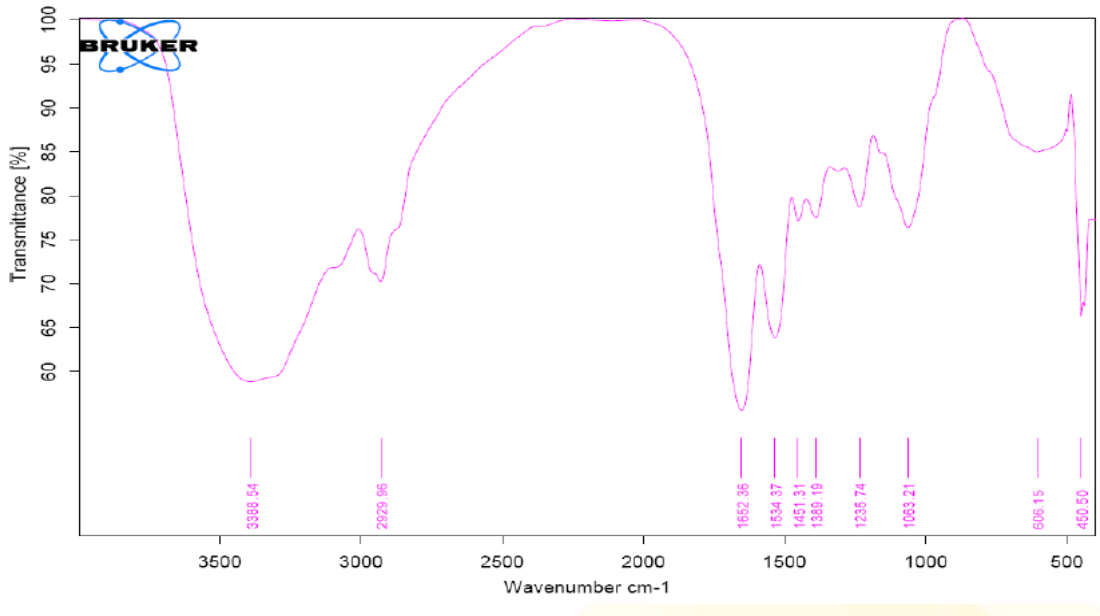
FTIR spectra of metal unloaded biomass of BRI 36 resulted in prominent peaks at 3304.03, 2930.39, 1653.60, 1540.35 and 1047.90 cm^{-1} as depicted in **Fig. 11** Other peaks were observed at 1400.86, 1333.60, 1239.94, 614.97, 436.18 cm^{-1}.

The absorption band assigned at 3400-3200 cm^1 indicates hydroxyl (-OH) and amine group. While stretching at 2930.39 cm^{-1} showed the presence of asymmetrical C-H stretching indicating aliphatic methylene group. Intensity of 1653.60 cm^{-1} is due to amide and carbonyl (–C=O) stretching. The cross peaks between 650 and 1750 cm^{-1} are attributed to variation in carbohydrates, lipids and proteins.

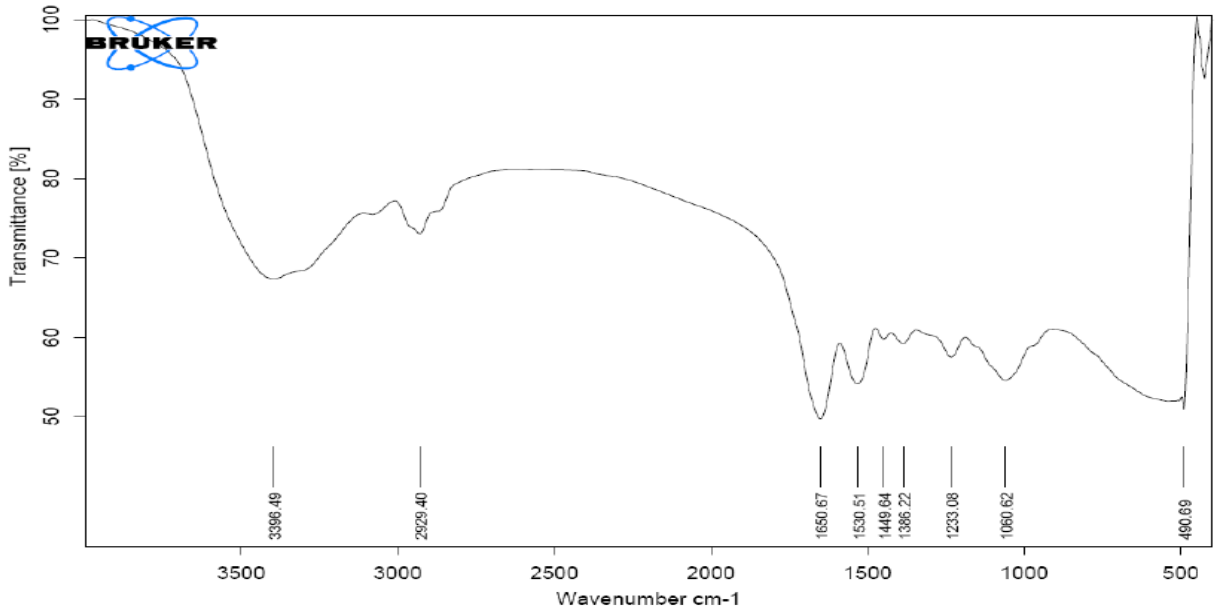
However, in Ni^{2+} and Cr^{3+} loaded biomass shifting of bands at 3300-3360 cm^{-1} , 1530-1550 cm^{-1}, 1030- 1050 cm^{-1} were observed indicating involvement of hydroxyl, carboxyl, and organic phosphate group respectively. Stretching of the band at 1630-1660 cm^{-1} shows interaction of metals with amide and carbonyl groups. In Ni^{2+} loaded sample additional shifts were observed at 2830 – 2930 cm^{-1} that may be due to carboxylic acid O-H stretch**.** Moreover, the band at 1431.31 cm^{-1} verified asymmetric C-H bend. In case of Cr^{3+} loaded samples the shifts observed at 1330-1390 cm^{-1} indicated O-H bending. Thus our observations are in good agreement with [33-36].



(a)



(b)



(c)

Figure 11. FTIR analysis: (a) metal unloaded, (b) Cr^{3+} loaded and (c) Ni^{2+} loaded biomass of *Kocuria* sp. BRI 36

CONCLUSION

Kocuria sp. BRI 36 biomass exhibited significant ability for Ni^{2+} and Cr^{3+} removal from aqueous solution by adsorption mechanism. Our observations suggested that biomass concentration of 0.8% demonstrates best performance as biosorbent at the end of 70 min at 30°C. The results fit into Langmuir isotherm model and are also supported by separation factor and surface coverage studies. Thus *Kocuria* sp. BRI 36 biomass could serve as promising candidate for bioremediation of Ni^{2+} and Cr^{3+}. The remarkable findings include i) presumably first report on *Kocuria* sp. for Ni^{2+} and Cr^{3+} adsorption, ii) requirement of reasonable amount of biomass with reusable applicability and iii) more than 70% metal removal at equilibrium conditions.

Conflict of interest

No conflict of interest was reported by the authors.

REFERENCES

[1] Hussein, H., Ibrahim, S.F., Kandeel, K., Moawad, H., 2005, "Biosorption of heavy metals from waste water using *Pseudomonas sp,"* Electron. J. Biotechnol., 7 (1).

[2] Brown, M.J. and Lester, J.N., 1982, "Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge", Water. Res, 16, pp. 1539-1548.

[3] Akhtar K., Akhtar M.W. and Khalid A.M., *2007, "*Removal and recovery of uranium from aqueous solutions by *Trichoderma harzianum",* Water. Res, *41,* pp. 1366-1378.

[4] Chen, X.C., Wang, Y.P., Lin, Q., Shi, J.Y., Wu, W.X. and Chen, Y.X., 1982, "Biosorption of copper (II) and zinc(II) from aqueous solution by *Pseudomonas putida* CZ1", Colloid. Surf. B Biointerfaces, *46,* pp. 101-107.

[5] Jalali, R., Ghafourian, H., Asef, Y.,Davarpanah, S.J. And Sepehr, S., 2002, "Removal and recovery of lead using nonliving biomass of marine algae", J. Hazard. Mater., 92, pp. 253-262.

[6] Mulik, A.R. and Bhadekar, R.K., 2017, "Heavy metal removal by bacterial isolates from the Antarctic Oceanic region", Int. J. Pharma Bio Sci, 8, pp. 535-43.

[7] Mulik, A.R., Kumbhar, P.K. and Bhadekar, R.K., 2017, "Production and partial characterization of pigments produced by *Kocuria sp* BRI 36: influence of heavy metals", Int. J. Pharam Pharm Sci, 10, pp. 137-145.

[8] Mulik, A.R. and Bhadekar, R.K., 2017, "Co-relation between heavy metal resistance and antibiotic susceptibility in halotolerant bacteria isolated from the Antarctic oceanic region", Int. J. Pharma Bio Sci, 8, pp. 302-306.

[9] El-Sharouny, E.E., Belal, M.A. and Yusef, H.H.,2013, "Isolation and characterization of two novel local psychrotolerant *Kocuria* spp. with high affinity towards metal cations biosorption", Life Sci J.,10, pp. 1721-1737.

[10] François, F., Lombard, C., Guigner, J.M., et al., 2012, "Isolation and characterization of environmental bacteria capable of extracellular biosorption of mercury", Appl. Environ. Microbiol, 78, pp. 1097–106.

[11] Pote, S., Chaudhary, Y., Upadhayay, S., Tale, V., Walujkar, S., Bhadekar, R., 2014, "Identification and biotechnological potential of psychrotrophic marine isolates", Eurasia. J. Biosci, 8, pp. 51-60.

[12] Ishii, S., Koki, J., Unno, H. and Hori, K., 2004, "Two Morphological Types of Cell Appendages on a Strongly Adhesive Bacterium, *Acinetobacter* sp. Strain Tol 5", Appl. Environ. Microbiol, 70, pp. 5026-5029.

[13] Oves, M., Khan, M.S., Zaidi, A., 2013, "Biosorption of heavy metals by Bacillus thuringiensis strain OSM29 originating from industrial effluent contaminated north Indian soil", Saudi. J. Biol. Sci, 20, pp. 121–129.

[14] Volesky, B. (1990) Biosorption of heavy metals. Eds. CRC Press, Boca Raton, pp 7–44.

[15] Horsefall, M. J. and Spiff, A. I., 2005, "Effect of Metal ion concentration on Biosorption of Pb^{2+} and Cd^{2+} by *Caladium Bicolor* (Wild Cocoyam)", Afr. J. Biotechnol, 4, pp. 191-196.

[16] Jobby, R., Jha, P. and Desai, N., 2015, "Sinorhizobium, a potential organism for bioremediation of nickel", Int. J. Adv. Res., 3.2, pp. 706-717.

[17] Mohamad, O. A., Hao, X. L., et al., 2012, "Biosorption of copper (II) from aqueous solution using non-living *Mesorhizobium amorphae* strain CCNWGS0123" Microbes Environ., 27, pp. 234–241.

[18] Sulaiman,I.S.C., Basri, M., Masoumi,H.R.F., Chee,W.J., Ashari, S.E. and Ismail, M., 2017, "Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of *Clinacanthus nutans* Lindau leaves by response surface methodology", Chem. Cent. J., 11.

[19] Butt, M., Ali, H., Chaudhary, H.J.,2015, "Biosorption of cadmium and chromium from water by endophytic *Kocuria rhizophila*: Equilibrium and kinetic studies", Desalination Water Treat., 2015, pp. 1–13.

[20] Kang, S., Lee, J., Kim, K., 2005, "Metal removal from wastewater by bacterial sorption: Kinetics and competition studies", Environ. Technol., 26, pp. 615–624.

[21] Kosa, A., Al-Zhrani, G., Salam, A., 2012, "Removal of heavy metal from aqueous solution by multi-walled carbon nanotubes modified with 8-hydroxyquinoline", Chem. Eng. J., 181, pp. 159-168.

[22] Pandey, A., Singh, P., Iyengar, L., 2007, "Bacterial decolorization and degradation of azo dyes", Int. J. Biodeteriotion. Biodegradation., 59, pp. 73–84.

[23] Fourest, E. and Roux, J.C., 1992, "Heavy metal biosorption by fungal mycelial byproducts: Mechanisms and influence of pH", Appl. Microbiol. Biotechnol., 37, pp. 399-403.

[24] Casas, A., Alvarez, F., Cifuentes, L., 2000, "Aqueous speciation of sulphuric acid-cupric sulphate solutions", Chem, Eng. Sci., 55, pp. 6223–6234.

[25] Gundogdu, A., Ozdes, D., Duran C., Bulut, V.N., Soylak, M., Senturk, H.B., 2009, "Biosorption of Pb(II) ions from aqueous solution by pine bark (Pinusbrutia Ten.)", Chem. Eng. J., 153, pp. 62–69.

[26] Farhan, S.N. and Khadom, A.A., 2015, "Biosorption of heavy metals from aqueous solutions by *Saccharomyces Cerevisiae",* Int. J. Ind. Chem., 6, pp. 119–130.

[27] Ashraf, M. A., Maah, M. J., and Yusoff, I., 2010, "Study of Mango biomass (Mangifera Indica L.) as a cationic biosorbent", Int. J. Environ. Sci., Tech. 7, pp. 581-590.

[28] Hall, K.R., Eagleton, L.C., Acrivos, A., Vermeulen, T., 1966, "Pore- and solid-diffusion kinetics in fixed-bed adsorption under constant-pattern conditions", Ind. Eng. Chem. *Fundam.*, 5, pp. 212-223.

[29] Wan Ngah, W. S., Endud, C. S. and Mayanar, E. R., 2002, "Removal of copper(II) ions from aqueous solution onto chitosan and cross-linked chitosan beads", React. Funct. Polym., 50, pp. 181-190.

[30] Gurgel, L.V.A., Gil, L.F., 2009, "Adsorption of Cu(II), Cd(II) and Pb(II) ions from aqueous single metal solutions by succinylated mercerized cellulose modified with triethylenetetramine", J. Carbohydr. Polym., 77, pp. 142-149.

[31] Shukla, S.R., Pai, R.S., 2005, "Adsorption of Cu(II), Ni(II) and Zn(II) on modified jute fibres", Bioresour. Technol., 96, pp. 1430–1438.

[32] Arıca, M.Y., Tüzün, İ., Yalçın, E., İnce, Ö., Bayramoğlu, G., 2005, "Utilisation of native, heat and acid-treated microalgae *Chlamydomonas reinhardtii* preparations for biosorption of Cr(VI) ions", Process Biochem., 40, pp. 2351–2358.

[33] Oh, S.E., Sedky, H. A., Hassan,J.H.J., 2009, "Biosorption of heavy metals by lyophilized cells of *Pseudomonas stutzeri",* World. J. Microbiol. Biotechnol., 25, pp. 1771–1778.

[34] Vijayaraghavan, K. and Yun, Y.S., 2008, "Bacterial biosorption", Biotechnol. Adv., 26, pp. 266-291.

[35] Yun,J., Im, J.S., Oh, A., Jin,D.H., Bae,T.S. . Lee, Y..S. et al., 2011, "pH-sensitive photocatalytic activities of TiO_2/poly(vinyl alcohol)/poly(acrylic acid) composite hydrogels", Mater. Sci. Eng., 176, pp. 276-281.

[36] Yu J., Yu, S., Teng, C., Song, T. et al., 2017, "Biosorption characteristic of *Alcaligenes* sp. BAPb.1 for removal of lead (II) from aqueous solution", 3 Biotech. 7, pp. 123.