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RESEARCH ARTICLE

Sinorhizobium, a potential organism for bioremediation of nickel.

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Abstract

Heavy metals represent a great environmental concern, because of their widespread use, distribution, and toxicity to human beings. The growing environmental awareness necessitates the development of effective and inexpensive methods for metal removal. In present study an attempt was made to examine the nickel tolerating abilities of *Rhizobium* sp. nodulating *Sesbania sesban.* The biosorption capability of the isolate was studied using synthetic nickel solution. Out of the 22 *Rhizobium* and *Sinorhizobium* isolates, *Sinorhizobium* sp. BEL5B (JX444691.1) tolerated the highest Ni concentration (3mM) and was used for further studies. The scanning electron micrographs revealed morphological changes and secretions of extra polymeric substances. The biosorption of nickel was highly influenced by parameters like pH, biomass dosage and metal concentration. The biosorption data followed the Langmuir and Freundlich isotherm models. The maximum biosorption capacity for nickel by BEL5B was found to be 25.13mg/g. FTIR analysis revealed the involvement of cell surface ligands in metal biosorption and alkali pretreatement with NaOH showed enhancement in biosorption capacity for nickel. Thus, on the basis of all these parameters studied a better insight was obtained into the tolerance mechanisms as well as the bioremediating capabilities of this well know organism commonly used as biofertilizer.

INTRODUCTION

Disposal of untreated industrial wastewater is the main cause of heavy metal pollution of the environment (Aksu and Kutsal, 1990). These heavy metals are causing a serious threat to vegetation, animals and mankind (Fan *et al*., 2008). Nickel is one of the major concerns because of its larger usages in developing countries. Nickel, like other heavy metals is non biodegradable and toxic at elevated levels. Wastewater containing nickel originates from the mining, metal industry, aircraft and motor vehicle industries, rechargeable batteries, printing, chemical industries, electroplating, pigments for paints or ceramics, electronic or computer equipment, leather tanning etc. (Volesky, 1990; Congeevaram *et al*., 2007). Nickel causes allergy in humans and if absorbed through food or water, it can cause impaired lung function and chronic bronchitis (Cempel and Nikel, 2006). Nickel has also been found to be carcinogenic (Volesky, 1990).

Microbial population has acquired a variety of mechanisms for adaptations to the presence of toxic heavy metals. These mechanisms may be genetic or just physiological adaptations (Rani *et al*., 2008). Bacteria have genes that determine resistance to many toxic heavy metals. Most of the times these genes are determined by the plasmids (Silver, 1996). The resistance to nickel in *Rhizobium* and *Bradyrhizobium* sp.was shown to be an operon mediated system, where the *ncc* operon was found to be responsible for high level combined nickel, cobalt and cadmium resistance, and the *nre* for low level nickel resistance.(Clemence *et al*., 2007; Abou-Shanab *et al*., 2007). The cell

surfaces of all microorganisms are negatively charged owing to the presence of various anionic structures. This gives bacteria the ability to bind metal cations which can be either extracellular or intracellular accumulation (Ahalya *et al*., 2011). The polysaccharide coating found in most forms of bacteria, or other extracellular structures such as capsules or slime layer are generally instrumental for this binding. (Rani *et al*., 2008). This feature of microbes can be exploited to physically remove heavy metals from solution through either bioaccumulation or biosorption.

Metal contaminated soils and water are being remediated using physical and chemical methods, but all these have high operating cost, additional requirement of pretreatment and it also generates secondary pollution (Volesky, 1990; Viraraghavan and Yan, 2001). Owing to the growing environmental awareness there has been emphasis on the development of environment friendly ways for decontamination procedures (Jooste, 2000). In this context microorganisms have emerged as a complementary, economical and ecofriendly system for controlling bioavailabilty of metals. Efforts are being made for decontamination of soil and water using fungi, yeast, bacteria and algae (Anjana *et al*., 2007; Awofolu *et al*., 2006; Sari and Tuzen 2008a, b)

In the present study *Sesbania sesban* was found to occupy the industrial and barren areas of Navi Mumbai. Soil amelioration and habitat processing properties of sesbanias are associated with its symbiotic partner i.e., rhizobia. An assessment of the functional traits of rhizobia associated with these would help in development of strategies to effectively utilise Sesbania-rhizobia symbiosis in various soil amelioration programs. (Sharma *et al* 2004). Hence the present study aims to explore nickel resistance among *Rhizobium* sp. isolated from root nodules of *S.sesban* and to study its potential in biosorption of nickel.

2. Materials and Methods

2.1 Isolation of *Rhizobium* sp.

Rhizobium cultures were isolated from root nodules of *Sesbania sesban* from various industrial areas and barren areas around Navi Mumbai. The root nodules were first washed under tap water. The healthy, undamaged nodules were then immersed for 5-10s in 95% ethanol (to break the surface tension and remove air bubbles from the tissue) and surface sterilized by soaking in sodium hypochlorite (2.5%v/v) solution for 2-4 min. Root nodules were then rinsed five times with sterile distilled water (DW). These root nodules were then crushed in a test tube using glass rod maintaining aseptic conditions. The exudates were further streaked on Congo Red Yeast extract Mannitol agar (CRYEMA) and incubated till growth appeared at $25^{\circ}C \pm 2^{\circ}C$ (Somasegaran and Hoben, 1994). Colorless /white/light pink colonies were taken for further studies . Identification of the isolates was carried out by 16SrRNA gene sequencing and the sequence data were deposited in NCBI.

2.2 Metal Resistance Studies

Filter sterilized stock solutions of nickel chloride (0.25M) were prepared. Each filter sterilized stock solution was added to Tryptone Yeast extract (TY) medium to the final concentration of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mM of nickel chloride for determination of MIC. Cultures were grown in TY broth until Log phase. 50µl of culture was spot inoculated onto the metal containing TY plates and incubated at 25ºC ($\pm 2^0C$). The observation for growth were taken at 2 days interval for one week. TY plates without metal were used as control. The lowest concentration that prevented growth was considered as MIC. All the experiments were carried out in triplicates. Strains that showed resistance in plate MIC were further used for broth MIC studies and the highest nickel tolerating strain was selected for further studies.

2.3 Scanning electron microscopy and EDS analysis

Alterations in cell-surface morphology in the presence of metals was detected by scanning electronic microscopy (SEM). Energy dispersive X-ray scanning (EDS) was employed to detect the metal identity. Isolate BEL5B was incubated with and without 3mM Nickel chloride till log phase. After centrifugation at 10000 rpm for 10 mins the pellets were treated as per Bagwell *et al*., (2008). The specimens were mounted onto the sample holder with carbon-conductive adhesive tapes and coated with gold using a sputter coater (Auto fine coater JFC-1600, JEOL) prior to viewing using a field-emission scanning electron microscope (FESEM JSM-7600F, JEOL) fitted with EDS analyser.

2.4 Screening for metal resistant genes

Amplification of 1141 bp fragment of *nccA* region belonging to the *ncc* operon coding for nickel, copper and cobalt resistance was carried out using forward primer *ncc*A1 5-ACGCCGGACATCACGAACAAG - 3 and reverse primer *ncc*A2 5-CCAGCGCACCGAGACTCATCA-3 as per methodology described by Abou-Shanab *et al*. (2007). *Ralstonia eutropha* obtained from IMTECH Chandigarh was used as a positive control for amplification of *ncc*A.

2.5 Nickel biosorption studies

2.5.1 Preparation of the bacterial biosorbent

The strain BEL5B was pre-cultured in TY medium at 25^0C ($\pm 2^0C$) on shaker at 120 rpm till log phase. The cells were harvested by centrifugation at 10,000 rpm for 6 min from early-stationary cultures (OD 600 = 1.2–1.5). The

biomass obtained was used for biosorption studies. (To correlate with dry biomass a weighed amount of wet biomass was accurately weighed and thereafter was oven dried at 80^0C till constant weight. The final weight was determined and used to calculate its dry weights)

2.5.2 Optimization of parameters for Nickel biosorption

To maximize the biosorption capacity different parameters like pH (2,3,4,5,6,7,& 8), Temperature (25,35,and 45) and Biomass dosage (1.25g/l – 20g/l) were optimized. Samples were taken from the solutions at predetermined intervals and centrifuged at 10000 rpm for 8 min. The nickel ions in the supernatant were measured by Inductively Coupled Plasma – Atomic Emission Spectrometer (ICP-AES, ACROS from M/s. Spectro, Germany). All the experiments were setup in triplicates and the observations were taken as average with Standard deviation.

2.5.3 Biosorption of Nickel at different initial concentration

Using the optimized condition of pH, Temperature and biomass dosage Nickel biosorption was assayed from 100 mg/L to 500mg/L. Samples were taken from the solutions at intervals and centrifuged at 10,000 rpm for 8 min. The nickel ions in the supernatant were measured as described earlier (2.5.2). All the experiments were setup in triplicates and the observations were taken as average with Standard deviation.

2.5.4Biosorption Isotherms

The biosorption capacity q (mg metal/ g dry cell) was calculated as q = (Ci - Ceq)/X, where Ci is the initial metal concentration (mg/L), Ceq is the residual metal concentration at equilibrium (mg/ L) and X is the biomass concentration (g dry cell/ L).

2.5.5 FTIR Analysis

For FTIR analysis 100mg/L of nickel concentration was used with a biomass dosage of 7.5g/L. After 120h the pellet was separated by centrifugation at 10,000 rpm for 8 min and dried at 60^0C for 18h. It was further powdered and used for FTIR analysis.

2.5.6 Effect of pretreatment of biomass

The biomass of BEL5B were pretreated in nine different ways, using 7.5g/l of wet biomass for every pretreatment reaction. The chemical and physical pretreatments were carried out for 30min. The untreated biomass, Autoclaved, Oven dried, Boiling water bath, 0.2N NaOH, 0.2N HCl, 0.2N NaCl, 1% CaCl2, 1% EDTA, 1% Triton X 100 was labelled as Type A,B,C,D,E,F,G,H,I, and J respectively. Data gathered was subjected to one-way analysis of variance (ANOVA) at 0.1% level of significance. Mean values were then compared using Tukey's Test. Statistical Package for the Social Sciences (SPSS) for Windows (Standard Version Release 16) software was used for statistical analysis.

3 Results

3.1 Isolation and Determination of MIC of the Isolates

In the present study a total of 22 *Rhizobium* and *Sinorhizobium* isolates were obtained. The plate and broth MIC studies revealed all the isolates to be resistant to 1mM of $NiCl_2$. While BEL5B was found to tolerate the highest $NiCl_2$ concentration (3mM). The 16s rDNA sequencing and BLAST analysis showed it to belong to the genus *Sinorhizobium*. The sequence was submitted to NCBI under the name *Sinohizobium* sp. BEL5B with the accession no. JX444691.1. The isolate BEL5B was used for further analysis.

3.2 Scanning Electron Microscopy and EDS analysis

FEGSEM micrographs and EDS spectra obtained for BEL5B grown without Ni (control) and exposed to 3mM Ni are presented in Fig. 1. The SEM micrographs revealed morphological changes in response to nickel stress. The cells displayed irregular shapes as well as cell aggregation. A slight increase in the length (0.87µm to 1.12µm) was also observed. Under nickel stress a mass production of extra polymeric substance was also evidence. The EDS analysis showed three distinct peaks of Ni 1KeV, 7.5KeV and 8.3KeV .

3.3 Amplification of the *ncc*A gene

All the 22 nickel resistant isolates were screened for the presence of *ncc*A. The *ncc*A primer pair yielded the expected ~ 1141 bp product with the positive isolate *R.eutropha* as well as with isolated SAR1 (Fig.2). But the extremely tolerant BEL5B isolate failed to yield amplification for *ncc*A gene. The sequencing of the 1141 bp product was tried, but failed for unknown reasons.

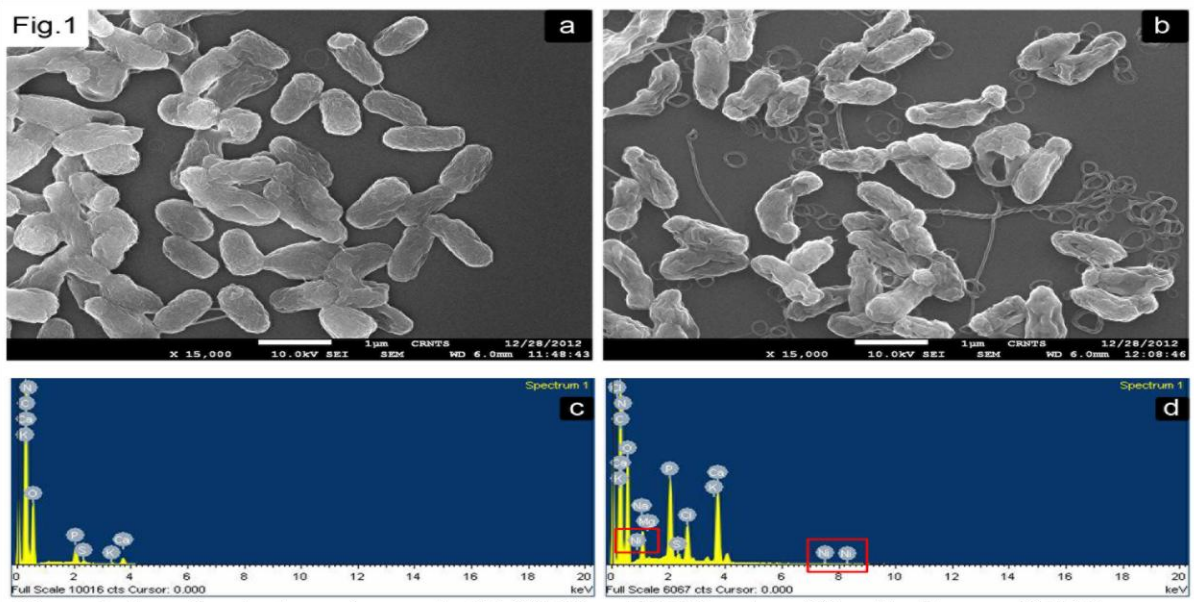

Figure 1: Scanning electron micrographs (15,000 X) and EDS Spectra of *Sinorhizobium* sp. BEL5B grown without (a, c) and with (b, d) nickel(3mM).(Nickel peaks are shown in red box).

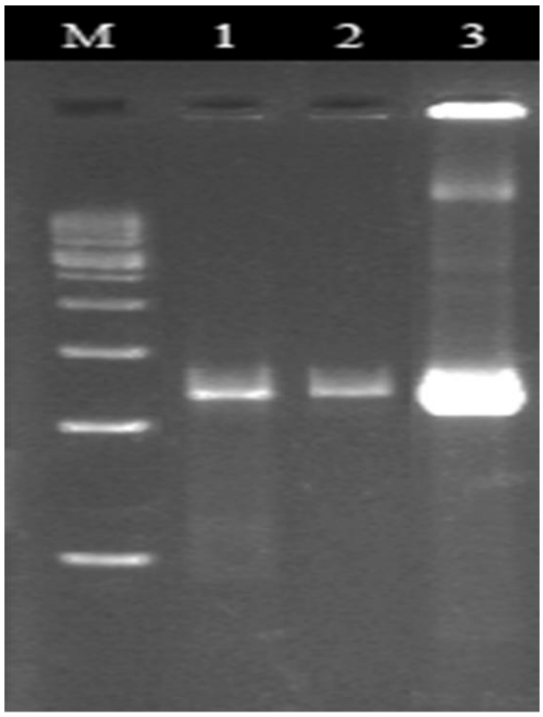

Figure 2: PCR amplification of *ncc*A. M - 500bp Ladder, Lane 1 – SAR1(50^0C Annealing), Lane 2- SAR1(50^0C Annealing), Lane 3- (*R.eutropha*)

3.4 Nickel Biosorption Studies

3.4.1 Optimization of parameters

The biosorption of nickel was found to be strongly dependent on the pH of the solution. The pH ranging from 4 - 8 showed about 80% biosorption of nickel (Fig.3a). At alkaline pH 9, precipitation of nickel ions was observed. For

further experiments, pH 6 was selected, which gave the highest (81%) biosorption. Biosorption studies done for different temperature ranged showed no significant difference, hence further experiments were carried out at 25^0C (± 2^0C). The effect of biomass dosage showed that % biosorption was directly proportional to biomass dosage, but a plateau was observed at higher concentrations of biomass (Fig.3 b). Hence, for further experiments a dosage of 7.5g/L was selected.

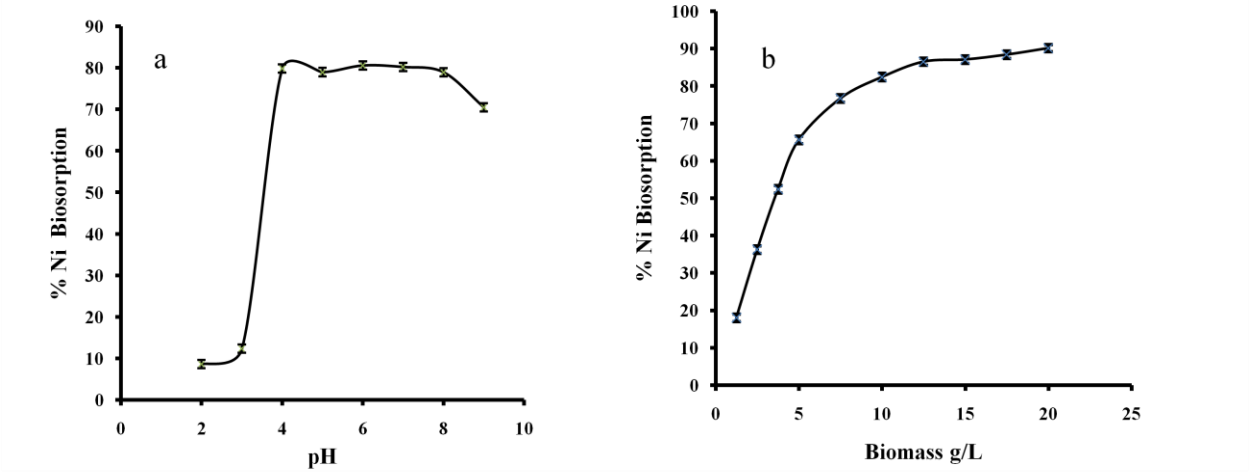

Figure 3a: Effect of pH. **b:**Effect of biomass dose on biosorption of nickel by BEL5B

3.4.2 Biosorption of Nickel at different concentration

Applying the optimized parameters the biosorption studies were extended to different concentration of Ni. A graph of % biosorption as well as metal uptake (mg/g) was plotted against the different Ni concentrations used. The results revealed a decrease in % biosorption with an increase in Ni concentration. On the contrary the uptake capacity was found to increase from 13.20 mg/g (calculated) for 100mg/L to 24.2 mg/g (calculated) for 500mg/L.(Fig.4)

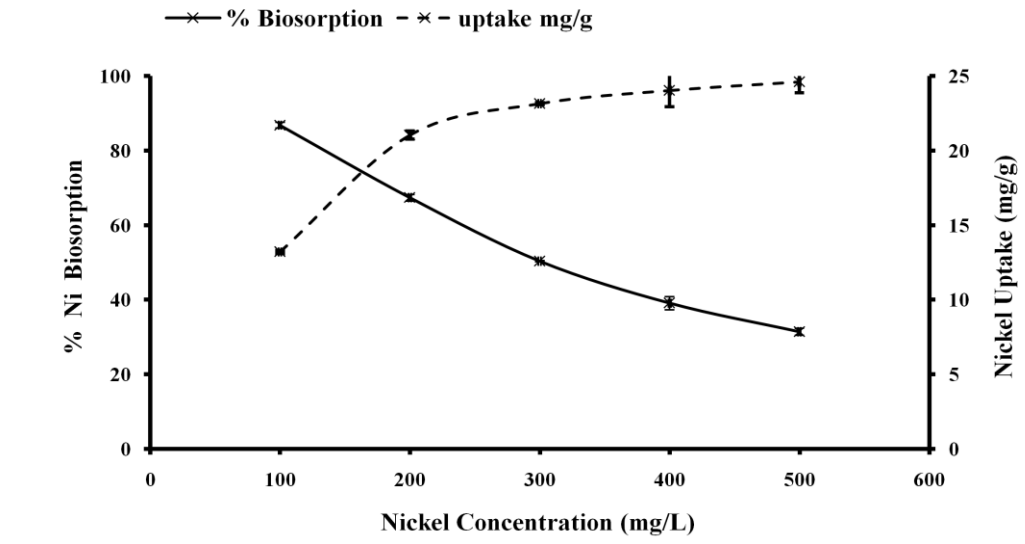

Figure 4: Effect of initial concentration of nickel on % biosorption and Uptake of nickel

3.4.3 Biosorption Isotherm

The biosorption isotherm indicates the adsorbate molecules distribute between the liquid phase and the solid phase at equilibrium. The analysis of the isotherm data by fitting them to different isotherm models is an important step to find the suitable model that can be used for design purpose (Yao *et al*., 2010). In the present study the Langmuir and Freundlich models were used to describe equilibrium biosorption isotherms.

Langmuir Model

This model accepts that biosorption occurs at specific homogeneous sites on the biomass and it is successfully used in many monolayer biosorption processes. The Langmuir isotherm is given by

$$qe = \frac{q_m K_L C_e}{1+K_L C_e}$$

The constants in Langmuir isotherm can be determined by plotting ($1/q_e$) versus ($1/C_e$)
and making use of above equation rewritten as: $1/q_e = 1/q_m + 1/q_m K_L C_e$

Freundlich Model

The Freundlich isotherm model was applied for biosorption on heterogeneous surfaces and for multilayer biosorption. It is given as:

$q_e = K_f C_e^{1/n}$

The logarithmic form of the equation becomes,

$\log q_e = \log K_f + 1/n \log C_e$

The calculated results of the Langmuir and Freundlich isotherm constants are given in Table1. The correlation coefficient obtained for both models indicated that biosorption of Ni by BEL5B could be described by both Langmuir and Freundlich isotherms under the concentration range studies (Fig 5 a and b).

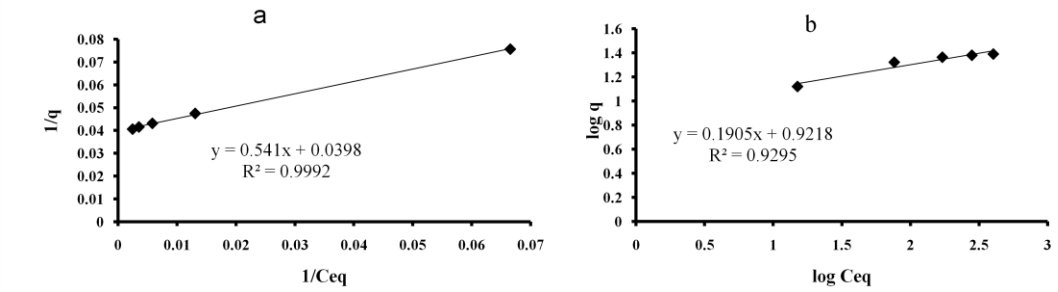

Figure 5: Langmuir (a) and Freundlich (b) isotherm of nickel b

Table 1: Isotherm Model Constants and Correlation Coefficients for biosorption of Nickel using *Sinorhizobium* sp. BEL5B

Langmuir Isotherm			**Freundlich Isotherm**		
$q_{m\ (mg/g)}$	K_L	R^2	K_f	N	R^2
25.13	0.07	0.9992	8.35	5.25	0.9295

3.4.4 FTIR Analysis

The FTIR spectra of the metal loaded and metal free biomass were compared to study the vibrational frequency changes in the functional groups of the adsorbents. The spectra of the adsorbents were measured within the range 450 – 4000cm^{-1} (Fig. 6). The FTIR spectra showed broadening and shifting of peaks observed in this region (3448.10 to 3435.65 cm^{-1}) after interaction with nickel.. Another important characteristic which was observed was the decrease in transmittance of the peaks in the nickel loaded biomass compared to the metal free biomass.

3.4.5 Effect of various pretreatment on bioadsorption of nickel

The studies of pretreatment showed enhancement to 83.89% ($p < 0.001$) in the biosorption capacity after NaOH (E) treatment (Fig.7) compared to 71.62% obtained for the untreated biomass. While no significant difference was observed for other pretreatments.The oven drying (C) and HCl(F) treatment was found to lower the biosorption capacity to 5.47% ($p < 0.001$) and to 50.66% ($p<0.001$) respectively.

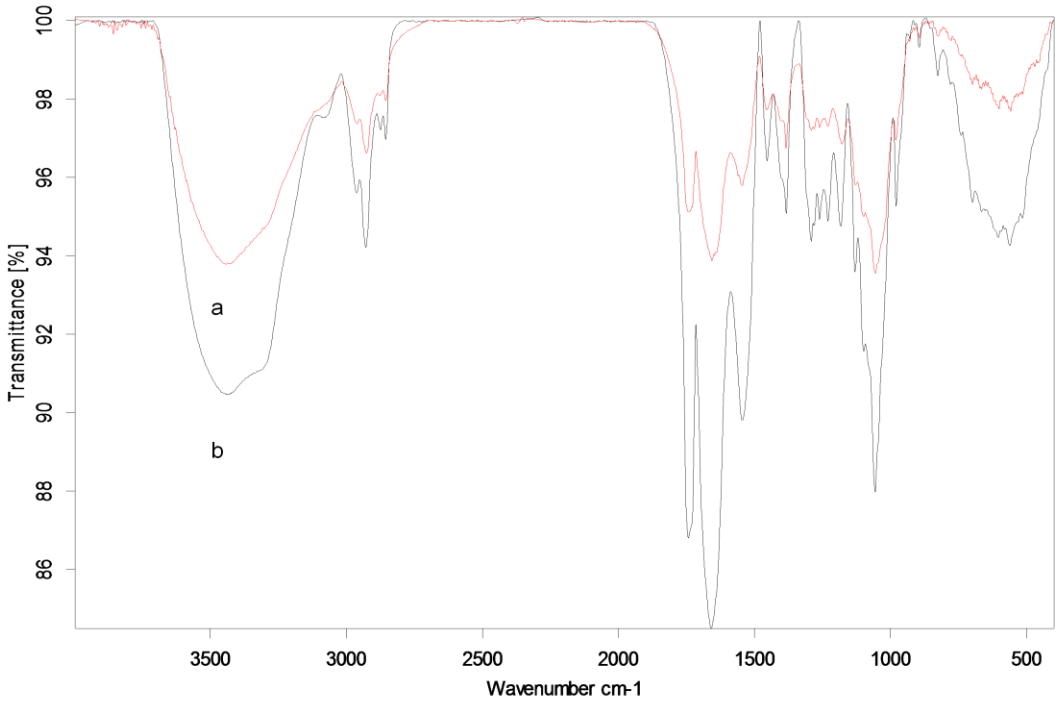

Figure 6: FTIR Spectra of (a) nickel free and (b) nickel loaded biomass of BEL5B

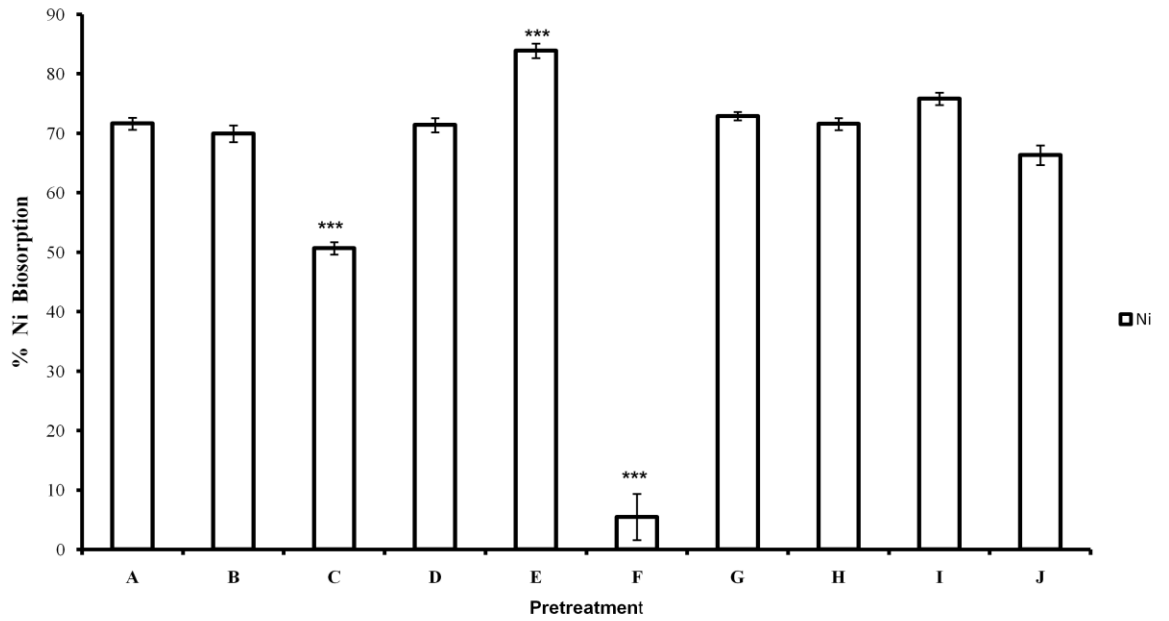

Figure 7: Effect of different pretreatments on nickel biosorption by *Sinorhizobium* sp. Values are mean of n = 3. Significantly different from control(A) ***P(0.001) by one-way (ANOVA) with Tukey comparison test. (A-untreated, B –autoclaved, C – oven, D – boiling, E – NaOH, F – HCl, G – NaCl, H – $CaCl_2$, I – EDTA, J – Triton X 100)

4 Disscusion

Rapid industrialization has brought about a marked increase in anthropogenic activities. Such industrial activities has lead to release of toxic metals into the soil, water and air, causing environmental pollution (Rani *et al*., 2008). Considering the importance of legumes in maintaining soil fertility and the conflicting reports on the effects of heavy metal on their micro-symbiont, some attention has been focused in recent years on the resistance of *Rhizobium* to these elements (Corticeiro *et al*., 2005). The present study explores the potential of gram negative, nitrogen fixing *Rhizobium* sp. for its metal resistance and metal removal capabilities. The rationale for the selection of *Rhizobium* is attributed to its well know field applications and its non pathogenic nature. Apart from this, its high exopolysaccharide producing ability is also instrumental for biosorption (Scott, and Palmer, 1988; Foster *et al*. 2000).

Sesbania sesban is commonly used as a green manure for soil restoration because of its root nodulating bacteria. Rhizobia nodulating *S. sesban* has shown a wide genetic diversity with isolates having sequence similarity to rhizobia belonging to genera *Rhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Allorhizobium* (Bala *et al*., 2002). All the 22 *Rhizobium* and *Sinorhizobium* isolates obtained from the various industrial areas of Navi Mumbai showed tolerance to nickel. The strain BEL5B was found to show highest tolerance to nickel (3mM).

The mechanisms of metal tolerance may depend on genetic as well as physiological adaptations (Rani *et al*., 2008).

The cell wall of bacteria represents the first defense system against any external stress (Langley and Beveridge, 1999). The morphological manifestations of nickel stress were explored by SEM analysis, which revealed an increase in size of the cells and secretion of extra polymeric substance after exposure of BEL5B to 3mM Nickel. Similar morphological changes like increased size were also demonstrated in phototrophic bacteria after exposure to metalloid oxyions as a protection system for bacteria facing a stressful environment (Nepple *et al*.,1999). Helmann *et al*. (2007) showed that the effect of metals on cells can be limited by binding metal ions to exopolysaccharide, thus the cells can survive the metal stress conditions and continue to perform their normal metabolic activities.

In order to understand the genetic mechanism of nickel tolerance screening for the *ncc* operon was carried out in all the strains and an amplification of *ncc*A gene was observed in another isolate SAR1. The absence of the *ncc* operon in BEL5B, which showed highest nickel tolerance could be due to divergence of the BEL5B operon in the primer binding site or a different order of the genes. While for SAR1 the *ncc* mediates resistance to nickel, cadmium and cobalt. Schmidt and Schlegel (1994) showed that this resistance complex probably works as a cation proton antiporter and is composed of a regulatory gene region *ncc*YXH followed by the structural region *ncc*CBA.

Rhizobium, being gram negative have an outer membrane which consists of lipopolysaccharides, lipoproteins, and phospholipids and carries a strong negative charge (Tortora *et al*.,2005). Due to which it can physically remove appreciable quantities of positively charged cationic metals from solution through either bioaccumulation or biosorption (Scott and Palmer, 1990).

The pH of the solution has a very significant effect on metal ion solubility and surface charge of the biomass (Guibal *et al*., 1994). It was observed that at low pH (below 3) the overall surface charge on the cells will be positive inhibiting the formation of links between divalent cationic metal ions and the cell surface (Kumar *et al*., 2010;Shetty and Rajkumar, 2009). As the pH increases the electrostatic repulsion decreases due to reduction of positive charge resulting in an increase in metal biosorption (Sari *et al*., 2009). The results showed pH 4 to 8 to be optimum for biosorption of nickel by BEL5B. Precipitation of nickel hydroxides was observed at pH 9 leading to a slight decrease in biosorption. Kumar *et al*. (2010) has observed that at the higher pH, ion exchange and metal hydroxide formation may become significant mechanisms in metal removal.

The biomass also affects the rate of biosorption of metals hence optimization of biomass is one important aspect. The number of available sites for biosorption depends on the amount of biosorbent used (Lahari *et al*., 2011). It was observed that though biomass dosage and biosorption was directly proportional owing to more number of available sites for adsorption of the metals (Burno *et al*., 2008), a plateau was obtained at higher concentrations of biomass. A biomass concentration of 7.5g/L was found to be optimum for the isolate under study.

The rate of biosorption depends on the initial metal concentration (Ahalya *et al*., 2005), hence biosorption study at different concentration of the metals was studied. The results revealed that increasing metal concentration increases the biosorbent capacity, but decreases percent biosorption probably due to saturation of adsorption sites (Ashraf *et al*., 2011; Singh and Gadi, 2012). At low concentrations, biosorbent sites take up the available metal more quickly. However, at higher concentrations, metal ions need to diffuse to the biomass surface by intraparticle diffusion and greatly hydrolyzed ions diffuse at a slower rate (Horsefall and Spiff, 2005).

There was a gradual increase of biosorption for nickel ions until equilibrium was attained. The Langmuir, Freundlich models are often used to describe equilibrium sorption isotherms. The results of the Langmuir and Freundlich, showed that the adsorption of nickel was correlated well with both the equations, under the concentration range studied. q_{max} which is a measure of the maximum adsorption capacity was found to be 25.13

mg/g. Table 2 summarizes the nickel biosorption capacities (q_m) using different microorganisms. BEL5B presents higher adsorption capacity than 2 out of 9 different adsorbents, reflecting a promising future for utilization of BEL5B in nickel ion removal.

Table 2: Comparison of Bisorption capacity of Sinorhizobium sp. BEL5B with other microbial biomass

Biomass Type	q_m (mg/g)	Reference
R. nigricans	1.0	Holan and Volesky, 1995
A. niger	1.1	Kapoor and Viraraghavan, 1998
A. niger 405	2.0	Kovacevic et al. 2000
Rhodococcus opacus	7.63	Cayllahua et al. 2009
E.cloacae	10.638	Pandiyan and Mahendradas 2011
Circinella sp.	18.66	Alpat et al 2010
Sinorhizobium sp. BEL5B	25.13	Present study
P.aeruginosa	26.316	Pandiyan and Mahendradas 2011
B. Subtilis	71.423	Pandiyan and Mahendradas 2011

An insight into the cell surface ligands involved in nickel biosorption by BEL5B was obtained using FTIR spectroscopy. The loading effect of nickel was seen as decrease in % transmittance as also observed by Joo *et al.* (2010). A peak in the region of 3500 – 3200 cm^{-1} is due to stretching of the N-H bonds of amino groups and the hydroxyl group (Sethuraman and Kumar, 2011). The shifting and broadening observed in this region indicates involvement of hydroxyl and amino acid functional groups on the cell surface of BEL5B in nickel biosorption. The involvement of hydroxyl and amine groups in biosorption of nickel was also reported in *Mucor hiemalis* (Shroff and Vaidya, 2011).The major locations of these functional groups were found to be in the lipopolysaccharide and exopolysaccharide layers of the isolates.

In order to increase the biosorption capacity modifications of the cell wall of the microbial biomass using different physical and chemical techniques have been explored extensively (Gupta *et al*., 2000). Metal affinity of biomass can be increased by pretreating the biomass with alkalies, acids, detergents and heat. When the *Sinorhizobium* sp. BEL5B was pretreated with NaOH, an increase in nickel bisorption capacity. On the other hand, a significant decrease was noticed after HCl and oven drying treatments. Similar enhancement in biosorption of $Cd^{2+,}$ Ni^{2+} and Zn^{2+} by *Penicillium digitatum* was observed after NaOH treatment (Galun *et al*., 1987). Alkali pretreatment removes surface impurities, ruptures the cell membrane, exposes available binding sites for metal biosorption and releases polymers such as polysaccharides that have a high affinity towards certain metal ions (Mittelman and Geesey, 1985; Loaec *et al*., 1997). This could be the reason for increase in biosorption of nickel after NaOH treatment. Similar results of enhancement of biosorption after alkali pretreatment was also observed by Yan and Viraraghavan (2000) and Shroff and Vaidya (2011) for *Mucor rouxii* and *Mucor hiemalis* respectively.

5 Conclusion

The results of the present study emphasize the potential of using *Rhizobium* sp. in bioremediation of nickel. Nickel resistance was identified in the *Rhizobium* and *Sinorhizobium* strains isolated from root nodules of *S. sesban* growing around the industrial and barren areas of Navi Mumbai. SEM analysis revealed an enhanced exopolysaccaride production in BEL5B under nickel stress. *Sinorhizobium* sp. BEL5B showing highest nickel tolerance was found to exhibit a good potential for nickel biosorption. The hydroxyl and the amino functional groups found on the lipo and exo polysaccharides layers of the cell surface were the major sites of nickel biosorption. Significant enhancement in the biosorption capacity after NaOH treatment of the biomass was detected. Thus, these results significantly contribute to better understanding the mechanism of nickel tolerance in Rhizobia isolated from *Sesbania*. Furthermore in view of the practical application, this preliminary study using rhizobia biosorption systems needs to be extended to additional experiments concerning industrial effluents and the use of the Sesbania – Rhizobia symbiosis in soil bioremediation still needs to be explored.

Acknowledgment

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