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Biosorption of Nickel by Live Biomass of *Trichoderma harzianum*

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ABSTRACT

Response of *Trichoderma harzianum* to different nickel concentrations was investigated by poison food technique. It was noticed that *T. harzianum* was moderately tolerant up to 60 mg/L, where the inhibition of mycelial growth was 33.3 %. Further increase in Ni concentration decreased the growth and total inhibition was observed at 200 mg/L. The chromium (VI) biosorption ability of *Trichoderma harzianum* was tested *in-vitro.* The organism was inoculated on Czapek Dox broth medium containing 50 mg/L of Ni salt. The metal residues were analyzed at different day's interval (4, 5, 6, 7 and 8 days). The effect of different pH and temperature on metal removal was also investigated. Results indicated that, at 7th day the metal removal reached the maximum level (90.2 %). Further incubation did not increase the metal uptake. A pH range of 4-5 and temperature of 30 $^{\circ}$C was optimum for Ni uptake by *T. harzianum* in the present study.

Key words: Nickel, *Trichoderma harzianum*, Biosorption, pH, Temperature

Nickel (Ni) is ubiquitous in nature (Boyle and Robinson, 1988). It occurs naturally in soil, sea salts, volcanic ash and particles in smoke from forest fires (Tripathi and Srivastava, 2007) in the form of sulfides, arsenides, antimonides and oxides. In some organisms, it is an essential constituent, like co-factors of some enzymes like hydrogenase, urease and methyl S-coenzyme M-reductase (Hausinger, 1987). However, at elevated concentrations it is a toxic metal to most of the organisms. Toxic levels of Ni inhibit growth and sporulation of various filamentous fungi and yeast (Babich and Stotzky, 1982) by reduction of RNA and protein synthesis (Puckett *et al*., 1979) whereas, a little of 0.34 µM Ni inhibits the growth of *Escherichia coli* (Abelson and Aldous, 1950). In case of human exposure, Ni cause dermatitis in sensitive individuals, injection of soluble salts causes nausea, vomiting and diarrhea.

Many industries such as electroplating, paint, pigments, batteries, mineral processing, porcelain enameling, copper sulphate manufacture and stem electric power plant discharge aqueous effluent containing high levels of Ni (Patel *et al*., 2006**;** Magyarosy *et al*., 2002; Norseth and Piscator, 1979). These various processes lead to accumulation of Ni to a toxic level in the environment, particularly in soil. Conventional methods like chemical precipitation, adsorption on activated carbon, electrodeposition, reverse osmosis etc are not often economically feasible or appropriate because of their high costs, particularly when metal ions are present at very low concentrations or in large solution volume (Bhattacharyya and Chen 1987; Padmavathy *et al*., 2003; El-Sherif *et al*., 2008) and often generate other wastes that require further disposal (Peter *et al*., 1985; Rostami and Joodaki, 2002). Due to these complications, an alternative method of metal removal which is economic and effective is of great importance.

The ability of microorganisms to take up metals has been demonstrated for some time (Gadd, 1993; Preetha and Viruthagiri, 2005). Various microorganisms such as bacteria (Elangovan *et al*., 2006), yeast (Salinas and Melorza, 2000), Fungi (Kogej and Pavko, 2001), algae (Chojnacka, 2007) have been reported to tolerate and remove heavy metals. Fungi are known to accumulate and detoxify metals by several mechanisms like valence transformation, extra or intra cellular precipitation and active uptake (Birch and Bachofen, 1990; Joho *et al*., 1995). This biological method of metal uptake offers several advantages over conventional methods of metal removing, like cost effectiveness, requirements of additional nutrients, efficacy etc (Alluri *et al*., 2007). In several previous studies, metal removal abilities of various fungi have been investigated like *Mucor rouxii* (Yan and Viraraghavan, 2000), *Rhizopus arrhizus* (Subudhi and Kar, 2008), *Polyporus squamosus* (Wuyep *et al*., 2007), *Aspergillus niger* (Tereshina *et al*., 1999), *Rhizomucor pulsillus* (Christov *et al*., 1999), *Phanerochaete chrysosporium* (Sing and Yu, 1998), *Aspergillus fumigatus* (Bhainsa and D'Souza, 1999), *Ganoderma lucidum* (Muraleedharan and Venkobachar, 1990). However, there is no sufficient data available on the metal removal capacity of *Trichoderma harzianum*. So in the present study an attempt was made to study the Ni removal ability of *T. harzianum in vitro*. The effect of different physical parameters like pH and temperature on metal removal was also investigated.

MATERIALS AND METHODS

Organism and culture conditions:

A strain of *Trichoderma harzianum* was obtained from center for advance studies in botany (CAS),

University of Madras, and was routinely maintained on readymade potato dextrose agar (PDA) of HI-MEDIA make.

Reagents:

Metal stock solution was prepared by dissolving Nickel chloride ($NiCl_2$) salt of SRL AR grade in double distilled water (DDW).

Sensitivity/Tolerance of T. harzianum to Ni:

In vitro sensitivity/tolerance of *T. harzianum* to different concentrations of Ni was determined by poisoned food technique (Dhingra and Sinclair, 1985). Appropriate quantity of the Ni stock solution was added to molten Czapek Dox agar (CDA) medium to get the required concentration (10, 20, 40, 60 ,100 and 200 mg/L) and poured in to sterilized petri plates after gentle shaking. Metal unamended medium served as the control. The plates were inoculated by placing 5 mm discs of 4 days old culture of *T. harzianum* and incubated at room temperature (27 ±2 °C). Inhibition of radial growth was measured based on colony diameter, by using the formula stated by Sundar *et al*. (1995).

Percent Inhibition (PI) = [(X-Y/X) x 100]

Where,

X = Radial growth (mm) of control plates

Y = Radial growth (mm) of treated plates

***Biomass preparation*:**

For preparation of the biomass, *T. harzianum* was inoculated in CDA plates and incubated at room temperature (27 ±2 °C). After 5 days, a small portion (0.5 mm) of the fungus was cut and transferred into 200 mL CD broth in a 500 mL Erlenmeyer flask and incubated at 27 °C in a shaker (150 rpm). After the 5-7 days period, the pellets thus formed were harvested from the medium, washed thrice with distilled water and stored at 4 °C till metal absorption studies.

Biosorption of Ni:

The biosorption experiment was conducted in Erlenmeyer flask (500 mL) containing 200 mL of Czapek Dox broth (CDB) and known concentration of Ni solution (50 mg/L) in triplicates. *T. harzianum* pellets (2 gm/flask) were inoculated in to the flask and incubated at different day's intervals (4, 5, 6, 7 and 8 days). Measurement of Ni residue in the growth medium was conducted with Perkin- Elmer AAnalyst, AA800 (Perkin- Elmer Corporation, Shelton, USA) atomic absorption spectrophotometer (AAS).

The adsorption isotherm was calculated by Freundlich and Langmuir isotherm pattern using the following formulas:

Freundlich isotherm:

$\log (x/m) = 1/n \log C + \log K$

Where, x/m is the amount of metals adsorbed (mg of Ni kg^{-1}), C is the equilibrium concentration in soil solution and n and K are the constants of adsorption isotherms. Values of log K represent the amount of metals adsorbed at unit concentration and 1/n represents the concentration gradient.

Langmuir adsorption isotherms:

$C/x = 1/ (K n) + C/n$

Where C= equilibrium concentration of Ni, x is the amount of metals adsorbed, K is the constant related to binding energy and n is the metals adsorption maxima. From a linear plot of C/x verses C, adsorption maxima was calculated, as the inverse of the slope and constant related to bonding energy was determined as slope (or) intercept.

Effects of pH and temperature on Ni removal:

To study the effect of different pH and temperature on Ni removal, CDB was amended with known concentration of Ni solution and adjusted to different pH (3,4,5,6 and 7) by using 0.1N HCl and 0.1N NaOH. *T. harzianum* was inoculated in the medium and incubated at different temperature (10 °C- 40 °C). Measurement of Ni content in the growth medium was measured as mentioned above at different day's intervals.

RESULTS AND DISCUSSION

Influence of Ni on mycelial growth of *T. harzianum* is presented on Table-1. It has been noticed that, *T. harzianum* is moderately tolerant to Ni. There was not much inhibition was noticed till 40 mg/L. At 60 and 100 mg/L the percentage inhibition was 33.3 % and 66 %, respectively while no growth was observed at 200 mg/L of Ni. Previous reports suggested that at higher concentrations Ni ions interact with cellular components like organic acids (Cataldo *et al*., 1988), nucleotides (Brintzinger, 1963), amino acids (Leberman and Rabin, 1957), phospholipids (Hendrickson and Fullington, 1965) etc. and result in disturbance of physiological and biochemical processes. Metal removal capacity of *T. harzianum* mycelium is given in Fig-1. It was observed that the metal removal to a certain extent was time dependent system. A progressive increase in Ni uptake was noticed with an increase in incubation days. The adsorption of nickel followed the typical Freundlich and Langmuir isotherm pattern (Table-2). In the case of Freundlich adsorption isotherms the plot made between log x / m verses log C was found to be straight line for Ni. The Freundlich coefficient 'K' is regarded as the hypothetical index of heavy metal sorbed from a solution having unit equilibrium concentration (Ghosal *et al*., 2003). Hypothetical index was found to be 2.605 in the present study. Considering the fit value (97.4 %), it is suggested that the adsorption of heavy metals by *T. harzianum* can use Freundlich adsorption isotherm to evaluate the adsorption characteristics. In case of Langmuir adsorption isotherm, the plot made between C/(x/m) and C was found to be straight line. In general, Langmuir adsorption isotherms were having fit value 93.9 % in the present study. Therefore, this isotherm can also be used to predict the metal adsorption characteristics of *T. harzianum.* In case of adsorption maxima, the value was higher (383.9) which indicated

the metal adsorption capacity of *T. harzianum* was better in case of Ni. The mycelial dry weights also indicated that the growth of the fungus in Ni amended medium as there was increase in dry mycelial weight as the incubation days extended (Fig.-2). The mycelium after the study was resuspended in water to observe any leakage of the adsorbed metals to the surroundings. No residues were detected in the water up to 10 days, which indicated the binding tendencies of the *T. harzianum* mycelium towards the tested nickel metal (data not shown). Maximum adsorption was achieved on 7th day (91%). Further incubation (8th day onwards) did not increased metal uptake. This may be due to the saturation of the fungal mycelia in metal uptake. Fourest and Roux, 1992 reported that metal-ion uptake of per gram of biosorbent increases as long as the biosorbent is not saturated.

Table 1: Sensitivity/Tolerance of *T. harzianum* to Ni

Ni Conc. (mg /L)	Radial growth (mm)
10	40.5 (10.0) [a]
20	39.4 (12.4
40	37.2(17.2)
60	30.0(33.3)
100	15.4(66.0)
200	0.0 (100)
Control[b]	45.0
CD at 5% [c]	0.60

a. Radial growth on PDA medium after 5days incubation on the treated plates. Values in the parenthesis indicate percent inhibition of the mycelial growth compared to the control plates.
b. Radial growth on PDA medium after 5 days incubation on control plates.
c. Critical difference.

pH is the most important biosorption parameter because it influences both metal speciation and cell surface metal binding sites (Hughes and Poole, 1991). The effect of different pH on Ni removal is presented in Fig.-3. At lowest pH tested in the present study (pH 2), no metal removal was observed. A gradual increase in pH showed increased Ni uptake capacity by *T. harzianum*. Similar kind of results was also noticed by other investigators (Tian-Wei *et al*., 2004; Wuyep *et al*., 2007). The low absorption ability at low pH is might be due to the competition of hydrogen ion with metal ion on the sorption site (Congeevaram *et al*., 2007). It has been reported that, sorption of heavy metals by the fungi is strongly pH dependent and biosorption rate increases with increase in pH (Huang *et al*., 1991; Zhang *et al*., 1998). Parvathi *et al*., 2007 reported increase in manganese removal with rise in pH by *Aspergillus niger*. Matheickal *et al*., 1999 suggested that metal binding increased with pH due to the decrease of hydronium ions in the system, as they also compete for binding sites. Metal removal was noticed between pH 3 to 5 in the present study with the maximum metal removal in pH 4 after 7th days of incubation. Several authors previously described an optimal pH around 4 is an ideal condition for metal removal (Tobin *et al*., 1984; Tsezos and Volesky, 1981). At pH value above 7, the metal uptake was reduced as metals exist as hydroxide colloids and precipitate at alkaline pH, resulting decrease in sorption rate (Liu *et al*., 2002) or due to osmotic changes and hydrolyzing effect (Nasseri *et al*., 2002; Zsljka Filipovic-Kovac *et al*., 2000).

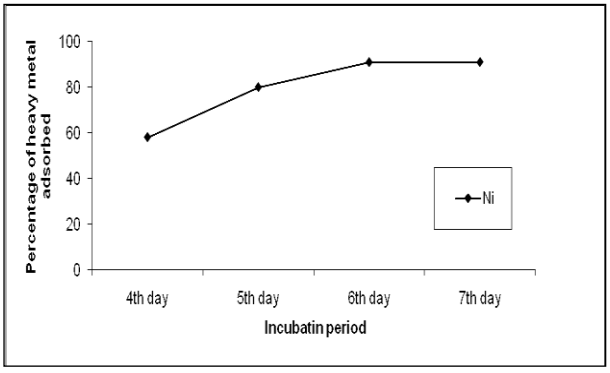

Fig. 1: Biosorption of Ni by *T. harzianum* at different days

Table 2: Constants and correlation coefficients for the Langmuir and Freundlich isotherms

Adsorption parameters	Ni
K	2.605
1/n	1.84
r value	0.974
Langmuir	
qmax	383.9
b	0.212
r value	0.939

r: fitness values of adsorption isotherm; **b**: binding energy; **K**: adsorption coefficient **qmax:** Adsorption maxima

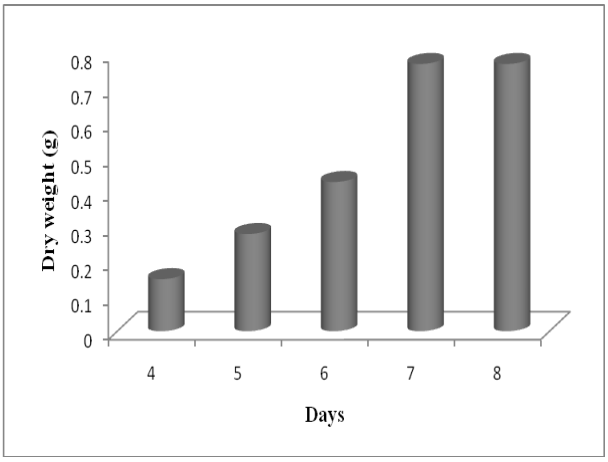

Fig. 2: Dry weight of *T. harzianum* at different days in Ni amended medium

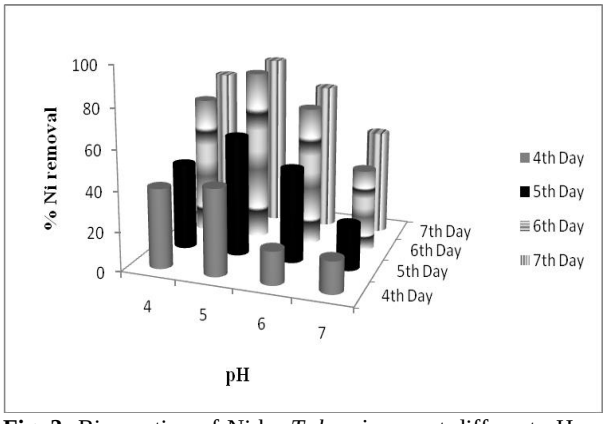

Fig. 3: Biosorption of Ni by *T. harzianum* at different pH

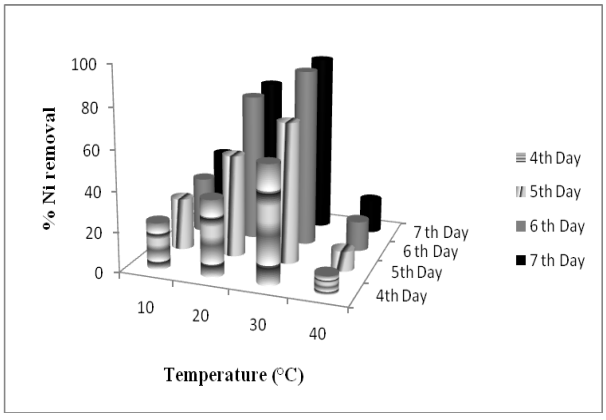

Fig. 4: Biosorption of Ni by *T. harzianum* at different temperature

Temperature plays a critical role in the biosorption of metal ions (Khambhaty *et al*., 2009). An increase in temperature showed increased metal removal to certain extent. The optimum temperature for maximum Ni removal was noticed at 30°C in the present study (Fig.-4). At this temperature, the metal removal was in the range of 90 %. The metal uptake was reduced drastically beyond this temperature. At 40°C the metal removal was reduced to 20 % whereas, no absorption was noticed at 50°C. Previous reports suggested that, temperature affects the biosorption capacity of fungi by influencing enzymatic system. In addition, the solubility of metals in the effluent is affected by temperature and its adsorption rate (Nouri Sepehr *et al*., 2005). Though temperature plays an important role for the growth of organisms, at elevated level, it damages the organisms by denaturing enzymes, transport carriers, integrity of cell membrane (Prescott *et al*., 2002), and also hinder compartmentalization of metal ions leading to reduced metal uptake (Faryal *et al*., 2007).

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