

Full Length Research Paper**Biosorption of Nickel Electroplating Effluent using *Rhizopus* species and its impact on the Growth of Cow Pea****J. Shantha and M. Poonkothai***

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ABSTRACT

The aim of the present investigation was to assess the impact of microbially treated nickel electroplating effluent on the growth of cow pea under optimal conditions. The metal tolerant fungal was isolated from effluent contaminated soil and was identified as *Rhizopus* species. Nickel removal by the *Rhizopus* species was efficient at 25% concentration of nickel electroplating effluent. Maximum removal of nickel was observed on seventh day of incubation at 30°C at pH 6. The biometric parameters of cow pea plants cultivated in microbially treated effluent showed higher growth when compared with untreated effluent and control.

Key Words: Nickel electroplating effluent, Biosorption, *Rhizopus* species, Cow pea.**INTRODUCTION**

Water pollution by industrial effluents has become a great concern in recent years. The contaminants present in the effluent are found to be toxic and hazardous not only to human beings but also to the surroundings (Patil and Hande, 2004). Heavy metals are most hazardous pollutants because of their non-degradable nature and property to affect all kinds of ecological system (Buccolieri *et al.*, 2005). Electroplating industry is one of the major contributors of heavy metal pollution in surface water (Siddigui *et al.*, 1999). Waste water from electroplating industries are characterized with the presence of strong toxic components as simple and complex cyanides of heavy metals, salts of hexavalent chromium, organic additions, mineral acids and bases (Stefanova, 2000).

In recent years the extent of nickel pollution has been recognized and various chemical and biological remediation techniques were investigated for its removal (McLaren and Clucas, 2001). Global input of nickel into the human environment is approximately 150,000 metric tonnes per year from natural sources and 180,000 metric tonnes per year from anthropogenic sources, including emissions from fossil fuel consumption and the industrial production, use and disposal of nickel compounds and alloys (Hostynek and Maibach, 2002).

Human exposure to highly nickel-polluted environments, produce a variety of pathologic effects such as skin allergies, lung fibrosis, cancer and other respiratory tract diseases (Kasprzal *et al.*, 2003). Major toxic effects of nickel include chromosomal damage, alteration of enzyme activity, inhibition of protein and RNA synthesis and decrease in ATP pool (Rajyalaxmi *et al.*, 2004). Fordsmann (1997) reported that the nickel toxicity in plants caused patchy discolorations, premature senescence, yellowing of old leaves, growth reduction and thus affecting the photosynthetic functions of higher plants.

The removal and recovery of heavy metals from waste water is important in the protection of the environment and human

health. A number of technologies such as chemical precipitation, evaporation, ozonation, flocculation, photolysis, adsorption and ion exchange processes have been used to remove metals from waste water. But the selection of an effluent treatment method is largely based on the concentration of heavy metal and the cost of treatment (Vijayaraghavan *et al.*, 2005). The use of microorganisms as biosorbents for removal of heavy metals is an attractive alternative to the existing methods for toxicity reduction and recovery of metal ions from industrial effluents because of their good performance and low cost (Deng *et al.*, 2005).

Hence the present study was aimed for the removal of nickel from the nickel electroplating effluent using *Rhizopus* species as biosorbent and also to study the impact of the microbially treated effluent on the growth of cow pea.

MATERIALS AND METHODS**Collection of sample**

The effluent contaminated soil and nickel electroplating effluent was collected from the discharge site of electroplating industry in Coimbatore district, Tamil Nadu. The soil samples were collected aseptically and used for further analysis.

Isolation and identification of metal tolerant fungi

One gram of the effluent spilled soil was weighed and transferred into 10ml of sterile distilled water and serially diluted. From each dilution 0.1ml of the sample was spreaded on Rose Bengal Chloramphenicol agar medium with the help of L-rod aseptically. The plates were incubated at room temperature for five days. After five days of incubation the well grown fungal colonies were isolated and subjected to identification macroscopically and microscopically using lactophenol cotton blue staining (Cappuccino and Sherman, 1999). The identified fungal isolates were screened for the nickel removal.

Preparation of culture

A mycelial disc of 1.2 cm diameter was obtained from 4 to 5 days old culture of the isolated fungal colonies and was transferred to sterile 100ml Sabouraud's dextrose broth (glucose - 2 g, peptone - 1 g and distilled water - 100 ml). The contents were incubated at room temperature for 5 days and analysed for their decolourisation efficiency.

Decolourisation assay

The isolated fungal colonies were screened for the removal of nickel by inoculating a loopful of those colonies into 100ml of different concentrations of nickel electroplating effluent (25%, 50%, 75% (effluent: water) and 100%) separately. In a 250 ml Erlenmeyer flask, 100ml of the effluent was taken and sterilized separately. After sterilization the fungal colonies were inoculated into the flask separately. The contents were incubated at room temperature for 7 days for decolourisation assay. The samples were centrifuged at 10,000 rpm for 10 minutes and the decolourisation was assessed by measuring the absorbance at maximum wavelength of 520 nm. The amount of nickel present in the effluent was determined by dimethyl glyoxime method (APHA, 1998). The fungal isolate which shows maximum uptake of nickel was selected for the study. The uptake of nickel was expressed in terms of percent decolourisation and calculated using the formula,

$$\text{Percent decolourisation} = \frac{A_i - A_f}{A_i} \times 100$$

A_i - Initial absorbance, A_f - Final absorbance

From the above study it was observed that in 25% concentration of effluent the removal of nickel was efficient and this concentration was taken for the further study.

Removal of nickel using the isolated fungus under optimal conditions

For the uptake of nickel from the effluent, different operational parameters such as pH, temperature and incubation time were optimized. One gram of the fungal isolate was inoculated separately into a series of 250ml conical flasks containing 25% concentration of nickel electroplating effluent. The pH was set at different ranges from 3 to 8 (3, 4, 5, 6, 7 and 8) by adjusting with 1N HCl or 1N NaOH. At each pH, the isolated fungal species was incubated at different temperatures (20°C, 25°C, 30°C, 35°C and 40°C) for different incubation periods (1, 3, 5, 7, 9 and 11 days). Glucose at a concentration of 1% was added which served as carbon source. At the end of the incubation period the samples were removed, centrifuged at 10,000 rpm for 10 minutes and the supernatants were analysed for the removal of nickel.

Impact of nickel electroplating effluent on biometric measurements of cow pea

Red soil for the pot culture experiment was sieved and mixed with sand in equal proportions. Cow pea (*Vigna unguiculata*, L.) seeds were collected from Tamil Nadu Agricultural University (TNAU), Coimbatore. Nine pots were set for the present investigation. Ten healthy seeds were sown in each pot and were kept under laboratory conditions. Thinning was done

on the seventh day after germination leaving only five plants per pot. They were watered twice a day. The plants were treated with,

T₁ - Control (Tap water)

T₂ - Untreated nickel electroplating effluent (25% concentration of nickel electroplating effluent)

T₃ - Treated nickel electroplating effluent (25% concentration of microbially treated nickel electroplating effluent)

Each treatment was replicated thrice. The plants were uprooted on 7th day and the biometric parameters such as germination percentage, vigour index, shoot length, root length, fresh weight and dry weight were analysed and subjected to one way ANOVA.

RESULTS AND DISCUSSION

Screening and isolation of reactive dye decolorizing fungi

About four morphologically distinct fungi were isolated from the nickel electroplating effluent contaminated soil by serial dilution technique. The fungal isolates 1,2,3,4 removed nickel at 60%, 30%, 35% and 39% respectively. Among them, the fungal isolate 1 showed higher per cent of nickel uptake and was selected for the present study and further subjected to identification.

Identification of fungal isolate

Based on the morphology and lactophenol cotton blue staining the fungal isolate was identified as *Rhizopus* species. The taxonomic position of the *Rhizopus* species (Isolate 1) was determined microscopically based on the conidial morphology, size and shape under low and high power objectives. The macroscopic identification of *Rhizopus* species shows rapidly growing white mycelium swarmed over the entire plate and the aerial mycelium was cottony and fuzzy. Microscopically, the spores are oval, colourless and brown. The mycelium is aseptate and gives rise to straight sporangiophores that terminate with black sporangium containing the sporangiospores. Roots like hyphae called rhizoids are present.

Uptake of nickel from the effluent using fungal isolate at different dilutions

The efficiency of nickel uptake by *Rhizopus* species was assessed 7 days after inoculation of fungal species in graded concentrations (25%, 50%, 75% and 100%) of the nickel electroplating effluent. *Rhizopus* species showed a decreasing trend in nickel uptake with increased concentration of nickel at 7 days of incubation (65%, 39%, 22% and 13% respectively). Maximum uptake of 65% of nickel was observed in 25% concentration of nickel electroplating effluent which indicates the presence of lesser amount of nickel in the effluent. Fungi play an important role in decolorization by adsorbing the heavy metal by entrapment and subsequent sorption onto the binding sites present in the cellular structure. Living cells of fungi are able to remove heavy metals ions from aqueous solutions (Volesky and Holan, 1995). Devi and Kausik (2005) reported that *A. flavus* was found to remove 44% of the colour in the undiluted dye effluent for 8 days of incubation. It was observed that among the different concentrations of effluent used, the lowest concentration (25%) was found to be more effective in the removal of nickel. The nickel content

found to be was 16.8 mg/l in 25% concentration of effluent, whereas in the effluent treated with *Rhizopus* species the nickel content was 2.8 mg/l which was within the limits prescribed by BIS (3 mg/l). Hence, this concentration was used for further studies.

Effect of pH on the uptake of nickel by *Rhizopus* species

Biosorption is analogous to an ion-exchange process and therefore, pH of solution influences the nature of biomass binding sites and metal solubility. It is observed from the Table 1 that the *Rhizopus* species was able to absorb the nickel ions over a wide range of pH and the maximum uptake of nickel was found to occur at pH 6 (62.15%). The variation of adsorption of nickel at various pH is on the basis of surface and metal chemistry in the solution. The decrease in removal of nickel above pH 6 is due to the formation of $\text{Ni}(\text{OH})_2$. Substantial precipitation of nickel as nickel hydroxide occurs at high pH values. The formation of hydroxide precipitate reduces the amount of free nickel ions, which accumulates on the organism. Similar reports has been reported for nickel removal using *S. cerevisiae*, where the outer cell wall consists of protein coat, which develops a charge by the dissociation of ionizable side groups of the constituent amino acids. The ionic state of ligands such as carboxyl, phosphate, imidazole, and amino groups will promote reactions with the positively charged metal ions. At low pH, cell walls ligands were closely associated with the hydronium ions $[\text{H}_3\text{O}^+]$ and restricted the approach of metal cations as a result of the repulsive forces of the sorbent (Congeevaram *et al.*, 2007).

The results of the present study coincides with the findings of Deepak *et al.* (2005) who reported that *Pleurotus florida* was found to grow at maximum pH 6.0 and 5.5 and *Pleurotus* species at pH 5.5 to 6.0 (Chang and Miles, 1989).

Effect of temperature on the uptake of nickel by *Rhizopus* species

Nickel uptake by *Rhizopus* species at different temperatures was studied and the results were shown in Table 1. It was observed that the uptake of nickel by *Rhizopus* species increased with increasing temperatures up to 30°C (63.91%), and no further increase in nickel uptake was noticed above this temperature. Temperature has also an influence on the biosorption of metal ions, but to a limited extent under a certain range of temperature, which indicates that ion exchange mechanism exists in biosorption to some extent. Biosorption process is usually not operated at high temperature because it will increase the operational cost. The decrease of biosorption capacity at higher temperature may be due to the damage of active binding sites in the biomass (Lau *et al.*, 1999).

The results of the present study coincides with the findings of Bai and Abraham (2001) who have reported higher chromium removal efficiency by *Rhizopus nigricans* biomass at 30°C. Uma and Sudha (2009) observed similar such result when they treated textile reactive dyes with *Trametes hirsuta*.

Effect of incubation time on nickel uptake by *Rhizopus* species

The results of varying incubation periods were carried out to find the possibility of the involvement of metabolic processes

in the uptake of nickel by *Rhizopus* species which was presented in Table 1. The rate of nickel uptake from the effluent by *Rhizopus* species showed a gradual increase from 1 to 7 days of incubation time and decreased after 7th day. Removal of nickel from the effluent by *Rhizopus* species was maximum at 7th day (93 per cent). *Phanerochaete chrysosporium* could decolourise the phenolic paper mill effluent within seven days of incubation (Ali *et al.*, 2007). Sukumar *et al.* (2008) also reported that decolourisation of the dye effluent by fungal culture was significantly increased with incubation period from the 2nd to 7th day.

Impact of nickel electroplating effluent on the biometric parameters of cow pea

The results of germination percentage, root length, shoot length, fresh weight, dry weight and vigour index of cow pea seedlings treated with tap water (control), 25% concentration of nickel electroplating effluent (T_2) and 25% concentration of microbially treated nickel electroplating effluent (T_3) on 7th day favoured the growth of the cow pea. The cow pea plants cultivated in microbially treated effluent showed higher growth and germination when compared to untreated effluent and control (Table 2).

The seeds of cow pea showed highest percentage of germination (93.3) in T_3 followed by T_1 (86.6) and decreased in T_2 (63.3) as shown in Table 2. The decrease in the germination percentage of T_2 plants could be attributed principally by physical constraints as well as biological harm caused to seeds by the physical and chemical constituents of effluents as opined by Debojit and Rao (1994). The increase in germination percentage in T_3 plants of the present study was in consonance with the findings of Jothimani and Elayarajan (2003) who reported the same in dye effluent treated with biological systems. The vigour index was maximum in T_3 (2109) followed by T_1 (1438) and minimum was observed in T_2 (367) as shown in Table 2.

The reduction in shoot and root length of seedlings grown using T_2 might be due to the presence of high amount of salts in the nickel electroplating effluent and also due to the salt stress and acidity which could have restricted the rooting and shooting (Evers *et al.*, 1997). Black gram and greengram seeds grown with microbially treated effluent have been reported to favour root and shoot length of the seedlings (Madhappan, 1993).

The reduction in the fresh and dry weight of the cow pea seedlings selected for the present study may be due to the toxic effect of nickel in the untreated nickel electroplating effluent which might have inhibited water uptake resulting in the retardation of plant growth (Ganesh *et al.*, 2006). Ramakrishnan *et al.* (2001) reported that the paddy seeds grown using sugar mill effluent treated with yeast increased the fresh and dry weight of the seedlings which was in accordance with the results of the present study.

Thus from the study it is concluded that the effluent can be used for irrigation after bioremediation with a potential fungal strain which is ecofriendly in nature.

Table 1 Percentage removal of nickel at different pH, temperature and incubation period by *Rhizopus* species

Ph	Removal of nickel (%)	Temperature (°C)	Removal of nickel (%)	Incubation period (days)	Removal of nickel (%)
3	32.05	20	40.82	1	10.71
4	43.33	25	53.64	3	21.53
5	50.12	30	63.91	5	56.40
6	62.15	35	58.73	7	64.38
7	57.03	40	42.56	9	60.82
8	46.67	45	26.03	11	43.25

Optimum pH – 6, Optimum Temperature – 30° C, Optimum Incubation period – 7 days

Values are mean of triplicates

Table 2 Biometric parameters in 7 days old seedlings of cow pea treated with different treatments

Treatments	Germination Percentage	Shoot length (cm)	Root Length (cm)	Fresh weight (g)	Dry weight (g)	Vigour index
T ₁	86.6	12	4.6	0.76	0.072	1438
T ₂	63.3	3.4	2.4	0.42	0.044	367
T ₃	93.3	14.8	7.8	0.91	0.102	2109
SED		0.6218	0.3830	0.0517	0.0075	
CD (5%)		1.4339	0.8831	0.1193	0.0173	

The values are mean of triplicates

T₁ - Control (Tap water),

T₂ - 25% concentration of nickel electroplating effluent

T₃ - 25% concentration of microbially treated nickel electroplating effluent

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