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A Study of Nickel Resistant Bacteria Isolated from Electroplating Industrial Effluent

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A B S T R A C T

Wastewater discharges from various industries including electroplating are responsible for increasing toxic heavy metals in the water bodies. These heavy metals which also contain nickel one of the pollutants of water and soil around contaminated sites. Nickel removal from effluent plays an important role in the reduction of environmental pollution. In the present study, twenty-four isolates were screened as nickel-resistant and effects of temperature, pH, incubation period and initial nickel concentration on bio removal efficiency of these isolates were studied. Nickel-resistant isolates were determined for MIC of nickel which was ranging from 400-2000 mg/L. Four isolates TIINi06, TIINi11, TIINi20, and TIINi23 showed higher MIC of nickel i.e., 2000 mg/L. Isolate TIINi06 was able to remove 100 mg/L of nickel completely in 3 days at 40°C. As heavy metals are non-degradable and toxic to the environment, such heavy metal-resistant microbes can be effective in the development of bioremediation technology.

***Key words*:** Nickel bioremediation, Electroplating effluent, Heavy metal toxicity, Water pollutants, MIC

Electroplating is a mostly used process for metallic article finishing. This metal processing method results in the generation of heavy metal pollutants in wastewater. These heavy metals are highly toxic as well as non-biodegradable. Heavy metal pollutants are one of the great concerns of the environment. As heavy metals are non-biodegradable hence these are causing biomagnifications in the environment. Nickel is present in effluents of electroplating industry, silver refineries, zinc base casting, storage battery industries, wire, and electrical parts. Nickel is the 24th most abundant element in the earth's crust and is present in the various parts of the biosphere. Nickel has properties of the hard and soft metal which can bind to other elements like oxygen, nitrogen, and sulfur. Even though nickel can exist in various oxidation states like -1, +1, +3, and +4, nickel in the +2 i.e., Ni (II) valence state is the prevalent oxidation state under environmental conditions [1]. A higher concentration of nickel causes cancer of the lungs, nose, and bone. Dermatitis (Ni itch) is the most frequent effect of exposure to Ni, such as coins and jewelry. Acute poisoning of Ni (II) causes nausea, vomiting, dizziness, and headache, rapid respiration, tightness of the chest, chest pain, dry cough and shortness of breath, cyanosis, and extreme weakness [2-3].

Steel processing units discharge nickel into the water bodies. Hence it is necessary to remove heavy metals like nickel from industrial wastewater before discharging it into the river water. Some chemical methods like electro-dialysis, reverse osmosis, ultra-filtration, and ion exchange are used for the removal of heavy metals from industrial wastewater. These methods are insufficient and also expensive. Many researchers have isolated and used various microbes including bacteria, fungi, and algae in the past few years for the bioremediation of heavy metals. These microbes can resist as well as remove highly toxic metals. These microbes have a great potential for the removal of heavy metals including nickel from wastewater. This bioremediation technology can be used to remove nickel in an eco-friendly manner [4]. The present study aimed to screen and characterizes nickel-resistant bacteria in the potential application of nickel bioremediation.

METHODS AND MATERIALS

Sample collection

An effluent sample was collected from the stainless-steel industry. The effluent sample was transported aseptically to a laboratory for further study.

Screening and identification of nickel resistant bacteria

The sample was serially diluted up to 10^{-5} in water. The diluted sample was spreaded on nutrient agar plates containing 100 mg/L Ni salt i.e., Nickel chloride. These plates were incubated for 2 days at 30°C [5]. Bacterial isolate which showed higher efficiency of nickel removal was identified by the 16S r-RNA sequencing method.

Determination of minimum inhibitory concentration

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These nickel-resistant isolates were determined for MIC of nickel. A stock solution of $NiCl_2$ was prepared in deionized distilled water. The sterile stock solution was added in increasing concentrations from 100, 200 up to 2500 mg/L in nutrient broth tubes. All these tubes were inoculated with an equal volume of an active culture of each isolate. All the tubes were incubated at 30°C for 2 days. The bacterial growth in terms of turbidity was determined by measuring optical density at 600 nm by using spectrophotometer.

Determination of bio-removal efficiency of nickel resistant isolates

Nickel-resistant isolates were inoculated in nutrient broth with 100 mg/L $NiCl_2$ and without metal salt as a control. The flasks were incubated at 30°C for 2 days. Initial and final samples were collected. The supernatant were taken after centrifugation at 6000 rpm for 10 mins. and concentration of Ni was determined by Atomic Absorption Spectrophotometer. The percentage of Ni removal by the isolate was calculated using the equation:

$$\text{Ni removal (\%)} = (C_o - C_e) / \text{x } 100 / C_o$$

Where,

C_o is the initial and C_e is the equilibrium concentrations of Ni (mg/L) in the solution, respectively [6].

Effect of various parameters on nickel bio-removal efficiency of isolates

Each nickel-resistant isolate was inoculated in separate flask containing 100 ml nutrient broth with $NiCl_2$ in a concentration of 100 mg/L and without metal salt as a control. The effects of different parameters were studied on bio removal efficiency of isolates. These parameters were included various incubation periods (1, 2, 3, 4, and 5 days), temperatures (10, 20, 30, 40 and 50°C), pH (5, 6, 7, 8 and 9), and initial nickel concentrations (100, 200, 300, 400 and 500 mg/L). Samples were collected interval during the study of each parameter. The supernatant was collected from samples after centrifugation. The bio removal efficiency of isolates was determined by measuring nickel concentration in each sample.

RESULTS AND DISCUSSION

Screening and identification of nickel resistant isolates

Twenty-four isolates were isolated on nickel-containing nutrient agar plates. These isolates were labeled as TIINi01 to TIINi24. In this study, among all isolates, TIINi06 could remove Nickel efficiently hence this isolates TIINi06 was identified by 16S r-RNA sequencing as *Bacillus cereus* with 99% similarity. Mahalingam *et al*. [7] reported that sixteen bacterial isolates were screened on a nutrient agar plate containing 100 ppm nickel and were characterized *Pseudomonas* spp., *Escherichia coli*, *Proteus* spp., *Staphylococcus* spp., *Salmonella* spp., and *Shigella* spp. In 2015, Jobby *et al*. [8] isolated *Rhizobium* cultures for nickel resistance study.

Minimum inhibition concentration (MIC) of nickel

There were twenty-four Ni resistant isolates were screened and determination of MIC by using $NiCl_2$ was carried out, results are shown in (Table 1). Four isolates TIINi06, TIINi11, TIINi20 and TIINi23 showed the highest MIC i.e., 2000 mg/L respectively in nutrient broth containing $NiCl_2$. MIC for $NiCl_2$ showed by all isolates varied from 400-2000 mg/L. Oves *et al*. [9] reported that *Bacillus thuringiensis* could survive at 1200 mg/L of nickel concentration. In 2016, Margaret *et al*. [10] investigated *Bacillus aerius, Pseudomonas*, and *Chryseo bacterium* sp. was able to tolerate nickel up to 1200 mg/L.

Table 1 MIC of nickel for nickel resistant isolates

S. No.	Isolates	$NiCl_2$ (mg/L)
1.	TIINi01	600
2.	TIINi02	1800
3.	TIINi03	1100
4.	TIINi04	600
5.	TIINi05	400
6.	TIINi06	2000
7.	TIINi07	1800
8.	TIINi08	1800
9.	TIINi09	1800
10.	TIINi10	400
11.	TIINi11	2000
12.	TIINi12	400
13.	TIINi13	1700
14.	TIINi14	1800
15.	TIINi15	400
16.	TIINi16	1800
17.	TIINi17	400
18.	TIINi18	1200
19.	TIINi19	1200
20.	TIINi20	2000
21.	TIINi21	1400
22.	TIINi22	1200
23.	TIINi23	2000
24.	TIINi24	1400

Nickel bio-removal efficiency of isolates

Four isolates (TIINi06, TIINi11, TIINi20, and TIINi23) which showed a higher MIC of Nickel were selected for nickel removal assay. Bacterial isolates TIINi06, TIINi11, TIINi20, and TIINi23 could remove 95%, 68%, 76%, and 89% respectively in 2 days of incubation. Banerjee *et al*. [11] stated that *Arthrobacter phenanthrenivorans* could show 47.62% removal efficiency for nickel in 3 days. The bio-sorption ability of *B. thuringenesis* OSM29 was highest i.e., 94% for nickel [9].

Effect of different parameters on Nickel removal efficiency of isolates

As shown in (Fig 1), nickel-resistant isolates showed most of Ni removal in 3 days in presence of 100 mg/L of nickel. Isolate TIINi06 showed a complete reduction of nickel in 3 days of incubation. Isolates showed an increase in nickel removal efficiency as the incubation period increased. (Fig 2) showed that, all isolates were able to remove maximum nickel in the media at 30-40°C. Isolate TIINi06 showed maximum nickel removal at 40°C in 3 days. As temperature increases from 10°C to 40°C, the nickel removal ability of these isolates was also increased and as an increase in temperature to more than 40°C, the nickel removal ability was decreased. In 2015, Aryal [12] stated that the maximum nickel uptake capacity of *Bacillus sphaericus* was at 40°C and pH 5. As shown in (Fig 3), at pH 7, all isolates showed maximum bio removal of nickel. In the range of pH 5 to 9, all isolates showed more or less nickel removal. Babar *et al*. [13] investigated the bioremediation potential of a nickel resistant *Bacillus altitudinis* MT422188, whose optimum growth parameters were showed at pH 7 and temperature 32°C. In (Fig 4) showed that, as the initial nickel concentration was increased in the media from 100 to 500mg/L,

the isolates efficiency of nickel removal was decreased. The initial nickel concentration and nickel removal efficiency of these isolates were reciprocal. Oves *et al*. [9] also stated that *B. thuringiensis* showed that as initial nickel concentration was increased from 25 mg/L to 150 mg/L; biosorption of nickel was decreased from 94% to 81.5%.

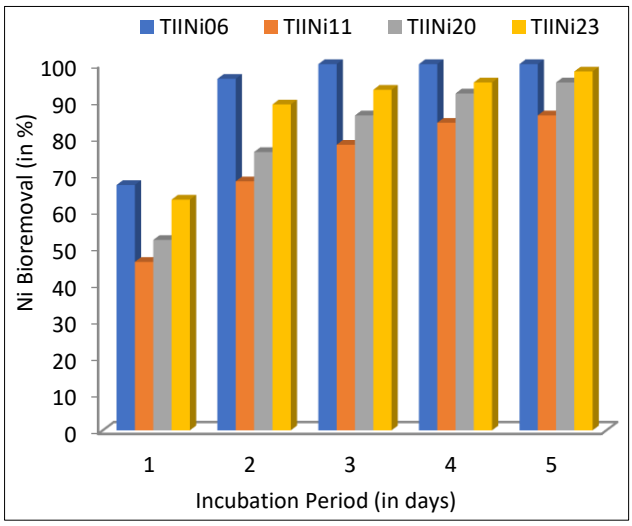

Fig 1 Effect of incubation period on Ni removal

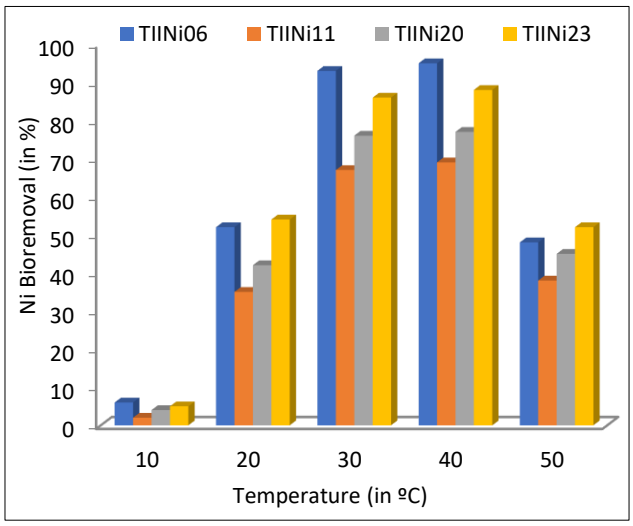

Fig 2 Effect of temperature on Ni removal

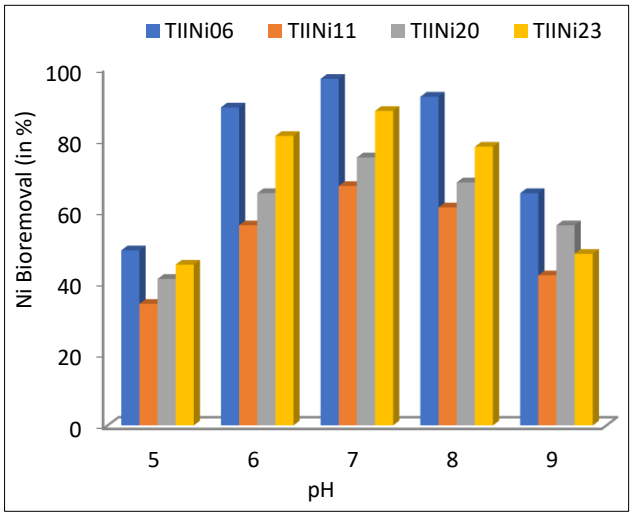

Fig 3 Effect of pH on Ni removal

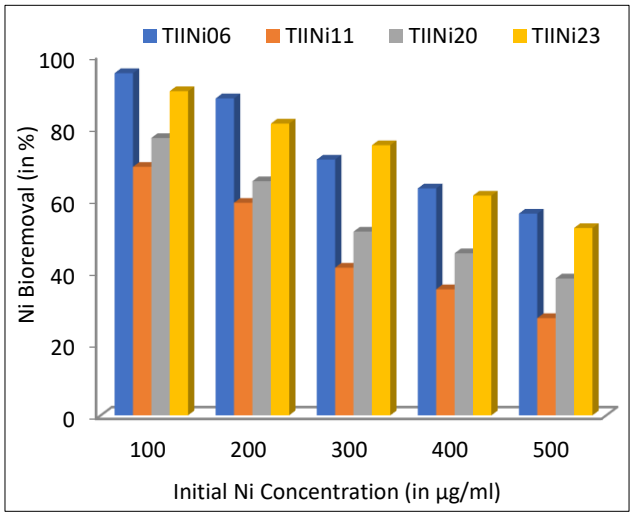

Fig 4 Effect of initial metal conc. on Ni removal

CONCLUSION

In the electroplating industrial effluent sample, twenty-four isolates were screened as nickel resistant bacteria. Among which four isolates showed maximum tolerance at a concentration of 2000 mg/L of nickel. Different parameters were studied for their effects on the removal efficiency of selected isolates. Isolate TIINi06 was able to remove 100mg/L of nickel completely within 3 days at pH 7 and 40°C. Nickel contaminated effluent can be discharged into the water bodies after the use of such bioremediation technology. All of these nickel tolerating isolates have a great perspective for decreasing nickel toxicity. This bioremediation technology is applicable for the removal of nickel from various contaminated sites.

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