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# Molecular Identification and Nickel Biosorption with the Dead Biomass of Some Metal Tolerant Fungi

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## Abstract

Nickel as a heavy metal is the fifth most abundant element on earth. It has many applications and usages. The present study was conducted to isolate metal tolerant fungal strains, molecular identification and evaluating the bioprocess efficiency by assessment the effect of some environmental conditions on biosorption capacity by the dead biomass of the selected fungi. Twelve fungal isolates obtained from polluted water of the nickel ($Ni^{2+}$) were screened, fungi under the isolation number MERV21569 and AHM21696 proved to be the best biosorbents. They removed $Ni^{2+}$ by 79.6% and 85.2% with uptake equal 4.33 and 4.75 µg/mL, respectively. According to the molecular identification of these selected isolates, they were designated as *Aspergillus sojae* MERV21569 and *Aspergillus terrus* AHM21696. It was found that the sorption isotherms for $Ni^{2+}$ by both fungi appeared well acceptable with both Freundlich and Langmuir's models. Both isolates were investigated and evaluated with different bioprocess factors including, initial concentrations of $Ni^{2+}$, different contact times and different initial pH of $Ni^{2+}$ solution with different process times. Metal resistant capacity of the selected fungi against ten heavy metals including $Cd^{2+}$, $Pb^{2+}$, $Cu^{2+}$, $Hg^{2+}$, $Ag^{+}$, $Cr^{6+}$, $Ni^{2+}$, $Zn^{2+}$, $Fe^{3+}$ and $Al^{3+}$, which are represent the highly poisonous heavy metals in wastewaters of different industries was determined. In waters polluted with heavy metals the maximum removal of $Ni^{2+}$ (100% for both strains) was achieved within 4 and 2 h contact time by using the dead biomass of *Aspergillus sojae* MERV21569 and *Aspergillus terrus* AHM21696, respectively.

**Keywords:** Heavy metals; Nickel ($Ni^{2+}$); Bio-sorption; Isotherms; *Aspergillus* sp.; Molecular identification

## Introduction

Wastewater during electroplating activities involves poisonous heavy metals like nickel, chromium, mercury, zinc, cadmium, phosphorus and copper which build up in the food chain and cause different pathological states. These heavy metals at elevated concentrations in the environment specifically in different water resources is of main concern in respect of their extremely toxicity to biological systems, bioaccumulation in them, non-environmental decomposable and menace to human, animal and plant life [1,2].

Nickel as a heavy metal is the fifth most abundant element on earth. It has many applications and usages, mainly in alloy preparations and as an ingredient of metal products and other industrial activities which raise its level in the environment, leading to continuous introduction of this element into the food chain. Thus effective removal of nickel from the environment is an increasing human health concern [3]. The allowable limits of $Ni^{2+}$ in drinking water and manufacturing waste are 0.01 mg/L and 2 mg/L, respectively [4]. Surpass $Ni^{2+}$ level in soil lead to phytotoxicity in plants and above allowable level (0.07 mg/L) is answerable for headache, vomiting, chest pain, cyanosis, bone dermatitis and lungs cancer, etc. in human [4].

A large number of microbial species, that including fungi (*Aspergillus niger*, *Rhizopus oryzae* and *Penicillium chrysogenum*); yeasts (*Saccharomyces cerevisiae* and *Rhodotorula mucilaginosa*) and bacteria (*Bacillus subtilis* and *Pseudomonas aeruginosa*) have the ability to adsorb different heavy metals **[5]**. The fungal strains may well suit this goal than other microorganisms; due to their great resistance toward most of heavy metals, wall binding ability and intracellular metals uptake abilities. Efficiency of biosorption response is improved by environmental conditions such as temperature, pH, ionic strength of the media and so on [2].

Thus, this study was conducted to evaluate $Ni^{2+}$ bio-removal capabilities by different sorption isotherms as well as evaluating the bioprocess efficiency by assessment the effect of some environmental elements on biosorption capacity by the biomass of the selected fungi.

## Materials and Methods

### Sampling and isolation processes

Water samples from polluted water of Damietta's drainages and canals sited near the industrial region of New Damietta were collected in clean and sterilized screw-cap glass bottles. To each sample, 372 mg/L of ethylene diamine-tetraacetic acid disodium salt (EDTA) was added as a chelating agent to decrease toxicity in samples until processing. For isolation of filamentous fungi a serial dilution method on Sabouraud dextrose agar (SDA) as previously described by El-Gendy et al. [6]. The plates were incubated at 28°C for 7 days. The relative frequency of incidence was considered as the number of species isolated from each sample divided by the total number of samples. The isolated species were categorized as very frequent (>20%), frequent (10-20%) or infrequent (<10%). Fungal isolates were identified according to previous keys [7-10]. Fungal strains were conserved on potato dextrose agar (PDA) and kept at 4°C. The most frequent occurrence fungi in sampling were

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subculture in potato dextrose broth (PDB) at 28°C for 7 days on a rotatory shaker at 180 rpm. The biomass was autoclaved for 15 min at 121°C. Then the biomass was recovered by filtering and it was dried at 50°C until dryness. The biomass was crushed to powder in mortal and pestle and then it was used for sorption studies [11].

## Reagents

All the chemicals were of analytical grade and were obtained from Merck. The stock solution (1 g/L) of Ni (II) was prepared by dissolving appropriate quantities of $NiCl_2$ salt in deionized water. The stock solution was diluted to required concentration by deionized water and the standard calibration was performed. The initial pH value of the Ni (II) solutions was adjusted to the required value with $HNO_3$ before mixing the biosorbent suspension. Fresh dilutions were prepared for each study.

## Genomic DNA preparation and 18S rDNA region sequencing

Genomic DNA samples of the most active biosorbent isolates were extracted and purified using the QIAGEN DNeasys Tissue Kit following the manufacturer's protocol (QIAGEN, Germany). Amplification of ribosomal DNA was achieved using puReTaq™ Ready-To-Go™ PCR Beads (GE Healthcare). For amplification of the nearly complete 18S rRNA gene, the primers NS1 5'- GTAGTCATATGCTTGTCTC -3' and NS8 5'- TCCGCAGGTTCACCTACGGA -3' were used. The PCR reaction was achieved with 20 ng of genomic DNA as the template in a 30 µL reaction mixture. The conditions for this PCR were initial denaturation (5 min at 94°C) followed by 35 cycles of primer annealing (1 min at 55°C), primer extension (1 min at 72°C) and denaturation (1 min at 94°C) and a final extension phase (10 min at 72°C) and then cooled at 4°C. PCR products were checked for correct length on a 1% Tris–borate-EDTA (TBE) agarose gel (1% agarose, 8.9 mM Tris, 8.9 mM borate, 0.2 mM EDTA), stained with ethidium bromide and visualized under UV illumination [12]. The purified PCR products sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, CA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, CA) [12].

Sequence data were edited with Lasergene Software SeqMan (DNAStar Inc.). Next relatives were determined by comparison to 18S rRNA genes in the National Center for Biotechnology Information NCBI GenBank database using BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov website) [13] to create a matrix using MEGA6 and ClustalW programs [14]. The tree topologies were evaluated by bootstrap analyses based on 1,000 replications with MEGA6 and phylogenetic trees were inferred using the neighbor-joining tree method [15].

## Biosorption experiment

In this study, biosorption ability of fungal species were determining in aerobic batch conditions. Adsorption was measured by dried fungal biomass in which weighed quantity of fungal biomass was placed in nickel solution of constant concentration. The mixture was then kept at room temperature, sample were withdrawn at different time intervals.

## Bioremoval of nickel under different adsorption isotherms

Adsorption isotherms are mathematical models that describe the distribution of the adsorbate species among liquid and adsorbent, based on a set of assumptions that are mainly associated to heterogeneity/homogeneity of adsorbents, the kind of coverage and possibility of interaction between the adsorbate species. Adsorption data usually described by adsorption isotherms, such as Langmuir and Freundlich isotherms. Langmuir equation represented as $qe=Q^o\ bCe/(1+bCe)$, where qe is the amount of biosorbed metal ions at time t (mg/g); Ce is the equilibrium concentration (mg/L); Q (mg/g) and b (L/mg) are the maximum biosorption capacity and energy of adsorption, respectively. Ka=1/Kd=b, ln Ka=ΔG max/RT (R is the gas constant, 8.314 J/mol K). The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless L constant separation factor called the equilibrium parameter, RL, which is used to predict if an adsorption system is "favorable" or "unfavorable by the following relationship $RL=1/(1+bC_0)$, where RL and C are the dimensionless constant separation factor or equilibrium parameter and initial metal ions concentration, respectively. The value of RL indicates the shape of isotherm to be either unfavorable (RL>1) or linear (RL=1) or favorable (0<RL<1) or irreversible (RL=0). The Freundlich expression is an empirical equation based on adsorption on a heterogeneous surface. The equation is commonly presented as: $q_{exp}=nCeKf$, where $q_{exp}$ is the amount of adsorbed metal ions at time t (mg/g), Ce is the equilibrium concentration (mg/L), Kf (mg/g) and n (g/L) are the equilibrium constants indicative of biosorption capacity and biosorption intensity [16].

## Influence of initial $Ni^{2+}$ concentrations

To evaluate the effect of initial $Ni^{2+}$ concentrations on adsorption process of $Ni^{2+}$ by dead fungal biomass, aliquots of 50, 100, 150 and 200 ppm concentration of nickel chloride solution was added to 250 mL Erlenmeyer flasks with a fixed biomass of 100 mg/L. The pH was adjusted to 5 with 0.1 N HCl and 0.1 N NaOH. The samples were mixed well by shaking. For sorption isotherm experiments, flasks were agitated on a rotatory shaker (180 rpm) at room temperature until no additional metal was removed (at different contact time 2, 4, 8 and 24 h). The samples were filtered through 0.45 mµ millipore filters. Triplicate samples were analyzed by A Perkin-2380 atomic absorption spectrophotometry. Samples also were taken from experimental controls which contain no biomass [6].

## Effect of pH at different contact time

A sorption of metal ions by dried mycelial biomass was determined at various pH values of 2.0, 4.0, 6.0, 7.0 and 8.0 to assess the optimum pH for the removal of nickel bioprocess by selected fungi in this study. The samples were shaken at 180 rpm on a rotatory shaker at room temperature. A fixed biomass of 100 mg/L was added to 50 mL of heavy metal solution containing $Ni^{+2}$ at an initial concentration of 200 mg/L for different contact time 1, 2, 4, 8, 12 and 24 h at room temperature. The pH was adjusted using 0.1N HCl and 0.1N NaOH after the solution had been in contact with the adsorbent. Triplicate samples were analyzed.

## Determination of resistance of selected fungi

Metals resistance level was determined in terms of minimum inhibitory concentration (MIC) by spotting inoculation of fungal inoculum ($10^6$ CFU/mL) on heavy metals supplemented agar plates and incubated at 30°C for 10 days. Presence or absence of growth was detected on the spotted area. MIC was defined as the lowest concentration of heavy metal/mL of medium inhibited the visible growth of the tested fungi.

## Factory effluents

This study was undertaken using the wastewaters resulted from painting industries in Egypt and their contents of $Ni^{2+}$ was evaluated

by using flame unit of atomic absorption spectrophotometer A Perkin-2380 atomic absorption spectrophotometry after digesting by microwave using standard protocols as described earlier by Hayat et al. [17] before removing studies by fungal bio-sorbets.

## Results and Discussion

### Evaluation and identification of $Ni^{2+}$ biosorbent isolates

The amount of $Ni^{2+}$ uptake (mg/L) and removal (%) obtained by the dead biomass of 12 fungal isolates were represented in Table 1. The biomass of the fungal isolates under the isolation number AHM21696 followed by MERV21569, AHM21693, AHM21694 and AHM21692 supported the highest uptake (4.75, 4.33, 4.27, 4.18 and 3.11 mg/L, respectively) with removal of $Ni^{2+}$ from aqueous solution equal to 82.2, 73.6, 53.08, 72.41 and 75.9%, respectively (Table 1). Consequently, two isolates under the isolation number AHM21696 and MERV21569 which exhibited the highest uptake and removal of $Ni^{2+}$ as the most cytotoxic heavy metals were selected for further adsorption isotherms studies. According to the international taxonomic keys [7-10], these strains were belonged to genus *Aspergillus*. Shoaib et al. evaluated $Ni^{2+}$ uptake ability of different fungal strains viz., *Aspergillus niger*, *A. terreus*, *A. flavus*, *Rhizopus arrhizus*, *Cunninghamella echinulata*, *Alternaria alternata* and *Trichoderma harzianum* through batch biosorption analyses and the maximum adsorption capability were recorded (7.69, 7.86, 7.5, 9.28, 4.69, 7.37 and 11.77 mg/g, respectively) [4].

| Isolate | $Ni^{2+}$ Uptake and removal | |
|---|---|---|
| | Uptake (mg/L) | Removal (%) |
| MERV21561 | 0.98 | 29.21 |
| MERV21562 | 1.77 | 50.19 |
| MERV21563 | 3.02 | 71.00 |
| MERV21564 | 3.00 | 69.18 |
| MERV21565 | 2.90 | 49.09 |
| MERV21596 | 4.33 | 79.60 |
| AHM21691 | 2.50 | 71.52 |
| AHM21692 | 3.11 | 75.90 |
| AHM21693 | 4.27 | 53.08 |
| AHM21694 | 4.18 | 72.41 |
| AHM21695 | 1.81 | 34.69 |
| AHM21696 | 4.75 | 85.20 |

**Table 1:** Removal and uptake of $Ni^{2+}$ from aqueous solution by dead biomass of fungal isolates.

### Molecular identification of the most active biosorbent isolates through 18S rRNA gene sequencing

The 18S rDNA regions of the most active biosorbent isolates (MERV21569 and AHM21696) were amplified and sequenced as well as matched with those in the NCBI Nucleotide Sequence Database by using BLAST algorithm. A relative analysis by MEGA6 software confirmed that 18S rDNA sequence from the most active biosorbent isolates had an important identity to a numbers of *Aspergillus* sp. The comparison of the most active biosorbent isolates MERV21569 and AHM21696 with sequences of the reference species of fungi within genomic database banks revealed 100% and 99.88% relationship with *Aspergillus sojae* JPDA1 and *Aspergillus terreus* ATE1, respectively. The phylogenetic tree achieved by applying the neighbor joining system is showed in Figures 1 and 2. Based on the analysis of 18S rDNA sequence together with their morphological and biochemical features, the most active biosorbent isolates MERV21569 and AHM21696 strains were identified and designated as *Aspergillus sojae* MERV21569 and *Aspergillus terreus* AHM21696.

### Evaluation of adsorption isotherms experiments

The Langmuir and Freundlich adsorption equations were used

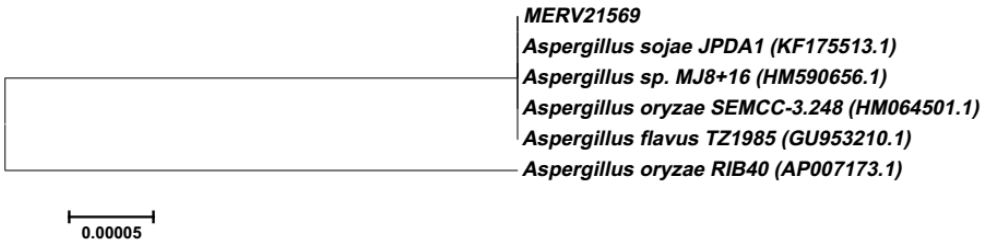


**Figure 1:** Phylogenetic analysis of 18S rDNA sequence constructed by neighbor-joining method for fungal strain MERV21569 and the highest similar fungi. A scale bar indicates the genetic distance in the NJ tree.

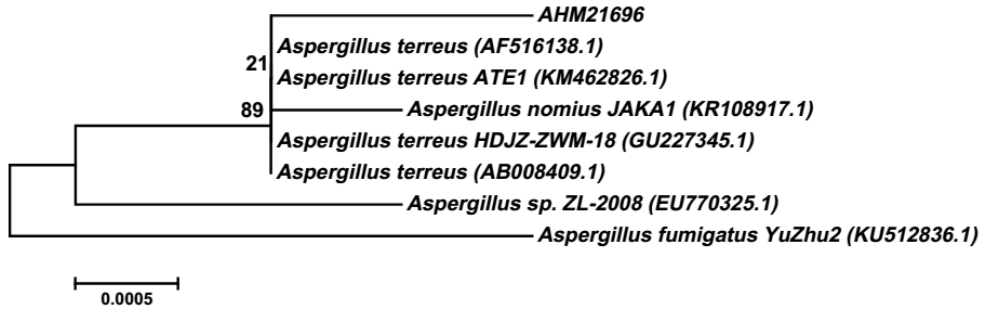


**Figure 2:** Phylogenetic tree of 18S rDNA sequence constructed by neighbor-joining method for fungal strain AHM21696 and the highest similar fungi. A scale bar indicates the genetic distance in the NJ tree.

for descripting the mathematical biosorption balance of $Ni^{2+}$ to dead biomass of *A. sojae* MERV21569 and *A. terreus* AHM21696 (Table 2). In Freundlich isotherm equation $Q_{exp}$, n constant and KF were founded to be (1.99 mg/g, 1.25 g/L and 0.058 mg/g, respectively) for *A. sojae* MERV21569 and (1.149 mg/g, 0.92 g/L and 0.03 mg/g, respectively) in the case of *A. terreus* AHM21696 (Table 2). These data display an appropriate relationship between the investigational amounts and those estimated by the Freundlich equation. Moreover, the maximum Langmuir constant b value for $Ni^{2+}$ (0.851 and 1.932 L/mg) as well as $Q_o$ (0.226 and 0.274 mg/g) for *A. sojae* MERV21569 and *A. terreus* AHM21696, respectively displays a downhill appropriate starting of the isotherm, the great binding consanguinity between the biosorbent and $Ni^{2+}$ that result in more stable adsorption product and the adsorption balance data suited very well to the Langmuir equation. Shahverdia et al. explained the balance sorption of $Ni^{2+}$ by dead fungal biomass of *A. awamori* by nonlinear regression analysis of Freundlich, Langmuir, Temkin and Redlich–Peterson isotherms to minimize the error apportionment structure between experimental balance data and theoretical isotherms [18].

## Effect of different concentrations of $Ni^{2+}$

Data in Table 3, show that when *A. sojae* MERV21569 was applied for removal of $Ni^{2+}$ at 50 and 100 ppm concentrations, they reduced removal by 93.2 % and 98.91% after treatment for 24 h and at 150 ppm it reduced by 98% and 100% after treatment for 8 and 24 h, respectively but at 200 ppm $Ni^{2+}$ concentration was reduced to 2.6 %, 0 % and 0 % after 4, 8 and 24 h of treatments (Table 3). On the other side, *A. terreus* AHM21696 at 50 ppm $Ni^{2+}$ was totally removed after treatment for 24 h and at 100 ppm $Ni^{2+}$ was reduced by 99.65 and 100 after 2 and 4 h of treatments, respectively but 100 % $Ni^{2+}$ removal was observed at $Ni^{2+}$ concentrations of 150 ppm and 200 ppm (Table 4). Greater premier concentration affords improved leading impose to overcome all mass transmit tolerance of metal ions between the aqueous and solid phase, resulting in greater probability of collision between metal ions and sorbents [1,2]. In agreement with our results Shantha and Poonkothai suggested that the effectiveness of $Ni^{2+}$ uptake by Rhizopus species was evaluated after 7 days of fungal species inoculation in graded concentrations (25%, 50%, 75% and 100%) of the $Ni^{2+}$ electroplating effluent [19]. Rhizopus species exhibited a decreasing trend in $Ni^{2+}$ uptake with increased concentration of $Ni^{2+}$ and maximum uptake of 65% of $Ni^{2+}$ was detected in 25% concentration of $Ni^{2+}$ electroplating effluent [19]. Mali et al. used dead biomass of *A. flavus* for the biosorption of zinc and nickel and they found that the biosorption capability for $Ni^{2+}$ was found to be 61.6% at room temperature and pH 5 with biomass concentration of 2 g/L having contact time of 60 min and solution concentration of 2 ppm [11].

| Strain | Freundlich isotherm equation | | | Langmuir isotherm equation | |
|---|---|---|---|---|---|
| | $Q_{exp}$ (mg/g) | n (g/L) | $K_F$ (mg/g) | b*10¹ (L/mg) | $Q_o$ (mg/g) |
| *A. sojae* MERV21569 | 1.991 | 1.25 | 0.058 | 0.851 | 0.226 |
| *A. terreus* AHM21696 | 1.149 | 0.92 | 0.030 | 1.932 | 0.274 |

**Table 2:** The Langmuir and Freundlich isotherm equations constants for biosorption of $Ni^{2+}$ from aqueous solution by selected fungal strains.

| $Ni^{2+}$ concentration (ppm) | Reduction of $Ni^{2+}$ after different contact time (h) | | | |
|---|---|---|---|---|
| | 2 | 4 | 8 | 24 |
| 50 | 72.94 | 78.13 | 86.50 | 93.20 |
| 100 | 78.06 | 88.69 | 92.24 | 98.91 |
| 150 | 85.54 | 91.84 | 98.00 | 100 |
| 200 | 89.21 | 97.40 | 100 | 100 |

**Table 3:** Reduction potentially of $Ni^{2+}$ by *A. sojae* MERV21569 at different concentrations of metal ion.

| $Ni^{2+}$ Concentration (ppm) | Reduction of $Ni^{2+}$ after different contact time (h) | | | |
|---|---|---|---|---|
| | 2 | 4 | 8 | 24 |
| 50 | 96.28 | 98 | 99.31 | 100 |
| 100 | 99.65 | 100 | 100 | 100 |
| 150 | 100 | 100 | 100 | 100 |
| 200 | 100 | 100 | 100 | 100 |

**Table 4:** Reduction potentially of $Ni^{2+}$ by *A. terrus* AHM21696 at different concentrations of metal ion.

## Influence of various pH on $Ni^{2+}$ removal and uptake

Because of pH is a decisive element in adsorption of metal ions that effects electrostatic binding of ions to matching functional groups, the $Ni^{2+}$ removal and uptake by dead biomass of both strains was examined in batch trials at different pH (2 ~ 8) at various contact times 1, 2, 4, 8, 12 and 24 h (Tables 5 and 6). Overall in the case of *A. sojae* MERV21569 after 1 h of treatment maximum reduction of $Ni^{2+}$ from aqueous solution 66.9% was detected at pH 6 compared to 30.22% and 59.18% reduction in $Ni^{2+}$ at pH 4 and 7, respectively. After 2 h of treatment pH 6 supported the maximum removal of $Ni^{2+}$ (89.21%) when compared to pH 4 and 7 (54.19% and 70.11%), respectively (Table 5). Interestingly, after 4, 8, 12 and 24 h of treatments at pH 6.0 corroborative the highest removal of $Ni^{2+}$ by 97.4%, 100%, 100% and 100% compared to 68.46%, 72.0%, 78.14% and 80.26% at pH 4 as well as 80.15%, 80.15%, 87.30% and 92.9% at pH 7.0 (Table 5). Our results are in line with the previous work of Shantha and Poonkothai who reported that maximum removal of $Ni^{2+}$ was observed at pH 6 by Rhizopus species [19].

On the other hand, for *A. terreus* AHM21696 the acidic pH 4 was optimal for the highest removal of $Ni^{2+}$ by 71.98% after 1 h of treatment and 100% reduction after 2, 4, 8, 12 and 24 h of treatments but lower or higher pH than optimal reduced $Ni^{2+}$ removal from aqueous solution (Table 6). Inhibition in bioremoval activities of $Ni^{2+}$ at all contact time applied in this study were noticed at pH 8 (Table 6). These results concur with the results of Shantha and Poonkothai who mentioned that biosorption is analogous to an ion exchange process and thus, pH of solution effects the nature of biomass binding sites and metal solubility and corroborative *Rhizopus* species to absorb the $Ni^{2+}$ over a extensive range of pH but the maximum uptake of $Ni^{2+}$ was found

| Time (h) | Removal of $Ni^{2+}$ (%) at different pH | | | | |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 7 | 8 |
| 1 | 12.63 | 30.22 | 66.90 | 59.18 | 51.90 |
| 2 | 31.50 | 54.19 | 89.21 | 70.11 | 70.00 |
| 4 | 46.91 | 68.46 | 97.40 | 80.15 | 78.23 |
| 8 | 58.06 | 72.00 | 100 | 80.15 | 81.00 |
| 12 | 62.17 | 78.14 | 100 | 87.30 | 90.62 |
| 24 | 78.40 | 80.26 | 100 | 92.90 | 75.34 |

**Table 5:** Reduction potentially of $Ni^{2+}$ by *A. sojae* MERV21569 at different pH.

| Time (h) | Removal of $Ni^{2+}$ (%) at different pH | | | | |
|---|---|---|---|---|---|
| | 2 | 4 | 6 | 7 | 8 |
| 1 | 38.45 | 71.98 | 70.42 | 34.8 | 22.15 |
| 2 | 49.18 | 100 | 100 | 86.72 | 31.90 |
| 4 | 48.91 | 100 | 100 | 79.54 | 39.04 |
| 8 | 37.06 | 100 | 100 | 77.12 | 25.19 |
| 12 | 57.00 | 100 | 83.15 | 66.05 | 17.85 |
| 24 | 49.11 | 100 | 70.80 | 45.23 | 10.91 |

**Table 6:** Reduction potentially of $Ni^{2+}$ by *A. terreus* AHM21696 at different pH.

| Metal ions | MIC (µg/mL) | |
|---|---|---|
| | ***A. sojae* MERV21569** | ***A. terreus* AHM21696** |
| $Cd^{2+}$ | 433 | 248 |
| $Pb^{2+}$ | 250 | 684 |
| $Cu^{2+}$ | 700 | 723 |
| $Hg^{2+}$ | 591 | 407 |
| $Ag^{+}$ | 725 | 692 |
| $Cr^{6+}$ | 803 | 457 |
| $Ni^{2+}$ | 1000 | 805 |
| $Zn^{2+}$ | 380 | 730 |
| $Fe^{3+}$ | 451 | 609 |
| $Al^{3+}$ | 638 | 710 |

**Table 7:** Minimum inhibition concentration (MIC) of different heavy metals ions against the selected fungal strains.

to occur at pH 6 [19]. The decrease in removal of $Ni^{2+}$ above pH 6 is because of the creation of $Ni(OH)_2$. Effect of incubation time on $Ni^{2+}$ removal by both strains under study was in line with those obtained by Shantha and Poonkothai on $Ni^{2+}$ removal by *Rhizopus* species in which the degree of $Ni^{2+}$ uptake from the effluent by *Rhizopus* species exhibited a gradual increase with increasing incubation time to a point [19]. Also in agreement with our results Mali et al. suggested that there is very less biosorption of nickel by *A. flavus* at lower pH such as 2.0 and subsequent increase in pH increase the degree of $Ni^{2+}$ biosorption and reaches an optimum at pH 5 within 50 min and solution concentration of 2 ppm [11].

## Determination of selected fungi resistance against different heavy metals

*Aspergillus sojae* MERV21569 and *A. terreus* AHM21696 displayed heavy metals tolerance with variable level as evident from the minimum inhibition concentration (MIC) values (Table 7). The MICs of $Cd^{2+}$, $pb^{2+}$, $Cu^{2+}$, $Hg^{2+}$, $Ag^{+}$, $Cr^{6+}$, $Ni^{2+}$, $Zn^{2+}$, $Fe^{3+}$ and $Al^{3+}$ were reported to be 433, 250, 700, 591, 725, 803, 1000, 380, 451 and 638 µg/mL against *A. sojae* MERV21569, respectively but toward *A. terreus* AHM21696 the MICs of these metal were equal to 248, 684, 723, 407, 692, 457, 805, 730, 609, 710 µg/mL, respectively. These data supported both strains derived from industrial area as powerful multi-metal resisted fungi. It appears that continuous exposures of these fungal strains grow in industrial region against heavy metals of wastewater might have exerted selection pressure on to fungal population resulting in the improvement of multi-metal tolerance of fungal strains. Furthermore, the study of Joshi was lead to screen out fungal tolerance to $pb^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Cr^{6+}$, $Cu^{2+}$, $Mn^{2+}$ and $Zn^{2+}$ as well as he described that SG1 isolate obtained through screening with maximum threshold level of resistance to $Mn^{2+}$, $Cu^{2+}$, $Co^{2+}$, $pb^{2+}$, $Ni^{2+}$, $Cr^{6+}$ and $Zn^{2+}$ (60, 5, 5, 4.5, 6 and 3.9 mg/mL, respectively) [20].

## Removal of $Ni^{2+}$ from real industrial wastewater

The biosorption activities of $Ni^{2+}$ from industrial wastewater (electroplating industry) was assessed by dead fungal biomass of *A. sojae* MERV21569 and *A. terreus* AHM21696 strains, individually under the enhanced conditions for each strain. The dead biomass of *A. sojae* MERV21569 removed heavy metal ion meaningfully and displayed same activity as was achieved with synthetic solution. The extreme removal of $Ni^{2+}$ (100% for both strains) was achieved within 4 and 2 h contact time by using the dead biomass of *A. sojae* MERV21569 and *A. terreus* AHM21696, respectively. Dias et al., reported that *A. terreus* was effectively applied as a metals biosorbent for the treatment of metallurgical effluents [5]. Maximal metal uptake values of $Ni^{2+}$ were reached in a culture medium containing 100% of effluent stream enhanced with 1% of glucose, after 6 days of incubation [5]. El-Gendy et al., mentioned that under improved conditions minor reduction in heavy metal ions observed in sorption potential from industrial effluents than from synthetic solutions could be because of different impurities existent in electroplating industrial effluents in the form of anions i.e., $SO_4^{2-}$, $NO_3^{-}$ and $Cl^{-}$ that may contend for binding sites on the fungal cell walls and then decrease uptake of metallic ions from industrial wastewater by fungal strain [2].

## Conclusion

The study of $Ni^{2+}$ biosorption by different dead biomass of fungal isolates derived from industrial region showed significant difference of $Ni^{2+}$ removal by two strains belonging to the genus *Aspergillus*. Thus *A. sojae* MERV21569 and *A. terreus* AHM21696 can be used to improve cost effective potential biosorbent for removing $Ni^{2+}$ from industrial effluents. Another very significant aspect of this investigation is the upcoming apply of these two fungal strains in biosorption of other heavy metal ions mostly existent in different industrial effluents.

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