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# Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates

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**Abstract**

Microorganisms play a significant role in bioremediation of heavy metal contaminated soil and wastewater. In this study, heavy metal resistant fungi and bacteria were isolated from the soil samples of an electroplating industry, and the bioaccumulations of Cr(VI) and Ni(II) by these isolates were characterized to evaluate their applicability for heavy metal removal from industrial wastewaters. The optimum pH and temperature conditions for both the growth and heavy metal removal were determined for each isolate. The optimal pH for fungal isolates was lower (5–5.2) than that for bacterial isolates (7). The observed effect(s) of pH was attributable mainly to organism-specific physiology because in all the tested cases the cellular growth positively correlated with heavy metal removal. Batch and tolerance experiments provided information for solid retention time (SRT) design and the lethal tolerance limits for the isolated microorganisms. Experimental results indicated that expanded SRTs (stationary phase) can be recommended while using the fungal and bacterial Cr-resistant isolates for removing chromium. In the case of Ni-resistant bacterial isolate, a non-expanded SRT was recommended for designing continuous-flow completely stirred (CFCS) bioreactor so that a mid-log phase of cellular growth can be kept during the bioaccumulation process. The tolerance data with a high range of heavy metal concentrations revealed the Cr-resistant isolates, especially the fungal one, could tolerate chromium toxicity at up to 10,000 mg $L^{-1}$ chromium. Result indicates the applicability of the isolated *Micrococcus* sp. and *Aspergillus* sp. for the removal of chromium and nickel from industrial wastewater.


*Keywords:* Fungi; Bacteria; Bioaccumulation; Metal bioremediation; pH; Temperature; Tolerance

## 1. Introduction

Chromium and nickel are released into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc., and the concentration levels of chromium and nickel in the environment widely varies. These two metals are of major concern because of their larger usages in developing countries and their nondegradability nature. Hexavalent chromium is highly soluble in water and carcinogenic to human. Ni(II) is more toxic and carcinogenic metal when compared with Ni(IV). Due to their toxic effects on living systems stringent limits have been stipulated for the discharge of chromium and nickel into the environment. According to ISI: Bureau of Indian Standard (BIS) the industrial effluent permissible discharge level of Cr(VI) and Ni(II) into inland water is 0.1 and 3.0 mg $L^{-1}$, respectively.

Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption [1,2] for heavy metal removal from waste streams are not cost effective [3] and hence biological approach has been considered as an alternative remediation for heavy metal contamination. Recently microbial systems like fungus, bacteria and algae have been successfully used as adsorbing agents for removal of heavy metals [4–7]. Microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant [8]. Different species of *Aspergillus*, *Pseudomonas*, *Sporophyticus*, *Bacillus*, *Phanerochaete*, etc., have been reported as efficient chromium and nickel reducers [9,10]. The response of microorganisms towards toxic heavy metals is of importance in view of

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their interest in the reclamation of polluted sites. In the present investigation, the ability of isolated fungal and bacterial strains towards remediation of chromium and nickel was evaluated by characterizing the bioaccumulation of these metals. Effect of temperature, pH, and tolerance to the heavy metals by the isolated organisms were carried out.

## 2. Materials and methods

### 2.1. Sampling

Soil samples were collected from a 30-year-old small scale electroplating industry at Sipcot, Vellore district, Tamil Nadu, India that uses chromium and nickel for metal plating. In the soil sample collected, the chromium and nickel concentration were approximately between 100 and 50 mg $L^{-1}$, respectively. Other reported literature values for contamination in soil are chromium: 4700 mg $kg^{-1}$ and nickel: 5100 mg $kg^{-1}$ [11]. The collected samples were stored at −80 °C before analysis.

### 2.2. Isolation of metal-resistant microorganisms

Chromium and Nickel resistant fungal and bacterial strains were isolated from the soil samples using fungal and bacterial medium [potato dextrose broth (PDB), and nutrient broth (NB)]. Potato dextrose broth and agar were prepared using 250 g of potato boiled in 100 ml of distilled water for 30 min and the filtrate is mixed with 2 g of dextrose and for agar plates 1.5 g of agar was added with this mixture. Nutrient broth and agar plates were prepared using peptic digest of animal tissue (5 $gL^{-1}$), beef extract (3 $gL^{-1}$), NaCl (5 $gL^{-1}$), and 1.5 g agar for 100 ml medium. To isolate metal resistant fungal and bacterial strains these medium were amended with 100 mg $L^{-1}$ Cr(VI) and 50 mg $L^{-1}$ Ni(II) and standard spread plate method was performed. The inoculated plates were incubated at room temperature (30–35 °C) for 48 h. After 48 h incubation larger identical colonies from each plate were isolated. These isolates were characterization and further employed for heavy metal removal and tolerance studies. Morphological, physiological, and biochemical characteristics of the isolated fungal and bacterial species is given in Tables 1 and 2, respectively.

Table 1
Morphological, physiological, and biochemical characteristics of the isolated fungal species

| Morphological/physiological/ biochemical characteristics | Isolated fungal strain |
|---|---|
| Colony diameter | 28 mm |
| Conidial color | Dark brown–black |
| Conidial shape | Globose |
| Vesicle shape | Globose |
| Conidiophore color | Brown |
| Mycelial color | Whitish |
| Colonial reverse | Whitish grey |
| Sterigmata color | Brown |
| No. of sterigmata | Present in two series |

Table 2
Morphological, physiological, and biochemical characteristics of the isolated bacterial species

| Morphological/physiological/ biochemical characteristics | Isolated bacterial strain |
|---|---|
| Gram stain | + |
| Cell shape | Cocci |
| Agar slant cultural characteristics | Soft, smooth, yellow growth |
| Florescence (UV) | − |
| Growth at temperature (°C) | 37 |
| Growth at pH | 5–11 |
| Indole test | − |
| Methyl red test | − |
| VP test | − |
| Citrate utilization | − |
| Starch hydrolysis | − |
| Lipid hydrolysis | − |
| Urease activity | + |
| Catalase activity | + |
| Oxidase activity | − |
| Nitrite reduction | − |
| $H_2S$ production | − |
| Acid production from carbohydrate | − |
| Gelatin liquefaction | + |
| Type strain | *Micrococcus* |

### 2.3. Heavy metal assay and biomass quantification

Chromium(VI) concentrations were determined by 1-5 diphenylcarbazide method [12] using spectrophotometer (Sanyo sp 65 UV–vis) at 540 nm and Ni(II) concentration was estimated using dimethylglyoxime (DMG) [13] at 366 nm. In case of Cr(VI) the applicable concentration limits is 100–1000 μg $L^{-1}$ using diphenylcarbazide method. The reaction is very sensitive with the molar absorptivity based on chromium being about 40,000 L $g^{-1}$ $cm^{-1}$ at 540 nm. The reaction with diphenylcarbazide is nearly specific to chromium. Estimation of Ni(II) using DMG is a very sensitive method and it can estimate as low as 1/1000 mg. The linear regression of the standard graph for the estimation of Cr(VI) and Ni(II) is 0.998 and 0.965, respectively. Fungal and bacterial biomass was quantified using spectrophotometer at 405 and 595 nm, respectively [8]. The initial and the final concentration of heavy metals used in batch mode studies were calculated by estimating the concentration of metals spectrophotometrically. From the difference in concentration the removal efficiencies of the microorganism has been calculated.

### 2.4. Optimization of pH and temperature on heavy metal removal

The fungal and bacterial isolates were inoculated into a series of 250 ml conical flasks containing either 100 mg $L^{-1}$ of chromium or 50 mg $L^{-1}$ of nickel. The pH was varied from 3 to 11 (3, 5, 5.2, 7, 7.5, 9, and 11). The pH of the medium was adjusted using dilute HCl or NaOH. To simultaneously search for optimal temperature, for each pH the represented cultures were incubated at different temperatures

(29, 30, 31, 32, 33, 34, 35, and 36 °C). The cultures were shaken in a rotary shaker (120 rpm) in a temperature controlled water bath. After 24 h incubation, heavy metal removal and biomass were measured. Based upon the heavy metal removal and biomass data, the optimal pH and temperature were determined.

### 2.5. Measurement of the kinetics of broth cellular growth and heavy metal removal

Fungal and bacterial isolates were added into a 250 ml flask containing either 100 mg $L^{-1}$ of Cr(VI) or 50 mg $L^{-1}$ of Ni(II). The flasks were mixed in a rotary shaker (120 rpm) at optimum pH and temperature for 26 h. During the incubation period, heavy metal concentration and biomass were monitored for every two hours interval until heavy metal removal attains a saturation level.

### 2.6. Protein expression assays in heavy metal-resistant microorganisms

Proteomes from the bacterial and fungal isolates were extracted and purified using microbial lyses method. Cell samples were taken from mid-log phases of cellular growth (at optical density of 0.3–0.4) under the conditions of the experiments to measure the kinetics of cellular growth and heavy metal removal. The isolated proteins were quantified by Bradford method and the pattern of proteomic expression was analyzed by 10% SDS-PAGE using Laemmli's method [14].

### 2.7. Heavy metal tolerance assays

To explore the tolerance of the isolates to the heavy metals, optimal culture conditions were used with varying initial heavy metal concentrations. To each freshly prepared growth medium, chromium was amended as Cr(VI) using potassium dichromate salt (Cr(VI) concentrations ranging from 100 to 10,000 mg $L^{-1}$), and Ni(II) using nickel sulphate (Ni(II) concentrations between 50 and 500 mg $L^{-1}$). After 24 h incubation, the biomass was measured. The extent of tolerance was compared and the "normalized" biomass was calculated, i.e., biomass at each heavy metal concentration per biomass using a control. All the experiments were carried out in triplicates.

## 3. Results and discussion

### 3.1. Optimal pH and temperature for heavy metals removal by the isolated species

In the pH range studied (3–11 for 100 mg $L^{-1}$ of chromium and 50 mg $L^{-1}$ of nickel), maximum removal of Cr(VI) (92%) and Ni(II) (90%) were observed around pH 5 in the case of *Aspergillus* sp. (Figs. 1 and 2). *Micrococcus* sp. reported a maximum removal for Cr(VI) (90%) and Ni(II) (55%) at pH 7.0 (Figs. 3 and 4). With increase in pH from two to four almost no bioaccumulation occurred in the case of both the metals for

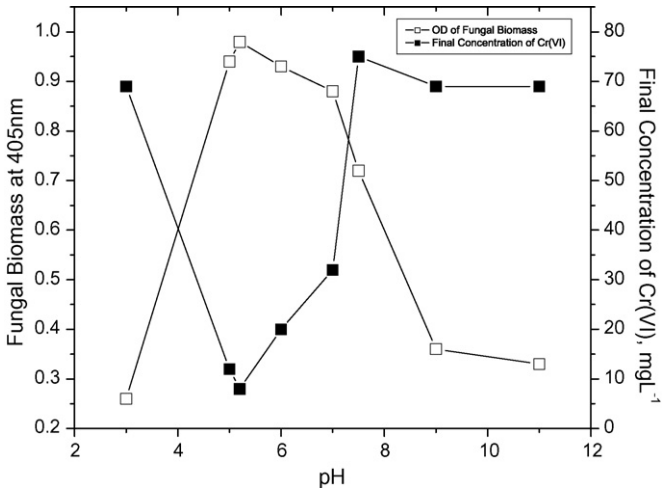


Fig. 1. Cellular growth and chromium removal by *Aspergillus* sp. in response to various pH. Temperature: 35 °C, incubation time: 24 h, concentration of Cr(VI): 100 mg $L^{-1}$.

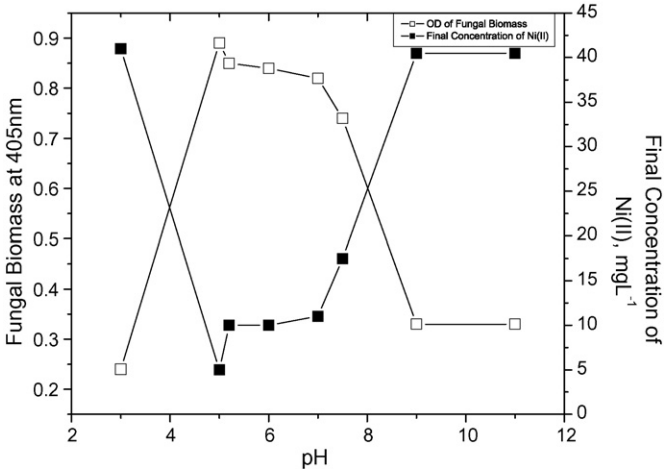


Fig. 2. Cellular growth and Ni(II) removal by *Aspergillus* sp. in response to various pH. Temperature: 35 °C, incubation time: 24 h, concentration of Ni(II): 50 mg $L^{-1}$.

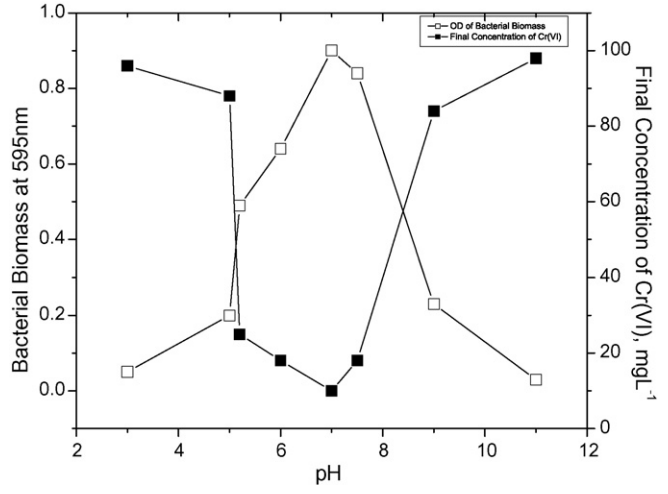


Fig. 3. Cellular growth and Cr(VI) removal by *Micrococcus* sp. in response to various pH. Temperature: 35 °C, incubation time: 24 h, concentration of Cr(VI): 100 mg $L^{-1}$.

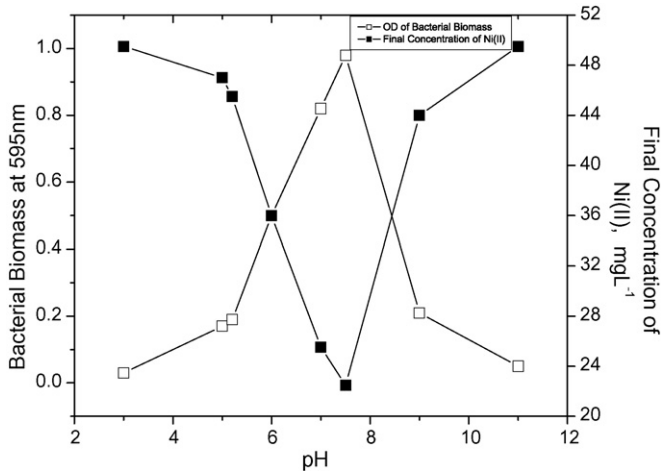


Fig. 4. Cellular growth and Ni(II) removal by *Micrococcus* sp. in response to various pH. Temperature: 35 °C, incubation time: 24 h, concentration of Ni(II): 50 mg $L^{-1}$.

the isolated *Aspergillus* and *Micrococcus* sp. Above pH 5, the percent removal for both the metals increased rapidly for both the isolated species. The low bioaccumulation capacity at pH values below five is attributed to the competition of hydrogen ion with metal ion on the sorption site. Thus, at lower pH, due to the protonation of binding site resulting from high concentration of proton, negative charge intensity on the site is reduced which results in the reduction or inhibition for the binding of metal ion. Most of the microbial surfaces are negatively charged due to the ionization of functional group, thereby contributing to metal binding. Fungal surfaces have a negative charge on pH range two to six. At low pH, some of the functional groups will be positive charged and may not interact with metal ions [9]. At acidic pH, the predominant species of Cr(VI) are $Cr_2O_7^{2-}$, $HCrO^-_4$, and $Cr_2O_4^{2-}$ and the surface of the sorbent becomes protonated and attracts anionic species of Cr(VI) [15]. Similar result is reported [9] for the removal of lead using *Penicillium digitatum* and *Rhizopus nigricans*. Low removal of nickel at lower pH range by *Pencillium chrysogenum* has also been reported [16]. Removal of copper and lead by *Micrococcus luteus* [17] also showed the same trend. The increase in percent removal of metal with increase in pH from two to five is due to the strong relations of bioaccumulation to the number of surface negative charge, which depends on the dissociation of functional group [18]. As the pH is increased above the zeta potential of the adsorbent, there is a reduction in the electrostatic attraction between the Cr(VI) species and the sorbent surface, with a consequent decrease in percentage bioaccumulation. The rate of chromium uptake and the extent were enhanced as the pH increases up to certain pH range. Prasenjit and Sumathi reported similar results using *A. foetidus* and *A. carbonarius* [8]. At low pH negligible removal of chromium may be due to the competition between hydrogen and metal ions. With further increase in pH, there is increase in metal removal, which may be due to the ionization of functional groups and an increase in the negative charge density on the cell surface. At higher alkaline pH values (8 and above), a reduction in the solubility of metals contributes to lower uptake rates. Nasseri et al. [19] reported the removal of chromium using *A. oryzae*, where maximum removal was observed at pH 5, which is suitable for the living cells of fungi and bacteria, and were able to grow significantly. With further increase in pH, the percent removal of metal was decreased. With increase in pH beyond five, the chromium removal rate decreased, which might be due to the osmotic changes and hydrolyzing effect. Similar results were also obtained in the case of nickel, where maximum removal is reported at pH 5.2. Hasan et al. [20] reported maximum removal of nickel in the pH range of 4.5–5.5. The variation of adsorption of nickel at various pH is on the basis of metal chemistry in solution and the surface chemistry of the sorbent. The pHmax where maximum removal occurs is related to the pka or the first hydrolysis product of the metal. The decrease in removal of Ni(II) above pH 5 is due to the formation of $Ni(OH)_2$. 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 to the organism. Similar reports has been reported for Ni(II) 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 [$H_3O^+$] and restricted the approach of metal cations as a result of the repulsive forces.

The range of optimal temperature values (30–35 °C) were comparable to the range of room temperature that was used when isolating the microorganisms (Section 2.2), suggesting that the selection of these isolates might have been influenced not only with the heavy metal(s) but also with the temperature used in the isolation procedure. The temperature of the adsorption medium could be important for energy dependent mechanisms in metal removal by microorganisms. Temperature is known to affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. These factors may simultaneously affect the binding sites on isolated fungal and bacterial species causing reduction in heavy metal removal. Energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature [21]. Bioaccumulation of chromium and nickel by bacterial and fungal species appears to be temperature dependent. Maximum removal of Cr(VI) and Ni(II) was observed at 35 and 30 °C for the isolated *Aspergillus* (Figs. 5 and 6) and *Micrococcus* sp. (Figs. 7 and 8). Similar results have been reported in the bioaccumulation of Cr(VI) by *S. equisimilis* and *A. niger* [22]. Although these data could not explain why the optimal pH for the fungal isolates was lower than that for the bacterial isolates, the positive correlation between heavy metal removal and cellular growth – consistently observed regardless heavy metals, pH and temperature – strongly suggests that the observed effect of pH on the heavy metal removal was attributed mainly to biological factor(s), in particular, organism-specific physiological one(s).

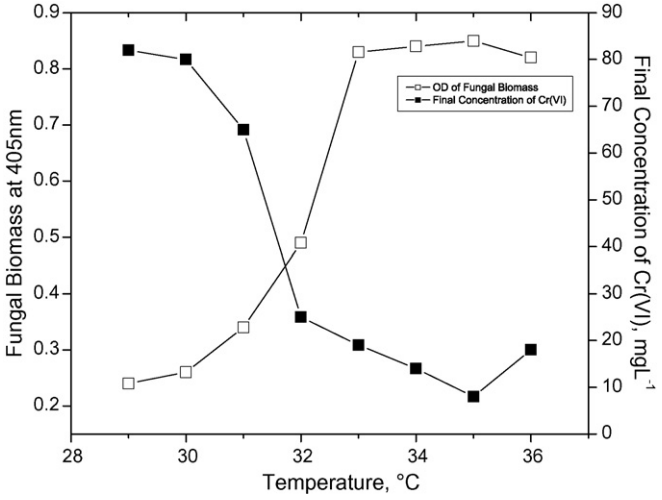


Fig. 5. Cellular growth and Cr(VI) by *Aspergillus* sp. in response to various to temperature. Concentration of Cr(VI): 100 mg $L^{-1}$, pH 5, incubation time: 24 h.

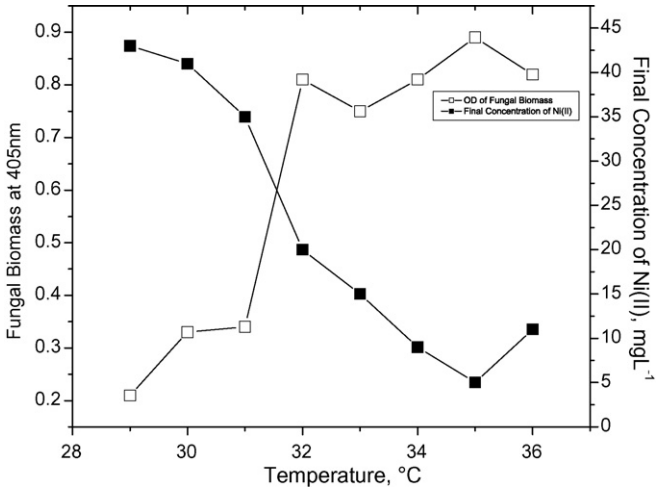


Fig. 6. Cellular growth and Ni(II) by *Aspergillus* sp. in response to various to temperature. Concentration of Ni(II): 50 mg $L^{-1}$, pH 5, incubation time: 24 h.

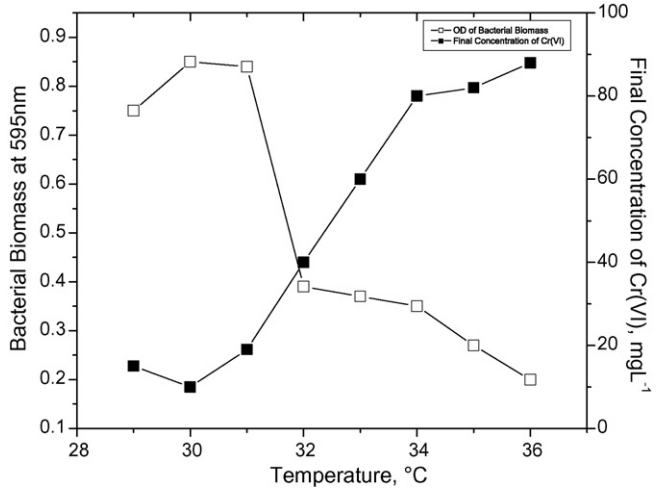


Fig. 7. Cellular growth and Cr(VI) by *Micrococcus* sp. in response to various to temperature. Concentration of Cr(VI): 100 mg $L^{-1}$, pH 7, incubation time: 24 h.

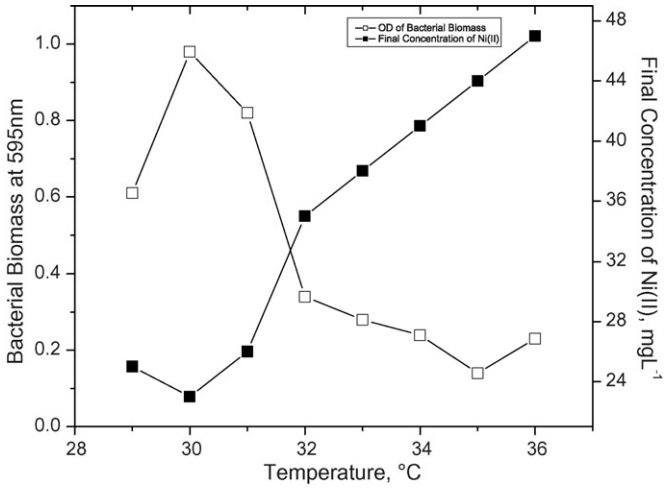


Fig. 8. Cellular growth and Ni(II) by *Micrococcus* sp. in response to various to temperature. Concentration of Ni(II): 50 mg $L^{-1}$, pH 7, incubation time: 24 h.

### 3.2. Kinetics of heavy metal removal and cellular growth

The time-course data for heavy metal removal and cellular growth were observed for each isolate under its optimal pH and temperature conditions. When these isolates are applied in removing heavy metal from industrial wastewater, information regarding the effect of growth phase will be important in designing solid (sludge) retention time (SRT) for continuous-flow completely stirred (CFCS) bioreactor, which is a general reactor type for wastewater treatment plants. In the fungal isolates, specific metal bioaccumulation (accumulative biosorption [removal] of each heavy metal per accumulative biomass) increased when cells were in stationary phases (Figs. 9 and 10) for *Aspergillus* species This trend was also observed when using the Cr-resistant bacterial isolate in removing Cr(VI) (Fig. 11). However, the Ni-resistant bacterial isolate exhibited reduced bioaccumulation when cells were in stationary phase (Fig. 12). Therefore, expanded SRTs (stationary phase) may be recommended using the fungal isolates in removing chromium and

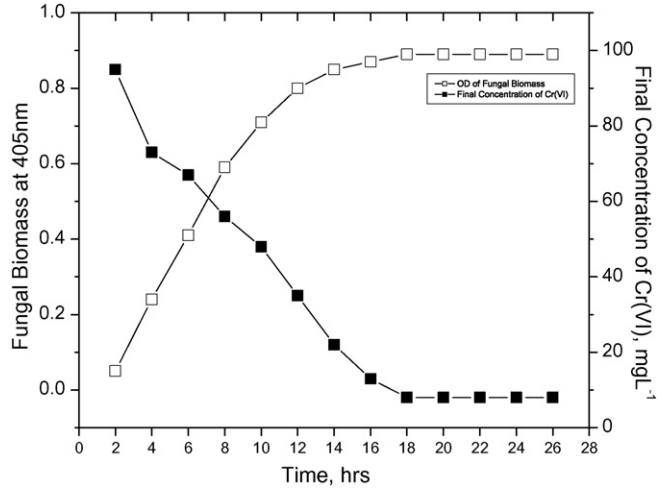


Fig. 9. Kinetics of cellular growth and Cr(VI) removal by *Aspergillus* sp. Concentration of Cr(VI): 100 mg $L^{-1}$, pH 5, temperature: 35 °C.

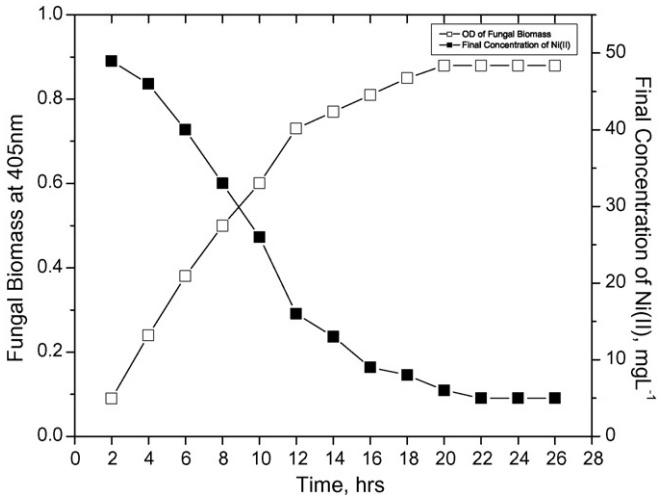


Fig. 10. Kinetics of cellular growth and Ni(II) removal by *Aspergillus* sp. Concentration of Ni(II): 50 mg $L^{-1}$, pH 5, temperature: 35 °C.

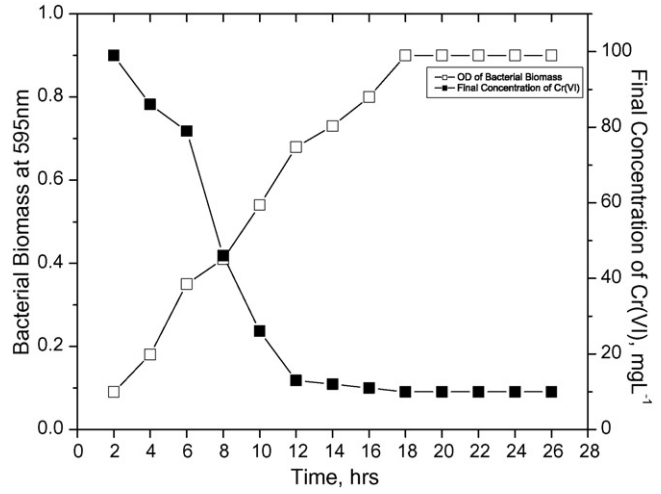


Fig. 11. Kinetics of cellular growth and Cr(VI) removal by *Micrococcus* sp. Concentration of Cr(VI): 100 mg $L^{-1}$, pH 7, temperature: 35 °C.

nickel from industrial wastewater as well as using the Cr-resistant and using the Ni-resistant bacterial isolate in removing nickel, however, a non-expanded SRT has to be designed for CFCS bioreactor so that a mid-log phase of cellular growth could be kept in the treatment system. The growth rate during the lag phase was very low because the isolated bacterial and fungal

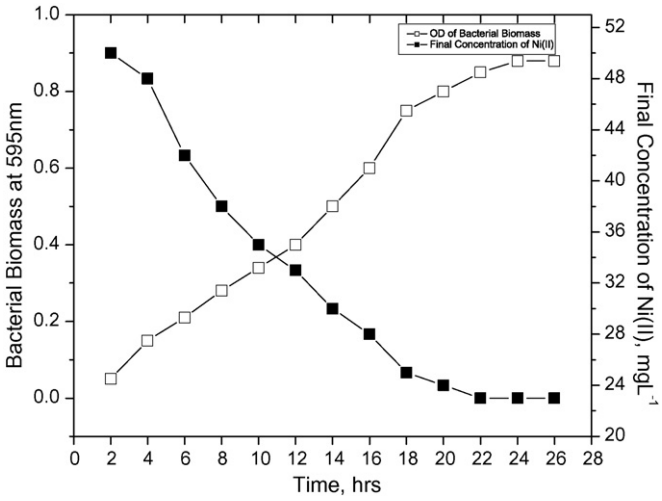


Fig. 12. Kinetics of cellular growth and Ni(II) removal by *Micrococcus* sp. Concentration of Ni(II): 50 mg $L^{-1}$, pH 7, temperature: 35 °C.

isolates was adapting with the environment. After this stage, the isolates grew in logarithmic form using the nutrients. In the third stage, the number of living and dead cells is fixed [19]. Similar studies have been reported by *Enterobacter cloacae* [23], *Bacillus circulans* [24]. Table 3 indicates that *Micrococcus* and *Asperigillus* sp. are more effective for the removal of Cr(VI) and Ni(II) when compared with other microbial biomass reported.

### 3.3. Proteomic expression assays in heavy metal-resistant microorganisms

Ten percent SDS-PAGE analysis showed the expression of proteins from the *Aspergillus* and *Micrococcus* species towards the exposure of chromium 100 mg $L^{-1}$ and nickel 50 mg $L^{-1}$ (Fig. 13). The cell samples were taken from the mid-log growth phase of the kinetics experiments, that is, the time required for cells to adapt heavy metal toxicity. When compared with controls (without heavy metals), both of the *Aspergillus* sp. expressed considerable amount of polypeptide (protein) on 93 kDa regardless the test heavy metals. This indicates the speculation that 93 kDa protein is involved in response to heavy metals and probably pervasively exists in heavy metal-resistant fungi, which remains to be further examined. Unlike the *Aspergillus* sp. the commonly expressed protein was not observed in the heavy metal accumulated *Micrococcus* species. In case of the Cr-

Table 3

| Metal | Microorganism/sorbent | Initial concentration (mg $L^{-1}$) | pH | % Removal | Time (h) | Reference |
|---|---|---|---|---|---|---|
| Cr(VI) | *Aspergillus foetidus* | 5 | 7 | 97 | 92 | [8] |
| Cr(VI) | Distillery sludge | 10 | 2–3 | 93 | 2 | [15] |
| Cr(III) | *Aspergillus oryzae* | 240 | 5 | 97 | 36 | [19] |
| Ni(II) | Malaysian rubber–wood ash | 20 | 5 | 65 | 4 | [20] |
| Cr(VI) | *Rhizopus nigricans* | 100 | 2 | 80 | 4 | [28] |
| Ni(II) | *Lemna minor* | 5 | 7–9 | 87 | 5 | [29] |
| Cr(VI) | *Micrococcus* species | 100 | 7 | 90 | 18 | Present study |
| Cr(VI) | *Aspergillus* species | 100 | 5 | 92 | 18 | Present study |
| Ni(II) | *Micrococcus* species | 50 | 7 | 55 | 20 | Present study |
| Ni(II) | *Aspergillus* species | 50 | 5 | 90 | 20 | Present study |

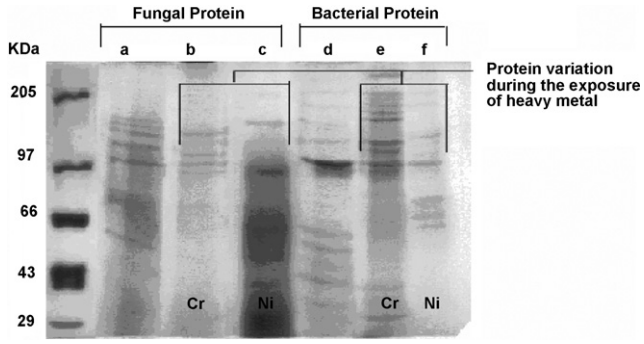


Fig. 13. SDS-PAGE protein expression patterns in heavy metal-resistant isolates from mid-log growth phase in the presence of chromium or nickel: (a) Protein isolated from fungi without heavy metal exposure; (b) Protein isolated from fungus that grows with Cr as supplement; (c) Protein isolated from fungus that grows with Ni as supplement; (d) Protein isolated from bacteria without heavy metal exposure; (e) Protein isolated from bacteria that grows with Cr as supplement; and (f) Protein isolated from bacteria that grows with Ni as supplement.

resistant *Micrococcus* species in the presence of chromium the organism express more proteins and a significant differential expression of some polypeptide was observed on 180 kDa. The Ni-resistant bacterial isolate with nickel expressed some proteins below 60 kDa. The variation in proteome expression patterns among the tested cases for the bacterial isolates was greater than that for the fungal isolates. This was probably attributed to a higher degree of functional diversity among bacteria.

### *3.4. Tolerance in response to widely varying heavy metal concentrations*

Initial metal ion concentration plays a role in determining the bioaccumulative capacity of the isolated *Aspergillus* and *Micrococcus* species. As the heavy metal concentrations increased, the cellular growth of all the isolates was inhibited (Fig. 14). Ni-resistant *Aspergillus* and *Micrococcus* species were sensitive to nickel exposure, while the Cr-resistant isolates were tolerant against chromium exposure. Among the tested isolates, the Cr-resistant *Aspergillus* sp. exhibited greater tolerance when treated with chromium. Cr-resistant *Aspergillus* sp. survived to a maximum level of 10,000 mg $L^{-1}$ of Cr(VI) (Fig. 15). Chromium-resistant *Micrococcus* sp. also survived to a concentration 8000 mg $L^{-1}$ of Cr(VI). These data suggest that the use of the Cr-resistant microorganisms is feasible for removing chromium of a wide range of chromium concentrations. It has been observed that an increase in the initial metal concentration results in an increase in the metal removal capacity of the biosorbent, which culminates in a plateau at very high metal concentration. The metal removal capacity of an organism touches its peak at these high metal concentrations. At low metal concentrations (as encountered in effluent samples) the biosorption capacity of the biosorbent is not fully utilized [25]. Similar results were obtained in the case of *Trametes versicolor* for $Cu^{2+}$, $Pb^{2+}$, and $Zn^{2+}$ removal [21], *Bacillus* for chromium removal [26], *Funalia trogii* for $Hg^{2+}$, $Cd^{2+}$, and $Zn^{2+}$ [15], *Bacillus firmus* for Pb, Cu, and Zn [27]. All these date clearly reveals the existence of a finite heavy metal reduction capacity possibly due to heavy metal toxicity toward cells.

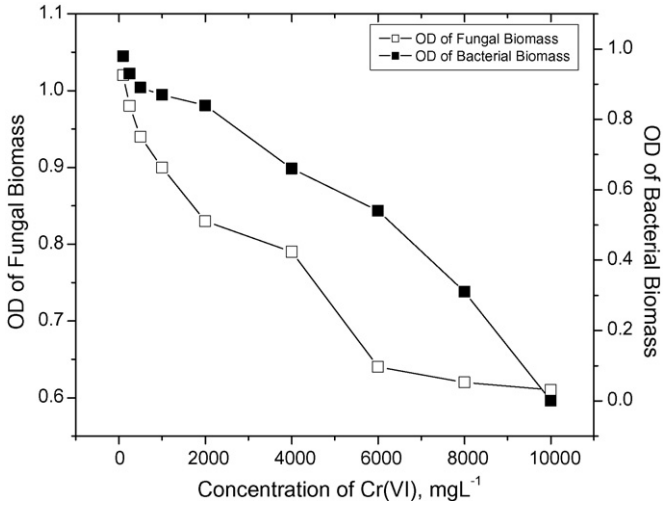


Fig. 14. Normalized biomass (measured at 24 h incubation time) in response to varying initial concentrations of Cr(VI). Cr(VI) concentration range: 100–1000 mg $L^{-1}$, temperature: 35 °C.

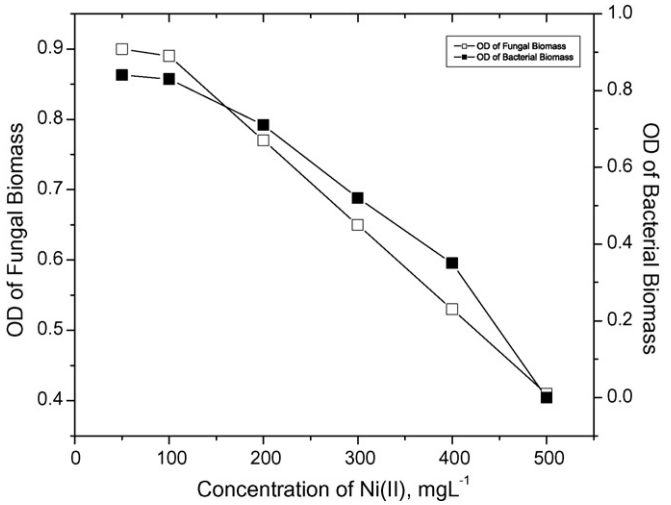


Fig. 15. Normalized biomass (measured at 24 h incubation time) in response to varying initial concentrations of Ni(II). Ni(II) concentration range: 50–500 mg $L^{-1}$, temperature: 35 °C.

## 4. Conclusion

In this study, Chromium- and Nickel-resistant microorganisms were isolated from heavy metal contaminated environments, and the applicability of their heavy metal removal from industrial wastewater was evaluated at a laboratory scale. The optimum conditions for both the growth and heavy metal removal were determined for each isolate. The optimal pH for fungal isolates was lower (5–5.2) than that for bacterial isolates. The observed effect(s) of pH on bioaccumulation was attributable mainly to organism-specific physiology, as indicated by the observed positive correlation between biomass and heavy metal removal. Furthermore, the kinetic and tolerance experiments provided information for SRT design and the lethal tolerance limits, which are important in designing CSCM bioreactors

for removing heavy metals of high concentrations. According to the kinetic data, expanded SRTs (stationary phase) was recommended while using the fungal isolates as well as when using the Cr-resistant bacterial isolate in the removal of chromium. When using the Ni-resistant bacterial isolate in removing Ni, however, a non-expanded SRT was recommended for designing CFCS bioreactor so that a mid-log phase of cellular growth could be kept in the treatment system. The tolerance data with the extremely high range of heavy metal concentrations revealed the Cr-resistant isolates especially the *Aspergillus* sp. can tolerate chromium toxicity up to 10,000 mg $L^{-1}$ chromium and maintaining 60% survival. The study demonstrated that the newly isolated *Micrococcus* sp. and *Aspergillus* sp. strains have potential application for the removal of chromium and nickel from industrial wastewaters. Further desorption studies can be carried out as the final approach for the management of heavy metal laden biomass as an environmental friendly method of disposal.

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