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Biosorption of cobalt by fungi from serpentine soil of Andaman

Arundhati Pal, Suchhanda Ghosh, A.K. Paul *

Microbiology Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata 700 019, India

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Abstract

Fungi belonging to *Aspergillus*, *Mortierella*, *Paecilomyces*, *Penicillium*, *Pythium*, *Rhizopus* and *Trichoderma*, isolated from serpentine soil of Andaman (India) were screened for cobalt-resistance. Eleven out of total 38 isolated fungi which tolerated >6.0 mM Co(II) were evaluated for cobalt biosorption using dried mycelial biomass. Maximum Co(II)-loading (1036.5 μM/g, 60 min) was achieved with *Mortierella* SPS 403 biomass, which removed almost 50% of 4.0 mM cobalt from the aqueous solution. Co(II)-sorption kinetics of *Mortierella* SPS 403 biomass was fast and appreciable quantities of metal [562.5 μM/g] was adsorbed during first 10 min of incubation. The metal biosorption capacity of the isolate was accelerated with increasing cobalt concentration, while it was reverse with increase of initial biomass. The optimum pH and temperature for Co(II) removal were 7.0 and 30 °C, respectively. However, Co(II)-uptake was inhibited in presence of other metals (Pb, Cd, Cu, Ni, Cr and Zn). Freundlich adsorption isotherm appropriately describes *Mortierella* SPS 403 biomass as an efficient Co(II)-biosorbent.

Keywords: Serpentine soil; Fungi; Cobalt-resistance; Cobalt biosorption; *Mortierella*; Bioremediation

1. Introduction

Industrial exploitation of cobalt in alloy production, electroplating, generation of gas turbines and petrochemical industries (Kuyucak and Volesky, 1989), results in discharge of cobalt wastes into the environment leading to variety of toxic effects on all living forms including plants, animals and microorganisms (Moore, 1994). It causes neurotoxicological disorders and genotoxicity in human beings and in chronic cases may cause cancer (Lison et al., 2001). Treatment of effluents and ground water polluted with heavy metals in general and cobalt in particular following biotechnological approaches is simple, relatively inexpensive and provides full-scale remediation over existing physicochemical technologies, most of which are either ineffective or uses costly chemicals (Ginisty et al., 1998).

Microorganisms by virtue of their wide degree of metabolic adaptability have shown resistance to several heavy metals including cobalt and many of them have the potential to sequester metal ions from aqueous solution (Gadd, 1990; Kapoor et al., 1999). The microbe-based technologies for removal of heavy metals from effluents and wastewaters may provide an alternative means of metal recovery for economical reasons and environmental protection. Both living as well as non-living microbial biomass can act as effective metal accumulator, but use of dead biomass is preferred since it is easy to handle, processes are growth independent and possess no harm while using pathogenic strains (Gadd, 1990). This phenomenon of passive uptake or entrapment of metal ions in cellular structure and subsequent binding of cations on to active sites using biomass is termed as 'Biosorption'.

Several studies have described the use of algae, fungi, bacteria and seaweeds as metal biosorbents (Viera and

* Corresponding author. Tel.: +91 33 2475 3681/2344 0509; fax: +91 33 2474 1042/2476 4419.
E-mail address: paulak@vsnl.com (A.K. Paul).

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Volesky, 2000), and the fungal biomass has been exploited as good sorbents of heavy metals (Gadd, 1990). However, reports on microbial cobalt biosorption are relatively few and in depth studies have only been made using *Rhizopus* (Suhasini et al., 1999), *Oscillatoria auguistissima* (Ahuja et al., 1999), *Pseudomonas halodenitrificans* (Ginisty et al., 1998) and *Ascophyllum nodosum* (Kuyucak and Volesky, 1989) biomass. The use of fungal biomass over other biosorbents could be economical for removal of metal ions because fungi can be grown in substantial amount without the use of sophisticated equipments and expensive chemicals. Moreover, continuous supply of fungal biomass as a byproduct is available from a variety of industrial fermentation processes (Kapoor et al., 1999). Search for newer biosorbents having specific priority for binding cobalt ions, therefore, remains a challenge for microbial biotechnologists.

Serpentine soils are metalliferrous ecosystem, which develop over rocks enriched with ferromagnesium minerals of nickel, cobalt and chromium. The occurrence and abundance of metal-resistant microflora in serpentine soil of New Caledonia (Amir and Pineau, 1998), Italy (Mengoni et al., 2001) and India (Pal et al., 2003, 2004) is well documented. But these unique microbial resources from naturally metal-percolated ecosystem have not been explored and exploited in bioremediation of heavy metal pollutants. In the present work, we report the isolation and enumeration of cobalt-resistance in serpentine mycoflora of Andaman, India and evaluation of their cobalt biosorption potential.

2. Methods

2.1. Isolation of cobalt-resistant fungi

Cobalt-resistant fungi were isolated from serpentine soil samples of Saddle hills, Chidyatapu and Rutland of Andaman Islands, India. The soils showed a cobalt content ranging from 255.2 to 433.4 mg/kg dry soil, while extractable Co varied from 45.0 to 245.0 mg (in 0.05 N EDTA) and 4.2 to 15.6 mg (in 1 N glacial acetic acid)/kg dry soil. Soil samples were serially diluted and plated on Czapek Dox agar plates amended with 1.0 mM Co(II) (as $CoCl_2$, $6H_2O$). Cobalt-resistant fungi were enumerated after 4–6 days of incubation at 28 °C and the pure cultures were maintained on slopes of Czapek Dox agar by subculturing at regular intervals. Micromorphological characteristics of the isolates were determined and their generic identities were confirmed following the Manual of Soil Fungi by Gilman (1957) and Manual of Penicillia (Raper and Thom, 1984).

2.2. Evaluation of cobalt-resistance

Minimum inhibitory concentration (MIC) of Co(II)-resistant fungi isolated from serpentine soil was determined following broth dilution method as described earlier (Pal et al., 2004). The fungi were allowed to grow at 28 °C in 20 ml Czapek Dox medium/100 ml flask containing 1.0–6.0 mM Co(II) and the minimum concentration of metal in the medium inhibiting complete growth was taken as the minimal inhibitory concentration (MIC).

2.3. Preparation of fungal biomass

Biomass of selected cobalt-resistant fungal strains was produced in Czapek Dox medium using shake flask method. The medium (50 ml/250 ml Erlenmeyer flask) was inoculated with 0.5 ml homogeneous spore suspension prepared in sterile Tween 80 (0.1% w/v) solution from 4 to 6 days old slant cultures. The flasks were agitated on a rotary shaker (120 rpm) for 5 days at 28 °C and the biomass was harvested by centrifugation at 6000*g* for 10 min using Remi R24 centrifuge and washed thoroughly with distilled water. The washed biomass was dried at 80 °C for 18–24 h, powdered, sieved (0.1 mm mesh) and stored in desiccators at room temperature until used for biosorption studies.

2.4. Biosorption studies

Cobalt biosorption was studied in 100 ml Erlenmeyer flask containing 20 ml metal solution in double distilled water following the procedure of Sag and Kutsal (1996) with minor modifications. Experiments were conducted using an initial Co(II) concentration (as $CoCl_2$, $6H_2O$) of 4.0 mM and a biomass level of 2 g/l. The flasks were agitated in a rotary shaker (120 rpm) at 30 °C for 120 min. Samples (1 ml) were withdrawn at regular interval, centrifuged at 10,000*g* for 10 min and the supernatant was filtered through Whatman (No. 42) filter paper. Residual Co(II) content of the supernatant was determined in a Varian Atomic Absorption Spectrometer (SpectrAA-20Plus) at 240.7 nm using air–acetylene flame. Calibration curve for cobalt was prepared using Atomic Absorption Standard solution (Sigma-Aldrich).

All experiments were performed in triplicates and results represent mean ± standard error.

3. Results

3.1. Evaluation of cobalt-resistance

In search of new biosorbents for cobalt, a total of 38 fungi were isolated from serpentine soil of Andaman,

Table 1
Minimum inhibitory concentration of cobalt-resistant fungi isolated from serpentine soil of Andaman

Fungi	Total isolate (s)	Number of isolate		
		MIC range (mM)		
		1.0–3.0	3.1–6.0	>6.0
Aspergillus	9	2	6	1
Mortierella	6	1	3	2
Paecilomyces	3	0	1	2
Penicillium	14	5	5	4
Pythium	2	0	1	1
Rhizopus	1	0	1	0
Trichoderma	3	1	1	1
Total	38	9	18	11

MIC of fungi was determined by broth dilution method in Czapek Dox medium.

India following dilution and plating on Czapek Dox agar amended with 1.0 mM Co(II). The fungal isolates belonged to the common genera, *Aspergillus* (9), *Mortierella* (6), *Paecilomyces* (3), *Penicillium* (14), *Pythium* (2), *Rhizopus* (1) and *Trichoderma* (3). Evaluation of minimum inhibitory concentration of cobalt shows that majority (about 47%) of the fungi tested had MIC values ranging between 3.1 and 6.0 mM Co(II). However, 11 (29%) of them were able to grow in presence of >6.0 mM Co(II) and were selected as potent cobalt-resistant strains (Table 1).

3.2. Screening for cobalt biosorption

The selected potent fungal isolates (11) were screened for biosorption of cobalt from aqueous solution using dried mycelial mass. It was revealed that biomass preparations of all fungal strains bind Co(II) ions from solution but at different degrees (Table 2). The sorption of Co(II) ions was rapid during the initial 15 min of incubation but continued up to 60 min irrespective of the fungal genera. In the early phase of biosorption (15 min), strains of *Penicillium* (SPS 106 and CTS 402), *Aspergillus* (SPS 202), *Mortierella* (SPS 403) and to some extent *Paecilomyces* (SPS 107) appeared to be more or less equally effective in the sorption of Co(II) ions from aqueous solution. At prolonged incubation (60 min), maximum Co(II) biosorption was achieved with *Mortierella* SPS 403 biomass which removed almost 50% of 4.0 mM cobalt from the aqueous solution and showed a metal loading capacity of 1036.5 μM Co(II)/g of biomass. *Mortierella* SPS 403 was, therefore, selected as the best sorbent for cobalt ions and optimal conditions for cobalt uptake were evaluated with this strain only.

Table 2
Screening of cobalt-resistant fungi for biosorption of Co(II)

Fungi	Co(II) biosorption, μM Co(II)/g biomass	
	Incubation (min)	
	15	60
Aspergillus sp. SPS 202	732.9 ± 13.7	902.1 ± 14.6
Mortierella sp. AND 604	49.7 ± 1.1	72.0 ± 2.3
Mortierella sp. SPS 403	747.4 ± 5.0	1036.5 ± 39.6
Paecilomyces sp. SPS 107	713.9 ± 15.0	761.5 ± 13.0
Paecilomyces sp. SPS 101	660.7 ± 7.5	734.1 ± 11.1
Penicillium sp. AND 104	651.2 ± 10.4	882.4 ± 16.4
Penicillium sp. SPS 102	648.7 ± 20.6	761.8 ± 25.2
Penicillium sp. SPS 106	776.1 ± 16.0	813.2 ± 16.0
Penicillium sp. CTS 402	738.8 ± 16.4	775.3 ± 1.1
Pythium sp. CTS 703	533.1 ± 12.6	670.6 ± 10.7
Trichoderma sp. SPS 404	370.4 ± 22.1	660.4 ± 15.0

Initial Co(II) concentration for biosorption was 4.0 mM.
Results represent mean of triplicate experiments ± standard error.

3.3. Biosorption kinetics

The dried mycelial mass of *Mortierella* SPS 403 showed fast kinetics of cobalt binding and adsorbed appreciable quantities of metal [562.5 μM Co(II)/g] within the first 10 min of incubation (Fig. 1A). However, the metal loading capacity of the biomass (μM/g) increased steadily with time and equilibrium was achieved after 60 min. Subsequent sorption experiments were conducted till 60 min of incubation.

3.4. Effect of cobalt concentration

The biosorption of Co(II) by *Mortierella* SPS 403 increased with increase of initial cobalt concentration in the sorption medium and reached a saturation value at 4.0 mM Co(II). Increase in cobalt concentration from 0.5 to 4.0 mM, significantly increased the metal loading capacity from 253.5 to 1036.8 μM Co(II)/g biomass, although, there was no noticeable increment in metal loading with further increase in Co(II) concentration to 5.0 mM (Fig. 1B).

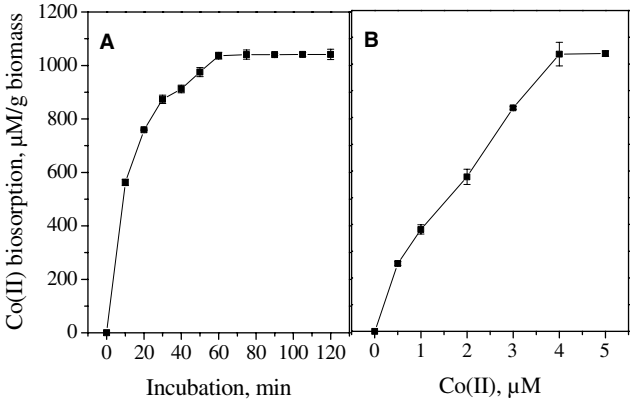

Fig. 1. Cobalt biosorption by *Mortierella* SPS 403 biomass as function of time (A) and Co(II) concentration (B). [A: the sorption medium (20 ml/100 ml flask) contained 4.0 mM Co(II) and 2.0 g/l biomass; incubation: 30 °C at 120 rpm. B: cobalt sorption was measured after 60 min incubation at 30 °C using 2.0 g/l biomass.]

3.5. Biosorption isotherms

Cobalt biosorption equilibrium was quantified with the help of standard adsorption isotherms such as Langmuir and Freundlich isotherms. The Langmuir model has the form:

$$Q_e = (a) \cdot (b) \cdot C_e / 1 + (b) \cdot C_e$$

and the Freundlich model has the form:

$$Q_e = K(C_e)^{1/n}$$

where, Q_e is the amount of metal ion biosorbed at equilibrium per unit weight of biomass; C_e is the metal ion concentration at equilibrium; a and b are the Langmuir model constants and K and n are the Freundlich model constants.

The linearised Langmuir and Freundlich adsorption isotherms for cobalt using *Mortierella* SPS 403 biomass are shown in Fig. 2A and B respectively. The adsorption constants were calculated from the respective isotherms along with correlation coefficients (Table 3). The constants for Langmuir model were found to be: '*a*' and '*b*' as 946.56 and 0.00096 respectively, while those for Freundlich isotherms were '*K*' as 3.516 and '*n*' as 1.4.

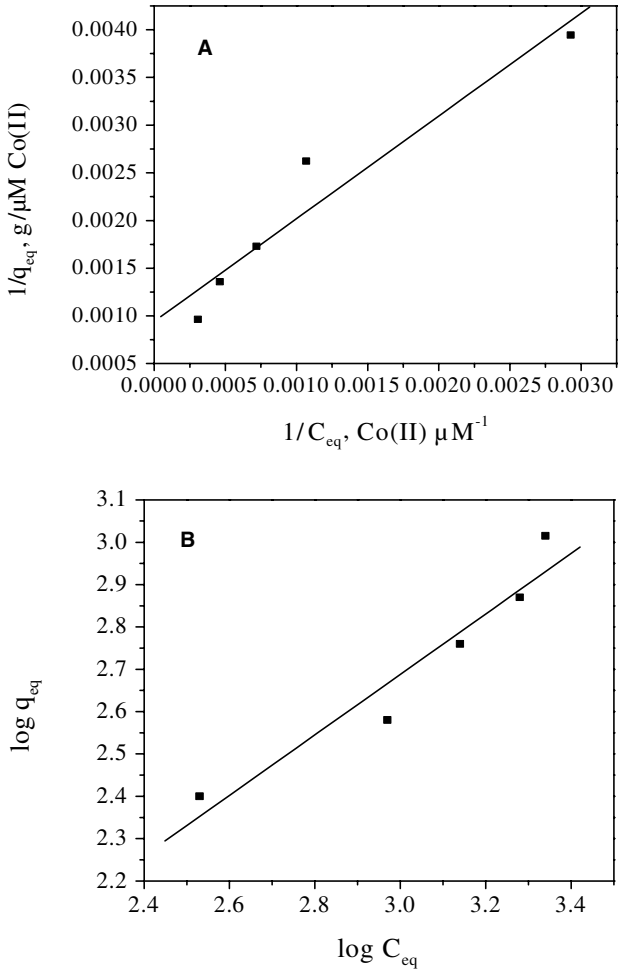

Fig. 2. The linearised Langmuir (A) and Freundlich (B) adsorption isotherms of Co(II) by *Mortierella* SPS 403 biomass.

Table 3
Equilibrium isotherms for cobalt biosorption on *Mortierella* SPS 403

Model	Equation	Isotherm constants	Correlation coefficient, r^2
Langmuir	$Q_{eq} = \frac{0.9087 C_{eq}}{1 + 0.00096 C_{eq}}$	$a = 946.5$ $b = 0.00096$	0.905
Freundlich	$Q_{eq} = 3.516 C_{eq}^{0.713}$	$K = 3.516$ $n = 1.401$	0.923

Q_{eq} is Co(II) adsorbed (μM/g biomass) at equilibrium.
C_{eq} is residual cobalt (μM) at equilibrium.

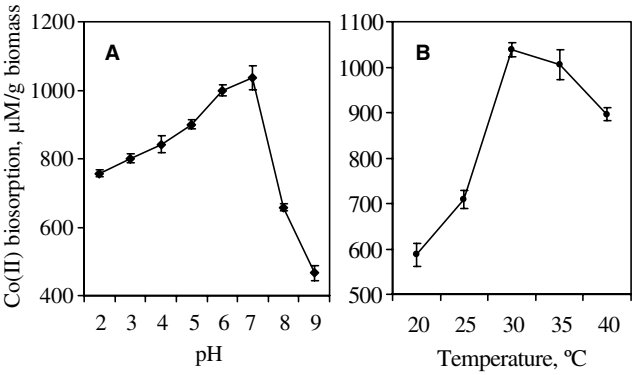

Fig. 3. Effect of pH (A) and temperature (B) on cobalt biosorption by *Mortierella* SPS 403 biomass. [Cobalt sorption was measured after 60 min incubation using an initial Co(II) concentration of 4.0 mM and biomass level of 2.0 g/l.]

3.5.1. Effect of pH and temperature

Biosorption of heavy metal ions from aqueous solution is strongly influenced by the pH and temperature of the solution. The optimum pH and temperature for Co(II) biosorption by SPS 403 biomass were 7.0 (Fig. 3A) and 30 °C (Fig. 3B) respectively. Under the above optimal conditions Co(II) loading capacity of the isolate was 1036.5 and 1038.8 μM Co(II)/g biomass respectively.

3.6. Effect of biomass concentration

Metal loading capacity on fungal biomass was greatly influenced by the initial concentration of dried mycelial mass used. The maximum cobalt adsorption was achieved with a biomass concentration of 0.5 g/l and sorption capacity declined sharply with increase in biomass level (Fig. 4).

3.7. Effect of additional ions

The presence of additional metal cations at equimolar concentration in the cobalt biosorption media inhibited

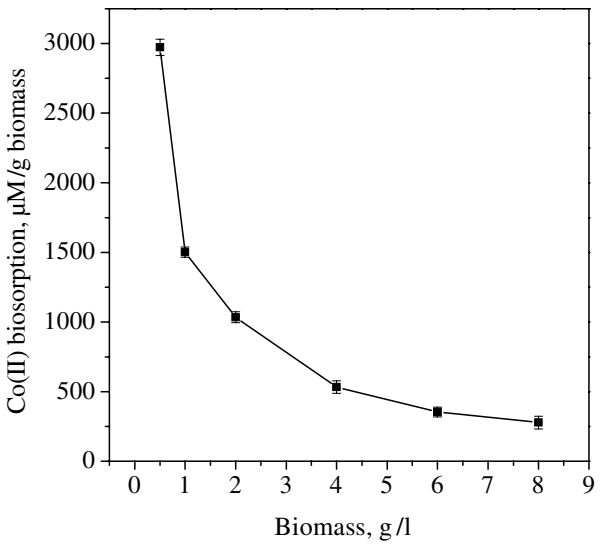

Fig. 4. Effect of initial biomass concentration on cobalt biosorption by *Mortierella* SPS 403. [Cobalt sorption was measured after 60 min incubation using an initial Co(II) concentration of 4.0 mM, variable concentration of biomass and incubated at 30 °C.]

sorption of Co(II) on to biomass of *Mortierella* SPS 403. The metal ions tested include Pb(II), Cu(II), Cd(II), Ni(II), Zn(II) (as chloride salt) and Cr(VI) (as potassium chromate). Results as summarized in Table 4 showed that Zn(II) was most inhibitory to Co(II) sorption by *Mortierella* SPS 403 showing an adsorption of merely 138.4 μM Co(II)/g biomass which represented 13.4% of the control set. Presence of Cr(VI) ions on the other hand were least inhibitory and the uptake of Co(II) ions by the biomass was retarded by about 15% only.

4. Discussion

Serpentine soils are metal-enriched ecosystem providing a metal-stressed habitat for evolution of metal-resistant microbiota (Amir and Pineau, 1998; Pal et al., 2003, 2004). The present study clearly established the occurrence of cobalt-resistant fungi in serpentine outcrops of Andaman, India, majority of which isolated represented the family Moniliaceae. The soil mycoflora as obtained by dilution–plating method on cobalt-supplemented media was dominated by metal-resistant members of the genera *Penicillium* and *Aspergillus* (Table 1) as it was evident in New Caledonian serpentine soils (Amir and Pineau, 1998). The selected 11 potent resistant strains showing MIC values >6.0 mM Co(II) represented members from all isolated genera with the exception of *Rhizopus*. This indicated that Co(II)-resistance phenotypes are well-spreaded among the representative fungal genera.

Aspergillus, *Penicillium* and *Rhizopus*, the common soil fungi, have already been exploited for the removal of heavy metals from aqueous solution (Kapoor et al., 1999; Say et al., 2003). The present findings undoubtedly indicate that fungi from natural metal-percolated environment are equally efficient in biosorbing Co(II) ions. The differences in Co(II) biosorption by different fungal genera may be attributed to wide variation in the chemical composition of their cell walls (Gadd, 1990). Earlier studies have indicated accumulation of Zn, Cd and Hg with *Mortierella isabellina*, but at considerably low levels (Krantz-Rulcker et al., 1996). In the present study, during long-term (60 min) exposure, the selected Co(II)-resistant *Mortierella* SPS 403 showed significant biosorption of Co(II) at high initial metal concentration which is generally encountered in industrial effluents (Table 2). However, in terms of rapid sorption of metal ions by the mycelial biomass during short-term (15 min) exposure, *Penicillium* SPS 106, CTS 402; *Aspergillus* SPS 202 and *Paecilomyces* SPS 107 were not inferior.

Cobalt sorption capacity on *Mortierella* SPS 403 biomass increased rapidly within 10–30 min of incubation but gradually reached equilibrium after 60 min (Fig. 1A). The initial phase of biosorption seemed to be active and was followed by passive sorption of Co(II) ions culminating in a state of equilibrium. In contrast, sorption equilibrium was reached after 2–4 h with biomass of *Aspergillus niger* (Kapoor et al., 1999) and *Penicillium canescens* (Say et al., 2003). Moreover, biosorption efficiency of the present *Mortierella* strain compared well with other cobalt biosorbents so far reported (Suhasini et al., 1999; Ahuja et al., 1999). The correlation coefficients (Table 3) of Langmuir and Freundlich isotherms (Fig. 2A and B) were significantly high and biosorption of Co(II) was better described with Freundlich isotherm similar to those of Tezos et al. (1988) and Kapoor et al. (1999).

The pH of the metal solution play an important role in the process of biosorption and low external pH generally decreases the rate and extent of metal biosorption (Gadd, 1990). The pH optima (Fig. 3A) for Co(II) biosorption by *Mortierella* SPS 403 corroborates the

Table 4
Effect of additional metal cations on cobalt biosorption by *Mortierella* SPS 403

Metals[a]	Co(II) biosorption,[b] μM Co(II)/g biomass	Biosorption, % of control
None (control)	1031.98 ± 10.3	100.00
Pb(II)	247.59 ± 16.7	23.9
Cu(II)	393.03 ± 8.4	38.0
Cd(II)	367.27 ± 29.3	35.6
Zn(II)	138.38 ± 8.1	13.4
Ni(II)	475.46 ± 7.2	46.0
Cr(VI)	873.08 ± 9.3	84.6

[a] The Co(II) concentration of the biosorption media was maintained at 4.0 mM, additional metals were added at equimolar concentration.
[b] Biosorption was measured after 60 min of incubation. Results represent mean of triplicate experiments ± standard error.

findings of Co(II) removal by *Rhizopus* PFB1 (Suhasini et al., 1999). However, it has been suggested by Zhou and Kiff (1991) that at low pH, hydronium ions compete with positively charged metal ions for amine group of chitin monomer, which acts as the active site on fungal biosorbents. At neutral pH, hydronium ion concentration is low leading to enhance binding of metal cations on to active site of fungal sorbent.

The optimum temperature for Co(II) uptake by *Mortierella* SPS 403 biomass (Fig. 3B) was similar to *Rhizopus* PFB1 (Suhasini et al., 1999). But contrary to *Rhizopus* PFB1, average adsorption efficiency of *Mortierella* SPS 403 was found to vary significantly with temperature. Unlike these organisms, high temperature (60 °C) optima have been reported for marine alga *A. nodosum* (Kuyucak and Volesky, 1989).

The Co(II) loading capacity (μM/g biomass) of *Mortierella* SPS 403 was inversely proportional to the amount of biomass (Fig. 4) present in the sorption medium. A phenomenon similar to this has also been noted for uptake of Cu(II) and Cr(VI) by *Aspergillus* (Al-Ashesh and Duvnjak, 1995). On the contrary, a steep increase in biosorption capacity of several metals was noticed with increase in algal biomass (Hamdy, 2000).

Presence of additional metal cations in the sorption medium, in general, was inhibitory to Co(II) biosorption by *Mortierella* SPS 403 (Table 4). This might be due to competition of divalent metal cations for complexation with the active binding sites of fungal biomass leading to decrease in Co(II) uptake. On the contrary, the partial non-inhibitory effect of Cr(VI) ions may be attributed to the difference in ionic radii of metal ions (Sag and Kutsal, 1996).

5. Conclusion

Broad screening of cobalt-resistant serpentine fungi for metal biosorption and studies on biosorptive properties of potent fungi *Mortierella* SPS 403 could serve as a basis for development of newer and efficient biosorbents for cobalt. However, detailed investigation on the mechanism of cobalt biosorption by SPS 403 biomass is essentially required for economical exploitation of the fungal isolate in future.

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