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# BIOACCUMULATION AND BIOSORPTION OF $Co^{2+}$ BY *NEUROSPORA CRASSA*

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## SUMMARY

Mycelial biomass of wild type and a $Co^{2+}$-resistant *N.crassa (cor)* was used to remove $Co^{2+}$ from aqueous media. Mycelia obtained from growth in nitrate N-medium ($NaN0_3$) was more effective than ammonium N-medium ($NH_4N0_3$), in removing $Co^{2+}$. $Co^{2+}$-resistant *N.crassa cor* was more efficient than wild type in removing $Co^{2+}$ from medium containing higher concentrations (500 mg/L). Metal removal was linear up to 12 h at which 35-60% $Co^{2+}$ is depleted from medium, reaching near saturation by 24 h (90% removal). $Co^{2+}$ removal was as efficient even from pure solutions and sodium azide inhibited the process up to 60%. Cell walls prepared from nitrate N-medium grown mycelia bound 3 - 5 fold more $Co^{2+}$ when compared to ammonium N-medium. The importance of bioaccumulation and biosorption in bioremediating toxic metal ions from effluents is discussed.

## INTRODUCTION

Cobalt is used in many industrial applications and hence development of alternate technologies for secondary recovery processes along with environmental concerns for decontamination of effluents has become necessary. Microorganisms are known to accumulate metals by two distinct processes: (i) bioaccumulation, an energy-dependent process, and (ii) biosorption, an energy-independent physical adsorption. Both the above principles have been investigated to determine their potential in applications to remove toxic metal ions from polluted waste waters and concentrate precious metals (Volesky, 1994; Gadd, 1993; Brierley *et al*, 1989; Akthar, *et al*, 1995, 1996; Akthar and Mohan, 1995). $Co^{2+}$ removal has been studied using algae and bacteria, employing both live and processed biomass preparations (Kuyucak and Volesky, 1989; Gadd, 1993; Garnham *et al*, 1993). $Co^{2+}$ uptake by *Neurospora crassa* is biphasic involving an initial rapid energy and temperature independent phase followed by slower uptake, sensitive to respiratory inhibitors (Venkateswerlu and Sastry, 1970). The importance of metal-resistant strains was first demonstrated using a nickel-resistant, hyperaccumulator strain of *N.crassa* which removed $Ni^{2+}$ more efficiently from media containing toxic concentrations than wild type (Kumar *et al*, 1992). In the present study mycelial biomass and cell wall preparations obtained from *N.crassa* cultures grown in medium with ammonium and nitrate N-medium are used to compare the efficiency in removing $Co^{2+}$ ions.

## MATERIALS AND METHODS

**STRAINS, MEDIA AND CULTURE CONDITIONS:** *N.crassa* wild type (FGSC # 4200) and $Co^{2+}$-resistant strain (FGSC # 7290) were grown on nitrate N-medium (Subramanyam *et al*, 1983) or ammonium N-medium (Venkateswerlu and Sastry, 1973). Culture conditions to obtain mycelial biomass and for $Co^{2+}$ removal were similar to those described earlier (Kumar *et al*, 1992).

**CELL WALL PREPARATION:** Cell walls from mycelia were prepared by the method of Schmit *et al* (1975).

**COBALT ESTIMATION:** $Co^{2+}$ removal from medium was followed by estimations at initial and final incubation time periods by Atomic Absorbtion Spectrophotometry [(AAS), Perkin-Elmer 2380]. $Co^{2+}$ content of mycelia was determined following acid digesion ($HNO_3$ : $HClO_4$ = 10:1) by AAS.

**Note:** Unless indicated the data shown are average values from three separate experiments. Variations up to 15% are observed.

## RESULTS AND DISCUSSION

Mycelial biomass of wild type and $Co^{2+}$-resistant (*cor*) *Neurospora crassa* was more efficient in removing up to 90% of $Co^{2+}$ from nirate N-medium compared to ammonium N-medium ( Fig 1 and 2 ). In the later medium only 45-50% of $Co^{2+}$ could be depleted in 2 d. *N.crassa cor* was 2-3 fold more efficient than wild type in $Co^{2+}$ removal, especially from medium containing higher concentrations (500-1000 mg $Co^{2+}$/L). However wild type *N.crassa* was as efficient or better than *cor* strain in removing $Co^{2+}$ from lower concentrations (5-50 mg $Co^{2+}$/L). Under the experimental conditions a maximal increase in biomass weight up to 20% was observed. $Co^{2+}$ removal increased with incubation time steadily and saturates around 24 h. Thereafter only a maximal increase of 5-10% was observed at 48 h. (Fig 3). Interestingly similar efficiencies of $Co^{2+}$ removal was observed from pure solutions of $Co^{2+}$ lacking medium constituents ( Fig. 4). To distinguish between the $Co^{2+}$ sorbed by the mycelia (biosorption) and cobalt actually taken up (bioaccumulation), the mycelia of *cor* strain were allowed to deplete $Co^{2+}$ from pure solutions in the presence of sodium azide (1 mM), a respiratory inhibitor. The results of Fig.4 clearly indicate that there is about 60 % suppression of $Co^{2+}$ uptake in presence of sodium azide, which represents the fraction of $Co^{2+}$ taken up by the control mycelia (bioaccumulation), while the remaining 40% is surface bound (biosorption). Biosorption was further confirmed by total leachability of the surface bound $Co^{2+}$ with either EDTA or 0.1 M HCl, after which the biomass was capable of rebinding almost identical quantities of $Co^{2+}$ (data not shown).

Since the surface bound $Co^{2+}$ represents a significant fraction of the $Co^{2+}$ depleted (40%), biosorption of $Co^{2+}$ from pure solutions was investigated using cell wall preparations from wild and $Co^{2+}$-resistant *N.crassa*. The results of table 1 indicate that cell walls prepared from mycelia grown on nitrate N-medium bound 4-5 fold more $Co^{2+}$ when compared to preparations from ammonium N-medium. In both the above cases wild type cell walls bound more $Co^{2+}$ than *cor* strain. At near saturation, binding of $Co^{2+}$ by cell walls constituted up to 4 - 5% on a dry weight basis (w/w).

**Table 1. $Co^{2+}$ binding by cell walls of *N.crassa*.**

| | $Co^{2+}$ [mg/100 mg dry wt.] Medium-N | |
|---|---|---|
| | $NH_4$ | $NO_3$ |
| Wild | 1.7 ± 0.25 | 5.6 ± 1.0 |
| *cor* | 0.8 ± 0.15 | 4.0 ± 0.7 |

Cell wall preparations (100 mg) were suspended in 10 ml solution containing $Co^{2+}$ (50 mM) for 1 h at 28°C. After incubation cell walls were separated by centrifugation at 5000 g for 5 min. The bound $Co^{2+}$ was eluted with EDTA (10 mM) and estimated by Atomic Absorption Spectrophotometry. Values shown are average of two experiments ± SD.

Fig. 1 **Removal of $Co^{2+}$ from nitrate N-medium**

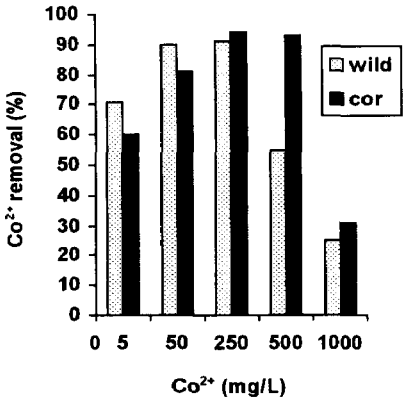


**Fig. 1** *N. crassa* mycelia (10 g ) was suspended in 250 ml of nitrate N-medium containing indicated $Co^{2+}$ and incubated in a rotary (100 rpm) shaker incubator for 2 d. $Co^{2+}$ remaining in medium was estimated by AAS.

Fig. 2 **Removal of $Co^{2+}$ from ammonium N-medium**

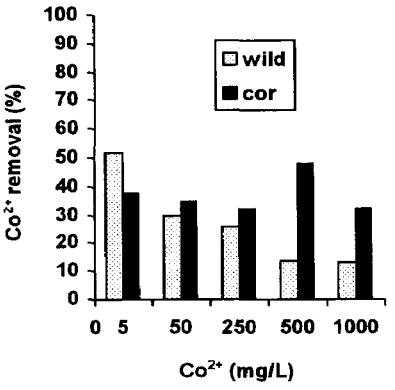


**Fig. 2** *N. crassa* mycelia (10 g ) was suspended in 250 ml of ammonium N-medium containing indicated $Co^{2+}$ for 2 d as in Fig. 1.

Fig. 3 **Kinetics of $Co^{2+}$ removal**

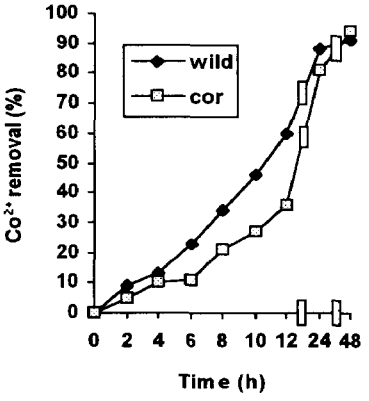


**Fig. 3** *N. crassa* mycelia (10 g ) was suspended in nitrate N-medium containing $Co^{2+}$ (250 mg/L) for indicated time periods. $Co^{2+}$ remaining in medium was estimated by AAS.

Fig. 4 **Effect of sodium azide on $Co^{2+}$ removal by *cor* strain**

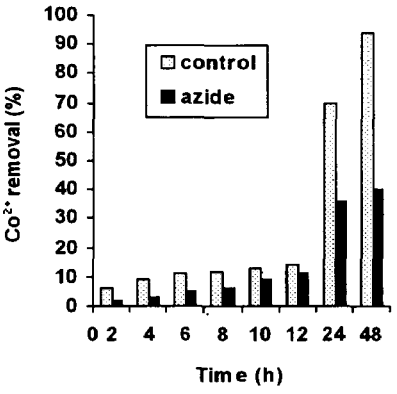


**Fig. 4** *N. crassa cor* mycelia (10 g ) was suspended in $Co^{2+}$ solution (250 mg/L) free of medium constituents. Sodium azide (1 mM) was included as shown against control. $Co^{2+}$ remaining was estimated by AAS.

In the present study removal of $Co^{2+}$ from medium was achieved more efficiently by *N.crassa* mycelia grown in medium containing nitrate as N-source compared to ammonium N-medium. *N.crassa cor* strain was more effective at higher concentrations of $Co^{2+}$ (> 250 mg/L) compared to wild type. This is in agreement with the earlier reported work on removal of $Ni^{2+}$ by a $Ni^{2+}$-resistant strain of *N.crassa* (Kumar *et al*, 1992). Metal-resistant strains in general have a superior ability to tolerate higher concentrations of toxic metal ions and hence are more desirable. In many applications it is not practicable to use live organisms which has necessiated the use of microbial biosorbents (Brierley *et al*, 1989). The superior ability of $Co^{2+}$ binding by cell wall preparations demonstrated herein has potential for application in both recovery and decontaminating precious/toxic metal ions. Efficient $Co^{2+}$ removal was also demonstrated from pure solutions lacking growth medium which has obvious advantages. $Co^{2+}$ removal does not appear to be related to growth, however active metabolism is necessary and hence inclusion of respiratory inhibitor resulted in 60 % decrease of $Co^{2+}$ removal. The source of nitrogen employed in growth medium is known to effect metal toxicities in *N.crassa*. $Co^{2+}$ and copper toxicities were reported to be enhanced in nitrate N-medium when compared to ammonium N-medium (Subramanian and Sarma, 1968; Venkateswerlu and Sastry, 1979). Metal toxicities in *N.crassa* and *Cunninghamella blakesleeana* were shown to result in altered composition of cell walls (Subramanyam *et al*, 1983; Venkateswerlu and Stotzky, 1986). In the present study cell wall composition with increased metal binding sites could probably result, due to nitrate nitrogen source of growth medium (compared to ammonium N-medium), which is the probable reason for the increased $Co^{2+}$ biosorption.


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