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Short Communication

Biosorption and Bioaccumulation of Thallium(I) and Its Effect on Growth of *Neosartorya fischeri* Strain

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Abstract

Little is known about thallium behavior in the environment, especially its interactions with microorganisms such as fungi. This article evaluates basic interactions (bioaccumulation, biosorption and growth inhibition) between thallium and the common heat-resistant fungal species *Neosartorya fischeri*. The results suggest that the *N. fischeri* strain is relatively resistant to elevated concentrations of thallium in cultivation media up to 1 $mg \cdot l^{-1}$. However, the toxic effect of thallium on fungal growth depends on the time of cultivation, and after 30-day cultivation growth inhibition was reduced. The bioaccumulation of thallium after 30-day cultivation by fungal strain was 35.74 $mg \cdot kg^{-1}$ and 432.91 $mg \cdot kg^{-1}$ for initial concentration 1.012 and 4.861 $mg \cdot l^{-1}$ of Tl(I) in medium, respectively. The biosorption capacity was calculated to be 11.77 mg·kg and 62.01 $mg \cdot kg^{-1}$ for initial concentration 1.012, and 4.861 $mg \cdot l^{-1}$ of Tl(I) in medium, respectively.

Keywords: thallium, fungi, biosorption, bioaccumulation, fungal growth

Introduction

Thallium (Tl), present in the natural environment in low concentrations, occurs mainly in potassium minerals and sulfides (e.g. pyrite) [1]. The dominant chemical species of thallium in natural waters is thallous cation [2]. Because the solubility of thallous compounds is relatively high, monovalent thallium is readily transported through aqueous environments. Along with its mobility, thallium is highly toxic to humans [3, 4]. The mobility of thallium in the environment plays an important role in determining the health risk of this element [5], which should make thallium and its compounds of particular scientific interest and environmental concern. This opinion is also supported by the fact that there is still little known about thallium behavior in the environment, especially about its interactions with microorganisms such as microscopic filamentous fungi, e.g. the effects of microorganisms on thallium mobility [6] and the effect of metal on microbial growth.

The aim of this study was to investigate the basic interactions between thallium and the fungal *Neosartorya fischeri* strain such as immobilization and bioaccumulation of thallium by native fungal biomass and its growth inhibition (toxicity).

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Materials and Methods

Isolation of Microorganism

A strain of *Neosartorya fischeri* was isolated from a soil sample after heating to 70°C for 30 min. in a Sabouraud agar (HiMedia, Mumbai, India) with Rose Bengal under laboratory conditions according to [7]. A strain of *Aspergillus niger* was isolated from a soil sample by serial dilution method according to [16]. Isolated fungi were maintained on Sabouraud agar.

Effect of Thallium on Fungal Growth

The 40 ml of sterilized Sabouraud maltose broth (HiMedia, Mumbai, India) was inoculated with a 5 ml spore suspension of 14-day-old culture spores cultivated on Sabouraud agar. This suspension was prepared from pure cultures of *N. fischeri* grown at room temperature at a ratio of 10^{-1} CFU. Then the cultivation media was enriched with 5 ml solution of Tl(I) to reach final concentration 1.012 or 4.861 mg·l^{-1} of Tl(I). The biomass was then harvested on day 1, 5, 10, 20 or 30 of cultivation, dried constant weight and measured. Also, the pH value of the cultivation media was determined.

To determine the effect of thallium in initial growth stages, the radial growth of *N. fischeri* strain on aseptic agar medium inoculated by 20 µl of spore suspension (10^{-1} CFU) was measured every 24 hours for 10 days.

There were three replicated runs for each experiment.

Biosorption of Thallium

Fungal biomass of the *N. fischeri* and *Aspergillus niger* strains was prepared by dynamic cultivation in 100 ml of Sabouraud medium inoculated with 10 ml of spore suspension. After the 4-day cultivation, pelletized fungal biomass with radius from 1 to 3 mm was separated from the cultivation media, and washed with a great amount of distilled water to remove any traces of cultivation media. Then the 2 g of the wet biomass was transferred into the 50 ml of thallium solution with concentration 0.101 mg·l^{-1} (5.06 µg) or 0.486 mg·l^{-1} (24.305 µg) of Tl(I). After one hour of contact time of the biomass with solution, the biomass was separated from solution by filtration, gently washed with deionized water and analyzed for the amount of biosorbed thallium. All experiments were replicated in three runs.

Bioaccumulation of Thallium

To study the bioaccumulation of thallium by microscopic filamentous fungus *N. fischeri*, 100 ml Erlenmeyer flasks containing 40 ml of Sabouraud medium were inoculated with a 5 ml spore suspension of 14-day-old culture spores cultivated on Sabouraud agar. This suspension was prepared from pure cultures of *N. fischeri* grown at room temperature at a ratio of 10^{-1} CFU. The SAB medium was autoclaved for 20 min at 121°C before inoculation. The SAB medium was then enriched with 50.4 or 243.05 µg of Tl(I) to reach concentrations of 1 or 5 mg·l^{-1} of thallium. After 30-day cultivation, the compact fungal biomass was separated from the SAB medium. The biomass was gently washed with deionized water and analyzed for total thallium concentration.

To determine the relative portion of active uptake of thallium by biomass on thallium removal, a 1 hour biosorption of thallium (50.4 or 243.05 µg of Tl(I)) by fungal biomass from 50 ml of Sabouraud medium was realized. The biomass was prepared by static cultivation on the 50 ml of Sabouraud medium, harvested after 30-day cultivation, washed with a great amount of water and used for the experiment as adsorbent. After one hour of contact time with the solution enriched with the desired amount of thallium, biomass was separated, gently washed with deionized water and analyzed for thallium. There were three replicated runs for each experiment.

Analytical Method

Total thallium in all fungal samples was determined after high-pressure digestion using a Perkin-Elmer (Überlingen, Germany) model Zeeman 3030 atomic absorption spectrometer equipped with a HGA 600 graphite furnace and an AS-60 autosampler.

Results and Discussion

Thallium is a highly toxic element, even more toxic than Ni or Cu and extremely mobile in aquatic environments [2]. Therefore, there were some previously published research articles dealing with the effect of thallium on the growth of aqueous biota, for example *Hyalella azteca* [8] or *Daphnia magna* [9], but none for microorganisms such as fungi or algae. Borgmann et al. [8] found out that a 25% reduction in survival of mentioned specie occurred at about 48 nmol·l^{-1} (9.804 µg·l^{-1}) and EC_{25} for growth was just about 7.153 µg·l^{-1}. Growth inhibition was also observed in a

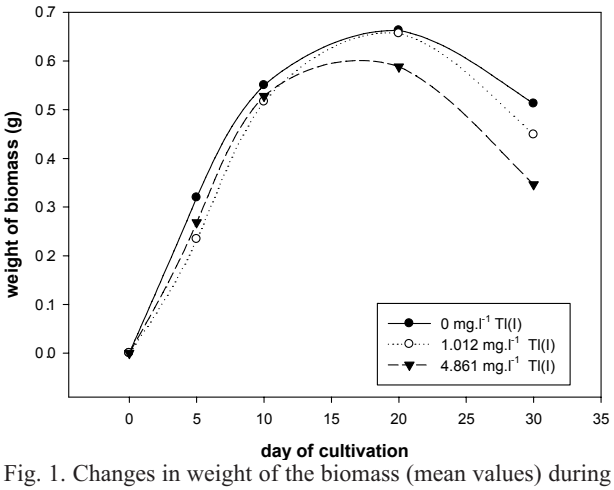

Fig. 1. Changes in weight of the biomass (mean values) during the 30-day cultivation of the *N. fischeri* strain in the presence of the different Tl(I) concentrations.

Table 1. Amount of biosorbed thallium onto biomass of two different fungal species (±SD).

Fungal species	Initial concentration of Tl(I) in 50 ml of aqueous solution [mg·l^{-1}]	Amount of Tl(I) in biomass after biosorption [µg]	Amount of removed Tl(I) from solution after biosorption [%]
Neosartorya fischeri	0.101	0.272±0.017	5.39
	0.486	1.810±0.183	7.4
Aspergillus niger	0.101	0.063±0.004	1.25
	0.486	0.188±0.037	0.77

recent experiment together with a slight decrease of pH value of culture media (Fig. 2). Thallium's effects on fungal growth of *N. fischeri* strain depend on time (Fig. 1). During 10-day cultivation, the little effect of Tl(I) on the radial fungal growth in the initial stages of colony growth was observed (Fig. 3). After 10-day cultivation, colony growth in the presence of 1 mg·l^{-1} Tl(I) was reduced. However, at higher thallous concentrations, the effect of thallium on fungal growth is not so significant (Figs. 1 and 3), probably due to some resistance mechanism. However, this high concentration of thallium in waters is extremely rare, but detected for example in groundwater in Lanmuchang area, China [10].

The bioaccumulation of Tl(I) by fungus *N. fischeri* is relatively high (Table 2). Bioaccumulation of thallium was reported by various authors, especially accumulation by brassicaceous plants [11, 12] that accumulated up to 40 mg·kg^{-1} of thallium; and microorganisms, which are capable of transforming thallium to dimethylthallium [13]. The bioaccumulation capacity for thallium of *N. fischeri* strain after 30-day cultivation was 35.74 mg·kg^{-1} and 432.91 mg·kg^{-1} for initial concentration 1.012 and 4.861 mg·l^{-1} of Tl(I) in medium, respectively. This is probably the result of chemical similarity of Tl(I) and K(I). Potassium uptake is necessary because of various potassium-dependent processes in cell metabolism. Since both ions are univalent with similar ionic radii, thallium is able to mimic potassium in its movement and intracellular accumulation [14]. This is confirmed by the results of the portion of the active accumulation on thallium removal (Table 2), which is relatively high. The results were calculated from the difference of thallium in biomass after 30-day cultivation and the amount of thallous ions adsorbed onto inactive fungal biomass surface (biosorption).

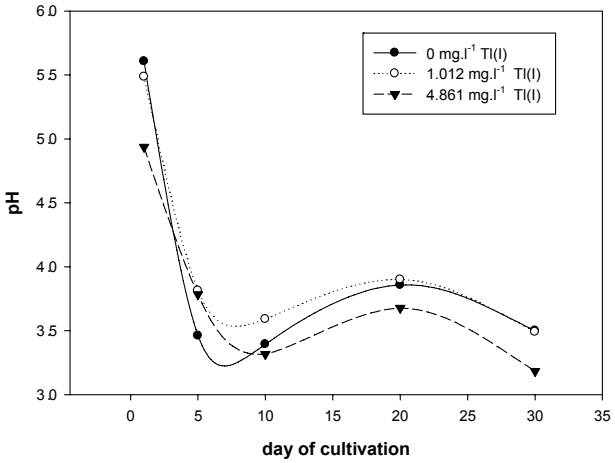

Fig. 2. Changes in pH of the cultivation media (mean values) during the 30-day cultivation of the *N. fischeri* strain in the presence of the different Tl(I) concentrations.

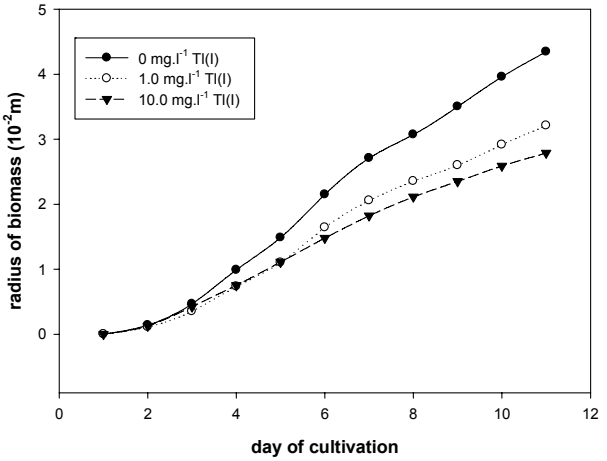

Fig. 3. Radial growth of biomass (mean values) in the presence of different concentrations of thallium.

The biosorption capacity was calculated to be 11.77 mg·kg^{-1} and 62.01 mg·kg^{-1} for initial concentration 1.012 and 4.861 mg·l^{-1} of Tl(I) in medium, respectively.

The biosorption was also evaluated using a lower concentration of Tl(I) and the pelletized biomass of *N. fischeri* strain. The results were surprisingly low (Table 1), probably resulting from some relation between initial concentration of Tl(I) in solution and adsorption capacity of the biomass. However, comparing biosorption capacity of *N. fischeri*

Table 2. Amount of bioaccumulated thallium by fungal biomass of *N. fischeri* strain during 30-day cultivation (±SD).

Initial amount of Tl(I) in 50 ml of cultivation media [µg]	Amount of Tl(I) in biomass after 30-day cultivation [µg]	Amount of removed Tl(I) after 30-day cultivation [%]	Amount of Tl(I) in inactivated biomass after 1 hour sorption [µg]	Relative portion of active accumulation on thallium removal [%]
50.6	15.786±2.952	31.19	6.035±0.352	61.77
243.05	142.963±14.579	58.82	31.785±5.489	77.77

strain and *A. niger* strain (Table 1), it is evident that the biomass of *N. fischeri* has a higher affinity for thallium than the previously reported common fungal biosorbent *A. niger* [15].

Conclusions

There is a lack of articles that compare biosorption and bioaccumulation of potentially toxic elements such as thallous cation by microorganisms. The results of this experiment have shown that uptake of the thallium by fungal biomass is highly enhanced when the active biomass is used, probably because of the chemical similarity of Tl(I) and K(I). Sorption of thallous cation onto fungal biomass is highly effective at higher concentrations of thallium in solution, but the sorption capacity markedly decreased when the lower concentration of thallium was used. However, the comparison between the commonly used fungal species *Aspergillus niger* for contaminants removal and *N. fischeri* has shown that the *N. fischeri* strain is more efficient in thallium removal from aqueous solutions. Also, the response of fungal growth on the presence of thallium in cultivation media was evaluated and it seems that the strain of *N. fischeri* is relatively resistant to Tl(I) concentrations up to 10 $mg \cdot l^{-1}$.

Acknowledgements

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