This method thus combined both high-resolution and non-radioactive cDNA-AFLP visualised on a fluorescence imager with the possibility of direct excision of differentially expressed cDNAs of interest.

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Comparison of the Heavy Metal Biosorption Capacity of Active, Heat-Inactivated and NaOH-Treated Phanerochaete chrysosporium Biosorbents

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Three different kinds of *Phanerochaete chrysosporium* (NaOH-treated, heat-inactivated and active) biosorbent were used for the removal of Cd(II) and Hg(II) ions from aquatic systems. The biosorption of Cd(II) and Hg(II) ions on three different forms of *Phanerochaete chrysosporium* was studied in aqueous solutions in the concentration range of 50–700 mg/L. Maximum biosorption capacities of NaOH-treated, heat-inactivated and active *Phanerochaete chrysosporium* biomass were found to be 148.37 mg/g, 78.68 mg/g and 68.56 mg/g for Cd(II) as well as 224.67 mg/g, 122.37 mg/g and 88.26 mg/g for Hg(II), respectively. For Cd(II) and Hg(II) ions, the order of affinity of the biosorbents was arranged as NaOH-treated > heat-inactivated > active. The order of the amount of metal ions adsorbed was established as Hg(II) > Cd(II) on a weight basis, and as Cd(II) > Hg(II) on a molar basis. Biosorption equilibriums were established in about 60 min. The effect of the pH was also investigated, and maximum rates of biosorption of metal ions on the three different forms of *Phanerochaete chrysosporium* were observed at pH 6.0. The reusability experiments and synthetic wastewater studies were carried out with the most effective form, i.e., the NaOH-treated *Phanerochaete chrysosporium* biomass. It was observed that the biosorbent could be regenerated using 10 mM HCl solution, with a recovery of up to 98%, and it could be reused in five biosorption-desorption cycles without any considerable loss in biosorption capacity. The alkali-treated *Phanerochaete chrysosporium* removed 73% of Cd(II) and 81% of Hg(II) ions from synthetic wastewater.

1 Introduction

Microbiological methods are being increasingly applied to the recovery or removal of metal ions from aqueous solutions. The uptake of metal ions onto surfaces and their subsequent translocation into the cells are well known natural processes, central to the accumulation of essential metals by microorganisms. Microorganisms have a range of metal-transport systems that are often highly specific for certain metals, and capable of accumulating metals against large concentration gradients. These adsorption and accumulation processes can

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be exploited in the accumulation of other metals [1–4].

Several researchers have reported that the biosorptive capacity of heat-inactivated cells might be greater, equivalent to or less than that of living cells [5,6]. However, the use of heat-inactivated biomass in industrial applications may offer some advantages (such as being less sensitive to heavy metal ion concentrations and adverse operating conditions) over the use of living cells [7]. The purpose of a pre-treatment is to improve the surface characteristics of the biosorbents in relation to their metal adsorbing mechanism. This may be in terms of increasing the negative charge on the cell surface by NaOH treatment [8], or opening of the available sites for the adsorption by acid treatment [9], and enhancing ion exchange by $CaCl_2$ treatment [10].

This communication deals with the adsorption characteristics of active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* biomass for Cd(II) and Hg(II) ions. In addition to the optimisation of the maximum adsorption conditions (i.e., pH, equilibrium time, temperature, initial metal ion concentration), adsorption isotherm modelling, desorption processes and removal of ions from synthetic wastewater samples were studied.

2 Materials and Methods

2.1 Microorganism and Media

The white-red basidiomycete *Phanerochaete chrysosporium*, ATCC-20696, was maintained by sub-culturing it on malt dextrose agar slants and adjusting the final pH to 4.5 at 25 °C.

2.2 NaOH Treatment of *Phanerochaete chrysosporium*

Phanerochaete chrysosporium was placed in a beaker together with 0.5 M NaOH solution, and the mixture was stirred at 400 rpm for 25 min at 25 °C. The pre-treated fungal preparations were then filtered and thoroughly washed several times with distilled, deionised water.

2.3 Biosorption Studies

The biosorption of Cd(II) and Hg(II) ions on the active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* from aqueous solutions was investigated in batch biosorption-equilibrium experiments. The effect of the medium pH and the initial concentration of metal ions on the biosorption rate and capacity were studied. The effect of the pH on the biosorption rate was investigated in the pH range of 3.0–7.0. The suspensions were brought to the desired pH by adding HCl or NaOH at the beginning of the experiment and were not controlled afterwards. Metal ion solutions (100 mg/L) and biosorbents were mixed using a magnetic stirrer at 400 rpm. The effect of the initial metal ion concentration on the biosorption was studied at pH 6.0 in the concentration range of 50–700 mg/L as described above.

2.4 Analytical Procedure

The biosorption of Cd(II) and Hg(II) ions from aqueous solutions was studied in batch systems. Nitrates of the metals were used. After the desired incubation period (of about 120 min), the aqueous phases were separated from the biosorbents, and the concentrations of the metal ions in these phases were determined. A Shimadzu Model AA-6800 Flame Atomic Absorption Spectrophotometer (Japan) was used. For mercury determinations, an MVU-1A (Mercury Vapour Unit) was employed.

The amount of metal ions adsorbed per unit mass of active, heat-inactivated and NaOH-treated preparations [mg metal ions/g] was obtained using the following expression:

$$Q = \frac{(C_o - C)\ V}{M} \tag{1}$$

where Q is the amount of metal ions adsorbed onto a unit of mass of the adsorbent [mg/g], C_o and C are the concentrations of the metal ions before and after the biosorption [mg/L], V is the volume of the aqueous phase [l] and M the amount of the adsorbent [g].

In order to determine the reusability of active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* biosorbents, consecutive adsorption-desorption cycles were repeated five times by using the same biosorbent. Desorption of the metal ions was performed with 10 mM HCl solution. The fungal preparations loaded with metal ions were placed in the desorption medium and stirred at 400 rpm for 60 min at 25 °C. The final metal ion concentrations in the aqueous phases were determined by applying the procedure described above. The desorption ratio was calculated from the amount of metal ions adsorbed on the biosorbents and the final metal ion concentration in the adsorption medium:

$$Desorption\ ratio = \frac{Amount\ of\ metal\ ions\ desorbed}{Amount\ of\ metal\ ions\ adsorbed} \tag{2}$$

3 Results and Discussion

3.1 Biosorption Rate

The changes in the amounts of Cd(II) and Hg(II) ions biosorbed by active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* with time were investigated. High biosorption rates were observed at the beginning and then the plateau values were gradually reached within 60 min.

3.2 Effect of the pH Value

In order to establish the effect of the pH value on the biosorption of Cd(II) and Hg(II) ions using active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* biomass, the batch equilibrium studies at different pH values were repeated in the range of 3.0–7.0. Fig. 1 illustrates the effect of the pH on biosorption. As can be seen from the figure, the maximum adsorption of Cd(II) and Hg(II) ions when using active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* biomass was observed at a pH of 6.0.

3.3 Effect of the Initial Metal Ion Concentration

Adsorption isotherms have been commonly used to describe the experimental results for the uptake of metal ions by biomass, since the initial rapid uptake is believed to be based on the binding of the metal ions to the cell wall. Many studies have shown that at low metal ion concentrations the mass of the metal ions accumulated (per unit of cell mass) is directly proportional to the concentrations of the ions in the solution [11].

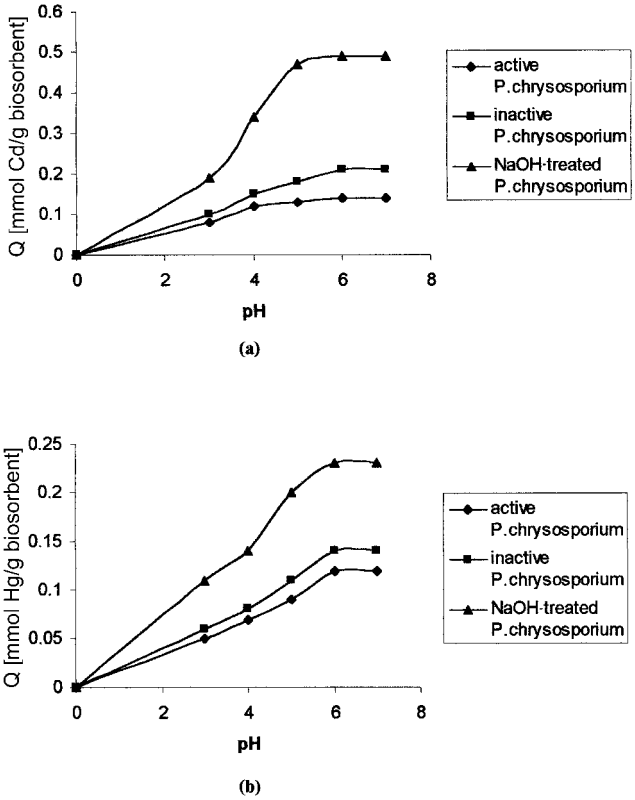

Figure 1. Effect of the pH on the biosorption capacities of active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* biomass for (a) Cd(II) and (b) Hg(II) ions.
Biosorption conditions: initial concentration of metal ions: 100 mg/L; volume of biosorption medium: 25 mL; temperature: 20 °C; biosorption time: 60 min.

Metal ion biosorption capacities of active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* biomass for Cd(II) and Hg(II) ions are presented in Fig. 2 as a function of the initial concentration of metal ions within the aqueous biosorption medium. Maximum amounts of metal ions adsorbed by each type of biosorbent are summarised in Tab. 1. The initial concentration was in the range of 30–700 mg/L.

As can be seen from Fig. 2 and Tab. 1, the order of the affinity of the biosorbents for the metal ions studied was found to be NaOH-treated > heat-inactivated > active, whereas the order of the amount of metal ions adsorbed, on the other hand, was established as Hg(II) > Cd(II) on a weight basis. However, when considered on a molar basis, the order changes to Cd(II) > Hg(II).

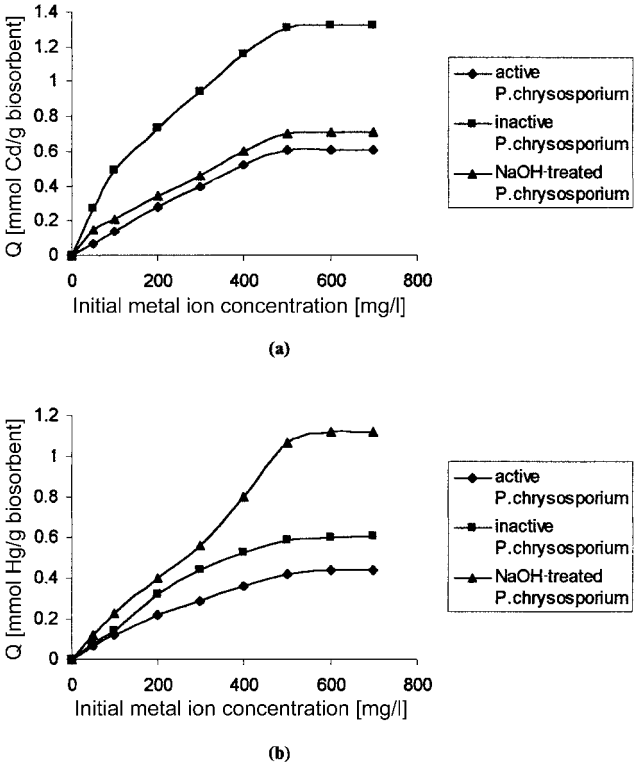

Figure 2. Biosorption capacities of active, NaOH-treated and heat-inactivated *Phanerochaete chrysosporium* biomass for (a) Cd(II) and (b) Hg(II) ions. Biosorption conditions: volume of the biosorption medium: 25 mL; pH 6.0; temperature: 20 °C; biosorption time: 60 min.

Table 1. Biosorption capacities of NaOH-treated, heat-inactivated and active biomass of *Phanerochaete chrysosporium*

Metal ion	NaOH-treated		Heat-inactivated		Active	
[mmol/g]	[mg/g]	[mmol/g]	[mg/g]	[mmol/g]	[mg/g]	[mmol/g]
Cd(II)	148.37	1.32	78.68	0.70	68.56	0.61
Hg(II)	224.67	1.12	122.37	0.61	88.26	0.44

3.4 Stability Constants of Metal Ion Biosorbents

The Hg(II) and Cd(II) ions binding properties of the NaOH-treated, heat inactivated and active biosorbents were investigated applying the method of Ruzic [12]. According to this data treatment, plotting $[M]_{ionic}/([M]_T - [M]_{ionic})$ *versus* $[M]_{ionic}$, a straight line should be obtained if one type of the complex is predominant (M_T is the total metal concentration and $[M]_{ionic}$ is the concentration of the labile metal species). From the slope, the metal binding capacity of the ligand (C_L) is determined. From the intercept according to the following equation, the apparent concentration stability constant (K_f) is defined as

$$[M]_{ionic}/[M]_T - [M]_{ionic}) = 1/K_f C_L \quad (3)$$

The straight-line relationship of the plot $[M]_{ionic}$ indicates that the interactions between Hg(II) and Cd(II) ions and biosorbents predominantly occurred with carboxylic groups of the fungal cell wall components. The ligand concentration (C_L) available for complexing Hg(II) and Cd(II) ions, and the concentration and stability constants (K_f) according to Eq. (3) were determined from the slope and the intercept and are listed in Tab. 2. The order of the stability constants of the biosorbents for both heavy metal ions was found to be NaOH-treated > heat-inactivated > active fungal biosorbent.

Table 2. Hg(II) and Cd(II) complexing capacity [C_L] and the stability constants [K_F] for the biosorbents

Biosorbents [mol/L]	Metal ion complexing capacity C_L [mol/L]	Stability constant K_f [L/mol]	R^2
Active (Hg(II))	$5.8 \cdot 10^{-7}$	$21.1 \cdot 10^{6}$	0.9993
Heat-inactivated (Hg(II))	$9.3 \cdot 10^{-7}$	$6.1 \cdot 10^{7}$	0.9987
NaOH-treated (Hg(II))	$2.8 \cdot 10^{-6}$	$2.8 \cdot 10^{9}$	0.9996
Active (Cd(II))	$6.5 \cdot 10^{-7}$	$19.1 \cdot 10^{6}$	0.9994
Heat-inactivated (Cd(II))	$6.9 \cdot 10^{-7}$	$5.3 \cdot 10^{7}$	0.9998
NaOH-treated (Cd(II))	$13.5 \cdot 10^{-7}$	$1.7 \cdot 10^{8}$	0.9991

3.5 Desorption and Reuse

The desorption of the adsorbed Cd(II) and Hg(II) ions was studied using NaOH-treated *Phanerochaete chrysosporium* biosorbent. The metal ions adsorbed were eluted with 10 mM HCl in a batch system. More than 98% of the adsorbed metal ions were desorbed. In order to show the reusability of the biosorbent, the adsorption-desorption cycle was repeated five times using the same preparation. The adsorption capacities did not change noticeably (a maximum of only 2% change was observed with the tested biosorbent) during the repeated adsorption-desorption operations. These results show that the NaOH-treated *Phanerochaete chrysosporium* could be repeatedly used in heavy metal adsorption studies without any detectable loss in the initial adsorption capacity.

3.6 Removal of Cd(II) and Hg(II) Ions from Synthetic Wastewater

In this group of experiments, the removal of Cd(II) and Hg(II) ions from synthetic wastewater by NaOH-treated *Phanerochaete chrysosporium* biomass was studied. Synthetic wastewater samples were prepared according to European Union Directive 91/271/EEC, entitled "Concerning Waste Water Treatment". These samples contain 0.10 mmol/L of Fe(III), Ni(II), Cr(III) and Al(III) ions in 1000 mg/L NaCl solution. These batch experiments were performed by using single (not mixed) solutions of the concerning ions. Metal ion solutions (200 mg/L) were prepared by means of synthetic wastewater and incubation with NaOH-treated *Phanerochaete chrysosporium* for 120 min at pH 6.0 and at a temperature of 20 °C. It was observed that within about 60 min 73% of Cd(II) ions and 81% of Hg(II) ions present were initially removed by NaOH-treated *Phanerochaete chrysosporium* biomass.

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