

The use of *Aspergillus niger* (Strain 4) biomass for lead uptake from aqueous systems

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Abstract

The potential of *Aspergillus niger* Strain 4 pellets to remove lead (Pb^{2+}) from solution was determined. *Aspergillus niger* Strain 4 was cultured in Currie's liquid medium as mycelial pellets for 5 d. Pellets were washed in water, and some were dried before exposure to varying concentrations of lead (Pb^{2+}) ion solutions. Various masses of dried mycelial material were exposed to different concentrations of Pb^{2+} solutions to determine the effect of biomass concentration on lead uptake. A mycelial biomass of 2 mg·ml⁻¹ was found to be optimal for Pb^{2+} uptake at all the lead concentrations tested. Drying of the mycelial pellets did not affect the uptake of Pb^{2+} . Scanning electron microscopy and energy dispersive X-ray micro-analysis of the fungal biomass, indicated that the lead was more or less evenly distributed within both the dried and undried mycelial pellets, and transmission electron microscopy confirmed that lead was present in the cell surface layers of the hyphal strands, i.e. the mechanism of uptake was determined to be biosorption onto the cell surface layers. *Aspergillus niger* Strain 4 pellets show potential for use in the removal of lead from industrial waste waters.

Introduction

Many heavy metals cause pollution in the environment and are toxic to living organisms. Conventional methods, such as filtration, chemical precipitation, ion exchange and electrolytic treatment, are becoming increasingly expensive to operate, and also have additional limitations (Wood, 1992). Micro-organisms have the potential for use as an alternative method of heavy metal removal from polluted waters and industrial effluents (Gadd and Griffiths, 1978).

Fungi are known to have good metal uptake systems (Gadd, 1986), with metabolism-independent biosorption being the most efficient mechanism (Tobin et al., 1994). Biosorption has been defined by Shumate and Strandberg (1985) as "a non-directed physico-chemical interaction that may occur between metal species and the cellular compounds of biological species". This may involve several chemical processes such as ion exchange, adsorption, co-ordination and covalent bonding, with the cell walls playing an important role, due to the presence of various uptake sites containing electronegative, anionic and N-containing groups (Tobin et al., 1994). Fungi often have greater tolerance than bacteria and algae towards metals and other adverse external conditions, such as low pH (Gadd, 1990). Some fungi produce spherical mycelial pellets with high metal uptake capacities, for example *Aspergillus*, *Penicillium* and *Rhizopus* species (Tobin et al., 1994). Immobilised *Rhizopus arrhizus* was found to effectively remove low concentrations of Cu^{2+} ions from aqueous solutions (Zhou and Kiff, 1991), a *Penicillium* sp. isolated from soil was found to accumulate large amounts of copper on the cell surface (Mitani and Mistic, 1991), and waste mycelium from several industrial fermentation plants (*A. niger*, *P. chrysogenum*, and *Claviceps paspali*) has been used to remove zinc ions from aqueous solutions (Luef et al., 1991).

Biomass-related technologies will not necessarily replace existing metal-ion removal treatments but may complement these chemical treatment processes. A knowledge and

understanding of the mechanisms controlling metal sorption by micro-organisms will aid in the optimisation of metal recovery processes (Fourest and Roux, 1992).

In this study, *A. niger* Strain 4 was cultured as submerged mycelial pellets, and then exposed to heavy metal solutions of Pb^{2+} to determine its metal uptake capacity over time. *A. niger* has previously been shown to be capable of removing Cu, Cd, Au, Ag, La and U from solution (Kapoor and Viraraghavan, 1995) but its ability to remove Pb from solution, as well as the mechanism of uptake, has not been demonstrated. Dried mycelial pellets were also used to investigate the effect of biomass concentration on metal uptake. Scanning and transmission electron microscopy and energy dispersive X-ray micro-analysis were used to investigate the uptake mechanism of the Pb^{2+} ions, i.e. whether intracellular uptake or simple biosorption to the cell surfaces was involved. A preliminary report on the ability of this fungus to take up lead has been published (Meyer et al., 1994). The quantitative and qualitative results obtained from a combination of these techniques and atomic absorption spectrophotometry should make it possible to determine the metal uptake potential of a specific biomass and to calculate the amount of biomass required to efficiently remove the metal ions present at known concentrations in a given volume of effluent.

Experimental

A previously isolated and identified *A. niger* (Strain 4) (Meyer et al., 1994) was used in all the experiments. Fungal pellets were cultured in Currie's liquid medium (Currie, 1917) for 5 d at 30°C on a rotary shaker at 200 r·min⁻¹. The resultant fungal pellets were washed twice in sterile distilled water, drained and weighed out into 100 ml Erlenmeyer flasks. To obtain the exact dry mass (i.e. 50, 100, 200 and 500 mg) some of the pellets were dried in an oven at 60°C under vacuum overnight before weighing. Aliquots (50 ml) of a lead nitrate solution (Pb^{2+} concentrations of 50, 100, 200 and 500 mg·l⁻¹), adjusted initially to approximately pH 4 with 1 M HCl and with no nutrients present, were added to each of the flasks containing the dried and undried mycelial pellets. These experiments were carried out in triplicate. The pellets were left in contact with the metal solutions for 24 h on a rotary shaker (200

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