Effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii*

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Abstract

Different chemicals were used to study the effect of pretreatment of *Mucor rouxii* biomass on bioadsorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}. Pretreatment with detergent and alkali chemicals such as NaOH, Na_2CO_3 and $NaHCO_3$ were found to improve or maintain the bioadsorption capacity in comparison with live *M. rouxii* biomass. Acid pretreatment using HCl, H_2SO_4 and $C_2H_4O_2$ resulted in a significant reduction in the bioadsorption capacity. Pretreatment using $CaCl_2$ and NaCl slightly reduced the bioadsorption capacity. All the pretreatment methods resulted in a reduction in biomass in comparison with autoclaved biomass. In addition, *M. rouxii* biomass pretreated with chemicals without autoclaving was still viable, even after boiling. To improve the bioadsorption capacity for metal ions by dead biomass, alkali pretreatment is an effective method, but the loss of biomass after the pretreatment should be taken into consideration while assessing the bioadsorption performance.

Introduction

Increased industrialisation and human activities have impacted on the environment through the disposal of waste containing heavy metals. Mine drainage, metal industries, refining, electroplating, dye and leather industries, domestic effluents, landfill leachate, agricultural runoff, and acid rain contribute such a kind of waste (Aksu and Kutsal, 1990). Micro-organisms including bacteria, algae, fungi and yeast are found to be capable of efficiently accumulating heavy metals (Gadd, 1987; Mullen et al., 1989; Atkinson et al., 1998). Bioadsorption mechanisms involved in the process may include ion exchange, co-ordination, complexation, chelation, adsorption and microprecipitation (Guibal et al., 1992; Fourest and Roux, 1992).

Both living and dead biomasses exhibit biosorption capacity (Brady et al., 1994); performance of living biomass in binding metal ions depends not only on nutrient and environmental status (Brierley et al., 1989), but also on cell age (Kapoor and Viraraghavan, 1995). In addition, living cells are subject to the toxic effect of heavy metals reaching a certain level, resulting in cell death. To overcome the disadvantages, non-viable or dead biomass is preferred in the removal of metal ions (Butter et al., 1998). In addition to ease of use and storage, dead biomass can be easily regenerated and reused (Spinti et al., 1995). Non-viable or dead biomass can be obtained through pretreatment of biomass (Butter et al., 1998). Physical pretreatment methods such as heating, autoclaving, freeze-drying and boiling and chemical pretreatment such as using acids, alkali and organic chemicals showed enhancement or reduction in metal bioadsorption, depending on the fungal strains and treatment procedures used (Galun et al., 1983; Huang et al., 1988; Kuyucak and Volesky, 1988; Paknikar et al., 1993; Kapoor and Viraraghavan; 1998). However, little was reported on the bioadsorption of heavy metals on fungi in the Mucorales order (Mullen et al., 1992; Guibal et al., 1992; Fourest et al., 1994; Tobin and Roux, 1998), let alone the effect of pretreatment on bioadsorption of heavy metals on live *M. rouxii*. The purpose of this investigation was to study the effect of pretreatment of *M. rouxii* on bioadsorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} in water.

Methods

A laboratory strain of *Mucor rouxii* (ATCC # 24905) was routinely maintained on Bacto potato dextrose agar (PDA). For experimental purposes, a liquid medium (YPG) (Bartnicki-Garcia and Nickerson, 1962) with a pH value adjusted to 4.5 was prepared, which comprises (in $g \cdot 1^{-1}$) the following: yeast, 3; peptone, 10; glucose (replaced by dextrose), 20. The cultures were grown at 23°C in the medium in conical flasks kept on a rotary shaker agitated at 125 $r \cdot min^{-1}$. All culture work was conducted aseptically. The fungi grew in a filamentous (moldlike) form under air, with fragmentation of some hyphae into spherical cells. They were harvested after 3 d of growth by filtering the growth media through a 150 μm sieve.

The harvested biomass was washed with generous amounts of deionised water. The live biomass so obtained will be referred to as Type A hereinafter. 50 g (wet mass) of Type A was then pretreated in different ways as listed in Table 1.

The biomass after each pretreatment was washed with generous amounts of deionised water, and then dried at 60°C for 24 h in a drying oven. In addition, prior to being autoclaved, biomasses that had been pretreated with alkali chemical such as NaOH, Na_2CO_3 and $NaHCO_3$ were washed with deionised water until the pH of the wash solution was in near neutral range (pH 6.8 to 7.2). Dried biomass was ground in a mortar and pestle.

Bioadsorption experiments were conducted using separate solutions containing Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} added in the form of $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_20$, $Ni(NO_3)_2.6H_20$, and $Zn(NO_3)_2.6H_20$ respectively. The solution prepared using distilled water had an initial metal concentration of 10 $mg \cdot \ell^{-1}$ and a pH of 5.0. Known amounts of biomass were contacted with each metal solution. The reaction mixture was agitated at 125 $r \cdot min^{-1}$ on a rotary shaker. After 15 h of contact time, filtrate was obtained by filtering the reaction mixture through an 0.45 μm polycarbonate filter and analysed for metal concentration. Metal concentrations were measured using a Varian AA-10 atomic absorption spectrophotometer. Bioadsorption experiments were carried out in duplicate and aver-

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