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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TABLE 1
PRETREATMENT METHODS APPLIED TO *M. ROUXII* BIOMASS (TYPE A)

Type	Solution (500 mℓ)	Duration (min)	Auto-clave*
B		30	✓
C	0.5N NaOH at 100°C	15	×
D	0.5N NaOH	30	✓
E	0.2N NaOH	30	✓
F	0.05N Na_2CO_3	30	✓
G	0.05N Na_2CO_3	30	×
H	0.1N $NaHCO_3$	30	×
I	Detergent (2.5 g)	30	×
J	1% (w) $CaCl_2$	30	✓
K	0.2N NaCl	30	×
L	0.1N $(NH_4)_2S_2O_8$	30	×
M	50% (vol/vol) C_2H_6SO	30	×
N	0.05N Na_2HPO_4	30	×
O	0.05N H_2SO_4	180	×
P	0.1N HCl	180	×
Q	0.1N $HClO_4$	30	×
R	10% (vol/vol) $C_2H_4O_2$	180	×
S	10% (vol/vol) H_3PO_4	30	×

*autoclaved for 30 min at 121°C and 18 psi; ✓ indicates that autoclaving is applied; × indicates that autoclaving is not applied.

age values were used in the analysis. Bioadsorption capacity, i.e. amount of metal ion (mg) bioadsorbed per g (dry mass) of biomass, was calculated using the following equation:

$$Q = \left(\frac{C_i - C_f}{m}\right)V$$

where:

Q = mg of metal ion bioadsorbed per g of biomass;
Ci = initial metal ion concentration, $mg \cdot \ell^{-1}$;
C_f = final metal ion concentration, $mg \cdot \ell^{-1}$;
m = mass of biomass in the reaction mixture, g;
V = volume of the reaction mixture, L.

Results

The effect of pretreatment of *M. rouxii* on bioadsorption of lead is shown in Fig. 1A. Live biomass was observed to possess a high lead bioabsorption capacity (17.13 $mg \cdot g^{-1}$). Bioadsorption of lead either maintained almost the same bioadsorption levels or decreased, depending on the pretreatment method in comparison with bioabsorption using live biomass. Pretreatment of live biomass using NaOH (with autoclaving), Na_2CO_3 (without autoclaving), and $NaHCO_3$ (without autoclaving) almost maintained or reduced to a small extent the bioadsorption of lead in comparison with live biomass (from 17.13 to 13.87 - 16.62 $mg \cdot g^{-1}$). However, pretreatment using Na_2CO_3 together with autoclaving reduced the bioadsorption capacity more than that without autoclaving, i.e. 10.89 and 15.10 $mg \cdot g^{-1}$ respectively. Pretreatment using NaOH with boiling but without autoclaving also reduced the bioadsorption of lead more than that without boiling but with autoclaving, i.e. 11.41 and 16.62 $mg \cdot g^{-1}$ respectively. Pretreatment using acids, i.e. H_2SO_4, HCl, $HClO_4$, $C_2H_4O_2$ and H_3PO_4, greatly reduced the bioadsorption of lead (from 17.13 to 0 - 9.76 $mg \cdot g^{-1}$). Pretreatment using detergent, C_2H_6SO, $CaCl_2$ and NaCl did not decrease the bioadsorption of lead to a great extent (from 17.13 to 7.87 - 14.44 $mg \cdot g^{-1}$), while autoclaving alone and $(NH_4)_2S_2O_8$ pretreatment significantly decreased lead bioadsorption in comparison with live cells (from 17.13 to 0 - 10.02 $mg \cdot g^{-1}$).

Figure 1B shows the effect of pretreatment of *M. rouxii* on biosorption of cadmium. Pretreatment using alkali chemicals, i.e. NaOH, Na_2CO_3, and $NaHCO_3$ (Types D, E, F and H) increased or maintained the bioadsorption of cadmium in comparison with live biomass (from 6.94 to 8.30 - 10.10 $mg \cdot g^{-1}$), with 0.45 time increase for Na_2CO_3 pretreatment. Pretreatment using detergent, C_2H_6SO, and $CaCl_2$ also increased the bioadsorption of cadmium (from 6.94 to 6.60 - 10.07 $mg \cdot g^{-1}$), with 0.45 time increase for detergent pretreatment. Chemical pretreatment using H_2SO_4, HCl, $HClO_4$, $C_2H_4O_2$ and H_3PO_4 resulted in a significant reduction in the bioadsorption of cadmium (from 6.94 to 0.32 - 0.94 $mg \cdot g^{-1}$). Autoclaving slightly decreased the bioadsorption of cadmium (6.94 to 5.04 $mg \cdot g^{-1}$). Na_2HPO_4 and $(NH_4)_2S_2O_8$ pretreatment completely inhibited cadmium bioadsorption

Figure 1C illustrates the effect of pretreatment on bioadsorption of nickel. Detergent pretreatment enhanced the bioadsorption of nickel by 16% in comparison with live biomass (from 5.24 to 6.07 $mg \cdot g^{-1}$). Pretreatment using alkali chemicals, i.e. NaOH, Na_2CO_3 and $NaHCO_3$, increased, maintained or slightly reduced the bioadsorption of nickel in comparison with live biomass (from 5.24 to 3.47 - 5.81 $mg \cdot g^{-1}$). Pretreatment using H_2SO_4, HCl, $HClO_4$, $C_2H_4O_2$ and H_3PO_4 marginally decreased the bioadsorption of nickel (from 5.24 to 0.24 - 2.11 $mg \cdot g^{-1}$). Biomass pretreated using $CaCl_2$, NaCl, C_2H_6SO, $(NH_4)_2S_2O_8$, and Na_2HPO_4 also reduced the nickel bioadsorption (from 5.24 to 0.56 - 3.84 $mg \cdot g^{-1}$). Autoclaving alone decreased the nickel bioadsorption by 68% in comparison with live cells (from 5.24 to 1.67 $mg \cdot g^{-1}$).

The effect of pretreatment on the bioadsorption of zinc is presented in Fig. 1D. In comparison with live biomass, bioadsorption of zinc on *M. rouxii* biomass pretreated using NaOH, Na_2CO_3 (without autoclaving), and $NaHCO_3$ resulted in an increase in zinc bioadsorption (from 4.89 to 5.63 - 6.28 $mg \cdot g^{-1}$), but Na_2CO_3 pretreatment with autoclaving reduced the bioadsorption (from 4.89 to 3.26 $mg \cdot g^{-1}$). Detergent pretreatment increased zinc biosorption by 32% (from 4.89 to 6.46 $mg \cdot g^{-1}$). Acid pretreatment using H_2SO_4, HCl, $C_2H_4O_2$, and H_3PO_4 significantly decreased zinc bioadsorption (from 4.89 to 0.36 - 0.92 $mg \cdot g^{-1}$). Pretreatment using $CaCl_2$, C_2H_6SO, NaCl reduced to a less extent the bioadsorption of zinc than that using acids (from 4.89 to 1.85 - 2.54 $mg \cdot g^{-1}$). Pretreatment using $HClO_4$, $(NH_4)_2S_2O_8$, and Na_2HPO_4 resulted in a complete inhibition of zinc bioadsorption.

It can be seen that, among all the pretreatment methods, almost all the methods using alkali chemicals and salts resulted in an improvement in bioadsorption capacities compared with autoclaving. For example, 0.5N NaOH pretreatment plus autoclaving (Type D) improved bioadsorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} by 66%, 76%, 189% and 120%, respectively, with the capacity increasing from 10.02 to 16.62 $mg \cdot g^{-1}$ for Pb^{2+}, 5.04 to 9.45 $mg \cdot g^{-1}$ for Cd^{2+}, 1.67 to 4.82 $mg \cdot g^{-1}$ for Ni^{2+} and 2.56 to 5.63 $mg \cdot g^{-1}$ for Zn^{2+}.

Figure 2 shows the effect of different pretreatment methods on biomass production. All the methods resulted in a loss of biomass, with a maximum of 63% loss for NaOH and boiling pretreatment. Other alkali pretreatment using NaOH and Na_2CO_3 also contributed to a high biomass loss (13 to 29%). A loss of 13 to 16% resulted from pretreatment using detergent, $CaCl_2$ and NaCl. Pretreatment

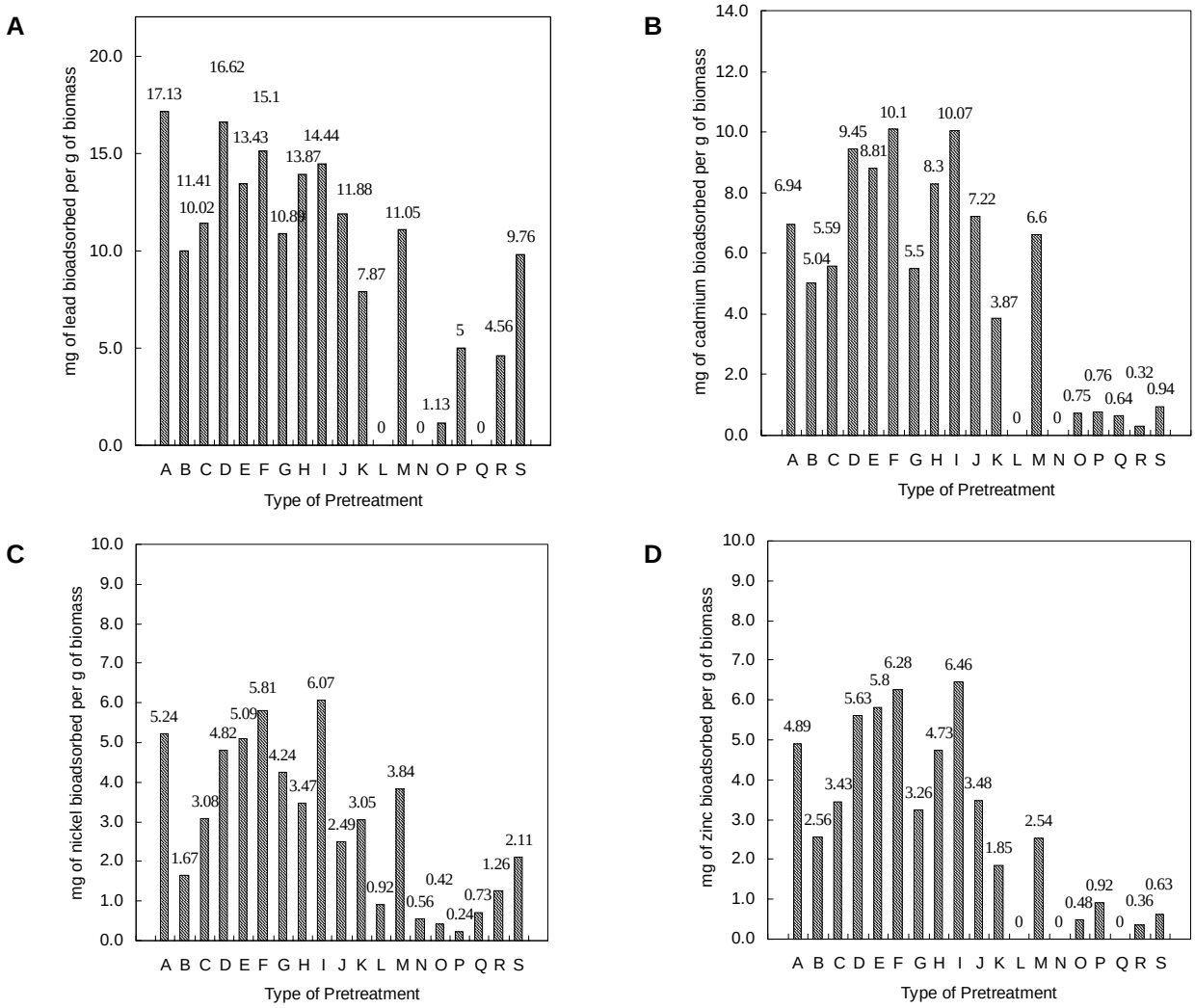

Figure 1
The effect of pretreatment on bioadsorption of heavy metals by a *biomass*

using HCl, $HClO_4$ and H_3PO_4 reduced the biomass by 11 to 16%. Other pretreatment methods resulted in a biomass loss of a lesser extent (1 to 8%).

Through examination on PDA, it was found that *M. rouxii* after pretreatment was still viable in the absence of autoclaving; this was also the case for biomass after NaOH and boiling pretreatment.

Discussion

Results from this study showed that *M. rouxii* live biomass had a high bioadsorption capacity for heavy metals, i.e. 17.13, 6.94, 5.24 and 4.89 mg·g^{-1} for Pb^{2+}, Cd^{2+}, and Ni^{2+} and Zn^{2+} respectively, while bioadsorption capacities of live *A. niger* for Pb^{2+}, Cd^{2+}, and Ni^{2+} were only 2.25, 1.31 and 1.75 mg·g^{-1} respectively (Kapoor and Viraraghavan, 1998). The difference may be ascribed to the larger surface area of *M. rouxii* biomass for adsorption, as mycelium of *M. rouxii* grows in the form of suspended growth, while in the case of *Aspergillus niger*, it grows in the form of pellets with a lower surface area.

The reduction of bioadsorption capacity of autoclaved *M. rouxii* biomass, in comparison with live biomass, may be attributed to the loss of intracellular uptake. In addition, Whistler and Daniel (1985) reported that heat treatment could cause a loss

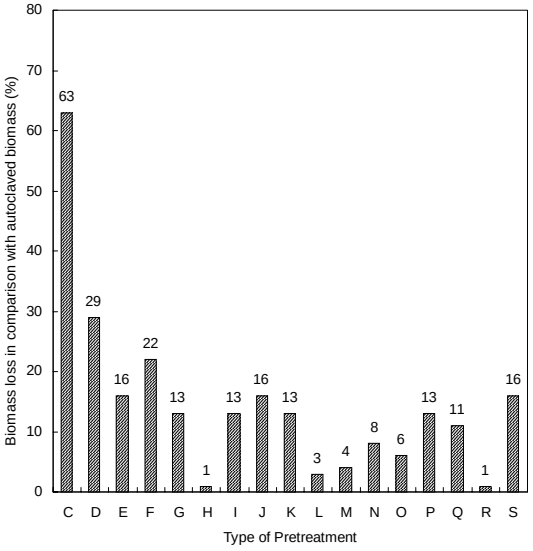

Figure 2
The effect of pretreatment on biomass production

TABLE 2
ACTUAL ENHANCEMENT (%) OF BIOADSORPTION OF HEAVY METALS BY SOME PRETREATED *M. ROUXII* BIOMASS IN COMPARISON WITH AUTOCLAVED BIOMASS

Metal ion	Pretreatment method									
	C	D	E	F	G	H	I	J	K	M
Pb^{2+}	-58	18	13	18	-5	37	25	0	-32	6
Cd^{2+}	-59	33	50	56	-5	63	74	20	-33	26
Ni^{2+}	-32	105	156	171	120	106	216	25	59	121
Zn^{2+}	-50	56	90	91	-11	83	120	14	-37	-5

of amino-functional groups on the fungal surface through the non-enzymic Browning reaction. Amino-functional groups are among the functional groups in the composition of polysaccharides which contribute to the binding of heavy metals (Loaec et al., 1997). Huang and Chiu (1994) found that because of the loss, cadmium bioadsorption of heat-treated *Rhizopus oryzae* was reduced. However, Galun et al. (1987) reported that *Penicillium* biomass pretreatment at 100°C for 5 min increased the bioadsorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} and the increase was attributed to the exposure of latent binding sites after pretreatment.

In the case of alkali pretreatment, bioadsorption capacity was significantly enhanced in comparison with autoclaving. In a study by Galun et al. (1987), NaOH treated *Penicillium digitatum* also showed enhancement in bioadsorption of Cd^{2+}, Ni^{2+} and Zn^{2+}. Removal of surface impurities, rupture of cell membrane and exposure of available binding sites for metal bioadsorption after pretreatment may be the reason for the increase in metal bioadsorption. McGahren et al. (1984), Brierley et al. (1985) and Muraleedharan and Venkobachar (1990) showed that alkali treatment of biomass may destroy autolytic enzymes that cause putrefication of biomass and remove lipids and proteins that mask reactive sites. Dow and Rubery (1977) found that cell walls of *M. rouxii* could be ruptured using NaOH treatment. Besides, the pretreatment could release polymers such as polysaccharides that have a high affinity towards certain metal ions (Mittelman and Geesey, 1985; Loaec et al., 1997). Most of the commercial detergents also contain alkalis as one of their ingredients (Prat and Giraud, 1964). This could be the reason why the alkaline detergent pretreatment resulted in an enhancement of bioadsorption of metal ions in the present study.

Acid pretreatment significantly decreased bioadsorption of heavy metals, which is in agreement with the observation of Kapoor and Viraraghavan (1998) in the case of *A. niger*. However, Huang and Huang (1996) reported that acid pretreatment can strongly enhance the adsorption capacity for *Aspergillus oryzae* mycelia. It should be noted that in their work, live biomass after acid pretreatment was directly used in bioadsorption of heavy metals instead of being autoclaved and dried. The difference in results after a specific pretreatment may be attributed to the different strains of fungi used and whether the biomass was live or dead when it is used in biosorption of metal ions. For example, pretreatment of *A. oryzae* by $HClO_4$ resulted in positive effects on the bioadsorption of Pb^{2+}, Cd^{2+} and Ni^{2+}, but it was not the case for the species of *R. oryzae* (Huang and Huang, 1996). In this study, the H^+ ions binding to the biomass after acid treatment may be responsible for the reduction in adsorption of heavy metals. The polymeric structure of biomass surface exhibits a negative charge due to the ionisation of organic groups and inorganic groups (Hughes and Poole, 1989). Bux and Kasan (1994) suggested that the higher the biomass electronegativity the greater the attraction and adsorption of heavy metal cations. Thus, the remaining H^+ ions on the acidic pretreated *M. rouxii* biomass may change the biomass electronegativity, resulting in a reduction in bioadsorption capacity.

All the pretreatment methods resulted in biomass loss in comparison with autoclaved biomass. Especially in the case of NaOH plus boiling pretreatment, the loss was up to 63%. Fourest and Volesky (1996) reported that up to 39% of biomass loss may result from pretreatment of *Sargassum fluitans* using NaOH. The mass loss of biomass during pretreatment may lead to some confusion during the quantitative assessment of the bioadsorption performance (Lee and Volesky, 1997). Taking into consideration the biomass loss after pretreatment in this study, it can be found that enhancement of bioadsorption after pretreatment will be offset to some extent by the loss. Table 2 shows the actual enhancement of some pretreatment to the bioadsorption in comparison with autoclaved biomass after considering the biomass loss. Even though alkali chemical (Type D, E, F and H), detergent (Type I) and $CaCl_2$ (Type J) pretreatment resulted in some biomass loss (from 1% to 29%), the pretreatment enhanced the biosorption capacities of the biomass for metal ions studied. For example, in the case of 0.5N NaOH plus autoclaving (Type D), the mass loss was 29% in comparison with the autoclaved biomass, but the actual enhancement of bioadsorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} was 18%, 33%, 105% and 56% respectively. Due to the heavy loss of biomass (63%) from boiling, NaOH solution boiling pretreatment (Type C) resulted in a significant reduction of bioadsorption of metal ions in comparison with autoclaved biomass. In other words, the increase in biosorption with the pretreatment was heavily offset by the big mass loss.

M. rouxii biomass was still viable after pretreatment if autoclaving was not used. Huang and Huang (1996) found that after acid treatment, *R. oryzae* was still viable, but *A. oryzae* was non-viable. Whether or not biomass is viable may depend on the treatment method and the strain of fungus to be used. If dead biomass is preferred in biosorption of metal ions, it may be ideal to include an autoclaving step in treatment.

It could be concluded that bioadsorption efficiency of dead biomass may be greater, equivalent to, or less than that of live biomass, depending on the pretreatment method applied. When non-viable biomass is to be used in the removal of heavy metals, alkali pretreatment is an effective method to improve the bioadsorption capacity for metal ions. It is necessary to carry out more detailed studies to understand why enhancement or reduction in adsorption capacity occurs under specific pretreatment conditions.

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