Standardization of variables involved in cadmium and zinc microbial removal from aqueous solutions

RESEARCH

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ABSTRACT

Water is an important natural resource for life and its chemical pollution is one of the most serious problems the current society is facing. Therefore, an environment-friendly method to reduce metal contaminants of aquatic ecosystems is required, and one potentially useful method for treatment of wastewater comprises the use of microbial biosorption. The objective of this work was to determine the effects of the main variables involved in cadmium and zinc removal by *Pseudomonas mendocina* (Ps-1) and *Saccharomyces cerevisiae* (Sc-10). Variables like pH, metal concentration, physiological age of the culture and state of the microbial biomass were assessed. The pH, metal concentration and the dead or inactive biomass had greater significant effect on the biosorption of cadmium and zinc by both microorganisms. The adjustment of the variables tested facilitated the increase of capture levels of the metal ions, which indicates that under the determined conditions *P. mendocina* (Ps-1) and *S. cerevisiae* (Sc-10) can be used in the decontamination of wastewater containing both heavy metals.

Keywords: microorganisms, biosorption, removal, cadmium, zinc, treatment variables

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RESUMEN

Estandarización de variables involucradas en la remoción microbiana de cadmio y cinc en solución acuosa. El agua es un recurso natural importante para la vida y su contaminación química constituye uno de los problemas más graves que enfrenta la sociedad actual. Un método amigable con el medio ambiente para disminuir contaminantes metálicos de ecosistemas acuáticos lo constituye la biosorción microbiana con aplicación en el tratamiento de aguas residuales. El objetivo de este trabajo fue determinar los efectos de las principales variables involucradas en el proceso de remoción de cadmio y cinc por *Pseudomonas mendocina* (Ps-1) y *Saccharomyces cerevisiae* (Sc-10). Se evaluaron las variables pH, concentración de los metales, edad fisiológica del cultivo y estado de la biomasa microbiana. El pH, la concentración de los metales y la biomasa muerta o inactiva presentaron un mayor efecto significativo sobre la biosorción de cadmio y cinc por ambos microorganismos. El ajuste de las variables ensayadas facilitó incrementar los niveles de captura de los iones metálicos, lo que indica que bajo las condiciones determinadas *P. mendocina* (Ps-1) y *S. cerevisiae* (Sc-10) pueden utilizarse en la descontaminación de aguas residuales que contienen ambos metales pesados.

Palabras clave: microorganismos, biosorción, remoción, cadmio, cinc, variables del tratamiento

*I*ntroduction

Environmental pollution by heavy metals compromises the quality of water resources, human health and ecosystems in general [1, 2]. The resistance of these inorganic compounds to biodegradation condition their permanence in nature, as well as their incorporation and accumulation through the food chain to toxic levels. Their toxicity supports the negative environmental impact they produce, even at very low concentrations [3, 4]. Cadmium affects the renal system, liver, blood, bone degeneration and can cause cancer [5]. On the other hand, the accumulation of zinc in organisms causes stress and toxic effects on cells [6, 7]. These damages indicate the need to remove these metals from the effluents, before being discharged to the receiving bodies, in particular to aquatic environments.

Water is an essential component for life and its contamination by metallic species has been recognized and reported by different authors [4, 8]. The application of biotechnological processes with microbial biomass is an ecological alternative for the protection of this natural resource. Metabolic activity and physiological responses of bacteria, yeasts, fungi and microalgae facilitate the removal of metals from contaminated effluents [9, 10]. On the other hand, cell structures favor the use of microorganisms as biosorbents, due to the diversity of cation sorption sites present in the cell wall [11].

The bioremediation of environments contaminated by heavy metals, through mechanisms such as bioaccumulation and biosorption allow the capture of cations by microbial cells [12-14]. These bioprocesses depend on the microorganism and on the environmental conditions. Different operational parameters such as: pH, ion and cell concentration, microorganism-metal contact time, biomass pretreatment and culture age, have a significant influence on metal bioremediation [4, 15], since they are involved in the sequestration of ions. Thus, it is necessary to study the experimental

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conditions that increase the natural capacities of microbial biosorbents to capture heavy metals. The objective of this work was to determine the effect of the adjustment of different environmental variables on zinc and cadmium removal by *Pseudomonas mendocina* (Ps-1) and *Saccharomyces cerevisiae* (Sc-10).

Materials and methods

Microorganisms used

The microorganisms *Pseudomonas mendocina* (Ps-1) and *Saccharomyce cerevisiae* (Sc-10) were used, from the Collection of Microbial Cultures of the Faculty of Biology, University of Havana and selected for having levels of zinc and cadmium ion removal above 20 mg/g, reported previously [16].

Metal solutions

Cd (II) and Zn (II) solutions were prepared in sterile bidistilled water, from salts of cadmium chloride tetrahydrate ($CdCl_2 \cdot 4H_2O$) and zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), both at the concentration of 1 mM/L, and their pH was adjusted to a value of 6.0.

Microbial culture

Bacteria was cultured in Nutrient broth and nutrient agar medium [17] and yeats in Yeast glucose medium (5 g yeast extract, 10 g glucose, 1 L distilled water; pH 7.0) [17]. Briefly, preinoculums were prepared by microbial inoculation of 50 mL of the respective liquid medium cultures in erlenmeyers of 250-mL effective volume. The flask were inoculated with a loop from solid medium cultures either in Nutrient agar (bacterium) and Yeast extract-glucose agar (yeast), and incubated at 30 ± 2 ºC for 4 h under agitation (100 rpm). Biomasses were propagated in erlenmeyers flasks of 500 mL effective volume, filled with 200 mL of the given liquid medium for each type of microorganism as used for preinoculation. The flasks were inoculated from each preinoculum at 5 % of the final volume. Two cultures were done per microorganisms, in order to provide enough material for the experiments of metal ion removal.

Biosorption experiments

Cultures in nutrient broth and yeast-glucose extract were obtained for the bacteria and yeast, respectively, which were incubated at 30 °C in an orbital screen (Infors HT, Labotron, Switzerland) at 100 rpm for 24 h. Each culture was precipitated and washed with double distilled water by centrifugation at 3500 *g* for 20 min in a Labofuge 200 centrifuge, Kendro Laboratory, Germany. Subsequently, the microbial biomasses were contacted with the individual metals solutions in a biomass-metal ratio of 2 g/L: 1 mM/L. The microorganism-metal suspension was stirred in orbital screen at 100 rpm at 28 °C, the pH maintained at 6.0 with 0.1 M HCl or 0.1 M NaOH. At 24 h the supernatant was harvested at 3200 *g* for 20 min, in Eppendorf Centrifuge (Sigma 1-14, Sigma Laborzentrifugen, Germany). After the biosorption experiments the residual metal was determined to 1 mL of the supernatant by atomic absorption spectrometry. In the control of the biosorption process solutions of the metals were used, at the concentrations established in each experiment, without adding the biomass and maintaining the same experimental conditions of the samples.

Residual metal analysis

Residual metal samples were analyzed on an air flame-acetylene atomic absorption spectrometer (Philips PU 9100X, The Netherlands). The lamp current used for 8 mA for Cd (II) and 10 mA for Zn (II). Wavelengths of 228.8 nm and 230.9 nm were used for Cd (II) and Zn (II), respectively. The instrumental conditions were optimized for achieving maximum sensitivity. Reagents of analytical purity or higher were used. Standard solutions of Cd (II) and Zn (II) were prepared by dilution of standard certified reference solutions of 1000 µg/mL; (Merk, Darmstadt, Germany). Deionized water with conductivity less than 0.05 µS/cm (Milli-Q Ultrapure Water, Millipore, Bedford, MA, USA) was used. All samples were analyzed according to the instructions of the equipment manufacturer.

The amount of metal captured by microbial biomass (q: mg of metal per gram of biomass, expressed as mg/g) was calculated by the mass balance equation for biosorbents, as reported in the literature [13].

Test of the biosorption of metals under different experimental conditions

The conditions established in the biosorption experiment, previously described, were maintained and only the analyzed factor was varied as appropriate, as follows.

pH

The initial pH of the solutions of the metals was adjusted to values of 5.0; 6.0 and 7.0 by using 0.1 M HCl or 0.1 M NaOH. Later on, the microbial biomass was added and in each biomass-metal slurry the initial pH value was maintained up to 24 h by addition of the acid or base as required.

Initial concentration of the metal

The solutions of the metals were used at the following concentrations 1; 1.5 and 2 mM/L.

Determination of the physiological age of the culture

The capture of metals was carried out with microbial biomass collected in two different physiological stages, corresponding to the 14 and 24 h of growth. Microbial growth in nutrient broth for bacteria and yeast-glucose extract for yeast, incubated at 30 °C in orbital screen (Infors HT, Labotron, Switzerland) at 100 rpm for 24 h, was estimated by a spectrophotometer (JENWAY 6305 Spectrophotometer, UK) at λ = 640 nm and at different time intervals of 2 h, against non-inoculated medium used as target. Growth curves were determined by relating absorbance, measured periodically, against time [18].

Biomass inactivation

Cell pellets collected by centrifugation, after 24 h of growth, were inactivated by dry heat at 60 °C in a furnace for 12 h and then brought into contact with the solutions of metals.

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Statistical analyses

All the experiments were run in triplicate. Experimental data were tested by ANOVA and normal distribution and variance homogeneity were determined by the Kolmogorov-Smirnov and Bartlett's tests, respectively. The amount of captured metal per gram of biomass (q mg/g) was evaluated by one-tailed ANOVA. Then, statistically significant results were analyzed by the Tukey's test run *a posteriori*, by comparing means at a significance level of 0.05. Results were processed with the aid of the Microsoft Excel® software and the statistical package Statistica for Windows, version 6.1. The error of the mean was expressed as standard deviation.

Results

Zinc and cadmium removal values (Table 1) show that the bacterium *P. mendocina* (Ps-1) and the yeast *S. cerevisiae* (Sc-10) showed capacity for capturing both metals. The bacterium reached removal values higher than 22 mg/g and yeast above 27 mg/g, which were higher in the capture of cadmium ions.

Figure 1 shows the influence of pH on the removal of metals by bacterium and yeast. The lowest zinc and cadmium capture was observed at pH 5.0 values, followed by an increase by both microbial biomasses at pH 6.0. The increase of this variable at pH 7.0 caused the cation capture values to decrease, except for cadmium which did not show significant differences in yeast removal at pH 6.0 and 7.0.

The effect of metal concentration in solution on the microbial removal process is shown in Table 2. The two microorganisms tested achieved the highest capture of zinc and cadmium at the concentration of 1.5 mM/L, with removal values of more than 34 mg/g of zinc and 41 mg/g of cadmium. Statistical analyses indicated that at the higher concentrations a different behavior is evident for each metal in both microorganisms, since zinc capture decreases, maintaining cadmium removal stable.

Figure 2 shows that zinc and cadmium capture levels by *P. mendocina* (Ps-1) did not show significant differences between the two physiological stages analyzed, corresponding to the final stage of exponential growth (14 h) and to the Stationary growth phase (24 h). A similar behavior was detected in cadmium removal by *S. cerevisiae* (Sc-10). Nevertheless, yeast reached statistically higher capture values against zinc ions with cells grown up to 24 h.

Cellular inactivation caused by dry heat pretreatment, applied to bacteria and yeast biomass, provided increases in zinc and cadmium ion sequestration. This favorable effect in the removal of metals is shown in Table 3, where it is observed that the dead biomass of *P. mendocina* (Ps-1) and *S. cerevisiae* (Sc-10) had higher capacity for the capture of cadmium ions.

Table 1. Removal of Cd (II) and Zn (II) from aqueous solution by microbial biomass

Microorganisms	Cd (II) q (mg/g)	Zn (II) q (mg/g)
Pseudomonas mendocina (Ps-1)	22.56 ± 0.25	25.36 ± 1.78
Saccharomyces cerevisiae (Sc-10)	34.26 ± 1.27	27.06 ± 0.92

Removal conditions: 28 °C, pH 6.0, 1 mM/L of metal in solution, 2 g/L biomass, agitation at 100 rpm for 24 h. q: mg of metal captured per g of biomass. Results are shown as means ± standard deviation (n = 3).

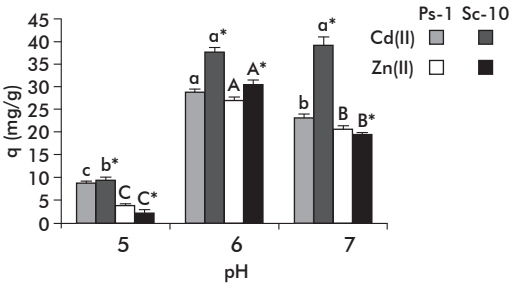

Figure 1. Effect of pH on Zn (II) and Cd (II) removal by microbial biomass of *Pseudomonas mendocina* (Ps-1) and *Saccharomyces cerevisiae* (Sc-10) in solution. Removal conditions: 28 °C, pH 6.0, 1 mM/L of metal in solution, 2 g/L biomass, agitation at 100 rpm for 24 h. q: mg of metal captured per g of biomass. Error bars stand for standard deviation (n = 3) of each pH determination. One-tailed ANOVA was applied to results of each microorganism. Different letters indicate statistically significant differences of q values as determined by the Tukey's test *a posteriori* ($p < 0.05$).

Table 2. Effect of the initial metal concentration on the ability of microbial biomass for removal of Zn (II) and Cd (II) from aqueous solution

Microorganisms	Metal initial concentration (mM/L)	Metal removal (mg/g microbial biomass)	
		Cd (II) q (mg/g)	Zn (II) q (mg/g)
Pseudomonas mendocina (Ps-1)	1.0	27.6 ± 2.63 B	23.7 ± 2.7 c
	1.5	41.7 ± 2.32 A	34.9 ± 1.1 a
	2.0	40.7 ± 1.17 A	28.4 ± 1.6 b
Saccharomyces cerevisiae (Sc-10)	1.0	36.4 ± 1.5 B*	25.6 ± 2.6 c*
	1.5	59.2 ± 2.5 A*	37.7 ± 0.5 a*
	2.0	57.2 ± 3.1 A*	30.2 ± 0.8 b*

Removal conditions: 28 °C, pH 6.0, 1 mM/L of metal in solution, 2 g/L biomass, agitation at 100 rpm for 24 h. q: mg of metal captured per g of biomass. Results are shown as means ± standard deviation (n = 3). Different letters stand for statistically significant differences among q values for the different concentrations of each metal ($p < 0.05$), according to the Tukey's test *a posteriori*.

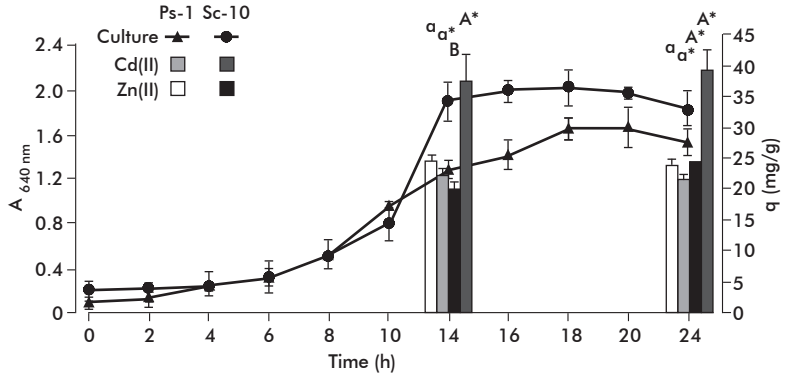

Figure 2. Influence of microbial culture physiology in the ability of *Pseudomonas mendocina* (Ps-1) and *Saccharomyces cerevisiae* (Sc-10) biomass for removal of Cd (II) and Zn (II) in solution. Removal conditions: 28 °C, pH 6.0, 1 mM/L of metal in solution, 2 g/L biomass, agitation at 100 rpm for 24 h. q: mg of metal captured per g of biomass. Error bars stand for standard deviation of three replicates of culture per physiological stage. One-tailed ANOVA was applied to results of each microorganism. Different letters indicate statistically significant differences of q values as determined by the Tukey's test *a posteriori* ($p < 0.05$).

Discussion

The removal of zinc and cadmium by bacteria and yeast is a consequence of interactions between cells and metal ions, based on the properties of each of the microbial species (biochemical, structural, physiological and genetic) and of the chemical characteristics of cations. The capture of these chemical contaminants may be bioaccumulation-mediated [1, 19], a mechanism dependent on cellular metabolism involving living cells, requiring transport systems that internalize the cations to cytosol [20] and by biosorption. In particular, the biosorption mechanism is present in living and dead cells; it is characterized as being passive and requires different functional groups such as carboxyls, hydroxyls, phosphates and amino, components of microbial cell walls. These negatively charged groups act as active metal binding sites through physicochemical interactions that favor the extracellular accumulation of cations [21].

Reductions in the capture of metals at pH 5.0 are related to the protonation of the functional groups. At this pH, competences are established between protons and cations with the ligands of the cell wall and extracellular capture of metal ions is limited. At higher pH values, the retention of metals in biomass is facilitated by the predominance of negative charges on the cell surface, as the amount of H+ decreases [22], which could explain the results at pH 6 and 7, as compared to the 5.0 value. Other authors have supported the increased availability of active sites in the cell wall by increasing pH in the biomass-metal suspension [23, 24]. Nevertheless, a decrease in the capture of the ions at pH 7 was detected, which may be due to the possible formation of hydroxylated complexes of metal ions [25]. Precipitation of metal ions as hydroxides at pH values above 6.0 has been corroborated in previous works [26, 27]. An exception in this behavior was obtained in cadmium capture by *S. cerevisiae* (Sc-10). Yeasts have the ability to accumulate intracellularly high cadmium concentrations by the presence of metallothioneins, cysteine-rich proteins that have high affinity for this ion, compared to others [28]. This capture mechanism may be less dependent on the chemistry of the metal in solution.

The results indicated that metal removal capacity increased and reached saturation with the increased initial concentration of zinc and cadmium ions in the aqueous solution. At low metal concentrations, the interactions between them and the binding sites are favored by the availability of free functional groups located in the cellular envelopes [29]. All this said, this behavior does not remain linear at higher concentrations, where ion retention by microbial biomass decreases or stabilizes due to saturation of the cell surface with cations [3, 30]. The lack of sufficient free functional groups for biosorption, as a consequence of their saturation, favors the availability of non-adsorbed cations in the aqueous solution [31].

Zinc and cadmium ion retention by *P. mendocina* (Ps-1) and *S. cerevisiae* (Sc-10) in different physiological stages or growth phases may be due to the extracellular and intracellular capture of metals. Similar results have been reported for other microbial species in metal capture [23, 32]. In extracellular accumulation, the interactions of ions with active groups of the cell surface [23] are fundamental and can occur throughout the cell cycle. Bioaccumulation requires cellular metabolism [33] and it is therefore associated with a more active physiological state. Metal removal by microorganisms is a complex process that depends on the age of the culture, among other factors [14, 23]. This biotic factor affects the capture of metals because of structural changes in the cell wall or due to the decrease of the entrance of ions into the interior of the cell [34], which can explain the decrease in zinc capture by yeast at 24 h of growth.

Table 3. Effect of dry heat inactivation of the microbial biomass on its ability to remove Cd (II) and Zn (II) from aqueous solution

Microorganisms	Cd (II) q (mg/g)		Zn (II) q (mg/g)	
	Inactivated biomass	Control	Inactivated biomass	Control
Pseudomonas mendocina (Ps-1)	53.96 ± 0.83 a*	21.56 ± 1.21b*	35.1 ± 3.34 a	23.70 ± 1.62 b
Saccharomyces cerevisiae (Sc-10)	54.70 ± 0.51A*	32.59 ± 2.02 B*	30.66 ± 0.28 A	25.06 ± 0.92 B

Removal conditions: 28 °C, pH 6.0, 1 mM/L of metal in solution, 2 g/L biomass, agitation at 100 rpm for 24 h. (q): mg of metal captured per g of biomass. Control: untreated, live biomass. Results are shown as means ± standard deviation (n = 3). Different letters stand for statistically significant differences among q values for the different concentrations of each metal ($p < 0.05$), according to the Tukey's test *a posteriori*.

The increase in metal capture by inactivated or dead cells, compared to the removal abilities of living cells, is the result of the effectiveness of the pre-treatment by dry heat. This physical method could remove impurities present on the cell surface, offer greater surface area and expose intracellular components from the rupture of cellular envelopes. In this manner, a greater exposure of functional groups constituting cation binding sites is facilitated [21, 35]. Pretreatment to microbial biomass by different physical and chemical methods has been supported in literature as an alternative to increase metal removal [35] and in the determination of biosorption mechanisms [36]. Cell inactivation could eliminate any potential harmful effect of *P. mendocina* for human health when applied in large amounts for bioremediation, since it is not a GRAS (generally regarded as safe) microorganism. This may also favor the use of *S. cerevisiae*, which is GRAS certified.

Conclusions

Pseudomonas mendocina (Ps-1) and Saccharomyces cerevisiae (Sc-10) have considerable capacity as biosorbents for the removal of Zn (II) and Cd (II) from aqueous solutions. In metal removal, it was very important to adjust factors influencing the process, which allowed increasing ion capture capacities in both microorganisms. The pH value 6.0 as well as the initial concentration of zinc (1.5 mM/L) and cadmium (1.5 to 2.0 mM/L) resulted in the most favorable operating conditions for removal. Cell inactivation was effective and the increased capture of Cd (II) in 2.5 and 1.7 times by bacterium and yeast, respectively, was highlighted in this condition. Application of dead biomass in the disposal of metal contaminants can be an economically viable, efficient and environment-friendly method to prevent environmental damage caused by zinc and cadmium.

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