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ORIGINAL PAPER

Heavy metal sorption by released polysaccharides and whole cultures of two exopolysaccharide-producing cyanobacteria

Roberto De Philippis · Raffaella Paperi · Claudio Sili

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Abstract The metal removal capacity of cultures of two capsulated, exopolysaccharide-producing cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936, were tested using copper (II) as the model metal. *C. capsulata* cultures removed the greatest amount of copper, with a maximum per unit of biomass (q_{max}) of 115.0 ± 5.1 mg copper g^{-1} of protein, compared with 85.0 ± 3.2 removed with *Nostoc* PCC7936 cultures. Water solutions of pure polysaccharides (RPSs) released into the culture medium by *C. capsulata* and *Nostoc* PCC7936 achieved q_{max} values of 20.2 ± 0.8 mg g^{-1} copper per polysaccharide dry weight with *C. capsulata* RPS and 11.0 ± 1.5 mg g^{-1} with *Nostoc* PCC7936 RPS. Cultures of the two cyanobacteria also removed Zn (II) and Ni (II), in both single-metal systems and in multimetal systems with Cu; in the various single-metal systems more copper was removed than Zn or Ni, while in the multimetal systems a smaller amount of each individual metal was removed but the overall amount of all metal ions sorbed or the amount of copper sorbed in the copper-only system was almost the same with *C. capsulata*, and slightly higher with *Nostoc* PCC7936.

Keywords Metal biosorption · Single-metal system · Multimetal system · Adsorption isotherm

Abbreviation
RPS released polysaccharide

Introduction

Heavy metals are one of the most widespread causes of pollution, both in water and in the soil, and increasing levels of heavy metals in the environment are causing mounting concern in public opinion (Forster and Wase 1997; Ledin 2000). Because of their chemical characteristics, heavy metals cannot be biodegraded by micro-organisms into non-toxic, eventually assimilable or volatile compounds, as is frequently the case with organic pollutants, but they remain in the environment, changing from one chemical state to another, and eventually accumulating in the food chain. For this reason, chemical and physicochemical methods have traditionally been utilized to remove heavy metals from polluted water bodies (Volesky 2001), but such methods have a number of disadvantages: they are not very efficient, in particular at low metal concentrations, and they are

R. De Philippis (✉) · R. Paperi
Department of Agricultural Biotechnology, University of Florence, Piazzale delle Cascine 24, I-50144, Florence, Italy
E-mail: roberto.dephilippis@unifi.it

C. Sili
Institute of Ecosystem Study, CNR, Via Madonna del Piano, I-50019, Sesto Fiorentino, FI, Italy

expensive (Volesky 1994, 2004) Recently, great interest has been aroused by a new technique, biosorption, which exploits the cell envelopes of microorganisms to remove metals from water solutions (Volesky 2001). Biosorption is a passive process of adsorption by either living or dead microbial biomass and it offers some interesting advantages such as (i) low operating cost, (ii) high efficiency in removing metals even from very dilute solutions, (iii) the possibility of recovering the valuable metals sorbed by the biosorbent, and (iv) a lower amount of metal-containing biological sludges that have to be disposed of after treatment (Kratochvil and Volesky 1998). Microbial cells can be viewed as natural ion-exchange material because they have many anionic groups on their cell surface (Kratochvil and Volesky 1998) and this enables them to fix metal ions, mainly by means of an ion-exchange mechanism (Crist et al. 1994; Schiewer and Volesky 1996). In this connection it has been suggested that exopolysaccharide-producing cyanobacteria could also be used for metal biosorption, since most of the polysaccharide envelopes that surround cyanobacterial cells are anionic (De Philippis et al. 2001), and recent studies have indeed reported that the capsulated biomass of two filamentous cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936, has a good metal sorption capacity (De Philippis et al. 2003).

The studies carried out so far have mostly examined the removal of single metals, and only a few have examined multimetal sorption systems (Sağ and Kutsal 1996; Sánchez et al. 1999; Sağ et al. 2000; Pradhan and Rai 2001; Mallick 2003), even though most metal-bearing effluents contain more than one metal (Kratochvil and Volesky 1998). Research has therefore failed to take due account of the possible competitive effects arising from the co-occurrence of a number of metals in solution (Volesky 2003).

The aim of the present study was to determine the metal-removing capacity of whole cultures of two exopolysaccharide-producing cyanobacteria, *C. capsulata* and *Nostoc* PCC7936, and of the released polzyaccharides (RPSs) from those cultures, in both single-metal and multimetal systems, with a view to determining the potential of the cultures or the RPSs for the biosorption of heavy metals from polluted water bodies.

Materials and methods

Cyanospira capsulata and *Nostoc* PCC7936 were axenically grown under laboratory conditions as reported in earlier studies (Vincenzini et al. 1990; De Philippis et al. 2000). The copper removing capacity of the two cyanobacteria was determined using 50 ml aliquots of seven-day-old bacterial cultures, when they were in the linear phase of growth. The aliquots were placed in small dialysis tubes (12 kDa cut-off), dialysed for 24 h against tap water and then dipped for 24 h in 490 ml copper (II) solutions at pH 5.5; a pH of 5.5 was kept constant throughout the experiments by adding 0.1 N HCl when needed. The experiments were carried out in a thermostatic chamber at 25 ± 1°C. Initial copper levels were adjusted to various concentrations ranging from 5 to 15 ppm, to obtain different Cu concentrations at the point of sorption equilibrium; the biomass concentration used in the experiments was in the range 200–400 mg l^{-1} protein, except in the tests on how copper uptake was related to the amount of biomass, where it was in the range 150–680 mg l^{-1} protein. Since the determination of the culture dry weight required very long procedures, due to the viscosity of the cultures themselves, the concentration of the sorbent material in the cultures was routinely determined as the amount of protein, according to the Lowry method (Herbert et al. 1971). Measurements on a large number of cultures of both *C. capsulata* and *Nostoc* PCC7936 showed that the protein content always comprised 40–42% of culture dry weight, which is determined from both the cells and the polysaccharide material. The amount of polysaccharide material released into the culture medium was determined by the phenol–sulphuric acid method (Dubois et al. 1956) after removal of the biomass by centrifugation (14,000×*g*, 20 min). The cultures of the two cyanobacteria were used in the experiments when the amount of RPS in the culture medium was broadly similar to the amount of protein in the biomass. The experiments with the released polysaccharides were carried out using water solutions of pure RPSs, obtained from whole cultures according to the method described by Vincenzini et al. (1990), at RPS concentrations ranging from 0.10 to 3.0 g RPS l^{-1}. Aliquots (50 ml) of the RPS solutions were placed in small dialysis tubes (12 kDa

cut-off), dialysed for 24 h against tap water, and then dipped for 24 h in 490 ml metal solutions at pH 5.5. The viscosity of the aqueous solutions of the RPSs was measured with a Brookfield LVT viscosimeter equipped with a small sample adapter and a cylindrical cell containing a coaxial cylindrical spindle. All experiments were done at least in triplicate and the data shown are the mean values ± standard deviation. The amount of metal removed from a metal solution was calculated as the difference in the metal concentration before and after contact with the cyanobacterial cultures or polysaccharide solutions, as determined with an atomic absorption spectrophotometer (Perkin Elmer, USA). The interference of the Zn (II) and Ni (II) ions competing with copper removal were assessed using initial concentrations of each metal of 5 or 10 ppm.

Preliminary sorption kinetics were determined to establish the exposure time necessary to reach an equilibrium in the sorption process; all the experiments described below were carried out by maintaining the cultures in contact with the metals for 24 h, a time more than sufficient to attain equilibrium.

The data for copper bioremoval were plotted according to isotherm of Langmuir and that of Freundlich, which are the two most commonly used adsorption models for single solute systems (Volesky 1994).

Langmuir adsorption isotherm

$$q = bC_e q_{max}(1 + bC_e)^{-1} \quad (1)$$

which was rearranged as:

$$C_e q^{-1} = (bq_{max})^{-1} + C_e(q_{max})^{-1} \quad (2)$$

where q is the amount of solute (metal) adsorbed per unit mass of adsorbent, expressed as mg g^{-1}; q_{max} is the amount of metal adsorbed at saturation, per unit mass of adsorbent; b is an equilibrium constant, related to the energy of adsorption; and C_e is the equilibrium (final) concentration of the metal in the solution, expressed as mg l^{-1}.

Freundlich adsorption isotherm

$$q = KC_e^{1/n} \quad (3)$$

which was linearized as:

$$\ln q = \ln K + n^{-1} \ln C_e \quad (4)$$

where q is the amount of metal adsorbed per unit mass of adsorbent, expressed as mg g^{-1}; K is a constant, related to the adsorbent capacity; n is a constant related to the energy of sorption; and C_e is the equilibrium (final) concentration of the metal in the solution, expressed as mg l^{-1}.

Results

Copper sorption with whole cultures of the two cyanobacteria started from the first minutes of contact between the metal in solution and the microbial biomass, and proceeded with linear kinetics until the metal removal capacity was saturated: after 9–10 h with *C. capsulata*, and after 12–14 h with *Nostoc* PCC7936 cultures, at this point no more metal ions were removed from the solution (Fig. 1).

The copper removal capacity of *C. capsulata* and *Nostoc* PCC7936 cultures was tested on final copper concentrations (i.e., concentrations reached when the system was in equilibrium) ranging from 2.0 to 12.3 mg l^{-1} (Fig. 2), values obtained by using various initial concentrations ranging from 5 to 15 mg l^{-1}; at the concentrations tested, the amount of copper removed increased with the increase of final metal concentration up to 5.7 mg l^{-1} for *C. capsulata*, and 7.7 mg l^{-1} for *Nostoc* PCC7936, at which point the metal removal capacity of the biomass was saturated. The maximum copper uptake (q_{max}) with these equilibrium concentrations was 115.0 ± 5.1 mg g^{-1} protein for *C. capsulata* cultures and 85.0 ± 3.2 mg g^{-1} protein for *Nostoc* PCC7936 cultures (Fig. 2). The experimental data were also plotted using the two most common adsorption isotherms, that of Freundlich and that of Langmuir [Eqs. (1–4) in Materials and methods]. In the resulting graphs, the linear regression of the data gave straight lines with r^2 values of 0.9651 (*C. capsulata*) and 0.9813 (*Nostoc* PCC7936) for the Langmuir model (Fig. 2), and 0.7555 and 0.9529 for the Freundlich model (data not shown), showing that the Langmuir model best fitted the experimental data.

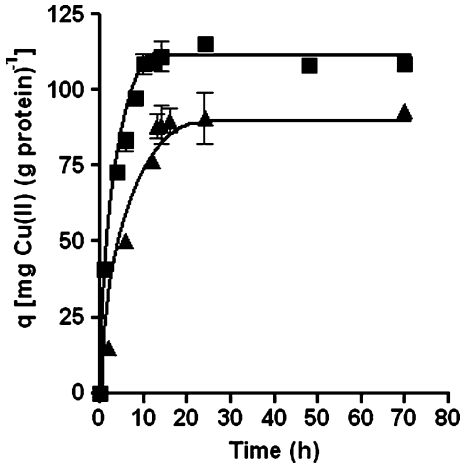

Fig. 1 Time course of copper sorption by *Cyanospira capsulata* (■) and *Nostoc* PCC7936 (▲) whole cultures. (*q*, copper adsorbed per unit mass of adsorbent, expressed as amount of protein; in this and in subsequent figures, symbols represent the mean of at least three replicates and bars represent the standard deviation; bars are shown only if they are larger than the size of the symbols)

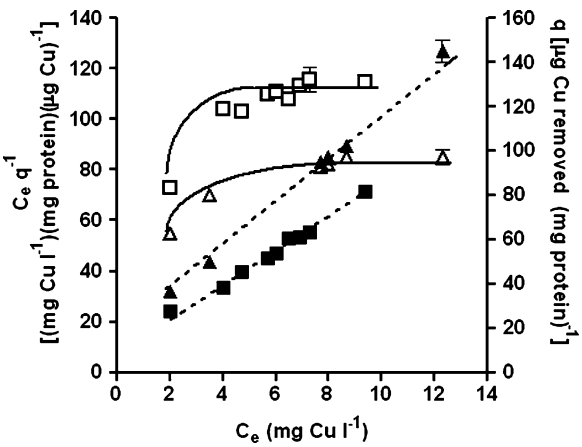

Fig. 2 Copper sorption isotherms (open symbols, right *y* axis) and Langmuir sorption isotherms (closed symbols, left *y* axis) of whole cultures of *Cyanospira capsulata* (□, ■) and *Nostoc* PCC7936 (△, ▲). (*q*, copper adsorbed per mass of adsorbent; C_e, equilibrium (final) concentration of copper in solution)

Using the Langmuir isotherm [Eq. (2) in Materials and methods] the theoretical value of q_{max} was calculated as 144.2 ± 9.1 mg copper g^{-1} protein for *C. capsulata* and 104.7 ± 7.2 mg copper g^{-1} protein for *Nostoc* PCC7936, and the value of parameter b as 0.5745 and 0.5029 for *C. capsulata* and *Nostoc* PCC7936 respectively. The copper removal capacity of the two cyanobacteria was also tested at seven

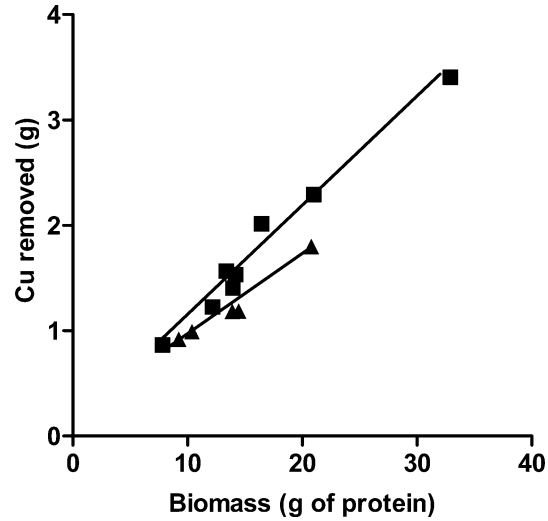

Fig. 3 Copper removed by whole cultures of *Cyanospira capsulata* (■) and *Nostoc* PCC7936 (▲), as a function of the amount of adsorbing biomass

culture concentrations, ranging from 150 to 680 mg l^{-1} of protein, with the same residual copper concentrations; in these experiments (Fig. 3), the average copper uptake was 110.4 ± 6.4 mg g^{-1} protein for *C. capsulata* and 85.8 ± 7.6 mg g^{-1} protein for *Nostoc* PCC7936.

The two cyanobacteria were also tested for their capacity to remove bivalent metallic cations of zinc and nickel, both in single-metal systems and in three-metal systems with copper. In the single-metal systems both cyanobacterial cultures had a lower affinity for Zn and Ni than for Cu, the q_{max} with *C. capsulata* being 41.5 ± 3.0 mg Zn removed per gram of protein and 55.4 ± 3.1 mg Ni, and the q_{max} with *Nostoc* PCC7936 being 32.6 ± 3.0 mg Zn g^{-1} protein and 37.3 ± 2.3 mg Ni (Fig. 4). In the multimetal systems, the q_{max} of each metal was lower than it was with the single-metal system: for copper it was only 61.7 and 77.7% of the copper q_{max} in the single-metal system with *C. capsulata* and *Nostoc* PCC7936 respectively; for Ni the q_{max} was 48.8% with *C. capsulata* and 49.9% with *Nostoc* PCC7936, and for Zn 29.9% with *C. capsulata* and 28.7% with *Nostoc* PCC7936, compared with the corresponding q_{max} in the single-metal systems (Fig. 4). However, when the mmol of metals sorbed per gram of protein was calculated with *C. capsulata*, the overall amount of all metal ions sorbed in a multimetal system was almost the same as that of copper ions sorbed in a copper-only

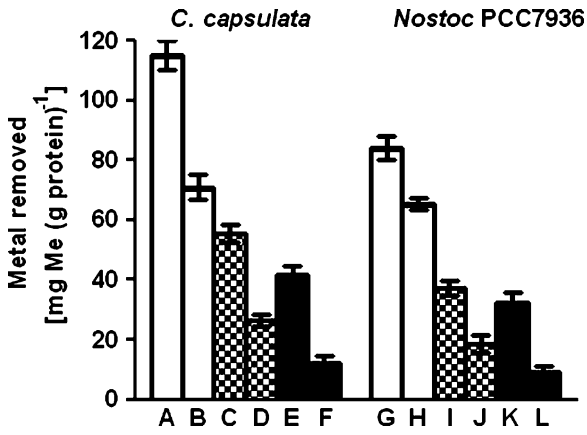

Fig. 4 Metal uptake by whole cultures of *Cyanospira capsulata* and *Nostoc* PCC7936 in single-metal and multimetal system containing Cu, Zn and Ni. Columns A and B, Cu removed by *C. capsulata* in the single-metal (A) and multimetal (B) system; columns C and D, Ni removed by *C. capsulata* in the single-metal (C) and multimetal (D) system; columns E and F, Zn removed by *C. capsulata* in the single-metal (E) and multimetal (F) system; columns G and H, Cu removed by *Nostoc* PCC7936 in the single-metal (G) and multimetal system; columns I and J, Ni removed by *Nostoc* PCC7936 in the single-metal (I) and multimetal (J) system; columns K and L, Zn removed by *Nostoc* PCC7936 in the single-metal (K) and multimetal (L) system

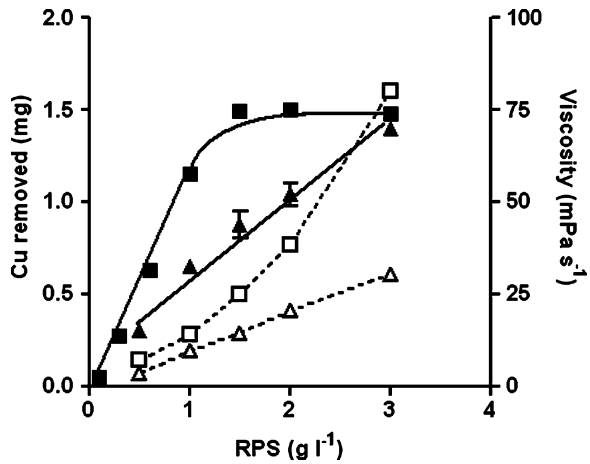

Fig. 5 Amount of copper removed (closed symbols, left *y* axis) and viscosity (open symbols, right *y* axis) in water solutions of pure RPSs produced by *Cyanospira capsulata* (■, □) and *Nostoc* PCC7936 (▲, △)

system (1.76 mmol of the three metals vs. 1.81 mmol of copper per gram of protein), whereas with *Nostoc* PCC7936 the amount of copper ions sorbed was 1.32 mmol g^{-1} protein, somewhat lower than the 1.49 mmol of all metal ions per gram of protein sorbed in the multimetal system.

The copper removal capacity of the polysaccharides released into the culture medium by the two cyanobacteria was tested using water solutions of the pure RPSs. Within the range of concentrations tested, the amount of Cu sorbed by the polysaccharide released by *Nostoc* PCC7936 increased linearly with the concentration of the polysaccharide, while the amount of metal sorbed by the RPS released by *C. capsulata* also increased linearly but only up to a concentration of 1.5 g l^{-1}, corresponding to the maximum copper uptake of about 1.5 mg. This uptake value did not increase further when the RPS concentration in the solution was increased to 3 g l^{-1} (Fig. 5). From the linear part of the graphs, the q_{max} values for the RPSs released by the two cyanobacteria were calculated to be 20.2 ± 0.8 mg copper removed g^{-1} RPS dry weight for *C. capsulata*, and 11.0 ± 1.5 mg copper removed g^{-1} RPS dry weight for *Nostoc* PCC7936. The RPS solutions also showed a significant increase in viscosity with increasing RPS concentration, especially in the case of the RPSs produced by *C. capsulata* (Fig. 5).

Discussion

The kinetics of the metal biosorption process, which is needed to establish the time required to reach the sorption equilibrium (Kratochvil and Volesky 1998), showed that: (i) the adsorption of copper by the two cyanobacteria was a single-phase process, in which the amount of metal removed increased by linear progression over time until the saturation point of the biomass was reached; and (ii) the time until saturation with *C. capsulata* and *Nostoc* PCC7936 cultures was much longer than that with the immobilized biomass of other cyanobacteria reported in other studies (Singh et al. 1989; Blanco et al. 1999; Pradhan and Rai 2000), and was also longer than the time required by the biomass of these cyanobacteria without the soluble RPS fraction (De Philippis et al. 2003). That metal biosorption was a single-phase process was already reported by Blanco et al. (1998) for *Phormidium laminosum* and by Singh et al. (1998) for *Microcystis*. It was explained on the supposition that it is mainly an ion-exchange mechanism that acts at the surface layer of the sorbent and hence does not require any induction time (Singh et al. 1998). In this connection, it should be noted that the polysaccharides of

C. capsulata and *Nostoc* PCC7936, are characterized by large amounts of negatively charged uronic acids (Vincenzini et al. 1990; De Philippis et al. 2000); as such they are quite compatible with metal adsorption by an ion-exchange mechanism. As regards the longer biosorption time until saturation, the slower biosorption rate of whole cultures of the two cyanobacteria as compared with that of the biomasses alone was most probably due to the fact that the diffusion of metal ions in the liquid phase is slow on account of the high viscosity of the culture medium. Indeed, Volesky (2001) stated that the rate of the biosorption process was mainly controlled by the interparticle mass transfer of metal ions, and this process is presumably slowed down by matrix viscosity.

The results indicated that the higher q_{max} of *C. capsulata* cultures makes them more useful for removing large quantities of copper, while *Nostoc* PCC7936, with its lower *b* value, corresponding to a greater affinity for the sorbed metal (Volesky 2004), is more suitable for removing copper at very low concentrations. It is also worth mentioning that, while the effectiveness of biosorbents reported by different laboratories may differ depending on the laboratory testing conditions (Volesky 2004), the metal removal capability of the two cyanobacteria studied were on a par with the best results obtained with other microbial biosorbents so far studied (Garnham 1997; Volesky 2004).

Both cyanobacterial sorbents removed metals from multimetal systems containing Cu, Zn and Ni, but more copper was consistently removed than Zn or Ni. A similar high rate of copper sorption was also found with a *Microcystis* sp capsulated biomass in a multimetal system containing Cu, Zn and Cd; this was explained as due to the high specificity that the active binding sites on the biomass had for copper (Pradhan and Rai 2001). However, in the present study the two biosorbents differed in the amount of metal ions they sorbed in the multimetal system. *C. capsulata* sorbed more or less the same amount of mmol of metal ions in the presence of the three metals together as it did in the presence of copper alone, indicating that there was no significant competition between the different metal ions while they were binding to the biomass active sites; *Nostoc* PCC7936 cultures on the other hand sorbed a slightly greater amount of mmol of metal per unit biomass in the three-component system, suggesting that there were synergistic interactions among the metal ions. Such interactions have been reported for some marine macroalgae as well (Volesky and Holan 1995).

The q_{max} values for copper with whole cultures of *C. capsulata* as calculated with the Langmuir isotherm was greater than that of *C. capsulata* without its RPSs by 18.8 mg g^{-1} protein; for *Nostoc* PCC7936 the difference in q_{max} between whole cultures and cultures lacking RPSs was 11.6 mg g^{-1} protein. (see De Philippis et al. 2003 for the q_{max} values of the biomass without RPS), showing that the RPSs dissolved in the culture medium gave a significant boost to metal sorption. It should be noted that these differences were broadly similar to the q_{max} values obtained with pure RPSs in solution, and that the copper uptake with RPSs from *C. capsulata* was much higher than that of other pure microbial polysaccharides (e.g. xanthan gum) that have been tested for their metal removal capacity (Gutnick and Bach 2000). However, here it should also be pointed out that the RPSs of *C. capsulata* showed a saturation of its metal removal capacity by increasing the concentration of the RPS in solution, probably because of the significant increase in the viscosity of the solution, which hindered the diffusion of the metal ions into the liquid phase.

It is concluded that whole cultures of exopolysaccharide-producing cyanobacteria are a promising new approach to the bioremoval of heavy metals. Though the sorption process with whole cultures was slower than that with a microbial biomass lacking RPSs, it had the important advantages of removing a greater amount of metal and of reducing the number of procedures needed to prepare the biosorbent.

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