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Cadmium, Zinc, Copper, Silver and Chromium(III) removal from wastewaters by *Sphaerotilus natans*

A. Lodi, C. Solisio, A. Converti, M. Del Borghi

Abstract Living cells of *Sphaerotilus natans* are used for heavy *metal's* (Cd, Zn, Cu, Ag, and Cr) removal from aqueous solutions simulating the polluting power of acid industrial wastewaters. At low metal concentrations (<25 mg/l) this microorganism is able to remove within 8–15 days Cd, Zn, Cu, and Ag with excellent yields (from 81 to 99%) often increasing with starting metal concentration. The yield observed for Cr(III) removal, never exceeding 60%, is not appreciably influenced by the starting biomass level; in addition, the time necessary to reach the equilibrium concentration is always remarkably longer (>30 days) than for the other metals. At much higher concentrations, the removal of all the metals is strongly affected in terms of both yield reduction and increase in the time necessary to reach the equilibrium concentrations. Under the hypothesis of mass transfer limitation, the kinetic study of batch runs suggests that metal diffusion from the bulk to the surface of *S. natans* clumps could be responsible not only for the simple biosorption of the tested metallic micronutrients or abiotic metals, but even for the cell penetration by ions of biological significance, like Mg^{2+} and Fe^{3+}.

1 Introduction

Heavy metals are often present together with organic pollutants in industrial wastewaters but may originate also from commercial and domestic activities. Metal plating, tanneries and industrial processes using catalysts yearly discharge worldwide huge amounts of these metals, the most abundant of which are chromium, manganese, iron, cobalt, copper, zinc, molybdenum, silver, mercury, cadmium and nickel. Although heavy metals get concentrated in the primary and secondary sludges during the treatment process of municipal wastewaters [1–3], at the high concentrations of industrial effluents, they exert toxic effects on the sludge microbial population and often cause upset [4].

To meet the water quality standards consistent with environment protection, industrial wastewaters need the simultaneous removal of both organic pollutants and metals. The treatment expenses could be partially or totally covered by the recovery of metals, mainly when precious metals or high metal concentrations are involved. Conventional technology for removing metals from solutions includes precipitation in the form of oxides, hydroxides, carbonates, or sulphides, and separation by settling. This approach, however, has several limitations, such as inefficient precipitation when metals are present as anions or are complexed, final concentration imposed by the solubility product, and formation of unsettleable particles [5]. It has long been recognized that adsorption is capable of removing metals in a wider pH range and to much lower levels than precipitation [6]. Other more expensive physico-chemical treatments, such as redox reactions or ion exchange, are justified only for particular recoveries.

Biological treatments arouse great interest because of their lower impact on the environment with respect to chemical treatments. It has recently been shown that heavy metal leaching using sulphur-oxidizing bacteria can be combined with activated sludge process, giving the so-called Simultaneous Sludge Digestion and Metal Leaching (SSDML) process [7]. The insertion of a separate section for biological metal removal in an aerobic wastewater treatment plant is also under consideration [8].

Five of the most important heavy metals commonly contaminating industrial wastewaters from electrolytic plants are considered in this study, namely, Cd, Zn, Cu, Ag, and Cr(III). Although zinc proved to be one of the less toxic heavy metals for anaerobic digestion [9], methane production rate [4] as well as human metabolism, mainly in children and in patients with metabolic disfunctions, are surely affected at high concentrations. Copper and zinc are also used in the paper manufacture, copper in the synthesis of pesticides and herbicides, zinc and cadmium in the processes for the productions of polymers, plastics, resins and fibers. Finally, chromium is a pollutant present in tannery wastes.

Many microorganisms are able to remove heavy metals from wastewaters but there is no agreement on the action mechanism of this phenomenon, which appears to depend on the microorganism used. For example, some authors observed that ions are removed from aqueous solutions in the presence of either living or dead cells [10, 11]. For this reason, two different mechanisms were proposed: a) intracellular uptake by an active process requiring an expenditure of energy [12], according to which cells behave as actual biological agents, and b) adsorption by

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complexation with negatively-charged groups of cell wall, where biomass can be considered as a simple adsorbent. Cation binding with cell wall polysaccharides of both procariotes and eucariotes can be schematized according to the mechanism [13]:

$$2NaR + Me^{2+} \rightarrow MeR_2 + 2Na^{+} \quad , \qquad (1)$$

where Me^{2+} is a bivalent metal ion and R the polysaccharide molecule.

Enterobacter cloacae reduced hexavalent chromium to insoluble trivalent chromium, thus promoting the indirect removal of this toxic metal from the medium [14], while the fungus *Rhizopus arrhizus* was able to remove La^{3+}, Cd^{2+}, UO^{2+} and Ag^{+} with satisfactory yields [15]. Although Norberg and Persson successfully employed *Zoogloea ramigera* for Cd, Cu and U removal [16], this microorganism, which forms around the cell a matrix of excreted negatively-charged polysaccharides, seems to be able to remove metals only temporarily [8]. Since a rapid release takes place after the achievement of maximum removal yield, the residence time of an eventual biological unit for metal removal from wastewaters should rigorously be controlled to ensure acceptable yields.

The filamentous bacterium *Sphaerotilus natans*, naturally present in sewage sludges and polluted waters [17], is tested in this study for the removal of Cd^{2+}, Zn^{2+}, Cu^{2+}, Ag^{+} and Cr^{3+} from pure solutions, at relatively high concentrations and acidic conditions simulating the composition of common industrial wastewaters. This choice has been dictated by the ability of this microorganism to form a protein-polysaccharide-lipid sheath, which is responsible for the binding and accumulation of metal ions in the form of a superficial mucilage layer. In this way, it accumulates Fe, Mg, Cu, Co, Cd, Ni and Cr [12, 18, 19]. In addition *S. natans* is characterized by a very long doubling time (6–10 h), by the presence of subpolar flagella that give motility and possibility to strike ions, and by optimum growth temperature and pH of 25–30 °C and 7.0, respectively. Finally, even though it is an aerobe, it has been shown to grow in the presence of dissolved oxygen concentrations as low as 0.1 mg/l.

2
Materials and methods

2.1
Microorganism

The cells of *Sphaerotilus natans* were obtained from National Collection of Industrial and Marine Bacteria Ltd (Aberdeen, Scotland) and grown for 36–48 h in shake flasks containing 0.5% yeast extract and 0.5% peptone in tap water and kept at 30 °C.

2.2
Metal uptake medium

Variable amounts of $CdSO_4$, $ZnSO_4$, $CuSO_4$, Ag_2SO_4, and $Cr_2(SO_4)_3$ were added to the growth medium up to the selected starting metal concentrations. Since the growth of *S. natans* is not affected by the presence of sulphates but was inhibited by chlorides [18], 5 M sulphuric acid has been used to ensure a starting pH of 5.0.

2.3
Operating conditions

Batch tests were carried out in 1 litre-shake flasks at 30 °C and 150 rpm. Concentrations of metals lower than 25 mg/l were initially used at a relatively low starting biomass level ($X_0 = 0.5$ g/l) in order to preliminarly screen the capability of *S. natans* cells to remove the selected metals in pure solutions. Although the pH increased up to 6.0–7.5 according to the runs, no adjustment of this parameter was operated in order to avoid any interference with the intrinsic biomass activity. Additional tests were then carried out increasing both starting metal and biomass concentrations in order to check the possible occurrence of inhibition due to high metal levels and to calculate the related toxicity thresholds. Triplicate flasks were used for each test, one of which was employed as control flask without inoculum. Simultaneous controls were also carried out using the same solutions in the absence of metal ions. Two millilitres of liquid phase were drawn everyday for determination of dissolved ions.

2.4
Analytical procedures

Samples were filtered through 0.2 μm-Millipore filters in order to remove solid particles and then were analyzed to determine the ion concentration by an atomic absorption spectrophotometer (Model 5000 Perkin-Elmer). Solids concentration was parallely measured by the dry weight method, while the content of metals adsorbed onto or bioaccumulated by cells (m) was determined by the same procedure after biomass dissolution with a mixture of 5 N HNO_3 and 5 N H_2SO_4 at 70 °C. Biomass concentration was calculated as the difference between the total solid content, previously determined, and the metal content of cells. Maximum metal uptake capacity (q) was determined by the decrease in metal concentration in the solution after addition of different amounts of biomass and equilibration.

3
Results and discussion

Although it is known that optimum acidity conditions for *S. natans* growth are met around neutrality, batch tests have been carried out at low pH values to avoid any interference of precipitation on biological removal of metals as well as to simulate the actual composition of industrial wastewaters from electrolytic plants.

3.1
Low starting metal concentrations

Metal levels lower than 25 mg/l were initially tested to avoid any interference due to possible metal inhibition on Cd, Zn, Cu, Ag, and Cr(III) removal kinetics. Figs. 1–5 show the progressive decrease of the concentrations of these metals in the solution versus time. The maximum removal rates were obtained at the beginning of each test and equilibrium concentrations higher than zero were achieved at the end of each run, whose values depended on both metal type and starting level.

Table 1 shows the most significant values of the related removal yields, Y, and equilibrium concentrations, C_f. A

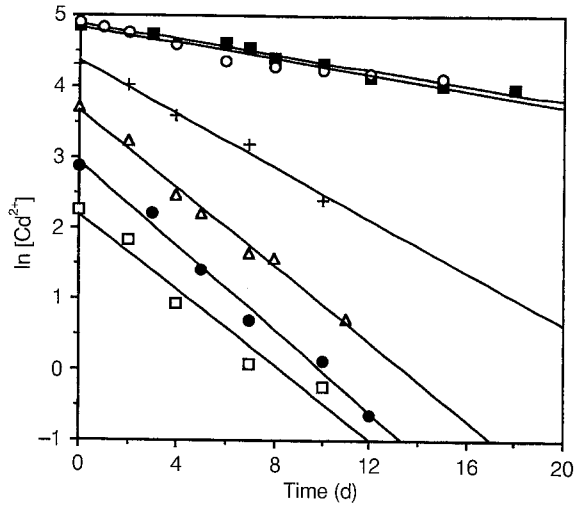

Fig. 1. Cd removal from aqueous solutions by living cells of *S. natans*. C_{mo} (mg/l): (□) 9.5; (●) 18.0; (△) 40.7; (+) 75.0; (■) 127.2; (○) 135.6

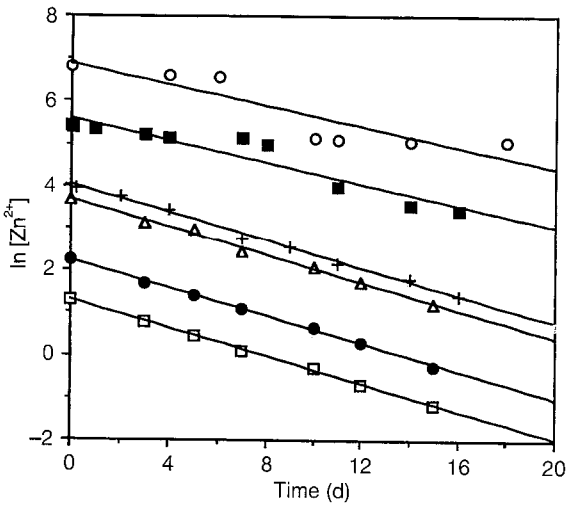

Fig. 2. Zn removal from aqueous solutions by living cells of *S. natans*. C_{mo} (mg/l): (□) 3.6; (●) 9.5; (△) 39.4; (+) 55.7; (■) 223.9; (○) 901.4

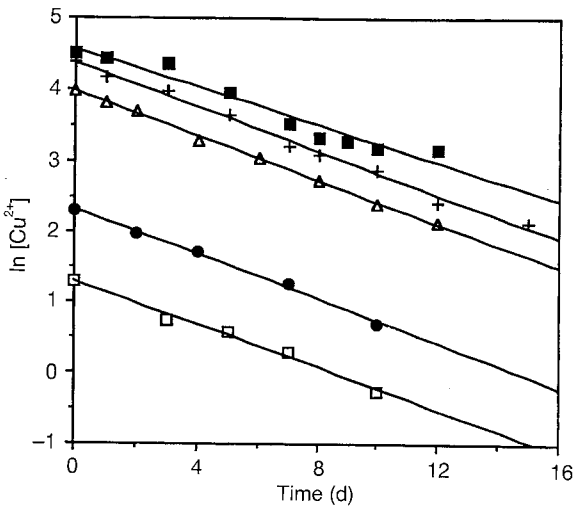

Fig. 3. Cu removal from aqueous solutions by living cells of *S. natans*. C_{mo} (mg/l): (□) 3.6; (●) 10.0; (△) 52.9; (+) 79.8; (■) 89.3

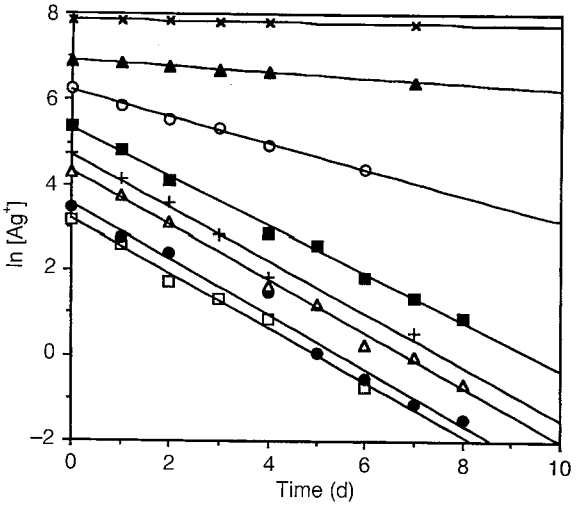

Fig. 4. Ag removal from aqueous solutions by living cells of *S. natans*. C_{mo} (mg/l): (□) 24.0; (●) 32.0; (△) 75.0; (+) 114.1; (■) 218.0; (○) 521.2; (▲) 996.9; (×) 2545

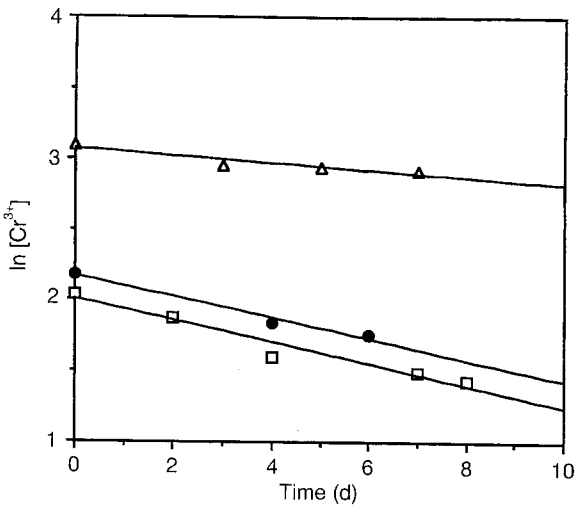

Fig. 5. Cr(III) removal from aqueous solutions by living cells of *S. natans*. C_{mo} (mg/l): (□) 7.7; (●) 8.9; (△) 22.0

yield of 46% was obtained after 8 days at C_{mo} = 7.7 mg/l for Cr removal, while even 30 days were necessary to increase the yield up to 58% (results not shown). No relevant change of maximum removal yield was observed when the contact time was prolonged further, thereby demonstrating that this microorganism is able to retain this metal for a reasonably long time, without any activity loss as that observed for *Zoogloea ramigera* [8] or *S. natans* in the presence of ethanol as inhibitor [19]. This behaviour should be considered as an important advantage in the design of an accessory biological unit for metal removal from industrial wastewaters, because it would allow to face eventual residence time oscillations, without the risk of efficiency fall as a consequence of metal release.

As far as chromium is concerned, if its uptake were the result of simple physical adsorption onto the cell sheath, an increase in the total amount removed of this metal as well as a corresponding decrease in final equilibrium concentration should be detected by increasing the

Table 1. Yields of metal removal, Y, and equilibrium concentrations, C_f, obtained for Cd, Zn, Cu, Ag and Cr(III) at different C_{mo} values

Cadmium					
C_{mo} (mg/l)	9.5	18.0	40.7	75.0	135.6
Y (%)	93.7	95.8	93.9	93.3	57.2
C_f (mg/l)	0.6	0.8	2.5	5.0	58.0
Zinc					
C_{mo} (mg/l)	3.6	18.0	55.7	223.9	901.4
Y (%)	94.5	95.8	94.6	86.6	82.2
C_f (mg/l)	0.2	0.8	3.0	30.0	160.4
Copper					
C_{mo} (mg/l)	3.6	10.0	52.9	63.0	89.3
Y (%)	80.8	90.0	88.6	88.9	73.3
C_f (mg/l)	0.7	1.0	6.0	7.0	23.8
Silver					
C_{mo} (mg/l)	24.0	32.0	75.0	218.0	997.0
Y (%)	96.9	99.2	99.3	99.1	47.8
C_f (mg/l)	0.7	0.3	0.5	2.0	520.4
Chromium(III)					
C_{mo} (mg/l)	3.5	7.7	8.9	22.0	
Y (%)	48.9	58.4	54.9	23.6	
C_f (mg/l)	1.8	3.2	4.0	16.8	

starting amount of biomass. This was not the case, the removal yield of chromium having not been appreciably influenced by a relevant inoculum increase, at the same starting metal concentration (C_{mo} = 22.0 mg/l). This result could be explained with the possible existence of a very low toxicity threshold beyond which the cell surface suffers a modification which prevents any further uptake of this metal, independently of the metal/biomass concentration ratio. As shown in Table 2, the maximum chromium(III) content of biomass, m (both adsorbed and eventually bioaccumulated), and the maximum uptake capacity, q, were at C_{mo} = 22.0 mg/l only 0.4% and 7.4 mg per gram of inoculum, respectively.

Table 2. Uptake capacity, q, and metal content of cells, m, obtained for Cd, Zn, Cu, Ag and Cr at different C_{mo} values

Cadmium					
C_{mo} (mg/l)	9.5	18.0	40.7	75.0	135.6
q (mg/g_{xo})	8.9	17.3	38.2	70.0	114.2
m (%)	0.50	0.96	2.13	3.91	4.33
Zinc					
C_{mo} (mg/l)	3.6	18.0	55.7	223.9	901.4
q (mg/g_{xo})	3.4	–	43.9	319.8	741.4
m (%)	0.22	1.10	3.46	10.8	21.8
Copper					
C_{mo} (mg/l)	3.6	10.0	52.9	63.0	89.3
q (mg/g_{xo})	5.9	10.0	93.8	112.0	130.6
m (%)	0.36	1.12	5.86	7.00	8.15
Silver					
C_{mo} (mg/l)	24.0	32.0	75.0	218.0	997.0
q (mg/g_{xo})	46.5	31.7	99.3	288.0	477.0
m (%)	1.29	2.03	4.51	15.8	34.5
Chromium(III)					
C_{mo} (mg/l)	3.5	7.7	8.9	22.0	
q (mg/g_{xo})	3.1	6.4	7.0	7.4	
m (%)	0.13	0.35	0.38	0.40	

More promising results have been obtained for the other metals under the same conditions (C_{mo} < 25 mg/l), whose maximum removal yields at X_0 = 0.5–1.0 g/l ranged from 90 to 97% within 8–15 days of contact time, depending on the metal under consideration, namely 90% for Cu, 96% for Cd and Zn, and 97% for Ag. Maximum percentages of these metals in the cells were around 1.0% on dry weight basis, namely 0.96% Cd, 1.10% Zn, 1.12% Cu, and 1.29% Ag. These preliminary results at low starting metal levels pointed out that uptake efficiency by *S. natans* decreases according to the following order: Ag > Zn ≅ Cd > Cu ≫ Cr.

The time resistance of this microorganism was then tested at higher concentrations of metals (25 < C_{mo} < 75 mg/l). All metals except Cr were removed at relatively high concentrations, with maximum yields ranging from 89 to 99%, within 5–15 days, depending on the metal. Hence, an increase in concentration up to the selected levels appreciably accelerated the process of metal uptake without affecting the yield. For Ag removal a yield increase from 97% to 99% was even observed. This behaviour is consistent with the hypothesis of Hatch and Menawat for uptake by *S. natans*, who put the observed first-order kinetics of Co, Mg, Cu, Fe, Cd, Ni, and Cr removals down to mass transfer limitation of diffusion. Biomass metal contents increased up to 3.9% Cd, 3.5% Zn, 7.0% Cu, and 4.5% Ag, which suggested to investigate removal capability of *S. natans* at higher concentrations.

3.2
High starting metal concentrations

Additional tests carried out at higher metal levels offered different results depending on the metal under consideration. The drastic reduction from about 94% to 57% observed for Cd removal yield at C_{mo} = 135 mg/l (Table 1) may be due either to saturation of biomass adsorption power or to possible inhibition of biomass activity due to high metal levels, with a toxicity threshold around 100 mg/l. In addition, the time required to reach the final equilibrium concentration, corresponding to the maximum removal yield, passed from 12 days for the tests at lower starting metal levels to about 20 days. Although the differences between the removal yields and the contact times to reach C_f of the last two series of tests are not so marked for both Zn and Cu, the hypothesis of a biologically active mechanism suggests the existence of a toxicity threshold located around 224–566 mg/l for the former metal and around 80 mg/l for the latter. The first value would be in reasonable agreement with the inhibitory threshold reported for Zn in anaerobic digester (400 mg/l) [9].

Zn concentration was then strongly increased up to C_{mo} = 2,545 mg/l; a lag-phase appeared in the curves of metal removal similar to the well-known substrate inhibition taking place in carbon-limited bioprocesses. During these tests and during the related simultaneous metal-free controls pH increased up to 6.0–7.5, as previously observed by Kuyucak and Volesky for Co [12]. Since this effect was progressively less marked when using

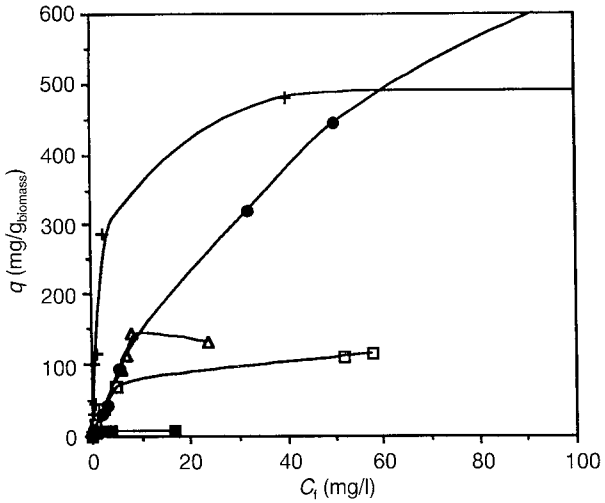

Fig. 6. Isotherms for metal uptake by living cells of *S. natans*. (□) Cd; (●) Zn; (△) Cu; (+) Ag; (■) Cr(III)

lower starting metal levels, it could probably be related to strong changes in the ionic strength of the solution as well as to the formation of salts and hydroxides binding the cell sheath.

3.3 Metal equilibrium uptake studies

Metal uptake capacity of *S. natans* cells, q, was evaluated for each metal by the related isotherm, plotted in Fig. 6 assuming that biomass concentration did not vary considerably during each test, i.e. considering biomass as a simple adsorbent. The curves of this figure show that uptake capacity increases with final equilibrium concentration up to a maximum value, q_{max}, which depends on the metal under consideration. Beyond this value the behaviour of Zn and Ag uptakes differs from those observed for common adsorbents (results not shown), exhibiting a progressive capacity decrease up to a new threshold. A possible explanation of this behaviour is that metal removal is on the whole a combination of two different phenomena: the physical adsorption onto the biomass, which is independent of both the state of biomass (living or not) and the starting metal concentration [12], and an active mechanism of biosorption, related to the cell activity of producing negatively-charged surface polymers and consequently strongly affected by metal toxicity. At very high equilibrium concentrations, Zn and Ag removal capacity by *S. natans* could be the result of the simple adsorption power in the absence of any residual biological activity. Both mechanisms would be consistent with the observation that biomass recycled from successive Zn removal tests progressively lost its capacity of removing ions, probably because of a decrease in the active surface of cells available for the adsorption (Table 3). In other words, the active mechanism of biosorption could not necessarily be associated to the introduction of metals into the cell.

From the values of C_f and q listed in Tables 1 and 2 respectively, the following scale of toxicity on *S. natans* can be deduced for the tested heavy metals: Cr(III) > Cd > Cu > Zn > Ag. It is quite different from that found by Hickey et al. for an anaerobic digester [4], probably because of the interplay and the different resistance of the various microorganisms present in that heterogeneous population. Unfortunately, the introduction of the concept of maximum adsorption power separated from the maximum uptake capacity does not allow any comparison among the data available in the literature for other heavy metals, because the related isotherms were either constructed using non-living biomass or stopped before the attainment of q_{max}.

Table 3. Zn removal by *S. natans* cells recycled in three successive batch removal tests

Test	1	2	3
C_{mo} (mg/l)	901.4	535.3	1536.5
Y (%)	82.2	81.4	21.2
C_f (mg/l)	160.4	99.8	1210.4
q (mg/g$_{xo}$)	741.4	453.9	326.1
t (d)[a]	12	13	20

[a] Time required to reach C_f

3.4 Kinetics of metal removal

The above data of Cd, Zn, Cu, Ag, and Cr(III) removals at different conditions are now used to carry out a kinetic study aiming, from one hand, at providing the parameters necessary to correctly design a unit for metal removal from industrial wastewater and, from the other hand, to provide further information on the actual mechanism involved in this process.

To this purpose, the kinetic approach followed by Hatch and Menawat [18] has been extended in this work to Zn^{2+}, Cr^{3+}, and Ag^{+} uptakes as well as to increased starting levels of Cd and Cu. The average first-order rate constants, k, calculated at low C_{mo} values for Cd (0.012 h^{-1}) and Cu (0.006 h^{-1}) are close to those calculated by the above authors (0.015 h^{-1} and 0.007 h^{-1}). In addition, as the values of the kinetic parameters listed in Table 4 show, no appreciable dependence of k on starting metal concentration is observed up to C_{mo} = 98 mg/l for Zn, 41 mg/l for Cd, 80 mg/l for Cu, 218 mg/l for Ag, and 9 mg/l for Cr. On the contrary, the relevant decrease in the regression coefficient at higher concentrations suggests that an inhibition phenomenon similar to substrate inhibition could be responsible for marked deviations from first-order kinetics. The yield of biomass produced was very low during these tests, which confirms the validity of having assumed a relatively constant biomass concentration during the above equilibrium uptake tests.

As suggested by the above authors, since first-order kinetics cannot be justified by growth limitation, which would imply an increase of rate constant with biomass growth, mass transfer limitation is the only reasonable explanation of the observed first-order kinetics. So, mass transfer to a spherical surface (*S. natans* clumps) can be described by the relationship:

$$-\frac{dC_m}{dt} = \frac{2\pi d_s D_m}{V_L}(C_m - C_{ms}) \quad , \qquad (2)$$

Table 4. First-order kinetic constant of metal removal by living cells of *S. natans*

Cadmium						
C_{mo} (mg/l)	9.5	18.0	40.7	75.0	127.0	135.6
$k \cdot 10^3$ (h^{-1})	11.1	12.3	11.4	7.8	2.2	2.3
r^2	0.960	0.991	0.991	0.987	0.968	0.922
Zinc						
C_{mo} (mg/l)	3.6	9.5	39.4	55.7	98.3	223.9
$k \cdot 10^3$ (h^{-1})	6.8	6.8	6.8	6.8	7.2	5.3
r^2	0.998	0.998	0.994	0.996	0.897	0.795
Copper						
C_{mo} (mg/l)	3.6	10.0	52.9	63.0	79.8	89.3
$k \cdot 10^3$ (h^{-1})	6.2	6.6	6.4	6.3	6.4	5.5
r^2	0.998	0.998	0.994	0.996	0.897	0.795
Silver						
C_{mo} (mg/l)	24.0	32.0	75.0	114.0	218.0	521.0
$k \cdot 10^3$ (h^{-1})	26.5	27.0	26.5	25.9	23.6	12.8
r^2	0.991	0.980	0.995	0.984	0.996	0.994
Chromium(III)						
C_{mo} (mg/l)	7.7		8.9		22.0	
$k \cdot 10^3$ (h^{-1})	3.2		3.1		1.05	
r^2	0.949		0.974		0.872	

r^2 = regression coefficient

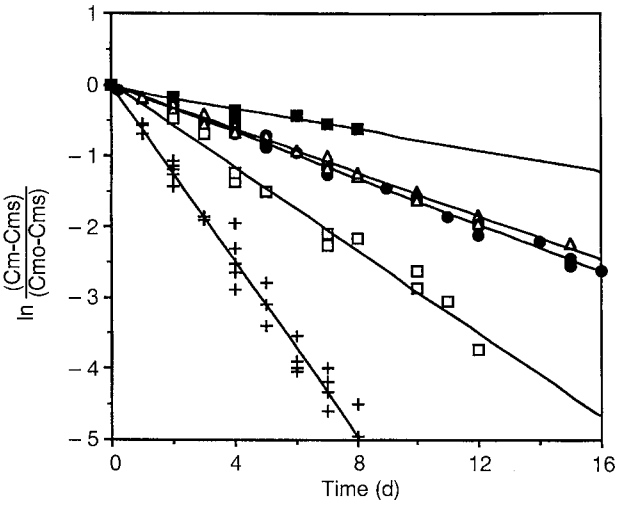

Fig. 7. Batch tests of metal uptake by *S. natans* cells. k_d and C_{ms} estimations by Eq. (3). (□) Cd; (●) Zn; (△) Cu; (+) Ag; (■) Cr(III)

where V_L is the liquid volume, C_m and C_{ms} the metal concentrations in the solution and at the surface of *S. natans* clump, D_m the approximate effective molecular diffusivity, and d_s the mean diameter of the clump. Integration of this equation gives:

$$\ln\left(\frac{C_m - C_{ms}}{C_{mo} - C_{ms}}\right) = -k_d t \ , \qquad (3)$$

where:

$$k_d = 2\pi d_s D_m / V_L \ , \qquad (4)$$

is the diffusion rate constant.

Making reference to the same hypotheses and conditions assumed by Hatch and Menawat [18], approximate D_m have been estimated for the different metals at various C_{mo} from the related values of k_d. These were in turn calculated from the straight lines resulting by application of Eq. (3) to the experimental data of C_m and t (Fig. 7), while C_{ms} values were estimated by regression analysis. The results of this analysis are listed in Table 5.

All the parameters calculated for Cu are in good agreement with those reported for the same metal ($k_d = 7 \cdot 10^{-3}$ h^{-1} and $C_{ms} \approx 0$) [18], while the extension of this approach also to higher concentrations of Cd has led to a value of k_d of only 0.012 h^{-1} (instead of 0.014 h^{-1}) and to a coincident value of C_{ms} (0.1 mg/l). D_m values calculated in this work are within a factor of 2 of the actual molecular diffusivities for these metals [20], which would confirm that the insolubilization process could actually be mass transfer limited.

Comparison of these results with those reported by Hatch and Menawat [18] shows that C_{ms} is always zero (except for Cd) for metallic micronutrients (Co, Zn and Cu) or metals not utilized by the cell and higher than zero for macronutrients (Fe and Mg). This behaviour suggests that the first kind of metals would reach the clump by simple diffusion and then would be adsorbed onto the sheath. No additional transfer by diffusion would be possible because of the absence of a concentration gradient between the surface of the clump and the cell inside. This could be the result of a sort of negative chemiotattic response of this microorganism to these heavy metals, which would not be able to enter the cell. The second class of metals (such as Mg and Fe), on the contrary, in addition to simple adsorption, could be subject to transfer by diffusion into the cell, made possible by positive values of C_{ms}, besides the presence of specific carriers.

Another interesting finding is that k_d sharply decreases with increasing the number of net positive charges of the ions, which means that the negatively-charged groups present on the cell surface are saturated by ion exchange more effectively by Cr^{3+} than Ag^+ ions. Zn^{2+} and Cu^{2+} show nearly coincident intermediate values not only be-

Table 5. Average diffusivities, D_m, and diffusion rate constants, k_d, calculated by Eqs. (3) and (4), under the hypothesis of mass transfer limitation

Metal	C_{mo}[a] (mg/l)	$k_d \cdot 10^3$ (h^{-1})	$D_m \cdot 10^5$ (cm^2/s)	r^2
Cadmium	9.5–40.7	12.2	4.06	0.975
Zinc	3.6–98.3	6.8	2.27	0.994
Copper	3.6–79.8	6.4	2.13	0.992
Silver	24–218	25.7	8.52	0.980
Chromium(III)	7.7–8.9	3.1	1.02	0.938

[a] Range of starting metal concentration within which the calculation has been worked out, r^2 = regression coefficient

cause both are provided with two net positive charges but likely also because they are characterized by very close atomic weights (65.4 and 63.5 g/mol, respectively).

The above mentioned anomalous behaviour of Cd^{2+} is now confirmed by a k_d value (0.012 h^{-1}) remarkably higher than those calculated for Zn^{2+} and Cu^{2+}, having the same number of charges, which is in contrast with what was expected from its much higher atomic weight (112.4 g/mol). An explanation of this strange result can be proposed taking in mind that the outer electrons of Cd^{2+} belong to a successive period of the periodic table of the elements with respect to Zn^{2+} and Cu^{2+}; since they are less strongly attracted by the nucleus, their interaction with the negative charges on the clump surface could take place at a remarkably longer distance, thereby reducing the diffusion length. The large size of Cd^{2+} ions could also imply, for the complete saturation of the negative charges, the existence of a thicker layer of ions around the clumps, thus providing a reasonable explanation also of the unexpected $C_{ms} > 0$ value. Anyhow, due to the insufficient concentration gradient between the surface and the cell inside, besides the absence of specific carriers for this metal, it is difficult to believe that it could actually pass through the cell wall.

4 Conclusion

Pure solutions of Cd, Zn, Cu, Ag, and Cr(III) sulphates at different starting concentrations were submitted to batch tests of metal uptake in the presence of living cells of *S. natans* at constant temperature. The growth of this microorganism under the tested conditions was so slow as to justify the assumption of a nearly constant biomass level in the system, which has been treated as a common biosorbent. The isotherms obtained for Cd, Zn, Cu, and Ag show that the uptake capacity increases with final equilibrium concentration up to a threshold depending on the type of metal. Beyond this value the capability of this microorganism to remove Zn and Ag decreases up to a new threshold, likely due to the toxicity exerted by heavy metals on biomass. The kinetic elaboration of the present results according to the hypothesis of mass transfer limitation demonstrates that metal concentration at the surface of *S. natans* cells is zero for metallic micronutrients or metals not utilized by the cell, thereby suggesting that metals reach the clump by simple diffusion without entering the cell inside, while Mg and Fe, besides the simple adsorption, could be transferred by diffusion into the cell in the presence of specific carriers. Approximate diffusivities calculated by this approach are in the same order of magnitude as the values reported in the literature, which suggests that mass transfer limitation could actually be the cause of the observed first-order kinetics.

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