Cadmium, Zinc and Chromium(III) Removal from Aqueous Solutions by *Zoogloea ramigera*

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A strain of *Zoogloea ramigera* was used in batch tests for Cd, Zn and Cr removal from solutions simulating the composition of industrial wastewaters. Living cells of this microorganism proved to remove metal ions from pure solutions more effectively (91–99%) than inactivated biomass (50% or less), thus confirming the predominant biological nature of this process. The removal yield strongly decreased when these metals were simultaneously present in the solution, probably because of a possible competition phenomenon. The evaluation of the related kinetic parameters would allow to design an accessory section for heavy metals removal in a wastewaters treatment plant.

Key words:
Zoogloea ramigera, heavy metals removal, industrial wastewaters.

Introduction

One of the most serious problems associated to biological treatment of industrial effluents is the sludge sensitivity to toxic heavy metals[1,2]. They have been treated up to now mainly by physico-chemical methods, such as chemical precipitation[3], adsorption[4], redox reactions, ion exchange, and so on. Nevertheless, the use of biological treatments has aroused great interest in the recent past because of their less impact on the environment as well as the possibility of recovering metals.

Many microorganisms are able to remove heavy metals either by intracellular uptake or by adsorption onto negatively-charged groups of cell wall. A good adsorber for metal removal by the latter way should exhibit: a) effective and quick uptake and release cycle; b) low cost; c) possibility of regeneration and recycling; d) capacity of flocculating; e) resistance against releasing reagents; f) selective release. Adsorption can take place either in the presence or in the absence of a carbon source as well as using either living or non-living biomass[5], which has given rise to suspicion about its mere biological nature. Molybdenum and cobalt have been removed by algae[5], uranium by algae, actynomycetes, a lot of bacteria and yeasts[6], lead and uranium by lyophilized cells of *Streptomyces longwoodensis*[7]. In addition, *Enterobacter cloacae* has reduced hexavalent chromium to insoluble trivalent chromium, thus promoting the indirect removal of this toxic metal from the medium[8], while the fungus *Rhizopus arrhizus* has proved able to remove, with satisfactory yields, La, Cd, U and Ag[9]. Finally, *Sphaerotilus natans* has removed Fe, Mg, Cu, Co, Cd, Ni and Cr[5,10,11] thanks to its ability of forming a protein-polysaccharide-lipid complex extending from the outer membrane of cells.

Norberg and Enfors have demonstrated that *Zoogloea ramigera*, a rod-shaped Gram-negative bacterium flocculating during late-log growth, is able to form a matrix around the cell consisting of excreted negatively-charged polysaccharides when cultivated in the presence of excess carbon source[12]. Although sensitive to low concentrations of heavy metals, this microorganism has been successfully employed for metal removal. An extensive study on *Z. ramigera* as biosorbent for Cd^{2+}, Cu^{2+} and UO^{2+} has been presented by *Norberg and Persson*[6], who have demonstrated that: a) both uptake and release phases are very rapid; b) the efficiencies of uptake and acid (HCl) release increase with repeating flocculations and with decreasing pH, respectively; c) a moderate mixing is required to minimize biomass loss during separation; d) uranium was already adsorbed at pH 3.5, Cu around pH 5.5 and Cd at pH 6.5.

This behaviour on the whole suggests us to use this bacterium for a continuous metal removal and recovery from wastewater in a common aerobic sewage treatment plant. The batch tests presented in this study have been carried out in order to gather the data necessary for the design of a biological treatment accessory device able to improve heavy metal removal.

Materials and methods

The microorganism used in this study is *Zoogloea ramigera* (NCIMB, 11941). It was cultivated in the following medium: tryptone 2.0 g l^{-1}; beef extract 0.5 g l^{-1}; sodium acetate 0.2 g l^{-1}; distilled water 1.0 litre (pH 7.0). The cells were cultivated at 25 °C in Erlenmeyer flasks, shaken at about 40 shakes min^{-1} under microaerophilic conditions

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(cotton caps), and then harvested after 120 h of incubation by centrifugation at 5000 rpm.

Batch tests were carried out in Erlenmeyer flasks containing heavy metals dissolved in solutions at pH 5.0 and having different initial concentrations; the inoculum was about 0.7 g l^{-1} (dry mass). Samples were withdrawn every 10 min during the first hour and every 30 min for the rest of each experiment to follow the decrease of heavy metal concentrations in the solutions. After microfiltration through 0.45 μm-Millipore filters, the liquid was analysed for metal concentration using an Atomic Absorption Spectrophotometer (Perkin-Elmer mod. 5000), while the entrapped mucilaginous residue was used for the determination of biomass concentration by dry mass. As expected, no appreciable growth was detected during the batch tests. Metal content of biomass was sometimes checked by the same procedure after dissolution of biomass with a mixture of 5 mol l^{-1} HNO_3 and 2.5 mol l^{-1} H_2SO_4 at 70 °C.

Two different sets of experiments were carried out. At first, the ability of the microorganism to remove single metal ions (Cd^{2+}, Zn^{2+}, and Cr^{3+}) was checked using the corresponding sulphates. Successively, a solution containing all three metals was tested to ascertain the existence of possible interaction among ions or competition. Additional tests were carried out using cells inactivated with a few drops of formic acid in order to compare the removal ability of dead biomass with the one of living cells.

Results and discussion

Since heavy metals are toxic to the biomass present in activated sludges, much effort is being increasingly addressed towards the selection of resistant microorganisms, which could be able to remove them. In particular, *Z. ramigera*, which is naturally present in activated sludges in the form of typical mucilaginous complexes, has proved to effectively remove several metals[6]. For this reason, it has been selected and tested in this work for the removal of Cd, Zn and Cr, either from pure solutions or from mixtures simulating the composition of industrial effluents (Table 1).

Table 1 – *Typical compositions of industrial effluents from metal treatment (data from ref. 16).*

pH	0.7— 2.0
Total solids (%)	11.6-19.1
Iron (%)	0.5— 5.8
Zinc (%)	0.1— 0.3
Lead (%)	≤ 0.02
Chromium (%)	≤ 6.2
Cadmium	present
Nickel	present

Figure 1 shows the experimental data of the concentrations of these metals in mixture, at pH 5.0, during batch tests which lasted up to 1.0 h. A rough comparison of these curves with the data of maximum specific growth rate reported in the literature for most of the microorganisms present in activated sludges[13] shows that the main problem for heavy metals removal by *Z. ramigera* in a sewage treatment plant is the large difference between the optimum residence times for metal uptake (20–60 min) and activated sludges (>> 60 min). This would suggest to operate a continuous process at the longer residence time.

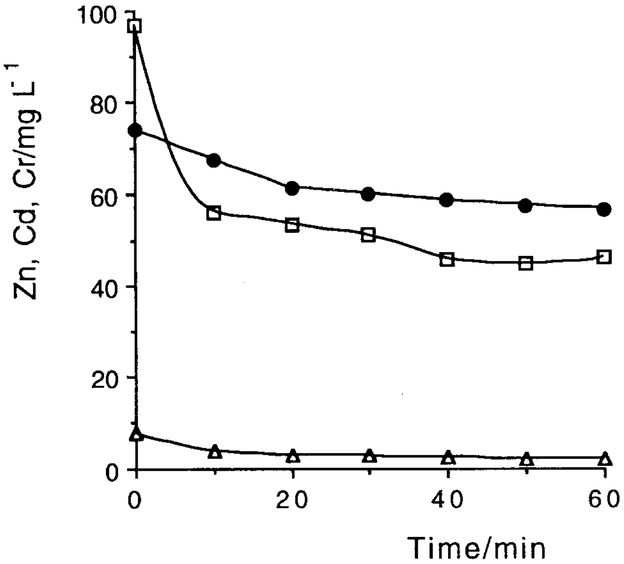

Fig. 1 – *Simultaneous Cd (●), Zn (□), and Cr(III) (Δ) removal by living cells of* Z. ramigera. *Data referred only to the 1st h of batch removal tests*

To verify whether biomass removal ability keeps constant during the time required to ensure a good efficiency of the activated sludge process, and then to correctly design a continuous sewage plant containing sludges enriched with this microorganism, a further set of batch tests lasting up to 4 days has been carried out using single metal solutions (Figures 2-4). The time behaviour of metal concentrations in the solution confirms the above results obtained in mixture, the optimum time for the removal of all three metals being about 30 min.

The results collected during the first hour of contact time compare reasonably with those presented by *Norberg and Persson* for Cd at a concentration about ten times higher[6]. In addition, a comparison with the above results shows that a reduction of about 90% of starting Cd level unables *Z. ramigera* to remove this metal at pH values progressively lowering. Nevertheless, for times longer than 10 h, an evident release of all metals can be observed. This result suggests that

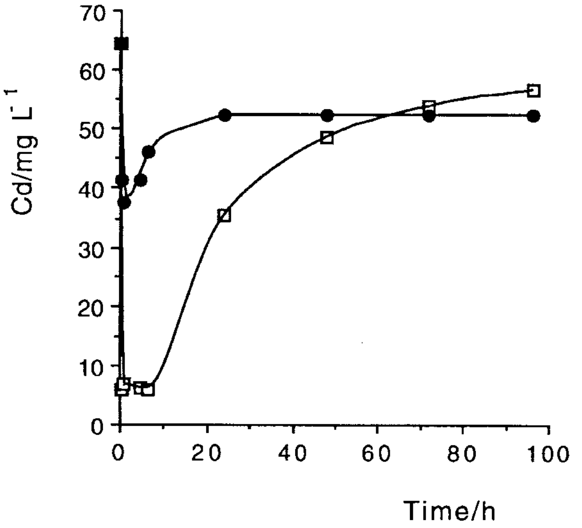

Fig. 2 – *Cd uptake and release by (□) living and (●) inactivated cells of* Z. ramigera

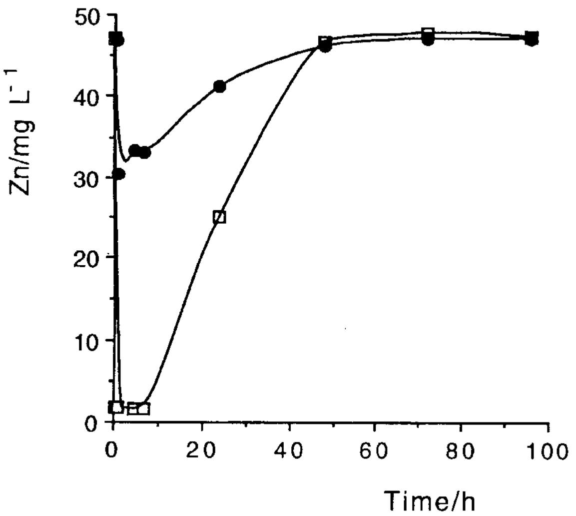

Fig. 3 – *Zn uptake and release by (□) living and (●) inactivated cells of* Z. ramigera

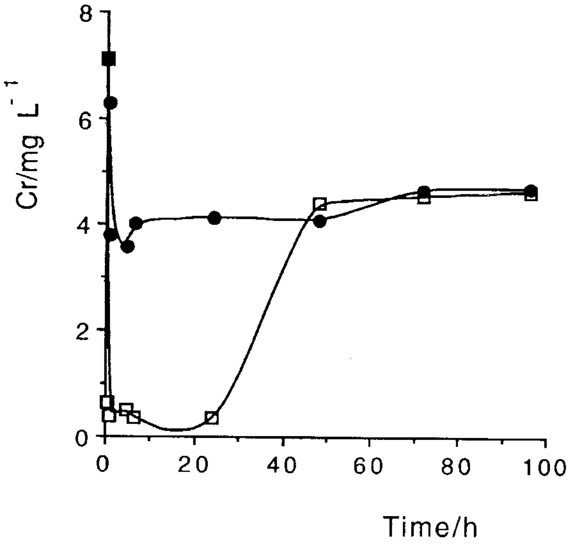

Fig. 4 – *Cr(III) uptake and release by (□) living and (●) inactivated cells of* Z. ramigera

the residence time usually employed in activated sludge plants is inconsistent with the requirements of the metal uptake process and would provide a possible explanation of the limited capacity of the traditional treatment plants to remove these pollutants[14,15]. Thus, a separate section, eventually connected with sludge recycle, could actually improve metal uptake by enriched sludges.

The removal capacity of the biomass inactivated with formic acid is also evident in Figures 2-4, while Table 2 summarizes the values of maximum removal yield obtained for all three metals after about 0.5-1.0 h of contact time. Although the observed yield was surely influenced by starting metal concentrations (intentionally selected to simulate the composition of typical industrial wastewaters), the results of Table 2 show that the living biomass is very effective in uptaking all three metals (91–99%), while the inactivated one is not able to ensure removal yields higher than 50%.

Table 2 – *Cadmium, zinc, and chromium removal yields (%) by* Z. ramigera *from single metal solutions, after 0.5–1.0 h of contact time.*

	Living biomass	Inactivated biomass
Cadmium	91	42
Zinc	99	36
Chromium	95	50

In addition, the removal from the solutions containing single metals is much more effective than when metals are simultaneously present in the wastewater; in fact, in the latter case removal yields of only 54, 70, and 23% can be calculated from the experimental data shown in Figure 1 for Zn, Cr, and Cd, respectively. This behaviour can be explained with a competition among the three metals for the same amount of adsorbent material (γ_{0X} = 0.7 g l^{-1}).

However, the most surprising result seems to be the resistance of *Z. ramigera* to chromium, that has been scarcely tested up to now. Chromium appeared to keep retained by the biomass for quite a longer time than the other two metals. To confirm this result, a further run of simultaneous removal of Cr, Zn, and Cd has been extended for more than 12 h (Figure 5). Both Cd and Zn were nearly completely released after 6 h while only 74% of the chromium uptaken during the first 20 min was released within 12 h. This behaviour can be the result of the resistance of the selected microorganism to chromium, at least at the low starting concentrations tested in this study. The yield observed for Cd removal compares with the 80% yield obtained by the above authors un-

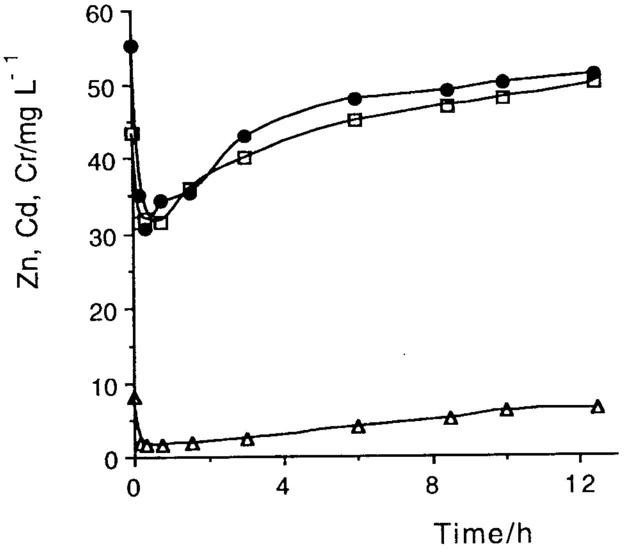

Fig. 5 – *Simultaneous Cd (●), Zn (□), and Cr (Δ) uptake and release by living cells of* Z. ramigera

Table 3 – *Metal levels in the cells of* Z. ramigera, *expressed as percentage with respect to total biomass dry mass.* γ_{0X} = *0.7 g* l^{-1}.

	After biosorption		After release	
	Living biomass	Inactivated biomass	Living biomass	Inactivated biomass
Zinc (%)	6.59	2.42	0.02	0.04
Time (d)*	1.0	1.0	–	–
Chromium (%)	0.97	0.49	0.36	0.35
Time (d)*	1.0	1.0	4.0	4.0
Cadmium (%)	8.29	3.92	1.14	1.76
Time (d)*	1.0	1.0	4.0	4.0

* Time at which maximum metal percentages in the cells were observed

der similar inoculum conditions, but at quite higher pH and starting metal concentration.

Table 3 shows the data of maximum concentrations of these metals in the cells after both phases of biosorption and acid release. Zn and Cd were retained after 1 day of contact time with living cells up to a relevant percentage of total biomass dry weight (6-8%), which suggests that both these metals could predominantly be uptaken by the cells. On the contrary, the low levels of Cr detected within the cells (<1%) could be ascribed to a physico-chemical precipitation promoted by simple adsorption onto biomass. This hypothesis would be consistent with the lower percentages of all three metals detected when inactivated biomass was used.

As the uptake phenomenon is probably due to metal accumulation onto the cell surface, an improvement of metal removal yield should be expected from an increase in cell mass concentration. For this reason, further experiments are being performed at both higher and lower inoculum levels.

Considering the large difference between the optimum residence times for metal removal and for municipal wastewater aerobic treatment, the accessory section of Figure 6, containing activated sludge enriched with *Z. ramigera*, can reasonably be added to a traditional wastewater treatment plant in order to improve heavy metal removal. The next work also will deal with the setting up and continuous testing of a laboratory-scale equipment simulating this process.

Conclusions

The present study confirms that *Zoogloea ramigera* is a very interesting microorganism to improve metal removal efficiency of traditional aerobic wastewater treatment plants. The opti-

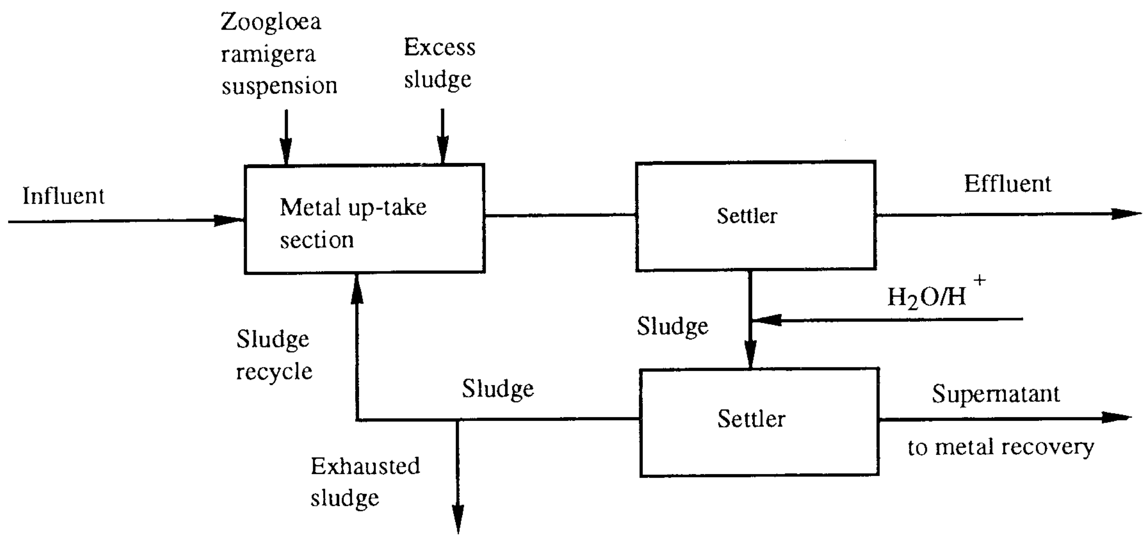

Fig. 6 – *Scheme of wastewater treatment plant provided with additional section for heavy metal removal*

mum residence time for Cd, Zn and Cr uptake by cells is always around 20–60 min, with no significant dependence on the fact that these metals are simultaneously present in the effluent or not, while the removal yield is appreciably higher when single metal solutions are used. Cd and Zn are rapidly removed with excellent yields, at optimum pH values progressively increasing with starting metal concentration, while after about 10 h both metals start to be released. On the contrary, chromium appears to keep retained by biomass for quite a longer time than the other two metals.

All three metals are simultaneously removed very effectively only by living biomass, whereas cells inactivated with formic acid are not able to ensure removal yields higher than 50%, which suggests that the removal process is the result of a combination of physico-chemical and biological events.

The addition of an accessory section, containing activated sludge enriched with *Z. ramigera*, is proposed to improve metal removal in a traditional wastewater treatment plant.

References

1. *Hickey, R.F., Vanderwielen, J., Switzenbaum, M.S.*, Wat. Res. **23** (1988) 533
2. *Hayes, R.F., Theis, T.L.*, J. Wat. Pollut. Control Fed. **50** (1978) 61
3. *Benjamin, M.M., Sletten, R.S., Bailey, R.P., Bennett, T.*, Wat. Res. **30** (1996) 2609
4. *Benjamin, M.M.*, Environ. Sci. Technol. **17** (1983) 686
5. *Kuyucak, N., Volesky, B.*, Biotechnol. Bioeng. **33** (1989) 809
6. *Norberg, A.B., Persson, H.*, Biotechnol. Bioeng. **26** (1984) 239
7. *Friis, N., Myers-Keith, P.*, Biotechnol. Bioeng. **28** (1986) 21
8. *Komori, K., Rivas, A., Toda, K., Ohtake, H.*, Biotechnol. Bioeng. **35** (1990) 951
9. *Tobin, J.M., Cooper, D.G., Neufeld, R.J.*, Biotechnol. Bioeng. **30** (1987) 882
10. *Hatch, R.T., Menawat, A.*, Biotechnol. Bioeng. Symp. **8** (1978) 191
11. *Converti, A., Fiorito, G., Zilli, M., Lodi, A., Del Borghi, M., Ferraiolo, G.*, Bioproc. Eng. **7** (1992) 325
12. *Norberg, A.B., Enfors, S.-O.*, Appl. Environ. Microbiol. **4** (1982) 1231
13. *Roels, J.A.*, Energetics and Kinetics in Biotechnology, Elsevier, Amsterdam, 1983
14. *Stephenson, T., Lester, J.N.*, Sci. Total Environ. **63** (1987) 199
15. *Stephenson, T., Lester, J.N.*, Sci. Total Environ. **63** (1987) 215
16. *Bridgwater, A.V., Mumford, C.J.*, Waste Recycling and Pollution Control Handbook, George Godwin Ltd, London, 1979