HEAVY METALS BIOSORPTION BY COMPOST-ASSOCIATED MICROORGANISMS: PRACTICAL APPLICATIONS

M.J. López, M.C. Vargas, F. Suárez, G. Guisado, J. Moreno
Unit of Microbiology. Department of Applied Biology. CITE II-B.
University of Almería.04120 Almería. Spain. mllopez@ual.es

ABSTRACT

The biosorption of a mixture of Zn^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} from aqueous solution by composted and uncomposted horticultural wastes was investigated. The involvement of natural microbiota from biosorbents in metals removal was also analysed. Physicochemical sorption of all metals occurred mainly during first hour contact between metal contaminated water and biosorbent. Microorganisms-mediated detoxification predominated for Cd^{2+} and Ni^{2+} at longer times. Temperature, pH and nutrients influenced this microbial activity. The modification of these factors increased removal of Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} by microorganisms. Biosorbents analysed have potential for low-cost remediation of heavy metals.

INTRODUCTION

Industry, mining and agricultural activities have lead to large scale contamination of the environment with toxic heavy metals. Several treatments are available for metal remediation, but most are expensive to apply and lack specificity (Lloys and Lovley, 2001). Biosorption is an alternative that offers many advantages including highly selective removal of toxic metals coupled with considerable operational flexibility (Eccles 1999). This method implies the use of natural substrates such as agricultural wastes or peat and involves physicochemical together with biological mechanisms for metal decontamination (Lloys and Lovley, 2001).

The present work is focussed on exploring the feasibility of using horticultural plant wastes (HW) and composted HW as biosorbents for the removal of a mixture of the heavy metals Zn^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} from aqueous solution. The possible enhancement of the metals adsorptive capacity by stimulation of microbial activity is also analysed. The utilisation of these natural products may provide effective, low-cost alternatives to chemical or other biological mechanisms for heavy metal removal. Such use would also afford a new market for waste products that are generated in abundance in the southeast of Spain and currently landfilled or burned.

MATERIALS AND METHODS

Horticultural plant wastes (HW) and mature compost (HWC) obtained from them were used as biosorbents for the removal of heavy metals from aqueous solutions. For HW preparation, sun dried pepper plant wastes were ground and sieved to 2 mm particle size. Compost with same particle size was obtained from melon plants composted for 4 months using aerated piles.

Metal uptake was examined by the addition of 0.5 g of biosorbent material to 50ml of metal solution in 250ml Erlenmeyer flasks containing 50 mg/l of each Zn^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} as nitrate salts. The pH of metal solutions was adjusted to 6.0 using 1N HNO_3. Flasks were incubated at 120 rpm and 30ºC for a maximum of 72 hours, unless otherwise indicated. All experiments were performed in triplicate and several controls were run concurrently to correct data or to allow for determination of the effects analysed. These controls included: biosorbent-free, microorganisms-free (sterile) and metals-free controls. For microorganisms-free controls preparation, biosorbent was autoclaved at 121ºC for 45 min and added of sterile filtered metal solution. Biosorbent-free controls included metals solutions but not biosorbent material. Metals-free controls consist on biosorbent and water.

The effect of initial pH, temperature and presence of nutrients on metals removal was examined. For pH assay, the initial metals solution was adjusted to desired values, 3, 5 and 6 using NaOH 1N or HCl 1N and not controlled afterwards. Incubation temperature varied from 25 to 40ºC. Nutrients experiments were performed by addition to metals solution of 1% (w/v) glucose, 0.02% (w/v) NH_4NO_3 or both together.

After the desired incubation period, microorganisms number and metals concentration were analysed. The number of microorganisms in suspension was analyzed by serial 10-fold dilutions in sterile saline solution (NaCl, 0.9 % (w/v)) and plate counts on nutrient agar medium (Difco) after 24-48 hours incubation at 30ºC. The concentrations of metal ions in aqueous phases separated from materials by filtration were measured by ICP-MS (inductively coupled plasma-mass spectroscopy).

RESULTS AND DISCUSSION

All metals analysed were removed to some extent from aqueous solution by the two bioadsorbents, but HW-compost showed a slightly higher ion-sequestering capability than HW (Table 1). About 50% of Cr, Ni, Zn and Cd and 80% of Cu and Pb were eliminated after one hour contact between adsorbent and solution. Similar removal levels were obtained by Senthilkumaar et al. (2000) after 2 hours. This metal adsorption was due to physicochemical processes as indicated by the comparable levels of residual metals in sterile and non-sterile samples. The disappearance of Ni and Cd after 72 hours was caused by the microbial activity but this activity did not have any effect in the concentration of the other metals.

Table 1. *Metal concentration in solution after different contact time with biosorbents**

Biosorbent	Contact time	Metal residual concentration (mg/l)					
	(h)	Cr(III)	Ni(II)	Cu(II)	Zn(II)	Cd(II)	Pb(II)
HW-Compost	1	21.9	23.4	7.9	20.5	24.6	5.8
	72	21.8	9.3	7.8	20.2	12.7	5.8
HW-Compost esterile	1	25.5	27.8	7.9	21.7	21.1	4.5
	72	25.3	21.9	7.9	21.7	13.5	4.5
HW	1	24.7	37.6	9.3	26.3	15.4	11.0
	72	24.5	24.8	9.1	26.0	9.9	10.8
HW esterile	1	29.3	37.4	9.6	31.4	16.7	11.1
	72	29.1	29.1	9.4	31.2	10.7	11.0

** Grey colour indicates significant variations at a level of $p<0.05$. Biosorbents: HW-compost (compost from horticultural wastes), HW Horticultural wastes. Conditions: Temperature 30ºC; pH 6, metal ion concentration Zn^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} 50 mg/l, biosorbent concentration 10mg/ml, agitation 120 rpm.*

Figure 1 shows the effect of metals on growth of naturally occurring biosorbents microorganisms. The presence of metals did not affect significantly the amount of microorganisms. Similar growth levels were obtained in suspensions of both biosorbents with and without metals.

Microbial numbers were higher in suspensions of plant wastes (HW) than in compost (HWC).

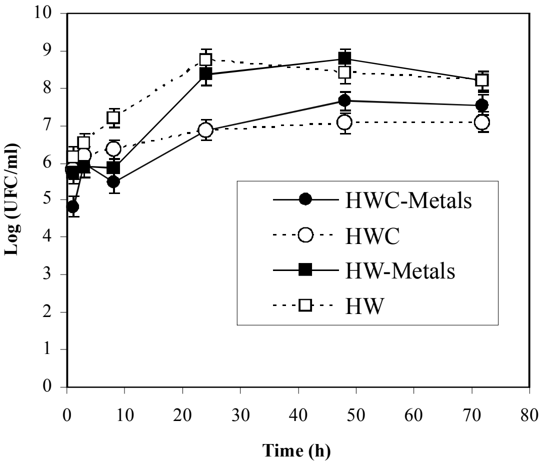

Figure 1. *Comparison of microbial growth on suspension of compost (HWC) and horticultural wastes (HW) with and without heavy metals in aqueous solution. Conditions: Temperature 30ºC; pH 6, metal ion concentration Zn^{2+}, Cr^{3+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} 50 mg/l, biosorbent concentration 10mg/ml, agitation 120 rpm.*

Medium pH affects the solubility of metal ions and the ionisation state of the functional groups but also influences microbial viability and growth. This factor had a clear effect on Cu, Zn and Pb removal (data not shown). The disappearance of these metals from solution increa-

Table 2. *Effect of nutrients addition on ions removal between 1 to 72 contact†*

Biosorbent	Nutrient added*	Removal (%)					
		Cr(III)	Ni(II)	Cu(II)	Zn(II)	Cd(II)	Pb(II)
HW-Compost	None	1.3	60.6	0.8	1.3	34.6	1.3
	Glucose	0.6	65.8	1.0	71.9	90.5	68.7
	NH_4NO_3	0.2	76.0	47.9	94.8	91.9	79.5
	GN	0.2	69.7	35.2	93.4	92.3	89.0
HW-Compost (sterile)	None	0.5	22.3	0.4	0.1	37.3	1.8
	Glucose	1.0	21.0	0.6	0.4	38.7	0.2
	NH_4NO_3	0.1	22.0	0.1	0.3	33.0	1.3
	GN	0.1	21.5	0.2	1.6	33.3	0.4
HW	None	1.4	15.2	7.8	2.0	1.0	9.2
	Glucose	0.4	31.6	4.6	61.3	14.7	24.4
	NH_4NO_3	1.6	36.7	5.2	73.5	33.0	53.2
	GN	1.0	29.6	8.0	38.2	40.9	51.9
HW (sterile)	None	0.4	22.3	0.5	0.7	0.4	0.4
	Glucose	0.5	20.7	1.0	2.0	0.8	0.7
	NH_4NO_3	1.0	25.6	1.7	0.4	1.2	2.6
	GN	1.7	18.7	0.9	0.9	1.3	1.3

*† Grey colour indicates significant variations at a level of $p<0.05$. Biosorbents: HW-compost (compost from horticultural wastes), HW Horticultural wastes. Conditions: Temperature 30ºC; pH 6, metal ion concentration 50 mg/l, biosorbent concentration 10mg/ml, agitation 120 rpm. * None: without additional nutrient; Glucose: 0.1% glucose; NH_4NO_3: 0.02%; GN: 0.1% glucose and 0.02% NH_4NO_3.*

sed at low pH. This higher removal efficiency was due to biological activity in the case of Cu and Pb. These ions decreased about 50% when initial solution was adjusted to 5 or 3 on non-sterilised materials but remained unchanged between 1 and 72 hours on sterilised samples. Low pH led to an increase of Zn removal without microorganisms involvement in compost samples. This metal was almost completely eliminated (89-95% removal) at pH 3 in sterilised and non-sterilised compost samples.

Pb was the sole ion affected by incubation temperature (data not shown). This metal was more effectively eliminated at higher temperatures (30 and 40ºC). The removal reached values of 80% and 50% for compost and HW samples, respectively. In both cases, sorption was achieved by microorganisms, since residual metal did not change in sterilised samples between 1 and 72 hours contact with biosorbent but decreased in non-sterilised samples.

The addition of nutrients to aqueous solutions had a marked effect on metals removal between 1 and 72 hours (Table 2). Microorganisms-mediated removal of Zn, Cd and Pb in the two biosorbents and Cu in compost increased when glucose and/or NH_4NO_3 were present in aqueous solution. The elimination of metals was superior in composted materials reaching values of 90%. It was also observed that microbial growth was higher in medium with additional nutrients.

CONCLUSIONS

The results show that the two sorbents assayed are capable of removing all above-mentioned metals, and consequently can be used as biosorbents for the effective decontamination of heavy metal ions from aqueous solutions. This biosorption capacity may be greatly enhanced by promoting growth of microorganisms presents in biosorbents. Variations of pH, temperature and addition of nutrients increase the range of metals removed and the amount of elimination.

Acknowledgements. This research has been funded by the Spanish “Ministerio de Ciencia y Tecnología” (CICYT project no. AGL2001-2815).

REFERENCES

Lloyd, J.R., Lovley, D.R. 2001. Microbial detoxification of metals and radionuclides. Curr. Opin. Biotechnol., 12: 248-253.

Eccles, H. 1999. Treatment of metal-contaminated wastes: why select a biological process?. Tibtech, 17: 462-465.

Senthilkumaar, S., Bharathi, S., Nithyanandhi, D. and Subburam, V. 2000. Biosorption of toxic heavy metals from aqueous solutions. Biores. Technol., 75: 163-165.