

Biosorption of Heavy Metals from Leachates Generated at Mine Waste Disposal Sites

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ABSTRACT

In the present paper, the efficiency of adsorbents and biosorbents for the removal of metal ions and the clean up of leachates, generated at two Romanian mine waste disposal sites is examined. Several adsorbents such as activated charcoal, molecular sieve, shell sand, diatomite, bentonite, kaolin, as well as biosorbents such as xanthan biopolymer and waste biomasses are assessed, in terms of metal ion removal efficiency, in laboratory glass columns, comprising layers of coarse sand and 3 layers of each adsorbent/biosorbent. The effect of immobilized bacterial cells (naturally occurring consortia of acidophilic heterotrophic and chemolithotrophic bacteria) was also studied. The experimental results show that shell sand, molecular sieve and waste biomass have a relatively high efficiency in removing most heavy metal ions present in low pH leachates. Regarding shell sand and for the experimental conditions used, the selectivity follows the order Pb>As>Cd>Ni>Cu>Zn>Al>Co>Mn. From the two types of the isolated from leachates inoculum used as immobilized biomass, only heterotrophic acidophilic bacteria had a positive effect on metal uptake in contrary to chemolithotrophic acidophilic bacteria. The sufficient metal removal efficiency attained even without the use of immobilized bacteria, is mainly due to the stimulation of naturally occurring microbiota by addition of culture media; this stimulation may be considered as a feasible alternative to the use of immobilized cells in biosorption applications, regardless of the type of adsorbent/biosorbent used and the metal ion in concern. © 2002 SDU. All rights reserved.

Keywords: Biosorption; Shell sand; Heavy metals; Leachates

1. INTRODUCTION

Intensive mining and processing activities at the Black Sea Coast and the West Carpathian Mountains in Romania, have resulted in the production of large volumes of mine wastes and tailings, which due to oxidation of the residual sulphide compounds exhibit a high potential of generating leachates with low pH (<2) and high concentration of heavy metals and other hazardous ions. A complex environmental characterization and risk assessment study (Petrisor *et al.*, 1998, Komnitsas *et al.*, 1998) has shown that leachates generated at both areas severely contaminate soils, surface- and groundwater.

At Baia, a small village on the Romanian Black Sea Coast, 65km north of the main harbor of Constanta, copper sulphide ores are processed and, as a result of these activities, wastes have been disposed of in three dumps occupying more than 1km² of land, part of which has been used for agricultural activities. These wastes are characterized by high acid generating potential due to their residual content of sulphide compounds. Due to a number of physico-chemical mechanisms, heavy metals are solubilized and leachates are produced, posing a high risk for

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contamination of soils and groundwater. Indeed, after periods of heavy rains acidic ponds are formed at the base of the dumps with a very low pH and elevated heavy metals concentration. Analysis of the sludge collected from the bottom of these ponds shows the presence of sulphate (jarosite type) compounds with elevated concentration of heavy metals.

Rosia Poieni is a vast mining area located in West Carpathian Mountains in Romania. As a result of mining activities over a period of 25 years, tens of millions of tons of sulphide wastes have been disposed of in several dumps; in addition, due to processing activities, a large tailings pond, with a depth of 126m and occupying an area of 3km² has been constructed to receive the produced low pulp density tailings. Leachates generated from the dumps, with a very low pH (1.5-2.0) and high concentration of heavy metals, flow directly into the tailings pond. Leakage from this pond, due to improper slope formation and non-impermeable base, contaminates severely Aries River (pH 3.5-4.0), a main Romanian water stream.

The metal uptake ability of several types of biomass to bind and remove reactive hazardous ions from waste streams has been known for some time and is referred to as biosorption (Hunt, 1986; Volesky, 1987 and 2001; Jain, 1990; Kratochvil and Volesky, 1998). This ability may provide the key to clean up, in a number of cases, leachates generated at mine waste disposal sites that are contaminated with heavy metals and other hazardous ions. Biosorption is considered as a potential alternative to conventional treatment methods (precipitation, oxidation, filtration, ion exchange, etc.) used for the removal of metal ions and the clean up of large volumes of industrial waste streams and contaminated waters.

The feasibility of biosorption as a cost effective process is mainly justified by the use of wastes, and therefore cheap materials, resulting among others from water purification installations, industrial and agricultural activities. Additional advantages include recovery of metals and reduction of the final waste volume after burning the exhausted biomass. The selectivity of the adsorbent depends mainly on the properties of the active sites at the material's surface. Since most of these materials are characterized by low density and poor mechanical properties immobilization, despite its potential disadvantages, is required prior to use in conventional fixed bed columns (Veglio, 1998).

Several metal binding mechanisms for the immobilization of metals on biosorbents, such as surface micro-precipitation, ion exchange, complexation and physical adsorption, have been already described by a number of researchers (Kuyucak and Volesky, 1986; 1988; Brierley *et al.*, 1989; Veglio and Beolchini, 1997). pH is one of the most important parameters related to biosorption of heavy metals, since it affects solution chemistry and therefore hydrolysis, complexation, precipitation and redox reactions as well as speciation and biosorption tendency of heavy metals (Yang and Volesky, 1999).

The use and efficiency of both living and dead bacteria, fungi and algae for biosorption and recovery of heavy metals, such as Cu, Cd, Hg, Ni, Au and Zn from aqueous streams, wastewaters and industrial effluents has been demonstrated in the number of studies (Ross and Townsley, 1986; Gadd, 1986; Ahlf, 1988; Brierley *et al.*, 1989; Falbo and Weaks, 1990; Turnquist *et al.*, 1990; Summers, 1992; Wilde and Benneman, 1993; Davis *et al.* 2000; Esposito *et al.*, 2001). The metal uptake efficiency may vary, depending on the type of the adsorbent, the quality of the contaminated stream, the retention time, the interactions between various metal ions present and the pH. Klimmek *et al.* (2001) have tested 30 strains of algae for the biosorption of Cd, Pb, Ni and Zn from aqueous solutions and stated that cyanophyceae *Lyngbya taylorii* exhibits the highest uptake capacity for all four metal ions studied, in the order of Pb>Ni>Zn>Cd. Biosorption may be benefited by the use of immobilized recombinant bacteria containing efficient metal binding proteins such as MT (Butt and Ecker, 1987; Gadd and White, 1989; Lodewyckx *et al.*, 2001).

Although the efficiency of biosorption has been demonstrated in a number of cases, there is still a need for research aiming to establish its potential regarding detoxification of metal bearing effluents in large-scale applications (Volesky, 2001). The use of new biosorbents, the potential of combining the adsorptive ability of some cheap compounds available in nature with the biosorption capability of microorganisms and the use of membrane reactors is one aspect that still remains to be evaluated (Barba *et al.*, 2000).

The present study investigates, in laboratory columns, the potential of biosorption for the clean up of leachates generated at two mine waste disposal sites in Romania and contaminated with heavy metals. Clean up efforts were focused on the use of several adsorbents and biosorbents as bio-filters, with and without the use of immobilized bacterial cells. The potential of combining surface retention and chemical precipitation of metal ions with the biosorption potential of existing and/or immobilized microorganisms is investigated. The stimulation of indigenous microbiota from adsorbents/biosorbents by the addition of culture medium as a cost-effective alternative to the use of immobilized bacterial cells was also examined.

2. MATERIALS AND METHODS

2.1. Leachates quality

Leachate samples from Baia were mainly collected after periods of heavy rains, while from Rosia Poieni just prior to the discharge point into the tailings pond. Leachates (chemical analysis seen in Table 1) were used, apart from biosorption studies, for bacterial isolation.

Table 1
Chemical analysis of leachates generated at Baia and Rosia Poieni

Ion	Baia (mg/l)	Rosia Poieni (mg/l)
Fe	9600	6705
Cu	462	941
Zn	173	288
Pb	0.80	0.91
Cd	0.40	1.87
As	n.d	8.63
Al	1620	2620
Mn	75	53.50
Ca	290	544
Mg	980	465.50
Co	6.37	5.56
Ni	4.30	6.70

n.d: not detected

2.2. Adsorbents/Biosorbents

A number of adsorbents and biosorbents, seen in Table 3, obtained from several Romanian industrial plants (cement plant, antibiotic plant providing the waste biomass of *Penicillium* sp., citric acid plant providing the waste biomass of *Aspergillus niger*, etc.), except for shell sand that was collected directly from Black Sea beaches, were used in the biosorption experiments.

2.3. Cells immobilized on adsorbents/biosorbents

For the biosorption experiments with Baia leachates, two types of bacterial inoculum were used, namely:

- Bacterial inoculum 1: acidophilic heterotrophic bacterial consortium represented by a mixed culture (natural consortium) of heterotrophic acidophilic bacteria, isolated from Baia acidic leachates and sludges;
- Bacterial inoculum 2: acidophilic heterotrophic and chemolithotrophic bacterial consortium consisting of a mixture of bacterial inoculum 1 and a mixed culture (natural consortium) of chemolithotrophic acidophilic bacteria isolated from Baia acidic leachates and sludges.

For the biosorption experiment with Rosia Poieni leachates, only one type of inoculum was used consisting of heterotrophic acidophilic bacteria isolated from these leachates.

For inoculum preparation, 750ml flasks incubated for 7-10 days on rotary shakers at 150rpm and 28°C. At the end of incubation period, the pH of the medium was lowered to 1.5-1.0 and the density of the cells varied between 4.5×10^3 and 2.5×10^{10} cells/ml. The culture media used for isolation of all types of bacterial inoculum, as well as the predominant bacteria species and the pH of the culture media are seen in Table 2.

Table 2
Characteristics of bacterial inoculum used in biosorption tests

Physiological group of bacteria	Predominant bacterial genera or species	Culture media used (Karavaiko <i>et al.</i> , 1988) / pH	pH of culture medium after 7-10 days inoculation
Heterotrophic acidophilic bacteria	- <i>Sulfobacillus</i> sp.	Golovacheva / 2.5	1.0
	- <i>Sulfidobacillus</i> sp.		
	- <i>Acetobacter acidophilum</i>	Brierley / 2.0	1.0
	- <i>Alcaligenes entrophus</i>	Manning / 2.8	1.5
Chemolithotrophic acidophilic bacteria	- <i>Pseudomonas putida</i>		
	- <i>Thiobacillus ferrooxidans</i>	9K / 2.5	1.5
	- <i>Leptospirillum ferrooxidans</i>	Waksman / 4.0	1.0
	- <i>Thiobacillus thiooxidans</i>		
	- <i>Thiobacillus thioparus</i>	March / 2.5	1.5

2.4. Trickling filter system

The trickling filter system used for all experiments performed comprised glass columns with a diameter of 35mm and a length of 300mm; a drop by drop flow rate was applied. Coarse sand that was intensively cleaned, washed with 0.1N HCl and distilled water and then dried at 105°C was added in the columns as an inert material. Each column comprised 3 layers of adsorbents/biosorbents; the thickness of each layer was 35mm and the weight varied between 5-10g depending on the type of the material used. Leachates were percolated through these columns three to five times using adequate pumps.

2.5. Experimental procedure

In order to pre-screen adsorbents/biosorbents, in terms of their metal uptake efficiency, leachates from Rosia Poieni, due to their higher metals content were used in the first series of experiments, where only a number of heavy metal ions were taken into account and no immobilized bacteria were used. 1 liter of leachates was percolated through each column three times. The adsorbents/biosorbents exhibiting the highest metal removal efficiency were selected and used, in the experiments that followed.

In the tests performed, with the use or without using immobilized bacteria, the trickling filter columns were initially flooded with 250ml of bacterial inoculum and left for 24 hours at 28°C in order to enable immobilization of bacteria on column fillings; then the liquid in excess was removed. The bacterial inoculum used had a high cells concentration, ranging between 10^9 and 10^{11} cells/ml. Then, all trickling filter columns were flooded with 250ml of pH 2.8 Manning medium and incubated for another 24 hours at 28°C, followed by removal of the liquid in excess. Finally a five time percolation of both Rosia Poieni and Baia leachates was initiated. In all tests performed, the initial leachates pH increased slightly from 1.5 to values close to 2.

Before and after the biosorption tests, all leachates were analyzed in terms of heavy metal content using Atomic Absorption Spectrophotometry (AAS). On this basis, the metal uptake was calculated and the biosorption efficiency of each material used was determined.

All tests were performed in duplicate, and analyses of metal ion concentration before and after the tests in triplicate.

3. RESULTS AND DISCUSSION

3.1. Pre-screening of adsorbents/biosorbents

Cheap and easy to procure adsorbents/biosorbents, such as shell sand, xanthan biopolymer or waste microbial biomass derived from industrial processes were compared, in terms of biosorption efficiency with well established materials such as activated charcoal and molecular sieve (Creek and Davidson, 2001). The data seen in Table 3 derive after three leachates percolations.

Table 3
Pre-screening of adsorbents and biosorbents

Adsorbent / Biosorbent	% metal uptake				
	Cu	Zn	Pb	Fe	Al
Activated charcoal	28.5±0.3	0	37.8±0.3	30±0.3	17.1±0.2
Molecular sieve	22.6±0.3	23±0.3	19.6±0.2	25.5±0.3	0
Shell Sand	38.5±0.4	29.5±0.3	61.5±0.6	60±0.6	23.5±0.3
Diatomite	22.6±0.3	0	45.3±0.5	27.5±0.3	10±0.1
Bentonite	0	3.8±0.1	36.2±0.1	50.3±0.5	29.5±0.3
Kaolin	0	0	0	17.5±0.2	0
Vulcan tuff	0	7.5±0.1	17.5±0.2	34.9±0.3	0
Xanthan biopolymer	10.0±0.1	4.5±0.1	50.5±0.5	17.3±0.2	5.9±0.1
Waste biomass of <i>Penicillium</i> sp.	28.5±0.3	0	30.8±0.3	27.5±0.3	5±0.1
Waste biomass of <i>Aspergillus niger</i>	28.5±0.3	20±0.3	37.4±0.4	32.5±0.3	5±0.1

From these preliminary experimental results the following conclusions can be drawn:

The innovative experimental set up is considered adequate and effective. This system was designed in such a simple way, by placing layers of adsorbents/biosorbents acting as filters over the stream of metal laden leachates, so that it can be easily transferred in larger scale experiments. Thus, drawbacks encountered in most basic biosorption studies, carried out in flasks on rotary shakers by using an artificially contaminated with a specific ion solution, are easily overcome. Similar column installations are mentioned in the literature, for the study of the biosorption efficiency of *Acacia nilotica* bark, but they are not identical to the one used in this experiments series, since in that case the entire column was packed with bark powder (Prasad *et al.*, 2001). This specific experimental setup with only three layers of adsorbent/biosorbent, is considered more convenient from a practical point of view.

The adsorbents/biosorbents tested exhibited a varying efficiency in removing hazardous ions from leachates, which was a function of the type of the material used and the ion in concern (Klimmek *et al.*, 2001). Some of them exhibited sufficient metal uptake efficiency for more than one ion. The results were quite surprising regarding the high efficiency of shell sand, which proved the most efficient from all materials tested while it is hardly mentioned in the literature for such purposes; metal uptake regarding Pb and Fe exceeded 60%. The metal uptake efficiency of shell sand is due to a combination of physical adsorption of ions through surface binding forces, with chemical precipitation caused by its high calcium carbonate content. The potential of shell sand for large-scale applications increases by considering the large availability of this material in many coastal areas in Romania. Good metal removal efficiencies were also displayed by the 2 waste biomasses examined and by the typical adsorbents activated charcoal and molecular sieve. However, all these materials exhibited lower metal removal efficiencies compared to shell sand. Bentonite, although used in a low pH system, exhibited a relatively high metal uptake, regarding Pb, Fe and Al, while diatomite showed a relative preference to Pb. Xanthan biopolymer, with a complicated biosynthesis and isolation process, was also quite efficient in removing Pb but exhibited a limited efficiency in the removal of all other metal ions. The least efficient material was kaolin, capable of only removing iron partially (17.5%).

Similar poor results were obtained when volcanic tuff was used. This low metal removal efficiency was expected though, since these materials are not considered as typical adsorbents, although they may display some surface-retention properties.

Therefore, based on parameters such as efficiency, availability and purchase cost, only shell sand, molecular sieve, activated charcoal and waste biomass of *Aspergillus niger* were used in the following experiments.

3.2. Assessment of adsorbents and biosorbents

In order to assess the pre-screened materials, in terms of their biosorption efficiency, additional experimental studies were performed with the use and without using immobilized bacterial cells; in both cases the indigenous microbiota was stimulated with the addition of culture medium. The use of living microorganisms (inoculum) was considered by taking into account their increased metal uptake potential compared to dead microbial biomass (Voicu *et al.*, 1999).

In this series of experiments, the metal laden leachates were percolated five times through each experimental column, in order to study also the effect of the increased retention period. The experimental results regarding clean up of Rosia Poieni and Baia leachates are seen in Tables 4 and 5 respectively.

Based on these data the following main conclusions can be derived:

1. All selected adsorbents and biosorbents were efficient, to a lesser or higher extent, in removing heavy metal ions present in leachates, with shell sand being the most efficient. The high metal uptake attained for a number of extremely hazardous ions, such as Pb, Cd, Ni and As confirms the potential of shell sand. In addition, the high metal uptake observed for Fe from both leachates confirms the potential of this treatment. Regarding Fe, although not listed as a hazardous ion, its removal from leachates is considered crucial. When present in high concentrations in wastewaters or leachates, it usually precipitates as hydroxide or jarosite, blanketing the bed of the streams and therefore causing serious impacts to aquatic life. Molecular sieve exhibited also sufficient metal uptake efficiency, however its use in field applications may be hindered due to its high purchase costs. Increased retention periods, in comparison to the initial experimental series, resulted in increased metal uptake. These experimental results are in conformity with previous studies, underlining the effect of heterotrophic acidophilic bacteria in biosorption studies (Avakyan, 1988; Johnson, 1998; Voicu *et al.*, 1998; 2000).
2. When immobilized bacterial cells were used (acidophilic heterotrophic bacterial consortium) a slight increase in metal uptake was recorded for most ions present. The average metal uptake was increased from 3.2%, for shell sand, to 8.5% for activated charcoal and molecular sieve. However, this increase, bearing in mind the cost of immobilisation, was not considered significant to justify the use of immobilised bacteria. On the contrary, average metal uptake was decreased by 3.1% in the case of biomass (Table 4). This may be explained by the incapacity of waste biomass to serve as immobilization support for bacteria, as well as by the presence of a rich indigenous microbial community in such biosorbents. Addition of culture medium in the columns with biomass may increase biosorption efficiency. It has to be mentioned though that Ca and Mg were not included in the calculations, since they are not considered as hazardous ions; besides the solution is enriched in Ca due to its solubilization from shell sand.
3. The mixture of acidophilic heterotrophic and chemolithotrophic bacterial consortia, used only for the clean up of Baia leachates (Table 5), had a detrimental effect on metal uptake. This is probably due to aggregation phenomena occurring at varying biomass concentration and to complexation of the organic compounds released, in relation with the presence of chemolithotrophic bacteria of *Thiobacillus* type.
4. One of the most important findings of this study is the stimulative effect of the acidophilic bacteria used for the clean-up of leachates at low pH, where biosorption rates usually decrease (Klimmek *et al.*, 2001). As demonstrated though, the use of acidophilic microbiota can increase biosorption efficiency even at low pH values.

Table 4
Metal uptake from Rosia Poieni leachates

Adsorbent/ Biosorbent	Experimental conditions	% Metal uptake											
		Fe	Cu	Zn	Pb	Cd	As	Al	Mn	Ca	Mg	Co	Ni
Shell sand	A	70.7±0.9	64.7±0.8	55.5±0.5	80.7±1.3	68.8±1.0	78.0±1.1	23.6±0.4	15.2±0.2	nc	14.6±0.2	38.3±0.4	68.1±0.9
	B	73.6±1.0	65.6±0.8	36.4±0.4	89.5±1.6	72.5±1.2	79.5±1.3	42.5±0.6	31.1±0.4	nc	33.6±0.4	37.3±0.4	67.4±0.9
Activated charcoal	A	34.3±0.4	38.2±0.4	0	46.5±0.5	18.2±0.3	28.5±0.4	17.1±0.4	20.4±0.3	21.4±0.4	19.7±0.3	18.0±0.2	53.7±0.8
	B	45.6±0.6	50.8±0.6	26.8±0.4	50.9±0.7	27.8±0.4	37.0±0.4	25.7±0.4	29.9±0.4	29.0±0.4	29.3±0.4	26.6±0.4	39.6±0.4
Biomass	A	42.5±0.6	31.8±0.4	20.8±0.4	51.0±0.6	21.4±0.4	32.4±0.4	15.8±0.2	20.8±0.3	2.5±0.1	19.3±0.3	19.4±0.3	8.2±0.1
	B	43.1±0.5	20.3±0.3	10.7±0.1	47.7±0.4	22.5±0.4	40.1±0.5	10.3±0.1	15.2±0.3	7.2±0.1	16.8±0.3	20.0±0.3	3.0±0.1
Molecular sieve	A	62.9±0.8	62.6±0.9	55.9±0.6	29.7±0.5	57.1±0.6	82.0±1.2	0	4.8±0.1	60.4±0.7	18.8±0.3	35.4±0.4	24.8±0.4
	B	69.8±0.9	69.3±0.9	40.1±0.4	28.6±0.3	64.3±0.8	82.0±1.3	15.8±0.2	20.3±0.4	60.2±0.8	28.5±0.4	44.0±0.6	64.8±0.8

A: Without using immobilized bacterial cells. Indigenous bacteria occurring in acidic waters were stimulated by the addition of culture medium

B: With the use of immobilized bacterial cells (heterotrophic acidophilic bacteria)

nc: not calculated due to additional solubilization of Ca from shell sand

Table 5
Metal uptake from Baia leachates

Adsorbent/ Biosorbent	Experimental conditions	% Metal uptake										
		Fe	Cu	Zn	Mn	Cd	Pb	Al	Ca	Mg	Co	Ni
Shell sand	A	59.4±0.7	27.1±0.2	9.8±0.1	13.3±0.9	45.0±0.5	37.5±0.4	27.8±0.4	nc	9.2±0.2	15.4±0.2	51.2±0.6
	B	60.9±0.7	36.1±0.3	20.8±0.2	12.0±0.2	50.0±0.6	37.5±0.4	32.1±0.4	nc	12.2±0.2	16.5±0.2	53.5±0.7
	C	29.2±0.3	11.7±0.2	17.3±0.2	13.3±0.2	42.5±0.5	37.5±0.4	16.7±0.2	nc	9.2±0.2	7.5±0.1	51.2±0.6
Activated charcoal	A	28.1±0.2	13.6±0.2	nc	22.7±0.3	37.5±0.4	12.5±0.2	6.2±0.1	6.9±0.1	15.3±0.2	12.7±0.2	32.6±0.4
	B	21.9±0.2	9.1±0.1	nc	17.3±0.2	35.0±0.4	12.5±0.2	8.0±0.1	20.7±0.3	16.4±0.2	11.1±0.2	16.3±0.3
	C	19.8±0.2	5.6±0.1	nc	13.3±0.2	25.0±0.4	12.5±0.2	3.7±0.1	13.8±0.2	7.1±0.2	6.3±0.2	9.3±0.1

A: Without using immobilized bacterial cells. Indigenous bacteria occurring in acidic waters were stimulated by the addition of culture medium

B: With the use of immobilized bacterial cells. Bacterial inoculum 1- acidophilic heterotrophic bacterial consortium

C: With the use of immobilized bacterial cells. Bacterial inoculum 2- acidophilic heterotrophic and chemolithotrophic bacterial consortium

nc: not calculated due to additional solubilization of Ca and Zn from shell sand and activated charcoal respectively

The next step of this biosorption study is the use of shell sand, or other low cost adsorbents/biosorbents, in field applications in order to examine their in situ efficiency for the clean-up of leachates generated at mine waste disposal sites. A feasible set up may include the installation, along the leachates route, of a system of biofilters comprising a number of layers of the selected materials, without supplementary addition of bacterial inoculum, but with previous addition of adequate culture media to stimulate acidophilic bacteria. The number of biofilters used can be determined by taking into consideration the optimum retention period required to maximize metal uptake, so that the treated leachates comply with the environmental regulations for safe disposal.

In addition, proper management of the metal laden biofilters is required in order to recover the adsorbed metals and reuse the regenerated materials, as demonstrated in Cr (VI) removal studies (Low *et al.*, 1997; 1999).

4. CONCLUSIONS

The present study investigates the clean-up of leachates generated at mine waste disposal sites, by combining the adsorptive ability of inexpensive and largely available compounds (shell sand, biopolymers, waste biomasses, etc.), used as barrier filters, with the biosorption potential of microorganisms immobilized on such filters. Established adsorbents, such as activated charcoal and molecular sieve were also tested for efficiency comparison.

From the adsorbent and biosorbent materials tested, sand shell, molecular sieve, activated charcoal and waste biomass of *Aspergillus niger* were efficient in removing most heavy metal ions from contaminated leachates. However, only shell sand shows an increased potential for field applications due to its large availability at no cost and high metal removal efficiency when used with or without immobilized bacteria. Regarding shell sand and for the experimental parameters used, in terms of quality of leachates and pH, the selectivity follows the order Pb>As>Cd>Ni>Cu>Zn>Al>Co>Mn.

When immobilized bacterial cells were used (acidophilic heterotrophic bacterial consortium) a slight increase in metal uptake was recorded for most ions present. However, this increase, bearing in mind the cost of immobilisation, was not considered significant to justify the use of immobilised bacteria.

The heterotrophic acidophilic bacterial consortium isolated from the examined leachates was effective in increasing metal uptake for all adsorbents/biosorbents tested, while the addition of chemolithotrophic bacteria decreased metal uptake to values lower than those seen in the control tests (without immobilized bacterial cells).

Stimulation of the indigenous microbial community from the adsorbents/biosorbents with the addition of culture medium increases metal uptake and can be considered as a cost-effective alternative to the use of immobilized bacterial cells. This stimulation has a beneficial effect on metal removal at low pH values, regardless of the type of adsorbent/biosorbent used and the metal ion in concern.

The simple and effective experimental set up used in this experimental study comprising several layers of materials in percolating column systems may be easily transferred in the field for large-scale applications, by installing a number of required barriers of adsorbents/biosorbents along the flow of leachates.

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