

**ELIMINATION OF HEAVY METALS FROM MICROBIAL CULTURES BY CHEMICAL AND BIOLOGICAL LEACHING UNDER DECREASED VALUES OF pH**František Kaštánek<sup>a</sup>, Vladimír Bizek<sup>b</sup> and Miroslav Matějka<sup>c</sup><sup>a</sup>Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic, 165 02 Prague 6-Suchbát<sup>b</sup>Ministry of the Environment of the Czech Republic, Prague 4<sup>c</sup>Prague Institute of Chemical Technology, 160 00 Prague 6**ABSTRACT**

The article compares experimental results of the effectiveness of the extraction of heavy metals contained in mixed microbial cultures forming activated sediments from communal sewage disposal plants. The methods used were elimination of the metals from the sediments based on their extraction with strong inorganic acids under pH 1, extraction with the aid of acid ion exchangers and simultaneous oxidation of the sludge with chlorine and ozone in the pH area 2.7 - pH 3 and a method of biological leaching through the effect of the micro-organism *Thiobacillus ferrooxidans* under pH 2. Relatively the most effective method appears to be extraction with acids under pH 1 when, with the exception of copper and lead, the other metals are extracted with great efficiency. Chrome is also well extracted with these low pH, in contrast, for instance, with the ion exchanger method. But this second case is good for the extraction of copper. Ni and Cd are well extracted by both methods. Biological leaching is shown to be the most suitable for the extraction of zinc, but it can also be used as an auxiliary method for disrupting the strong bonds even of sulphides of other heavy metals with their receptors on the surface of cell membranes. It seems from our results that the majority of metals are mainly bound just on the surface of the cells, with the exception of copper, which evidently penetrates into the volume of the cells, from which it can be partially washed out only after disrupting the cell walls through the effect of strong oxidative agents. It is stated, on the basis of evaluation of the experiments that successive application of all three methods could be practically utilizable as an effective way of extracting heavy metals from sludge from sewage treatment plants.

## INTRODUCTION

The technology of communal sewage treatment produces a large quantity of mixed microbial cultures, concentrated into what is called activated sludge. That sludge can be considered as a weak suspension of solid particles (1-3 % approx.) in water. The composition of activated sludge is not uniform and varies according to the locality, though it also changes within a given locality if there are changes in the composition of the waste water, or in the temperature, or the technological parameters under which the activation was carried out (residence time of distribution and the total residence time, the loading of the sludge both with organic substances and with heavy metals). The types of bacteria occurring most frequently in activated sludge are: *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Chromobacterium*, *Azotobacter*, *Micrococcus*, *Bacillus*, *Alkaligenes*, *Anthrobacter*, *Acinetobacter*, *Nocardia* and so on. Apart from bacteria the following are also present in smaller amounts in activated sludge: fungi, moulds, yeasts, nitrification bacteria, then of higher organisms various protozoa, ciliata, fibrous microorganisms and so on.

From the point of view of the further use of sludge from biological sewage plants, which amounts to tons daily, the decisive parameter is the content of heavy metals. Obviously the liquidation of these sludges by incineration is extremely uneconomical. With the present continuing irreversible loss of good-quality agricultural soils, their most suitable use seems to be the recycling of the high-quality organic substrate represented by these sludges back into the soil. So the content of heavy metals is a crucial criterion for the possibility of their purposeful use<sup>1</sup>. Unfortunately the microorganisms of sludge very actively adsorb and absorb the heavy metals present in waste waters and are typical accumulators of them<sup>2-7</sup> (indeed the branch of biometallurgy is based on this activity of microorganisms<sup>8-11</sup>).

The elimination of heavy metals from microbial sludge, particularly in view of their possible penetration into the

cells, will therefore be very difficult. It may be said that this theme has not so far been satisfactorily solved anywhere and that there is not sufficient fundamental research in this field.

The aim of this study is to present quantitative findings on the possibilities of eliminating heavy metals caught in mixed cultures of activated sludge of varying age by three independent methods of extraction:

- a) leaching with organic and inorganic acids,
- b) extraction of metals in the presence of oxidative agents and granular polymer ion exchanger, bound on a magnetic carrier,
- c) a bioremedial method making use of the ability of microorganisms to transfer some sulphides of heavy metals not soluble in water to soluble sulphates.

Included in the study is a brief survey of the mechanisms of accumulation of heavy metals, which enables a discussion on the effectiveness and specific effect of the extraction methods chosen.

### **Findings to date on the accumulation of metals**

#### *The mechanism that is not dependent on the metabolism of microorganisms*

This includes a number of interactions of metals with a surface structure of living and dead cells on the basis of

- the effect of electric forces, the reaction of positive ions with negatively charged components of the cell walls
- physical adsorption, including van der Waals forces
- the effect of chemical reactions of the ions of heavy metals with the cell acceptors on the surface of the cells.

In individual types of microorganisms the adsorption may take place in all the ways given, depending for instance on the composition of the cell walls, which differs in various types of microorganisms<sup>12,13</sup>. The speed of the bonds is typical of these accumulations (the sorption is complete in 5-10 minutes) and the reversibility of the process.

The main heavy metals that are preferentially sorbed by this mechanism are  $\text{Hg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and possibly also  $\text{Cd}^{2+}$ . According to the interpretation of our recent results, it may be judged that  $\text{Zn}^{2+}$  is probably also bound by this mechanism.

*The mechanism of intracellular accumulation depending on metabolism*

Accumulation depending on metabolic processes of the cell is generally a slower process than adsorption. The transport mechanisms ruling the accumulation of heavy metals have not been entirely investigated. But it can be said that the quantity of metals taken into a cell can significantly exceed the quantity taken in by physical-chemical sorption. With active acceptance at the cases of high residence time of the sludge intracellular concentration reaches several thousand times higher values in the cell than in the surrounding ambience. Bacterial communities are capable in a very concentrated environment of accumulating, for instance up to 30 %  $\text{Cu}^{2+}$ , in relation to dry matter, or up to 25  $\mu\text{g}/(\text{mg}$  of protein) of toxic ion  $\text{Cd}^{2+}$ <sup>7</sup>. What happens to the metals in the cell has been equally little investigated. It appears that of heavy metals, for instance  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$  are located in vacuoles, where they may be bound to low-molecular polyphosphates,  $\text{Cd}^{2+}$  is most probably bound to cell protein<sup>14</sup>.

Many bacteria produce a large quantity of extracellular polymers, which form a capsule or aggregates round the cells. These polysaccharides often bind metal cations in large quantities<sup>15</sup>.

*Conclusions resulting from ideas on the manner of binding metal ions in cells*

In spite of a certain amount of inconsistent literary information, it has been shown that

- the ions Pb, Cu and probably also Hg show a tendency towards surface binding. Gentler, non-invasive (non-destructive for cells) solubilization processes will be suitable for the

removal of these metals from microbial biomasses

- the accumulation of highly toxic Cd evidently goes through a complicated mechanism, including both surface adsorption and binding on cellular substance. To remove this metal it will be necessary to use methods connected with the partial destruction of the cell substance, that is disruption of the cell walls.

From studies that have so far been devoted to the dynamics of sorption of metals on cellular substance it is clear that the decisive factor will be the time spent by the microbial biomass in the conditions of the reduction medium, when even the ions of the metals not having a tendency to penetrate into the volume of the cell substance will be sorbed on the surface of the cells as sulphides of heavy metals that are very difficult to eliminate.

It has been formerly ascertained that an acid medium can suppress both biosorption and the intracellular reception of metals. With the dilution of 0.1 mol/l of mineral acid the adsorbed Cu and Cd can be removed from the surfaces of most organisms composing aeration sludge. With a greater concentration of acids of over 1 mol/l the cell walls are most likely destroyed. Organic chelation agents are effective desorbents, for instance EDTA, 8-hydroxyquinoline (to remove the Cu) and nitrilotriacetic acid and diethylenetriamin pentaacetic acid (to remove the Cd). It is usually necessary to work with pH 2 - 3. Carbonates are also a potential means for the elimination of some metals (U) and in this case the conditions are not significant enough to disrupt the biomass.

In this study methods have been chosen that could be used experimentally even in the practical use of aeration sludge.

## **EXPERIMENTAL PART**

*Microbiological composition of mixed bacterial biomasses (Prague sludge)*

An average amount of  $1.3 \cdot 10^6$  cells/ml of sludge was determined in the samples. No lipolytic, proteolytic, amyolytic

or cellulolytic activity was found. Two main tribes have been found, which were typical gram-negative rods. The tribes were classified as *Escherichia coli* and *Klebsiella pneumoniae*.

#### *Extraction of heavy metals with inorganic acids*

The following results are averaged from several independent measurements (the metal content varied in samples taken at different times). Experiments were made with three different kinds of sludge:

- 1) thin slurry, taken from a Prague sewage treatment plant
  - a) after anaerobic stabilization, 20 days of the average time of being kept under anaerobic conditions
  - b) fresh, so-called return sludge (4 hours old)
- 2) dry granulated sludge (exposed to thermal processing), produced by the firm Tiefbauamt Stadt Zurich, Switzerland.

The content of the dry matter was determined gravimetrically, by evaporation on a water bath and drying to constant weight at 105°C. The metal content was determined by atomic absorption spectroscopy. The composition of the sludge is given in Tables 1 and 2.

Leaching was carried out with hydrochloric and sulphuric acids. It was ascertained at the same time that any use of organic acids is ineffective.

Table 1  
Composition of thin slurry from a Prague sewage plant, ppm, related to dry matter

Metal	Zn	Pb	Cu	Fe	Ni	Cr	Hg	Cd
Contents	1860	174	450	41500	119	90	3.5	7.0

Table 2  
Composition of granulated sludge, ppm, related to dry matter

Metal	Zn	Pb	Cu	Fe	Ni	Cr	Hg	Cd
Contents	910	220	560	50000	40	75	2	3

#### *Leaching of thin slurry with hydrochloric acid*

17.5 ml of 35 % hydrochloric acid was added to 1000 ml of thin slurry of the composition shown in Table 1, the mixture was stirred for 2 hours at a temperature of 25°C. The resultant concentration of acid was 0.2 mol/l, concentration after completion of the leaching was 0.12 mol/l, which corresponds to the consumption of approximately 7.5 mol of acid to 1000 g of dry matter. After the completion of the leaching the mixture was centrifuged, the water extract filtered and the insoluble part

washed with distilled water. In both phases the concentration of the metals was determined. 200 ml of the extract was further precipitated with 0.9 g of calcium hydroxide and water solution and the precipitate was analysed for the content of metals. The results of the experiments are given in Tables 3 - 5.

Table 3  
Composition of dry matter, %

Property	Starting sludge	Sludge after leaching
Dry matter	1.07	0.46
Combustible substances	56	71
Non-combustible substances	44	29

Table 4  
Analysis of the dry matter, ppm

Metal	Sludge after leaching	Precipitate
Zn	370	9500
Pb	170	420
Cu	950	64
Fe	9300	195000
Ni	39	310
Cr	19	390
Cd	4	-

Table 5  
Analysis of the solution, ppm

Metal	Original solution after filtering off the solid components	Leach	Solution after precipitation
Zn	0.04	18.2	0.06
Pb	0.03	1.06	0.25
Cu	0.24	0.42	0.34
Fe	0.55	400	0.91
Ni	0.05	1.08	0.48
Cr	0.005	0.77	-

*Conclusions resulting from leaching with hydrochloric acid*

- Less than half the quantity of the original sludge occurs in the course of the leaching of a well sedimenting solid phase,

which contains an increased amount of combustible organic components and, with the exception of copper and lead, a decreased amount of metals.

- In view of the decrease of dry matter (as a result of the decomposition of the carbonates present) it can be judged that copper practically does not leach, lead leaches to approximately 50 %, other metals pass into the solution to 85-90 %, which is an important finding, especially because of the quantity of Cr eliminated (determined totally  $\text{Cr}^{3+}$  and  $\text{Cr}^{6+}$ ). In view of the fact that part of the metals did not leach even in the course of two hours mixing into a strongly acid solution (initially pH 0.7, finally pH 1.1),, it can be stated in connection with the development of hydrogen sulphide in the initial stage of the leaching, that metals are bound on the surface of microbial biomasses in the form of little soluble sulphides.

- Metals in a leach can be precipitated with a high yield by calcium hydroxide.

#### *Leaching of granulated sludge with sulphuric acid*

The use of granulated dried sludge (i.e. after application of the thermal process during the course of the process of drying and granulation) was motivated by an attempt to ascertain the possible influence of thermic destruction of microbial biomass on the effectiveness of leaching.

The granulated sludge was spread out and 158 g of dry matter was mixed with 1422 g of water. 31 ml of concentrated sulphuric acid was added. The content of dry matter in the mixture dropped in the course of the leaching from the original 10 % to 7 %, with an increase in the amount of combustible components from 52 % to 55 %. The composition of the dry matter after leaching and the extract are given in Table 6.



Table 6  
Leaching of granulated sludge, ppm

Metal	Dry matter after leaching	Extract
Cd	2	0.19
Pb	230	2.51
Cr	34	2.22
Ni	19	2.79
Cu	580	1.55
Zn	260	115
Fe	13000	4900

*Conclusions resulting from leaching of granulated, thermally processed sludge*

- Approximately 70 % of a well sedimenting solid phase occurs from the original sludge in the course of the leaching, containing roughly the same amount of combustible organic substances as the original sludge and, with the exception of copper and lead, a decreased amount of metals.

- Thermal processing of sludge, causing the destruction of the cells (cracking of the cell walls) does not contribute significantly to increasing the extractability of heavy metals with acid leaching.

*Conclusions from the leaching of microbial biomasses with inorganic acids*

- It was ascertained that hydrochloric and sulphuric acids are capable of leaching part of the metals present in microbial biomasses of activated sludge. The degree of leaching is minimal in the case of copper, low in the case of lead. In other metals it ranges between 60-90 %. It can be justifiably assumed that the unleached part of the metals is present in the solid phase in the form of little soluble sulphides bound on the surface of the microbial biomasses, rather than inside the cells (the thermal process, leading to partial destruction of the cells, had no influence on the effectiveness of the leaching).

- It was ascertained that in the course of acid leaching there is a dissolution of part of the sludge that is by no means negligible (more than 50 % in the case of hydrochloric acid, approximately 30 % in the case of sulphuric acid), while with the use of HCl there is preferential dissolution of the incombustible components of the sludge.

- The consumption of leaching acid amounts to 8 mol to the kilogram of dry sludge with the use of HCl and 4 mol to the kilogram with the use of sulphuric acid.

### **Extraction of metals in the presence of oxidation agents and granular polymer ion exchanger**

The stage of mere extraction of metals from the microbial biomass with acids, without their previous treatment, showed that the effectiveness of this method is very limited.

Research on the elimination of heavy metals from thin activated slurry was carried out by a process that mainly consists in the simultaneous leaching of metals from the microbial biomass and their elimination from the liquid phase in an acid medium in the presence of an oxidative agent and a small quantity of granular polymer ion exchanger. The addition of the ion exchanger causes a shift of the extraction balance in the direction of increasing the concentration gradient of the metal ions between the surface of the microbial biomass and the surrounding solution, with simultaneous fixation of the heavy metals on the regeneratable ion exchanger.

Three different types of cation exchanger were used:

- a strongly acid cation exchanger (Ostion KS, producer Spolchemie, Ústí nad Labem, Czech Republic)
- a weakly acid cation exchanger (Lewatit CNP 80, Bayer AG, Germany)
- chelation iminodiacetate cation exchanger (Lewatit TP 207, Bayer AG, Germany).

Fractions with average grains over 0.5 mm were used.

### *Fresh microbial biomass*

The concentration of metals in fresh microbial biomass is given in Table 7.

Table 7  
Content of heavy metals in fresh microbial biomass, ppm in dry matter

Metal	Cu	Zn	Pb	Cd	Ni	Cr
Content	415	1393	241	9.8	92	214

### *Use of chlorine as an oxidative agent*

Procedure: Samples of the microbial biomass (return sludge, age of sludge 4 hours after being taken from the return pipe) to the amount of 500 ml (pH 6.8) were bubbled through with gas chlorine for 2 minutes. Before the introduction of the catex the pH was adjusted

- a) in the strongly acid catex to pH 3
- b) in the weakly acid catex to pH 4.1.

Then 7.5 ml of catex was added to the sample (the cation exchangers were measured in H<sup>+</sup> form), strongly acid and chelation catex was added in H<sup>+</sup> form, weakly acid carboxylic catex was added in Na<sup>+</sup> form. The mixture was stirred for 40 minutes at a temperature of 20-25°C.

#### Sample A:

After 40 minutes of mixing the sample of sludge with strongly acid catex the acidity was adjusted to pH 3 and the catex removed (the leaching proceeded in intervals of pH 3 - pH 2.3). The biomass was in contact with the solution without the catex for a further 60 minutes.

#### Sample B:

After 40 minutes of mixing with the chelation catex the pH was adjusted to pH 3.1 (the leaching proceeded mostly at a value of pH 2.65). The biomass was in contact with the solution without the catex for a further 60 minutes.

#### Sample C:

After dosing with chlorine the pH was increased to 4.1 and weakly acid carboxylic catex was added. After 40 minutes of stirring the pH was adjusted to 4.1 (the leaching proceeded within the range of pH 3.5 - 4.1). After the removal of the cation exchanger the biomass was in contact with the solution for a further 60 minutes.

The results are given in Tables 8 - 11.

Table 8  
Content of heavy metals in sample A after leaching, ppm in dry matter

Metal	Cu	Zn	Pb	Cd	Ni	Cr
Content	38.6	14.7	104	3.3	20.7	148
Decrease, %	90.7	99.0	56.8	66.4	77.4	30.1

Table 9  
Content of heavy metals in sample B after leaching, ppm in dry matter

Metal	Cu	Zn	Pb	Cd	Ni	Cr
Content	36.9	16.9	102.4	3.85	23.9	161.0
Decrease, %	91.1	98.8	57.5	60.8	75.9	24.8

Table 10  
Content of heavy metals in sample C after leaching, ppm in dry matter

Metal	Cu	Zn	Pb	Cd	Ni	Cr
Content	7.0	18.2	121.3	3.0	23.6	145.5
Decrease, %	98.3	98.7	49.6	69.5	74.2	32.1

Table 11  
Content of heavy metals in the effluent (ml/l) after removal of the ion exchanger and the leached sludge

Metal	Sample A	Sample B	Sample C
Cu	4.54	2.59	3.89
Zn	0.97	5.03	4.86
Pb	0	0.46	0
Cd	0	0.1	0
Ni	0.3	0.5	0.5
Cr	0.125	0.18	0.125

*Conclusions from the leaching of fresh sludge in the presence of dissolved chlorine and catex*

- A great decrease in the content of metals in the dry matter of the leached sludge was attained with all the cation

exchangers used.

- The greatest effectivity of decrease of the content was found in the cases of copper and zinc (98-99 %).

- The least effectivity of leaching was ascertained in chrome: 25-32 %. Its elimination evidently requires the use of extremely acid leaching solutions.

- The residue concentration of very toxic cadmium in the dry matter of the microbial biomass was decreased to a value that is satisfactory for instance even to the exacting norms of the European Community.

- The residue contents of heavy metals in the effluent are very low.

*Chemical leaching in the presence of the oxidative agent ozone - comparison of standard cation exchangers and cation exchangers immobilized on a magnetic carrier*

The aim of the experiment was to ascertain whether oxidation has any significant influence on the disruption of bonds between metal-surface of microbial particles. In this case, instead of the very aggressive chlorine as an oxidative agent, the more acceptable ozone, usual in waste water treatment technologies, was proposed.

Cation exchangers used:

- standard type (Ostion KS, producer Spolchemie, Ustí nad Labem, Czech Republic)
- strongly acid cation exchanger, anchored onto the carrier with a magnetic substance  $\text{CrO}_2$  (ICI Australia + CSIRO Melbourne, Australia).

*Fresh (return) activated sludge*

A 500 ml sample of mixed microbial suspension (thin slurry) was gassed through with a mixture of ozone and air (concentration of ozone 24 mg/l of air) rate of flow of air 0.5 l/min.

Sample OA: The ozone was introduced for 20 minutes.

Sample OB: The ozone was introduced for 40 minutes.

In both samples the suspension was acidified after the end of the ozonization with HCl at pH 2.7 and 1.5 % of standard strongly acid catex in H<sup>+</sup> form was added. The acidified suspension with the cation exchanger was stirred for 40 minutes (the extraction of heavy metals took place over this period at pH 2.2 - pH 2.7).

Sample OM: A sample of 500 ml of original microbial suspension (of thin slurry) was ozonized for 20 minutes, then 1.5 % of strongly acid catex was added in H<sup>+</sup> form immobilized on a magnetic carrier. The acidified suspension with the catex was stirred for 40 minutes, after which the catex was removed with a permanent magnet. The extraction was carried out at pH 2.7 - pH 3.0.

The results are given in Table 12.

Table 12

The influence of ozonization on the extraction of heavy metals, concentration in ppm in dry matter

Sample OA			
Metal	Starting suspension	After leaching	Decrease, %
Zn	1640.3	585.4	64.3
Pb	134	62	53.7
Cd	18.4	12.9	29.9
Ni	89.1	72.9	18.2
Cr	551.4	238.1	56.8
Sample OB			
Zn	1640.3	920.9	43.9
Pb	134	69.2	48.4
Cd	18.4	9.4	48.9
Ni	89.1	48.0	46.1
Cr	551.4	236.2	57.2
Sample OM			
Zn	1640.3	651	60.3
Pb	134	83.4	37.8
Cd	18.4	10.3	44.0
Ni	89.1	35.0	60.7
Cr	551.4	333.7	39.6

*Conclusions resulting from the experiments with ozonization*

Increased doses of ozone led to significant improvements only in the leaching of nickel and cadmium. If we compare the effects of leaching metals through ozonization and through chlorination, we find a greater effect of extraction only in the case of chrome. In the case of all the other metals the leaching is more effective with the use of chlorination. Magnetic catex (which has the same functional group ( $-\text{SO}_3\text{H}$ ) as standard catex) is more effective for leaching nickel and cadmium than standard catex. In the case of chrome it is difficult to make a quantitative comparison of effectiveness, as if the magnetic catex (which is composed on the basis of Cr) is not completely quantitatively separated, the residue content of chrome in the sludge may be apparently increased.

On the whole it may be said that the effectiveness of magnetic catex is comparable with standard catex, which is an important conclusion from the point of view of the technological utilization of this method (possibility of continuous separation of catex in the course of extraction of metals).

Oxidation of the suspension is an important positive factor influencing the effectiveness of the extraction.

*Old sludge after anaerobic stabilization (20 days)*

Procedure: 500 ml of sample, the sample ozonized for 20 minutes, suspension acidified at pH 2.8 - pH 3, 1.5 % of magnetic catex added in  $\text{H}^+$  form. After 40 minutes of stirring the catex was separated. The results are given in Table 13.

Table 13  
The effectiveness of the metal extraction after previous ozonization in old sludge (20 days under anaerobic conditions), ppm in dry matter

Metal	Starting sludge	After leaching	Decrease, %
Cu	594	618.8	-
Zn	2428	1649	32.1
Ni	123	65	47
Cd	32.6	23	29.4
Cr	326	244	25
Pb	300	267	11.1

The effectiveness of the elimination of metals from a mixed microbial suspension after standing under anaerobic conditions is lower than in the case of fresh sludge. This shows the irreversible fixation of heavy metals (evidently in the form of sulphides) on the microbial substance. The quantity of the bound sulphide depends on how long the sludge is maintained under anaerobic conditions. This conclusion is important from the point of view of further technological applications of acid extraction methods (the sludge should be treated when it is as fresh as possible, i.e. return sludge).

### Bioremedial methods

The basis of the method consists in transforming the insoluble sulphides of heavy metals into the more easily soluble sulphates, through so-called bacterial leaching.

Dry granulated sludge was used, which was ground to a fine powder and 150 g of powder mixed with 1500 ml of distilled water, in two flasks. Acidity was adjusted to pH 2. Both flasks were put into shakers and shaken (125 rpm) at a temperature of 30°C for 13 days, after which the contents of both flasks were left without shaking for a further 10 days. A constant value of pH 2 was maintained throughout the whole experiment.

Acidophilic sulphide bacteria *Thiobacillus ferrooxidans* (CCM 3973) were used as microorganism, cultivated aerobically at 30°C in a medium with the composition (all to 1000 ml of water, pH 2): 0.4 g  $(\text{NH}_4)_2\text{SO}_4$ , 0.1 g  $\text{K}_2\text{HPO}_4$ , 0.4 g  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ , 33.3 g  $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ . The results are given in Table 14.

Table 14  
Results of bacterial leaching of granulated sludge after 23 days of leaching  
W, mg/l, means the water phase, S, mg/kg dry matter, means the microbial phase

Metal	Initial state		After leaching	
	W	S	W	S
Zn	0	719	63	92
Cu	0	522	22	290
Pb	0	289	0.1	280



It is clear that, as a result of microbial leaching there is a release of Zn and Cu into the solution. This fact is important especially for the possibility of the extraction of Cu from old (rotted) sludge, in which copper is very strongly fixed onto the bacterial biomass and is practically impossible to eliminate even with very strongly acid leaching (without the simultaneous oxidation with strong oxidants).

## CONCLUSION

Acid leaching of bacterial biomasses is a very effective method of eliminating heavy metals from sludge, in the case under study from sludge composed mainly of bacterial biomass. But at the same time it is clear that none of the methods studied of acid leaching of a mixed bacterial biomass retaining heavy metals is sufficient by itself for the quantitative extraction of these metals, i.e. it does not make it possible to attain residue values of heavy metals such as would enable the sludge, after simple extraction, to be used e.g. in agricultural soil.

The small differences in the effectiveness of extraction between cells with undisrupted walls and disrupted cell walls after thermal processing, when temperatures rose well over 100°C, and when it could be supposed that even the interior of the cells would be accessible to acid leaching, show that the heavy metals are evidently mostly fixed in the sludge on the surface of the cells as sulphides that are very difficult to eliminate with acids, even with the simultaneous effect of strong oxidative agents. It can however be deduced from our results that the successive combination of all these methods, for which a strongly acid area of leaching under values of pH 3 is typical, for instance in the sequence: oxidation with chlorine in the presence of cation exchangers (pH 3) - release of part of the bound sulphides bacterially (pH 2) and final extraction in a strongly acid medium (pH 1), could be practically utilizable for the elimination of most of the heavy metals, especially the highly toxic  $\text{Cd}^{2+}$  and  $\text{Cr}^{6+}$  from these

systems. In this way it would be possible to make use of large quantities of waste waterworks sludge for agricultural purposes as an organic fertilizer. Our preliminary laboratory experiments simulating such a sequence have shown that it is possible to achieve the required residue contents of heavy metals in this way (in the case of highly contaminated sludge the concentration of Pb remains rather high, but a very strong bond of lead sulphide to the cells can be assumed and therefore the safe fixation of this metal in the soil).

What must be considered important is the finding that for the possible technological application of these acid extractions the freshest possible biomass must be treated, that has not undergone anaerobic rotting of the sludge in a strongly reducing medium while being stored.

#### REFERENCES

1. Dean R.B., Suess M.J.: Waste Management and Research 3, 251 (1985).
2. Matthew P.J., Andrews D.A., Critchley R.F.: Methods for the application and incorporation of sludge in processing and use of sewage sludge (P.L. Hermite, Q.H. Ott, Eds.). p. 244, D. Reidel, Dordrecht, Holland 1984.
3. Macaskie L.E., Dean A.C.R.: Environ. Technol. Lett. 3, 49 (1982).
4. Baldry M.G.C., Dean A.C.R.: Microbios Lett. 15, 105 (1980).
5. Houba C., Remacle J.: Appl. Environ. Microbiol. 47, 1158 (1984).
6. Mann H., Fyfe W.S., Kerrich R.: Tox. Assess. 3, 1 (1988).
7. Dunn G.M., Bull A.T.: Eur. J. Appl. Microbiol. Biotechnol. 17, 30 (1983).
8. Rossi G. in: Proc. 5th European Congress Biotechnol., Vol. 1, p. 552, Copenhagen 1990.
9. Korenovsky A.A., Kazavaiko G.I. in: Ibid. p. 561.
10. Boogerd F.C., Boon M., Meeder T.A., Bos P. in: Ibid. p. 558.
11. Beveridge T.J., Murray R.G.E.: J. Bacteriol. 141, 876 (1980).

12. Trevors J.T., Stratton G.W., Gadd G.M.: *Can. J. Microbiol.* 32, 447 (1986).
13. Strandberg G.W., Shumate S.E., Parrot J.R.: *Appl. Environ. Microbiol.* 41, 237 (1981).
14. White C., Gadd G.M.: *FEMS Microbiol. Ecol.* 38, 277 (1986). *J. Gen. Microbiol.* 133, 727 (1987).
15. Lester J.N., Sterritt R.M., Rudd T., Brown M.J. in: *Microbial Methods for Environmental Biotechnology* (J.M. Grainger, J.M. Lynch, Eds.), p. 197, Academic Press, London 1984.