

ORIGINAL PAPER

Bioleaching of hazardous waste

Klára Drobíková*, Lucia Rozumová, Hana Otoupalíková, Jana Seidlerová

Nanotechnology centre, VSB-Technical University of Ostrava, 17. listopadu 15, 708 33 Ostrava-Poruba, Czech Republic

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Landfill represents the least environmentally-friendly method of waste disposal because of possible pollution to the environment. Dangerous wastes pose the greatest problems and are often disposed of by combustion. This process reduces their volume but entails the formation of new types of dangerous waste. The present study focuses on the possibilities of the removal of the hazardous properties of waste originating from hazardous waste incinerators (three types of bottom ash and charcoal from flue gas cleaning) by bioleaching. Toxic pollutants originating from waste could be removed by bioleaching with *Acidithiobacillus ferrooxidans*. The effectiveness of bioleaching was evaluated on the basis of the pollutant content in the aqueous leachates. For studying the relation between the efficiency of bioleaching and the binding of pollutants in the waste, Tessier's sequential extraction was used. A comparison of bioleaching efficiency and the results of sequential extraction shows that bioleaching can be used to remove elements which are in an exchangeable form or are bound to carbonates, meaning that they are bound in bio-available forms. Bacterial activity was also shown to change the bonds of pollutants in wastes, leading to increased solubility of the pollutant. © 2015 Institute of Chemistry, Slovak Academy of Sciences

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Introduction

Landfill represents the least environmentally-friendly method of waste disposal because of possible pollution to the environment. Over recent decades, the wastes have either been recycled or their properties have been modified by an appropriate treatment so as to render them usable as secondary materials. Dangerous wastes are the most problematic wastes; most of these are disposed of by thermal methods such as incineration, gasification and pyrolysis. These processes reduce their volume, but also lead to the formation of new types of dangerous waste, for example, fly ash, bottom ash and the products of cleaning waste gases. These products are evaluated in accordance with the applicable EU legislation. European Committee for Standardization (2002) stipulates the criteria for the acceptance of waste at landfills and the limit values for inert, non-hazardous and hazardous materials.

The pollutants in wastes are bound in various chemical compounds which are not necessarily harm-

ful to the environment. Sequential leaching or extraction can be used to distinguish the bonds of different forms of a given pollutant. In general, a multiple extraction is carried out, using any number of steps and any number of extraction agents. The aggressiveness of the extraction agent increases with each stage. Tessier is considered to be the founder of speciation analysis (Tessier et al., 1979; Tessier & Turner, 1995; Batley, 1989). He categorised pollutants in four forms and a residual fraction: (i) exchangeable ions; (ii) forms bound to carbonates; (iii) forms bound to Mn and Fe oxides; (iv) forms bound to organic matter; (v) forms in the residual fraction.

The speciation analysis procedure was developed gradually, distinguishing between forms bound to Mn oxides, hydrated Fe oxides (denoted as amorphous) and Fe-oxides (denoted as crystalline).

Exchangeable ions are bound by electrostatic forces onto the material surface or by ions weakly adsorbed on the surface of mineral or organic particles. Ions bound in soluble minerals also belong to

*Corresponding author, e-mail: klara.drobikova@vsb.cz

this group (Spear et al., 1998). This means that ions in this group are readily leached by water or weak electrolyte solutions. The carbonate-bound elements form relatively stronger bonds and can be released by slightly acidic solutions. Unlike the carbonates, the oxidic phase is regarded as part of a residual fraction. Mn and Fe oxides have a great capacity to bind heavy metals to their surface. The secondary oxides are presumed to be formed by co-precipitation, adsorption, ion exchange, penetration into the grid or by forming surface complexes. The type of bonds which exchangeable ions form with Mn and Fe oxides is dependent on the reaction conditions. The mechanism of forming bonds can include several processes. The compounds formed can create a coating on the surface of oxides, which can be separated by various reducing agents. Organic substances represent an important part of the bio-available materials for which the speciation analysis was developed. Industrial waste can also contain organic substances, in which the toxic substances can be directly bound to the surface or in the form of organometallic compounds; these latter can be released by oxidising agents. Another significant group of toxic phases can be firmly bound in the remaining part of the matrix in the form of oxides, carbides, silicates or clay materials. These phases can be released only after complete decomposition by strong mineral acids or fusion.

Since 1979, when the speciation analysis was first applied, the stated procedure has been modified in terms of the number of steps and agents used by many authors. Tessier's procedure used for sediments was modified, for example, by (Campanella et al., 1995). Subsequently, the speciation analysis has also been used for studying the forms of pollutants in soil, sediments (Borovec, 2000; Venditti et al., 2000; Cauwenberg & Maes, 1997; Száková et al., 1997; Calvet et al., 1990; Zeihen & Brummer, 1989; Shuman & Hargrove, 1985; Sposito et al., 1982), geological materials (Tossavainen & Forsberg, 2000; Hall et al., 1996), in coal and its ash (Bartoňová et al., 2001; Querol et al., 1996; Buchholz & Landsberger, 1995; Raclavská et al., 1995; Belevi et al., 1992) and in fly ash from waste incinerators (Tan et al., 1997; Kirby & Rimstidt, 1993; Theis & Padgett, 1983). Some studies compare speciation analysis with leaching procedures such as United States Environmental Protection Agency (US EPA), or with leaching by acid solution (Buchholz & Landsberger, 1995). Recently, the speciation analysis has been applied to metallurgical waste (Kulveitová, 1999; Polyák et al., 1995). The sequence extraction procedure developed by Polyák et al. (1995) distinguishes seven forms of pollutants: (i) exchangeable ions; (ii) forms bound to carbonates; (iii) forms bound to Mn oxides; (iv) forms bound to organic matter; (v) forms bound to amorphous Fe oxides; (vi) forms bound to crystalline Fe oxides; (vii) forms bound in residual fraction.

In the study by Kulveitová, Polyák's speciation analysis procedure was applied to blast furnace sludge and sludge from steelworks (Kulveitová, 1999); the way in which cadmium, lead and zinc form their bonds was also studied. Due to the time-demanding preparation of extracts, a shortened procedure distinguishing only three forms of pollutants was designed: (i) exchangeable and easily extracted forms; (ii) forms bound to organic matter; (iii) forms bound in residual fraction.

This work studies the preferential binding of Cd, Pb, Zn in the three forms referred to above. By applying the shortened sequential extraction procedure, only the amount of leached zinc was comparable to the original full procedure (Kulveitová, 1999; Kulveitová et al., 1997, 2000). Understanding of how pollutants form bonds in waste can significantly simplify the choice of technology to be used in order to remove a given pollutant. This study uses speciation to evaluate the efficiency of bioleaching.

The dangerous wastes either have to be disposed of at special landfills or treated by solidification, vitrification and bitumination, as demonstrated by Visvanathan, 1996; Batchelor, 2006; Malviya & Chaudhary, 2006; Derie, 1996; Fuoco et al., 2005; Kavouras et al., 2003; Piantone et al., 2003, so that their hazardous properties will be reduced. Ashes free from undesirable contaminants can be used in construction, especially in the preparation of concrete, mortar, cement, aerated concrete and synthetic stones. Then they are used for producing bricks or interior elements; they can also be used as a base layer or material for road construction, etc. Toxic pollutants from waste could be removed by bioleaching which is widely used in the treatment of ores and mineral separation. *A. ferrooxidans* is one of the most commonly used bacteria.

A. ferrooxidans has been an economically significant bacterium in the leaching of sulphide ores since its discovery in 1947 by Colmer and Hinkle as described by Fečko et al. (2004). The discovery of *A. ferrooxidans* led to the development of a new branch of metallurgical sciences known as bio-hydrometallurgy. This branch deals with all aspects of the microbial extraction of metals from minerals, solid wastes and acid mine water as described by Corkhill & Vaughan, 2009; Drogui et al., 2003; Rohwerder et al., 2003; Olson et al., 2003. *A. ferrooxidans* and *Acidithiobacillus thiooxidans* convert insoluble metal sulphides into soluble metal sulphates. Currently, bioleaching is mainly used in recovering copper, uranium and gold from ores. Bioleaching also has some potential for the detoxification of industrial waste products, sewage sludge and soil contaminated by heavy metals as detailed by Narayan and Sahana (2009). As described by Bayat and Sari (2010a, 2010b), bioleaching techniques using *A. ferrooxidans* have been used to recover heavy metals (Zn, Cu, Ni, Pb, Cd and Cr) from drained sludge formed during electroplating with no sulphide or sul-

phate compounds. Bioleaching processes have been used to mobilise metals from printed circuit boards (Ivanus, 2010; Yang et al., 2009; Choi et al., 2004) and batteries (Mishra et al., 2006). There have also been many studies related to bioleaching of the waste from mining Cu (Dopson et al., 2009) in different mining areas from Europe to Africa (Andráš et al., 2008; Trois et al., 2007) and Chile (Escobar et al., 2009). *A. ferrooxidans* and *A. thiooxidans* were used to recover metal from waste petroleum catalysts as described by Pradhan et al. (2010) and to recover heavy metals from metallurgical waste (Kovohute, Pribram, Czech Republic) as described by Fečko et al. (2011). Finally, bioleaching is used to reduce the concentration of toxic elements in lignite ash (Jekić et al., 2007). Some authors treated metallurgical wastes by bioleaching to remove lead (Fečko et al., 2011), cadmium and zinc (Štěrbová et al., 2004; Kratošová et al., 2012). Information on the application of bioleaching to fly or bottom ash is limited, hence bottom ash originating from incinerating chemical, medical and industrial wastes was chosen for this study. The study also deals with a bioleaching treatment of charcoal used for cleaning flue gases.

Experimental

Two types of dangerous waste (charcoal and bottom ash) from dangerous waste incinerators were studied. The charcoal was used for cleaning the flue gas released while incinerating wastes from health services I (sample A). The three bottom ashes originated from incinerating health service waste II (sample B), chemical waste (sample C) and industrial waste (sample D).

The chemical compositions of solid samples were determined by X-ray fluorescence spectroscopy (energy disperse spectrometer SPECTRO X-LAB, Germany), the morphology and shapes of waste particles were studied by electron microscopy (PHILIPS XL-30 raster electron microscope with RTG spectrometer EDAX, PHILIPS XL series30, The Netherlands). The phase compositions were determined by X-ray diffraction analysis (ID 3003/URD-6 diffraction unit Freiburger Präzissionsmechanik/Seifert Roentgen, GE Inspection Technologies-Seifert Roentgen, Germany) in laboratories in the Institute of Geological Engineering, Technical University of Ostrava (Ostrava, Czech Republic). The phenol index was determined by UV-VIS spectrometry (LAMBDA 11, Perkin-Elmer, Germany). The concentration of soluble substances (TDS) was determined gravimetrically. The concentrations of As, Mo, Se and Sb were determined by GF-AAS (UNICAM 989 QZ, CHROMSPEC, United Kingdom), the concentrations of Cd, Ni, Pb and Zn were determined by F-AAS (UNICAM 969, CHROMSPEC) and the concentrations of other elements were determined by AES-ICP (Spectro Ciros Vision). The proportion of

mercury in the solid samples and leachates was determined using an atomic absorption spectrometer (AMA 254, ALTEC, Czech Republic). The concentrations of selected anions in the leachates were determined by ionic chromatography (WATERS 431, Waters, USA) and the pH of leachates was measured using a Gryf 158 (GRYF HB, Czech Republic). All the analytical methods used are validated with the combined standard uncertainty estimated.

The water extracts from the samples studied prior to and after bioleaching were prepared in accordance with European Committee for Standardization (2002) characterising wastes (Czech Office for Standards, Metrology and Testing (2003)). Deionised water was used to prepare water extracts in the solid/liquid ratio 1 : 10. The solid phase of wastes was separated by membrane filters with a pore size of 0.45 μm . The extracts were then stabilised using concentrated nitric acid and analysed by ICP-AES and GF-AAS or F-AAS.

The proportions of bio-available elements and elements bound to iron and manganese oxides were determined using the three steps of Tessier's sequential extraction as follows: (i) exchangeable: the sample was extracted using 1 M MgCl_2 , at pH 7.0 under continuous agitation at ambient temperature for 1 h; (ii) bound to carbonates: the residue from the first step was leached with 1 M NaOAc adjusted to pH 5.0 with HOAc at ambient temperature. Continuous agitation was maintained and the time needed for complete extraction was 5 h; (iii) bound to iron and manganese oxides: the residue from the second step was extracted with 0.04 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 25 vol. % HOAc . The latter experiments were performed at $(96 \pm 3)^\circ\text{C}$ with occasional agitation over 6 h.

After subsequent cooling, the solid phase was separated from the extract by membrane filters with a pore size of 0.45 μm (Tessier et al., 1979).

Bioleaching (or bacterial leaching) was applied in order to remove pollutants (Drobíková, 2010; Rozumová, 2010). Bioleaching was carried out dynamically, using a batch reactor at ambient temperature with the presence of an activation medium. The sample of waste was ground to a particle size of less than 0.05 mm and treated by bioleaching, using *A. ferrooxidans* and medium 9K of Silverman and Lundgren. The medium is a mixture of two solutions at a ratio of 1 : 1. The former contains $(\text{NH}_4)_2\text{SO}_4$, KCl , K_2HPO_4 , $\text{Ca}(\text{NO}_3)_2$, $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ and distilled water and the latter contains 0.5 M H_2SO_4 , $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ and distilled water. Leaching was performed at ambient temperature and pH was within the range of 1.8–2.0. Leaching was carried out for one month under continuous aeration of the suspension. 100 g of the sample was mixed with 1L of Silverman 9K and with *A. ferrooxidans*. Subsequently, the suspension pH was adjusted by 10 M H_2SO_4 . After one month, the solid phase was filtered using a filter with

Table 1. Chemical composition of solid wastes

Element	Sample A	Sample B	Sample C	Sample D
	mass %			
Al	< 0.21	5.03	5.48	< 0.05
Br	1.21	0.05	0.01	0.15
Ca	12.9	12.3	13.9	0.56
Cl	13.9	2.49	4.51	2.65
Fe	0.17	22.3	7.39	0.72
K	0.41	0.50	0.87	1.0
Mg	< 0.20	1.85	1.74	< 0.59
Mn	0.01	0.16	0.22	0.01
Na	6.90	1.22	4.35	11.1
P	< 0.02	0.33	0.72	0.09
S	0.58	1.78	1.20	1.07
Si	0.07	8.39	9.09	0.77
	mg kg ⁻¹			
Ba	88.3	6157	21440	5163
Cd	29	44.8	3625	635
Cr	< 7.4	2642	4027	70.5
Cu	82.2	1007	3374	567
Mo	< 3	353	232	477
Pb	628	116	9133	4229
Zn	6086	5420	5048	11790
Se	2.9	< 3.1	< 4.5	182

a pore size of 0.45 μm and washed with distilled water until neutral pH was achieved. Next, the samples were dried in a laboratory oven and analysed.

Results and discussion

Chemical and phase composition of solid wastes

The compositions of waste samples are shown in Table 1. The chemical analyses disclosed a variation in the composition of samples. Despite sample A be-

ing a different type of sample, it contains a similar amount of Ca as samples B and C. Sample A contains the highest amount of Cl (14.0 mass %) and approximately 7 mass % of Na. Samples B and C contain similar amounts of Al (5.0–5.5 mass %), Si (8.5–9.1 mass %), Mn (about 0.2 mass %) and Mg (about 1.8 mass %) but they have different contents of Fe, Na and Cl. Sample D contains the highest amount of Na (11.1 mass %) and has a similar content of Cl as sample B (approximately 2.5 mass %). Table 1 shows that the samples also contain dangerous elements. Sample C contains the highest amount of Ba (2.1 mass %), sample B and D contain about 0.55 mass %. The highest Zn content is in sample D (1.18 mass %) and the other samples (A, B, C) contain similar amounts of Zn (0.5–0.6 mass %). Samples B and C contain more Cu and Cr than samples A and D, but sample D has a high content of Se (182 mg kg⁻¹). Each sample contains different amounts of Cd and Pb.

The results of phase analysis (Table 2) show that the amorphous phase is the predominant phase in the samples. Halite and silicon dioxide were found in samples A and C. The different contents of Fe in samples B and C were also affected by the content of hematite. Fe is also bound in magnetite in sample B. In each sample Ca is bound in a different type of compound.

Scanning electron microscopy

An example of the outcome of scanning electron microscopy (SEM) analysis is presented in Fig. 1. The particles of charcoal (sample A) are aggregated into larger units and are approximately < 50 μm . The particles of fly ashes B and C are very similar although the samples were created by the incineration of different kinds of waste. Samples B and C consisted of particles of various sizes and shapes (Figs. 1b and 1c). In comparison with samples A–C, sample D consisted mostly of very small particles (less than approximately 20 μm).

Table 2. Mineralogical composition of wastes studied

Phase	Formula	Sample A	Sample B	Sample C	Sample D
amorphous	–	xxx	xxx	xxx	xxx
anhydrite	CaSO ₄	–	x	–	–
bassanite	2CaSO ₄ · H ₂ O	x	–	–	–
calcite	CaCO ₃	–	x	–	–
calcite magnesian	(Ca, Mg)CO ₃	–	–	x	–
C3A cubic	–	x	–	x	–
halite	NaCl	xx	–	–	xx
hematite	Fe ₂ O ₃	–	xx	x	–
magnetite	Fe ₃ O ₄	–	xx	–	–
plagioclase	NaAlSi ₃ O ₈ –CaAl ₂ Si ₂ O ₈	–	–	x	–
portlandite	Ca(OH) ₂	xx	–	–	–
quartz	SiO ₂	–	x	x	x
sanidine	(K, Na)(Si, Al) ₄ O ₈	–	x	x	–

xxx – High abundance, xx – medium abundance, x – minor abundance.

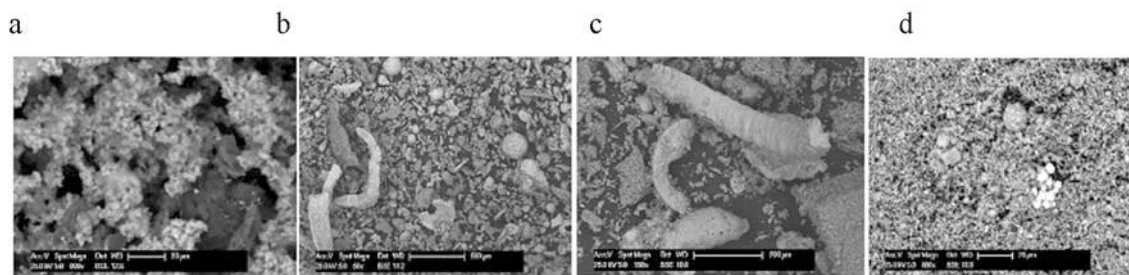


Fig. 1. SEM images of charcoal and fly ash particles of sample A (a), sample B (b), sample C (c) and sample D (d).

Table 3. Concentration in aqueous extract

Parameter	Before BL				After BL				Leaching limited values		
	Sample A	Sample B	Sample C	Sample D	Sample A	Sample B	Sample C	Sample D	Inert	Non-hazardous	Hazardous
	Concentration in aqueous extract/(mg L ⁻¹)										
TDS	87500	3400	5800	70500	2210	2520	2770	646	400	6000	10000
pH	11.7	11.4	10.7	6	3.8	4.9	4.6	2.9	–	–	–
As	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.05	0.2	2.5
Ba	4.06	0.17	0.32	0.76	0.06	< 0.05	< 0.05	< 0.05	2	10	30
Cd	< 0.005	< 0.005	< 0.005	51	0.011	4.88	< 0.005	0.11	0.04	0.1	0.5
Cr total	< 0.06	2.38	11.3	< 0.06	< 0.03	< 0.03	< 0.03	< 0.03	0.05	1	7
Cu	0.17	< 0.03	< 0.06	9.79	0.1	0.65	0.1	0.92	0.2	5	10
Hg	0.025	< 0.001	0.003	0.004	< 0.001	< 0.001	< 0.001	< 0.001	0.001	0.02	0.2
Mo	0.12	2.13	1.6	3.81	< 0.01	< 0.01	< 0.01	0.044	0.05	1	3
Ni	< 0.03	< 0.03	< 0.03	0.61	< 0.02	0.33	0.63	< 0.02	0.04	1	4
Pb	31	< 0.05	0.08	3.7	0.84	0.26	0.08	2.88	0.05	1	5
Sb	0.032	< 0.01	0.019	0.21	< 0.01	0.017	< 0.01	0.034	0.006	0.07	0.5
Se	0.025	< 0.01	0.13	2.8	< 0.01	< 0.01	< 0.01	0.014	0.01	0.05	0.7
Zn	15.5	0.15	0.66	1009	5.52	2.78	2.93	11	0.4	5	20
chloride	39400	825	2220	20500	26	23	27	15.6	80	1500	2500
fluoride	< 300	< 100	< 100	< 300	< 1	< 1	18.8	< 1	1	15	50
sulphate	852	756	546	18700	1470	1530	1650	380	100	2000	5000
phenol in.	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	0.1	–	–

Aqueous leaching

Aqueous leachates of the wastes studied were evaluated in accordance with European Committee for Standardization (2002) (Table 3). Table 3 shows that, prior to bioleaching, only sample B met the requirements of Council Directive 2003/33/EC and was classified as dangerous. The concentrations of Cr and Mo in the leachate exceeded the limit values for non-hazardous waste. Leachates prepared from the other three samples (A, C and D) exceeded the limit parameters for hazardous wastes. Only the total concentration of Cr in the leachate prepared from sample C exceeded the limit value. The concentrations in the leachate prepared from sample A exceeded the limit parameters for Pb and chloride and TDS. Most limit parameters were exceeded in the leachate of sample D (concentration of Cd, Mo, Se, Zn, chloride, sulphate and TDS). Accordingly, samples A, C and D have to be treated prior to deposition in landfills. Bioleaching was chosen as the method of treating selected wastes.

Although sample B met the requirements of Council Directive 2003/33/EC for landfills, the method of bioleaching was used for treatment of this sample also.

Efficiency of bacterial leaching

The efficiency of bioleaching of the wastes studied was evaluated by a water-leaching test. Table 3 shows the values of the parameters monitored in leachates prepared from wastes after bioleaching. The values of all the exceeded parameters decreased in the leachates prepared from samples A, C and D. These three samples were less hazardous after bioleaching because they no longer exceeded the limit values for dangerous wastes. The value of TDS clearly indicated that bioleaching reduced the amount of soluble compounds in all samples. The changes in pH resulted from the presence of the residual amount of the Silverman 9K activation medium used during bioleaching, which enhanced the solubility of pollutants. Bioleaching decreased the soluble concentration of chloride and zinc

Table 4. Proportion of leached pollutants prior to and after bioleaching (BL) and in fraction of sequential extraction for sample A

Element	Aqueous extract/mass %		Sequential extraction/mass %			
	Before BL	After BL	step I	step II	step III	steps IV + V
Ba	46.0	0.7	43.4	22.4	10.5	23.7
Cd	< 0.5	< 0.5	4.6	92.1	2.3	< 0.5
Cr	8.1	4.1	3.5	3.7	90.8	2.0
Cu	2.1	1.2	< 0.5	98.1	< 0.5	0.9
Mo	40.0	3.3	8.8	75.9	14.2	1.1
Pb	49.4	1.3	5.5	63.2	25.6	5.7
Zn	2.6	0.9	< 0.5	63.0	9.4	27.1

in samples A and D, and that of Cr and Mo in sample C. After bioleaching, it was possible to classify samples A, C and D as wastes acceptable at landfills for hazardous waste. After bioleaching sample B, the concentration of Cr and Mo in the leachate decreased whereas the concentration of Cd increased, hence the limit value for dangerous waste was exceeded. Prior to bioleaching, the concentration of Cd was lower than 0.005 mg L^{-1} and after bioleaching it was higher than 4 mg L^{-1} , which means that the sample did not comply with the criteria for acceptance at a hazardous waste landfill.

The effect of bioleaching on a similar type of bottom ash (samples B, C and D) was different. Accordingly, the monitored parameters of water extract prepared from the samples after bioleaching could be divided into three groups: (i) parameters which remain stable prior to and after bioleaching (As, phenol index); (ii) parameters with decreased value after bioleaching compared with that prior to bioleaching (pH, Ba, Hg, Mo, chloride, fluoride, TDS); (iii) parameters which change differently for each sample (Cd, Cr total, Cu, Ni, Pb, Sb, Se, Zn, sulphate).

Bonding of pollutants

The sequential extraction of the samples studied was performed in order to explain the differences in leachability of selected elements prior to and after bacterial leaching. To evaluate the bonds of pollutants and bioleaching efficiency, the concentrations of pollutants measured in the extracts were converted to their contents in the solid phase. Next, the proportion of pollutants leached into the extraction agent was calculated (Eq. (1)):

$$\eta = (w_1/w_s) \times 100 \% \quad (1)$$

where w_1 (mass %) is the content of pollutant leached into the extract, then converted to the solid phase and w_s (mass %) is the content of pollutant in the dry matter.

Tables 4–7 show the proportion of selected pollutants leached from waste samples prior to and after bacterial leaching, and in three steps of sequential ex-

traction. The amount of pollutant in fraction IV and V was calculated (Eq. (2)):

$$\begin{aligned} \eta(\text{steps IV} + \text{V}) &= \\ &= 100 - \eta(\text{step I}) - \eta(\text{step II}) - \eta(\text{step III}) \end{aligned} \quad (2)$$

where $\eta(\text{step I})$, $\eta(\text{step II})$ and $\eta(\text{step III})$ (all in %) are the portion of pollutant leached into the extract in accordance with Tessier's procedure in steps I, II and III respectively.

Table 4 presents a comparison of the proportions of pollutants in different forms and the leachable proportion of pollutants prior to and after bioleaching. The results of sequential extraction show that more than 40 mass % of Ba was in exchangeable form but only up to 10 mass % of Cd, Cu, Pb and Zn were found in that form. The predominant amounts of Cd, Cu, Mo, Pb and Zn were bound to carbonates. After bioleaching, the leachable part of Ba, Cr, Mo, Pb and Zn decreased distinctively. This means that the monitored pollutants were removed by the bioleaching, i.e. that part of the pollutants corresponded to exchangeable and carbonate forms meaning that the exchangeable and carbonate forms of pollutants in sample A were affected by *A. ferrooxidans*.

The sequential extraction proved that all the monitored pollutants with the exception of Mo were found in an exchangeable form (Table 5). These results corresponded to their concentration (extracted proportion) in the leachate. The amount of Mo found in exchangeable form was in good agreement with the portion released into the aqueous extract (6.0 mass %). The sequential extraction showed that almost all the Pb content was bound to iron and manganese oxides. Over 90 % of the remaining monitored elements were bound to organic matter and residual fractions (fractions IV and V, respectively). Nevertheless, bioleaching resulted in a change in Cd bonds which after bioleaching changed into the water-soluble form. The slightly acidic pH of the extract (pH 1.8–2.0) probably led to the higher solubility of Cd compounds in a leachate.

All the monitored pollutants in sample C with the exceptions of Mo and Cd were in exchangeable forms (Table 6). These results correspond to the amount of

Table 5. Proportion of leached pollutants prior to and after bioleaching (BL) and step sequential extraction for sample B

Element	Aqueous extract/mass %		Sequential extraction/mass %			
	Before BL	After BL	step I	step II	step III	steps IV + V
Ba	< 0.5	< 0.5	< 0.5	1.0	< 0.5	98.0
Cd	< 0.5	99.9	< 0.5	1.4	< 0.5	97.6
Cr	0.9	< 0.5	0.6	0.5	7.4	91.5
Cu	< 0.5	0.7	< 0.5	< 0.5	2.6	96.4
Mo	6.0	< 0.5	5.3	2.5	6.3	85.9
Pb	< 0.5	2.3	< 0.5	1.9	96.1	1.5
Zn	< 0.5	0.5	< 0.01	6.0	7.4	86.6

Table 6. Proportion of leached pollutants prior to and after bioleaching (BL) and step sequential extraction for sample C

Element	Aqueous extract/mass %		Sequential extraction/mass %			
	Before BL	After BL	step I	step II	step III	steps IV + V
Ba	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	98.5
Cd	< 0.5	< 0.5	7.2	22.1	21.8	48.9
Cr	2.8	< 0.5	< 0.5	< 0.5	3.0	96.0
Cu	< 0.5	< 0.5	< 0.5	< 0.5	2.1	96.9
Mo	6.9	< 0.5	8.5	< 0.5	7.3	83.7
Pb	< 0.5	< 0.5	< 0.5	1.6	10.9	87.0
Zn	< 0.5	0.6	< 0.5	6.3	15.9	77.3

Table 7. Proportion of leached pollutants prior to and after bioleaching (BL) and step sequential extraction for sample D

Element	Aqueous extract/mass %		Sequential extraction/mass %			
	Before BL	After BL	step I	step II	step III	steps IV + V
Ba	< 0.5	< 0.5	< 0.5	< 0.5	0.7	98.3
Cd	80.3	< 0.5	87.9	5.8	4.7	1.6
Cr	< 0.5	< 0.5	< 0.5	1.9	19.3	78.3
Cu	17.7	1.6	14.7	7.3	50.8	27.2
Mo	8.0	< 0.5	4.3	3.3	22.7	69.7
Pb	0.9	0.7	2.9	8.8	17.6	70.7
Zn	85.6	0.9	59.5	8.1	23.1	9.3

pollutants leached into the aqueous extract. Only cadmium (22 mass %) and a negligible amount of Zn (≈ 6 mass %) were found in carbonates. More than 70 mass % of Zn and 87 mass % of Pb were bound to organic matter and residual fractions (steps IV + V). Approximately 3 mass % of Cr bound to iron and manganese oxides was removed by bioleaching. Bioleaching did not cause any changes in the chemical bonds of pollutants in sample A, as compared to the previous sample B. A small amount of pollutants in bioavailable form and in carbonates was also removed. The leachate prepared from the sample after bioleaching did not contain any pollutants, although the pH of leachate was slightly acidic (pH 4.6).

Table 7 shows the results of sequential extraction and the amounts of leachable pollutants prior to and after bacterial leaching. Sample D contains 90 mass % of Cd in exchangeable form. Taking into con-

sideration the combined standard uncertainty of Zn determination (6 mass %), the amount of Zn in bioavailable forms (about 70 mass %) corresponds to the amount of Zn leached into the extract (75 mass %). The results for Cu and Mo were very similar. Bioleaching removed most of the Cd and Mo in bioavailable forms as well as almost all of Cu and Zn. The sample after bioleaching did not exceed the limit concentrations for dangerous waste.

Conclusions

The study sought to assess the suitability of bioleaching for removing toxic elements from dangerous waste. Three kinds of bottom ash from different types of dangerous waste incinerators (waste from health services II, chemical and industrial wastes) and charcoal after cleaning hospital waste flue gas were selected

for testing. Based on the evaluation of subsequent hazards of waste as defined by European Committee for Standardization (2002), it was found that, after bioleaching, the charcoal used for cleaning flue gas and the bottom ash from chemical and industrial waste incinerators lost their dangerous properties. Because of the change in Cd bonds to a water-soluble form, the properties of the bottom ash from health service waste incinerators worsened. A comparison of bioleaching efficiency and the results of sequential extraction show that bioleaching could be used to remove elements which are in exchangeable form or are bound to carbonates. However, bacteria can change the bonds of pollutants in residual fractions and also those bound to the organic phase of a material. This change does not lead to their removal, but vitiates the parameters characterising dangerous properties – the content of pollutant in the aqueous leachate.

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