The role of iron in metal-bearing waste bioleaching was studied. Four various types of waste (printed circuit boards (PCBs), Ni-Cd batteries, alkaline batteries and Li-ion batteries) were treated by bioleaching using the acidophilic bacteria *A. ferrooxidans* and *A. thiooxidans* (separately or in mixture). Role of main leaching agents (Fe$^{3+}$ ions or sulphuric acid) was simulated in abiotic experiments. Results showed that oxidation abilities of Fe$^{3+}$ ions were crucial for recovery of Cu and Zn from PCBs, with the efficiencies of 88% and 100%, respectively. To recover 68% of Ni from PCBs, and 55% and 100% of Ni and Cd, respectively, from Ni-Cd batteries both oxidation action and hydrolysis of Fe$^{3+}$ were required. The importance of Fe$^{2+}$ ions as a reducing agent was showed in bioleaching of Co from Li-ion batteries and Mn from alkaline batteries. The efficiency of the processes has increased by 70% and 40% in Co and Mn bioleaching, respectively, in the presence of Fe$^{2+}$ ions. Based on the results we suggest the integrated biometallurgical model of metal-bearing waste recycling in the effort to develop zero-waste and less energy-dependent technologies.

**Keywords:** bioleaching; e-waste; acidophilic bacteria; iron

1. Introduction

Microorganisms are capable of transforming many metals that occur in multiple valence states by catalysing redox reactions. Many of them consequently promote the mobilisation or immobilisation of metals in ecosystems due to their dissolution or precipitation [1]. In the case of metal mobilisation by autotrophic microorganisms the oxidation and/or reduction, often combined with production of inorganic acids, are the main reactions involved in bioleaching processes. Bacterial strains, the most often used in bioleaching, belong to genus *Acidithiobacillus*. They can obtain energy oxidising both reduced sulphur compounds and ferrous ion [2]. The acidophilic bacteria are well-known as an important part of sulphur cycle in biosphere. In the symbiosis with sulphate-reducing bacteria they catalyse weathering processes of sulphides [3,4]. In the past much attention was paid to the sulphur oxidation reaction due to bioenergetic considerations [5]. Therefore, for long time sulphur has been considered a key element for bacteria used in metal bioleaching. Regeneration of Fe$^{3+}$ ions by microorganisms was important for maintaining a high redox potential necessary for sulphur oxidation providing low pH values of the system [6]. However, the geomicrobiological characterization of Rio Tinto River proved the importance of the iron cycle for the existence of this ecosystem. Based on the studies of microorganisms living in such extremely acidic environment Johnson [7] and Amils [2] have found that not sulphur but iron is a key element in proper functioning of that ecosystem.

Nowadays, there is an increasing amount of new results on utilization of acidophilic microorganisms in bioleaching of various kinds of metal-bearing wastes with high metal bioleaching efficiencies [8]. Several research works have been undertaken to recover metals from different metal-containing solid waste using acidophilic bacteria (Tab. 1). However, sulphur or sulphidic compounds are generally not main parts of such waste. Metals present are mostly in the metallic form or as a part of non-sulphidic compounds.

Sulphur is not naturally present in waste materials and even if it is added into the bioleaching systems its amount is not sufficiently high to play a key role.

The present studies were carried out to point out the iron importance as well as the possibility to use its oxidised and reduced forms to create a cyclic bioleaching process connecting different waste mimicking the natural conditions. And in such way, to close the loop and facilitate development of zero-waste and less energy-dependent technologies and direct our thinking a step further toward a so-called ‘circular economy’ (EU Communication „Towards the circular economy“). Basis for these technologies we can find in nature, in natural ecosystems, since the success of each ecosystem depends on its recycling ability. Because a system without recycling will only last while the limiting factor (substrate or electron acceptor) is available [5].
Exploitation of acidophilic bacteria for waste bioleaching

| Used bacterial culture                                           | Type of waste | Leaching efficiency (%) | References |
|-----------------------------------------------------------------|---------------|-------------------------|------------|
| Acidithiobacillus ferrooxidans and A. thiooxidans               | PCBs          | Zn – 88.9%, Cu – 99%, Pb – 88.9% | [9]        |
| Acidithiobacillus ferrooxidans and A. thiooxidans               | TV circuit boards | Cu – 24%-84%           | [10]       |
| Acidithiobacillus ferrooxidans, A. thiooxidans and               | PCBs          | Cu – 65-95%             | [10]       |
| Leptospirillum ferrooxidans                                     |               |                         |            |
| Sulfobacillus sp.                                               | Converter slag | Zn – 37.7%, Cu – 81.6%  | [11]       |
| Mixed culture of acidithiobacillus bacteria                     | PCBs          | Cu – 96.8%, Al – 88.2%, Zn – 91.6% | [12]       |
| Acidithiobacillus ferrooxidans and A. thiooxidans               | Lithium-ion battery | Li – 80%, Co – 90%     | [13]       |
| Sulfobacillus thermosulfidoxidans and Thermoplasma acidiphilum | PCBs          | Ni – 82%, Al – 75%, Zn – 80%, Cu – 85% | [14]       |
| S. thermosulfidoxidans and S. acidophilus                       | PCBs          | Zn – 85%, Al – 80%, Cu – 90%, Ni – 82% | [14]       |

In this research work we focused on the metal bioleaching from one group of metal-bearing waste, particularly electrical and electronic waste (e-waste) as it belongs among the one of the fastest growing waste group with the estimated production about 40-50 million tonnes per year [15]. Also, according to metal amount, this waste represents valuable material because of its high metal content. Iron and steel constitutes about 50% of e-waste, non-ferrous metals represent in general 13% [16] but some kind of e-waste, e.g. PCBs contain about 40% of various metals including precious metals.

This paper presents an experimental study conducted to determine the influence of iron on different metal bioleaching from four various types of e-waste (PCBs, alkaline and Li-ion batteries) in which biochemical processes from different parts of iron cycle were applied.

2. Material And Methods

2.1. Bacteria

The acidophilic bacterial cultures of A. ferrooxidans and A. thiooxidans, used in the experiments were recovered from the acid mine drainage water in Smolník and obtained from the Institute of Geotechnics of Slovak Academy of Sciences in Košice, Slovakia. The mixture of A. ferrooxidans and A. thiooxidans was used for metal recovery from PCBs, alkaline and Li-ion batteries. The pure culture of A. ferrooxidans was cultured in 9K medium and used for recovery of metals from alkaline and Ni-Cd batteries. The pure culture of A. thiooxidans was cultured in Waksman and Joffe medium and used for metal recovery from alkaline batteries. The mixture of the bacteria A. ferrooxidans and A. thiooxidans was cultured in the medium containing all nutrients necessary for their growth. The medium for mixed culture composed of KCl – 0.1 g, (NH4)2SO4 – 2.0 g/l, K2HPO4 – 0.25 g/l, MgSO4.7H2O – 0.25 g/l, FeSO4.7H2O – 44.2 g/l, sulphur – 4 g/l and distilled water, 1000 ml [17].

2.2. Material characterization

Four different metal-bearing solid wastes were used in this study: PCBs, spent Ni-Cd batteries, alkaline batteries and Li-ion batteries. The PCBs were crushed and sieved to obtain a mesh size of less than 1 mm. Analysis of the electronic scrap before bioleaching revealed the presence of Cu (19.21%), Zn (1.17%), Ni (0.32%), and Al (1.73%). Alkaline batteries, Ni-Cd and Li-ion batteries were manually cut up into different portions. To obtain the electrodes, the steel case was manually opened and rolled electrodes were separated. The active electrode material, cathode and anode powders were physically removed from the metal grid, ground and sieved to obtain a mesh size of less than 40 μm. Analysis of the Ni-Cd batteries before bioleaching revealed the content of cadmium of 6.5% and 38% for the cathode and anode, respectively. The content of nickel was 47.1% and 22.2% for the cathode and anode, respectively. Analysis of the alkaline batteries showed the presence of Mn and Zn to be 21.4% and 20%, respectively. In the Li-ion batteries the content of Li and Co was 5% and 58%, respectively.

2.3. Leaching experiments

The leaching experiments were carried out in 250 ml Erlenmeyer flasks containing 200 ml of leaching media. The initial pH was adjusted to 1.5 with 10M H2SO4 or 10% NaOH. To each medium 2 g of waste powders were added and simultaneously 5 ml pure culture of A. ferrooxidans, 5 ml A. thiooxidans or 10 ml of their mixture were added to the bioleaching media according to the experimental protocol. The samples for analysis were regularly withdrawn on days: 1, 3, 7, 10, 13, 21, and 28. All experiments were conducted in triplicates.

The PCBs bioleaching was undertaken using the mixed bacterial culture of A. ferrooxidans and A. thiooxidans. The abiotic leaching experiments were carried out in the single H2SO4 solution and also in Fe2(SO4)3 solution. The H2SO4 was prepared by dilution 10M H2SO4 in deionised water. The single Fe2(SO4)3 medium composed of the deionised water and iron(III) sulphate (44.63 g/l of Fe2(SO4)3.9H2O).

The Ni-Cd batteries bioleaching was undertaken in 9K medium using A. ferrooxidans. The abiotic leaching experiments were carried out under the same conditions as in the case of PCBs abiotic leaching.
The bioleaching of alkaline batteries was carried under three different conditions:
- bioleaching using *A. ferrooxidans* in 9K medium,
- bioleaching using *A. thiooxidans* in Waksman and Joffe medium,
- bioleaching using the mixture of *A. ferrooxidans* and *A. thiooxidans* in their growth medium.

Li-ion batteries bioleaching experiments were carried out in two different media using mixed bacterial culture of *A. ferrooxidans* and *A. thiooxidans*:
- rich-nutrient medium (medium for the mixture of *A. ferrooxidans* and *A. thiooxidans* as mentioned above),
- low-nutrient medium (the low-nutrient medium composed only of diluted sulphuric acid at pH 1.5 and a trace amount of elemental sulphur).

### 2.4. Analytical determinations

The pH values were measured throughout the experimental periods by pH meter GRYF 208 L using a combined electrode. The leachant (5 ml) was periodically taken and filtrated. Concentration of the metals in each filtrate was determined by Atomic Absorption Spectrometry (AAS), Perkin Elmer 3100. Chemical composition of the initial metal-bearing waste and the final leaching residues were determined by AAS and X-ray analysis (URD-6 (Rich. Seifert-FPM, SRN), radiation source Co).

### 3. Results and Discussion

#### 3.1. Oxidative and hydrolytic abilities of iron

**3.1.1. Metal dissolution from PCBs**

In general, metals in PCBs occur in various forms; copper and nickel in metallic forms, zinc either in metallic form or alloy, aluminium in metallic form as well as a compound of Al₂O₃. The effect of iron on bioleaching of particular metals differs depending on the type of the metal. According to published results [18] metals in PCBs can be divided into two groups. The first group involves metals which dissolution requires only presence of Fe³⁺ ions and dissolution of metals from the second group requires both Fe³⁺ ions and protons. In order to demonstrate the importance of iron in dissolution of metals involved in the first group the copper bioleaching using the mixture of acidophilic bacteria *A. ferrooxidans* and *A. thiooxidans* was chosen and compared with abiotic leaching in diluted H₂SO₄ and in a solution of Fe³⁺ ions (Fig. 1A).

Those abiotic leaching systems were designed to simulate the influence of the most important leaching agents produced by metabolic activities of the bacteria during bioleaching. The results revealed that the highest rate of copper dissolution was reached in the single Fe³⁺ leaching system. As it can be seen in Fig. 1A, maximum copper leaching efficiency (88%) was reached on day 14 and with further increase in time no copper extraction was observed. It might be due to exhaustion of available Fe³⁺ ions.

Reaction of metal dissolution by Fe³⁺ ions as important oxidative agents can be expressed as follows (Eq. 1):

$$\text{Fe}^{3+} + \text{M}^{0} + \text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{M}^{2+} + \text{H}^+$$ (1)

In comparison with abiotic Fe³⁺ leaching system the bioleaching showed a slow and steady increase on copper dissolution, which might be related to a gradual oxidation of Fe²⁺ ions to Fe³⁺ ions during bacterial activity (Eq. 2):

$$2\text{Fe}^{2+} + 0.5\text{O}_2 + 2\text{H}^+ \rightarrow 2\text{Fe}^{3+} + \text{H}_2\text{O}$$ (2)

However, at the end of the experimental period the copper bioleaching efficiency (88.4%) was the same as that in the single Fe³⁺ abiotic leaching system. Regarding copper recovery in the H₂SO₄ system without presence of Fe³⁺ ions only 9% of Cu dissolved. The positive influence of iron as an oxidative agent on copper bioleaching was also observed by other authors [14,19]. The highest metal extraction in the presence of Fe³⁺ ions was also observed in the case of zinc (100%) dissolution [17].

Given the dissolution of metals involved in the second group the leaching process can be expressed by Eq. (3) (M-metal):

$$\text{M}^{0} + \text{Fe}^{3+} + \text{H}^+ + \text{O}_2 \rightarrow \text{M}^{2+} + \text{Fe}^{2+} + \text{H}_2\text{O}$$ (3)
As the example of the second group of metals, dissolution of metallic nickel, under the same conditions as those in copper dissolution, is shown in Fig. 1B. The results revealed that all nickel was completely dissolved (100%) in the bioleaching system, where both ferric ions and protons were present whilst significantly lower Ni leaching efficiency 68% and 36% was reached in the presence of only Fe$^{3+}$ ions and H$_2$SO$_4$, respectively.

### 3.1.2. Dissolution of metals from Ni-Cd batteries

The important influence of Fe$^{3+}$ ions was also confirmed in the case of metal dissolution from Ni-Cd batteries. Metals in the Ni-Cd batteries occur in metallic forms as well as in the form of hydroxides, oxohydroxides. The percentage of particular forms differs in anode and cathode [20,21]. To elucidate the significance of iron in Ni and Cd bioleaching from Ni-Cd batteries the metal dissolution from cathode in the same leaching systems as used for PCBs bioleaching and abiotic leaching is shown in Fig. 2. As it can be seen the maximum leaching efficiency of 100% and 55% for Cd and Ni recovery, respectively, was reached in bioleaching as well as abiotic leaching in the presence of Fe$^{3+}$ ions.

The results showed the important role of protons in metal dissolution. Abiotic leaching in diluted H$_2$SO$_4$ involves neutralization reaction (Eq. 4, M – metal) needed for dissolution of metal hydroxides.

\[
\text{M(OH)}_2 + \text{H}_2\text{SO}_4 \rightarrow \text{MSO}_4 + \text{H}_2\text{O} \quad (4)
\]

Metal concentration in the solution increased gradually followed by the pH increase. The higher pH values lead to slowdown of the leaching process and finally at pH 7 the metal dissolution did not continue. In the single Fe$^{3+}$ abiotic leaching system the maximum leaching efficiency of both metals (100% Cd and 55% Ni) was reached at very low pH (2.5 and 2 respectively).

This finding indicated that not only oxidative capacity of Fe$^{3+}$ ions but also their hydrolytic capacity resulting in sufficient amount of protons significantly influenced the metal leaching process (Eq. 5).

\[
\text{Fe}_2(\text{SO}_4)_3 + 6\text{H}_2\text{O} \rightarrow 6\text{H}^+ + 3\text{SO}_4^{2-} + 2\text{Fe(OH)}_3 \quad (5)
\]

The comparable results of metal dissolution in bioleaching and abiotic leaching in the presence of Fe$^{3+}$ ions as well as very low pH values throughout the metal leaching indicate that Fe$^{3+}$ ions due to their oxidative and hydrolytic abilities play the key role in the leaching processes.

The importance of iron was also confirmed in the previous research work concerning of Ni-Cd batteries leaching in abiotic environment of ferric iron [20]. The authors reported that an increase of Fe$^{3+}$ concentration in the solution lead to higher leaching rates as well as leaching efficiency of nickel and cadmium.

### 3.2. Reducing abilities of iron

On the contrary to above mentioned experiments showing significant oxidative and hydrolytic effects of iron in metal dissolution from waste, the results of experiments dealing with bioleaching of alkaline and Li-ion batteries revealed that reduction effects of iron can significantly enhance bioleaching efficiency, as well.

#### 3.2.1. Metal leaching from alkaline batteries

In spent Zn-Mn (alkaline) batteries zinc occurs in the form of ZnO, manganese is present in the form of Mn$_2$O$_3$ or Mn$_3$O$_4$ containing in acids soluble MnO and insoluble MnO$_2$. ZnO is readily soluble in the presence of acids such as H$_2$SO$_4$, HCl or HNO$_3$, whereas Mn is dissolved only partially [22,23].

Based on the results (Fig. 3) it is also obvious that acid produced by metabolic activity of bacteria is necessary in zinc bioleaching (Eq. 6):

\[
\text{ZnO} + \text{H}_2\text{SO}_4 \rightarrow \text{ZnSO}_4 + \text{H}_2\text{O} \quad (6)
\]

The recovery of zinc was 98% no matter what the bacterial species were used (Fig. 3A). The importance of iron as a reducing agent is visible from the results of Mn dissolution (Fig. 3B).
Whereas in the presence only of sulphuric acid generated by pure bacteria of *A. thiooxidans* Mn partially dissolved (40%) a much higher Mn extraction efficiency (80%) was reached using the mixed culture of bacteria as well as the pure culture of *A. ferrooxidans*, in the media where ferrous ions were present. In acidic environment ferrous ions were able to reduce insoluble Mn(IV) to soluble Mn²⁺ (Eq. 7):

\[
\text{MnO}_2 + 2\text{Fe}^{2+} + 4\text{H}^+ \rightarrow \text{Mn}^{2+} + \text{Fe}^{3+} + \text{H}_2\text{O}\tag{7}
\]

The findings indicate that Fe²⁺ ions play a crucial role in the process as reducing agents able to reduce Co³⁺ ions to Co²⁺ ions (Eq. 8):

\[
\text{Co}_3\text{O}_4 + 6\text{H}_2\text{SO}_4 + 6\text{FeSO}_4 \rightarrow 3\text{CoSO}_4 + 3\text{Fe}_2(\text{SO}_4)_3 + 6\text{H}_2\text{O} + 0.5\text{O}_2\tag{8}
\]

According to published results utilization of pyrite (FeS₂) as energy source seems to be more suitable [24]. Sulphuric acid as well as Fe²⁺ ions are produced as a result of bacterial oxidation of pyrite. Ferrous iron can consequently act as reducing agent. The importance of Fe²⁺ ions as reducing agent was also confirmed in experiments dealing with chemical, abiotic leaching of alkaline batteries [24].

### 3.2.2. Metal leaching from Li-ion batteries

In spent Li-ion batteries Li and Co occur in the form of lithium cobalt dioxide. Li occur in the form of Li₂O and Co in soluble form of CoO and insoluble form of Co₂O₃ [23,25]. The important influence of Fe²⁺ ions on Li and Co bioleaching is evident from behaviour of the metal dissolution in two different bioleaching systems – rich nutrient medium containing all necessary nutrients including Fe in the form of Fe²⁺ ions and a low nutrient medium containing only diluted sulphuric acid and elemental sulphur as the only energy source for bacterial growth (Fig. 4). As it can be seen from Fig. 4 and 5 much higher efficiency of Li and Co bioleaching was reached in media where Fe²⁺ ions were present in spite of low pH values in both bioleaching systems. The Li and Co recovery was found to enhance by 55% and 70% (total 85% and 83%, respectively).

Despite the fact that Li can dissolve by the mechanism of acid leaching this process without reduction of Co would not be effective since both metals are part of the same oxidic compound. To increase the leaching efficiency of both metals the presence of acid as well as reducing agent is necessary.
3.2.3. The importance of iron in waste bioleaching

Utilization of acidophilic bacteria in biohydrometallurgy was usually associated with sulphur. It was generally accepted that sulphur is a main source of energy for their metabolism [26]. However, nowadays it seems that the iron is much more important in metabolisms of those bacteria [27]. Even so important that acidophilic bacteria are considered to be main producers of acidic conditions in nature [5]. Since Fe³⁺ ions are stable only up to pH 3 it is obvious that acidic environment enable them to utilize such energy source [2].

Bacteria utilize iron as an electron donor in aerobic respiration. Ferric iron produced in this process serves as a strong oxidation agent able to oxidise present metals [1]. However, iron can be also used as a good electron acceptor in anaerobic respiration chains of bacteria resulting in production of Fe²⁺ ions which serve as reducing agents for metals which dissolve in their reduced forms [28,29].

Third role of iron making it so interesting for bacteria is its hydrolytic ability. Due to the hydrolysis of ferric iron a constant pH is maintained in the system what is a property of interest for the integrity of bacterial membranes. Constant low pH is, however, also important in metal bioleaching from waste. According to our results as well as published data [30, 31] it is evident that iron plays an important role not only in bioleaching of metals from sulphide ores but also in bioleaching of metals from waste where they occur in metallic or oxidic form. Similarly as in nature, e.g. in ecosystem of Rio Tinto River, where it was found that not sulphur but iron is the element with central and critical role [32], it seems that also bio-processing of metal-bearing waste is under the control of iron.

Iron, particularly Fe³⁺ ions, acted as an important oxidation agent in bioleaching of metals from PCBs and Ni-Cd batteries where metal oxidation took place. In the case of Ni-Cd batteries, significant hydrolytic role of Fe³⁺ ions was confirmed. They supplied protons enhancing hydroxide dissolution. Different efficiencies of Ni dissolution from PCBs and Ni-Cd batteries indicate that electrochemical mechanisms will be also involved in the nickel dissolution (e.g. galvanic leaching). The leaching efficiency in the presence of more electropositive copper occurring in PCBs was significantly higher in comparison with nickel bioleaching efficiency from Ni-Cd batteries under the same conditions. However, in Ni-Cd batteries only more electronegative Cd was present.

Two mechanisms related to crystallographic structure of the mineral substrate were suggested to explain bioleaching of sulphides – so called thiosulphate mechanism and polysulphide mechanism [33, 34]. But similarly in metal bioleaching from waste we found that some metals such as Cu and Zn only required the oxidation action of ferric ions as it is in the thiosulphate mechanism but others such as Ni required both ferric ions and protons as it is in polysulphidic mechanism. This phenomenon needs further study which could shed light on the mechanisms of waste bioleaching with composition different from conventional sulphidic ores typically processed by bioleaching.

Under anoxic conditions iron might be reduced by the same bacteria, e.g. A. ferrooxidans. Reduced forms of iron, as we showed in the experiments, also participate in metal dissolution from waste. In the case of Co in Li-ion batteries and Mn in alkaline batteries, as well, the presence of Fe²⁺ ions was crucial in enhancing the metal bioleaching efficiency.

Based on the obtained results we suggest coupling of iron-oxidising and iron-reducing activities in integrated biometallurgical model of metal-bearing waste recycling in which the iron cycle microorganisms play a central role (Fig. 6).

Depending on conditions bacteria are able to oxidise iron to Fe³⁺ ions (aerobic conditions) or reduce iron to Fe²⁺ ions (anaerobic conditions). Iron oxidation and production of Fe³⁺ ions represents one part of iron cycle which can be used to recover metals requiring oxidation or low pH for their bioleaching. Such way can be used for processing of waste such as PCBs, Ni-Cd batteries etc. The other part of the iron cycle is iron reduction to Fe²⁺ ions under anaerobic conditions. These ions consequently act as the reducing agents for metals which require reduction to form soluble compounds, e.g. in the case of Co in Li-ion batteries or Mn in alkaline batteries.

Proposed model can lead to the development of new technologies based on iron recycling in the effort for sustainable, zero waste technologies. Such technologies in industrial application can have significant impact on the increase of waste recycling efficiency as well as important decrease of processing costs.

4. Conclusion

Microbial processes are widely applied to recover metals from sulphidic ores. Recently they have become very promising in the treatment of metal-bearing waste. Although mechanisms of metal bioleaching of sulphidic ores are in general well-known, mechanisms of waste bioleaching are still uncovered. Based on the obtained results it is evident that iron plays an important role not only in bioleaching of metals from sulphidic ores but also in bioleaching of metals from waste materials.
Iron influenced bioleaching of studied waste by three main activities:

- as an oxidising agent – oxidises metals (e.g. Cu, Zn, Ni) from PCBs, Ni-Cd batteries,
- hydrolytic activity – supplies sufficient amount of protons and maintain constant low pH, necessary for metal dissolution,
- as a reducing agent – reduces metals (e.g. Co, Mn) which dissolve in reduced form enhancing their bioleaching efficiency.

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