Application of biological method for removing selected heavy metals from sewage sludge

Joanna Willner, Agnieszka Fornalczyk
Silesian University of Technology, Department of Metallurgy and Recycling, ul. Krasińskiego 8, Katowice, Poland

Corresponding author: Agnieszka.Fornalczyk@polsl.pl (Agnieszka Fornalczyk)

Abstract: The aim of the study was to evaluate the application of bioleaching technique to reduce content of selected heavy metals (Zn, Cu) in sewage sludge, and hence to indicate possibilities for metals recovery from this type of waste. Bioleaching experiments were carried out with mixed bacteria Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans, using leaching media: ferrous sulfate with different concentrations of Fe²⁺ (2 g/dm³ and 9 g/dm³) and sulfuric acid (VI). Dynamics of the increase in zinc concentration in biological systems was almost identical for both 9 g Fe²⁺/dm³ and 2 g Fe²⁺/dm³ in samples. However, higher values of Cu concentration were achieved using a medium with iron(II) salt 9 g/dm³ than in a 2 g/dm³ solution. Bioleaching with 9 g Fe²⁺/dm³ allowed for a nearly 20-fold reduction of zinc content and a 2-fold reduction in copper content in sewage sludge. Using 9g/dm³ ferrous sulfate bioleaching could dissolve 94.8% Zn and 58.9%, whereas chemical leaching dissolved 47.3% Zn and 4.2% Cu.

Keywords: bioleaching, sewage sludge, copper, zinc, Acidithiobacillus

1. Introduction

Sewage sludge is characterized by high hydration (over 99% for raw sludge, 80-55% for dehydrated sludge), high content of phosphorus and potassium nitrogen compounds and high content of organic compounds (aldehydes, ketones, organic acids, hydrocarbons). Organic compounds present in sewage sludge are easily broken down and are responsible for the release of odours. In addition, sewage sludge is characterized by a diverse content of heavy metals and microbial contaminants in which parasite eggs may occur. The above-mentioned features of municipal sewage sludge are shaped differently, depending on the type of sewage, the method of their treatment and the way sewage sludge is processed (Błaszczyk and Krzyśko-Łupicka, 2014; Skoczko et al., 2014).

The method of sewage sludge management is selected depending on their physicochemical composition and hygienic and sanitary condition. The simplest and often used method of sludge management is their direct use as fertilizers. However, such sludges may contain heavy metals, the excessive amount of which limits the possibilities of their utilization in the agricultural industry. Heavy metals in municipal sewage come from domestic sewage, surface runoff and as a consequence of corrosion of sewage pipes. However, the high concentration of these metals is the result of the share of industrial wastewater in the total mass of municipal wastewater.

Metals can be removed from sewage sludge by the method of chemical leaching with inorganic and organic acids (Veeken and, Hamelers, 1999; Marchioretto et al., 2002; Ukiwe et al., 2008), ion exchange, adsorption or chemical extraction (Zagury et al., 1999; Babel et al, 2006; Lee et al., 2006; Chen et al., 2005, Karwowska et al., 2014). As a result of these operations, an environmentally safe material is obtained and, at the same time, metals are recovered. The limitation in the use of conventional metal separation techniques for sewage sludge are primarily high costs and operational difficulties. Biohydrometallurgical methods can become an alternative method to eliminate these obstacles. The
potential of microorganisms is used in these processes, and these methods are part of the series of methods used in the sewage treatment plant not only for sewage but also sewage sludge.

The commercial use of biohydrometallurgical methods in the recovery of metals (Cu, Au, Ni, Co, Zn) from primary raw materials - low-percentage ores and concentrates is well-known. Research on the possibility of metal extraction from waste materials with the participation of microorganisms was also carried out for various types of waste (mineral waste, sludge, slag, catalysts, lithium batteries, electronic scrap, LCD panels) (Mrazikova et al., 2013; Willner et al., 2018), including sewage sludge (Pathak et al. 2009a; Wen et al., 2021; Ghavidel et al., 2017).

The bioleaching processes of sewage sludge mainly use acidophilic bacteria of the genus Acidithiobacillus: Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans (Tyagi, 1993, Chan, 2003). It is assumed that the process using ferric bacteria, compared to sulfur bacteria, is more desirable due to the lack of residual sulfur in the sludge after the metal bioleaching process, which further reduces the risk of secondary pollution (Wong et al. 2002). A. ferrooxidans oxidize Fe²⁺ to Fe³⁺, produce SO₄²⁻ ions and sulfuric acid (VI), which leads to acidification of the precipitate and dissolution of heavy metals (Wong and Gu 2008). The results of work on the removal of heavy metals from sewage sludge with the participation of A. ferrooxidans, showed that the efficiency of the metal removal process is primarily influenced by parameters such as: pH, oxidation-reduction potential (Eh), sulfate production, sludge density or mixing time (Bayat and Sari 2010, Pathak et al. 2009a).

It has also been shown that the addition of 4.0 g Fe²⁺/dm³ increases the solubility of metals after 10-16 days (Xiang et al. 2000). Table 1 presents an overview of selected results of heavy metal bioleaching from sewage sludge. The degree of removing heavy metals varies depending on the sludge type, mobility of metals, their chemical bond in sewage sludge (Fuentes et al., 2004) and applied microorganisms. However, it is clear, that in comparison to chemical leaching, bioleaching process is more effective.

| Metals concentration (mg/kg dry sludge) | Main process parameters | Results (Reference) |
|----------------------------------------|-------------------------|---------------------|
| Zn - 1,732                             | indigenous iron-oxidizing bacteria isolated from the sludge, bioleaching time 12 days, sludge 2 % (w/w), FeSO₄·7H₂O 20 g/dm³, inoculum 10 % (v/v), 30°C, agitation. | Bioleaching: 88.5% Zn, 79.9% Cu, 33.2% Pb, 50.1% Cr | Chemical leaching: 80.2 % Zn, 21.8 % Cu, 10.9 % Pb, 10.5 % Cr (Wen et al., 2013) |
| Cu - 472                               | indigenous iron-oxidizing microorganisms, bioleaching time 16 days, sludge 20 g/dm³, | Bioleaching: 69% Zn, 52% Cu, 46% Cr, 45% Ni | Chemical leaching: 16% Zn, 10% Cu, 12% Cr, 12% Ni (Pathak et al., 2009) |
| Ni - 294                               | FeSO₄ [g/dm³]: 5, 10, 15, 20, inoculum 10 % (v/v), 28°C, agitation. |  |  |
| Zn - 1310                              |  |  |  |
| Cr - 332                               | A. ferrooxidans, bioleaching time 15 days, sludge 0.1 dm³, inoculum 10 % (v/v), 28°C, shaker. | Bioleaching: 71.9% Cd, 92.5% Mn, 89.1% Zn | Chemical leaching 22.0% Cd, 25.0% Mn 14.2% Zn (Ghavidel et al., 2017) |
| Cd - 4.32-9.27                         |  |  |  |
| Mn - 400-1090                          |  |  |  |
| Zn - 2800-5600                         |  |  |  |

Due to possible presence of heavy metals in sewage sludge (which is an obstacle to their agricultural use), studies on the effectiveness of copper and zinc reduction present in sewage sludge in the bioleaching process with mixed bacteria A. ferrooxidans and A. thiooxidans were carried out. A mixed bacterial culture was selected, ensuring both the availability of the oxidizing agent Fe³⁺ as well as the production of sulfuric acid (VI) by oxidation of S²⁻ and reduced sulfur compounds (S₂O₃²⁻, S²⁻) to SO₄²⁻.

Sewage sludge from one of the Polish wastewater treatment plants has been tested. In the technological process implemented there, the sludge is neutralized by means of mesophilic methane
fermentation in a separate closed fermentation chamber, then the sludge is directed to open fermentation chambers, mechanically dewatered on a belt press and hygienized with the addition of lime. The work analyzes the impact of selected physicochemical factors on the effectiveness of the Cu and Zn bioleaching process (taking into account the type of leaching medium used), considering the impact of environmental pH and oxidation reduction potential (Eh). Determining the impact of the indicated parameters on the rate of the metal bioleaching reaction and metabolic activity of microorganisms is the basis for describing the process, determining its efficiency and application possibilities.

2. Materials and methods

2.1. Characterization of sewage sludge

The research was carried out on sewage sludge taken directly from conveyor of the mechanical dewatering press. The collected sludge sample was stored at 4°C. Then the sludge was dried (6 h, temperature 75°C) and ground in a laboratory mill into a fraction with particle size <1 mm (Fig. 1). The sieve analysis was carried for the grain size of 1 mm, 0.5 mm and <0.5 mm. Table 2 presents the granulometric composition of the sewage sludge fraction together with the content of copper and zinc in a given grain fraction.

![Fig. 1. Sewage sludge material used in the tests: (A) raw sewage sludge (immediately after collection), (B) sewage sludge after drying and milling (prepared for testing)](image)

Table 2. Percentage grain size distribution of crushed sewage sludge material with Cu and Zn content

| Grain size, mm | Content, % | Cu, % | Zn, % |
|---------------|------------|-------|-------|
| 1             | 40.6       | 0.0214| 0.2068|
| 0.5           | 27.8       | 0.0217| 0.2027|
| <0.5          | 31.6       | 0.0206| 0.2059|

Composition analysis did not reveal a visible tendency to accumulate copper and zinc in individual fractions from sieve analysis. Therefore, all fractions of ground sewage sludge were mixed, homogenised and used in the research (Cu: 0.021%, Zn: 0.204%). No exceeding of the limit values specified in the Regulation of the Minister of the Environment (from February 6, 2015 on municipal sewage sludge - Journal of Laws of 2015, item 257) was observed.

2.2. Microorganisms

Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans bacteria was used in the research. Bacterial strains have been isolated the source of mineral water coming from Głębokie and Łomnica (Nowy Sącz, Poland). The composition of medium for growth of iron- and sulphur-oxidizing bacteria was as follows (g/dm³): (NH₄)₂SO₄-2.0; KCl-0.1; MgSO₄ · 7H₂O-0.25; KH₂PO₄-0.25; FeSO₄·7H₂O-44.2 and S⁰-10 (Mrazikova et al., 2013). To increase cell activity, the culture was passaged several times using fresh mixed medium. A mixed culture of A. ferrooxidans and A. thiooxidans was adapted to the sewage sludge components for a period of 4 weeks. Adaptation was carried out by adding small portions of sewage sludge to mixed medium, regularly every 3 days. Cell density of bacteria (cell density/cm³) was estimated by the visual count method using an optical microscope (Zeiss Axio Imager A2, Germany), and a Thoma chamber with a depth of 0.1 mm and an area of 0.0025 mm². Biological leaching tests were
carried out in a thermostat at 30°C while maintaining optimal growth conditions for *A. thiooxidans* bacteria: pH in the range 1.8-2.5, temperature 30-50°C and pH 1.5-2.5; temperature 28-37°C for *A. ferrooxidans* (Valdés et al., 2008).

2.3. Bioleaching of metals

The experiment was performed in Erlenmeyer flasks (300 cm³) with 200 cm³ of the mixed medium and 10% (v/v) inoculum with an initial density of bacteria approximately 10⁶-10⁷ cells/cm³. Preliminary tests of bioleaching of sewage sludge with the solid to liquid ratio (S/L) in the amount of 50-100g/dm³ showed the pH increase to pH> 3 (within 6 days), which did not provide optimal growing conditions for bacteria and generated the possibility of jarosite (HFe₂(SO₄)₃(OH))₉ precipitation, inhibiting the metals dissolution from sludge (Jensen and Webb, 1995). Additionally, at higher S/L ratio, the reduction in pH decreased due to higher buffering capacity of the sludge, and more time for attaining the pH values necessary for solubilization of metals is required (Pathak et al., 2009). Therefore, bioleaching was carried out with sludge concentration of 25 g/dm³, in thermostat (temperature 29±1°C) for 30 days. In order to determine the impact of the availability of iron(II) ions for bacteria and the possibility of using sulfuric acid (VI) as leaching agent, three different leaching media (initial pH 1.8) were used: 1) mixed medium - 9K with Fe²⁺ concentration of 9 g/dm³; 2) modified mixed medium 2K with Fe(II) concentration of 2 g/dm³; 3) H₂SO₄ solution without Fe(II) addition (Fe(II) 0.0 g/dm³). H₂SO₄ solution was prepared by dissolution of concentrated (96%) H₂SO₄ in distilled water to obtain the final pH value of 1.8. To maintain the same experimental conditions, elemental sulphur in the amount of 1g/100 cm³ was added to the sulfuric acid solution (VI). In parallel, chemical leaching experiments (controls: bacteria-free) were conducted while maintaining similar experiment conditions. Concentration of metals (Cu, Zn) in the solutions was analysed periodically and determined by atomic absorption spectrophotometer (AAS). All of the experiments were conducted at least twice.

2.4. Analytical methods

To determine content of metals in the sample before and after bioleaching, hot aqua regia dissolving method was applied. Aqua regia was prepared freshly with analytical reagent grade HCl (35% m/v) and HNO₃ (69% m/v) obtained from APM Poland S.A. (POCH™ brand from Avantor™. 0.5 g of sewage sludge was dissolved in 30 cm³ of aqua regia for 1 h. The concentrations of dissolved metal ions in all samples were determined by atomic absorption spectrophotometer (SOLAAR M6-UNICAM Atomic Absorption). The correctness of the method was checked through certified analysis reference material (The Institute of Non-Ferrous Metals) at appropriate wavelengths characteristic of the element.

The pH and Eh of the samples were monitored using a digital pH-Eh-meter (Hydromet, model CP-505 pH lab, Poland) calibrated with a low pH buffer (pH and Eh accuracies are ± 0.003 and ± 0.2 mV, respectively).

3. Results and discussion

3.1. Variation of pH during Zn and Cu extraction from sewage sludge

The variation of pH during 30 days of the experiment is shown in Fig. 2. For both biological and chemical tests, a significant increase in the pH value was observed in the initial phase of the process (1st-3th day). Over the next days, the pH increased for chemical systems (pH 3-6) or maintained a constant value for biological tests (pH 2.3). It was related to the nature of the material being tested, the possibility of alkaline components (fertilizer and organic components) releasing, the buffering capacity of sludge (Ghavidel et al., 2017) which finally affected the increase in pH in chemical samples. To neutralize alkaline character of sewage sludge, more acid was needed, which was provided by bacteria. In systems with bacteria it takes few days for pH to start slightly decreasing and it is evident, that low pH (pH arround 2) remaine only in biological tests with access to Fe²⁺ and is stimulated by the microorganisms’ activity. Reactions occurring in biological systems (involving bacteria), for the copper case, may occur as follows:

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Fe^{2+} + O_2 + 4H^+ \xrightarrow{Bacteria} Fe^{3+} + 2H_2O
\]
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$$Fe^3+ + Cu \rightarrow 2Fe^{2+} + Cu^{2+}$$

(2)

$$6Fe^3+ + 12H_2O \rightarrow 2HF_{e3}(SO_4)_2(OH)_6 + 10H^+$$

(3)

$$S^0 + H_2O + 1.5O_2 \xrightarrow{\text{bacteria}} SO_4^{2-} + 2H^+$$

(4)

Fig. 2. Change of pH in biological (b) and control (c) tests with 1) 9K medium, 2) 2K medium 3) H$_2$SO$_4$ solution

The sample with the H$_2$SO$_4$ solution had the highest pH increase, where the rise was observed from an initial value of pH 1.8 to final pH 6.7. This was expected, when there are no bacteria in the samples, Fe$^{2+}$ but only the addition of S$^0$, which oxidation with atmospheric oxygen only, did not ensure maintaining a low pH. In addition, with the amount of Fe$^{2+}$ increasing, the pH of samples decreased. A higher concentration of Fe$^{2+}$ (9g/dm$^3$) promotes oxidation Fe$^{2+}$ to Fe$^{3+}$ by chemical route, and the hydrolysis reactions (3) accompanying the leaching process can also cause the self-acidification effect.

In biological tests with 9K and 2K medium, after the initial increase in pH (pH 2.3), the samples were gradually acidified to pH 1.9 in the final phase of bioleaching. A decrease in pH in samples inoculated with bacteria indicates the adaptation of bacteria to the environment and their gradual use of Fe$^{2+}$ as an energy source. In addition, the presence of A. thiooxidans bacteria promotes the oxidation of S$^0$ to H$_2$SO$_4$, leading to a gradual increase in acidity and a decrease in pH in systems with 9K and 2K medium. The biological system with H$_2$SO$_4$ solution had different course of pH changes. Despite the presence of bacteria and availability of S$^0$, the dynamics of pH changes over 30 days is analogous to that in control samples. The lack of addition of nutrients: Mg, K, Ca, and above all the lack of access to the necessary nutrient substrate of A. ferrooxidans - Fe$^{2+}$ - contributed to the disappearance of the activity of microorganisms in the acid solution and visible alkalization of the sample.

3.2. The Eh changes from sewage sludge

The changes of Eh, which is a function of concentrations of ferric and ferrous iron and could be used to indicate bacterial activity, is shown in Fig. 3. In 9K and 2K sample with bacteria, after an initial decrease in Eh (first 8 days), this parameter increased from 353 mV to 430 mV for 9K and 484 mV for 2K. The increase in Eh combined with a low pH (Fig. 2) during the bioleaching process is an indicator of the growth of microorganisms and indicates the course of the biological reaction. However, the lower range of Eh rather than standard Eh values (600-700 mV) corresponding to high bacterial activity, indicates some bacteria limitation, inhibited by chemical nature of sewage sludge.

In control tests (2K-c) and sulfuric acid solution a decrease in Eh was observed from +250 mV to values in the range from -250 mV to -300 mV and finally rise to -40 mV. A similar nature of changes in the course of oxidation-reduction properties of the environment was characterized by the H$_2$SO$_4$ sample inoculated with bacteria. Extreme environmental conditions: the alkaline nature of the solution with increasing pH to pH$>$ 4.5, the lack of bacterial access to nutrients in the H$_2$SO$_4$ solution negatively affected bacteria, resulting in a reduction in biomass growth and reduction of oxidative capacity. Oxidation of Fe$^{2+}$ by A. ferrooxidans drops sharply at pH above 2.5 (Pesic et al., 1989; Nakamura et al., 1986), and at pH$>$ 4.5 bacterial growth disappears. The result is a sharp Eh decrease to negative values and the leaching process is analogous to control samples - by chemical means. Eh changes in chemical
tests are mainly related to the content of organic compounds in sewage sludge and negative Eh values indicate the course of reduction processes accompanied by the decomposition of organic matter (Wilk and Gworek, 2009).

Fig. 3. Eh variation in biological (b) and control tests (c) with 1) 9K medium, 2) 2K medium 3) H\textsubscript{2}SO\textsubscript{4} solution

3.3. Degree of Zn and Cu leaching from sewage sludge

The dissolution rates of Zn and Cu in biological and chemical samples are shown in Fig.4 and 5. For zinc, the concentration of this metal in the initial leaching phase decreased in both biological and control tests. For example, in the control solution and the biological system acidified with H\textsubscript{2}SO\textsubscript{4}, the zinc concentration gradually decreased from the initial value in the range of 5.07-8.89 mg/dm\textsuperscript{3} to the value of about 0.40 mg/dm\textsuperscript{3} on the 16th day of leaching. Over the next 14 days, the concentration of Zn in these solutions remained at 0.46-0.67 mg/dm\textsuperscript{3}. Also, in the case of copper for all control solutions and in the biological sample with H\textsubscript{2}SO\textsubscript{4} solution, the copper concentration gradually decreased from the initial value in the range of 0.25-1.16 mg/dm\textsuperscript{3} to the value of about 0.03 mg/dm\textsuperscript{3} in 30th day. The rapid decrease in the concentration of Zn is associated with a change in pH, the main factor determining the solubility of metals. The solubility of metal compounds decreases with increasing pH, which is reflected in the course of pH changes accompanying control tests (Fig 2). However, the pH depended on the initial concentration of Fe\textsuperscript{2+} in the leaching medium, which higher concentration contributed to maintaining a lower pH in chemical tests. Additionally, it has been reported that the solubilization of Cu is strongly dependent on Eh (Theis and Hayes, 1978; Couillard et al., 1993) than from pH and to solubilize Cu, Eh of the medium should be more than 250 mV (Theis and Hayes, 1978). This may be the reason for a gradual decrease in copper concentration in chemical solutions for which Eh was negative or oscillated around +250 mV or even below.

Biological systems 9K-b and 2K-b were characterized by a visible rise of Zn and Cu extraction from sewage sludge. The maximum increase in the concentration of Zn was achieved on 23rd day in the range 13.22–12.46 mg/dm\textsuperscript{3}. Increase in the concentration of Cu from value 2.63 to 7.61 mg/dm\textsuperscript{3} (9K-b) and from 1.281 to 5.09 mg/dm\textsuperscript{3} (2K-b) was likewise achieved. The increasing concentration of zinc in biolo-

Fig. 4. Change of zinc and copper concentration in the biological (b) and control (c) tests with 1) 9K medium, 2) 2K medium 3) H\textsubscript{2}SO\textsubscript{4} solution
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gical samples is almost identical for both 9 g/dm$^3$ and 2 g/dm$^3$ Fe(II) and correlates with the acidic medium pH, promotes the release of metal from sewage sludge. Higher Cu concentration was reached using medium with Fe$^{2+}$ 9 g/dm$^3$ than 2 g/dm$^3$. The accompanying course of Eh changes for these samples (480 mV and 370-400 mV, respectively), indicates a stronger effect of Eh than pH on the dissolution of Cu from sewage sludge. Similar tendencies were observed by Pathak et al. (Pathak et al., 2009), who indicated that high Eh of more than 400 mV was favoured the Cu solubilization, when using 10-20 g/dm$^3$ of ferrous sulfate, instead of 5g/dm$^3$ and value Eh of 350 mV.

The analysis of the tested material showed the content of zinc in the sewage sludge batch at the level of 2040 mg/kg and the copper content at the level of 212 mg/kg. Based on the content of metals in raw sample of sewage sludge and in residues after biological and chemical leaching, removal of Zn and Cu was calculated. Using bacterial medium with 9 g/dm$^3$ and 2 g/dm$^3$ of Fe$^{2+}$, a total of 94.8% and 94.7% of zinc was recovered respectively, compared to 47.3% and 8.7% of Zn solubilization in the controls. For copper, 58.9% dissolution using 9g Fe$^{2+}$/dm$^3$ and 35.8% using 2gFe$^{2+}$/dm$^3$ was achieved, while in the chemical it was 4.2% and 5.1%. The reduction of Zn and Cu content in sewage sludge was significantly limited for chemical and biological systems using H$_2$SO$_4$. Table 3 shows the comparison of leaching efficiency, in chemical and biological leaching of copper and zinc from sewage sludge.

| Type of leaching medium | Zn content, mg/kg | Cu content, mg/kg |
|-------------------------|------------------|------------------|
|                         | After bioleaching | After leaching   | After bioleaching | After leaching   |
| 9K                      | 105              | 1075             | 87               | 203             |
| 2K                      | 107              | 1862             | 136              | 201             |
| H$_2$SO$_4$ solution    | 1444             | 2017             | 198              | 210             |

4. Conclusions

Based on the tests of zinc and copper leaching from sewage sludge using A. ferrooxidans and A. thiooxidans bacteria, a greater efficiency of biological extraction of Zn and Cu was demonstrated in comparison to chemical systems. The bioleaching process resulted in a nearly 20-fold reduction of the zinc content in the sewage sludge and a 2-fold reduction in copper. It corresponds to 94.8% and 94.7% dissolution of Zn in 30 day using 9 g Fe$^{2+}$/dm$^3$ and 2 g Fe$^{2+}$/dm$^3$ in biological medium respectively, compared to 47.3% and 8.7% removal in the control samples. For copper it was 58.9% dissolution of Cu using 9g Fe$^{2+}$/dm$^3$ and 35.8% using 2g Fe$^{2+}$/dm$^3$, while in the control leaching it was 4.2% and 5.1%, respectively. Our research indicates the potential for the use of acidophilic bacteria to reduce heavy metals in sewage sludge. Biological processes may become very promising in the treatment of that specific type of waste, enabling their agricultural application. Although further research discussing reduction of a wider range of metals found in sewage sludge (especially those listed in the Regulation of the Minister of the Environment), should be carried out.

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