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Bioleaching using *Acidithiobacillus Thiooxidans* – an option for element recovery from highly alkaline waste incineration ash?

Introduction

A stable supply of raw materials required for ongoing technological development is a key issue for the EU economies. The increasing consumption of everyday goods that require raw materials for their production and a constant decrease in natural resources are the greatest challenges in the Anthropocene. EU countries can supply no more than 9% of the required raw materials (EU 2014, 2017). Growing concerns over the supply of mineral resources on

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the one hand and the sustainable economy, where the protection of natural resources is one of the key goals, on the other, force us to search for alternative sources of economically important elements (EU Commission 2020). Waste stream materials such as bottom ash (BA) of municipal-waste incineration and fly ash (FA) from the incineration of sewage sludge are often considered as novel anthropogenic waste-based substitutes for natural resources (Kasina et al. 2020), thus maximizing the protection of natural resources and returning elements into the production cycle by recycling and reusing, while simultaneously fulfilling the assumptions of a close-loop economy. BA from municipal-waste incineration and FA from sewage-sludge incineration are known to contain valuable metals; however, the standard recovery methods are not fully satisfactory (Kasina et al. 2021), which results in considerable amounts of valuable elements being immobilized in these waste streams and thus removed from the natural cycle of elements and the possibility of being excluded from accumulation in anthropogenic deposits by improper management of incineration residues. The selection of a cheap and proper leaching method that enables the most effective extraction of elements is a significant challenge. Additionally, an important issue here is the possibility of usage in the decontamination of waste, which can find a plethora of uses after this process.

Nowadays, various leaching methods are available, among which bioleaching is gaining interest due to its low environmental costs (Mikoda et al. 2019); however, the efficiency still needs to be improved (Naseri et al. 2019). Among the major groups of bacteria that are involved in the bioleaching process, chemolithoautotrophic acidophiles such as *Acidithiobacillus thiooxidans* are widely and successfully used in extracting metals (Yang et al. 2019) due to their autotrophy and high-tolerance heavy metals (Fazzini et al. 2011).

A. thiooxidans use metal-sulfide phase and elemental sulfur as a substrate to produce sulfuric acid and promote leaching by reducing pH and increasing metal dissolution (Bosecker 1997) as a widely employed practice. These bacteria strains have been applied for the extraction of metals from waste materials including metallurgical slags (Potysz et al. 2016), FA and BA from incinerations processes (e.g., Funari et al. 2015; Mäkinen et al. 2019), spent coin cells (Naseri et al. 2019), sewage sludge (Pathak et al. 2009) and mine tailings (Lee et al. 2015). Microbial leaching using *A. thiooxidans* of industrial waste allowed the recovery of metals such as Al, Zn, Cu, Cd, Mn, Ni, Sn, Co, Li, V, Mo, Au, Ag and Pt (Gómez-Ramírez et al. 2020, and references therein, Kamizela et al. 2021; Pacholewska et al. 2003; Willner et al. 2018; Rouchalova 2020).

There are still some important questions that arise. Firstly, whether this strain is sufficiently effective to cause metal removal from highly alkaline materials; next, if and how the bacteria inhabit the material and in what quantities, and finally what the lifetime of the bacteria is in this synthetically provided environment. The experiments are made in a time range from three days up to thirty days (Schonborn and Hartman 1978; Tyagi et al. 1988). To respond to these questions, a bioleaching test with a duration of twenty-eight days was applied. In addition to laboratory leaching tests, biological staining using DAPI (4,6'-diamidino-2-phenylindole) solution was used to observe, via fluorescence microscopy, the manner in which the bacteria inhabit ash. These observations were complemented with imaging using a scanning electron microscope. Due to the chosen methodological approach, the presented study completes current studies, usually based only on extraction efficiency results. It is a much needed step towards a better understanding of the bioleaching course.

1. Materials and methods

1.1. Characteristics of ash

For the bioleaching experiments, FA from a sewage-sludge incineration plant (SZ01), where sludge dried to 36% of the original mass is incinerated in a fluidized-bed boiler (supplier PyrofluidTM) operating at 850–900°C and BA from a municipal-waste incineration plant (KRZ7) where a grate furnace operates at 850–1000°C (supplier Doosan Lentjes) were used. The FA (SZ01) is composed of quartz, whitlockite, feldspar, amorphous phase, hematite and Fe-PO_4 as the major phases. In the case of BA, one sample used for bioleaching was split; one subsample was powdered to >75 μm grain size (KRZ7) and a second was prepared as particles of around 5 mm in diameter (KRZ7f). The BA is composed of the amorphous phase, quartz, calcite, anhydrite, pyroxene (diopside), mullite and feldspar as the major components. The FA and BA solutions were highly alkaline with pH levels of 9.4 and 10.5, respectively.

Detailed chemical composition of the selected elements of both materials has already been published (Kasina et al. 2020, 2021); however, a brief characteristic of the samples is shown in Table 1.

1.2. Bioleaching

For the bioleaching procedure (Figure 1), chemolithoautotrophic acidophiles *A. thiooxidans* were used. Prior to leaching, the bacteria was grown in a glass Erlenmeyer flask in a Waksman liquid medium consisting of 0.5 g $(NH_4)_2SO_4$, 0.06 g $MgSO_4 \cdot 7H_2O$, 0.025 g K_2HPO_4 and 0.025 g KCl dissolved in 250 ml of deionized water and 10 mg of elemental sulfur and plugged with a cotton plug to avoid contamination while still ensuring aerobic conditions. The pH of the medium was adjusted to 2.5 using 1 M H_2SO_4. The pre-grown inoculum (2% v/v) was added to flasks containing growth medium and 2% ash (one FA sample, and two BA samples, one powdered and one in the form of 0.5 cm diameter pieces, comprising three sets of samples in total), following the procedure described in Mikoda et al. (Mikoda et al. 2019). The biotic experiment was accompanied by a control experiment, where both the medium and ash were included, but with the absence of the microorganisms. All samples were placed on the orbital shaker, which worked constantly during the duration of the experiment at 100 rpm at room temperature. The experiment lasted twenty-eight days;

Table 1. Chemical characteristic of starting samples (selected elements)

Tabela 1. Charakterystyka chemiczna próbek wyjściowych (wybrane pierwiastki)

Chemical composition	FA SZ01 (mg kg^{-1})	BA KRZ7 (mg kg^{-1})
Al	41,600	30,400
Ba	1,204	1,385
Ca	90,300	99,300
Cd	7	9
Co	27	58
Cr	1047	308
Cu	666	451
Fe	110,800	243,00
K	15,000	7,600
Mg	20,900	10,700
Mn	900	500
Na	5,300	54,400
Ni	120	34
P	78,208	3,011
Pb	138	366
Si	163,790	282,519
Sr	535	381
V	62	26
Zn	4,472	1,232
S_{tot}	7,900	4,700

after every seven days, the pH was measured and one set of samples was collected for imaging analyses. The pH of the solution was measured using an Elmetron CP-401 pH-meter, which, prior to measurement, was calibrated using standard buffering solutions (pH = 4, pH = 7 and pH = 9). The concentrations of elements in the leachates were measured using inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer, ELAN 6100) at the AGH University of Science and Technology, Krakow, Poland, according to 17294-2 ISO Standard. The leaching efficiency was calculated according to the following formula:

$$X\,(\%) = \Big(\big(C\,(\mathrm{mg/L}) \cdot L/S\,(\mathrm{L/kg})\big) / \big(C_{total}\,(\mathrm{mg/kg})\big)\Big) \cdot 100\% \tag{1}$$

↳ C – refers to the metal concentration in the solution,

L/S – indicates the liquid/solid ratio,

C_{total} – refers to the total metal concentration in the sample.

1.3. Microscopic observations

Microscopic observations were performed to observe if and how the microorganisms inhabited FA and BA (Figure 1).

A multipurpose Nikon Eclipse Ni-U microscope equipped with Nikon's bandpass fluorescence filter cube suitable for DAPI-stained samples was used to evaluate the relative content of bacteria on the surface of the ash samples.

For the fluorescence microscopy (FL), observation samples were stained with DAPI, which enabled the observation of nucleic acid on the surface of the ash samples. The solution was prepared as follows: 5 mg DAPI was dissolved in 2 ml of deionized water to obtain a DAPI stock solution. Even though the DAPI solution immediately and completely dissolves in water, 15 mins of sonication was applied. The combination of DAPI staining and DAPI filter caused the observation of blue-to-turquoise fluorescence emission from observed microorganisms. Camera exposure times varied from 0.6 to 4 s, because of changes in the intensity of illumination and the contrast of the image in various samples.

A Hitachi S-4700 field emission scanning electron microscope (FE-SEM) combined with a Noran energy dispersive spectrometer (EDS) was used to observe how bacteria colonize the surface of ash. Observations using FE-SEM were performed using the secondary electron mode at 20 kV.

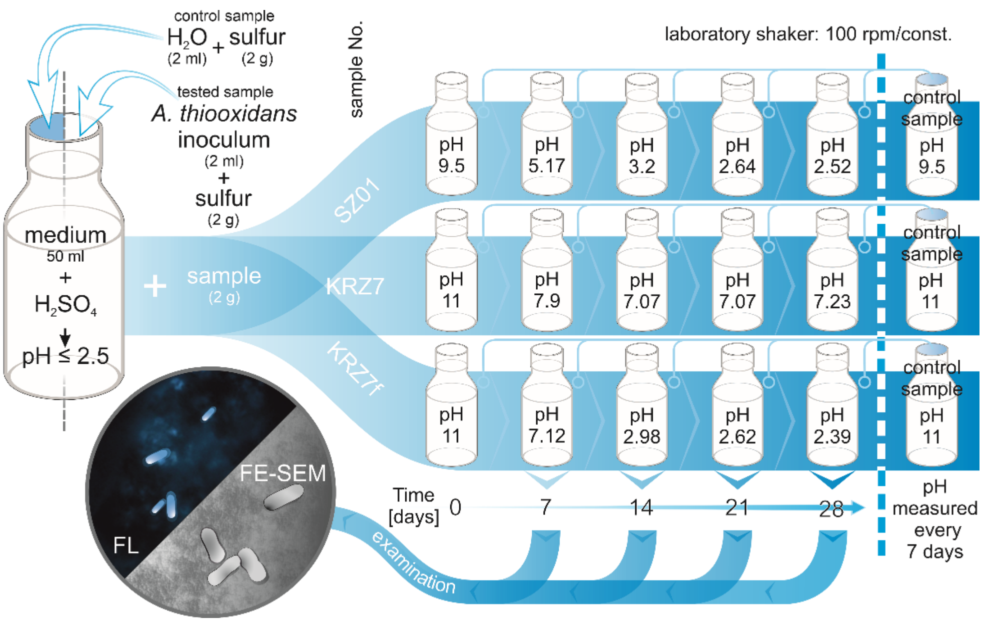

Fig. 1. The overview of the experimental set-up

Rys. 1. Schemat przebiegu eksperymentu

1.4. Sample preparation

Every seven days, ash samples for the microscopic observations were collected using sterile tools and sterile conditions to avoid contamination. The ash was placed on a microscope glass slide (25 × 75 mm/approx. 1 mm) using a laboratory spatula, which prevented the transfer of elemental sulfur floating in the medium and sedimented on the ash surface, thus enabling the sampling of undisturbed powders (SZ01 and KRZ) and BA fragments (KRZ7f). To preserve the bacteria structure, the samples were fixed with methanol and dried at room temperature. Directly prior to FL observations, the samples were stained with DAPI.

2. Results and discussion

In bioleaching using *A. thiooxidans*, two mechanisms were recognized:

1) Metal sulfides (MS) were oxidized directly by *A. thiooxidans* into soluble metal sulfates via the following reaction:

$$MS + 2O_2 \rightarrow MSO_4 \text{ [14]} \tag{2}$$

In both studied samples, the overall total sulfur content was low and did not exceed 1% (0.79% and 0.47% for the FA and BA, respectively), which also confirms that the MS content was low. Therefore, this mechanism of bioleaching was excluded in the case of the studied samples.

2) *A. thiooxidans* oxidize elemental sulfur, which was added to the medium as an energy source for bacteria, into sulfuric acid as follows:

$$S_0 + H_2O + 1.5O_2 \rightarrow H_2SO_4 \tag{3}$$

As a result of oxidation reduction in pH in the medium occurs that enhances metal solubility (Wen et al. 2021).

Even though a decrease in pH was observed during the experiment (Figure 2), the extraction levels remained low (Figure 3). The question arises as to what extent the pH decrease was caused by bacterial metabolic activity or by sulfur addition. The pH in the solution of our control sample reduced after the addition of sulfur and remained stable during the experiment, whereas in the samples with a medium containing bacteria, the pH constantly decreased over time, as shown in Figure 2. Thus, we may assume that the leaching of elements was due to bacterial activity rather than through the addition of acid.

Due to the low sulfur concentration in the starting samples (0.47% and 0.79% for the samples KRZ7 and SZ01, respectively), the supplementation of sulfur had to be performed to provide an energy source for the bacteria to oxidize elemental sulfur and enhance metal solubility.

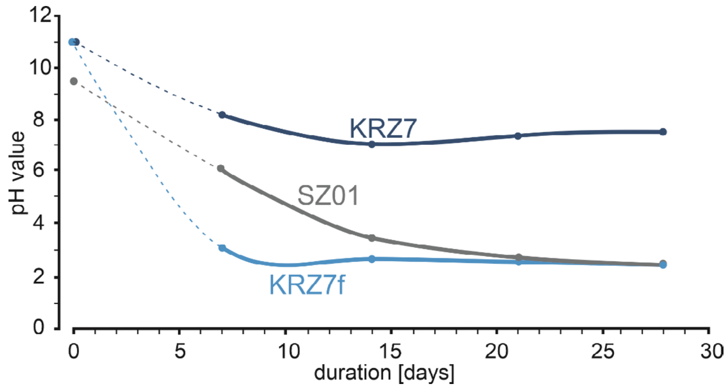

Fig. 2. Changes of pH values during bioleaching (points – pH values measurements)

Rys. 2. Zmiany wartości pH w trakcie procesu bioługowania (kropki – punkty pomiarowe).

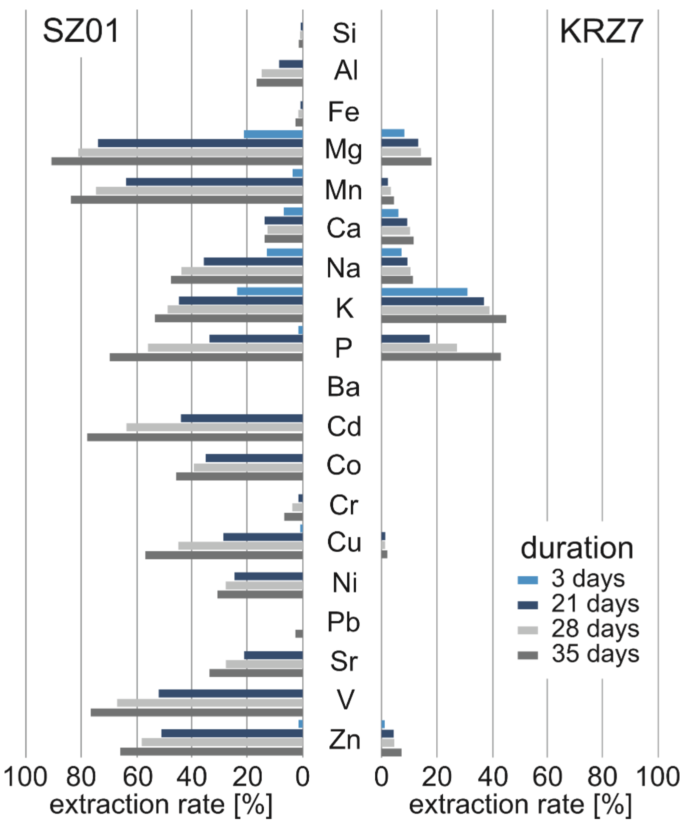

Fig. 3. The release of elements from FA (SZ01) and BA (KRZ7)

Rys. 3. Efektywność uwalniania pierwiastków z popiołu lotnego (SZ01) i dennego (KRZ7)

In the case of FA sample SZ01, a satisfactory extraction rate (>50%) was measured in the case of Mg, Mn, V and Zn after twenty-one days of the experiment, and for P and Cd after twenty-eight days. The extraction rate for other studied elements hardly reached the level of 40% in the case of Cu, Co, Na and K. The failure of Pb extraction may be related to the fact that after the addition of elemental sulfur, $PbSO_4$ could precipitate. $PbSO_4$ is characterized by low solubility (Lo et al. 1990), and thus the efficiency of Pb extraction was significantly lower than in the case of other elements. A similar situation was observed in the case of Ba, which precipitates as $BaSO_4$, and is also characterized by low solubility. The solubility of Cr, as suggested by Fang and Zhou (Fang et al. 2006), is strongly accelerated at low pH. Even though at the beginning of the experiment the pH was adjusted to 2.5, the dissolution of alkaline mineral phases led to the increase of the pH value, which remained lower than the pH of the initial samples (9.43) but still high enough to disable the effective bacterial extraction of elements. Funari et al. (Funari et al. 2015) pointed out that high Cl content and Na, Ca and K salts are responsible for acid consumption during bioleaching, thus influencing the leaching efficiency. Yang et al. noted that even moderate concentrations of NaCl can inhibit bacterial growth. In the studied samples, the presence of Ca, Na and K in the leachates was connected with the dissolution of sulphates, carbonates, chlorides and amorphous material that contained these elements, pointing unequivocally elements to the elements' donors in the leachates (Kasina et al. 2021).

Wen et all noticed a decreasing order in the leachability efficiency that follows Zn > Cu > Cr > Pb and is in agreement with this study, although the extraction rates that were measured here were much lower.

In the BA sample KRZ, the extraction failed. The concentrations of leached elements were barely at the level of a few percent except for P and K where it reached 27% and 39%, respectively. This failure was most likely dependent on the high pH value of the initial samples, which could not be lowered only by the adjustment at the beginning of the experiment. The higher pH was observed in case of KRZ7 than in the case of KRZ7f. This can be explained by the fact that KRZ7 as a powder sample had a much larger specific surface and therefore a larger solid reactant surface.

The obtained results are consistent with microscopic observations where the relative number of bacteria increased slightly over time. After seven days of the duration of the experiment, no microbes were observed in the FA and BA samples both in FL and SEM (Figures 4 and 5). After fourteen days, dispersed but not dense bacterial colonies were observed in the FL, usually at the particle edges in FA SZ01 (Figure 4). From the SEM-EDS studies, we learned that microorganisms chose to inhabit flat, smooth surfaces of quartz grains for example. They were present in the form of rods with a size not exceeding 1.5 µm. In the case of BA sample KRZ7 (Figure 5), bacteria was dispersed and in a low relative amount. In the SEM, no bacteria were found. After twenty-one days, not much had changed in terms of the relative amount of bacteria and the manner of their surface inhibition in comparison with the experiment with a duration of fourteen days (Figures 4 and 5). Interestingly, after twenty-one days, we were already able to observe well-developed extracellular polymeric substance on the bacteria surface. After twenty-eight days, a higher relative number of bacteria in the samples was observed which was more densely distributed, not only on the particle's edges but also on their surface. The SEM observations of FA sample SZ01 indicated the presence of two groups of bacteria, different in size and shape. Not only were rods with a size around 1.5 µm present but also thin elongated rods with a size of 2 µm (Figure 4).

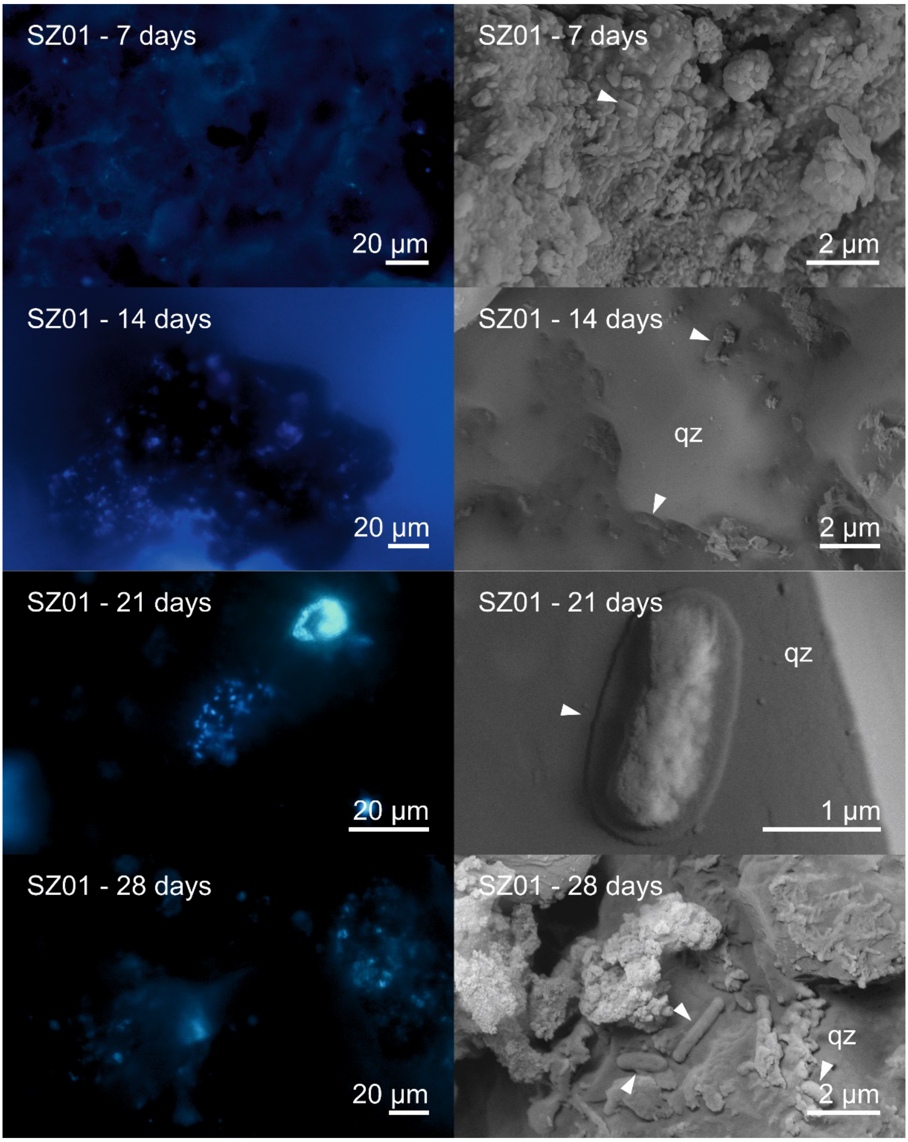

Fig. 4. Bacterial colonization of FA particles (SZ01) in FL (left side) and in the SEM (right side) after 7, 14, 21 and 28 days; arrows indicate bacteria, qz – quartz

Rys. 4. Zasiedlanie popiołów lotnych (SZ01) przez bakterie – (lewa strona) mikroskopia fluorescencyjna, (prawa strona) mikroskopia skaningowa – po 7, 14, 21 i 28 dniach. Strzałki wskazują bakterie, qz – kwarc

Unfortunately, the SEM observations of the BA sample were not successful. Nevertheless, these observations are in agreement with the result of chemical analyses of the leachates and the measured extraction rates for selected elements.

Different images were obtained from the larger BA pieces (Figure 6). Here, bacteria was already observed after seven days of the bioleaching experiment both in the FL and in the

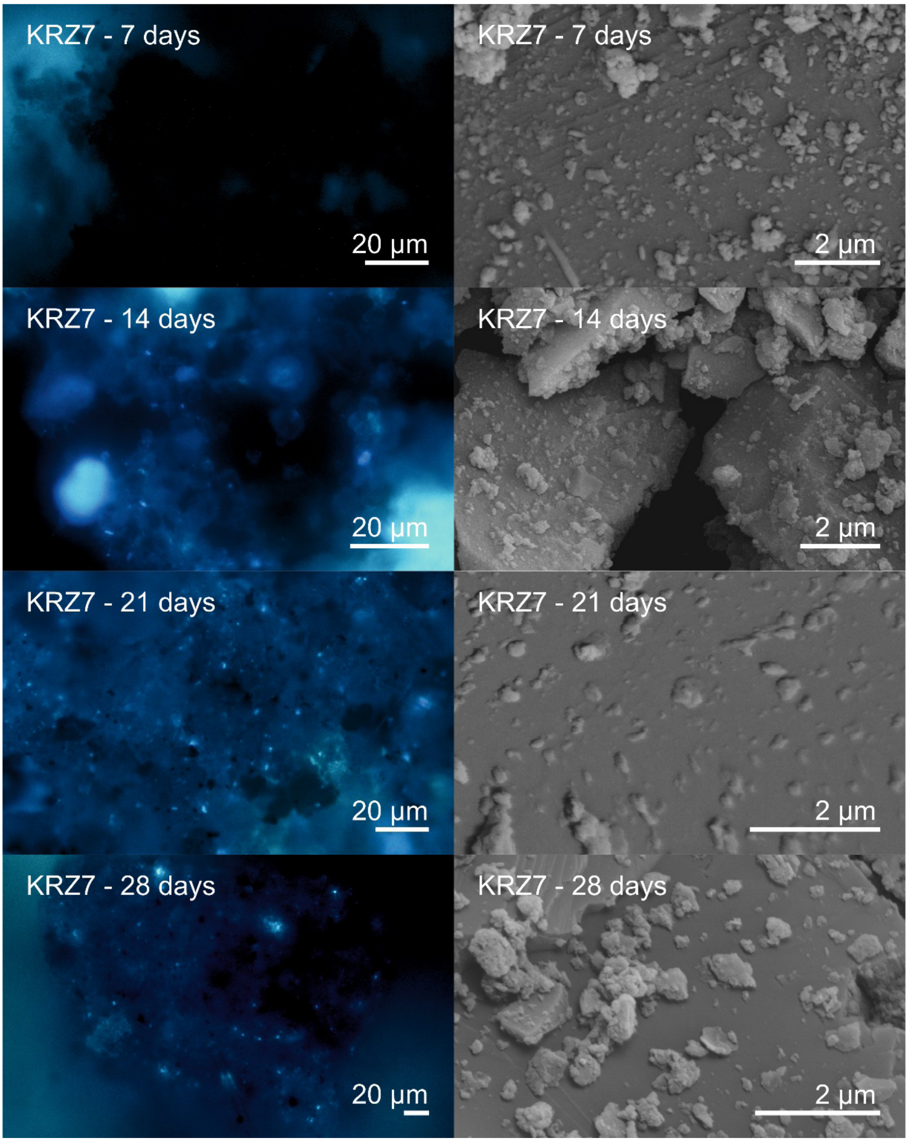

Fig. 5. Bacterial colonization of BA particles (KRZ7) in FL (left side) and in the SEM (right side) after 7, 14, 21 and 28 days; arrows indicate bacteria, qz – quartz

Rys. 5. Zasiedlanie popiołów lotnych (SZ01) przez bakterie – (lewa strona) mikroskopia fluorescencyjna, (prawa strona) mikroskopia skaningowa – po 7, 14, 21 i 28 dniach. Strzałki wskazują bakterie, qz – kwarc

SEM. After seven days, they were widely distributed, although present in relatively high amounts. They were 1.5 µm and rod-shaped, and as in previous observations, for inhabitation, they chose flat, rather smooth grains. As the duration of the experiment continued, the relative number and density of microbes increased. Their sizes also increased, and in many

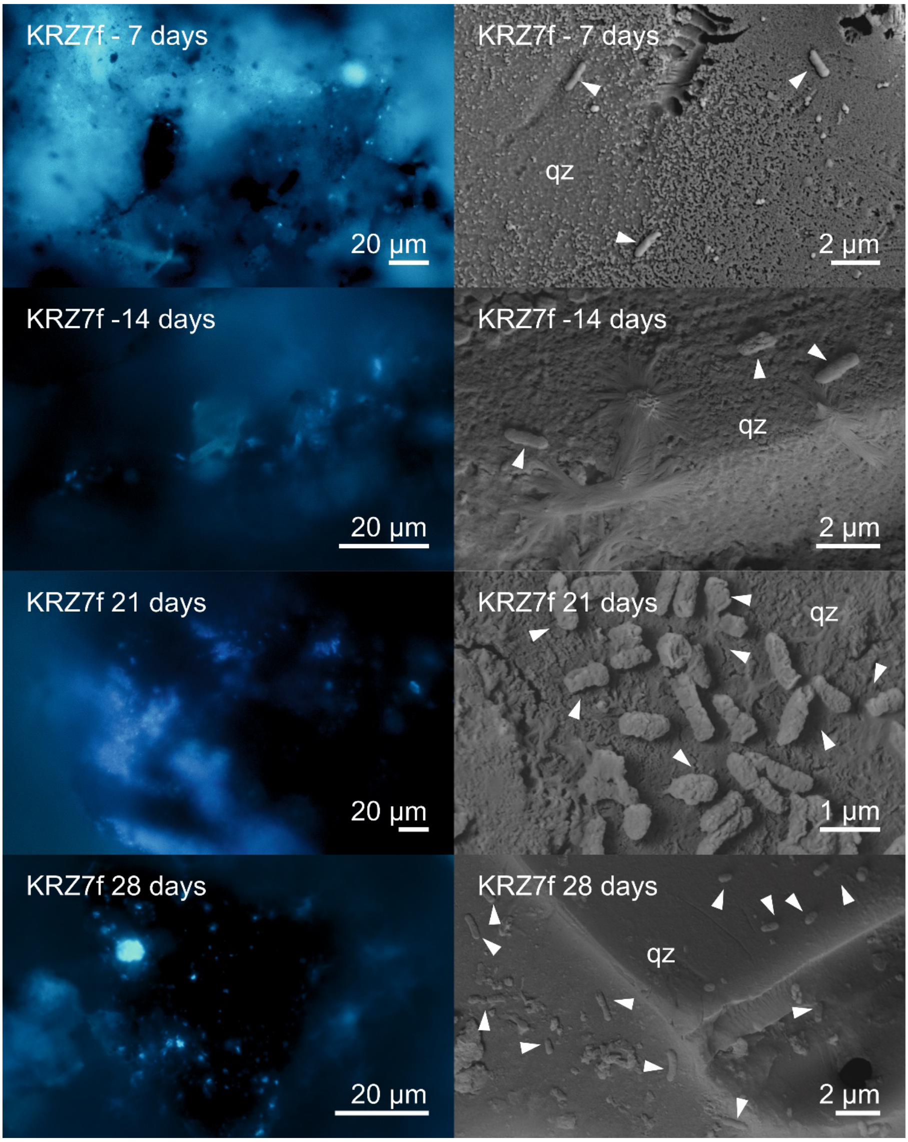

Fig. 6. Bacterial colonization of BA fragment (KRZ7-f) in FL (left side)
and in the SEM (right side) after 7, 14, 21 and 28 days; arrows indicate bacteria, qz – quartz

Rys. 6. Zasiedlanie fragmentów popiołu dennego (KRZ7-f) przez bakterie –
(lewa strona) mikroskopia fluorescencyjna, (prawa strona) mikroskopia skaningowa –
po 7, 14, 21 i 28 dniach. Strzałki wskazują bakterie, qz – kwarc

cases, sights of reproduction were seen such as in Figure 5 after twenty-one and twenty-eight days. Since bioleaching on fragments of samples is not a standard procedure, the concentrations of elements in leachate for this sample were not measured, and thus it has not been proven whether bioleaching using *A. thiooxidans* would be more efficient than in the case of powdered samples; however, taking into consideration the relative abundance of microorganisms, it may be assumed to be the case. In further studies, this measurement will also be performed. The control samples were free of microorganisms.

Chemical and mineralogical composition impacts the effectiveness of bioleaching. Low concentration of sulfur and sulfide minerals, and low solubility due to the constant alkalization of solution due to the dissolution of minerals make it impossible to obtain the required conditions for microbial growth and activity, although the pH range used in the field is in the range of 0.5–6 (Gu et al. 2017).

As suggested by Bosecker (Bosecker 1997), the highest efficiency can only be obtained when the leaching conditions represent the optimal growth conditions for bacteria. Due to the lack of knowledge on reaction kinetics, it is difficult to estimate other reasons for bioleaching efficiency in the studied samples. We may also assume that using a buffer solution to keep the acid conditions more stable could cause improvement in the efficiency of metal recovery and this will thus be further tested.

Effective use of this biotechnological solution does not need to imply that it is the bacteria that are directly responsible for bioleaching, but it is enough if they increase the effectiveness of chemical leaching by easing the maintaining of lower pH. The fact that the bacteria positively influenced the lower pH can be noticed by comparison with the blank samples.

Conclusions

The results of bioleaching using *A. thiooxidans* of FA from sewage sludge incineration and BA from municipal solid waste incineration are far from being satisfactory in contrast to many other studies where the extraction rate is much more effective. In the case of FA where the leachability for most elements was at the level of 50%, pre-washing or ash acidification would be required to obtain more satisfactory results, or an increase of the initial inoculum concentration would be required.

The failure in the case of BA can be related to the fact that the samples subjected to bioleaching were highly alkaline, and even though prior to the experiment the pH of the leachate was adjusted to provide the best conditions for bacterial growth and their metabolic activity, the dissolution of alkaline phases caused an increase in pH. Therefore, the differences in achieved results and those reported in the literature can be related to the low solubility of metals caused by the high pH of the system or/and high salts content responsible for the alkalization of the solution, thus retarding the leaching process. In the case of the BA powdered samples, pre-treatment of the samples, before bioleaching through pre-washing or acidification would generate additional cost thus, it was assumed that in the case of the samples characterized by high alkalinity, searching for bacterial or fungal strains that require high pH values for their metabolism is reasonable and will be a direction to follow in the future.

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REFERENCES

Bosecker, K. 1997. Bioleaching: metal solubilization by microorganisms. *FEMS Microbiology Reviews* 20(3–4), pp. 591–604, DOI: 10.1111/j.1574-6976.1997.tb00340.x.

Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions on the 2017 list of Critical Raw Materials for the EU. [Online:] https://ec.europa.eu/transparency/regdoc/rep/1/2017/EN/COM-2017-490-F1-EN-MAIN-PART-1.PDF [Accessed: 2017-09-13].

European Commission. Study on the EU's list of Critical Raw Materials – Final Report Brussels, Belgium, 2020.

European Commission. Report on Critical Raw Materials for the EU 2014. In Report of the Ad-Hoc Working Group on Defining Critical Raw Materials; Raw Materials Supply Group: Brussels, Belgium, 2014.

Fang, D. and Zhou, L.X. 2006. Effect of sludge dissolved organic matter on oxidation of ferrous iron and sulfur by Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans. *Water, Air, & Soil Pollution* 171, pp. 81–94, DOI: 10.1007/s11270-005-9014-9.

Fazzini et al. 2011 – Fazzini, R.A.B., Levican, G. and Parada, P. 2011. Acidithiobacillus thiooxidans secretome containing a newly described lipoprotein Licanantase enhances chalcopyrite bioleaching rate. *Applied Microbiology and Biotechnology* 89(3), pp. 771–780, DOI: 10.1007/s00253-010-3063-8.

Funari et al. 2015 – Funari, V., Salminen, J., Mäkinen, J., Revitzer, H. and Brag, R. 2015. Metal recovery from municipal solid waste incinerators fly ash by acid leaching and bioleaching. *Fifteenth International Waste Management and Landfill Symposium* S. Margherita di Pula, Cagliari, Italy; 5–9 October 2015.

Gómez-Ramírez et al. 2020 – Gómez-Ramírez, M., Moreno-Villanueva, F. and Rojas-Avelizapa, N.G. 2020. Acidithiobacillus thiooxidans DSM 26636: An Alternative for the Bioleaching of Metallic Burrs. *Catalysts* 10, DOI: 10.3390/catal10111230.

Gu et al. 2017 – Gu, X.Y., Wong, J.W.C. and Tyagi, R.D. 2017. Bioleaching of Heavy Metals From Sewage Sludge for Land Application. *Current Developments in Biotechnology and Bioengineering* 241–265, DOI: 10.1016/b978-0-444-63664-5.00011-3.

Kamizela et al. 2021 – Kamizela, T., Grobelak, A. and Worwag, M. 2021. Use of Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans in the Recovery of Heavy Metals from Landfill Leachates. *Energies* 14, DOI: 10.3390/en14113336.

Kasina et al. 2020 – Kasina, M., Kowalski, P.R., Kajdas, B. and Michalik, M. 2020. Assessment of Valuable and Critical Elements Recovery Potential in Ashes from Processes of Solid Municipal Waste and Sewage Sludge Thermal Treatment. *Resources* 9(11), pp. 131, DOI: 10.3390/resources9110131.

Kasina et al. 2021 – Kasina, M., Kajdas, B. and Michalik, M. 2021. The leaching potential of SS, and MSWI incineration ashes in terms of landfilling safety and potential reuse. *Science of the Total Environment* 791, pp. 15, DOI: 10.1016/j.scitotenv.2021.148313.

Lee et al. 2015 – Lee, E., Han, Y., Park, J., Hong, J., Silva, R.A., Kim, S. and Kim, H. 2015. Bioleaching of arsenic from highly contaminated mine tailings using Acidithiobacillus thiooxidans. *Journal of Environmental Management* 147, pp. 124–131, DOI: 10.1016/j.jenvman.2014.08.019.

Lo, K.S.L. and Chen, Y.H. 1990. Extracting heavy metals from municipal and industrial sludges. *Science of the Total Environment* 90: 99-116, DOI: 10.1016/0048-9697(90)90189-2.

Mäkinen et al. 2019 – Mäkinen, J., Salo, M., Soini, J. and Kinnunen, P. 2019. Laboratory Scale Investigations on Heap (Bio)leaching of Municipal Solid Waste Incineration Bottom Ash. *Minerals* 9(5), pp. 290, DOI: 10.3390/min9050290.

Mikoda et al. 2019 – Mikoda, B., Potysz, A. and Kmiecik, E. 2019. Bacterial leaching of critical metal values from Polish copper metallurgical slags using Acidithiobacillus thiooxidans. *Journal of Environmental Economics and Management* 236, pp. 436–445, DOI: 10.1016/j.jenvman.2019.02.032.0.

Naseri et al. 2019 – Naseri, T., Bahaloo-Horeh, N. and Mousavi S.M. 2019. Environmentally friendly recovery of valuable metals from spent coin cells through two-step bioleaching using Acidithiobacillus thiooxidans. *Journal of Environmental Economics and Management* 235, pp. 357–367, DOI: 10.1016/j.jenvman.2019.01.086.

Pathak et al. 2009 – Pathak, A., Dastidar, M.G. and Sreekrishnan, T.R. 2009. Bioleaching of heavy metals from sewage sludge: a review. *Journal of Environmental Economics and Management* 90, pp. 2343–2353, DOI: 10.1016/j.jenvman.2008.11.005.

Potysz et al. 2016 – Potysz, A., Lens, P.N.L., van de Vossenberg, J., Rene, E.R., Grybos, M., Guibaud, G., Kierczak, J. and van Hullebusch, E.D. Comparison of Cu, Zn and Fe bioleaching from Cu-metallurgical slags in the presence of Pseudomonas fluorescens and Acidithiobacillus thiooxidans. *Applied Geochemistry* 68, pp. 39–52, DOI: 10.1016/j.apgeochem.2016.03.006.

Pacholewska, M. 2003. Microbial leaching of blende flotation concentrate using Acidithiobacillus Ferrooxidans and Acidithiobacillus Thiooxidans. *Physicochemical Problems of Mineral Processing* 37, pp. 57–68

Rouchalova et al. 2020 – Rouchalova, D., Rouchalova, K., Janakova, I., Cablik, V. and Janstova, S. 2020. Bioleaching of Iron, Copper, Lead, and Zinc from the Sludge Mining Sediment at Different Particle Sizes, pH, and Pulp Density Using Acidithiobacillus ferrooxidans. *Minerals* 10(11), pp. 1013, DOI: 10.3390/min10111013.

Tyagi et al. 1988 – Tyagi, R.D., Couillard, D. and Tran, F. 1988. Heavy metals removal from anaerobically digested sludge by chemical and microbiological method, *Environmental Pollution* 50(4), pp. 295–316, DOI: 10.1016/0269-7491(88)90194-7.

Wen et al. 2012 – Wen, Y.M., Wang, Q.P., Tang, C. and Chen, Z.L. 2012. Bioleaching of heavy metals from sewage sludge by Acidithiobacillus thiooxidans – a comparative study. *Journal of Soils and Sediments* 12, pp. 900–908, DOI: 10.1007/s11368-012-0520-2.

Willner et al. 2018 – Willner, J., Fornalczyk, A., Gajda, B. and Saternus, M. 2018. Bioleaching of indium and tin from used LCD panels. *Physicochemical Problems of Mineral Processing* 54(3), pp. 639–645, DOI: 10.5277/ppmp1878.

Yang et al. 2019 – Yang, L., Zhao, D., Yang, J., Wang, W., Chen, P., Zhang, S. and Yan, L. 2019. Acidithiobacillus thiooxidans and its potential application. *Applied Microbiology and Biotechnology* 103, pp. 7819–7833, DOI: 10.1007/s00253-019-10098-5.

BIOLEACHING USING *ACIDITHIOBACILLUS THIOOXIDANS* – AN OPTION FOR ELEMENT RECOVERY FROM HIGHLY ALKALINE WASTE INCINERATION ASH?

Keywords

bioleaching, *Acidithiobacillus thiooxidans*, sewage-sludge incineration ash, municipal-waste incineration ash, waste resources

Abstract

Bioleaching research considers both the bio- and anthroposphere in the search for novel ways to recover resources and elements, which is important to the concept of sustainable development. Since the efficient, cost-effective and simple recovery of resources is of increasing importance in the circular economy model, the bioleaching of metals is a method currently gaining interest. The process is also of importance considering the need for the neutralization of waste materials/resources that allow for their safe storage and use. In this study, *Acidithiobacillus thiooxidans* bacteria, which is commonly found and widely utilized in the bioleaching process due to its high tolerance to heavy metals, was used in a twenty-eight-day experiment. The manner in which bacteria inhabit incineration residues was observed using fluorescence optical microscopy and scanning electron microscopy. The concentration of elements in incineration residues and in the post-reaction solutions was measured using inductively coupled plasma mass spectrometry and the efficiency of element recovery was calculated based on the results. Municipal waste incineration bottom ash and sewage sludge incineration fly ash were considered in the experiment. The extraction rates were far from satisfactory, with the average 20 and 50% for bottom ash and sewage sludge ash, respectively. The obtained results were consistent with microscopic observations where the relative number of bacteria increased only slightly over time in the sewage-sludge fly ash and was barely observed in the bottom ash of municipal-waste incineration.

BIOŁUGOWANIE Z WYKORZYSTANIEM *ACIDITHIOBACILLUS THIOOXIDANS* – ROZWIĄZANIE DLA ODZYSKIWANIA PIERWIASTKÓW Z WYSOKOALKALICZNYCH POPIOŁÓW ZE SPALANI ODPADÓW?

Słowa kluczowe

bioługowanie, *Acidithiobacillus thiooxidans*, popiół ze spalania osadów ściekowych, popiół ze spalania odpadów komunalnych, surowce odpadowe

Streszczenie

Badania procesów bioługowania to analiza przenikania się bio- i antroposfery, w poszukiwaniu nowych sposobów na odzyskiwanie zasobów i pierwiastków. Granica tych sfer ma duży potencjał dla wdrażania modelu zrównoważonego rozwoju. Ze względu na fakt, że efektywne, tanie oraz proste metody odzyskiwania surowców mają rosnące znaczenie w gospodarce o obiegu zamkniętym,

bioługowanie metali jest metodą, która obecnie cieszy się coraz większym zainteresowaniem. Jest to również proces istotny w kontekście neutralizacji surowców odpadowych umożliwiającej ich bezpieczne magazynowania i zastosowanie. W przeprowadzonym badaniu powszechnie występujące bakterie *Acidithiobacillus thiooxidans*, które są często używane w procesach bioługowania ze względu na fakt ich wysokiej tolerancji na metale ciężkie, zostały wykorzystane w 28-dniowym eksperymencie. Sposób i zmienność w czasie, w jaki bakterie zasiedlają osad po spalaniu, został zaobserwowany przy pomocy fluorescencyjnej mikroskopii optycznej i skaningowej mikroskopii elektronowej. Stężenie pierwiastków w osadach po spalaniu i w poreakcyjnych roztworach zostało zmierzone przy użyciu indukcyjnie sparowanej plazmowej spektrometrii mas, a efektywność odzyskiwania pierwiastków została obliczona na podstawie uzyskanych wyników. Popioły po spalaniu odpadów komunalnych oraz popioły lotne ze spalania osadów ściekowych zostały ujęte w eksperymencie. Stosunki ekstrakcji pierwiastków były dalekie od zadowalających ze średnią 20 oraz 50% dla popiołów po spalaniu odpadów komunalnych oraz popiołu z osadów ściekowych. Pozyskane wyniki są w zgodzie z obserwacjami dokonanymi przy pomocy mikroskopii, gdzie relatywna liczba bakterii wzrosła niewiele we wspomnianym czasie w przypadku popiołów ściekowych i była w zasadzie niedostrzegalna w przypadku popiołów dennych.