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Research article

Leachability of metals from waste incineration residues by iron- and sulfur-oxidizing bacteria

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ABSTRACT

Hazardous waste disposal via incineration generates a substantial amount of ashes and slags which pose an environmental risk due to their toxicity. Currently, these residues are deposited in landfills with loss of potentially recyclable raw material. In this study, the use of acidophilic bioleaching bacteria (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, and *Leptospirillum ferrooxidans)* as an environmentally friendly, efficient strategy for the recovery of valuable metals from incineration residues was investigated. Zinc, Cobalt, Copper, and Manganese from three different incineration residues were bio-extracted up to 100% using *A. ferrooxidans* under ferrous iron oxidation. The other metals showed lower leaching efficiencies based on the type of culture used. Sulfur-oxidizing cultures *A. ferrooxidans* and *A. thiooxidans*, containing sulfur as the sole substrate, expressed a significantly lower leaching efficiency (up to 50%). According to ICP-MS, ashes and slags contained Fe, Zn, Cu, Mn, Cr, Cd, and Ni in economically attractive concentrations between 0.2 and 75 mg g^{-1}. Compared to conventional hydrometallurgical and pyrometallurgical processes, our biological approach provides many advantages such as: the use of a limited amount of used strong acids (H_2SO_4 or HCl), recycling operations at lower temperatures (~30 °C) and no emission of toxic gases during combustion (i.e., dioxins and furans).

1. Introduction

Incineration is one of the most widely applied processes worldwide to reduce the volume and mass of municipal solid waste (MSW). In 2012, the global generation of MSW reached approximately 1.3 billion tons per year. With an increasing world population, the generated waste will further increase, reaching a level of around 2.2 billion tons per year in 2025 (Hoornweg and Bhada-Tata, 2012). Besides material recycling (75%), incineration is still one of the most common waste treatment strategies in the EU, accounting for approximately 70%, followed by landfilling (57%) and composting (43%) (Eurostat Waste Statistics, 2017). In this study, ashes and slags from a state-of-the-art municipal waste incineration plant (MWIP) in Austria (EVN, Dürnrohr) were investigated for microbial metal recovery. Although the waste incineration tackles the principle of waste to energy and reduces the waste volume up to 80% (Wei et al., 2011), there are still residuals like ashes and slags accounting for approximately 80–90% of the total residual mass (EVN Abfallverwertung, 2017; Zhu et al., 2018). These residues contain economically attractive concentrations of valuable metals such as Cu, Zn, Cd, Ni, Mn, and others (Table 2). However, due to their heavy metal content, ashes and slags are declared as hazardous waste (European Parliament and Council, 2008), ending most frequently up on landfills and underground disposal sites. Different attempts have been made to prevent these resources from being landfilled by using them, for example, as additives for various construction materials (Blasenbauer et al., 2020; Cristelo et al., 2020; Joseph et al., 2018). Nevertheless,

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using ashes and slags in construction still means a loss of valuable resources. Krebs et al. considered these residues as "artificial ores" for the approach of microbial recovery (Krebs et al., 1997).

In this study, the potential of chemo lithotrophic acidophiles to recover metals from waste incineration slags and ashes was investigated. Solubilization of metals by microbial action called "bioleaching" or "biomining" is an already well-established process and finds various applications in mineral industries (Banerjee et al., 2017; Schippers et al., 2013; Sethurajan et al., 2018; Werner et al., 2018). Furthermore, different studies have been made on the applicability of bioleaching to other processes like remediation of contaminated soils (Akinci and Guven, 2011), metal recovery from sewage sludge (Pathak et al., 2009) and other industrial residues (Mishra and Rhee, 2010, 2014; Solisio et al., 2002). The commonly used bioleaching bacteria are extremely acidophilic and mesophilic microorganisms (pH < 3), known to obtain energy by the oxidation of Fe(II), elemental sulfur and reduced inorganic sulfur compounds (RISCs) (Quatrini and Johnson, 2018; Rohwerder et al., 2003). Although sulfates are end products of bacterial oxidation, the combination of the degree of acidity and redox conditions represents a key factor influencing the reaction course and the mechanism of bacterial leaching of sulfide minerals. Thus, the major role of bioleaching microorganisms is the regeneration of Fe^{3+} (Eq. (1)) and the production of sulfuric acid according to equation (2) or directly from (iron) sulfides (Borilova et al., 2018). Bacterial leaching of metal oxides from solid materials (i.e., ashes and slags) is mainly facilitated by biogenic production of inorganic and organic acids and the secretion of complexing agents (Vestola et al., 2010).

$$2Fe^{2+} + 2H^{+} + 0.5O_2 \xrightarrow{\substack{A.\ ferrooxidans \\ L.\ ferrooxidans}} 2Fe^{3+} + H_2O \tag{1}$$

$$S^0 + 1.5O_2 + H_2O \xrightarrow{\substack{A.\ ferrooxidans \\ A.\ thiooxidans}} SO_4^{2-} + 2H^{+} \tag{2}$$

For the first time, three different incineration residues, arising in higher quantities during incineration such as slag, kettle-ash, and filter-ash, were assessed for bioleaching by pure cultures of iron- and sulfur-oxidizing bacteria in this study. The bioleaching efficiency was examined using pure cultures of three bacterial species: iron-oxidizing *Leptospirillum ferrooxidans,* sulfur-oxidizing *Acidithiobacillus thiooxidans*, and iron- and sulfur-oxidizing *Acidithiobacillus ferrooxidans*. The influence of various factors comprising heavy metal content, media composition, sulfur-addition, and cellular adaptation, was evaluated and the positive effect of iron-oxidizing bacteria on metal recovery was demonstrated.

2. Material and methods

All chemicals were of analytical grade unless otherwise specified and purchased from Sigma-Aldrich (Sigma-Aldrich, Vienna, Austria). Deionized water was used for preparation of cultivation media and stock solutions. Cell number determination was done by counting bacterial cells in a Neubauer improved haemocytometer with 0.01 mm depth (BRAND GmbH, Wertheim, Germany) under an Olympus BX43 optical microscope (Olympus, Hamburg, Germany). For pH measurements, a Mettler Toledo S220 pH meter with a combined glass electrode was used.

2.1. Waste incineration ashes and slags

Three different ashes and slags were obtained from a local municipal waste incineration plant (MWIP) operated by the energy producer EVN (EVN Waste Processing, Dürnrohr, Austria). The investigated site shows a capacity of around 500,000 t waste per year. After incineration of residual and hazardous waste, about 1/4 of the initial volume is remaining as so-called kettle-slag. Additionally, around 20 kg t^{-1} of ash (kettle-ash) are separated from the off-gas after incineration in the kettle. The third primary residue is filter-ash (around 30 kg t^{-1}) arising by a final filtration *via* fabric filter elements (Fig. 1) In total, around 300 kg residues per ton of incinerated waste arise, accounting for a total annual residual output of around 150,000 t (EVN Abfallverwertung, 2017). Prior to experiments, all ashes and slags were sieved to reach a particle size of less than 2.8 mm. Especially in case of slag, it was previously reported, that the concentration of extractable heavy metals is increasing within a particle size <2 mm (Abramov et al., 2018). All samples were washed three times in double-distilled water (10% w/v) and filtrated through a Whatman® cellulose filter paper with a pore size of 11 μm to remove water-soluble salts (Wang et al., 2009). Prior to use, ashes and slags were dried at 60 °C for 48 h to remove the remaining water.

2.2. Bacteria and growth conditions

Following bacteria were obtained from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ) and used in this work; *Acidithiobacillus ferrooxidans* DSM 583, *Leptospirillum ferrooxidans* DSM 2705, and *Acidithiobacillus thiooxidans* DSM 504. Ferrous iron-oxidizing *L. ferrooxidans* was cultivated in DSMZ medium 882 containing 20 g l^{-1} of ferrous sulfate heptahydrate with pH adjusted to 1.8 with sulfuric acid. Ferrous iron- and sulfur-oxidizing *A. ferrooxidans* was cultivated either in DSMZ medium 70 containing 33.3 g l^{-1} of ferrous sulfate heptahydrate with pH adjusted to 1.4 or in a basal salts medium (Wakeman et al., 2008) containing 10 g l^{-1} of elemental sulfur with an initial pH 4.5. Sulfur-oxidizing *A. thiooxidans* was cultured in DSMZ medium 35 containing 10 g l^{-1} of elemental sulfur with an initial pH 4.5. Bacteria were cultured at 30 °C in 250-ml Erlenmeyer flasks containing 90 ml of selected media and 10 ml of inoculum with shaking at 130 rpm until cell density of 1×10^8 cells ml^{-1} was reached.

2.3. Adaptation of A. ferrooxidans to slags and ashes

Ferrous iron- and sulfur-oxidizing *A. ferrooxidans* was adapted to increasing concentration of slag, kettle-ash, and filter-ash in a two-step process prior to the batch tests. First, 10 ml of ferrous iron-grown *A. ferrooxidans* culture was inoculated into 90 ml of the DSMZ 70 medium containing additionally 10 g l^{-1} of elemental sulfur and 1 g of ash/slag (1% w/v) in 250-ml Erlenmeyer flasks and incubated at 30 °C with shaking at 130 rpm. After two weeks of incubation, 10 ml culture from the first adaptation step was used to inoculate 90 ml of fresh DSMZ 70 medium containing additionally 10 g l^{-1} of elemental sulfur and 2 g of ash/slag (2% w/v) in 250-ml Erlenmeyer flasks. After further two weeks of adaptation, culture from the second adaptation step was used as an inoculum for batch tests.

2.4. Bioleaching batch tests

Batch tests with selected acidophiles were carried out in biological duplicates as previously described (Kremser et al., 2020) with some adaptations according initial media pH and substrate addition. Tests were performed in a total of five variants (Table 1 and Fig. S1). The overall pulp density in all experiments was 10 g l^{-1}. In order to determine the difference between microbial and chemical leaching, one abiotic control (BLANK) containing only medium, was performed in parallel for each mode. Furthermore, one chemical control containing 0.05 M sulfuric acid only (same sulfuric acid concentration as in the used medium) was performed for each incineration residue. All 100-ml batch tests (bacteria and abiotic controls) were allowed to leach in 250-ml Erlenmeyer flasks at 30 °C with shaking at 130 rpm for two weeks. The pH values were measured at beginning and end of each incubation. Aliquots for elemental analysis were collected at the end of the experiment. Prior to elemental analysis and pH measurement, leachates were

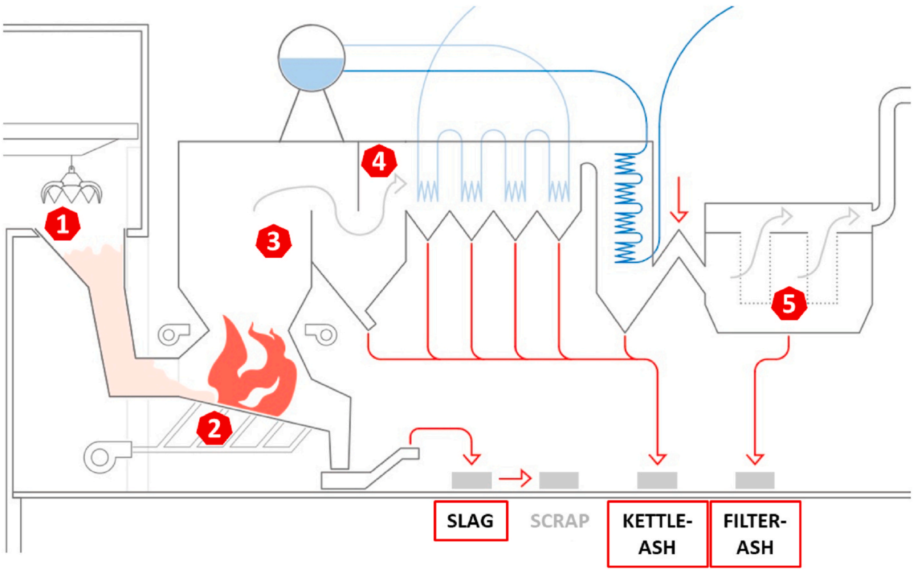

Fig. 1. Process scheme of the municipal waste incineration plant located in Dürnrohr, Austria. The waste is loaded into the incinerator (1) and incinerated in the kettle (2). The exhaust air is passing by a gravity separation step (3 and 4), followed by fabric membrane filtration (5). The main residual fraction (SLAG) is separated after incineration in the kettle, followed by the first filtration step (KETTLE-ASH) and the filtration through fabric filter elements (FILTER-ASH). The exhaust air is finally cleaned through a liquid washing procedure and ammonia (not shown) (EVN Abfallverwertung, 2017).

Table 1
Setup of batch experiments with different iron- and sulfur-oxidizing bacteria.

Organism	Initial pH	$FeSO_4$	S^0	Inoculum [% v/v]	Pulp density [g l^{-1}]
L. ferrooxidans	1.8	+	–	10	10
A. ferrooxidans	1.4	+	–	10	10
A. ferrooxidans	1.4	+	+	10	10
A. ferrooxidans	4.5	–	+	10	10
A. thiooxidans	4.5	–	+	10	10

*A more detailed description of bath experiments is shown in the supplementary material (Fig. S1).

Table 2
Metal content in three different MWIP substrate types determined by ICP-MS analysis.

Element	SLAG	KETTLE-ASH	FILTER-ASH
	c_F ± SD (mg g^{-1})	c_F ± SD (mg g^{-1})	c_F ± SD (mg g^{-1})
Fe	75.8 ± 1.3	39.01 ± 0.91	29.77 ± 0.72
V	0.058 ± 0.001	0.064 ± 0.002	0.027 ± 0.001
Cr	0.744 ± 0.015	1.105 ± 0.097	0.365 ± 0.012
Mn	1.766 ± 0.055	2.762 ± 0.077	1.201 ± 0.042
Co	0.148 ± 0.004	0.063 ± 0.002	0.061 ± 0.002
Ni	0.401 ± 0.010	0.291 ± 0.009	0.219 ± 0.005
Cu	5.81 ± 0.11	2.32 ± 0.06	8.89 ± 0.18
Zn	5.98 ± 0.10	24.32 ± 0.54	61.8 ± 1.6
Pb	1.12 ± 0.03	5.72 ± 0.24	15.2 ± 0.4
Sb	0.393 ± 0.007	2.759 ± 0.047	5.296 ± 0.069
Cd	0.007 ± 0.008	0.169 ± 0.005	0.669 ± 0.013

*Note: c_F indicates metal concentration; SD means standard deviation (n = 3).

filtrated through a CHROMAFIL® Xtra Nylon filter (Macherey-Nagel GmbH) with a pore size of 0.45 μm to remove solid particles.

2.5. Sample characterization

For ICP-MS analysis, samples of ashes and sludges were dried, grinded, and homogenized prior to decomposition. To determine elements in slags and ashes, samples were decomposed by a four-step procedure. 1 g of each sample was leached in a mixture containing nitric acid and hydrogen peroxide and evaporated to dryness. Next, samples were leached in hydrofluoric acid and evaporated to dryness, followed by leaching in perchloric acid and evaporation to dryness. Finally, samples were leached in hydrogen chloride and evaporation to dryness. Decomposed samples were afterwards analysed using inductively coupled plasma mass spectrometry (ICP-MS). To minimize the matrix effect and to get the best LOD, all samples were diluted with MiliQ water by factor 100, and a solution of Sc (400 μg l^{-1}) was used as internal standard before analysis. Equally, microbial leachates were diluted with MiliQ water by factor 100 before ICP-MS analysis. Sc, as internal standard (400 μg l^{-1}), was used to suppress the possible matrix effect. For quantification, a set of calibration solutions was prepared.

X-ray diffraction analysis was used to determine mineral phases of the used incineration residues. Samples were pulverized in a Retsch PM 100 planetary ball mill with agate grinding jar and balls, and homogenized with 10 wt% of zincite (ZnO). Zincite was used as an internal standard for the quantification of the amorphous phase. Powder X-ray diffraction (XRD) analysis was conducted using a Panalytical X'Pert PRO MPD diffractometer with reflection geometry equipped with a cobalt tube (λKα = 0.17903 nm), Fe filter, and 1-D RMTS (X'Celerator) detector. Step size: 0.017° 2Θ, time per step: 320 s, angular range: 4–100° 2Θ, total scan duration: 247 min. Acquired data were processed using Panalytical HighScore 4 plus and Bruker AXS Topas 4 software. Quantitative phase analysis was done by the Rietveld method.

To determine the particle size distribution in the slag, a separation using a stacked sieve tower was performed in order to fractionate the material into 5 fractions (I: >8.0 mm, II: 2.8–8.0 mm, III: 1.4–2.8 mm, IV: 1.0–1.4 mm and V: <1.0 mm).

2.6. Calculation of the leaching efficiency

Leaching efficiency was calculated by the ratio of the metal concentration in the leachate to the metal content of the untreated ash/slag. To minimize the effect of trace amounts of dissolved metals included in culture media and inoculum, leaching efficiency calculation equation (3) was modified as follows:

$$L_{eff}[\%] = \frac{((c_L - c_M)^*V_B) - (c_C{}^*V_C)}{c_F{}^*M_F}{}^*100 \tag{3}$$

where c_L is the metal concentration in the leachate, c_M is the metal concentration in the culture media, V_B is total batch volume, c_C is the metal concentration in the inoculum, V_C is the inoculum volume, c_F metal concentration in the untreated ash/slag, and M_F is total mass used for the leaching experiments.

2.7. *Statistical evaluation*

Results of the bioleaching efficiency under different experimental conditions were evaluated using a *t*-test. Differences between corresponding groups were detected based on duplicates. A significance level of 0.05 was used to evaluate significance of differences. To characterize a dominant effect of a defined culture and compare it with another one, the following majority criterion was applied: a significantly higher leaching effect of one bacteria culture is evident if the leaching efficiency for 4 out of 7 metals, or 5 out of 8 metals in the case of all waste materials was higher compared to another culture or blank.

3. Results and discussion

3.1. *Characterization of MWIP ashes and slags*

In a first step, the particle size distribution of the coarse slag was measured resulting in a distribution of 0.42% (Phase I), 26.34% (Phase II), 35.46% (Phase III), 15.07% (Phase IV) and 22.71% (Phase V). As mentioned in section 2.1, more extractable heavy metals are present in slag particles <2 mm. Therefore, all fractions <2.8 mm (73.2% of the total material) were used for metal analysis and bioleaching experiments. The chemical composition of the tested slag and the two ashes was measured via ICP-MS and revealed notable differences in metal content (Table 2). The predominant metal in the slag was Fe with a content of around 76 mg g^{-1}. Other precious metals such as Zn, Cu, and Ni were found in contents ranging from 0.4 to 6.0 mg g^{-1}. More toxic heavy metals such as Pb, Mn, and Cd were present in lower amounts (0.01–1.77 mg g^{-1}) compared to both types of ashes. Fe content was decreasing with every gravity separation and membrane filtration step ($76 > 40 > 30$ mg g^{-1}) resulting in the lowest concentration in the final filter-ash. A different trend can be observed on heavy metals such as Cu, Zn, Pb, and Cd. The content of these metals was increasing over the filtration process, reaching its maximum of around 8.9, 61.8, 15.2, and 0.7 mg g^{-1} for Cu, Zn, Pb, and Cd, respectively. The content of Fe and Cd in both ashes was in agreement with previous studies on waste incineration fly ashes, whereas concentrations of Cu, Zn, and Pb were significantly higher for the tested filter-ash (Krebs et al., 1999; Wang et al., 2009).

In incineration ashes and slags, metals are mainly present in their oxide forms (Funari et al., 2017; Wang et al., 2009), which was also proved by phase analysis. Mineral phase analysis revealed that minerals like quartz, hematite, calcite, magnetite, anhydride, akermanite, and gehlenite are the most abandoned amongst all three different residues but the concentration of the different minerals differs within the residues (Table 3 and Fig. S2). The results obtained are in arrangement with previous studies on mineral phases in waste incineration residues (Abramov et al., 2018).

3.2. *Bioleaching efficiency – Effect of adaptation and sulfur addition*

In order to test the effect of adaptation to ashes and slags on the bioleaching efficiency, *A. ferrooxidans* was gradually exposed to increasing MWIP substrate concentration (up to 2% w/v). During batch tests in DSMZ medium 70, the leaching efficiency was compared between the adapted culture, the same adapted culture supplemented with 10 g l^{-1} of elemental sulfur, and a non-adapted culture. Bioleaching of the slag indicated a higher efficiency in some cases using the adapted *A. ferrooxidans* cultures supplemented with elemental sulfur, in addition to ferrous iron (Fig. 2A). Metals such as V, Cu, and Zn seemed to be leached more efficiently (up to 100%). Within experimental error (using the *t*-test for the culture pairs), no significant differences between adapted and non-adapted *A. ferrooxidans* cultures were observed. Furthermore, the difference between the adapted *A. ferrooxidans* culture supplemented with elemental sulfur (in addition to ferrous iron) and any other non-adapted iron-oxidizing cultures was only insignificant ($P > 0.05$). This was detected in all tested waste materials (Fig. 2 A - C) and indicated no effect of both adaptation and elemental sulfur addition.

Table 3
XRD mineral phase analysis of the three waste incineration residues.

Mineral name	Idealized chemical formula	SLAG	KETTLE-ASH	FILTER-ASH
		[wt. %]		
Quartz	SiO_2	8.3	1.5	1.4
Cristobalite	SiO_3			1
Akermanite	$Ca_2MgSi_2O_7$	2.3		3.8
Gehlenite	$Ca_2Al(AlSi)O_7$	0.8	3.2	6.2
Magnetite	Fe_3O_4	3.7		0.6
Maghemite	γ-Fe_2O_3		1.7	0.2
Hematite	α-Fe_2O_3	0.7	3.3	2.3
Clinopyroxene	$(Ca,Na)(Mg,Fe,Al,Ti)(Si,Al)_2O_6$	1.5		
Ferrosilicon	FeSi		1.2	
Alkali feldspar	$(Na,K)AlSi_3O_8$	1.2	2.5	
Larnite	β-Ca_2SiO_4	7.3		
Sylvite	KCl		1.6	2.3
Halite	NaCl			15.8
Anhydrite	$CaSO_4$	0.6	13.5	5.1
Bassanite	$2CaSO_4 \cdot H_2O$		4.9	
Gypsum	$CaSO_4 \cdot 2(H_2O)$	1.3		
Ettringite	$Ca_6Al_2(SO_4)_3(OH)_{12} \cdot 26(H_2O)$	0.2		
Salammoniac	NH_4Cl		1.5	1.3
Calcite	$CaCO_3$	7.2	5.4	4.2
Amorphous		64.9	59.8	55.8

*Minerals in higher concentrations are written in bold letters.

It is apparent that sulfur-oxidation provided an additional energy source and acidifies the culture, which should be positive for metal extraction. The effect of sulfur addition is evident by comparing the resulting pH of the cultures at the end of the two-week incubation (Table S1). However, all the variants (including the controls) were adjusted at the beginning for acid pH and these acid values were spontaneously kept, although alkalization may be observed in case of the controls (pH 2.2–2.8). Under studied conditions, there was no significant difference between iron-oxidizing cultures ($P > 0.05$). In addition to above mentioned majority criterion to evaluate a dominant culture effect, the bacterial adaptation facilitated a more effective bioleaching of Mn and Ni independent of the elemental sulfur supplementation. High standard deviations in the leaching efficiency of some metals most likely result from the inhomogeneity and the relatively low concentration of coarse slag. With an increasing concentration, the negative impact of this effect should decrease. Zn and V could be leached from kettle-ash to a higher efficiency (~100 and 40%, respectively) by the adapted culture supplemented with elemental sulfur. The same trend could be observed for Zn in bioleaching of the filter ash (Fig. 2C).

By comparing the leaching efficiencies of the different metals for all three types of MWIP substrates, filter-ash showed the highest recovery rates for all tested metals, followed by kettle-ash and slag. One explanation for the differences in leaching efficiency might be the particle sizes of the materials. Ash particles are tiny (less than 1 mm), resulting in an increased surface area per unit volume for both chemical and enzymatic reactions. An increased leaching efficiency of the blank for most of the tested metals compared to chemical leaching indicates that leaching in medium at low pH in combination with ferrous-iron leads to a more effective metal extraction.

3.3. *Bioleaching efficiency*

Metal recovery batch experiments were performed in various media with all MWIP substrate types using the following bacteria *A. ferrooxidans*, *L. ferrooxidans*, and *A. thiooxidans*. pH values for the chemical (medium only) and biological leaching experiments were monitored, and leaching efficiencies were compared based on observed

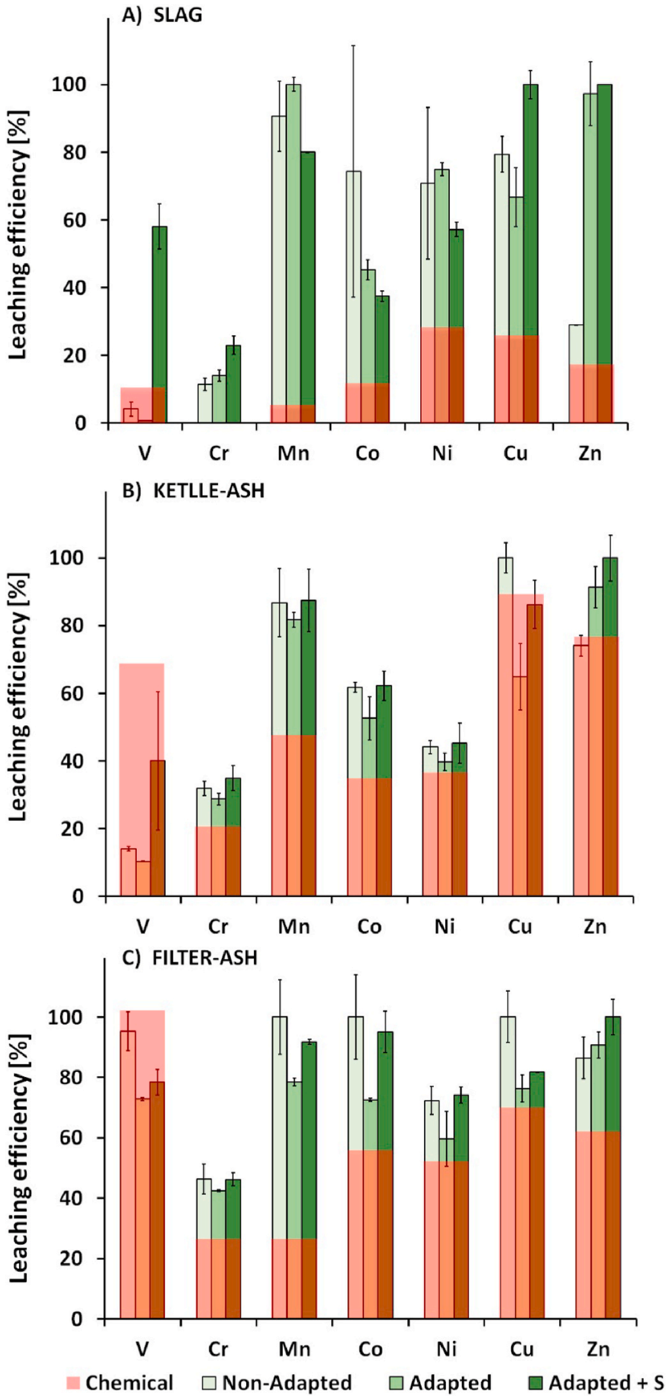

Fig. 2. Bioleaching of incineration residues with *A. ferrooxidans* – efficiencies for three types of residues, namely SLAG (A), KETTLE- (B), and FILTER-ASH (C). The amount of chemical leaching related to the sulfuric acid in the medium (red) is shown together with the three different batch tests comprising non-adapted (light-green), adapted (green), and adapted with elemental sulfur addition (dark-green). Error bars indicating the standard deviation of the biological duplicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

metal concentrations.

3.3.1. *Leptospirillum ferrooxidans*

Exclusively ferrous iron-oxidizing bacterium *L. ferrooxidans* was tested in a medium containing ferrous sulfate as the only energy source. The medium was acidified with sulfuric acid to ensure the stability of soluble ferrous iron, as in the case of *A. ferrooxidans* in the above-mentioned iron-containing medium. Therefore, chemical leaching of abiotic controls expectedly increased. *L. ferrooxidans* successfully leached Mn and Cu up to 100% of all three MWIP substrate types (Fig. 3A). Leaching efficiencies of Zn, Ni, and Cr were lower compared to *A. ferrooxidans*, and only a little difference between chemical and biological leaching was found. According the significance criteria mentioned in chapter 3.2., leaching efficiency of *L. ferrooxidans* was only insignificantly different compared to the other iron-oxidizing bacterium *A. ferrooxidans*.

The initial low pH of the medium did not result in a significant increase in pH of abiotic controls (∼2.6–3.4), which facilitated metal solubilization also in absence of bacteria (Fig. 4). *L. ferrooxidans* lacks the ability of metabolizing RISCs (Hallmann et al., 1992), leading to an elevated pH value as a result of the oxidation of ferrous iron to ferric iron. Therefore, maximum values after two weeks between 2.1 and 2.3 were reached. Although iron oxidation results in increasing of pH, a subsequent Fe^{3+} hydrolysis decreases pH. Thus, some acid solution is kept, in contrast to blank or chemical leaching, although 2.1–2.3 is higher than the pH of cultures with elemental sulfur.

3.3.2. *Acidithiobacillus ferrooxidans*

According to before mentioned significance criteria, biological leaching by *A. ferrooxidans* in medium containing both ferrous iron and elemental sulfur was significantly higher compared to both set-ups containing elemental sulfur only. Leaching efficiencies reached up to 100% for Mn, Co, Cu, and Zn, respectively (Fig. 3B and S3). Due to the presence of sulfuric acid in the medium (0.05 M H_2SO_4), chemical

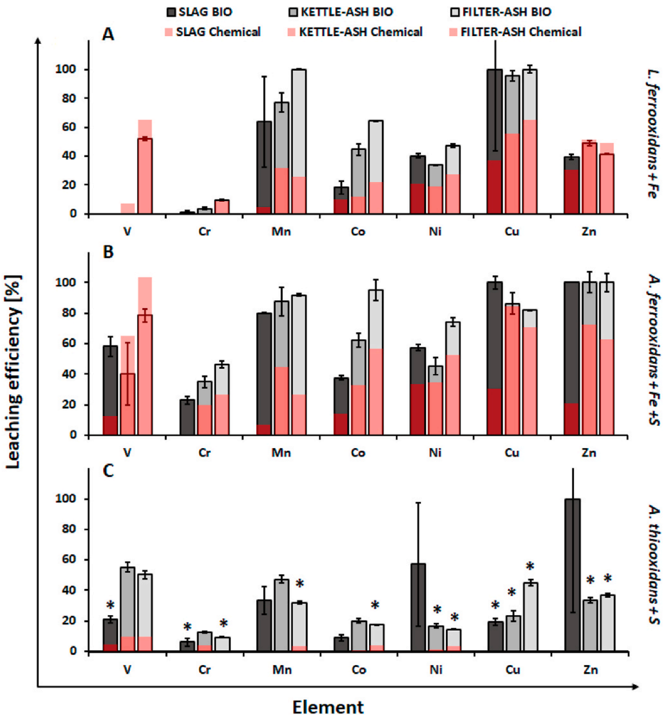

Fig. 3. Efficiency of chemical (red bars) and biological (grey bars) leaching of incineration residues by *L. ferrooxidans* in medium containing ferrous iron (A), *A. ferrooxidans* in medium containing ferrous iron and elemental sulfur (B), and *A. thiooxidans* in medium containing elemental sulfur (C). Three different types of substrates are shown in dark grey (slag), grey (kettle-ash), and light grey (filter-ash). Error bars indicate the standard deviation of the biological duplicate. Stars above the bars indicate a significant decrease ($P < 0.05$) in the metal extraction yield between *A. ferrooxidans* in medium containing ferrous iron plus elemental sulfur (B) and *A. thiooxidans* in media containing elemental sulfur only (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

leaching for most of the metals also showed good efficiencies. Most likely, Fe^{2+}2+ in the medium is chemically oxidized by either atmospheric oxygen or by the incineration residues to Fe^{3+}, which contributes to a higher solubility of other metals in an acidic metal-rich environment. It can also be observed by comparing the pH after two-week leaching (Fig. 4). pH of abiotic controls is comparatively low (~2.2–2.9) but still higher compared to bacterial cultures supplemented with ferrous iron and elemental sulfur (~1.6–1.8).

Bioleaching efficiency of *A. ferrooxidans* was further tested using elemental sulfur as only energy source. Under these conditions, bacteria oxidized elemental sulfur to produce sulfuric acid. Biological leaching efficiencies of V, Mn, Cu, and Zn reached up to 50%, whereas chemical leaching was not detectable (Fig. S3). Compared to other iron-oxidizing cultures, under studied short-term conditions, *A. ferrooxidans* utilizing elemental sulfur decreased the pH during the two-week incubation to around 1.7. In contrast, controls without bacteria reached maximum values between 6.9 and 8.4 over the same period (Fig. S4). Most metals tend to precipitate at neutral and alkaline pH (Blais et al., 2008), making the solubilization more difficult.

3.3.3. *Acidithiobacillus thiooxidans*

The predominant sulfur-oxidizing bacterium *A. thiooxidans* is known for its efficient RISCs metabolism to generate sulfuric acid (Wang et al., 2019). Therefore, all three types of MWIP substrates were leached by *A. thiooxidans* in a medium containing elemental sulfur as only energy source. Initial pH of the media in biological and abiotic control batch tests was not adjusted with sulfuric acid. It resulted in an apparent difference between chemical and biological leaching due to the absence of acid in the abiotic control and the ability of *A. thiooxidans* to produce sulfuric acid. Biological leaching efficiencies of V, Mn, Cu, and Zn reached a maximum between 37 and 55%, whereas chemical leaching was not detected (Fig. 3C). As expected, the bioleaching efficiency was in the same range as obtained by *A. ferrooxidans* with elemental sulfur as the only energy source. However, according to significance criteria, biological leaching was significantly lower for most of the tested metals compared to all iron-containing set-ups. It confirmed a fundamental role of iron and its contribution to the leaching. A comparison of final pH values demonstrated sufficient elemental sulfur oxidation by *A. thiooxidans* (Fig. 4). After two weeks of incubation, *A. thiooxidans* cultures showed the lowest pH of all batch tests (pH ~ 1.5), whereas the pH of the abiotic controls increased up to 7.5.

3.3.4. *Susceptibility of incineration residues for sulfur- and iron oxidizing bacteria*

By comparing bioleaching efficiencies of pure bacterial cultures with studies working with mixed acidophilic cultures or fungal bioleaching (Table 4), it is clearly visible that in the case of waste incineration residues (i.e. filter ash), pure cultures of iron-oxidizing bacteria were more efficient. All investigated metals, except Cr, could be leached more effectively by pure *A. ferrooxidans* or *L. ferrooxidans*, compared to *A. thiooxidans*, a mixed acidophilic culture or fungal bioleaching with *A. niger*. A possible explanation for these findings can be derived from a study by Abramov and co-workers. In their study they showed, that metals incorporated in mineral phases as mentioned in section 3.1 show an increased leachability under oxidizing and reducing conditions compared to acid extraction (Abramov et al., 2018). In case of *A. ferrooxidans* and *L. ferrooxidans*, an effective bio-oxidation of Fe^{2+} to Fe^{3+} is creating such kind of environment which furthermore leads to an increase in redox potential. A high redox potential can further facilitate the metal extraction from solid wastes (i.e. incineration residues), without being significantly affected by the added elemental sulfur (Gu et al., 2018). In contrast, solely acid generating bacterium *A. thiooxidans* is lacking an iron-oxidizing metabolism, explaining the lower metal extractions yields. Other effects, like a higher heavy metal tolerance (Cabrera et al., 2005; Navarro et al., 2013), resulting in uninhibited metabolism, and faster growth of iron-oxidizing bacteria (Bas et al., 2013) may contribute to higher leaching efficiencies of pure iron-oxidizing bacteria. Additionally, the presence and increased production of biosurfactants by *A. ferrooxdians* in iron-containing medium compared to media containing sulfur only, might contribute positively to metal extraction. Induction of biosurfactant formation in iron-oxidizing cells is considered based on interaction of iron-oxidizing metabolism with the waste materials. The positive effect of biosurfactants in bioleaching processes has been reported previously (Rangarajan and Narayanan, 2018; Sekhon et al., 2012; Shekhar et al., 2015). Our results using the tested waste materials may indicate this effect, which could be related to the metabolism of bacteria that actively oxidize iron. It could be confirmed by further research.

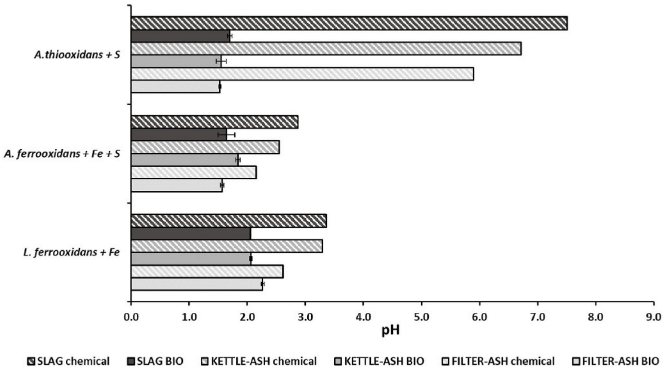

Fig. 4. Final pH of chemical (striped bars) and biological (filled bars) leaching of incineration residues by *A. thiooxidans* in medium containing elemental sulfur, *L. ferrooxidans* in medium containing ferrous iron, and *A. ferrooxidans* in medium containing ferrous iron and elemental sulfur. Three different types of substrates are shown in dark grey (slag), grey (kettle-ash), and light grey (filter-ash). Error bars indicate the standard deviation of the biological duplicate.

4. Conclusion

This study demonstrates the high potential of bioleaching for metal recovery from waste incineration residues, which usually end up in landfills or find use in construction materials. Different iron- and sulfur-oxidizing acidophiles were compared for leaching efficiency and culture medium composition. Kettle- and filter-ash showed the highest

Table 4
Comparison of bioleaching efficiencies [%] for certain metals from waste incineration fly ash. Pure bacterial cultures from the present study were compared to results obtained by a mixed acidophilic bacteria culture and bioleaching by the fungus *A. niger*.

Element	Present study			Funari[a]	Wang[b]
	L.f.	A. f.	A.t.	Mixed acidophilic bacteria	*A. niger*
	Leaching efficiency [%] ± SD			Leaching efficiency [%] ± SD	
Cu	100 ± 2.6	81.8 ± 0.1	45 ± 1.8	74 ± 12	56
Zn	41.3 ± 0.2	100 ± 5.9	36.8 ± 1.0	91 ± 0.1	62
Ni	47.1 ± 1.4	74.2 ± 2.7	14.3 ± 0.4	66 ± 11	–
Co	64.4 ± 0.2	95.1 ± 6.9	17.3 ± 0.5	55 ± 17	–
Mn	100 ± 0.4	91.8 ± 0.8	32 ± 1.0	87 ± 16	50
Cr	9.1 ± 0.5	46.3 ± 2.2	9.3 ± 0.4	63 ± 6	20
V	51.7 ± 1.1	78.4 ± 4.3	50.2 ± 2.4	52 ± 6	–

[a] (Funari et al., 2017).
[b] (Wang et al., 2009).

concentrations of valuable metals such as Cu, Cr, Mn, Zn, and Cd. Both types of substrates were leached more efficiently by all tested bacteria compared to the slag. In addition, metal extraction by iron-oxidizing bacteria was significantly higher in media containing iron in addition to sulfur. Bioleaching using only sulfuric acid produced by sulfur-oxidizing strains resulted in an efficiency of about 50% for most of the metals studied. However, the combination of a low pH and the presence of iron in the lixiviant resulted in nearly 100% efficiency for some metals and therefore appears to be optimal for applying biological leaching on waste incineration residues. Furthermore, the acidic environment, especially for long-term bioleaching period to increase the leaching efficiency, can be easily maintained by adding elemental sulfur to the medium, thereby producing sulfuric acid by strains that oxidize both iron and sulfur such as *A. ferrooxidans*. The application of iron- and sulfur-oxidizing acidophiles could significantly contribute to the recovery of economically attractive metals from the final products of the waste treatment process, as well as their decontamination and subsequent use in construction or environmentally friendly landfill.

CRediT authorship contribution statement

Klemens Kremser: Methodology, Conceptualization, Investigation, Data curation, Visualization, Writing - original draft. **Sophie Thallner:** Conceptualization, Investigation, Data curation, Writing - review & editing. **Dorina Strbik:** Investigation, Visualization, Data curation. **Sabine Spiess:** Investigation, Writing - review & editing. **Jiri Kucera:** Investigation, Data curation, Validation, Writing - review & editing. **Tomas Vaculovic:** Data curation, Visualization. **Dalibor Vsiansky:** Data curation, Visualization. **Marianne Haberbauer:** Conceptualization, Writing - review & editing. **Martin Mandl:** Formal analysis, Visualization, Writing - review & editing. **Georg M. Guebitz:** Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.111734.

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